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SCHOOL OF ANIMAL BIOSCIENCES
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Doctoral Thesis

Genetic identification of Greek bovine breeds/populations using molecular markers

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Genetic identification of Greek bovine breeds/populations using molecular markers

Γενετική ταυτοποίηση των Ελληνικών φυλών/πληθυσμών βοοειδών με τη χρήση μοριακών δεικτών

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Genetic identification of Greek bovine breeds/populations using molecular markers

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Abstract

The autochthonous cattle population of Greece is like that bred throughout the Balkan Peninsula and they consist of the Brachyceros ("Shorthorn") and the Podolian or Steppe type. In the middle of the 20th century, eight indigenous breeds of cattle were reported in Greece. Today, four of them are considered officially extinct (Tinos, Andros, Chios, Corfu), three as threatened (Brachykeros, Katerini, and Sykia), and one (Kea) as a rare breed.

During the introduction of domesticated cattle to Europe, the east Mediterranean coast and the southern Balkans played a decisive role. Greek cattle breeds originate from a geographical area near the center of domestication with a Mediterranean climate. The breeding of these breeds is characterized by the absence of performance records and thus low use of artificial selection, also by poor feeding and housing conditions as well as by the rare veterinary service. Most of these populations come from the last remains of formerly large populations and are bred mainly in mountainous areas and/or islands with poor infrastructure. Finally, these populations are reproductively isolated due to geographic distances and physical barriers without the use of artificial insemination. The Greek local cattle breeds have decreased to small numbers and are currently at risk of extinction due to socio-economic reasons, geographic isolation and crossbreeding with commercial breeds.

This study represents the first comprehensive genome-wide analysis of 11 indigenous cattle populations from continental Greece, Greek islands and Cyprus and compares them with 104 international breeds using more than 46,000 single nucleotide polymorphisms (SNPs). The following local breeds from Greece and Cyprus were sampled in our analysis: (**i**) from mainland Greece: Greek Brachyceros breed (n = 97), Katerini breed (n = 20), Prespa cattle (n = 10), Rodope cattle (n = 12), Sykia breed (n = 16), (**ii**) from the islands: Kea breed (n = 97), Agathonisi cattle (n = 6), Crete cattle (n = 11), Kastelorizo cattle (n = 4), Nisyros cattle (n = 7) and (**iii**) Cyprus cattle (n = 5).

Several parameters of genetic diversity (e.g., heterozygosity and allelic diversity) were estimated and indicated a severe loss of genetic diversity for the island populations compared to the mainland populations, which is mainly due to the declining size of their population in recent years and subsequent inbreeding. Greek Brachyceros breed present high level in almost all parameters similar with Buša and Anatolian Breeds. This high inbreeding status also resulted in higher genetic differentiation between island and mainland Greek breeds compared to the breeds from the remaining geographic groups. Supervised and unsupervised cluster analyses revealed that the phylogenetic patterns in the indigenous Greek breeds were consistent with their geographical origin and historical information regarding shared ancestry with breeds of Anatolian or Balkan origin. Greek island populations are placed close to the root of the tree as defined by Gir and the outgroup Yak, whereas the mainland breeds share a common historical origin with Buša. Unsupervised clustering and D-statistics analyses provided strong support for *Bos indicus* introgression in almost all the investigated local cattle breeds along the route from Anatolia up to the southern foothills of the Alps, as well as in most cattle breeds along the Apennine peninsula to the southern foothills of the Alps. Cyprus, Kastelorizo, and Agathonisi cattle populations showed a higher indicine ancestry compared to other populations from Greece and the Balkans.

All investigated Cyprus and Greek breeds present complex mosaic genomes, as a result of historical and recent admixture events between neighbor and well-separated breeds. While the contribution of some mainland breeds to the genetic diversity pool seems important, some island and fragmented mainland breeds suffer from a severe decline of population size and loss of alleles due to genetic drift. Conservation programs that are a compromise between what is feasible and what is desirable should focus not only on the highly diverse mainland breeds but also promote and explore the conservation possibilities for island breeds.

Scientific area: phylogenetic

Keywords: Genetic structure, diversity, Greek cattle breeds

Genetic identification of Greek bovine breeds/populations using molecular markers

Τμήμα Επιστήμης Ζωικής Παραγωγής
Εργαστήριο Γενικής και Ειδικής Ζωοτεχνίας

Περίληψη

Ο τοπικός πληθυσμός βοοειδών της Ελλάδας μοιάζει με τα βοοειδή που εκτρέφονται σε όλη τη Βαλκανική Χερσόνησο και αποτελείται από τον Βραχυκερατικό και τον Ποδολικό ή Στεππικό τύπο. Στα μέσα του 20ου αιώνα αναφέρθηκαν στην Ελλάδα οκτώ αυτόχθονες φυλές βοοειδών. Σήμερα, τέσσερις από αυτές θεωρούνται επισήμως εξαφανισμένες (Τήνου, Άνδρου, Χίου, Κέρκυρας), τρεις απειλούμενες (Βραχύκερατικής, Κατερίνης και Συκιάς) και μία (Κέας) σπάνια φυλή.

Κατά την εισαγωγή των εξημερωμένων βοοειδών στην Ευρώπη, οι χώρες της Νοτιοανατολικής Μεσογείου και τα νότια Βαλκάνια έπαιξαν καθοριστικό ρόλο. Οι ελληνικές φυλές βοοειδών προέρχονται από μια γεωγραφική περιοχή κοντά στο κέντρο της εξημέρωσης με μεσογειακό κλίμα. Η εκτροφή αυτών των φυλών χαρακτηρίζεται από την απουσία γενεαλογικών στοιχείων καθώς και καταγραφών παραγωγικών αποδόσεων και συνεπώς χαμηλή χρήση τεχνητής επιλογής. Ως επί το πλείστον διατρέφονται κατά το μεγαλύτερο μέρος του χρόνου σε υποβαθμισμένους ορεινούς βοσκοτόπους με ελλιπείς εγκαταστάσεις στέγασης καθώς και με ελάχιστη ή καθόλου κτηνιατρική φροντίδα. Οι περισσότεροι από αυτούς τους πληθυσμούς προέρχονται από τους τελευταίους απογόνους μεγάλων πληθυσμών του παρελθόντος και εκτρέφονται σε ορεινές περιοχές ή/και νησιά, κυρίως με κακές υποδομές. Τέλος, είναι αναπαραγωγικά απομονωμένοι πληθυσμοί λόγω γεωγραφικών αποστάσεων και φυσικών φραγμών χωρίς τη χρήση τεχνητής γονιμοποίησης. Οι ελληνικές τοπικές φυλές βοοειδών έχουν μειωθεί σε ελάχιστους ή πολύ μικρούς πληθυσμούς και σήμερα κινδυνεύουν να εξαφανιστούν λόγω κοινωνικοοικονομικών αιτίων, γεωγραφικής απομόνωσης καθώς και ανεξέλεγκτων διασταυρώσεων με ξένες φυλές υψηλών αποδόσεων.

Αυτή η μελέτη αντιπροσωπεύει την πρώτη ολοκληρωμένη ανάλυση σε επίπεδο γονιδιώματος 11 πληθυσμών αυτόχθονων βοοειδών από την ηπειρωτική Ελλάδα, τα ελληνικά νησιά και την Κύπρο, οι οποίοι συγκρίνονται με 104 φυλές, διεθνείς ή αυτόχθονες άλλων φυλών, σε περισσότερους από 46.000 μονονουκλεοτιδικούς πολυμορφισμούς (SNPs). Στην ανάλυσή μας ελήφθησαν δείγματα από τις ακόλουθες τοπικές φυλές από την Ελλάδα και την Κύπρο: (**i**) από

την ηπειρωτική Ελλάδα: η Ελληνική Βραχυκερατική φυλή (n = 97), η ποδολική φυλή Κατερίνης (n = 20), τα τοπικά βοοειδή Πρέσπας (n = 10), τα τοπικά βοοειδή Ροδόπης (n = 12), η ποδολική φυλή Συκιάς (SYK; n = 16), (**ii**) από τα νησιά: η φυλή Κέας (n = 97), τα τοπικά βοοειδή Αγαθονησίου (n = 6), τα τοπικά βοοειδή Κρήτης (n = 11), τα τοπικά βοοειδή Καστελόριζου (n = 4), τα τοπικά βοοειδή Νισύρου (n = 7) καθώς και (**iii**) βοοειδή Κύπρου (n = 5).

Υπολογίστηκαν αρκετές παράμετροι γενετικής ποικιλομορφίας (π.χ. ετεροζυγωτία και αλληλομορφική ποικιλομορφία), οι οποίες έδειξαν σοβαρή απώλεια γενετικής ποικιλότητας στους πληθυσμούς των νησιών σε σύγκριση με τους πληθυσμούς της ηπειρωτικής χώρας, η οποία οφείλεται κυρίως στη μείωση του μεγέθους του πληθυσμού τους τα τελευταία χρόνια και στην επακόλουθη ομομιξεία. Η ελληνική Βραχυκερατική φυλή παρουσιάζει υψηλά επίπεδα γενετικής και αλληλομορφικής ποικιλομορφίας σε όλες σχεδόν τις παραμέτρους με τιμές παρόμοιες με τις Ανατολικές φυλές και τις φυλές Buša.

Τα υψηλά επίπεδα αναπαραγωγικής απομόνωσης οδήγησαν επίσης σε υψηλότερη γενετική διαφοροποίηση μεταξύ των νησιωτικών και ηπειρωτικών ελληνικών φυλών σε σύγκριση με τις φυλές από τις υπόλοιπες γεωγραφικές ομάδες. Οι εποπτευόμενες και μη εποπτευόμενες αναλύσεις ομαδοποίησης αποκάλυψαν ότι τα φυλογενετικά πρότυπα στις αυτόχθονες ελληνικές φυλές ήταν σε συμφωνία με τη γεωγραφική τους προέλευση και τις ιστορικές πληροφορίες σχετικά με την κοινή καταγωγή με φυλές από την Ανατολή ή με φυλές βαλκανικής προέλευσης. Οι πληθυσμοί των ελληνικών νησιών τοποθετούνται κοντά στη ρίζα του δέντρου όπως ορίζεται από τα είδη Gir και Yak, ενώ οι φυλές της ηπειρωτικής χώρας μοιράζονται μια κοινή ιστορική προέλευση με την Buša. Οι μη εποπτευόμενες αναλύσεις ομαδοποίησης και η ανάλυση D-statistics παρείχαν ισχυρές ενδείξεις για την παρουσία γονιδίων του *Bos indicus* σε όλες σχεδόν τις τοπικές φυλές βοοειδών που ερευνήθηκαν κατά μήκος της διαδρομής από την Ανατολή μέχρι τις νότιες παρυφές των Άλπεων, καθώς και στις περισσότερες φυλές βοοειδών κατά μήκος της χερσονήσου των Απεννίνων προς τους πρόποδες των Άλπεων. Οι πληθυσμοί βοοειδών της Κύπρου, του Καστελόριζου και του Αγαθονησίου παρουσίασαν υψηλά επίπεδα παρουσίας του *Bos indicus*, παρόμοια με αυτά των ανατολικών φυλών.

Όλες οι φυλές της Κύπρου και της Ελλάδας που ερευνήθηκαν παρουσιάζουν ένα πολύπλοκο γονιδιωματικό μωσαϊκό ως αποτέλεσμα ιστορικών και πρόσφατων γεγονότων πρόσμιξης μεταξύ γειτονικών φυλών μιας γεωγραφικής έκτασης που εκτείνεται σε όλη την περιοχή της Νοτιοανατολικής Ευρώπης, συμπεριλαμβανομένης της Ευρωπαϊκής Ασίας. Αν και η συμβολή

ορισμένων ηπειρωτικών φυλών στη δεξαμενή γενετικής ποικιλότητας φαίνεται σημαντική, ορισμένες νησιωτικές και κατακερματισμένες φυλές της ηπειρωτικής χώρας υποφέρουν από σοβαρή μείωση του μεγέθους του πληθυσμού τους και απώλειας αλληλομόρφων λόγω της τυχαίας γενετικής παρέκκλισης.

Τα προγράμματα γενετικής διατήρησης, που θα μπορούσαν να εφαρμοστούν για τη προστασία των τοπικών φυλών, θα πρέπει να αποτελούν έναν συμβιβασμό μεταξύ του εφικτού και του επιθυμητού και να επικεντρώνονται όχι μόνο στις φυλές της ηπειρωτικής χώρας που έχουν υψηλά επίπεδα ποικιλομορφίας αλλά να διερευνούν και τις δυνατότητες διατήρησης των νησιωτικών φυλών.

Επιστημονική περιοχή: Φυλογενετική

Λέξεις κλειδιά: Γενετική δομή, ποικιλομορφία, Ελληνικές φυλές βοοειδών

Δήλωση έργου

Ο κάτωθι υπογεγραμμένος, Παπαχρήστου Δημήτριος δηλώνω ότι το κείμενο της μελέτης αποτελεί δικό μου, μη υποβοηθούμενο πόνημα. Υποβάλλεται σε μερική εκπλήρωση των απαιτήσεων για την απόκτηση Διδακτορικού του Γεωπονικού Πανεπιστημίου Αθηνών. Δεν έχει υποβληθεί ποτέ πριν για οιοδήποτε λόγο ή για εξέταση σε οποιοδήποτε άλλο πανεπιστήμιο ή εκπαιδευτικό ίδρυμα της χώρας ή του εξωτερικού.

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Chapter 1: Introduction and Background

The cultivation of plants and the domestication of animals as a process known as the "First Agricultural Revolution", was a fundamental and necessary prelude to human civilization. It is the Neolithic era in which man transforms from hunter and gatherer to settled village farmer (Bocquet-Appel, 2011). The transition from the Mesolithic to the Neolithic era is related to the climatic changes resulting from the retreat of the ice from the Last Ice Maximum (Higgs, 1964).

In human history to date, only a few species of large animals have been domesticated. Livestock domestication took place mainly in three regions: *Region 1* is the Fertile Crescent and its eastern margin, towards the Indus Valley, *region 2* is in East Asia (China and countries South of China), and *region 3* is in the Andean chain of South America (**Figure 1.1**). The domestication of goats, sheep, cattle, pigs, and buffalo was held in two Asian regions, whereas llamas and alpacas were domesticated in South America (Bruford et al., 2003).

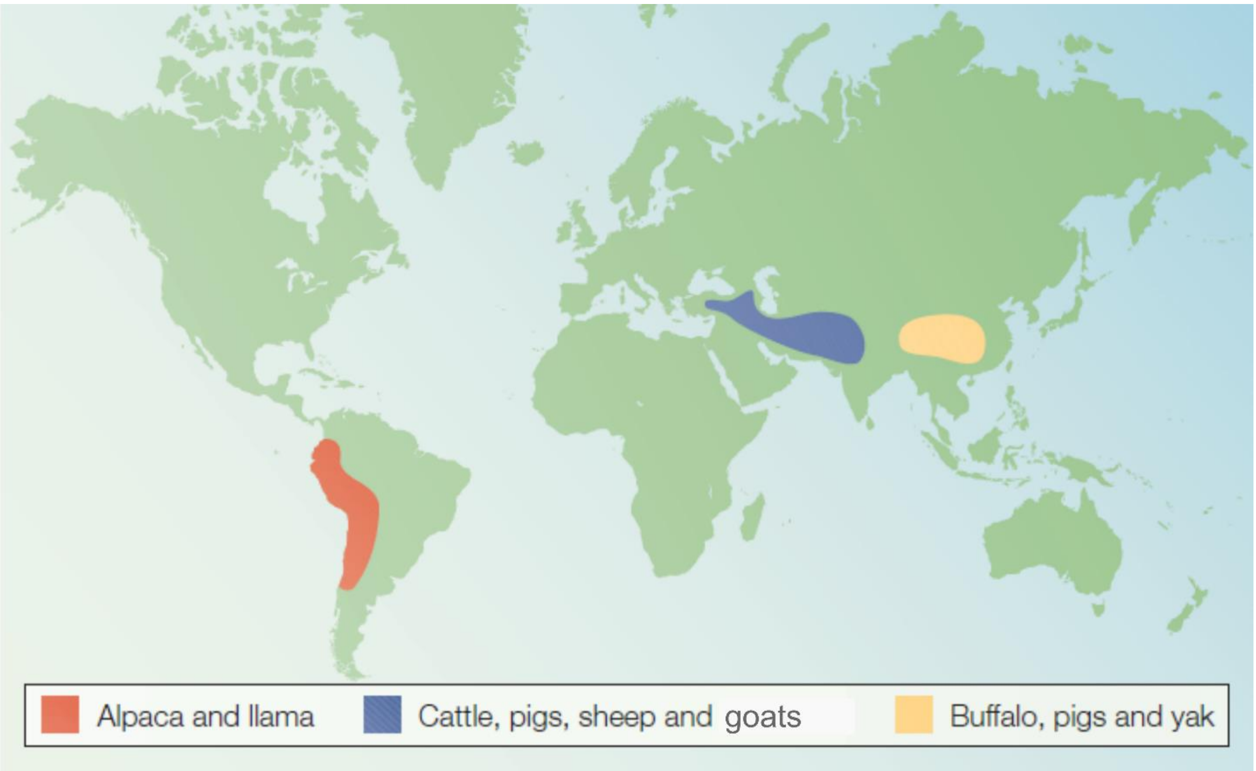


Figure 1.1. The principal centers of animal domestication. The putative global sites where ancestral populations of modern livestock were domesticated (Bruford et al., 2003).

The study of the domestication process as well as the study of livestock species' expansion contributes to our understanding of human history (Ajmone Marsann et al., 2010). This is because allowed the development of the agricultural field, which is a critical point for the development of human culture and because success in the domestication process of animals and plants facilitated a gradual change in human behavior contributing to the emergence of more complex societies (Diamond J., 2002).

1.1 Domestication, migration routes and the genomic mosaic of cattle

The genus *Bos* belongs to the subfamily Bovidae and includes several species, such as *Bos taurus* (taurine), *Bos indicus* (indicine/zebu), *Bos frontalis* (gayal), *Bos gaurus* (gaur), *Bos javanicus* (banteng), and *Bos grunniens* (yak). All these species are domesticated independently by humans. The gaur (*Bos gaurus*), American bison (*Bison bison*) and European bison known as wisent (*Bison bonasus*), are the only three extant *Bos* species that are not domesticated (Wu et al., 2018).

The ancestors of today's cattle (Taurine and Zebu or Indicine) descend from the extinct wild ox (*Bos primigenius*), known as "aurochs". The clade of *Bos primigenius* separated from ancestors of today's cattle ~ 250,000 years ago to less than 1 million years ago in South and Southwest Asia (MacHugh, 1997; Achilli, 2008). According to Zeuner (Zeuner, 1963) between the late Pleistocene and early Holocene (12,000 Years ago), the most common species of cattle was the "aurochs" (*Bos primigenius*), which ranged from Northern Africa to the coasts of Eurasia in the Atlantic and Pacific and from the northernmost tundra to India and Africa (**Figure 1.2**). Their disappearance occurred at different periods in the past. While "aurochs" became extinct in Southern Sweden around 4,500 BC, and in Portugal had probably become extinct in the Chalcolithic or Bronze Age (Castaños, 1991) in Jutland, they survived until 500 BC (Aaris- Sørensen, 1999), and the last recorded bull of this wild species died in 1627 AD in Poland (Wright, 2013).

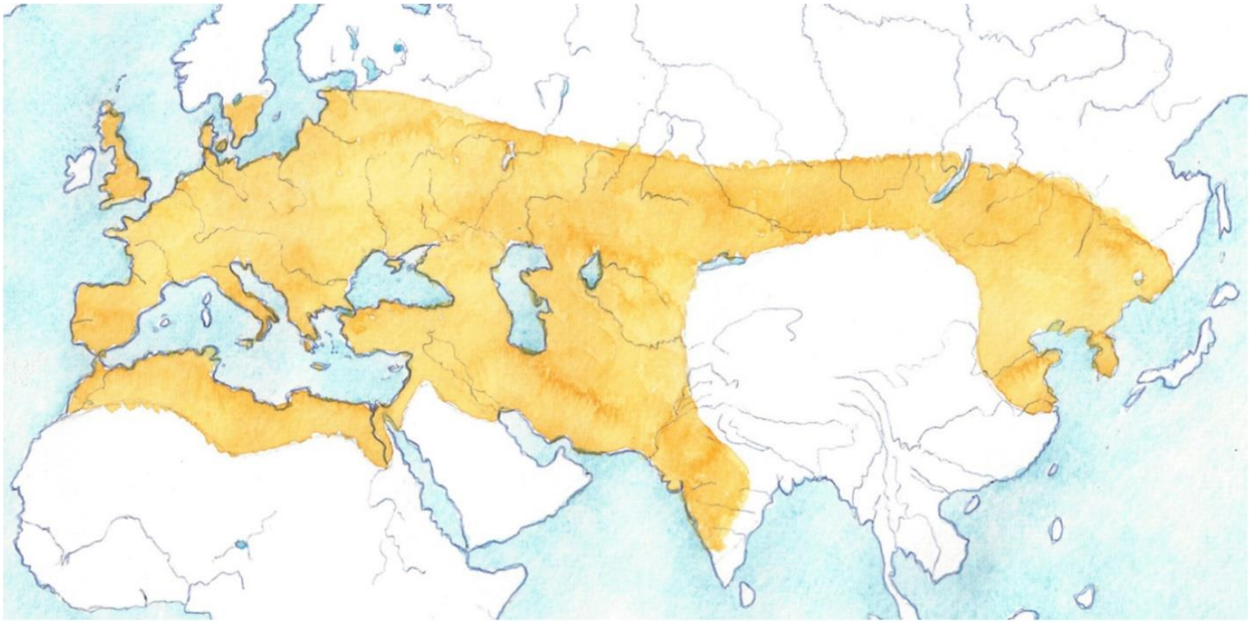


Figure 1.2. Distribution of *Bos primigenius* ca. 12,000 Years ago (Map by Marleen Felius) (Felius M. et al., 2014)

Archaeological and genomic data show that the ancestors of taurine cattle *(Bos taurus*) were domesticated from *Bos primigenius primigenius* (taurus), like goats and sheep, in the Fertile Crescent during the Neolithic period, more than 10,000 years ago (Bruford et al., 2003; Ajmone-Marsan et al., 2010; MacHugh et al., 2016). About 1,500 years later, a second domestication event took place in the Indus Valley (today's Pakistan) by *Bos primigenius nomadicus* that gave rise to the extant indicine cattle (*Bos indicus*), often also called zebu cattle (Loftus et al., 1994; Ajmone-Marsan et al., 2010). It was supposed that *B. primigenius nomadicus* ranged over the Indian subcontinent during Pleistocene and Holocene periods and that some of their populations almost certainly survived into Neolithic times to give rise to *B. indicus* (Chen et al., 2009). Mainly the presence of a hump in the acromial region and the floppy rather than upright ears of indicine cattle (Grigson, 1991) distinguish these two domesticated subspecies. Generally, indicine cattle can withstand high temperatures compared with taurine breeds (Chen et al., 2018), while European taurine cattle have been subjected to more intensive selection for milk and meat production, as well as docility and ease of handling (McTavish et al., 2013). Although a third independent domestication event of *Bos taurus* in West Africa has been debated for many years, recent comparative analyses between scenarios consistently favor a model with only two domestication events (Pitt et al., 2018).

In early pastoral societies, the Cattle species become the major source of milk, meat, and fiber while provided draught power allowing a further development of plant cultivation as well as human convoys in their constant relocations. Thus, the phylogeographic patterns of cattle genetic diversity should mirror human activities (Kidd & Cavalli-Sforza, 1974). Cattle husbandry in contrast with keeping small ruminants requires larger lands for grazing as well as work force and organization for housing, feeding, and food production. These may have contributed to the first unequal division of labor and resources in early human societies (Ajmone-Marsan et al., 2010). Finally, in China during the middle period of the Shang Dynasty (about 1450 BC), cattle gradually became the most significant animal sacrifice in ritual activities (Yuan et al., 2007).

After their domestication, both taurine and indicine cattle dispersed colonizing the world giving many populations (or breeds) locally adapted. This intense mobility is associated with growth in human population size, encouraging them to move out of domestication areas, often accompanied by cattle (Pitt et al., 2018).

The expansion of the domesticated *Bos taurus* followed western and eastern routes. Westward is a route from the Fertile Crescent through Turkey to the Balkans, Northern Italy, and Europe, either along the Danube River or the Mediterranean (coast of Dalmatia). Another westward route suggested by patterns of gene flow from African to European taurine north across the Mediterranean, particularly at the Strait of Gibraltar to the Iberian Peninsula and from Tunisia into Sicily (Cymbron et al., 1999; Beja-Pereira et al., 2006) (**Figure 1.3**).

Mitochondrial DNA (mtDNA) analysis of cattle remains from five archaeological sites in Northern China supported an eastward migration of *Bos taurus* into Northern China or Mongolia during the late Neolithic period between 5000 and 4000 YBP (years before present) (Payne & Hodges, 1997; Cai et al., 2013). The presence of taurine cattle in East Asia clearly predates the Silk Road (2000 YBP), which is considered the main axis between Europe and China and is proposed as the migration route of cattle to East Asia (Decker et al., 2014).

A similar expansion to that of *Bos taurus* also occurred with *Bos indicus* (Decker et al., 2014), with strong evidence of movements from India to China and Southeast Asia as well as Africa and the Americas. Evidence retrieved from the archaeological sites of Harappa and Mohenjo-daro indicates that domestic zebu was widespread throughout the Indus Valley region; 5,000 YBP (Fuller, 2006). MtDNA sequences surveyed from 19 Asiatic countries and archaeological data suggest that *Bos indicus* may have dispersed from the Indian subcontinent to East Asia at a later stage, from 3,500 to 2,500 YBP (Higham, 1996; Chen et al., 2010; Cai et al., 2013). The presence of cattle both taurine and indicine led the hybridization between them in Central China (Lai et al., 2006).

Archaeological and genomic data also support the indicine introgression in Anatolian cattle (Loftus et al., 1999; Decker et al., 2014). The presence of indicine genes at some European taurine breeds above the Mediterranean (Greece and South Italy) and the absence of them in Iberian Peninsula (Upadhyay & Bortoluzzi et al., 2019; Flori et al., 2019; Papachristou et al., 2020) is consistent with two separate introductions of *Bos taurus* in Europe, one from the Middle East potentially by the Romans which captured East African taurines in which indicine introgression had already occurred and the second from western Africa into Spain which included African taurines with no indicine introgression. It was this second group of cattle which likely radiated from Spain into Southern France and the Alps (Decker et al., 2014).

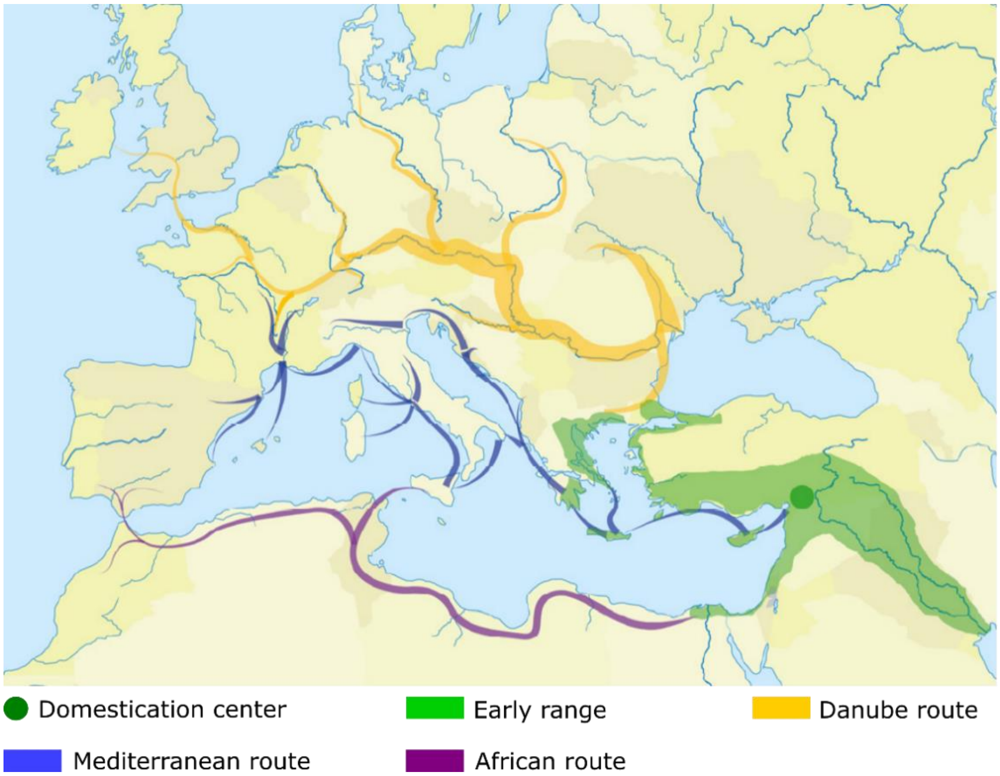


Figure 1.3. Neolithic migration of domestic cattle in Europe (Map by Marleen Felius) (Felius M. et al., 2014)

Furthermore, the cattle genomic mosaic is supplemented in many areas by admixture events between different species of the genus *Bos* as well as between local aurochs and ancestors of domesticated cattle. While it is known that there is no reproductive isolation between the taurine and zebu, the two types of cattle can be hybridized, and their offspring are completely fertile. However, studies showed that domestic cattle crossed with other *Bos* species broke reproductive isolation by hybridization or backcrossing, resulting in genetic introgression among *Bos* species (Chen et al., 2018; Wu et al., 2018). East cattle breeds highlight the contribution of other *Bos* species to this genome architecture and vice versa and may have enriched the gene pool of domestic cattle thereby helping them to adapt to local environments (Li et al., 2022). Gao et al. (2017) quantified Banteng and Gayal introgression into Southern Chinese cattle while gene introgression between the Yak and Tibetan cattle was also detected from whole-genome sequencing analysis (Wu et al., 2018) and another study quantified the proportion of bovine introgression (~1.3%) in the Yak genome (Medugorac et al., 2017).

MtDNA studies applied to samples of ancient cattle and modern *Bos taurus*, support a small initial domesticated pool (about 80 effective number of wild female aurochs) (Bollongino et al., 2012) followed by episodes of successive migrations from the Near East to Western and Northern Europe resulting in a gradual decline in genetic diversity (Troy et al., 2001; Scheu et al., 2015).

However, domesticated cattle did not entirely replace wild cattle in either their social or economic role. For example, in the geographic area known as Levant or Levante, 10,3 thousand years ago have identified both wild and domestic cattle (Horwitz et al., 1999), indicating that well-established hunting strategies persisted despite the introduction of domestic cattle. Also, as farmers settled in the regions that harbored native European aurochs, sporadic interbreeding might have taken place between domestic cattle and native European aurochs (Cubric-Curik et al., 2022), which persisted in some regions until the Middle Ages (Upadhyay & Lenstra, 2017). Park et al. (2015) using genome-wide single nucleotide polymorphisms (SNPs) identified significant enrichment of British aurochs' alleles in north European cattle breeds. In addition, Upadhyay et al. (2017) reported a high frequency of aurochs-specific-derived alleles in the Iberian cattle and northwestern European breeds and low frequency in Italian and Balkan cattle which may indicates no or very limited contact between the ancestor of these cattle breeds and the British aurochs.

As it becomes apparent, analyses of hybridization and archaeological data are revealing a richer and more complex history of modern breeds and are constantly providing new insights into the explanation of their genetic diversity as well as their genetic adaptations to different environmental conditions, including extremely cold and hot climates.

1.2 The local and "Cosmopolitan" European cattle breeds

A generally recognized factor involved in the origin of breeds is the introduction of domesticated species into new habitats and ecological niches outside their original range. Through this process, subpopulations of a species were genetically isolated from the rest and selected for their adaptability to new sets of ecological factors (Köhler-Rollefson, 1997). Beyond this well-known process, various cultural and socioeconomic processes shape and influence the genetic structure of breeds.

Several studies have shown that local breeds near the domestication center of *Bos taurus* show higher values of genetic polymorphism than the more selected breeds of Northern and Western Europe (Ramljak et al., 2018; Papachristou et al, 2020). The gradual decrease in genetic diversity as distance from the center of domestication increases confirms a sequential founder effect (Taberlet et al., 2011).

The evolution process of the European livestock breeds and especially the more "cosmopolitan" ones, from one point onwards is quite different from that of the Greek and more generally the breeds of Southeastern Europe. From the time of domestication and for about 10,000 years farmers exercised low-intensity selection, favoring the reproduction of individuals with better phenotypes. This resulted in the gradual adaptation of livestock populations to local environments, a situation which persists in much of Africa and South Asia (Felius et al., 2014). This changed abruptly about two hundred years ago with the Industrial Revolution and the intensification of animal husbandry, which led to the development of many specialized breeds with derived traits and uniform appearance. European cattlemen began forming closed herds, which they developed into breeds.

It is the era of the appearance of the "concept of breed" and genealogical books in industrialized Western countries. Since then, local populations received much stronger selection followed by standardization of morphology and performance. All animals of the same breed gradually acquired the same phenotypic and productive characteristics. More importantly, gene flow between different phenotypes (i.e., between different breeds) was severely reduced (Taberlet et al., 2011). The process of selecting certain populations for the purpose of clear production direction while marginalizing other populations concerns the whole of Europe. Initially, the areas (Northern Europe) in which this process was developed as well as in the areas (Balkans) where cattle breeding was not systematically developed with high-yielding breeds replacing the local ones (Kantanen et al., 1999; Tapio et al., 2006; Medugorac et al., 2009; Papachristou et al., 2020).

However, several local cattle breeds have retained the primitive features of their wild ancestors. Several studies (Medugorac et al., 2009; Ramljak et al., 2018) have recognized these breeds as a valuable resource of genetic variation. Most of these cattle breeds are hardier than commercial breeds and endure adverse environmental conditions and extensive management with low quality forage better (Sæther et al., 2006). On the other hand, several of these breeds have declining effective population sizes, which erode their genetic diversity (Tapio et al., 2006). Among these local breeds, many podolian or steppe breeds are seriously endangered in various European countries (Ivankovic et al., 2014; Ilie et al., 2015). Podolian cattle include a group of very ancient European breeds phenotypically close to the aurochs (*Bos primigenius*), with a grey coat color and long horns. Nowadays, significant phenotypic differences are observed between the podolian breeds. The breeds with big horns (such as Hungarian Grey, Greek Katerini, Podolsko, Slavonian Syrmian, and Maremmana) are considered as the only true podolian breeds closer to the ancient ancestors. However, some local breeds (i.e., Podolica Italiana, Turkish Grey, Greek Sykia, and other Balkan breeds) do not necessarily show long horns but maintain some distinctive podolian traits. According to many traditional notes the name “podolian” refers to a common ancestral origin in Podolia (the modern Western Ukraine). However, place of origin and timing of spread out of the source area are both debated (Zsolnai et al, 2020).

The recording and study of cattle breeds worldwide is of increasing interest with the so-called local and/or rare breeds having the largest number of unknown statuses. There are an estimated 1,423 breeds of cattle, with 159 listed as extinct and 50% of these in developing countries (FAO, 2022). However, there is a possibility that many breeds have become extinct without ever being recorded (FAO, 2000; FAO, 2022). This global trend (Taberlet et al., 2008; Medugorac et al., 2011; Ramljak et al., 2018) also broadly reflects the situation of Greek cattle local breeds (Papachristou et al., 2020). The current loss of genetic resources is not only about the extinction of traditional breeds, but also about the loss of genetic diversity within breeds. Still, with the development of artificial insemination over the past 50 years, very few males participate in breeding programs. Consequently, breeds with a worldwide distribution such as Holstein Friesian show an extremely small effective population size. This process leads to genetic drift and loss of alleles, possibly responsible for its sharp decline in fertility (Pryce et al., 2004).

Breeding and reproduction practices associated with these local breeds differ significantly from those of high-yielding breeds: (i) they are in many cases quite undifferentiated from each other; ii) they have undergone low-intensity artificial selection; (iii) there are no systematic and standardized records characteristics; (iv) pedigree records are incomplete or non-existent; (v) breeding associations are either non-existent or newly formed and are for conservation purposes only; while (v) the necessary infrastructure for such a record is rudimentary consequently; (vi) there are no classic breed patterns, but a breed is defined by common ancestry, history and local culture. Therefore, these local indigenous cattle populations of Southeast Europe do not meet all the requirements to be registered as breeds. These are populations that rarely meet the notions of phenotypic distinctiveness and homogeneity as shown by registered breeds from Western countries (Köhler-Rollefson, 1997). However, to avoid confusion between the terms breed, strain, and population, the term 'breed' only with the adjectives 'indigenous', 'local', or 'rare' or without an adjective will be used.

1.3 The formation of the Greek cattle breeds

During the introduction of domesticated cattle to Europe, the Mediterranean coast and the southern Balkans played a decisive role. From the even later phases of the Neolithic, at sites in Greece and Bulgaria there is a clear increase in the frequency of cattle use (Sampson, 2018; Conolly et al., 2012). Greece and Cyprus, located close to the center of cattle domestication (**Figure 1.3**), i.e., in the Near East, representing an important crossroad for the dispersal of human groups and their herds from Anatolia to Europe (Ripoll, 2013; Peters et al., 2014; Arbuckle et al., 2014). Historically, the Southern Balkan peninsula has been characterized by the free movement of people and animals, especially in the areas near the current borders, from almost the Neolithic period throughout Antiquity, the Roman, Byzantine and Ottoman empires to almost 40 years before today (Ripoll, 2013; Lenstra & Felius, 2014). Throughout the Balkan region, the seasonal movement of herds and people for the exploitation of pastures was a common practice for centuries (Chang, 1993; Hadjigeorgiou, 2011). Migration events enhancing gene flow between domesticated cattle populations, genetic drift, physical isolation due to geographical barriers during the above historical periods as well as low-intensity artificial selection led to the formation of well-adapted local breeds of cattle in rather marginal and harsh environments (Ripoll, 2013; Simčič et al., 2015). The indigenous cattle breeds of Southeast Europe present remarkable differences in body size, habitus, production traits, longevity and reproduction compared with most breeds of central and Northwest Europe. Regarding body size and exterior, Greek cows with wither's height varying between 95-125 cm and body weight of 200-300 kg (i.e., about half of the body weight of most high-yielding cattle breeds) are in a way comparable to the small cattle have been reported in Southeast Europe since the Late Bronze and Iron Ages (Becker, 1986). The high fitness of these small animals, which are well-adapted to the climatic and environmental constrains, is confirmed by their high reproduction ability and longevity in challenging environments.

The autochthonous cattle population of Greece is likely bred throughout the Balkan Peninsula (Hatziolos B., 1941) and was formed by the Brachyceros ("*Shorthorn*") and the podolian or steppe type. In the middle of the 20th century, eight indigenous breeds of cattle were reported in Greece. Today, four of them are considered extinct (Tinos, Andros, Chios, Corfu), three as threatened (Brachykeros, Katerini, and Sykia), and one (Kea) as a rare breed (Bizelis 2019; Domestic Animal Diversity Information System (DAD-IS).

The bulk of Brachyceros animals were found on both sides of the Pindos Mountain range and further South of it - in Epirus, in Western Greek Macedonia, in Aetoloakarnania, in the island of Kefalonia (Hatziolos, 1941), and in Peloponnese (Karantounias, 1963). Also, the Brachyceros type of animals prevailed in the Aegean islands and in Crete (Karantounias, 1963). The cattle breeds Katerini and Sykia belong to the steppe type. The most genuine representative of the steppe type in Greece is the Katerini breed which was in the plains of Thessaly and the Katerini region. The Sykia steppe breed was located mainly around Halkidiki and its body size was characterized as intermediate between the Brachyceros and Katerini breeds (Hatziolos, 1941).

As can be seen in **Figure 1.4**, the Brachyceros type in the Middle of the 20th century predominated throughout the mainland as well as in Crete as a working animal. The steppe type, as a heavier body type, was found in the plains of Thessaly and central Macedonia as a working animal too.

The improved type (purebred or crossbred animals) was located around the urban centers and was used for milk and meat production. Finally, an important role in the local economies was played by the now-extinct island breeds (Corfu, Andros, and Tinos) as well as the endangered Kea cattle. These island breeds were used for dual production purposes, for milk production, and as draft animals (Papadopoulos, 1934).

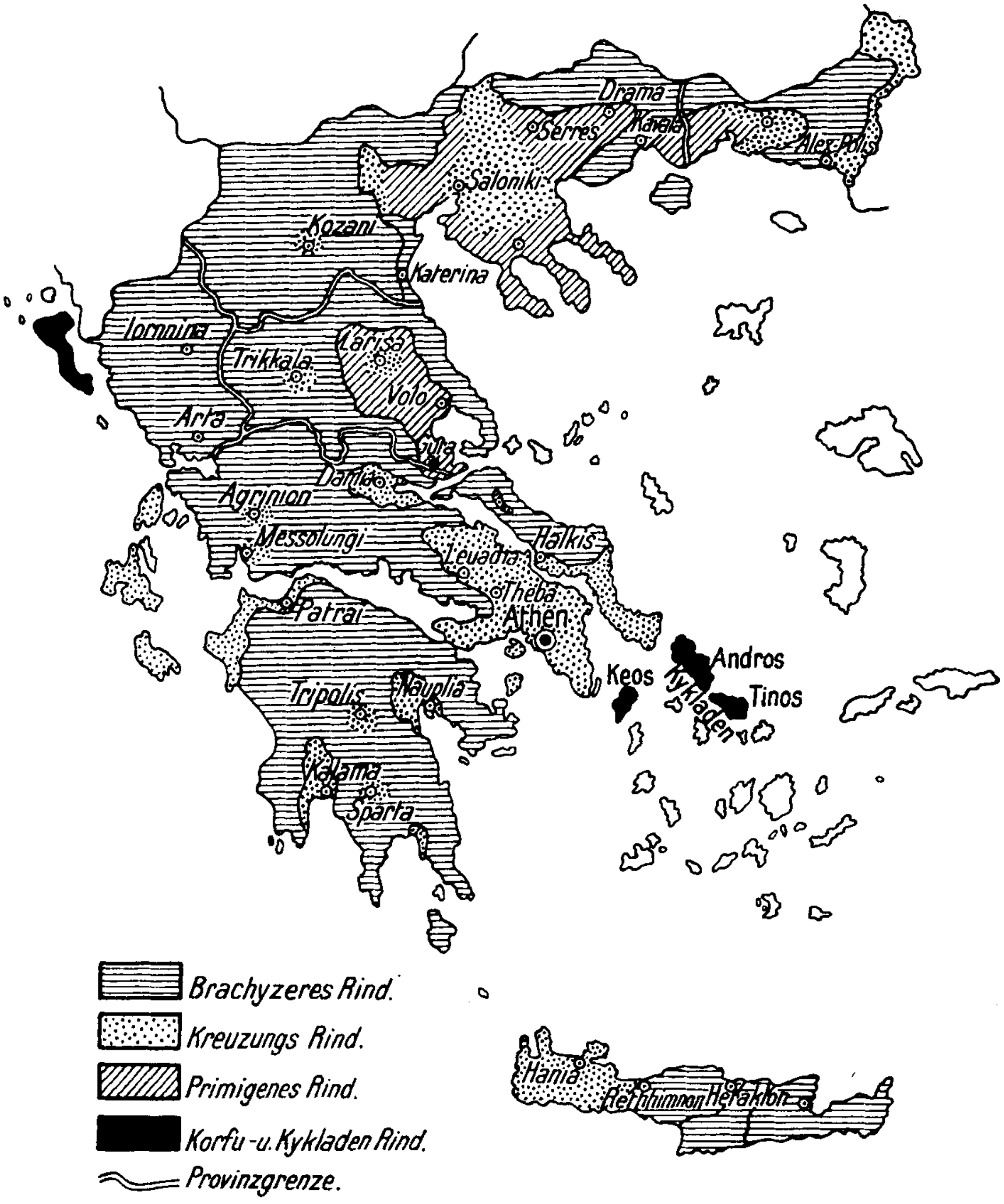


Figure 1.4. Geographical origin and distribution of indigenous breeds. In parallel lines Brachyceros type, in inclined lines the steppe type (Katerini, Sykia), in solid black the island breeds, and in dots Improved population (with imported breeds). Map by Papadopoulos (1934). Translation from German. Brachyzeres Rind: Brachyceros Cattle, Kreuzungs Rind: Crossbreeding cattle, Primigenes Rind: Primigenous cattle, Korfu-u.Kykladen Rind: Corfu and Cyclades cattle, Provinzgrenze: Provincial border

In the context of improving domestic cattle breeding yields, since 1910 the Ministry of Agriculture introduced bulls (mainly of Alpine, French, and Balkan origin) to the islands of Kea, Tinos, Andros, and Corfu as well as to the regions of Greek Macedonia - Thrace. In the Aegean islands, there are reports of crossbreeding of local animals with Alpine breeds since the time of the Venetians (Dimitriadis, 1900; Papadopoulos, 1946). Particularly, after the 1960s, with the implementation of artificial insemination in Greece and Cyprus, many indigenous populations were crossed with highly selected commercial breeds (Zervas & Boyazoglou, 1977; Constantinou, 1985; Mason, 1988). However, despite efforts to upgrade local cattle through crosses with higher-yielding breeds, their ofsprings have retained their primitive phenotype. This is due to the unfavorable geographical relief of the country, which creates unsuitable conditions for high-yielding breeds, with their spread and repeated crossing with local animals not favored. Thus, despite the repeated introduction of improved genetic material, to the extent that cattle breeding is practiced extensively, the genetic material reverts to the phenotype of the indigenous animals. Of the various foreign breeds imported only Tarentaise and Schwytz are reported to have thrived. The Bulgarian Iskar breed also thrived as a work animal (Hatziolos, 1941).

Until the World War II, the main productive direction of cattle breeding in Greece was draft power. Of the approximately 1,000,000 cattle in 1937, 50% of individuals were used as working animals, and the rest for meat and milk production. This fact is also due to the gender ratio, which was approximately 50% in females and 50% in males. Thus, in draft animals, males were calculated at 73.15% and females at 26.85%, while in animals for the purpose of milk/meat production, it was observed the reverse proportion with males at 26.6% and females at 73.4% (Statistique Annuale Agricole et d ‘elevage de la Grece, 1937).

Exact data on the ratio between indigenous breeds and others (improved and/or crossbred) do not exist. By studying various sources, some statistics about the whole cattle population could be seen. Thus, in 1966 out of the total population of 1,092,305 cattle approximately 40% were autochthonous unimproved cattle. Six years earlier (1960), out of a total of 1,074,286 cattle, approximately 72% were indigenous unimproved cattle (Karantounias, 1967). In the prefecture of Epirus in 1962, the number of cattle amounted to 56,163, of which 56.9% were indigenous unimproved (Exarchos, 1965).

In 2019 of the 530,061 cattle reared in Greece, indigenous cattle are estimated to be <1% (Hellenic Statistical Authority, 2019. www.statistics.gr). These local breeds have been in constant danger mainly since the 1970s. In fact, in some extreme cases, the current population size of some island local populations consists of only a few animals (Bizelis et al, 2021).

Official information on the population of the indigenous breeds of the Brachyceros, Sykia, and Katerini exist since 1986, which is not complete for all of them. According to Domestic Animal Diversity Information Systems (DAD-IS) the Greek Brachyceros breed reached a population of about 1,000 individuals in the early 2000s. The steppe type of autochthonous cattle faced the risk of extinction and only in recent years there seemed to be an interest in the reconstitution of the Katerini and Sykia breeds (**Figure 1.5**).

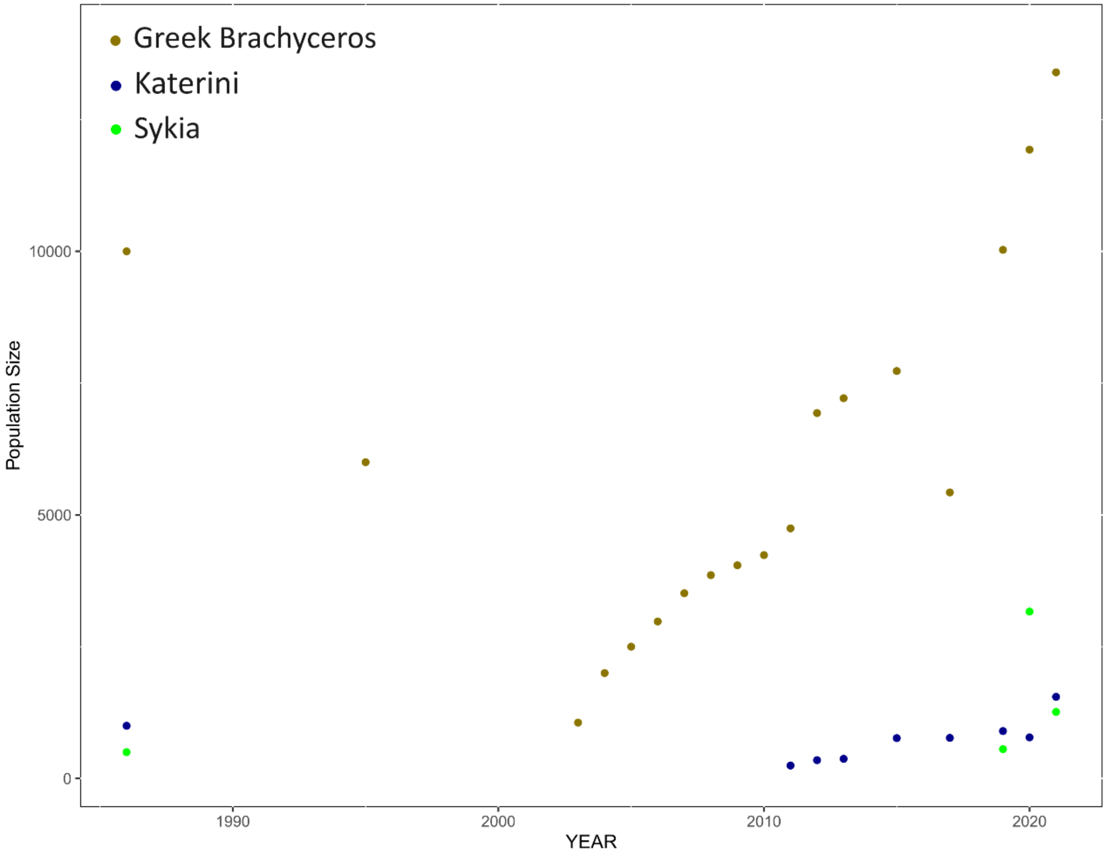


Figure 1.5. Changes in the population size of the Greek indigenous cattle populations (Brachyceros, Katerini, Sykia) from 1986 to the present.

In Greece and Cyprus, economic and social conditions, as well as geomorphological and climatic reasons did not allow the development of local high-yielding cattle populations. Local breeds were mainly raised in mountainous areas and/or in areas with poor infrastructure. The latter combined with their constant replacement by foreign breeds, led them to reproductive isolation, fragmentation, and gradual depletion of genetic diversity in these breeds (FAO, 2015; Papachristou et al., 2020). Population fragmentation is known to have deleterious consequences in the long term by increasing genetic drift and inbreeding, and by reducing fitness (Frankham et al., 2002). Thus, while in the cosmopolitan breeds of high-performance fragmentation occurs due to high intensity of artificial selection, in the local breeds of Greece, Cyprus, and in general South-Eastern Europe it occurs due to natural obstacles with parallel abandonment or replacement and crossing with foreign breeds.

1.4 SNP arrays

Recent technological advances allow affordable use of DNA arrays able to scan several thousand genome-wide markers for all the major livestock species. Advantages of SNPs as genetic markers are the availability of fast, reliable, and reproducible high-throughput or high-density genotyping protocols and substantially lower costs per data point (Lenstra et al., 2012). Such information has been used in several studies to successfully identify the complex relationships with wild populations by introgression from local aurochs into domesticated cattle, indicine introgression within *Bos taurus* breeds, the genomic divergence of *B. indicus* (zebu) cattle from the Indus Valley region, signatures of selection as well as association of allelic variants to quantitative traits in a variety of cattle breeds (Scheu et al., 2015; Bomba, 2015; Verdugo et al., 2019; Ghoreishifar et al., 2020; Schmidtmann et al., 2021).

In addition, SNPs panels enable a reliable description of the genetic diversity and population structure in cattle breeds. In addition, the growing availability of genomic tools provides the opportunity to investigate gene flow and genetic closeness among livestock populations on a molecular basis (Schmidtmann et al., 2021). Thus, valuable insights into historical breeding strategies are gained as well as to explore the potential for the estimation and conservation of livestock genetic diversity (Eusebi et al., 2020).

Finally, genetic markers (SNPs) can be used to identify the breeding strategies in breeds as well as to estimate inbreeding, which is traditionally measured through the pedigrees from genealogical books. The genomic tools can either estimate inbreeding (gametic correlation approach) with no bias or correct its values in past generations (case of ROHs), especially in cases where the recording of mating is incomplete or completely absent.

1.5 The aim of the study

Neutral and functional genetic diversity captures the role of a basic information bank that provides the long-term capacity of single species to persist in and adapt to abrupt and gradual abiotic changes (Steffen et al., 2015). This long-term capacity is of fundamental to the biosphere, but it is significantly diminished by human, mostly agricultural, activities. Therefore, agriculture is the driving force that causes degradation of complex ecosystems but, on the other hand, should provide the long-term capacity to meet the needs for food and energy in the face of continuous human population growth. The neutral and functional genetic diversity consists of the basis for sustainable development that is trimmed by various demographic, evolutionary and breeding forces in domestic species.

As mentioned earlier, these forces differ substantially and diametrically between the regions of Europe. Greek cattle breeds originate from a geographical area near the center of domestication with a Mediterranean climate. The breeding of these breeds is characterized by the absence of performance records and thus low use of artificial selection, also by poor feeding and housing conditions as well as by the rare veterinary service. Most of these populations come from the last remains of formerly large populations and are bred mainly in mountainous areas and/or islands with poor infrastructure. Finally, these populations are reproductively isolated due to geographic distances and physical barriers without the use of artificial insemination. The above description is a more or less common practice for all the indigenous breeds of Southeast Europe. On the other hand, the local breeds from central and northern Europe are far removed from the domestication center and kept in temperate climates. The breeding of these breeds is under strong artificial selection based on performance records and hosted in favorable environments managed through interventional husbandry strategies. Also, these breeds are represented by the high number of breeding animals interwoven by artificial insemination and sophisticated breeding programs. Finally, are kept in overlapping areas but are reproductively isolated by breeding organizations (pure breeding). The extent of differentiation of cattle breeds is examined here, through a dataset that includes representatives of these from across the European continent.

Studies of genetic diversity and phylogeny in recent years have been conducted on many domesticated cattle breeds (Gautier et al., 2010; Simčič et al., 2013; Rothammer et al., 2013; Ramljak et al., 2013; Simčič et al., 2015; Kukučková et al., 2017; Browett et al., 2018; Mastrangelo et al., 2018). Knowledge of the genetic diversity within and between Greek local breeds is considered a crucial issue for improving their effective use in terms of sustainable animal husbandry in harsh and less intensive farming contexts as well as the implementation of further conservation programs (Groeneveld et al., 2010). Since indigenous breeds show adaptability to their local environment and remarkable longevity, the gene pool of unselected local breeds may represent a valuable resource of genes (Medugorac et al., 2009). However, with few exceptions (Cymbron et al., 2005; Beja-Pereira et al., 2006; Lorenzo et al.,2018; Flori et al., 2019), little research has been conducted investigating the genetic diversity, genetic relationships, and ancestry of indigenous cattle from Southeast Europe relative to Greece and Cyprus.

Thus, using single nucleotide polymorphism (SNP) array technology, our objectives were: (i) to obtain unbiased estimates of the neutral genetic diversity of Greek and Cyprus cattle populations, which represents the first comprehensive genome-wide analysis for these breeds; (ii) to assess the different sources of genetic variation within breeds/populations, as well as their level of differentiation; (iii) to predict recent admixture patterns of the highly selected and competitive breeds with the unselected and heterogeneous native breeds from Greece and Cyprus; (iv) to predict the historical patterns of admixture in Greek and Cyprus cattle breeds and their path of expansion towards the southern foothills of the Alps; (v) to build an objective basis for the implementation of conservation programs for breeders' associations and national and international organizations, as solution to the uncontrolled mating and interbreeding of certain rare breeds at high risk of extinction and (vi) to identify the evolutionary forces responsible for their present structure.

1.6 Description of Greek and Cyprus local cattle breeds

Below it is presented the history, the origin, the current population situation as well as data on the breeding of the Greek breeds under study. These are five island populations (Agathonisi Cattle -AGT, Crete Cattle- CRT, Nisyros Cattle - NSY, Kastellorizo Cattle - KAS, and the Kea Breed - KEA) and five mainland breeds (Greek Bracyceros breed - GRB, Prespa Cattle - PRG, Rhodope Cattle - ROG, Sykia Breed - SYK, and Katerini Breed - KTR). From the mainland breeds, Sykia and Katerini are the representatives of the Greek steppe type, while the population of Prespa and Rhodope are strains of the Brachyceros type. In addition, an extensive historical reference is made to the Cypriot Cattle – CYP. This is a bibliographic review as well as an update on their current situation.

Finally, data are presented on the population evolution of the local Cyprus cattle (**Table 1.1**), of the Greek island populations (**Table 1.2**) as well as a table with the most important phenotypic, productive, and reproductive traits of the above breeds (**Table 1.3**).

1.6.1 Cyprus cattle (CYP)

Origin, history and sampling: The indigenous Cyprus cattle historically consisted of two distinct native types, namely the "Mesaoria" i.e., of the plain area and the "Paphos" i.e., of the mountain areas. The "Mesaoria" type was characterized by a heavy animal with a thin coat, reddish brown, brown-red skin color and whitish color on the abdomen. This type was suitable for the plain areas (Pitcairn, 1935). The Paphos type was a small animal with a rough coat with colors in all shades of brown, but black animals were also found. This type was adapted to the mountains and hills. Nowadays the above types are considered as a unique population. For the present study five samples were collected, four samples from Evrichou and one from Kambia.

Breed standards, productivity characteristics and reproduction: The indigenous Cyprus cattle are characterized by low growth rate and low productivity, while their milk production is only sufficient to feed the calf of each cow (Pitcairn, 1935). It is assumed that some local cattle were crossed in 1912 with Devon cattle, but the products of these crosses never came into favor among farmers for raising draught cattle (Bevan, 1919). Thus, the use of native cattle remained stable during time, and consisted in their exploitation as a source of power to perform agricultural work (plowing, threshing) but also to transport people and products. Because of the agricultural mechanization after the World War II, the breeding of these animals became unprofitable. Common characteristics for both types are a hump, a relatively large dewlap, a black tuft at the end of the tail and the white-gray ring surrounding the muzzle. At the bottom of the limbs and towards the base of the claws, the coat is a fading light white/yellowish color.

The animals graze on natural pastures from March to October and are fed with green barley, maize, and alfalfa, if they are available. During winter, draught animals are housed in rather primitive stables and a ration of vetches, oats and chopped straw is provided. Breeding is carried out throughout the year, but efforts are made to arrange for cows to calf during the season from January to April when green forage is plentiful. The calves suckle for 6 to 7 months (Bevan, 1919).

Breeding status: According to the official records (Cypriot Ministry of agriculture), the number of animals in 2020 was 1,244, from a former number of 27,500 in 1960 and 52,916 in 1907 (Pitcairn, 1935) (**Table 1**). These animals are distributed across 91 farms or kept by smallholders.

Figure 1.6. Cyprus cattle (photos provided by I. Bizelis)

Table 1.1. Population evolution of the Cypriot local cattle breed. The last official census took place in 1975. New censuses have been systematically carried out since 2008 (Cypriot Ministry of agriculture).

Year	**1960**	**1965**	**1970**	**1973**	**1974**	**1975**	**2008**	**2010**	**2012**	**2014**	**2016**	**2018**	**2020**
Animals	27,500	26,000	16,000	14,500	7,000	5,000	746	807	1,102	1,397	1,384	1,324	1,244

1.6.2 Greek Brachyceros breed (GRB)

Origin, history and sampling: The Greek Brachyceros breed belongs to the group of Illyrian Brachyceros cattle (*Bos taurus brachyceros*), the most common cattle in the Balkan region in the past (Keller, 1911; Karadounias, 1967). It resembles a dairy type of animal (Keller, 1909). The resistance to adverse weather conditions and their minimal nutritional needs allowed their spread throughout Greece. The hardiness of the breed allowed its widespread in the bovine population of the country. For this study, ninety-seven samples were collected from four farms: (i) Aetochori (Karditsa), (ii) Lepenou (Aetoloakarnania), (iii) Kefalonia island, (iv) Pagoneri (Drama).

Breed standards, productivity characteristics and reproduction: These are free-range cattle feeding in mountainous and semi-mountainous pastures throughout the year. Their diet is mainly based on native vegetation, and especially in winter or in adverse conditions they receive supplementary nutrition and shelter.

The overall external features suggest they are unimproved dairy type cattle. The body size is small with a small head, long neck, and small dewlap. The horns are thin with a small forward curve. The trunk is rather short, the chest and thorax have small width. The back and the loin are narrow, too. The pelvis is narrowly inclined. The udders are small and hairy. The overall development of muscles is rather limited. The coat color is variable (blond, silver-footed, dark-gray, brown, dark brown, black). The color of the muzzle, horns and hoofs is usually black and there is a white ring around the muzzle (mealy marking). The animals are well adapted and thrifty with a slow growth rate, long life expectancy and high reproductive performance. Formerly, the breed was bred primarily for work. Milk and meat productivity were of secondary importance. Nowadays they are bred almost exclusively for their meat, although the meat production capacity is small. The slaughter age is 20 months at a slaughter weight of 160–180 kg with a carcass yield of about 45%.

The calf is weaned at approximately 6 months but in many cases is completed over a long period. Milk output amounts to 500-1200 litters per lactation period, with a 4.5% fat content. Generally, it is a frugal, hardy adaptable animal with minimal demands (Bizelis et al, 2021).

With the improvement of environmental conditions (stable, nutrition) as well as through an applied systematic selection, the Brachyceros breed can be the basis for the creation of animals with a higher genetic value, useful for small farmers, especially in mountainous areas (Dimitriadis, 1933).

Breeding status: Nowadays, the breeding areas of Brachyceros cattle are the mountainous areas of Epirus, Thessaly, Aetoloakarnania, Kefalonia island and Greek Macedonia. As it has been said above, the breed is under constant pressure, just like the other Greek local breeds from the 60's onwards. In 2003, it reached 1,060 animals, but according to Centre of Animal Genetic Resources (CAGR, 2019) the total population in 2019 was 10,027 in 168 farms with 7,822 females and 1,625 males. Today its adult population is around 13,500 animals (Domestic Animal Diversity Information Systems (DAD-IS)) (**Figure 1.6**). However, the estimated purebred animals are approximately 4,000. In 2016, an Association of Brachyceros cattle breeders was created named the "Greek Shorthorn Cattle Breeders' Association" (Kazoglou, 2015; Tsaprailis & Kazoglou, 2017) with the aim of recording and monitoring the breed's population and implementing breeding programs.

Figure 1.7. Bulls and cows from a herd of Greek Brachyceros (photos by A. Tsaprailis (above) and I. Bizelis (below))

1.6.3 Greek Prespa Cattle (PRG)

Origin, history and sampling: The Greek Prespa cattle are kept in the homonymous region near the borders with Albania and North Macedonia. Based on their phenotypic characteristics, the animals are attributed to the Greek Shorthorn subtype (Greek Brachyceros). The relative isolation of the Prespa region increased because of economic and political factors that arose in the post-war period and lasted until the end of the millennium. Nevertheless, due to the cross-border nature of the Prespa area and the similarity of the Brachyceros animals found on both the Greek and Albanian sides (Buša Cattle), movements of animals between at least these two neighboring areas should not be excluded during the long or recent past as well as gene flow between the Prespa Cattle and the Greek Brachyceros breed (Kazoglou et al., 2010).

For this study, ten samples were collected: (a) seven from Agios Achilios, a small island in the Mikri Prespa lake and (b) three from Florina region.

Breed standards, productivity characteristics and reproduction: Colors vary between gray, grayish blue, and reddish or dark brown. The withers height varies between 120–125 cm for males and 95–105 cm for females, while body weight ranges from 230 to 250 kg for males and 120 to 150 kg for females. In the past, they were bred for work, milk, and meat but today, only for meat production.

Breeding status: A small population (65 to 70 individuals) is currently found near Prespa (Psarades, Agios Achilios) and in the Florina region (Kazoglou et al., 2010; Grünenfelder & Trivizaki, 2014). In 2006, 400 to 500 animals of a similar population were recorded in Albania.

Figure 1.8. Bull (left) and cow (right) of the Greek Prespa cattle (photos by I. Bizelis)

1.6.4 Greek Rhodope Cattle (ROG)

Origin, history and sampling: Greek Rhodope cattle are small shorthorn animals. Officially, they are not a recognized breed, although they are reported as a distinct population from the middle of previous century. The Brachyceros, Buša and Anatolian-Podolian types of breeds are considered to have contributed to the creation of the Rhodope breed. Psaltis (Psaltis, 1931) differentiated the population into mountain and plains forms of Greek Thrace. The mountain type was largely kept by the Pomak minority but also in the Greek villages of the Rhodope prefecture, hence its name. It was smaller than the plains' type and darker including black. Nowadays, the population has survived around the Rhodope Mountains. During the last 40 years, the breed was crossbred with European cosmopolitan breeds and became nearly extinct. The breed was used for work, milk, and meat production.

For this study, twelve samples were taken from one herd in the Lagada region, north of Thessaloniki.

Breed standards, productivity characteristics and reproduction: Greek Rodope cows have coats of red-brown to yellow color, but various shades of dark brown are also found. The bulls are of dark color, commonly black. The withers height of a cow is 100–110 cm with an average body weight ranging from 200 to 250 kg. The average body weight of a bull is around 400 kg.

Breeding status: The population is considered as threatened by extinction because there are only 250 animals in a pure herd of the original breed type.

Figure 1.9. Greek Rodope cattle (photos by I. Bizelis)

1.6.5 Sykia breed (SYK)

Origin, history and sampling: Sykia is one of the two Greek strains belonging to the steppe type. However, contrary to those of Katerini, are not typical representatives of the steppe cattle group. According to earlier reports, it is conjectured that the original population stems from crosses of indigenous podolic type cattle and the Brachyceros breed. This combination produced smaller steppe type type cattle compared to other podolic breeds but larger than Brachyceros. Its name derives from Sykia village in the Sithonia region of Chalkidiki (Hatziolos, 1931). The breed's features resulted from its adaptation to the environment and climate conditions in Chalkidiki and its ability to utilize the region's natural resources.

Samples were collected from sixteen individuals from two farms in two regions. More specifically: (a) 12 animals were sampled from village Sochos, central Macedonia and (b) 4 animals from the Serres, central Macedonia.

Breed standards, productivity characteristics and reproduction: The breed has a small body size with wither's height in cows and bulls from 106 to 116 cm and from 108 to 120 cm, respectively. The body weight of cows and bulls is 160–240 kg and 180–280 kg, respectively (Mason, 1988). The primitive appearance is obvious as the front part of the body which is more developed than the back. The animals are strong, resistant, thrifty, and well adapted to the extensive farming conditions for exploitation of the poor vegetation in the semi-mountainous areas. The horns are long, lyre-shaped, or crowned with light color at the base, while the tips are black. They are cylindrical and relatively thin, of medium length in bulls and longer in cows. The length of the outer curvature of the horn's ranges from 27 to 47 cm.

The coat color varies from silver-gray to dark black with mixed yellow-brown hair. Darker shades of hair exist on the cheeks, the neck, the front legs, the lower abdomen, and the back legs from the pelvis down. There is a lighter coloration on the ribs, the forehead, the nose, the paralumbar fossa and the rump. On the back, a dark stripe is often present. The muzzle and the hooves are black. There is a silver-grayish ring around the nose. The skin is loose in a dewlap with several folds in the bulls. The tuft of the tail is darker in color. The animals were used for work (e.g., plowing, sowing, transporting etc.). Nowadays, they are bred exclusively for meat. Although milk yield is low, additional milking in the spring can give on average 4 kg of milk per day additional to the amount consumed by the calf.

Breeding status: Until 1923, there was a breeding center for Sykia cattle, which provided animals for draught purposes throughout the peninsula of Chalkidiki including Mount Athos. At that time, the number of animals was ~13,000. After 1922, the extension of arable land and the resulting shrinking pastures led to a decrease in the number of cows in the area. According to the breeding center, only 1,330 animals were left in 1936 (Hatziolos, 1931). Improved breeds, which were better suited to intensive breeding, gradually filled the gap caused by the declining numbers of this ancient local breed, and this practice has continued until today.

Until recently, the Sykia cattle strain was considered to have disappeared. In 2005, only a few females remained in four flocks. In 2008, a small nucleus of 80 individuals was discovered in Olympiada, Chalkidiki, a farm with Sykia animals in the village of Sochos (80 individuals), a farm near Ierissos (40 individuals), and a nucleus consisting of 10 animals in Perdika (Epirus) by Amalthia. In 2019 according to data supplied by CAGR, 583 animals (516 female and 67 male), had been declared and today's adult population seems to have increased to 1,263 animals in 2021 (Domestic Animal Diversity Information Systems (DAD-IS)) (**Figure 1.5**). Nonetheless, it is estimated that the number of purebred animals does not exceed 200.

Figure 1.10. Bull (left) and cows (right) from the Sykia breed (photos by I. Bizelis (left) and P. Koutsouli (right)).

1.6.6 Katerini breed (KTR)

Origin, history and sampling: Katerini is one of the two Greek cattle strains belonging to the steppe or podolic breeds. The other one is the Sykia cattle. Some similarity is observed between Katerini cattle and drawings of cattle found in frescoes, on coins and other archaeological findings of the Minoan and Mycenaean periods (Keller, 1909). The white circle around the muzzle as well as the lyre-shaped horns are characteristic signs of a primitive breed. The breed was formed in the region of Katerini as working cattle and from there spread to many areas. The main population was reared in central Macedonia before the 1940s, while another smaller type of Katerini was restricted to Magnesia, Thessaly (Goura type) (Hatziolos, 1941). The latter population is considered as a more isolated population due to geographical boundaries.

Samples were collected from twenty individuals from two farms in two regions. More specifically: (a) 10 animals were sampled from the Anavra/Goura region (Magnesia, south Thessaly) and (b) 10 animals from the Trikala region (West Thessaly).

Breed standards, productivity characteristics and reproduction: In the past, the breed was used for working purposes. Nowadays, it is bred exclusively for its meat. Katerini cattle attain the greatest size of all Greek indigenous cattle breeds. Height at the withers for bulls is 115-125 cm and 110-120 cm for cows with average weights of 375 kg and 280 kg, respectively. The breed is medium to large sized with a more developed anterior part of the body and strong legs. The coat color is gray or gray silver, mostly dark gray to blackish. The bulls are very dark to solid black without a dorsal stripe. The animals carry long horns with a characteristic lyre shape and black tips. The conformation of the back is often defective with a notable dip between the withers and the posterior. The long tail ends in a dense tuft and the limbs are strong with black hooves.

Katerini oxen are mentioned as the best suited and strongest for work (pulling of carts, plowing etc) and known for their stamina and their frugal character. The breed is characterized as slow maturing. Females are usually bred at 20 months of age. The calf is reared for six months and usually consumes all milk. Milk production is low, estimated at 500 to 700 liters during a 6-month period. The meat is very tasty but viewed as of mediocre quality due to its toughness. Katerini cattle have a life span of 15-20 years.

Breeding status: The geographic distribution of the breed formerly included the region of Katerini and other plains of Macedonia where it is mainly crossed with other breeds and especially with Greek Brachyceros. Nowadays, its breeding area is restricted to the Thessaly plains (Trikala-Kalambaka) and the Anavra/Goura plateau in Magnesia Thessaly. According to CARG (2019), around 900 animals are recorded with 697 females and 45 males and today's adult population is 1,549 animals (Domestic Animal Diversity Information Systems (DAD-IS)) (**Figure 1.5**). However, the purebred population is currently estimated at less than 400 animals distributed in four herds (Bizelis I. et al., 2021).

Figure 1.11. Bull (left) and cows (right) of the Katerini cattle (photos by V. Lekkas)

1.6.7 Agathonisi Cattle (AGT)

Origin, history and sampling: The animals reared in Agathonisi (an island of the Dodecanese group) are considered to belong to the Greek Brachyceros type with an amalgamation of podolic (steppe) or even Anatolian breed influences (Bizelis et al., 2021). They are well adapted to the island's dry and warm conditions and poor pastures. Crossbreeding with other cattle breeds has not been reported for at least the last 30 years.

For the present study, six samples from one herd were collected on Agathonisi island.

Breed standards, productivity characteristics and reproduction: Height at the withers is about 110– 115 cm and body weight ranges from 100 to 150 kg, with bulls reaching 200 kg or more if they fed properly and cows reach 160-180 Kg. Horns usually are short to very short, with dark tips. White muzzle rings of variable intensity are present in most but not all animals. Colors are always solid without spotting and usually range from shades of red, brown-red, grey to black. The cows are not milked, and they are used only for meat production. Because of the isolation of the Agathonisi Island, it is difficult for farmers to buy animal feed and sell livestock products beyond its shores.

Breeding status: A small population was detected in August 2014, which since then has been fluctuating between 30 and 40 individuals spread between two farms in Agathonisi. On the neighboring island, Lipsi, there are also about 20 - 30 animals that originated from the same cattle of Agathonisi population.

Figure 1.12. Agathonisi cattle from homonymous island (Left and Right) and Lipsi (down) (photos by Amalthia).

1.6.8 *Kastelorizo cattle (KAS)*

Origin, history and sampling: The native cattle of Kastelorizo (Megisti island) constitute a very small population spread across three different island localities (Ahladiotis, 2015). Temporarily, 3 to 4 animals were kept on the islet of Ro. The animals have been isolated for more than 80 years.

There are multiple speculations about their origin. It is possible that the animals' ancestors were imported from the Turkish coast (Western Asia Minor). Another possibility is that they arrived from Astypalea or Kalymnos as many residents of these islands moved to Kastelorizo in the past, along with their livestock and entire households. It is likely that the current animals on the island descended from all above cattle populations. There is no mention of Kastelorizo cattle in Greek bibliographical sources as is the case with most Aegean Island cattle.

Four samples were collected from two regions of Kastelorizo island (Aheres and Avlonia).

Breed standards, productivity characteristics and reproduction: This population resembles many of the old shorthorn native cattle that were used for work, milk, and meat production (Vezzani, 1929). Height at the withers for bulls is 112 cm and 101 cm for cows with average body size of 167 cm and 147 cm, respectively. The coat color is brown, black, or grayish white with black or white rings around the eyes. The muzzle is black with a white ring. The horns are short, white with black tips turning to the front having a length of approximately 14 cm. The hooves are black, and the tail end is brownish to solid brown. The body score of all animals is poor: only three animals were assigned a score of 2 on the scale (1–5), while all other animals were assigned the lowest score of 1 on the scale. The cows are not milked but kept for their meat.

Breeding status: The population is under risk of extinction with only 4 bulls and 12 cows.

Figure 1.13. Bull (left) and a bull with cows (right) of Kastelorizo cattle (photos by Y. Achladiotis)

1.6.9 Nisyros cattle (NSY)

Origin, history and sampling: The animals were described as primitive. Their origin is essentially unknown. An often-quoted view is that they originated in Western Asia Minor and arrived with refugees in 1922. According to Manetti (1922), cattle from the western coast of Asia Minor that belonged to an unimproved steppe type of low economic value and low productivity was imported into the Archipelagos and the islands.

Seven samples of Nisyros cattle were collected for this study.

Breed standards, productivity characteristics and reproduction: In terms of size, they are considerably larger than Agathonisi cattle and appear to have stronger bodies and a squarer body outline. The height at the withers of adult cows is estimated at 120–130 cm or even reaches 140 cm. Cows usually reach 180–250 kg and bulls 250-300 Kg live weight. Slaughter weight is ~200–250 kg. Presently, they are not milked. The well-fed bulls can reach a live weight of 600 kg in 2 years. Horns are variable in length and shape. Overall, they are longer and larger/thicker compared to typical short horns but shorter than classic steppe type horns. Body color occurs in ranges from gray/black and solid black, to cinnamon, solid beige/orange, or red/orange with darker facial markings and/or stripes. Some individuals have visible (light) dorsal or eel stripes along the top line/back. Many animals have white rings around the muzzle (mealy markings/deer muzzle).

Breeding status: According to Amalthia records, the population in 2021 consists of at least 35-40 animals of both sexes in two farms.

Figure 14. Bulls and cows from a herd of Nisyros cattle (photos provided by St. Dellepiane)

1.6.10 Kea breed (KEA)

Origin, history and sampling: The name of the KEA breed derives from the island of Kea (Cyclades group). Old sources classified the breed as a shorthorn type (Keller, 1911; Hatziolos, 1941). The creation of breed, started on 1909, when crossbreeding carried out between local cows and Schwyz bulls (Papadopoulos, 1946). Until the 1930's, bulls from foreign breeds such as Simmental or Sziget were also imported to a lesser degree. The result was the formation of a breed with improved milk production, while maintaining the original characteristics of the Brachyceros breed. Their adaptability as draught animals to the harsh, rocky conditions of the island remained intact (Papadopoulos, 1946). The Kea cattle were larger than the corresponding Brachyceros in mainland Greece (Greek Brachyceros) and the Cretan type (Messaras cattle). In 1946, the total number of cattle on the island was approximately 1,200 animals. After the 1940s, Kea cattle spread to other islands of the Aegean (e.g., Kythnos, Sifnos, Paros, Naxos, etc.). In Makronissos, the Kea cattle were also well adapted to even tougher environmental conditions with poor grazing and lack of fresh water. In the 1960s, 70 animals were transported to Trifylia in the Peloponnese. During that decade, the modernization of farming in parallel with the intensive efforts to improve the milking potential of the breed led to a gradual reduction in its use. The breed no longer exploited the natural pastures and became more expensive to maintain. In the early 1970s, the above facts led to the inevitable diminishment of the pure breed and its original type.

Ninety-seven samples from six different locations (a. Kea Island, b. Makronissos island, c. Kythnos island, d. Paros Island, e. Salamina island and f. Trifylia-Peloponnese area) were collected for this study.

Breed standards, productivity traits and reproduction: The coat color fluctuates from blond to dark brown with gray-brown color prevailing. The color is solid with various gradations from light shades mixed with blond to dark or almost black. There are darker and lighter gradations depending on the part of the body and the viewed angle. A white ring encircles the muzzle. The face and the exterior of the ears are darker. Almost all animals have a light dorsal stripe along the spine. The tips of the thin and short horns and the hooves are dark-colored. The coat is thin to moderate and glossy in the summer but coarser in the less-improved animals. The animals are kept for meat and milk and spontaneous for work whereas the animals are well adapted to the island conditions of poor pastures and rocky terrain. The annual milk production is nearly 1500 liters. According to old reports, Kea cows produced on average 16 – 25 kg of milk per day immediately after calf weaning (Hatziolos, 1941; Papadopoulos., 1946).

Breeding status: Presently, there are 50 animals in Kea, a few in Kythnos, Makronissos, Trifylia and Salamis islands. The total number of Kea cattle is estimated to be less than 100.

Figure 1.15. Kea cattle in Makronissos Island (left), in Kea Island (right) (photos by I. Bizelis) and in peloponnese at 1960 (down)

1.6.11 Crete cattle (CRT)

Origin, history and sampling: These cattle represent the only currently surviving indigenous breed on the island of Crete, derived probably from an ancestral population of cattle bred in Crete for centuries. The presence of cattle from the Neolithic period is also indicated by pictorial depictions of them in a hunting scene (Younger, 1995) as well as by wall paintings and seals that represented a sport of the Minoan era where the athlete performed jumps on a bull (Ταυροκαθάψια in Greek).

Old sources (Keller, 1911) state that in 1850 the population of indigenous cattle of Crete amounted to approximately 60,000 individuals, characterizing two types of cattle: (a) the type of Messara (Messara is a plain in the Herakleion prefecture), which was improved through small-scale selection by the breeders, and (b) the mountainous shorthorn type, which is like the examined current population. Messara cattle are extinct today (a detailed report on Messara cattle can be found in Manetti (1922). In a study of 1934, Papadopoulos mentions that in Crete

45,411 cattle were counted that were reared with a primitive type of animal husbandry with improper management. As in many other regions of Greece, after the World War II, the local population was upgraded with bulls and semen of foreign breeds (mainly Jersey and Schwyz) (Settas, 1963).

For this study, eleven animals of the mountainous type were sampled from three herds in the Chania region.

Breed standards, productivity characteristics and reproduction: Raised in a dry environment with poor pastures, the Cretan cattle breed shows great adaptability and seems to be quite resistant to adverse conditions. The extensive production system that utilizes the area's pastures is ideal for these animals and economically beneficial. The breeding purpose was mainly for work. Nowadays, they are bred only for meat. According to Kalaisakis (1948), the meat production capacity of the mountain cow of Crete was equal to 110 kg and a yield of 41%.

In terms of morphological characteristics, they seem to belong to the shorthorn type. According to a recent study (Koutsouli, 2022), the following emerged. The individuals of the cattle population of Mountain Crete are small. The withers height of males (bulls) is 103.7 ± 3.9 cm and of females (cows) 106 ± 3.6 cm. Mean body weight is 218.5 ± 42.7 and 205 ± 28.5, respectively. The horns are weak, thin, short, pointing upwards, downwards, or forwards, white in color ending in black tips and in some animals, they may have a crown shape. The head is thin and ends in a small snout. The muzzle has a white ring. In addition, adult animals usually have dark rings around the eyes. The size of the head is symmetrical with the body. The body consists of well-developed muscles and is compact and long. On the back, there is a straight white line. In male animals, a small hump (bulge) is observed in the withers. The limbs are long and thin, the tail is long with the tuft reaching to the ground and the breast is small and usually hairy. The coat is short and dense. The color of the coat is dark brown, brown, or yellow-brown, as some animals tending to black are also observed. In general, the coloring is uniform (monochrome) without spots and spots. There are usually mild discolorations on various parts of the body and darker shades around the eyes, cheeks, nape, back, and limbs.

Breeding status: Today, there are 35 animals approximately, which are distributed among four small-scale breeders of which 3 are in the prefecture of Chania and one in the prefecture of Lasithi. (AMALTHIA, Network for the Protection of Greek Indigenous Farm Animals, 2018, Bizelis, personal communication). The largest herd has 15 animals. All cattle descended from a single private herd formed in 1983 with animals bought from the peninsula in West Crete.

Figure 1.16. Cow (left) and bull (right) of Crete cattle (photos by V. Lekkas (left) and I. Bizelis (right)), cattle of Crete in agricultural work using a plough (down).

Table 1.2. Evolution of bovine population sizes on the islands during the last sixty years (Hellenic Statistical Authority, Greek Payment Authority of Common Agricultural Policy (C.A.P.))

Islands		**Number of farms**				**Number of animals**						
	Year	**1951**	**1961**	**1991**	**2001**	**1951**	**1961**	**1991**	**2001**	**2011**	**2016**	**2021**
Agathonisi		-	-	4	3	-	-	14	30	23	18	40
Crete		26,280	20,061	402	226	40,374	35,058	2,670	2,207	1,683	1,821	3,236
Kastelorizo (Megisti)		-	-	6	3	-	-	46	26	18	18	10
Kea		865	987	91	132	1,745	2,544	669	1,504	652	1,165	823
Kythnos		-	-	125	54	-	-	688	309	52	92	103
Nisyros		-	-	16	26	-	-	251	590	159	332	214
Paros		1,012	742	106	221	2,279	1,857	415	1,533	1,100	1,029	907

Table 1.3. Phenotypic, productive, and reproductive traits of Greek local breeds.

Traits		Cattle populations/Breeds										
		CYP	AGT	CRT	NSY	GRB	KAS	KEA	KTR	PRG	ROG	SYK
Phenotypic traits	Withers height (cm)		110- 115	104,9	123	90 - 110		126.4				
	Male			106	125		112.5		123.5	120-125	100-110	108-120
	Female			103,7	122		101.6		113.5	95-105	103-113	106 - 116
	Stature (cm)											
	Body length (cm)			146,9				142.3				
	Body weight, 12 months weight (kg)											
	Male	342	200 - 240	218,5	250 -300	300	167	500	375	230 -250	400	375
	Female	307	160 - 180	205	180 -250	200 - 220	147	300	280	120 - 150	230	280
	Birth weight (kg)											
	Male	31				14 - 16	13					
	Female	30					11					
	Chest girth (cm)							166				151
	Chest depth (cm:					54		63.6	60.4			60.2
	Rump length (cm)							46.7				39.9
Productive traits	Lactation (days)	Usually not milked			Usually not milked	180	Usually not milked	150 - 270	180			180
	Milk yield (kg/year)					500 – 1200		1500	500-700			500
	Fat content (%)					4.5		3.7				
	Protein content (%)											
	Carcass weight (kg)					160 -180		130-180	130-180			130 - 180
	Dressing percentage (%)					45%						
	Other characteristics	Suitable for work, resistance, longevity, adaptability			Resistance, longevity, adaptability, easy calving	Resistance, longevity, adaptability, easy calving	Resistance, longevity, adaptability, easy calving	Suitable for work, resistance, longevity, adaptability, easy calving	Suitable for work, resistance, longevity, adaptability, easy calving			Suitable for work, resistance, longevity, adaptability
Reproductive traits	Sexual maturity (months)	15				12		12-15				12-15
	Age of mating (months)					18		17-20				17-20
	Fertility (calves/year)				1	1		1	1			1
	Breeding time (years)											
	Lifetime (years)					20-25		15 - 20	15-20		15-20	15-20
	Economic maturity											
	Conservation	Endangered-maintained			Endangered	Endangered-maintained	Endangered	Endangered-maintained	Endangered-maintained			Endangered-maintained
	Estimated number of pure-bred individuals	1384	60	35	40	4000 -5000	20	100	400	90	250	200

Chapter 2. Genetic Analyses

2.1 Sampling

To the present study, hair or blood from 285 individuals from 10 indigenous Greek populations as well as the Cypriot Cattle were sampled. The sampling areas are illustrated in **Figure 2.1** and each breed are described in detail in **Chapter 1.6**. The following local breeds from Greece and Cyprus were sampled in our analysis: (**i**) from mainland Greece: Greek Brachyceros breed (GRB; n = 97), Katerini breed (KTR; n = 20), Prespa cattle (PRG; n = 10), Rodope cattle (ROG; n = 12), Sykia breed (SYK; n = 16), (**ii**) from the islands: Kea breed (KEA; n = 97), Agathonisi cattle (AGT; n = 6), Crete cattle (CRT; n = 11), Kastelorizo cattle (KAS; n = 4), Nisyros cattle (NSY; n = 7) and (**iii**) Cyprus cattle (CYP; n = 5). The samples of the present study were completed by whole-genome genotypes for GRB (n = 19) and CYP (n = 9) reported by Flori et al., 2019 and SYK cattle (n = 5) reported by Verdugo et al., 2019 (**Table 2.1**).

Additionally, for comparison purposes, genetic information of 104 international breeds based on genetic, historical, and geographic criteria was included. The sampling area of each breed is shown in **Figure 2.2**. More specifically, the large dataset of the above-selected breeds belonged to eight main geographic groups (Minor Asia, Southeast Europe, East Podolian, Tyrrhenian (Apennin-Sicily-Sardinia-Corse), Alpine, France, Iberian, and Northwest European breeds) plus an outgroup that included Gir (GIR), Yak (YAK) and N'Dama (NDA).

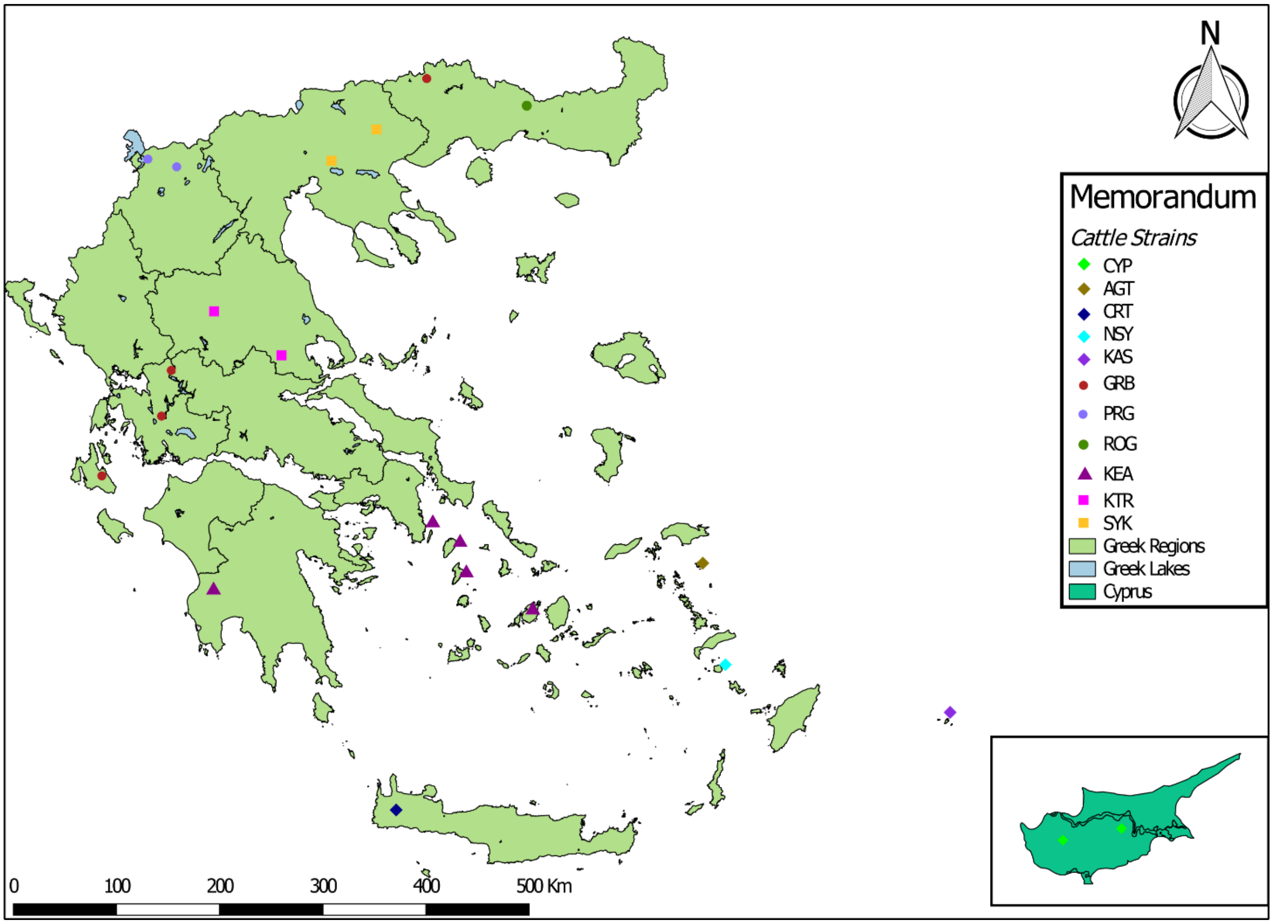


Figure 2.1. Sampling area of the Greek indigenous cattle populations as well as the Cypriot cattle.

All the above breeds were included in this study because the geographical origin of some of them have obvious geographical proximity to Greece and Cyprus or because according to various literature reports (see **Chapter 1: introduction**), they have influenced the genetic pool of local breeds from Greece and Cyprus through long-term interbreeding events in the past and probably to this day. In addition, the creation of an increasingly complete data set (from as many breeds as possible covering a large geographical area) reveals in each case the actual demographic scenarios that have contributed in the past or recently to the present levels of diversity of each breed.

For example, the long-term crossbreeding of indigenous shorthorn cattle (GRB) and some breeds from the Alpine group (eg, OBV, BBV and TGV) led to the formation of the KEA breed. However, the GRB and the shorthorn Buša cattle of the neighboring Balkan countries (Southeastern European group) probably have the same origin. Katerini (KTR) and Sykia (SYK) breeds are assumed to share a common ancestry with breeds of the Eastern Podolia steppe cattle geographic group. In addition, the AGT and NSY strains are assumed to share ancestry with some Podolian steppe or/and eastern origin populations, while CYP and KAS may have a common Anatolian and Zebu origin.

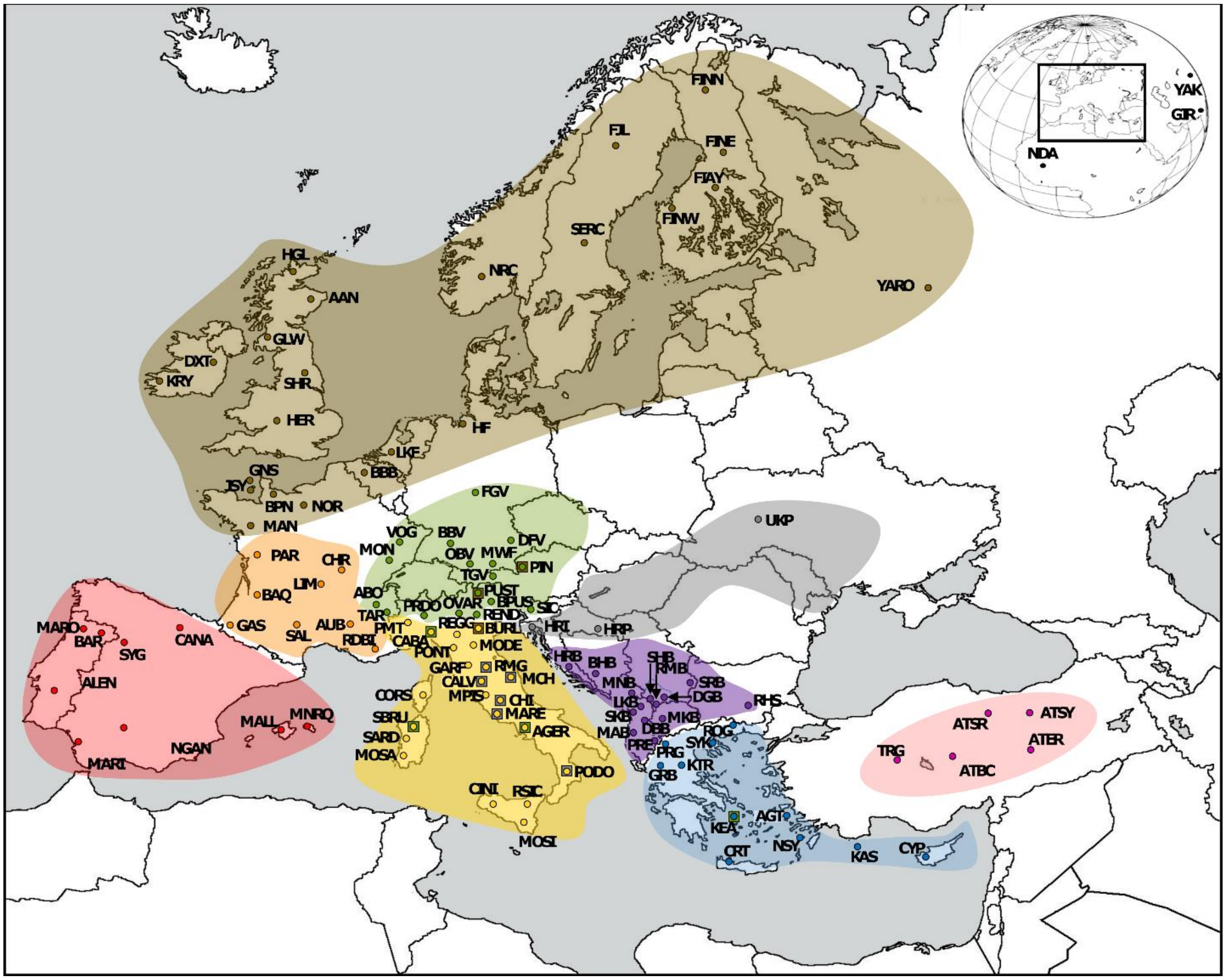


Figure 2.2. Origin of the breeds used in the analyzed dataset. Special square marks represent the influence of East-Podolian (grey), Alpine (green) and North-West (olive green) groups.

In addition to *Bos taurus* breeds originating from Europe and Asia Minor, YAK populations from Mongolia, NDA from West Africa representing African *Bos taurus*, and GIR originating from India, but they are raised in Brazil representing *Bos indicus* cattle, were used as outgroups in phylogenetic analyses. These 115 breeds from 10 geographic breed groups participating in our analysis are described in **Table 2.1**. The grouping of breeds was chosen to be displayed by geographic origin (color-coded by geographic group). Considering previous literature reports of various breeds and to indicate some known genetic similarities with other breeds or groups, a symbol was added to them to improve visualization. More specifically, these symbols combine the color of the group from which they are geographically descended with the color of the group with which they have some known genetic similarity. Thus the BURL breed (from the Tyrrhenian group) and the PUST and PIN (from the Alpine group) display an additional symbol with a color indicating a known influence from the Northwest group, the KEA breed (from Greece) and CABA, AGER and SBRU (from the Tyrrhenian group) show an extra color indicating influence from the Alpine group and some local breeds from Italy (RMG, MCH, CALV, CHI, MARE and PODO) show an extra color which indicates possible Podolian influence (Appuhn, 2010).

In all the following analyses when the foreign breeds are included, the populations always take the color of **Figure 2.2**, while if only the Greek breeds are included, they take the color and symbol of **Figure 2.1**.

2.2 DNA isolation and SNP's quality control

DNA isolation was performed using a commercial kit (QIAamp DNA MiniKit, QIAGEN) according to the manufacturer's instructions. For SNP genotyping, the Illumina BovineSNP50 BeadChip array was used following standard procedures (http://www.illumina.com).

Quality control was applied to obtain high quality data. Quality control for genomic data is about removing individuals and markers with little information. Thus, individuals with missing genotypes, more than 5%, were removed. Regarding markers, quality control is based on the frequency of alleles as well as the amount of missing data. Markers with a low frequency (MAF<0.02) on the minor allele usually do not provide sufficient information and in some cases are considered monomorphic and thus excluded. In addition, SNPs that were genotyped in a percentage less than a limit of the samples (call rate < 90%) were removed. Finally, SNP's that were mapped to unknown or breed chromosomes according to the *Bos taurus* genome assembly UMD 3.1 (http://www.cbcb.umd.edu/research/bos_taurus_assembly.shtml#1) and that deviate from Hardy – Weinberg equilibrium within breed (P ≤0.01) were excluded. Thus, a genetic database of 46,678 SNPs for 3,457 individuals was created.

Table 2.1. Sample description. Group allocation, RGB (color) code assigned to the pre-defined groups in the paper, breed names, breed code, number of sampled and genotyped individuals (N), number of genotyped and unrelated individuals used for estimation of diversity parameters (Nd), number of genotyped and unrelated individuals used for Admixture Analysis (N_Adm), current breeding purposes as well as sporadic or recent past breeding purposes in parenthesis, breed origin and source of the samples or genotypes used in this study (Source).

Group	RGB code (color)	Breed	Code	N	Nd	N_Adm	Breeding purposes	Breed origin	Source of genotypes
Out-Groups	43/0/0 (black)	Yak	YAK	26	26	0	milk, work, beef	Mongolia	Decker JE et al., 2009 ; Medugorac et al., 2017
		Gir (Zebu)	GIR	30	24	24	milk, work, beef	India (Brasil)	Eggen, pers comm.; Verdugo et al., 2019
		N'Dama	NDA	42	27	27	milk, work, beef	Burkina Faso/Guinea	Eggen, pers comm.; Verdugo et al., 2019; Decker JE et al., 2009
Minor Asia	212/0/170 (pink)	Anatolian East Red cattle	ATER	20	17	17	milk, (beef)	Turkey	Decker JE et al., 2014 ; Verdugo et al., 2019
		Anatolian Black cattle	ATBC	43	37	37	milk	Turkey	Ramljak J et al.,2018
		Anatolian South Red cattle	ATSR	21	17	17	milk, beef	Turkey	Decker JE et al., 2014 ; Verdugo et al., 2019
		Anatolian South Yellow cattle	ATSY	8	7	7	milk	Turkey	Decker JE et al., 2014
		Turkish Grey cattle	TRG	8	8	8	milk, beef, work	Turkey	Upadhyay MR et al., 2017
Greece and Cyprus	0/112/192 (blue)	Cyprus cattle	CYP	14	12	9	beef, (work)	Cyprus	This study; Flori et al., 2019
		Agathonisi cattle	AGT	6	6	3	beef, (work)	Greece	This study
		Crete cattle	CRT	11	11	1	beef, (work)	Greece	This study
		Nisyros cattle	NSY	7	7	5	beef, (work, milk)	Greece	This study
		Greek Brachyceros cattle	GRB	116	41	32	beef, (work, milk)	Greece	This study; Flori et al., 2019
		Kastelorizo cattle	KAS	4	4	1	beef, (work)	Greece	This study
		Kea cattle	KEA	97	27	20	beef, milk, (work)	Greece	This study
		Greek Prespa cattle	PRG	10	9	7	beef, (milk, work)	Greece	This study
		Greek Rodope cattle	ROG	12	9	8	beef, (work, milk)	Greece	This study
		Sykia cattle	SYK	21	17	12	beef, (work, milk)	Greece	This study; Verdugo et al., 2019
		Katerini cattle	KTR	20	19	11	beef, (work, milk)	Greece	This study
South East Europe	112/48/160 (dark blue)	Rhodopean Shorthorn	RHS	24	17	17	beef, milk	Bulgaria	Ramljak J et al.,2018
		North Macedonian Buša	MKB	42	22	22	beef, milk	North Macedonia	This study; Ramljak J et al.,2018
		Serbian Buša	SRB	58	20	20	beef, milk	Serbia	Upadhyay MR et al., 2017 ; Ramljak J et al.,2018

		Albanian Prespa cattle	PRE	39	29	29	beef, milk	Albania	Ramljak J et al.,2018
		Red Metochian Buša	RMB	26	17	17	beef, milk	Kosovo	Ramljak J et al.,2018
		Sharri Buša	SHB	21	17	17	beef, milk	Kosovo	Ramljak J et al.,2018
		Dilagjini Buša	DGB	21	21	21	beef, milk	Kosovo	Ramljak J et al.,2018
		Dibra Buša	DBB	25	25	25	beef, milk	Albania	Ramljak J et al.,2018
		Middle Albanian Buša	MAB	43	43	43	beef, milk	Albania	Ramljak J et al.,2018
		Lekbian Buša	LKB	27	27	27	beef, milk	Albania	Ramljak J et al.,2018
		Skodra Buša	SKB	14	14	14	beef, milk	Albania	Ramljak J et al.,2018
		Monte-Negro Buša	MNB	23	19	19	beef, milk	Montenegro	Upadhyay MR et al., 2017 ; Ramljak J et al., 2018
		Bosnian Buša	BHB	18	18	18	beef, milk, (work)	Bosnia & Herzegovina	Ramljak J et al.,2018
		Croatian Buša	HRB	28	28	28	beef, milk, (work)	Croatia	Ramljak J et al.,2018
East Podolian	128/128/128 (grey)	Croatian Istrian cattle	HRI	30	28	28	beef, (work)	Croatia	Ramljak J et al.,2018
		Croatian Podolian cattle	HRP	24	24	24	work, beef	Croatia	Ramljak J et al.,2018
		Ukrainian Podolian cattle	UKP	24	21	21	milk	Ukraine	Upadhyay MR et al., 2017
Tyrrhenian	255/212/42 (light yellow)	Podolica	PODO	25	25	25	beef, milk, work	Italy	Mastrangelo S et al., 2018
		Cinisara	CINI	30	30	30	milk	Italy	Mastrangelo S et al., 2018
		Modicana Sicily	MOSI	29	29	29	milk, (beef)	Italy (Sicily)	Mastrangelo S et al., 2018
		Rossa Siciliana	RSIC	24	24	24	milk	Italy (Sicily)	Mastrangelo S et al., 2018
		Modicana Sardinia	MOSA	28	28	28	milk, beef	Italy (Sardinia)	Mastrangelo S et al., 2018 ; Flori et al., 2019
		Sarda	SARD	30	30	30	beef	Italy (Sardinia)	Mastrangelo S et al., 2018 ; Flori et al., 2019
		Sardo-Bruna	SBRU	10	10	10	beef, milk	Italy (Sardinia)	Mastrangelo S et al., 2018
		Corsican cattle	CORS	33	30	30	beef	France (Corsica)	Flori et al., 2019
		Agerolese	AGER	22	22	22	beef, milk	Italy	Mastrangelo S et al., 2018
		Maremmana	MARE	51	34	34	beef, (work)	Italy	Upadhyay MR et al., 2017 ; Mastrangelo S et al., 2018 ; Flori et al., 2019
		Chianina	CHI	18	12	12	beef	Italy	Decker JE et al., 2009 ; Upadhyay MR et al., 2017
		Mucca Pisana	MPIS	23	15	15	work, beef, milk	Italy	Mastrangelo S et al., 2018
		Calvana	CALV	24	24	24	beef	Italy	Mastrangelo S et al., 2018
		Marchigiana	MCH	23	21	21	beef, (work)	Italy	Decker JE et al., 2009; Mastrangelo S et al., 2018

		Romagnola	RMG	54	18	18	beef	Italy	Eggen, pers comm.; Mastrangelo S et al., 2018
		Garfagnina	GARF	23	23	23	milk, beef	Italy	Mastrangelo S et al., 2018
		Pontremolese	PONT	24	13	13	beef	Italy	Mastrangelo S et al., 2018
		Modenese	MODE	23	23	23	beef, milk	Italy	Mastrangelo S et al., 2018
		Cabannina	CABA	22	22	22	milk	Italy	Mastrangelo S et al., 2018
		Reggiana	REGG	26	26	26	milk	Italy	Mastrangelo S et al., 2018
		Piedmontese	PMT	34	16	16	beef, (milk)	Italy	Eggen, pers comm.; Decker JE et al., 2009; Mastrangelo S et al., 2018
		Burlina	BURL	24	24	24	milk, beef	Italy	Mastrangelo S et al., 2018
Alpine	102/153/0 (green)	Pezzata Rossa D'Oropa	PRDO	23	23	23	milk	Italy	Mastrangelo S et al., 2018
		Ottonese-Varzese	OVAR	43	31	31	milk, beef, work	Italy	Mastrangelo S et al., 2018
		Rendena	REND	24	24	24	milk, (beef)	Italy	Mastrangelo S et al., 2018
		Bará-Pustertaler	BPUS	24	24	24	milk, beef	Italy	Mastrangelo S et al., 2018
		Pustertaler	PUST	24	24	24	milk	Austria/Italy	Mastrangelo S et al., 2018
		Cika	SIC	26	26	26	milk, beef	Slovenia	Ramljak J et al.,2018
		Pinzgauer cattle	PIN	29	29	29	milk, beef	Austria/Italy	Ramljak J et al.,2018
		Tiroler Grauvieh	TGV	50	50	50	milk, beef	Austria/Italy	Ramljak J et al.,2018
		Murnau-Werdenfelser	MWF	46	46	46	beaf, milk	Germany	Ramljak J et al.,2018
		Original Braunvieh	OBV	35	35	35	milk, beef	Germany/Switzerland	Ramljak J et al.,2018
		Braunvieh	BBV	50	50	50	milk, (beef)	Germany/Switzerland	Ramljak J et al.,2018
		Fleckvieh	DFV	50	50	50	milk, beef	Germany/Austria	Ramljak J et al.,2018
		Gelbvieh	FGV	50	50	50	milk, beef	Germany	Ramljak J et al.,2018
		Vosges cattle	VOG	18	18	18	milk	France	Gautier M et al., 2010
		Abondance	ABO	22	22	22	milk	France	Gautier M et al., 2010
		Montbéliarde	MON	28	28	28	milk (beef)	France	Gautier M et al., 2010
		Tarentaise	TAR	37	37	37	milk (beef)	France	Ramljak J et al.,2018
France	250/140/0 (orange)	Raco di Biou	RDBI	29	29	29	beef	France	Gautier M et al., 2010
		Salers	SAL	26	26	26	beef	France	Gautier M et al., 2010
		Aubrac	AUB	22	22	22	beef	France	Gautier M et al., 2010
		Limousin	LIM	73	48	48	beef	France	Eggen, pers comm.; Decker JE et al., 2009; Ramljak J et al.,2018

		Charolais	CHR	52	39	39	beef	France	Eggen, pers comm.; Decker JE et al., 2009; Gautier M et al., 2010
		Parthenaise	PAR	17	17	17	beef	France	Gautier M et al., 2010
		Blonde d'Aquitaine	BAQ	35	33	33	beef	France	Eggen, pers comm.; Ramljak J et al.,2018
		Gascon	GAS	22	22	22	milk	France	Gautier M et al., 2010
Iberian	250/140/0 (red)	Menorquina	MNRQ	30	30	30	beef, (work)	Spain (Menorca)	Flori et al., 2019
		Mallorquina	MALL	30	30	30	beef	Spain (Majorca)	Flori et al., 2019
		Negra Andaluza	NGAN	32	14	14	beef	Spain	Flori et al., 2019
		Casta Navarra	CANA	30	30	30	beef	Spain	Flori et al., 2019
		Marismeña	MARI	22	22	22	beef	Spain	Flori et al., 2019
		Alentejana	ALEN	11	10	10	beef, work	Portugal	Upadhyay MR et al., 2017 ; Verdugo et al., 2019
		Barrosa	BAR	14	14	14	beef, milk, work	Portugal	Ramljak J et al.,2018
		Maronesa	MARO	20	19	19	beef, (work)	Portugal	Upadhyay MR et al., 2017 ; Ramljak J et al., 2018
		Sayaguesa	SYG	11	11	11	milk	Spain	This study; Upadhyay MR et al., 2017
North West Europe	128/98/0 (green olive)	Bretonne Black Pied	BPN	15	15	15	milk, (beef)	France	Gautier M et al., 2010
		Normande	NOR	30	30	30	beef	France	Gautier M et al., 2010
		Maine-Anjou	MAN	20	20	20	beef	France	Gautier M et al., 2010
		Blanc Bleu Belge	BBB	45	45	45	beef	Belgium	Ramljak J et al., 2018
		Dutch Belted cattle	LKF	22	22	22	milk, beef	Netherlands	Ramljak J et al., 2018 ; Upadhyay MR et al., 2017
		Holstein	HF	50	50	50	milk	Germany	Upadhyay MR et al., 2017
		Guernsey	GNS	31	16	16	milk	Channel Islands	Eggen, pers comm.; Decker JE et al., 2009;
		Jersey	JSY	52	49	49	milk	Channel Islands	Gautier M et al., 2010; Decker JE et al., 2014; Ramljak J et al.,2018
		Hereford	HER	65	41	41	beef	England	Eggen, pers comm.; Decker JE et al., 2009; Ramljak J et al.,2018
		Shorthorn	SHR	14	13	13	beef	England	Decker JE et al., 2014
		Kerry	KRY	16	14	14	milk	[35]	Verdugo et al., 2019

🟫		Dexter	DXT	22	16	16	beef, milk	Ireland	This study, Decker JE et al., 2009
		Galloway	GLW	40	40	40	beef	Scotland	Decker JE et al., 2014 ; Ramljak J et al.,2018
		Angus	AAN	66	48	48	beef	Scotland	Eggen, pers comm.; Decker JE et al., 2009
		Highland	HGL	28	27	27	beef	Scotland	Upadhyay MR et al., 2017 ; Ramljak J et al., 2018 ; Verdugo et al., 2019
		Norwegian Red cattle	NRC	56	34	34	milk	Norway	Eggen, pers comm.; Decker JE et al., 2009; Ramljak J et al.,2018
		Swedish Red cattle	SERC	25	24	24	milk	Sweden	Upadhyay MR et al., 2017
		Fjaell cattle	FJL	24	22	22	milk	Sweden	This study; Upadhyay MR et al., 2017
		Finnish Ayrshire	FIAY	53	42	42	milk, (beef)	Finland	Decker JE et al., 2009; Decker JE et al., 2014; Iso-Touru T et al., 2016
		Eastern Finncattle	FINE	40	20	20	milk, beef	Finland	Iso-Touru T et al., 2016
		Western Finncattle	FINW	40	35	35	milk, beef	Finland	Iso-Touru T et al., 2016
		Northern Finncattle	FINN	25	18	18	milk, beef	Finland	Iso-Touru T et al., 2016
		Yaroslavskaya	YARO	20	20	20	milk	Russian Federation	Iso-Touru T et al., 2016

2.3 Haplotyping and unified additive relationships (UAR)

A hidden Markov Model (HMM) implemented in the program Beagle software package (v 5.0) was used for imputation of missing genotypes (Browning & Browning, 2016) and haplotype phasing (Browning & Browning, 2007). To improve the efficiency of phasing and imputation, genotyping data of all available bovine animals that stored in SQL database of Ludwig Maximilian University of Munich (Prof. Medugorac Ivica) were considered in the in-house database, which includes many pairs and trios from other projects.

Genome-wide relationship matrix between individuals was estimated according to Yang et al. (2010). This method later was named by Powell et al. (2010) as unified additive relationship (UAR) matrix. This process uses an arbitrary base population in which the average relationship between all pairs of individuals is zero (0), including the relationship of an individual with itself. This methodology implemented in the R package *snpReady* (Granato et al., 2018) and applied to 46,678 SNP genotypes of 3,457 animals.

Analyses of diversity, phylogeny, and population structure require samples that are representative of each breed and unrelated as possible. In the Greek island populations (AGT, CRT, NSY, KAS), sampling was done in a few animals, which although they are closely related to each other, no further choice of sampling could be done. To create a subset of the most unrelated Greek and Cyprus animals required for phylogenetic analyses the family structure was reduced removing extremely highly related animals (relationship coefficient > $\overline{UAR}_{\text{(Breed)}}$). In addition, to keep the most representative animals, a more robust method was applied to exclude those individuals (outliers) that show a high genetic relationship with foreign breeds and/or individuals. Multivariate analysis implemented in mvoutlier R package (Filzmoser, 2004) was used to find these individuals. The multivariate outlier analysis and the reduction of family structure within breeds rely on the genome-wide additive genetic relationships stored in the UAR matrix. This procedure applied in GRB, KEA, ROG, PRE, and SYK.

Multivariate outlier analysis to find outliers is also used for local breed conservation purposes. According to Akcakaya et al. (2007) the individuals worth keeping in a local breed should be without or with few foreign haplotypes. The identification of admixed individuals in a population under conservation is based on the identification of admixture signatures (Ramljak et al., 2018) caused by the introduction of foreign haplotypes. Consequently, purebred individuals will show higher cumulative genetic relatedness to individuals from the same population than admixed ones, while they will show lower relatedness to some individuals from distant or foreign populations. In addition, the proportion of foreign alleles will increase in admixed individuals, which is reflected in the increased proportion of semi-private alleles. Finally, an increased number of a person's network connections with people of foreign origin indicates possible admixture.

While it is accepted that each of the above parameters provides an indication of admixture, a one-dimensional approach to finding outliers is not considered safe. Thus, a multivariate method is considered capable of distinguishing extreme values of a distribution from values belonging to a different distribution (Filzmoser et al., 2005). The parameters used for the multivariate analysis are as follows:

1. The genetic distance to the own population (**$D_{UAR(W)}$**), which can be defined as $D_{UAR(W)} = -\ln(mUARi + mUARmp)$. *mUARi* is the mean UAR of individual *i* to all members of the metapopulation, and *mUARmp* is the mean UAR for the entire metapopulation.
2. The highest UAR with a particular animal of a foreign breed (**max-UAR1(B)**).
3. The second highest UAR with a particular animal of a foreign breed (**maxUAR2(B)**). maxUAR2(B) serves as a confirmation of maxUAR1(B), i.e., a randomly increased maxUAR1(B) association with a single foreign animal will be degraded by maxUAR2(B). However, two consecutive high genetic relationships with foreign animals (maxUAR1(B), maxUAR2(B)) are a strong indication of an admixed animal.
4. The highest average UAR with all animals of foreign breeds (**max- UAR(P)**). Firstly, the mean UAR of every i of the metapopulation with all members of breed p outside of the metapopulation (mUAR(i,p)) was estimated. The maxUAR(P) is the highest average UAR of member i with a particular breed outside of the metapopulation.
5. The number of connections to foreign animals in the Nearest Neighbour Graph (**k-NN(B)**). The estimation of Nearest Neighbour Graph was obtained with the program NetView (Steinig et al., 2016) with mk-NN = 100. Then, for each member i of the metapopulation, from the total number of connections, the number of connections with animals of the

same population was removed. The number of connections with animals outside of the breed was used as k-NN(B) parameter in the mvOutlier test.

6. The relative number of semiprivate alleles observed in the particular animal (***nspAA***). Semi-private alleles (*spA*), characterized as the alleles that exist only in two populations. For each animal i and each foreign population p number of semi-private alleles was counted. The mean, standard deviation (SD) as well as the number of sample size correction for nspA(i,p) of each source population p were used to determine the most influential source population. Thus, the sum of alleles displayed by member i of each breed with the source population were used as the *nspAA* parameter. This parameter provides an indication of the direction of gene flow that cannot be obtained from symmetric matrices (UAR, DPS). Admixed individuals in a recipient population will be characterized by an increased proportion of $spA_{admixture}$ originating from and distributed evenly among individuals of a donor population.

Finally, the dataset used in subsequent diversity and phylogenetic analyses included the 2,858 most representative and unrelated animals. The starting and optimized sample sizes for each breed are listed in columns N and Nd (see **Table 2.1**).

2.4 Haplotype diversity

To design the Illumina Bovine SNP50 BeadChip (Illumina), only five taurine breeds and one indicine breed were considered (Matukumalli et al., 2009) with the reference genome assembly obtained from taurine Hereford (The Bovine HapMap consortium, 2009). Consequently, a large proportion of the included markers are poorly informative for breeds not included in the BeadChip development, especially for the local breeds of Southeast Europe. Thus, to reduce the possible ascertainment bias, a 4-SNP-block approach as described previously (Simčič et al., 2015; Papachristou et al., 2020) was adopted. Specifically, 4-SNP blocks (haplotypes) that spanned less than 150 kb and had an inter-marker distance shorter than 50 kb were defined, leading to a compromise between the maximum number of SNPs and the minimum recombination probability within the block (Ramljak et al., 2018). In total, 5,756 SNP blocks were considered as multi-allelic markers and their haplotypes as alleles in the subsequent unbiased allelic diversity and heterozygosity analyses. Hereafter, SNP blocks are also referred to as multi-allelic markers.

2.5 Genetic diversity

To determine the genetic diversity, the values for the following indicators were calculated: total number of alleles (*nA*), mean number of alleles per block (*mA*), observed (H_O) and expected heterozygosity (H_E) (Nei, 1987), number of private alleles (*npA*; alleles observed only in one population), frequency of private alleles (*fpA*) and number of common alleles (*ncA*; observed in all subpopulations). In addition, the number of semi-private alleles (*nspA*) or rare alleles, defined as the alleles observed in two populations only was estimated. This definition of rare alleles largely implies private alleles introgressed from a donor breed into a recipient breed at a lower frequency by crossing (Simčič et al., 2015) as described previously and can be used as an indicator of the amount of gene flow between subpopulations (Slarkin 1985; Barton & Slatkin, 1986).

To avoid the effect of population size on the measurement of the number of haplotypes, the allelic richness index (*AR*) (El Mousadik & Petit, 1996) was calculated. The *AR* is determined based on the lowest number of people per population. In the dataset, the lowest number of individuals is 4 in the KAS population.

To improve the presentation and discussion of the summary statistics related to diversity, these statistics standardized and then plotted onto a map with a tessellated projection using the R-script available with the package *Tess* (http://membres-timc.imag.fr/Olivier.Francois/TESS_Plot.html).

2.6 Inbreeding coefficient

The consequences of inbreeding as the result of mating among relatives, on the performance or life ability of progeny, has attracted the interest of many scientists (Bjelland et al., 2013; Charlesworth & Willis, 2009) and consists of a “mysterious danger” for many breeders. Inbreeding by fate in small or/and isolated populations, where few dominant males participate in the reproductive process has a stronger effect on the survival ability of species compared with inbreeding by selection in breeding programs of high-performance breeds. This is explained by the risk of extinction of these local breeds, some of them may carry valuable genes for future survival (González-Recio et al., 2007; Mc Parland et al., 2007). The inbreeding level in a population could be limited using specific mating strategies, but with the assumption that there is a sufficient population size.

The measurement of the inbreeding coefficient as the probability that a pair of alleles to be Identical By Descent (IBD), could be calculated by pedigree information but especially for local breeds where the pedigrees are incomplete or absent completely, the genomic measurement of inbreeding is the only solution. The analysis of extensive molecular data, such as SNP markers, offers a more complete estimate of IBD alleles (Alemu et al, 2011; Marras et al., 2015). Such approximations are provided through the construction of a genome wide relationship matrix as well as the identification of Runs of Homozygosity (ROH) (Ferencakovic et al, 2011; Purfield et al., 2012). Unlike the estimation of inbreeding which calculated from the diagonal elements of a genome wide relationship matrix giving an unbiased estimation, the ROH-based estimation makes it possible to distinguish between recent and ancient inbreeding (Keller et al., 2012). Although ROH can arise for several reasons, the primary cause of ROH is believed to be inbreeding (Gibson et al., 2006). As recombination will break long chromosome segments, it is expected that long autozygous segments in an individual genome would be found when there is a recent common ancestor and shorter segments would be found when the common ancestor is more distant (Broman & Weber, 1999). Hence, the longer the homozygous segments are, the more recent the inbreeding. The length and the distribution of Runs of homozygosity give an assessment of the temporal origin of inbreeding (McQuillan et al., 2008; Curik et al., 2014) as well as the demographic history of population (Purfield et al., 2012; Bertolini et al., 2018).

2.6.1 Analysis of Runs of Homozygosity (ROH) and Genomic Inbreeding (FROH)

The Runs of Homozygosity estimated using consecutive runs (window-free) method according to (Marras et al., 2014) implemented in the R package “detectRUNS” (Biscarini et al., 2018). Sliding windows were not used to detect ROH to avoid the introduction of artificial ROH that were shorter than the window (Ferencakovic et al., 2013b). The following parameters was used to mitigate false positive results: (i) the minimum length of a ROH was set at 4 Mb, (ii) the maximum distance between adjacent SNPs was 1 Mb, (iii) one heterozygous and up to one missing genotypes were allowed in a ROH and (iv) the minimum number of SNPs that constituted a ROH (l) was calculated as was initially proposed by Lencz et al., 2007 and applied by Purfield et al., 2012 to studies on cattle breeds using the following function:

$$l = \frac{log_e \frac{a}{ns*ni}}{log_e(1-het)}$$

Where ns = the number of genotyped SNPs per individual; ni = the number of genotyped individuals; α = the percentage of false positive ROH (set to 0.05 in our study); and het = the mean heterozygosity across all SNPs. Calculated l was equal to 58.

ROHs classified into three length classes (4–8 Mb, 8–16 Mb, and > 16 Mb) and identified as $ROH_{4\text{-}8Mb}$, $ROH_{8\text{-}16Mb}$, $ROH_{>16Mb}$. The ROH-based inbreeding coefficient (F_{ROH}) was calculated only for ROH length >4 Mb following Ferenčaković et al. (2013b) who found that BovineSNP50 BeadChip was not sensitive enough for the precise determination of length segments < 4 Mb. Finally, the ROH-based inbreeding for ROH segments 4-8, 8-16 and >16 was estimated as $F_{ROH\ (4\text{-}8Mb)}$, $F_{ROH\ (8\text{-}16Mb)}$ and $F_{ROH\ >16Mb}$. The inbreeding was calculated for each animal using the method proposed by McQuillan et al. 2008, as the proportion of the genome lying in ROH of a certain minimal length relative to the overall autosomal genome covered by SNPs on the chip. In this study, there were 2.48 Gb covering 29 chromosomes ($F_{ROH} = L_{ROH}/ L_{AUTOSOME}$).

Under several assumptions and approximations, it was expected that, “remote” $F_{ROH\ (4\text{-}8Mb)}$, was related to the proportion of the autozygosity originating from ancestors that were from 6–7 to 12–13 generations ago while “close” $F_{ROH\ (8\text{-}16Mb)}$, from ancestors that were born 3–6 generations ago (Curik et al. 2014). Following the same principles, $F_{ROH>16Mb}$ was related to the proportion of the autozygosity originating from ancestors that were born in less than 3 generations ago.

2.6.2 Inbreeding from genome wide relationship matrix

According to Wright (1922), the inbreeding coefficient has been defined in terms of correlations between the parents’ uniting gametes and require a past base population. This definition is directly related to the method of calculation of inbreeding coefficient by Yang et al (2010) which was based on the correlation between uniting gametes with the base population and is indirectly defined by the set of individuals used to estimate the allele frequencies (Wang, 2014). Contrary to the definition inbreeding as IBD probabilities, this measure can take negative values and behave more like correlations. **Also, this method gives more weight to homozygosity at rare alleles** (Keller et al., 2011). The inbreeding of individual (i) was calculated as fi= $UAR_{(i,i)}$ – 1.

2.7 Subpopulation differentiation

For the estimation of differentiation among subpopulations, the G_{ST} and D_{EST} parameters from multiallelic markers were calculated. The G_{ST} estimator proposed by Nei (1973) is the most widely used applied statistical measure of differentiation and is equivalent to Wright's fixation index (F_{ST}) for two alleles. To address concerns about the reliability of this classical measure (G_{ST}) (Jost et al., 2018), the true population differentiation D_{EST} by Jost (2008) was predicted as the harmonic mean of D values across loci using the approach described by Crawford et al. (2010). The D_{EST} estimator, which is analogous to the classical G_{ST} for multi-allelic loci, is unbiased and more suitable when the level of gene diversity (H_E) is high.

In this approach, the dataset of the genotypes for 5,756 multi-allelic SNP blocks in 115 breeds was used.

2.8 Past effective population size based on linkage disequilibrium.

Effective population size (Ne) of a real population X can be defined as the size of a hypothetical ideal population that will result in the same amount of genetic drift as in the (actual) population (Wright, 1931). It is an important population parameter that helps to explain how populations have evolved (Falconer & Mackay, 1996).

The recent and historic effective population size was estimated from genomic data to overcome the absence or incomplete pedigree data. The calculation of Ne was based on LD according to Corbin et al (2012) formula as implemented in *SNeP* R package (Barbato et al., 2015). Linkage disequilibrium was estimated according to Hill and Robertson's (1968) squared correlation formula. The effective population size (Ne) was estimated for all breeds with a sample size greater than 8. The estimation was performed on the SNPs with minimum and maximum distances equal to 20,000 and 10,000,000 bp, respectively, and by applying a recombination rate correction (Sved & Feldman, 1973) and a sample size correction (Weir & Hill, 1980).

The most recent effective population is represented by Ne_5, (i.e., five generations ago), the effective population size in preindustrial times (i.e., 50 generations or 250 years ago) is represented by Ne_{50}, and in times close to domestication (10,000 years ago) by Ne_{2000}. To improve the presentation and discussion of the effective population size across time and space, these values standardized and then plotted Ne_5, Ne_{50} and Ne_{2000} onto a map with a tessellated projection using the R-package Tess as described above.

2.9 Cluster analysis

Four phylogenetic and population structure analyses to infer relationships between animals and breeds was applied. Two of these analyses rely on bi-allelic SNP genotypes and two on multi-allelic SNP-block genotypes. In addition, two of these analyses represent supervised clustering and two represent unsupervised clustering.

2.9.1 Supervised phylogeny of 115 cattle breeds

To elucidate the phylogenetic relationships among the studied populations, two supervised methods were evaluated. One used maximum likelihood on biallelic markers and the other used a distance-based method on haplotype blocks.

Maximum likelihood method. Maximum likelihood (ML) method implemented in the TreeMix program (Pickrell & Pritchard, 2012) was used to infer the population mixtures. In this approach, the dataset of SNPs genotyped in 115 breeds (**Table 2.1**) was used and YAK was set as outgroup to root the tree.

Distance-based method. The second supervised approach used the allele frequencies of 5,756 haplotype blocks of 115 breeds to estimate the Nei's unbiased D_A-distances (Nei et al., 1983). Then, the D_A - distance matrix was used to reconstruct the neighbor-net network (Bryant &

Moulton, 2004) and the neighbor-joining tree (Saitou & Nei, 1987). The construction of neighbor-net network was made with SplitsTree4 (Huson & Bryant, 2006) software and the neighbor-joining (NJ) tree with the FigTree 1.4 software (Rambaut, 2018) (http://tree.bio.ed.ac.uk/software/figtree/). In the NJ the YAK was set as outgroup to root the tree while for checking the reliability of the tree the nonparametric bootstrap for 1000 resamplings according to Felsenstein et al (1985) was used.

2.9.2 Unsupervised population structure analyses

In this type of analysis, to infer the breed ancestry a distance-based method and a model-based method at individual level was used.

Distance-based method. The following approach relies on the estimation of the proportion of genome-wide shared SNP-block alleles among all pairs of individuals. It is about the so-called Identity by State values (IBS). For the calculation of IBS values is not required the estimation of allele/genotype frequency which makes it valid in the case as the sample number is small. The proportion of shared alleles matrix (PS) of 5,756 haplotype blocks for all pairs of 2,858 animals, as a measure of similarity between two individuals was constructed. These values were calculated averaged over loci according to Bowcock et al (1994) following the bellow equation from Gao & Martin (2009).

The average allele sharing between i and j is defined as

$$PS = \frac{1}{L}\sum_{l=1}^{L} d_l$$

Where:

d = 0 if individual i and j have two alleles in common at the l -th locus,
d = 1 if individual i and j have only a single allele in common at the l -th locus,
d = 2 if individual i and j have no allele in common at the l -th locus

Then the PS matrix transformed into a matrix of genetic distances between all pairs of individuals by D_{PS}= -log (*PS*). The D_{PS} matrix is symmetric, non-negative, and hollow (i.e., has zero diagonal). Then Multidimensional scaling analysis (MDS) to the D_{PS} matrix was applied. MDS analysis is considered suitable when a dissimilarity matrix (D_{PS}) is used as the input file, as opposed to PSA analysis which uses a correlation matrix as the starting matrix (Gao & Martin, 2009). The basic idea of this analysis is to create a map from any measure of proximity between individuals. The main output is a spatial representation, consisting of a geometric configuration of individuals as points. In the case of population stratification, this configuration reflects the hidden structure of the data which means that the greater the dissimilarity (or the less similarity) between the two individuals, as shown by their proximity value, the further or closer they should be in the spatial map (Kruskal, 1978).

R programming provides several ways to perform metric MDS, here analysis was made by function "cmdscale ()". This function follows the analysis of Mardia (1979) and provides a classical approach of metric multidimensional scaling which also known as principal coordinates analysis (Gower, 1966). The function comes with the default distribution of R.

As it has been said the D_{PS} distances are calculated at the individual level. Because the dataset consists of 2,858 individuals, simple visualization of the results shows successive overlaps, particularly of undifferentiated breeds' individuals. For this reason, the MDS analysis is also presented at the breed level, according to the following procedure.

Firstly, the mean MDS coordinates of all the individuals were calculated, which correspond to the center of each breed symbol (circle), and then the standard deviation (SD) around that center was estimated.-Specifically, for each breed, the spatial distance of each individual to the group center by applying the Pythagorean theorem was calculated, assuming that the hypotenuse is the distance between the center of the breed symbol and the position per individual. Then, the SD of these spatial distances was estimated. Finally, the SD was used as the radius around the breed center symbol, as a proxy for spatial dispersion of animals of each breed. For visualization purposes, plot dimensions were proportionally adjusted in R, considering a 1-inch (= 0.254 cm) length as the longest radius.

The calculation of the mean D_{PS} within breed gives information for in-breed uniformity. Mean D_{PS} values were standardized and then plotted as a tessellated projection onto a map, using the R-package Tess as described above.

Model-Based method. In the second unsupervised analysis, to investigate the pattern of ancestry among individuals the admixture algorithm based on SNP genotypes was used. This model – based estimation of ancestry was implemented in program **Admixture 1.3** (Alexander et al., 2009). This approach in terms of the global ancestry paradigm (Pritchard & Connelly, 2001) converges in an appropriate k number of ancestral/underlying populations, each of which contributes a proportion over the present individual's genome. The determination of the appropriate number of ancestral populations in the Admixture program is assessed through the cross-validation process (Alexander & Lange, 2011). This procedure defines a prediction error for each value of K. When the estimated prediction error is minimized then suggests the most appropriate K. For this data set performed 10 cross-validations and estimated the cross-validation error for each K. For this purpose, clustering under the assumption that the number of clusters is equal to K was conducted, with K ranging from 1 to 115, i.e., the number of breeds plus 1. Since the admixture analysis does not need an outgroup, a reduced data set that excludes YAK was used. Also, because the program is sensitive to the presence of related individuals, giving very little or no information about the origin of these, further reducing the animal numbers of Greek cattle breeds was chosen (**Table 2.1**).

To mitigate the effects of LD, as the Admixture program does not consider it, an LD pruning according to an association threshold was applied and then only the remaining SNPs were used for the analysis. More specifically in PLINK v2.0 program (Chang, Purcell et al., 2015) the following command was applied.

```
“--indep-pairwise 50 10 0.1”
```

The command targets for removal one of a pair of SNPs that has an r^2 value of greater than 0.1 with any other SNP within a 50-SNP sliding window (advanced by 10 SNPs each time).

To illustrate the results of the admixture analyses, the R package *pophelper* was used (Francis, 2017).

2.10 D-Statistics

D-statistics has been a very powerful weapon for scientists looking at various prehistoric admixture scenarios and how they leave their signature on the current human genome (Green et al., 2010; Wall et al. 2013). The various methods of analyzing and inferring admixture events as implemented in the popular programs ADMIXTURE and STRUCTURE are not considered suitable for determining ancient gene flow. The D-statistics is a common tool for determining ancient admixtures and according to Soraggi et al (2017) "is based on the assumption that testing the correctness of a hypothetical genetic relationship of four populations involves evaluating specific coincidences of alleles between groups". In fact, the knowledge of ancient admixtures through the study of the genome helps us to discover demographic facts that explain the history of breeds and their current genetic variability.

Thus, this methodology is also an important tool for explaining the current genetic structure of the Greek breeds. As it has been mentioned above, the Greek territory but also more broadly the regions of Southeastern Europe are close to the domestication center of the *Bos taurus* and were a key transit center for the peoples of antiquity. Furthermore, the Balkan cattle population is located closer to current Anatolian cattle that carry both indicine and African taurine ancestry (Decker et al., 2014) which could indirectly shape the distribution of rare and private alleles in the cattle population of Balkan (Simčič et al., 2015).

In the present study, the investigation of historical admixture between taurine and indicine via D-Statistics (Green et al. 2010) was calculated using the qpDstats tool of the AdmixTool software package (Patterson et al., 2012). In this methodology, three populations and an outgroup (breed) that is genetically equally distant from the other three are considered. In this way, the outgroup serves as a reference point in measuring the gene flow of the other three populations Thus a set of three populations P1, P2, P3, and an outgroup O was created (((P1, P2),P3).O) (**Figure 2.3**). The numbers of shared alleles between P1 and P3 (BABA) and, P2 and P3 (ABBA) calculated by assuming that allele “A” represents the ancestral allele and allele “B” the derived allele. Significant excess of either «ABBA» or «BABA» indicates admixture between P2 and P3 or P1 and P3 populations, respectively.

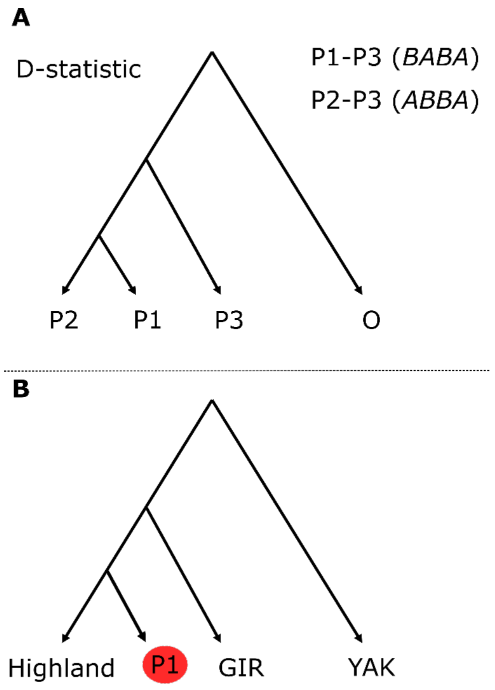


Figure 2.3 (A and B). Schematic explanation of the methodology "The four-population test" (D-statistic) with the presence of an Outgroup (A). Demonstration of the phylogeny used for our analysis (B).

To investigate the influence of *Bos indicus* on the 115 European *Bos taurus* breeds the following scheme was created. YAK was used as an outgroup population (O) and GIR as a representative population of *Bos indicus* (P3, the source of admixture). The Highland breed originating from the most northwestern part of Europe (Scotland) was chosen as P2 and, therefore, as the *Bos taurus* breed with the lowest or no level with *Bos indicus*. The remaining breeds were tested as P1 (Figure 1B).

D values were calculated according to Patterson et al (2012).

Numerator (Num_i)= P(BABA) – P(ABBA)
Denominator (Den_i)= P(BABA) + P(ABBA)

$$D=\frac{\Sigma_i \widehat{Num}_i}{\Sigma_i \widehat{Den}_i}$$

To present the gradient of *Bos indicus* genes in European taurine cattle, the values standardized and then plotted the D-values onto a map with a tessellated projection using the R-package Tess. The D-values with Z > |3| were considered as significant and indicated on the map.

Chapter 3. Results

3.1 Heterozygosity and allelic diversity parameters

To illustrate a possible ascertainment bias of the SNP chip data, the tessellated projection of the observed heterozygosity estimations based on multi-allelic blocks (H_O) and bi-allelic SNPs ($H_{O[SNP]}$) were presented side-by-side in **Figure 3.1**. This shows that $H_{O[SNP]}$ suggests a high level of genetic diversity in some Alpine and Northwest European breeds, whereas H_O highlights breeds from Southeast Europe and Anatolia as having the highest level of diversity. The heterozygosity estimates based on multi-allelic SNP blocks and on biallelic SNPs are highest for the Southeast European Buša breeds. The only diversity parameter, which indicates a higher diversity in the central Europe group than in the Minor Asian group, is the observed heterozygosity estimator based on bi-allelic SNPs ($H_{O[SNP]}$) (**Figure 3.1**). The ascertainment bias of SNP chip data was further highlighted by the scatterplot of $H_{O[SNP]}$ versus H_O in **Figure 3.1**. Both $H_{O[SNP]}$ and H_O are estimators of the true diversity. Therefore, the diversity of the breeds placed above the overall trend line (e.g., Northwest Europe) is overestimated by $H_{O[SNP]}$ and the diversity of the breeds placed below this line (e.g., Minor Asia) is underestimated by $H_{O[SNP]}$. Thus, genetic, and allelic diversity estimators based on multiallelic blocks were selected for annotation. (**Table 3.1, Figure 3.2)**.

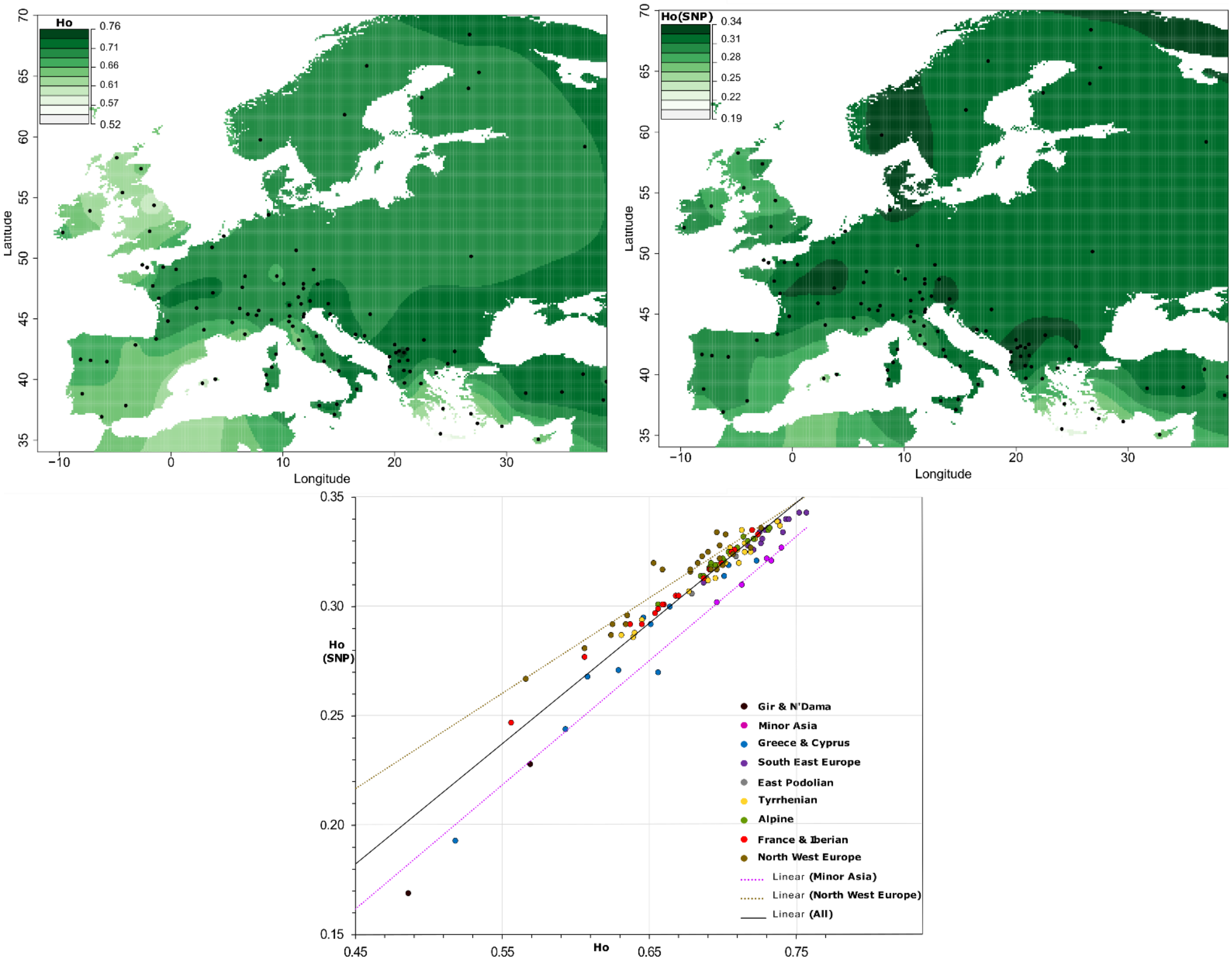


Figure 3.1. Tessellated projection and value distribution plot of observed heterozygosity estimated based on multi-allelic SNP-blocks (H_O) and bi-allelic SNPs ($H_{O[SNP]}$). For the breed position see Figure 2.2.

In total, 590 common alleles were detected among the 115 studied breeds, which represents only 0.7% of the total number (80,720) of alleles. All estimators of genetic diversity for the breeds studied here were highly differentiated among the predefined geographical groups (**Table 3.1, Figure 3.1,** and **Figure 3.2)** reflecting the large geographical extent occupied by the breeds of the data set. For the study of Greek cattle at the geographical group level, the populations are analyzed separately in two sub-groups (mainland and island populations) due to the great variation observed between them in all estimators. The *Greek mainland subgroup* consist of Greek Brachyceros breed - GRB, Greek Prespa cattle - PRG, Greek Rodope cattle - ROG, Sykia breed-SYK, Katerini breed - KTR. The *Greek Island subgroup* consist of Agathonisi Cattle - AGT, Crete Cattle - CRT, Nisyros cattle - NSY, Kastelorizo cattle - KAS, Kea breed – KEA.

3.1.1 Allelic Diversity parameters

The geographic group of Minor Asia displays the highest average value for all the estimators of allelic diversity used, i.e., for: total number of alleles ($\overline{nA}$ (Minor Asia) = 44,046), number of alleles per haplotype block ($\overline{mA}$ (Minor Asia) = 7.65), number of private ($\overline{npA}$ (Minor Asia) = 191.7), number of semi-private alleles ($\overline{nspA}$ (Minor Asia) = 234.8) and allelic richness ($\overline{AR}$ (Minor Asia) = 4.18) (**Table 3.1**). The geographic subgroup of Greek island populations takes the lowest average values for all allelic diversity estimators, for: total number of alleles ($\overline{nA}$ (Greek island populations) = 24,445), number of alleles per haplotype block ($\overline{mA}$ (Greek island populations) = 4.25), number of private ($\overline{npA}$ (Greek island populations) = 15), number of semi-private alleles ($\overline{nspA}$ (Greek island populations) = 18.8) and allelic richness ($\overline{AR}$ (Greek island populations) = 2.93). After the Minor Asia breed group, the highest values shared between the subgroup of Greek mainland breeds and Southeast Buša breeds. More specifically the Greek mainland breeds, for: total number of alleles ($\overline{nA}$ (Greek mainland breeds) = 40,211), number of alleles per haplotype block ($\overline{mA}$ (Greek mainland breeds) = 6.99), number of private ($\overline{npA}$ (Greek mainland breeds) = 87.2), number of semi-private alleles ($\overline{nspA}$ (Greek mainland breeds) = 123) and allelic richness ($\overline{AR}$ (Greek mainland breeds) = 3.92) (**Table 3.1**).

At breed level in almost all parameters of allelic diversity (*nA*, *mA*, *npA*, *nspA*) the Anatolian Black cattle takes the highest values with *nA* (Anatolian Black cattle) = 51,955, *mA* (Anatolian Black cattle) *= 9.03*, *npA* (Anatolian Black cattle) *= 294*, *nspA* (Anatolian Black cattle) *= 362.* At the unbiased index of Allelic Richness (*AR*) the Red Metochian Buša takes the highest value with *AR* (Red Metochian Buša) = 4.28. The lowest values in almost all parameters of allelic diversity (*nA*, *mA*, *nspA, AR*) show the Crete Greek Island breed with *nA* (Crete) = 12,688, *mA* (Crete) *= 2.2*, *nspA* (Crete) *= 8 and AR* (Crete) = 2.02. Only, at the number of private alleles the Italian Agerolese -AGER presents the lowest value with, *npA* (Agerolese) = 2. The Cyprus Cattle takes low values for: total number of alleles ($\overline{nA}$ = 27,328), number of alleles per haplotype block ($\overline{mA}$ = 4.75), number of private ($\overline{npA}$ = 32), number of semi-private alleles ($\overline{nspA}$ = 55) and allelic richness ($\overline{AR}$ = 3.41) (**Table 3.1**).

Table 3.1. Parameters of genetic diversity in the 115 examined cattle populations with 46,678 SNPs and 5,756 SNP blocks. ***Nd***, number of genotyped animals; ***nA*** and $\overline{nA}$, total and mean number of observed alleles within subpopulation; mA and $\overline{mA}$, number and mean number of alleles per haplotype block (mA = nA/ 5,756); HO and $\overline{Ho}$, average observed heterozygosity within subpopulation and in group; ***HE*** and $\overline{H_E}$,average expected heterozygosity within subpopulation and in group; Hdef, measurement of heterozygosity deficiency; ***npA*** and $\overline{npA}$, number and mean number of private alleles; nspA and $\overline{nspA}$, number and mean number of alleles present only in two subpopulations; fpA, average frequency of private alleles, ***F*** and $\overline{F}$, inbreeding coefficient per breed and per group; ***AR*** and ***AR*** , number and mean value of allelic richness; ***Ne₅*** and $\overline{Ne_5}$, effective population number for five generations back per breed and per group; ***Ne50*** and ***Ne2000***, effective population number for fifty and two thousand generations back. Abbreviations are indicated in Table 2.1.

Group	***Breed***	***Nd***	***nA***	$\overline{nA}$	***mA***	$\overline{mA}$	H_O	$\overline{Ho}$	H_E	$\overline{H_E}$	***npA***	$\overline{npA}$	***nspA***	$\overline{nspA}$	***fpA***	***F***	$\overline{F}$	***AR***	$\overline{AR}$	Ne_5	$\overline{Ne_5}$	***Ne50***	***Ne2000***
Out-Groups	YAK	26	9193		1.60		0.232		0.099		79		70		0.406	1.056		1.29		54		123	
	GIR	24	24111		4.19		0.486		0.464		78		139		0.041	0.647		2.63		48		304	3966
	NDA	27	32136		5.58		0.569		0.574		188		185		0.032	0.334		3.15		67		524	2737
Minor Asia	ATER	17	41775		7.26		0.733		0.725		147		158		0.031	0.106		4.17		40		349	4338
	ATBC	37	51955		9.03		0.730		0.746		294		362		0.015	0.112		4.25		96		831	5040
	ATSR	17	40859		7.10		0.713		0.719		152		185		0.034	0.142		4.12		40		337	4274
	ATSY	7	29941		5.20		0.696		0.683		43		64		0.076	0.161		4.08		-		-	-
	TRG	8	31406	**44046**	5.46	**7.65**	0.740	**0,725**	0.695	**0,727**	28	**191.7**	65	**234.8**	0.069	0.071	**0.117**	4.10	**4.18**	15	**64**	125	2952
Greece & Cyprus	CYP	12	27328		4.75		0.629		0.627		32		55		0.115	0.261		3.41		18		119	2753
	AGT	6	17302		3.01		0.593		0.504		17		23		0.206	0.329		2.74		-		-	-
	CRT	11	12688		2.20		0.518		0.373		11		8		0.376	0.457		2.02		8		53	1753
	NSY	7	19634		3.41		0.608		0.551		11		16		0.188	0.242		2.97		-		-	-
	KAS	4	17185		2.99		0.656		0.520		23		19		0.141	0.261		2.99		-	-	-	-
	KEA	27	33145	**24445**	5.76	**4.25**	0.646	**0.610**	0.622	**0.543**	16	**15.0**	23	**18.8**	0.026	0.150	**0,250**	3.31	**2,93**	28	-	160	2791
	GRB	41	49079		8.53		0.701		0.742		142		208		0.021	0.108		4.18		85		618	4170
	PRG	9	26666		4.63		0.704		0.643		13		31		0.073	0.096		3.58		15		109	2950
	ROG	9	25912		4.50		0.723		0.602		20		29		0.100	0.094		3.36		15		114	3099
	SYK	17	37037		6.43		0.664		0.698		67		71		0.050	0.149		3.91		29		210	3429
	KTR	19	37105	**40211**	6.45	**6.99**	0.651	**0,686**	0.685	**0,700**	54	**87.2**	74	**123.0**	0.045	0.175	**0,126**	3.80	**3,92**	26	**50**	192	3441
South East Europe	RHS	17	41278		7.17		0.741		0.734		70		104		0.034	0.058		4.22		38		308	3905
	MKB	22	45364		7.88		0.721		0.744		106		156		0.025	0.076		4.27		51		425	3932

	SRB	20	44355		7.71		0.726		0.742		101		120		0.028	0.063		4.27		46		396	4099
	PRE	29	44126		7.67		0.752		0.733		73		92		0.025	0.028		4.12		58		420	3933
	RMB	17	42940		7.46		0.757		0.738		105		127		0.032	0.027		4.28		40		345	3825
	SHB	17	39748		6.91		0.727		0.724		84		104		0.039	0.062		4.13		34		275	3664
	DGB	21	36322		6.31		0.729		0.700		30		41		0.052	0.053		3.86		31		228	3398
	DBB	25	37724		6.55		0.725		0.703		34		41		0.038	0.060		3.87		34		257	3298
	MAB	43	51066		8.87		0.738		0.745		148		180		0.013	0.042		4.20		97		712	3748
	LKB	27	44381		7.71		0.743		0.729		85		105		0.024	0.034		4.10		54		406	3709
	SKB	14	35556		6.18		0.717		0.707		33		48		0.048	0.070		4.00		25		205	3312
	MNB	19	41711		7.25		0.731		0.730		65		81		0.032	0.045		4.16		41		337	3679
	BHB	18	30993		5.38		0.687		0.642		21		44		0.064	0.113		3.47		23		154	3161
	HRB	28	48122	**42800**	8.36	**7.44**	0.745	**0,733**	0.745	**0,726**	106	**82.2**	147	**106.5**	0.020	0.027	**0.051**	4.26	**4.10**	68	**51**	583	3916
East Podolian	HRI	28	40285		7.00		0.709		0.704		25		58		0.039	0.074		3.88		49		322	3414
	HRP	24	29711		5.16		0.679		0.639		16		31		0.092	0.129		3.38		26		149	2959
	UKP	21	31176	**34188**	5.42	**5.94**	0.689	**0,693**	0.656	**0,669**	27	**22.6**	38	**43.4**	0.053	0.109	**0.103**	3.50	**3.61**	29	**36**	160	2925
Tyrrhenian	PODO	25	43858		7.62		0.715		0.731		75		95		0.023	0.074		4.13		56		434	3756
	CINI	30	43242		7.51		0.715		0.727		44		77		0.025	0.075		4.06		60		434	3882
	MOSI	29	37742		6.56		0.690		0.688		26		46		0.021	0.112		3.75		51		300	3546
	RSIC	24	40468		7.03		0.739		0.719		21		41		0.024	0.041		4.02		45		342	3632
	MOSA	28	39565		6.87		0.719		0.707		22		39		0.037	0.068		3.91		53		349	3816
	SARD	30	44722		7.77		0.710		0.726		76		90		0.021	0.059		4.08		66		533	3633
	SBRU	10	32211		5.60		0.697		0.689		15		18		0.050	0.080		3.95		21		173	3226
	CORS	30	44708		7.77		0.722		0.724		120		119		0.022	0.050		4.06		67		535	3572
	AGER	22	34676		6.02		0.705		0.688		2		15		0.023	0.072		3.76		32		222	2966
	MARE	34	37470		6.51		0.695		0.690		44		47		0.028	0.105		3.74		53		294	3367
	CHI	12	32476		5.64		0.702		0.677		21		42		0.042	0.093		3.81		24		166	2928
	MPIS	15	21614		3.76		0.639		0.539		7		8		0.152	0.173		2.82		15		86	2203
	CALV	24	28886		5.02		0.640		0.617		4		19		0.057	0.172		3.24		29		146	2841

	MCH	21	37548		6.52		0.711		0.696		39		50		0.040	0.077		3.86		45		282	3311
	RMG	18	34890		6.06		0.677		0.667		158		153		0.032	0.127		3.69		34		191	3245
	GARF	23	26502		4.60		0.645		0.618		13		16		0.077	0.156		3.21		22		121	2833
	PONT	13	25804		4.48		0.631		0.599		3		18		0.103	0.170		3.20		17		104	2466
	MODE	23	35203		6.12		0.705		0.687		27		31		0.045	0.069		3.75		35		239	3111
	CABA	22	36019		6.26		0.715		0.693		21		22		0.027	0.052		3.81		38		258	3132
	REGG	26	38053		6.61		0.724		0.699		52		56		0.046	0.049		3.83		45		293	3503
	PMT	16	39962		6.94		0.737		0.719		171		171		0.033	0.029		4.12		36		294	3284
	BURL	24	36125	**37025**	6.28	**6.43**	0.713	**0,700**	0.696	**0,688**	11	**45.1**	19	**55.7**	0.047	0.077	**0.087**	3.81	**3.79**	40	**44**	267	3014
Alpine	PRDO	23	33445		5.81		0.685		0.673		15		17		0.038	0.088		3.64		38		250	2912
	OVAR	31	39534		6.87		0.731		0.706		30		29		0.035	0.038		3.87		48		330	3409
	REND	24	33939		5.90		0.687		0.671		11		23		0.053	0.092		3.63		44		233	3181
	BPUS	24	38621		6.71		0.717		0.703		25		31		0.032	0.052		3.88		42		321	3335
	PUST	24	31615		5.49		0.701		0.667		16		17		0.059	0.075		3.57		32		197	3075
	SIC	26	39920		6.94		0.732		0.714		28		39		0.032	0.035		3.97		49		355	3562
	PIN	29	38594		6.71		0.714		0.701		27		39		0.024	0.066		3.84		50		309	3408
	TGV	50	38939		6.76		0.700		0.690		36		45		0.023	0.071		3.72		67		344	3187
	MWF	46	35855		6.23		0.710		0.675		21		25		0.039	0.058		3.58		45		252	3128
	OBV	35	36925		6.42		0.695		0.682		17		26		0.028	0.074		3.68		66		333	3390
	BBV	50	35287		6.13		0.656		0.643		10		15		0.010	0.100		3.39		65		233	2797
	DFV	50	40079		6.96		0.692		0.684		26		41		0.012	0.075		3.69		98		445	3123
	FGV	50	37494		6.51		0.704		0.682		15		36		0.032	0.067		3.65		64		311	3148
	VOG	18	34749		6.04		0.721		0.688		12		22		0.039	0.045		3.80		33		245	3128
	ABO	22	33385		5.80		0.706		0.666		21		18		0.040	0.054		3.60		37		240	3005
	MON	28	31738		5.51		0.691		0.647		11		15		0.065	0.083		3.43		36		209	3003
	TAR	37	37339	**36709**	6.49	**6.38**	0.692	**0,700**	0.680	**0,680**	18	**20.5**	47	**30.0**	0.022	0.079	**0.070**	3.66	**3.67**	65	**56**	342	3228
France	RDBI	29	26163		4.55		0.606		0.578		25		34		0.174	0.200		2.99		28		127	2615
	SAL	26	36384		6.32		0.668		0.667		26		40		0.043	0.117		3.62		58		321	2942
	AUB	22	37489		6.51		0.691		0.685		32		49		0.028	0.079		3.79		51		349	2840

	LIM	48	42891		7.45		0.708		0.705		65		75		0.015	0.066		3.85		106		563	3274
	CHR	39	43444		7.55		0.720		0.719		67		63		0.018	0.061		3.97		87		482	3340
	PAR	17	36898		6.41		0.724		0.701		52		53		0.035	0.044		3.93		34		262	3242
	BAQ	33	40280		7.00		0.706		0.700		53		73		0.021	0.065		3.83		69		426	3291
	GAS	22	37288	**38387**	6.48	**6.67**	0.699	**0,691**	0.687	**0,683**	49	**48.9**	65	**58.9**	0.028	0.071	**0.087**	3.80	**3.73**	49	**67**	317	3071
Iberian	MNRQ	30	26609		4.62		0.637		0.605		28		27		0.173	0.166		3.12		29		128	2868
	MALL	30	20486		3.56		0.556		0.521		14		26		0.211	0.276		2.65		19		81	2456
	NGAN	14	35429		6.16		0.660		0.702		56		67		0.053	0.143		3.96		28		221	3208
	CANA	30	31527		5.48		0.645		0.614		16		36		0.151	0.158		3.24		31		168	2801
	MARI	22	30016		5.21		0.656		0.622		22		42		0.116	0.147		3.30		27		148	2686
	ALEN	10	29018		5.04		0.654		0.655		98		86		0.080	0.166		3.67		19		136	3104
	BAR	14	32044		5.57		0.659		0.663		29		54		0.051	0.145		3.68		31		199	2738
	MARO	19	35039		6.09		0.687		0.677		40		65		0.037	0.108		3.73		45		265	3147
	SYG	11	30662	**29205**	5.33	**5.07**	0.670	**0,639**	0.667	**0,621**	43	**31.3**	35	**43.2**	0.063	0.129	**0.169**	3.75	**3.32**	20	**28**	149	2802
North West Europe	BPN	15	33384		5.80		0.726		0.689		28		46		0.055	0.047		3.83		26		200	3033
	NOR	30	34001		5.91		0.698		0.664		38		61		0.055	0.091		3.55		43		234	3072
	MAN	20	34940		6.07		0.683		0.664		23		22		0.032	0.123		3.64		44		244	2941
	BBB	45	39395		6.84		0.698		0.690		40		46		0.028	0.103		3.72		67		329	3110
	LKF	22	31859		5.53		0.678		0.658		10		25		0.057	0.129		3.54		34		187	2810
	HF	50	37888		6.58		0.696		0.685		14		29		0.011	0.127		3.65		72		279	2877
	GNS	16	28903		5.02		0.634		0.623		89		65		0.039	0.175		3.36		29		159	2606
	JSY	49	31507		5.47		0.624		0.619		20		28		0.020	0.185		3.23		74		223	2641
	HER	41	36659		6.37		0.653		0.676		55		64		0.028	0.229		3.57		79		227	2286
	SHR	13	25626		4.45		0.566		0.575		4		15		0.039	0.300		3.12		27		128	2131
	KRY	14	27681		4.81		0.678		0.644		57		67		0.083	0.144		3.46		22		131	2698
	DXT	16	31660		5.50		0.625		0.667		32		26		0.065	0.202		3.64		31		185	2841
	GLW	40	34358		5.97		0.635		0.652		24		36		0.047	0.186		3.45		82		275	2744
	AAN	48	35756		6.21		0.659		0.668		30		42		0.026	0.188		3.53		83		249	2617

	HGL	27	28798		5.00		0.606		0.605		33		47		0.045	0.231		3.18		57		181	2524
	NRC	34	36853		6.40		0.702		0.686		20		39		0.033	0.101		3.70		57		268	3016
	SERC	24	35396		6.15		0.690		0.678		32		35		0.069	0.113		3.68		47		239	2839
	FJL	22	35464		6.16		0.700		0.680		67		74		0.083	0.093		3.72		42		242	2946
	FIAY	42	37043		6.44		0.686		0.677		19		46		0.018	0.111		3.61		65		281	2942
	FINE	20	38958		6.77		0.692		0.711		78		100		0.048	0.097		3.99		41		321	3608
	FINW	35	38966		6.77		0.696		0.693		59		81		0.040	0.095		3.77		56		316	3372
	FINN	18	32166		5.59		0.719		0.670		27		42		0.067	0.069		3.64		27		190	3140
	YARO	20	36065	**34908**	6.27	**6.06**	0.707	**0,672**	0.686	**0,666**	58	**34.9**	69	**47.0**	0.068	0.080	**0.142**	3.78	**3.58**	39	**57**	246	3265

3.1.2 Heterozygosity

In the actual heterozygosity (H_O), the geographic groups of Southeast Europe, Minor Asia, Alpine and Tyrrhenian show the highest values respectively ($\overline{Ho}_{\text{(S-E-Europe)}}$ = 0.733, $\overline{Ho}_{\text{(minor Asia)}}$ = 0.725, $\overline{Ho}_{\text{(Tyrrhenian)}}$ = 0.7, $\overline{Ho}_{\text{(Alpine)}}$ = 0.7). The subgroup of Greek island breeds takes the lowest values $\overline{Ho}_{\text{(Greek Island subgroup)}}$ = 0.61, the rest geographic groups take intermediate values including the Greek mainland subgroup with $\overline{Ho}_{\text{(Greek mainland subgroup)}}$ = 0.686 (**Table 3.1**).

In the expected heterozygosity (H_E) the geographic groups of Minor Asia, Southeast Europe, and the Greek mainland subgroup present the highest values with $\overline{\boldsymbol{H_E}}_{\text{(minor Asia)}}$ = 0.727, $\overline{\boldsymbol{H_E}}_{\text{(S-E-Europe)}}$ = 0.726, $\overline{\boldsymbol{H_E}}_{\text{(Greek mainland subgroup)}}$ = 0.7 respectively. The subgroup of Greek island breeds takes the lowest values $\overline{\boldsymbol{H_E}}_{\text{(Greek Island subgroup)}}$ = 0.543, the rest geographic groups take intermediate values (**Table 3.1**).

At breed level the highest value of H_O takes the Red Metochian Buša with $H_{O\text{ (Red Metochian Buša)}}$ = 0.757, and the highest value of H_E takes the Anatolian Black cattle with $H_{E\text{ (Anatolian Black cattle)}}$ = 0.746. In addition, very high H_E values are shared by the Greek Brachyceros breed ($H_{E\text{ (Greek Brachyceros breed)}}$ = 0.742) with most of the neighboring Buša breeds and Italian PODO. At both indexes the lowest values takes the Crete Greek Island breed with $H_{O\text{ (Crete)}}$ = 0.518 and $H_{E\text{ (Crete)}}$ = 0.373. The Cyprus cattle takes intermediate values among Balkan, Minor Asia breeds and the subgroup of Greek island breeds $H_{O\text{ (Cyprus)}}$ = 0.629 and $H_{E\text{(Cyprus)}}$= 0.627 (**Table 3.1**).

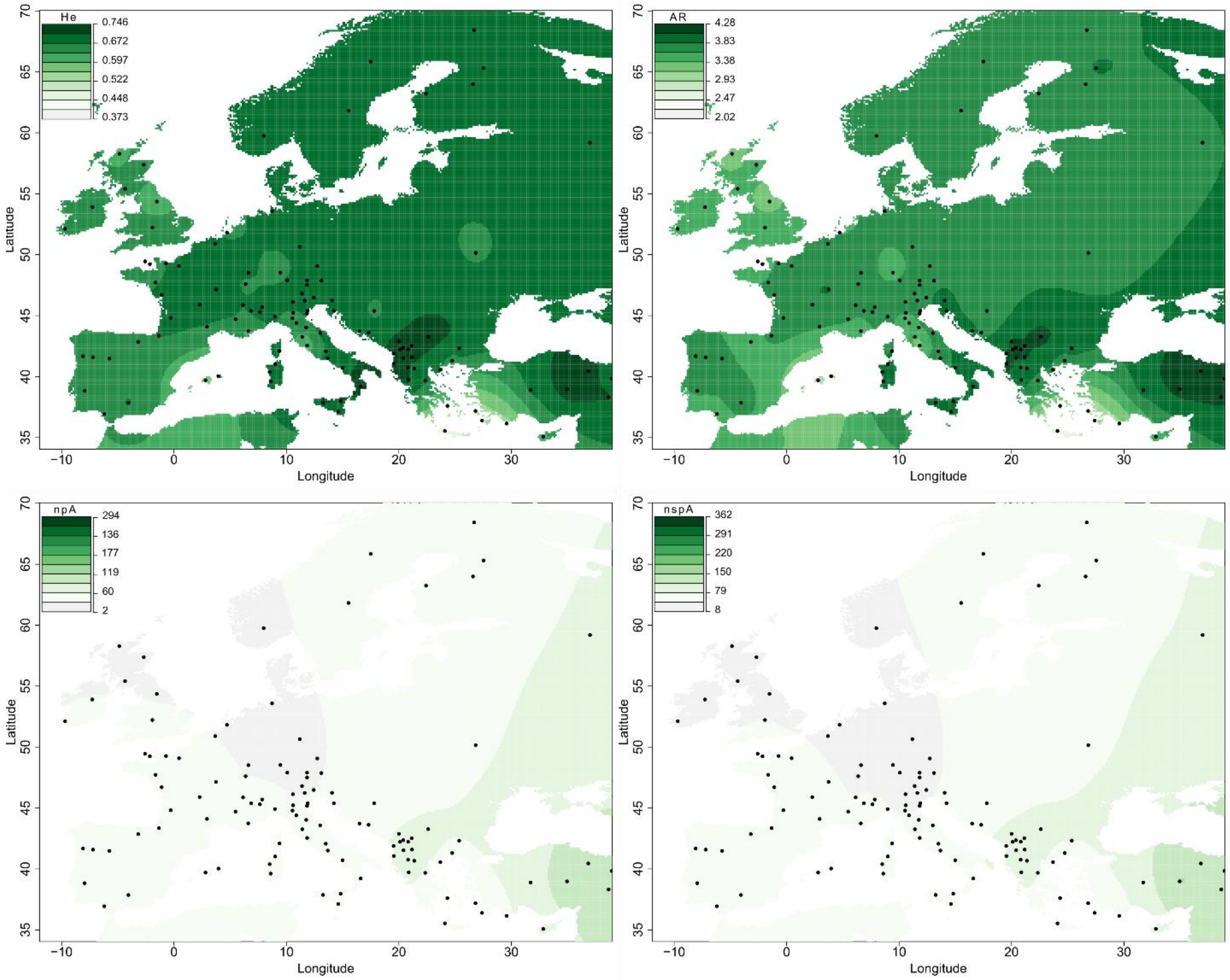


Figure 3.2. Tessellated projection. Spatial geographic presentation of the herein estimated diversity parameters (H_E, AR, *npA*, *nspA*). For the breed position see *Figure 2.2*.

Within the Greek and Cyprus cattle breeds, CRT exhibited the lowest values for all genetic diversity parameters and, at the same time, the highest frequency of private alleles (*fpA* = 0.376) as well as the highest average inbreeding coefficient (*F* = 0.457). For GRB, most of the diversity estimates had the highest values but the frequency of private alleles (*fpA* = 0.021) and the inbreeding coefficient (*F* = 0.108) were low. Generally, all the analyzed island populations except the KEA breed, which was sampled on the island of Kea and on mainland, had very high levels of inbreeding and very low diversity parameters (**Table 3.1**). GRB and the Southeast European Buša cattle shared similar values for almost all diversity parameters.

The tessellated projection of diversity statistics provided strong support for a high allelic diversity in breeds from Anatolia and part of Southeast Europe. Based on **Figure 3.2**, a Southeast to Northwest gradient of genetic diversity as we move away from the center of cattle domestication could be inferred, which is interrupted by the genetic diversity parameters of the Greek island breeds. However, if the Greek island breeds are excluded, this possible Southeast to Northwest gradient of genetic diversity remains consistent.

3.2 Unified Additive Relationships (UAR)

The UAR matrix consists of the genetic relationship between 2,858 individuals. Thus, information can be extracted regarding the genetic closeness between different breeds (**Table 3.2, Table 3.3**) and individuals as well as the degree of kinship of the individuals that make up each breed (UAR relationship within breed) (**Figure 3.3**). Finally, from the diagonal as has been said, of this matrix the inbreeding coefficient is inferred (**Table 3.1** and **Figure 3.3**).

3.2.1 UAR relationships between breeds

From the original matrix of Unified Additive relationships between individuals (2858 x 2858) was constructed the genetic relationships matrix at breed level as $\overline{UAR}_{\text{(all individuals breed A; all individuals breed B)}}$ (115 x 115). Because the demonstration of such a table is impossible, the representation of part of it was chosen. More specifically, the four largest relatedness values for each breed are presented in descending order and the two smallest genetic relatedness values in ascending order (**Table 3.2**). Furthermore, the genetic relationship between each breed and the geographic breed groups is shown (**Table 3.3**).

As can be seen in the kinship matrix (**Table 3.2**), all Greek breeds as well as the Cypriot cattle show the highest closeness values with the representative of the *Bos indicus* (GIR). In fact, the Greek island populations (Agathonisi, Kastelorizo) and the Cypriot cattle show very high values. The remaining breeds with which the Greek breeds show a high affinity are from Minor Asia, the Cypriot cattle, or the Greek island population of Kastelorizo. An exception to this is the island breed Kea, with a known Alpine influence, which shows the highest value of genetic affinity with Alpine breeds (Braunvieh).

Table 3.2. For the 113 breeds, the four highest and two lowest genetic relationships based on the UAR table are presented at the breed level in descending order. Breed abbreviations are indicated in Table 2.1.

Breed	MaxUAR_1 (Value)	MaxUAR_1 (Breed)	MaxUAR_2 (Value)	MaxUAR_2 (Breed)	MaxUAR_3 (Value)	MaxUAR_3 (Breed)	MaxUAR_4 (Value)	MaxUAR_4 (Breed)	MinUAR_1 (Value)	MinUAR_1 (Breed)	MinUAR_2 (Value)	MinUAR_2 (Breed)
GIR	0.4034	**ATSY**	0.4	CYP	0.3926	KAS	0.3708	ATSR	-0.099	**SHR**	-0.091	DXT
ATER	0.3684	**GIR**	0.176	ATSY	0.1716	KAS	0.1676	ATSR	-0.056	**SHR**	-0.051	HER
ATBC	0.3515	**GIR**	0.1669	ATSY	0.1657	KAS	0.1614	CYP	-0.051	**SHR**	-0.048	HER
ATSR	0.3708	**GIR**	0.1974	CYP	0.1791	ATSY	0.1732	KAS	-0.056	**SHR**	-0.054	HER
ATSY	0.4034	**GIR**	0.1872	CYP	0.1838	KAS	0.1791	ATSR	-0.059	**SHR**	-0.054	HER
TRG	0.2361	**GIR**	0.1196	ATSY	0.1168	KAS	0.1124	ATSR	-0.047	**SHR**	-0.039	HER
CYP	0.4	**GIR**	0.1974	ATSR	0.1872	ATSY	0.1851	KAS	-0.058	**HGL**	-0.056	GLW
AGT	0.3442	**GIR**	0.171	KAS	0.1614	ATSY	0.1608	CYP	-0.056	**SHR**	-0.049	HER
CRT	0.2028	**GIR**	0.1067	CYP	0.1053	ATSY	0.1042	KAS	-0.048	**SHR**	-0.043	HER
NSY	0.1319	**GIR**	0.0724	KAS	0.068	CYP	0.0645	ATSY	-0.03	**HGL**	-0.028	SHR
GRB	0.1079	**GIR**	0.0694	KAS	0.0656	ATSY	0.0641	CYP	-0.033	**SHR**	-0.03	AAN
KAS	0.3926	**GIR**	0.1851	CYP	0.1838	ATSY	0.1732	ATSR	-0.064	**SHR**	-0.058	HER
KEA	0.0456	**BBV**	0.0291	OBV	0.0279	SBRU	0.0246	AGER	-0.028	**GIR**	-0.017	JSY
PRG	0.0611	**GIR**	0.0425	KAS	0.0411	ATSY	0.04	ATER	-0.025	**SHR**	-0.023	AAN
ROG	0.1618	**GIR**	0.0929	KAS	0.0887	ATSY	0.0837	CYP	-0.046	**SHR**	-0.037	MAN
SYK	0.1124	**GIR**	0.0604	ATSY	0.0571	KAS	0.0565	ATSR	-0.031	**SHR**	-0.027	AAN
KTR	0.1435	**GIR**	0.079	KAS	0.0768	ATSY	0.0751	CYP	-0.035	**SHR**	-0.033	AAN
RHS	0.1243	**GIR**	0.0705	KAS	0.0696	ATSY	0.0654	ATER	-0.033	**SHR**	-0.028	AAN
MKB	0.0916	**GIR**	0.0586	KAS	0.055	ATSY	0.052	ATER	-0.032	**SHR**	-0.027	AAN
SRB	0.055	**GIR**	0.040	KAS	0.039	ATSY	0.037	ATER	-0.029	**SHR**	-0.025	AAN
PRE	0.05	**GIR**	0.037	KAS	0.035	ATSY	0.032	ATER	-0.026	**SHR**	-0.021	AAN
RMB	0.056	**GIR**	0.046	KAS	0.044	ATSY	0.042	ATER	-0.029	**SHR**	-0.025	AAN
SHB	0.047	**GIR**	0.041	KAS	0.039	ATSY	0.037	ATER	-0.03	**SHR**	-0.022	AAN
DGB	0.011	**RMB**	0.0091	ROG	0.009	HF	0.0087	HRP	-0.01	**OBV**	-0.01	BBV
DBB	0.057	**JSY**	0.0231	MAB	0.0204	SKB	0.0195	LKB	-0.016	**MON**	-0.015	DFV
MAB	0.062	**JSY**	0.0231	DBB	0.023	LKB	0.021	SKB	-0.017	**DFV**	-0.016	SHR
LKB	0.043	**JSY**	0.0231	MAB	0.02	DBB	0.018	SKB	-0.019	**SHR**	-0.016	AAN
SKB	0.046	**JSY**	0.021	MAB	0.02	DBB	0.018	LKB	-0.014	**MON**	-0.014	SAL
MNB	0.013	**RMB**	0.0112	TGV	0.011	SHB	0.011	ROG	-0.020	**SHR**	-0.015	AAN
BHB	0.031	**ATER**	0.0301	KAS	0.03	ATSY	0.028	ATSR	-0.025	**SHR**	-0.023	SERC
HRB	0.015	KAS	0.014	ATER	0.014	ATSY	0.013	ROG	-0.025	**SHR**	-0.020	AAN

HRI	0.44	**RMG**	0.038	MARE	0.030	MCH	0.026	PODO		-0.031	**SHR**	-0.025	AAN
HRP	0.038	**GIR**	0.036	KAS	0.0297	ATSY	0.028	ATSR		-0.029	**SHR**	-0.024	AAN
UKP	0.027	**GIR**	0.026	ATSY	0.025	HRP	0.0249	ATER		-0.022	**SHR**	-0.021	AAN
PODO	0.055	**RMG**	0.045	**GIR**	0.041	MARE	0.037	MCH		-0.032	**SHR**	-0.026	SERC
CINI	0.064	**MOSI**	0.055	MOSA	0.051	**GIR**	0.044	RSIC		-0.027	**SHR**	-0.022	AAN
MOSI	0.117	**MOSA**	0.101	RSIC	0.072	**GIR**	0.064	CINI		-0.036	**SHR**	-0.030	AAN
RSIC	0.101	**MOSI**	0.069	MOSA	0.044	CINI	0.036	**GIR**		-0.025	**SHR**	-0.022	FIAY
MOSA	0.117	**MOSI**	0.068	RSIC	0.058	**GIR**	0.055	CINI		-0.036	**SHR**	-0.031	AAN
SARD	0.0257	**OBV**	0.026	SBRU	0.016	CORS	0.0155	REND		-0.057	**GIR**	-0.018	CYP
SBRU	0.082	**OBV**	0.061	BBV	0.043	REND	0.038	CABA		-0.073	**GIR**	-0.037	CYP
CORS	0.016	**SARD**	0.014	MCH	0.013	MOSA	0.012	MOSI		-0.033	**GIR**	-0.0215	SHR
AGER	0.117	**BBV**	0.034	SBRU	0.031	0BV	0.03	HF		-0.070	**GIR**	-0.034	CYP
MARE	0.056	**GIR**	0.05	RMG	0.043	MCH	0.041	PODO		-0.033	**SHR**	-0.027	HER
CHI	0.177	**CALV**	0.081	MCH	0.047	RMG	0.03	MPIS		-0.019	**MON**	-0.019	DFV
MPIS	0.034	**CALV**	0.03	CHI	0.02	MCH	0.018	OBV		-0.032	**GIR**	-0.021	SHR
CALV	0.177	**CHI**	0.094	MCH	0.056	RMG	0.045	**GIR**		-0.033	**SHR**	-0.031	AAN
MCH	0.094	**CALV**	0.081	CHI	0.063	RMG	0.047	**GIR**		-0.030	**SHR**	-0.030	AAN
RMG	0.063	**MCH**	0.056	CALV	0.055	**PODO**	0.05	MARE		-0.031	**SHR**	-0.026	AAN
GARF	0.02	**OBV**	0.015	SBRU	0.01	REND	0.09	PONT		-0.037	**GIR**	-0.021	SHR
PONT	0.05	**TAR**	0.018	PRDO	0.016	ABO	0.015	REGG		-0.051	**GIR**	-0.024	SHR
MODE	0.017	**REND**	0.0153	OBV	0.016	SBRU	0.015	TGV		-0.052	**GIR**	-0.024	SHR
CABA	0.045	**OBV**	0.038	SBRU	0.033	BBV	0.030	REND		-0.070	**GIR**	-0.033	CYP
REGG	0.025	**OVAR**	0.016	DFV	0.015	PONT	0.014	PRDO		-0.047	**GIR**	-0.020	CYP
PMT	0.017	**TAR**	0.017	AUB	0.162	GAS	0.016	BPUS		-0.069	**GIR**	-0.028	CYP
BURL	0.091	**HF**	0.069	LKF	0.043	BBB	0.041	NRC		-0.078	**GIR**	-0.039	ATSY
PRDO	0.066	**DFV**	0.064	ABO	0.06	MON	0.053	TAR		-0.076	**GIR**	-0.041	KAS
OVAR	0.025	**REGG**	0.0194	OBV	0.019	SBRU	0.017	REND		-0.053	**GIR**	-0.021	ATSR
REND	0.052	**OBV**	0.043	SBRU	0.038	BBV	0.030	CABA		-0.074	**GIR**	-0.038	CYP
BPUS	0.05	**VOG**	0.0472	PRDO	0.047	ABO	0.045	DFV		-0.080	**GIR**	-0.040	CYP
PUST	0.043	**PIN**	0.033	VOG	0.028	SIC	0.025	BPUS		-0.067	**GIR**	-0.033	CYP
SIC	0.033	**DFV**	0.031	PIN	0.028	PUST	0.024	PRDO		-0.061	**GIR**	-0.027	CYP
PIN	0.043	**PUST**	0.039	HF	0.031	SIC	0.018	BURL		-0.063	**GIR**	-0.029	CYP
TGV	0.036	**OBV**	0.0286	REND	0.029	SBRU	0.025	BBV		-0.075	**GIR**	-0.038	CYP
MWF	0.024	**OBV**	0.0229	TGV	0.023	REND	0.022	SBRU		-0.071	**GIR**	-0.037	CYP
OBV	0.082	**SBRU**	0.059	BBV	0.052	REND	0.045	CABA		-0.078	**GIR**	-0.040	CYP
BBV	0.117	**AGER**	0.061	SBRU	0.059	0BV	0.046	KEA		-0.079	**GIR**	-0.041	CYP

DFV	0.075	**MON**	0.067	ABO	0.066	PRDO	0.048	TAR		-0.085	**GIR**	-0.045	CYP
FGV	0.044	**DFV**	0.039	MON	0.037	ABO	0.035	PRDO		-0.077	**GIR**	-0.038	CYP
VOG	0.05	**BPUS**	0.046	MON	0.0454	ABO	0.045	DFV		-0.078	**GIR**	-0.041	CYP
ABO	0.067	**DFV**	0.064	PRDO	0.059	MON	0.056	TAR		-0.080	**GIR**	-0.045	KAS
MON	0.075	**DFV**	0.06	PRDO	0.059	AB0	0.047	TAR		-0.081	**GIR**	-0.045	KAS
TAR	0.056	**ABO**	0.053	PRDO	0.05	PONT	0.048	DFV		-0.080	**GIR**	-0.043	KAS
RDBI	0.036	**AUB**	0.034	SAL	0.03	BAQ	0.029	GAS		-0.080	**GIR**	-0.040	AGT
SAL	0.09	**AUB**	0.068	LIM	0.067	BAQ	0.061	GAS		-0.084	**GIR**	-0.046	KAS
AUB	0.09	**SAL**	0.067	LIM	0.067	BAQ	0.063	GAS		-0.085	**GIR**	-0.043	CYP
LIM	0.068	**SAL**	0.067	AUB	0.062	BAQ	0.051	GAS		-0.079	**GIR**	-0.041	KAS
CHR	0.069	**SHR**	0.049	MAN	0.032	SAL	0.028	AUB		-0.058	**GIR**	-0.039	KAS
PAR	0.028	**AUB**	0.026	SAL	0.022	LIM	0.022	BAQ		-0.075	**GIR**	-0.039	CYP
BAQ	0.067	**AUB**	0.0665	SAL	0.062	LIM	0.058	GAS		-0.079	**GIR**	-0.039	KAS
GAS	0.063	**AUB**	0.061	SAL	0.058	BAQ	0.051	LIM		-0.073	**GIR**	-0.037	CYP
MNRQ	0.013	**MALL**	0.011	BURL	0.0083	SBRU	0.0077	LKF		-0.066	**GIR**	-0.031	KAS
MALL	0.013	**MNRQ**	0.0122	BAR	0.012	ALEN	0.011	NGAN		-0.067	**GIR**	-0.030	KAS
NGAN	0.052	**MARI**	0.0497	SYG	0.0496	BAR	0.049	ALEN		-0.053	**GIR**	-0.022	KAS
CANA	0.052	**MARI**	0.048	BAR	0.046	NGAN	0.044	MARO		-0.053	**GIR**	-0.021	SHR
MARI	0.054	**BAR**	0.052	NGAN	0.0518	CANA	0.051	ALEN		-0.047	**GIR**	-0.021	ATSY
ALEN	0.052	**SYG**	0.518	BAR	0.051	MARI	0.049	NGAN		-0.044	**GIR**	-0.016	ATBC
BAR	0.094	**MARO**	0.058	SYG	0.054	MARI	0.052	ALEN		-0.054	**GIR**	-0.021	ATBC
MARO	0.094	**BAR**	0.056	SYG	0.049	MARI	0.048	ALEN		-0.058	**GIR**	-0.021	ATBC
SYG	0.058	**BAR**	0.056	MARO	0.052	ALEN	0.05	NGAN		-0.059	**GIR**	-0.021	ATSY
BPN	0.047	**SHR**	0.039	MAN	0.027	KRY	0.026	DXT		-0.078	**GIR**	-0.038	KAS
NOR	0.031	**SHR**	0.03	MAN	0.029	BBB	0.025	HF		-0.080	**GIR**	-0.040	CYP
MAN	0.28	**SHR**	0.105	BBB	0.089	AAN	0.086	SERC		-0.091	**GIR**	-0.055	KAS
BBB	0.16	**SHR**	0.105	MAN	0.0642	SERC	0.0641	NRC		-0.081	**GIR**	-0.046	KAS
LKF	0.069	**BURL**	0.068	HF	0.054	BBB	0.052	SHR		-0.075	**GIR**	-0.041	KAS
HF	0.091	**BURL**	0.068	HF	0.058	BBB	0.053	NRC		-0.071	**GIR**	-0.039	KAS
GNS	0.052	**JSY**	0.026	DXT	0.021	KRY	0.0205	GLW		-0.084	**GIR**	-0.039	ATSY
JSY	0.062	**MAB**	0.057	DBB	0.052	JSY	0.046	SKB		-0.084	**GIR**	-0.049	CYP
HER	0.057	**HGL**	0.056	DXT	0.0542	KRY	0.054	GLW		-0.084	**GIR**	-0.058	KAS
SHR	0.280	**MAN**	0.160	BBB	0.139	AAN	0.126	SERC		-0.099	**GIR**	-0.064	KAS
KRY	0.108	**SHR**	0.073	KRY	0.067	MAN	0.0669	AAN		-0.081	**GIR**	-0.053	KAS
DXT	0.083	**SHR**	0.073	KRY	0.066	AAN	0.064	GLW		-0.091	**GIR**	-0.053	CYP
GLW	0.064	**HGL**	0.064	DXT	0.060	KRY	0.057	AAN		-0.090	**GIR**	-0.056	CYP

AAN	0.139	**SHR**	0.089	MAN	0.067	KRY	0.066	DXT		-0.081	**GIR**	-0.051	ATSY
HGL	0.069	**GLW**	0.061	KRY	0.060	DXT	0.057	HER		-0.088	**GIR**	-0.058	CYP
NRC	0.174	**SERC**	0.126	FIAY	0.109	SHR	0.072	MAN		-0.079	**GIR**	-0.048	KAS
SERC	0.174	**NRC**	0.144	FIAY	0.126	SHR	0.086	MAN		-0.086	**GIR**	-0.050	KAS
FJL	0.133	**FINN**	0.041	FINW	0.040	FINE	0.023	YARO		-0.062	**GIR**	-0.027	CYP
FIAY	0.144	**SERC**	0.126	NRC	0.064	SHR	0.056	KRY		-0.083	**GIR**	-0.050	CYP
FINE	0.061	**FINW**	0.05	FINN	0.04	FJL	0.03	FIAY		-0.057	**GIR**	-0.023	CYP
FINW	0.068	**FINN**	0.061	FINE	0.03	FIAY	0.034	FIAY		-0.065	**GIR**	-0.029	CYP
FINN	0.133	**FIAY**	0.068	FINW	0.05	FINE	0.029	FIAY		-0.062	**GIR**	-0.029	CYP
YARO	0.029	**FINE**	0.0274	HF	0.0271	FINW	0.026	FINN		-0.050	**GIR**	-0.022	CYP

The same kinship pattern with the Greek breeds is shown by all the breeds of Minor Asia as well as most of the breeds of the South-East Europe group. Exception in this is the majority of the Albanian populations (DBB, MAB, LKB, SKB), the Kosovar DGB, and the MNB from Montenegro, which show a particularly high affinity with populations from the Northwest group (mainly Jersey) and breeds from Alpine group (**Table 3.2**). Also, a similar pattern of kinship with the Greek breeds is presented by the podolian breeds (East Podolian group), apart from the Croatian HRI, which shows high values of relationship with Italian breeds (RMG, MARE, MCH, PODO) of known podolian influence. Also of interest is the fact that most breeds of known podolian influence (PODO, CALV, MARE, RMG, MCH) of the Tyrrhenian group, as well as the local breeds of Sicily (CINI, MOSI, RSIC) and the MOSSA breed from Sardinia show significant levels of affinity with GIR.

The rest of the breeds, in general, show the highest values of genetic affinity with breeds of the same geographic breed Group. Exceptions are some breeds of Northern Italy that show a significant genetic relationship with the neighboring Alpine breeds, as well as some cosmopolitan breeds (Jersey, Holstein, Braunvieh) that show a high affinity with breeds of geographically distant breed groups. The latter is because these famous breeds have played the role of donors in many populations.

Regarding the values of lower genetic affinity, it is observed that the local breeds of groups Asia Minor, the Greek and Cyprus, Southeast Europe as well as the podolian and local breeds of the Tyrrhenian, who show high values of relatedness with the representative of the *Bos indicus* (GIR), they get the lowest value with the isolated island breeds of Great Britain (SHR or HJL). The opposite path is seen in the breeds of the Alpine, French, Iberian, and Northwest geographic groups which show the least affinity with GIR.

Among 112 breeds the highest genetic relatedness was observed between two breeds of the Northwest geographic group in Shorthorn - SHR and Maine Anjou - MAN with $UAR_{\text{(Shorthorn - Maine Anjou)}} = 0.28$ and the lowest between Shorthorn and Kastelorizo from Greek island subgroup with $UAR_{\text{(Shorthorn - Kastelorizo)}} = -0.064$.

Table 3.3. Genetic relatedness index *UAR*. Pairwise values among 112 Greek breeds and 10 geographic groups. The cell numbers greater than or equal to zero are presented with a green gradient and negative ones with yellow without gradient. Breed abbreviations are indicated in Table 2.1

	UAR	Minor Asia	Greek Islands	Greek Mainland	S-E Europe	East Podolian	Tyrrhenian	Alpine	France	Iberian	N-W Europe
Minor Asia	**ATER**	0.153	0.092	0.062	0.026	0.025	0.001	-0.031	-0.036	-0.021	-0.038
	ATBC	0.146	0.090	0.060	0.025	0.022	0.001	-0.030	-0.035	-0.021	-0.035
	ATSR	0.154	0.095	0.062	0.024	0.022	0.001	-0.033	-0.036	-0.016	-0.040
	ATSY	0.160	0.101	0.067	0.027	0.025	0.002	-0.034	-0.038	-0.021	-0.041
	TRG	0.112	0.063	0.048	0.021	0.021	0.003	-0.023	-0.026	-0.013	-0.028
Greece and Cyprus	**CYP**	0.165	0.102	0.063	0.023	0.021	-0.00001	-0.037	-0.038	-0.016	-0.042
	AGT	0.141	0.078	0.059	0.024	0.021	0.001	-0.030	-0.036	-0.020	-0.036
	CRT	0.092	0.058	0.040	0.017	0.015	0.001	-0.024	-0.028	-0.015	-0.031
	NSY	0.056	0.047	0.025	0.008	0.003	0.000	-0.008	-0.017	-0.012	-0.019
	KAS	0.162	0.084	0.068	0.028	0.026	0.001	-0.035	-0.039	-0.021	-0.042
	KEA	-0.010	-0.006	-0.001	-0.003	-0.002	0.001	0.007	-0.006	-0.007	-0.011
	GRB	0.059	0.040	0.035	0.019	0.018	0.005	-0.017	-0.019	-0.009	-0.019
	PRG	0.037	0.024	0.025	0.014	0.011	0.002	-0.010	-0.012	-0.009	-0.012
	ROG	0.079	0.049	0.037	0.020	0.018	0.004	-0.019	-0.023	-0.011	-0.025
	SYK	0.054	0.035	0.031	0.0134	0.013	0.003	-0.012	-0.017	-0.009	-0.019
	KTR	0.069	0.042	0.038	0.017	0.018	0.004	-0.018	-0.019	-0.011	-0.023
South East Europe	**RHS**	0.062	0.038	0.034	0.018	0.016	0.003	-0.015	-0.019	-0.010	-0.018
	MKB	0.050	0.031	0.029	0.017	0.016	0.004	-0.012	-0.016	-0.007	-0.017
	SRB	0.035	0.022	0.022	0.014	0.012	0.004	-0.006	-0.010	-0.006	-0.015
	PRE	0.030	0.019	0.019	0.013	0.010	0.002	-0.008	-0.012	-0.007	-0.011
	RMB	0.040	0.025	0.027	0.018	0.018	0.004	-0.012	-0.015	-0.005	-0.013
	SHB	0.035	0.022	0.024	0.017	0.016	0.004	-0.010	-0.013	-0.005	-0.013
	DGB	0.005	0.002	0.006	0.006	0.003	-0.002	-0.004	-0.007	-0.003	-0.0002
	DBB	0.008	0.004	0.007	0.010	0.003	-0.002	-0.009	-0.010	-0.005	-0.0001
	MAB	0.013	0.005	0.011	0.013	0.006	-0.0001	-0.010	-0.011	-0.005	-0.002
	LKB	0.014	0.007	0.012	0.014	0.008	0.001	-0.007	-0.010	-0.004	-0.006
	SKB	0.005	0.001	0.008	0.011	0.004	-0.001	-0.008	-0.009	-0.004	0.001
	MNB	0.006	0.004	0.008	0.008	0.007	0.003	0.002	-0.005	-0.004	-0.008
	BHB	0.028	0.017	0.019	0.014	0.015	0.002	-0.011	-0.013	-0.004	-0.012
	HRB	0.013	0.008	0.011	0.009	0.009	0.003	0.001	-0.005	-0.004	-0.011
East Podolian	**HRI**	0.015	0.008	0.013	0.007	0.018	0.011	-0.004	-0.009	-0.005	-0.016
	HRP	0.032	0.017	0.020	0.014	0.025	0.002	-0.013	-0.015	-0.008	-0.014
	UKP	0.023	0.013	0.014	0.010	0.018	0.004	-0.010	-0.012	-0.005	-0.011
Tyrrhenian	**PODO**	0.027	0.016	0.018	0.009	0.017	0.014	-0.008	-0.011	-0.005	-0.018
	CINI	0.026	0.015	0.014	0.006	0.009	0.013	-0.010	-0.012	-0.006	-0.015
	MOSI	0.033	0.018	0.017	0.007	0.011	0.018	-0.015	-0.017	-0.009	-0.021
	RSIC	0.018	0.010	0.009	0.003	0.007	0.015	-0.007	-0.005	-0.006	-0.017
	MOSA	0.029	0.017	0.016	0.007	0.011	0.017	-0.010	-0.013	-0.007	-0.021
	SARD	-0.015	-0.008	-0.003	-0.001	-0.0004	0.007	0.008	0.005	0.002	-0.009
	SBRU	-0.028	-0.010	-0.010	-0.006	-0.006	0.004	0.023	0.005	-0.003	-0.011
	CORS	-0.003	-0.003	0.004	0.005	0.005	0.007	-0.002	0.002	0.003	-0.009
	AGER	-0.030	-0.010	-0.010	-0.008	-0.011	-0.001	0.014	-0.002	-0.007	0.000
	MARE	0.031	0.017	0.019	0.010	0.024	0.012	-0.015	-0.016	-0.007	-0.018
	CHI	0.015	0.007	0.011	0.005	0.015	0.019	-0.013	-0.012	-0.008	-0.009
	MPIS	-0.010	-0.006	-0.006	-0.002	0.001	0.008	0.005	-0.003	-0.008	-0.012
	CALV	0.027	0.015	0.017	0.008	0.020	0.022	-0.015	-0.016	-0.008	-0.021

	MCH	0.029	0.017	0.019	0.010	0.022	0.019	-0.013	-0.014	-0.007	-0.019
	RMG	0.024	0.012	0.016	0.007	0.026	0.016	-0.012	-0.014	-0.007	-0.019
	GARF	-0.010	-0.008	-0.002	-0.001	0.001	0.006	0.002	-0.005	-0.004	-0.009
	PONT	-0.018	-0.010	-0.006	-0.003	-0.003	0.002	0.012	0.006	-0.002	-0.010
	MODE	-0.018	-0.009	-0.004	-0.001	0.000	0.003	0.011	0.003	-0.003	-0.009
	CABA	-0.026	-0.013	-0.010	-0.005	-0.006	0.003	0.017	0.005	-0.002	-0.007
	REGG	-0.015	-0.010	-0.006	-0.002	-0.001	0.003	0.010	0.002	-0.002	-0.007
	PMT	-0.023	-0.016	-0.009	-0.002	-0.003	0.002	0.010	0.012	0.004	-0.004
	BURL	-0.033	-0.023	-0.018	-0.007	-0.013	-0.010	-0.002	-0.005	-0.002	0.024
Alpine	**PRDO**	-0.034	-0.022	-0.018	-0.010	-0.010	-0.002	0.033	0.023	-0.005	-0.016
	OVAR	-0.018	-0.011	-0.006	-0.002	-0.002	0.004	0.011	0.007	-0.002	-0.009
	REND	-0.028	-0.014	-0.011	-0.006	-0.005	0.004	0.020	0.005	-0.006	-0.011
	BPUS	-0.034	-0.023	-0.019	-0.010	-0.012	-0.002	0.026	0.017	-0.002	-0.006
	PUST	-0.025	-0.016	-0.011	-0.004	-0.006	-0.001	0.019	0.005	-0.002	-0.007
	SIC	-0.021	-0.014	-0.009	-0.003	-0.003	-0.0001	0.018	0.007	-0.003	-0.007
	PIN	-0.025	-0.016	-0.012	-0.005	-0.006	-0.002	0.008	-0.002	-0.002	0.004
	TGV	-0.029	-0.015	-0.013	-0.004	-0.007	0.001	0.019	0.007	-0.006	-0.011
	MWF	-0.027	-0.018	-0.013	-0.007	-0.008	-0.00005	0.017	0.005	-0.005	-0.010
	OBV	-0.031	-0.013	-0.014	-0.008	-0.006	0.007	0.021	0.005	-0.006	-0.013
	BBV	-0.034	-0.004	-0.009	-0.010	-0.011	0.006	0.015	0.001	-0.009	-0.015
	DFV	-0.037	-0.024	-0.022	-0.012	-0.013	-0.005	0.034	0.022	-0.006	-0.014
	FGV	-0.033	-0.023	-0.019	-0.010	-0.012	-0.005	0.021	0.012	-0.005	-0.005
	VOG	-0.034	-0.022	-0.019	-0.010	-0.012	-0.003	0.027	0.019	-0.003	-0.006
	ABO	-0.036	-0.024	-0.020	-0.012	-0.012	-0.003	0.033	0.024	-0.005	-0.014
	MON	-0.037	-0.026	-0.022	-0.012	-0.012	-0.004	0.032	0.020	-0.006	-0.013
	TAR	-0.034	-0.024	-0.020	-0.011	-0.012	-0.001	0.028	0.022	-0.003	-0.012
France	**RDBI**	-0.033	-0.025	-0.015	-0.010	-0.011	-0.006	0.004	0.026	0.015	-0.008
	SAL	-0.039	-0.030	-0.022	-0.014	-0.016	-0.006	0.018	0.054	0.003	-0.010
	AUB	-0.037	-0.026	-0.020	-0.013	-0.014	-0.004	0.020	0.054	0.005	-0.012
	LIM	-0.036	-0.026	-0.018	-0.012	-0.014	-0.005	0.014	0.046	0.004	-0.007
	CHR	-0.032	-0.024	-0.020	-0.012	-0.014	-0.008	0.006	0.022	-0.001	0.012
	PAR	-0.045	-0.035	-0.027	-0.017	-0.020	-0.014	-0.010	0.002	-0.005	0.048
	BAQ	-0.030	-0.022	-0.015	-0.006	-0.007	-0.002	0.004	0.020	0.005	0.005
	GAS	-0.034	-0.024	-0.019	-0.011	-0.011	-0.004	0.014	0.047	0.007	-0.009
Iberian	**MNRQ**	-0.031	-0.023	-0.017	-0.010	-0.010	-0.003	0.013	0.043	0.009	-0.009
	MALL	-0.027	-0.019	-0.015	-0.008	-0.013	-0.004	0.001	-0.001	0.006	0.001
	NGAN	-0.027	-0.019	-0.014	-0.008	-0.011	-0.005	-0.002	0.001	0.010	-0.007
	CANA	-0.018	-0.015	-0.008	-0.005	-0.005	-0.004	-0.004	0.009	0.039	0.002
	MARI	-0.015	-0.014	-0.009	-0.005	-0.006	-0.005	-0.006	0.009	0.036	-0.007
	ALEN	-0.016	-0.013	-0.007	-0.005	-0.006	-0.006	-0.007	0.005	0.040	-0.001
	BAR	-0.010	-0.011	-0.006	-0.003	-0.002	-0.004	-0.005	0.007	0.037	0.00006
	MARO	-0.018	-0.015	-0.008	-0.005	-0.003	-0.004	-0.006	0.008	0.046	0.0002
	SYG	-0.018	-0.015	-0.009	-0.004	-0.004	-0.004	-0.006	0.008	0.044	0.0002
North West Europe	**BPN**	-0.018	-0.013	-0.008	-0.004	-0.005	-0.003	-0.004	0.008	0.040	0.001
	NOR	-0.033	-0.025	-0.016	-0.005	-0.010	-0.005	-0.002	0.006	0.003	0.020
	MAN	-0.034	-0.027	-0.018	-0.008	-0.012	-0.007	-0.004	0.003	-0.002	0.019
	BBB	-0.039	-0.030	-0.023	-0.013	-0.017	-0.013	-0.013	-0.007	-0.003	0.041
	LKF	-0.035	-0.026	-0.019	-0.008	-0.014	-0.009	-0.010	-0.008	-0.0003	0.030
	HF	-0.034	-0.025	-0.019	-0.011	-0.017	-0.009	-0.012	-0.014	-0.005	0.027
	GNS	-0.035	-0.024	-0.014	-0.002	-0.007	-0.004	-0.003	0.001	0.003	0.014
	JSY	-0.039	-0.029	-0.021	0.008	-0.014	-0.011	-0.008	-0.004	-0.004	0.006
	HER	-0.049	-0.038	-0.026	-0.015	-0.019	-0.015	-0.009	0.002	0.001	0.028

	SHR	-0.054	-0.042	-0.034	-0.022	-0.027	-0.020	-0.019	-0.006	-0.010	0.064
	KRY	-0.042	-0.035	-0.024	-0.013	-0.017	-0.014	-0.012	-0.003	0.002	0.043
	DXT	-0.046	-0.032	-0.024	-0.012	-0.017	-0.013	-0.007	-0.002	0.002	0.039
	GLW	-0.047	-0.035	-0.026	-0.013	-0.016	-0.013	-0.010	-0.003	0.004	0.032
	AAN	-0.046	-0.037	-0.029	-0.018	-0.023	-0.017	-0.014	-0.006	-0.003	0.041
	HGL	-0.046	-0.035	-0.024	-0.013	-0.014	-0.013	-0.009	-0.002	0.003	0.029
	NRC	-0.041	-0.032	-0.025	-0.015	-0.018	-0.015	-0.014	-0.010	-0.006	0.047
	SERC	-0.044	-0.034	-0.027	-0.016	-0.021	-0.016	-0.014	-0.008	-0.004	0.050
	FJL	-0.018	-0.014	-0.005	0.0003	-0.002	-0.006	-0.009	-0.009	0.0003	0.021
	FIAY	-0.044	-0.034	-0.026	-0.015	-0.018	-0.015	-0.013	-0.008	-0.003	0.044
	FINE	-0.014	-0.012	-0.004	0.002	-0.001	-0.005	-0.007	-0.007	-0.00001	0.017
	FINW	-0.020	-0.017	-0.008	-0.001	-0.004	-0.007	-0.008	-0.008	0.001	0.020
	FINN	-0.018	-0.015	-0.007	-0.001	-0.004	-0.007	-0.009	-0.009	-0.001	0.023
	YARO	-0.014	-0.011	-0.005	-0.00002	-0.002	-0.005	-0.006	-0.007	0.000	0.015

By comparing the average genetic relatedness among the 112 breeds to each geographic group, a phylogeographic pattern in which breeds tend to take values of relatedness proportional to their geographic proximity is strongly apparent.

As can be seen in **Table 3.3**, all Greek breeds including Cypriot cattle as well as the populations from Anatolia show the highest closeness value with the Minor Asia geographic group and then the values decrease until they reach zero in the Tyrrhenian group. The genetic relationship with the other groups takes a negative value. An exception again is the insular breed KEA that shows a positive relationship mainly with the Alpine group. A similar kinship pattern is shown by the Buša populations of the Southeast geographic group, the podolian breeds of the East Podolian breed group as well as some breeds of the Tyrrhenian geographic group. These are the well-known podolic influenced breeds (CALV, CHI, MARE, RMG, MCH), the local Sicilian breeds (CINI, MOSI, RSIC) as well as the Corsican MOSA.

3.2.2 UAR relationship within breed and Inbreeding Coefficient ($F_{(UAR)}$)

The Southeast Europe cattle group shows the lowest value of inbreeding ($\bar{F}_{(S\text{-}E\text{-}Europe)} = 0.051$) followed by the Alpine group with ($\bar{F}_{(Alpine)} = 0.07$) and Tyrrhenian and French geographic groups with $\bar{F}_{(Tyrrhenian)} = \bar{F}_{(French)} = 0.087$ respectively.

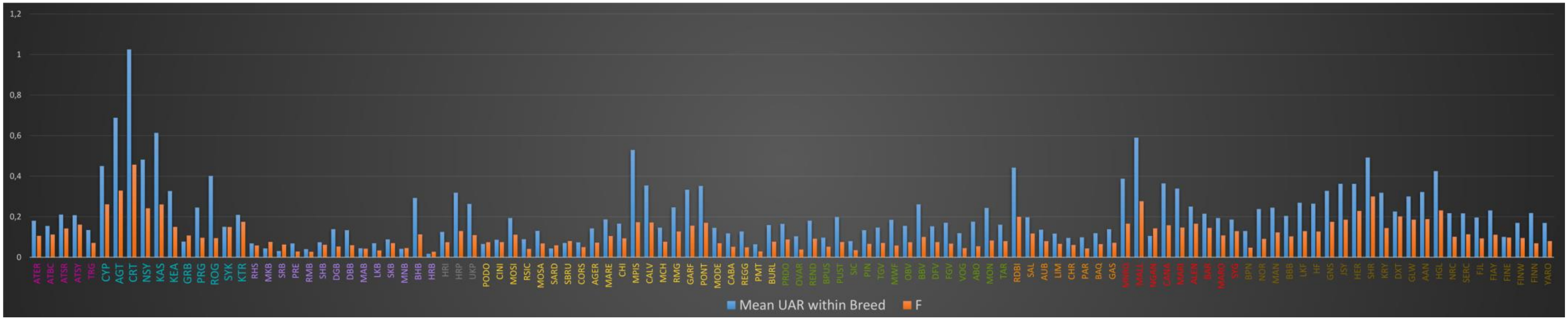


Figure 3.3. Mean Unified Additive Relationships within breed (blue) and inbreeding at breed level (orange). Breeds are colored according to the geographic group to which they belong. Breed abbreviations and geographic groups' colors are indicated in Table 2.1.

The Greek Island geographic subgroup shows extremely high level of inbreeding ($\bar{F}_{\text{(Greek Island subgroup)}}$ = 0.25) and followed by the Iberian and North-west Europe groups with $\bar{F}_{\text{(Iberian)}}$ = 0.169 and $\bar{F}_{\text{(N-W-Europe)}}$ = 0.142 respectively. The rest of breed groups take intermediate values including Greek mainland subgroup with ($\bar{F}_{\text{(Greek mainland subgroup)}}$ = 0.126) (**Table 3.1**).

As in the indices of genetic diversity (**Table 3.1**, **Figure 3.1**, and **Figure 3.2**) and in the inbreeding coefficient, there is great variability in the breed values (**Figure 3.2**). Among the 112 breeds, the population of Crete gets the highest value with $F_{\text{(Crete)}}$ = 0.457 and the lowest the Red Metochian Buša – RMB with $F_{\text{(Red Metochian Buša)}}$ = 0.027. There are populations with very significant levels of inbreeding (>0.16) and others with very low levels (<0.05). In the first category belong all the Greek island populations, the Cyprus cattle as well as various local breeds with small population sizes from all geographical groups except the geographic groups of Buša and the Alpine breeds. This category also includes some isolated cosmopolitan breeds from the Northwest geographic group (Guernsey, Jersey, Hereford, Shorthorn) as well as the French (Raco di Biou). In the second category belong various local breeds mainly of the Southeast and the Alpine geographic groups (**Figure 3.3**).

Within the Greece and Cyprus geographic group, the highest value is obtained by the population of Crete while the lowest values are obtained by the populations of Rhodope -ROG, Prespa -PRG and Greek Brachyceros -GRB with $F_{(Rhodope)} = 0.094$, $F_{(Prespa)} = 0.096$, $F_{(Greek\ Brachyceros)} = 0.108$ respectively. The Cyprus cattle shows high level of inbreeding with $F_{(Cyprus)} = 0.261$. Similarly high values to the Cyprus cattle are shown by the island breeds of Great Britain (Shorthorn, Hereford, Highland) as well as the Iberian Mallorquina with $F_{(Shorthorn)} = 0.3$, $F_{(Hereford)} = 0.229$, $F_{(Highland)} = 0.231$, $F_{(Mallorquina)} = 0.276$ respectively (**Table 3.1**, **Figure 3.3**).

Most breeds show an average within-breed genetic relatedness of less than 0.25. Exceptions to this are some of the isolated breeds of Great Britain, the Channel Island breeds as well as some local populations of the Tyrrhenian and Iberian geographic groups. Regarding the Greek breeds as already said, limiting the average genetic affinity within the breed was not always possible, especially in the islands where the population size was often very close to the sampling size. However, even in the case of mainland Rhodope cattle, the sampling concerns animals from a very small and isolated population within which mating are between relatives.

In general, the magnitude of the average genetic relatedness within the breed and the level of inbreeding at the breed level show a proportional relationship. Of interest are the local breeds Greek Brachyceros – GRB, Italian Podolica - PODO, Serbian Buša - SRB, and Monte-Negro Buša - MNB showing higher levels of inbreeding than the average consanguinity (**Figure 3.3**). In the case of the GRB, it is a confirmation of the existence of subpopulations, with intensive action of inbreeding within subpopulations and low genetic affinity between them.

3.3 Analysis of Runs of Homozygosity (ROH) and Genomic Inbreeding (F_{ROH})

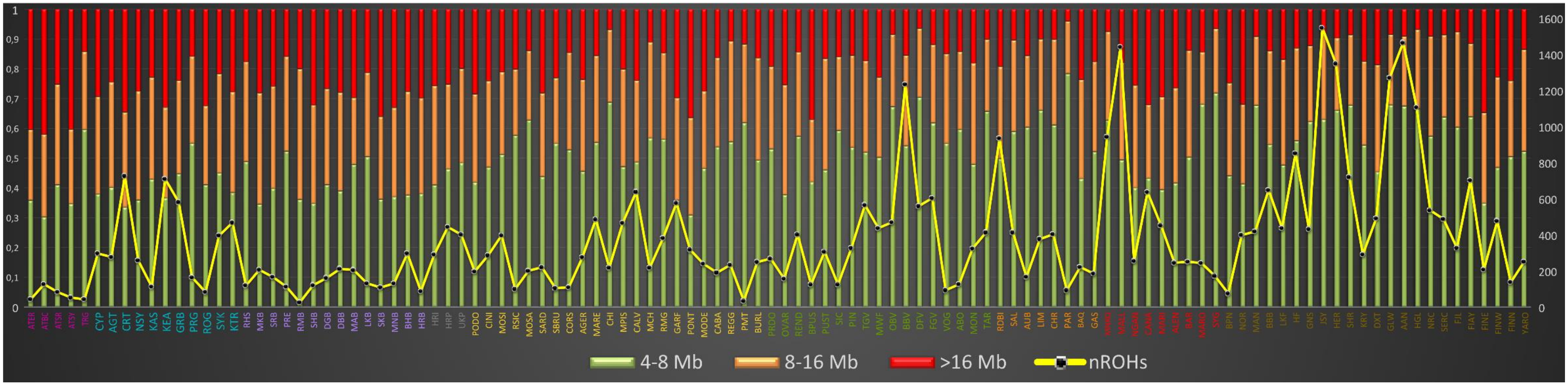


Figure 3.4. Total number of detected ROH and distribution of ROH classes among the 112 cattle breeds. Total number of detected ROH and distribution of ROH classes among the studied cattle breeds. X-axis – Cattle Breeds; Y-axis – distribution of ROH classes of different lengths, left; the total ROH counts in each breed are shown graphically by black circles adjoined by values corresponding to the ROH numbers, right; The distribution ROH classes of different length is shown by a bar dendrogram: green to 4–8 Mb, orange to 8–16 Mb, red to more than 16 Mb. Breeds are colored according to the geographic group to which they belong. Breed abbreviations and geographic groups' colors are indicated in Table 2.1.

In a total of 112 breeds, ROHs were found in all breeds, with intermediate ROH segments (4-8 Mb) being predominant for 104 breeds, including all breeds of Greek and Cyprus geographic group apart of NSY and CRT, with a frequency ranging from $f_{(SHB)} = 0.284$ (SHB - Buša breed) to $f_{(PAR)} = 0.783$ (PAR -French breed). Large ROH segments (8-16 Mb) dominate in 3 breeds i.e., in the Greek island NSY $f_{(NSY)} = 0.365$ and in the Buša breeds MKB and RMB with $f_{(MKB)} = 0.373$ and $f_{(RMB)} = 0.44$ respectively. Finally, in 5 breeds the largest ROH segments (>16 Mb) dominate, in 3 from Minor Asia geographic group (ATER, ATBC, ATSY) with frequencies $f_{(ATER)} = 0.405$, $f_{(ATBC)} = 0.421$, $f_{(ATSY)} = 0.4$ respectively, in the Greek island Crete (CRT) with $f_{(CRT)} = 0.345$ and in the Italian PONT with $f_{(PONT)} = 0.365$ (**Figure 3.4**).

A large variation is also observed in the number of ROH segments (> 4Mb). The Jersey breed gets the highest value (1,547) while the lowest value gets the RMB Buša breed with only 25 ROH segments. Very high values are also obtained by some Western and Northern

European breeds such as BBV (1,234), MALL (1,441), HER (1,348), GLW (1270), AAN (1465), and HGL (1107), while very low values are obtained by 3 of the Minor Asia geographic group i.e., ATER (42), ATSY (52), TRG (42) as well as the Italian PMT (34). Within the Greek and Cypriot Geographic Group, CRT breed shows the most ROH segments (726), followed by KEA (709) and Greek Brachyceros (581), while ROG and KAS show the fewest segments (83 and 112 respectively).

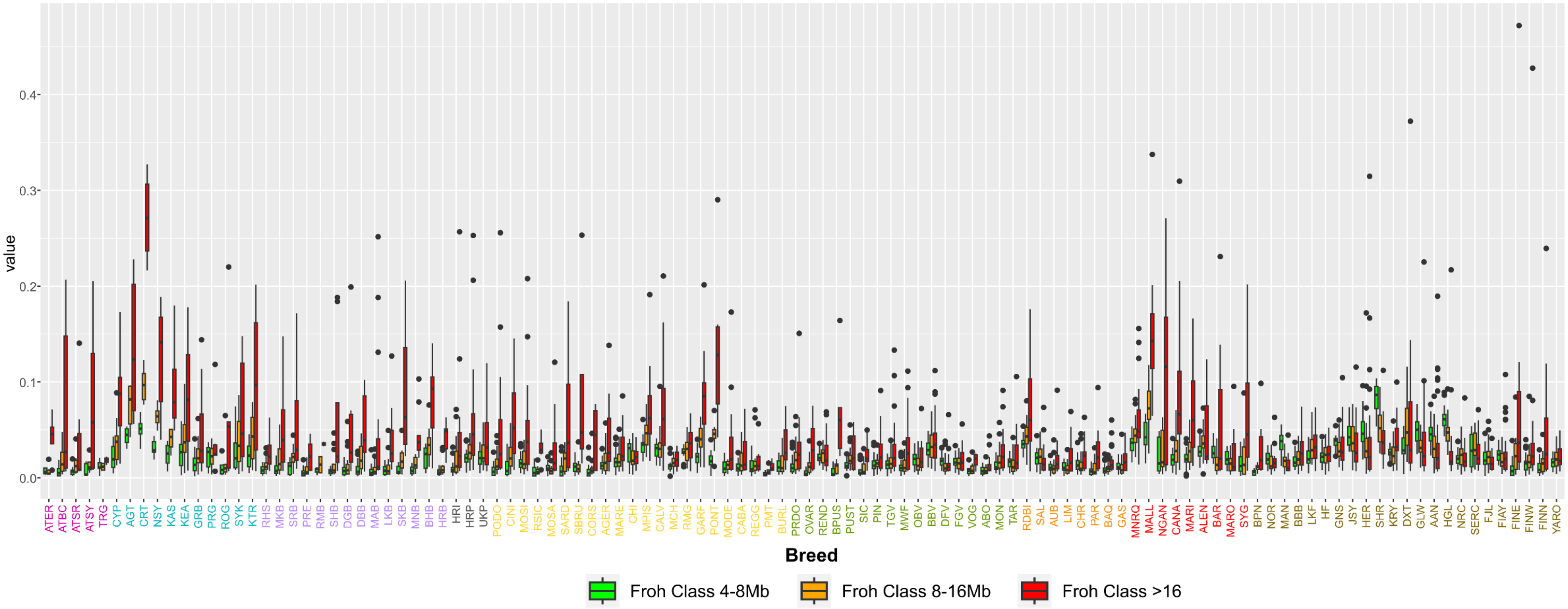


Figure 3.5. Boxplots showing the level of genomic inbreeding ($F_{ROH\ 4\text{-}8\ Mb}$, $F_{ROH\ 8\text{-}16\ Mb}$, $F_{ROH\ >16\ Mb}$) per breed. Breeds are colored according to the geographic group to which they belong. Breed abbreviations and geographic groups' colors are indicated in Table 2.1.

As shown in **Figure 3.5**, in most breeds the level of inbreeding for the class F_{ROH}>16 is higher than the other two. This shows that in these breeds the intensity of consanguinity increases as we reach the present. This tendency is stronger in the local/indigenous breeds mainly of the Balkan peninsula, Asia Minor, and of the Tyrrhenian group. The highest average value for this class is obtained by the Greek island breeds (Crete-CRT, Agathonisi-AGT, and Nisyros-NSY), the Italian PONT, and the Iberian MALL and NGAN. The values in descending order are as follows $F_{ROH>16Mb\ (CRT)} = 0.270$, $F_{ROH>16Mb\ (MALL)} = 0.143$, $F_{ROH>16Mb\ (AGT)} = 0.135$, $F_{ROH>16Mb\ (PONT)} = 0.124$, $F_{ROH>16Mb\ (NSY)} = 0.123$ and $F_{ROH>16Mb\ (NGAN)} = 0.115$.

In contrast to the above, many cosmopolitan breeds show a decrease in the autozygosity ratio of the closest ancestors ($F_{ROH>16Mb}$ and $F_{ROH\ 8\text{-}16Mb}$) compared to the most distant ($F_{ROH\ 4\text{-}8Mb}$). This is probably due to mating control in recent decades to mitigate inbreeding. Some of them are Jersey, Guernsey, Hereford, Shorthorn, Angus, Fleckvieh, Original Braunvieh, and Chianina. Several local breeds of the North-West group follow the same trend.

As regards the breeds of the Greek and Cyprus geographic group, in all classes, they show high levels of inbreeding, especially regarding the island populations. Only the population of Prespa seems to maintain stable mean values in all three classes.

The lowest level of genomic inbreeding was observed for Piedmontese for three classes, with mean inbreeding coefficients ranging from 0.004 for $F_{ROH4\text{-}8Mb}$ to 0.007 for $F_{ROH8\text{-}16Mb}$ and 0.12 $F_{ROH>16Mb}$. The highest correlation value between the two inbreeding coefficients was observed between F_{UAR} and $F_{ROH>\ 4Mb}$ equal to 0.91.

3.4 Effective population size

The weighted mean of the current effective population size (Ne_5) is relatively small and ranges from 28 in the Iberian to 67 in the French geographic group, the Minor Asia geographic group also gets a similar value (64). The Greek mainland subgroup and Southeast Europe geographic groups showed similar intermediate average values with $Ne_{5\ (Greek\ Mainland\ breeds)}$ = 50 and $Ne_{5\ (Southeast\ Europe)}$

= 51 respectively. In Greek mainland subgroup only GRB showed a larger Ne_5 ($Ne_{5\,(GRB)}$= 85) than the weighted average of the group (**Table 3.1**).

Table 3.4. Mean effective population size per group for 8 geographic groups, Greek Mainland subgroup and Cyprus Cattle. $\overline{Ne_5}$, effective population number for five generations back; $\overline{Ne_{50}}$, effective population number for fifty generations back and $\overline{Ne_{2000}}$, effective population number for two thousand generations back.

Group	$\overline{Ne_5}$	$\overline{Ne_{50}}$	$\overline{Ne_{2000}}$
Minor Asia	64	549	4513
Cyprus	18	119	2753
Greek Mainland breeds	50	364	3675
South East Europe	51	399	3704
East Podolian	36	219	3124
Tyrrhenian	44	299	3313
Alpine	56	300	3167
France	67	386	3108
Iberian	28	158	2821
North West Europe	57	246	2863

Going back in the past, the effective population size in the Buša group and in the Greek mainland subgroup increased faster than in other European cattle groups. Consequently, during the pre-industrial time (Ne_{50} 250 years ago), the effective population size was clearly larger for the Southeast Europe Buša group (Ne_{50} = 399) than for other European breed groups. Only the Minor Asian group showed a larger value (Ne_{50} = 549) during pre-industrial times (**Table 3.4**).

A comparable trend was also observed for effective population size 10,000 years ago (Ne_{2000}), with the Minor Asia geographic group having the largest effective population size (4.513). The Buša breeds and Greek mainland subgroup following closely with similar values (3.704 and 3.675 respectively). The effective population size of the remaining geographic groups for the same period appears to decrease as we move northwest (**Table 3.4**).

The values for the GRB and Buša group were comparable and differed from those obtained in the other Greek cattle breeds. The 11 indigenous CRT animals sampled on the island of Crete showed the highest inbreeding level and the smallest effective population size (Ne_5, Ne_{50} and Ne_{2000}) in the entire dataset (**Table 3.1** and **Figure 3.6**). The tessellated projections provide strong support to the observation that as we go back in time the effective population size increases in regions closer to the domestication center and decreases in Northwest Europe (**Figure 3.6**). Finally, it is worth mentioning that, in general, the evolution of the effective population size of the local breeds of Southern Italy and Sardinia follows a similar trend to the breeds of the Balkans and Minor Asia. As *Ne* is inversely correlated to the extent of LD, our results suggest that the level of LD is high in the fragmented breeds under extinction pressure, which is most probably caused by uncontrolled inbreeding.

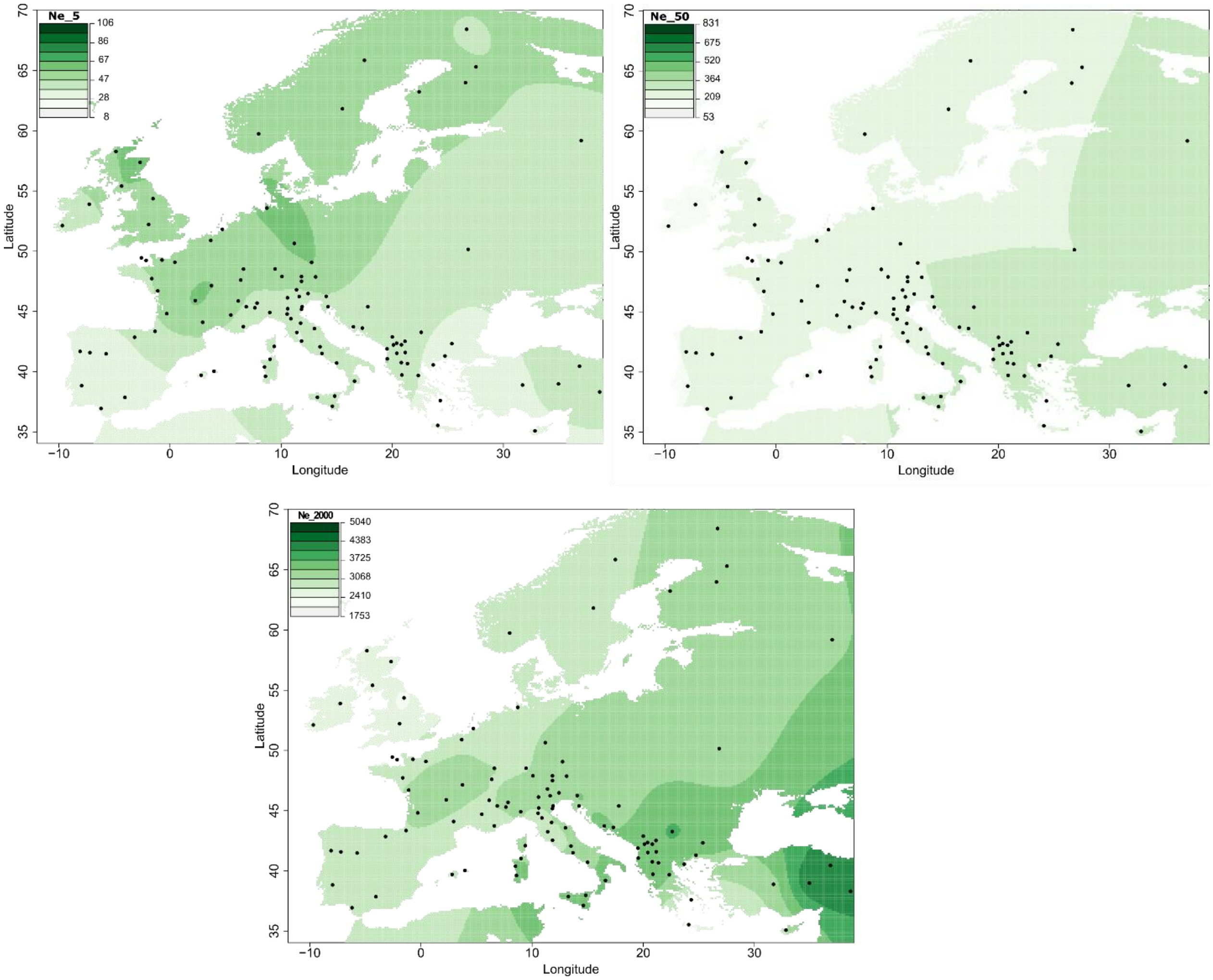


Figure 3.6. Tessellated projection. Spatial geographic presentation of the estimated effective population number (Ne5, Ne50, Ne2000). For the breed position see Figure 2.2.

3.5 Genetic differentiation between breeds and breed's homogeneity

To assess the genetic differentiation between the 112 breeds (YAK, GIR and NDA excluded) as well as within the geographical groups, the G_{ST} and D_{EST} indices were calculated based on the multi-allelic markers. The degree of differentiation within breed was also estimated as the average genetic distance (*Dps*) of the individuals that make it up. The latter is a measure of the homogeneity of each breed.

The D_{EST} values between 112 cattle breeds ranged from 0.001 in two Anatolian breeds (ATSY - ATBC) to 0.462 (Creta - Hereford) while G_{ST} ranged from 0.004 in two Anatolian breeds (ATER - ATBC) to 0.409 (Creta - Mallorquina). The correlation coefficient between G_{ST} – D_{EST} is 0,974 but D_{EST} showed better diversification when the level of gene diversity is high. For this reason, both D_{EST} and G_{ST} were presented (**Table 3.5**), but mainly D_{EST} was commented as the chosen population differentiation values because this parameter is independent of heterozygosity.

Breeds and breed groups with high allelic diversity and high heterozygosity also showed a very low level of differentiation in both indices. For example, the high diversity Minor Asia group showed an average differentiation to each other of only 0.006 and the Buša breeds a value of 0.050. On the other hand, the Greek island subgroup were highly differentiated ($\overline{D_{EST\ (Greek\ island\ subgroup)}}$= 0.321), followed by the Iberian group $\overline{D_{EST\ (Iberian\ group}}$ = 0.189) and the East Podolian group $\overline{D_{EST\ (East\ Podolian\ group)}}$ = 0.180) (**Table 3.5**). Similar trends were also observed when all breeds from one predefined group were compared to all the other breeds, i.e., the level of differentiation was lowest for the Buša breeds (0.113) and highest for the Greek and Cyprus breeds (0.273) (**Table 3.5**).

Within the Greek and Cyprus group, low pairwise D_{EST} values were obtained between two mainland breeds i.e., GRB-SYK (D_{EST} = 0.073) and high values were obtained between two island breeds i.e., CRT-AGT (D_{EST} = 0.413). It is remarkable that the CRT breed showed the highest differentiation level among all the investigated breeds ($\overline{D_{EST\ (CRT-All\ breeds)}}$ = 0.391). Again, the GRB and Buša breeds shared comparable $\overline{D_{EST}}$ values.

Table 3.5. Genetic differentiation indexes. D_{EST} and G_{ST} values among 10 geographic breed groups and all breeds in two first columns. $\overline{D_{EST}}$ and $\overline{G_{ST}}$ in each geographic breed group in two last columns. Numbers indicating maximum and minimum values among geographic breed groups and all breeds as well as in geographic breed group are in green and grey respectively for each of the two indexes.

	D_{EST} (All Breeds)	**G_{ST} (All Breeds)**	$\overline{D_{EST\ (breed\ group)}}$	$\overline{G_{ST\ (breed\ group)}}$
Minor Asia	0,137	0,082	0,006	0,014
Greek Island	0,273	0,177	0,321	0,231

Greek Mainland	0,165	0,115	0,144	0,123
S-E Europe	0,113	0,070	0,050	0,034
East Podolian	0,178	0,108	0,180	0,108
Tyrrhenian	0,147	0,093	0,125	0,081
Alpine	0,148	0,092	0,110	0,071
France	0,145	0,092	0,093	0,065
Iberian	0,189	0,122	0,189	0,130
N-W Europe	0,181	0,111	0,172	0,108

The genetic differentiation between the breeds of the Greek and Cyprus geographic groups and the rest geographic cattle groups is presented in **Table 3.6**. As can be seen, almost all the breeds show the lowest differentiation with breeds of the Asia Minor geographic group. It is characteristic that their differentiation with the Buša group is lower than the Greek Mainland subgroup. Then as we move to the northwest the genetic differentiation values increase. An exception to this trend is mainly the Keas breeds which shows very low differentiation values with distant geographic groups (mainly Alpine).

Indicative of the degree of isolation as well as the level of inbreeding of the Greek island breeds is the fact that all mainland populations, as well as the island CRT, NSY, and KEA, show their greatest differentiation with the Greek island subgroup (**Table 3.6**).

Table 3.6. Genetic differentiation index D_{EST}. Pairwise values among 11 Greek breeds and 10 breed groups. Numbers indicating maximum and minimum values for each Greek breed are in green and grey respectively for each of the two indexes. Abbreviations are indicated in Table 2.1.

D_{EST}	***Minor Asia***	***Greek Island***	***Greek Mainland***	***S-E Europe***	***East Podolian***	***Tyrrhenian***	***Alpine***	***France***	***Iberian***	***N-W Europe***
CYP	0,102	0,284	0,202	0,186	0,246	0,234	0,261	0,258	0,279	0,291
AGT	0,191	0,321	0,264	0,252	0,298	0,289	0,309	0,310	0,332	0,337
CRT	0,345	0,401	0,374	0,364	0,388	0,382	0,394	0,394	0,409	0,418
NSY	0,195	0,311	0,235	0,205	0,259	0,235	0,238	0,244	0,277	0,275
KAS	0,111	0,284	0,202	0,182	0,244	0,232	0,258	0,256	0,283	0,291
KEA	0,208	0,325	0,221	0,178	0,231	0,200	0,192	0,202	0,241	0,238
GRB	0,036	0,212	0,106	0,046	0,124	0,097	0,117	0,114	0,156	0,152
PRG	0,115	0,261	0,152	0,108	0,179	0,153	0,161	0,159	0,201	0,195
ROG	0,151	0,287	0,189	0,170	0,227	0,211	0,226	0,228	0,257	0,258
SYK	0,075	0,237	0,130	0,086	0,158	0,131	0,143	0,144	0,184	0,183
KTR	0,092	0,250	0,143	0,110	0,178	0,155	0,172	0,171	0,207	0,208

In addition, average allele sharing distance (Dps) between animals within a breed as a measure of the breed-level differentiation was used. Then the standardized Dps values plotted in tessellated projection (**Figure 3.7**).

As shown the highest DPS values were observed for most of the Buša and Anatolian breeds together with the Greek GRB and the Italian PODO breed. High prices are also shown by local breeds of the islands of the Tyrrhenian group (CORS, SARD, SCINI), the Italian PMT, the Finnish FINE as well as the Greek SYK. The lowest values were observed for Greek island breeds (except KEA), Italian MPIS, mainland Greek ROG, French RDBI and Iberian Breeds MNRQ and MALL. Low values are also shown by some cosmopolitan and/or isolated breeds such as BBV, GNS, JSY, and SHR (**Figure 3.7**).

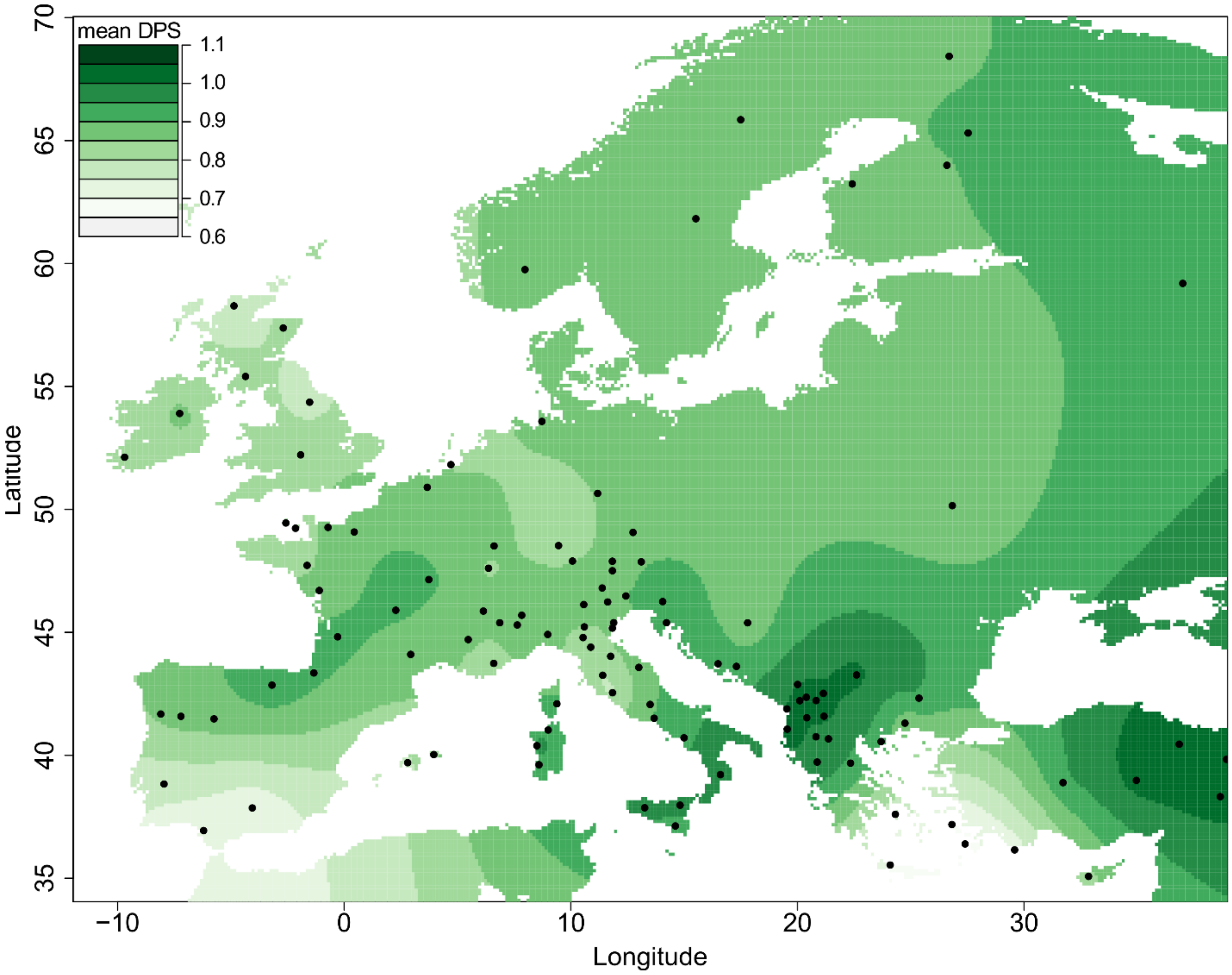


Figure 3.7. Tessellated projection. Spatial geographic presentation of the estimated allele sharing distance matrix (DPS) among breeds using multi-allelic SNP-blocks. For the breed position see Figure 2.2.

3.6 Genetic distances and clustering

3.6.1 Supervised phylogeny

Nei’s genetic distance D_A was estimated based on multiallelic SNP blocks and presented the values as a neighbor-net (**Figure 3.8**) and as a neighbor-joining tree routed by YAK (**Figure 3.9**). In both figures to improve the visibility of the main part of the trees, the branch length for YAK as well as the branch length between *Bos indicus* and *Bos taurus* breeds were shortened (**Figure 3.9**).

In **Figure 3.8**, the Central, Western, and North-Western European breeds are generally seen to be placed on the basis of their geographical origin creating distinct breed groups (Alpine, French, Iberian, and Northwest breed group) on the Neighbor network's right side. On the other hand, the Buša breeds, together with the breeds of the Greece and Cyprus geographic Group, the East Podolian breeds, the Anatolian breeds as well as the populations of the outgroup occupy the right part of the Neighbor network. The Tyrrhenian geographical group's breeds were observed that they are placed on both sides.

Among the Greek and Cyprus group, the island breeds CYP, AGT and KAS were placed close to the representative of *Bos indicus* (GIR). This is also the case for the Anatolian breeds, which form a cluster with the aforementioned breeds. Interestingly, the GRB breed from the mainland Greek group, is positioned within the cluster of the Buša breeds with short branches. On the opposite, for the island breeds CRT, AGT, KAS and NSY, long branches result from a high inbreeding level. KTR and SYK, which represent Greek podolian cattle, are placed between the TRG and Italian podolic breeds, Corsican MOSA and the local breeds from Cicily (CINI, MOSI, RSIC). Also, some Albanian and Kosovar Busa breeds (DBB, MAB, SKB, DGB) with Jersey influence (**Table 3.2**) placed together with JSY between Northwest Europe breeds and East podolian (UKP, HRP).

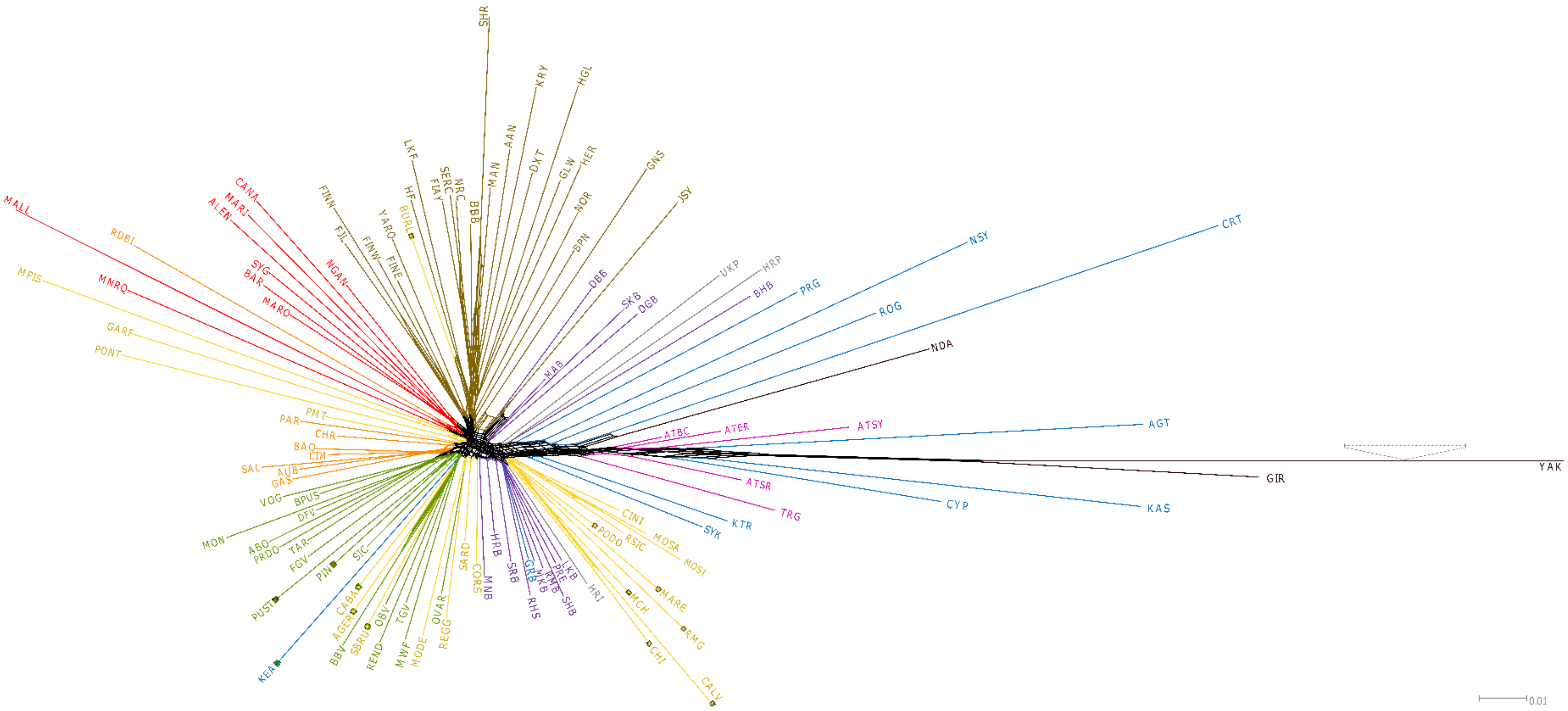


Figure 3.8. Neighbor-network based on pairwise Nei's D_A genetic distances among 115 breeds. Special square marks represent the influence of East-Podolian (grey), Alpine (green) and North-West (olive green) groups. Dotted lines indicate the shortened branch length of Yak to improve visibility.

The KEA is the only Greek breed that is grouped together with some breeds from the Alpine geographic group, because of the crossing that took place between indigenous Greek cattle and some breeds of Alpine cattle during the creation of the breed in Kea Island (**Table 3.2 and 3.3**). This cluster also includes some Italian breeds with Brown-Swiss influence (CABA, AGER and SBRU) (**Table 3.2 and 3.3**). Also, the Italian BURL was placed in the distant Northwest geographic group because of Holstein influence (**Table 3.2 and 3.3**) (**Figure 3.8**).

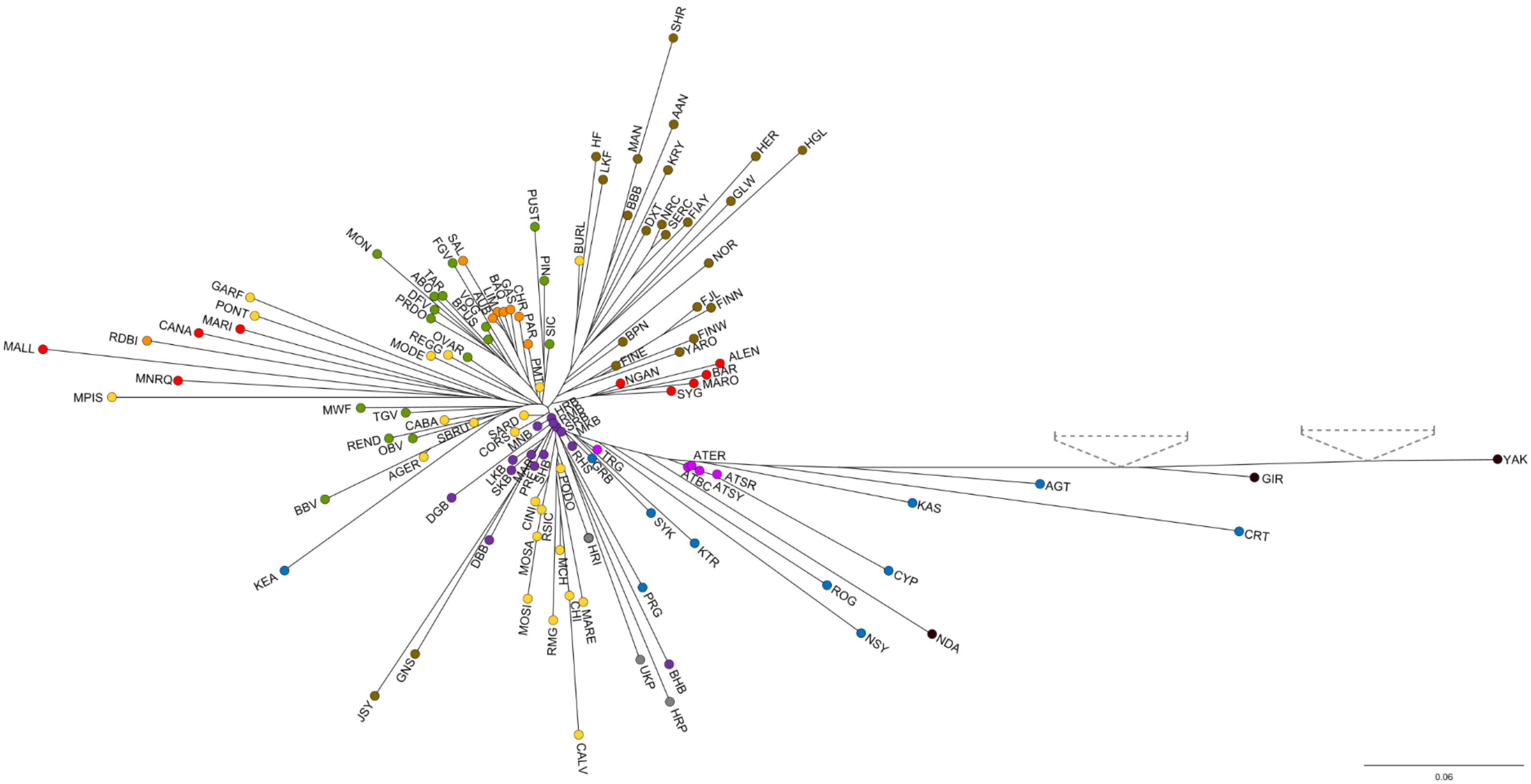


Figure 3.9. Phylogenetic tree. Neighbor-joining tree based on Nei's genetic distance D_A using multi-allelic SNP blocks. Mongolian yak (YAK) was used as a root. Dotted lines indicate the reduced length of YAK and GIR to improve visibility. Special square marks represent the influence of East-Podolian (grey), Alpine (green) and North-West (olive green) group.

Neighbor-joining tree clearly showed that less differentiated breeds (**Table 3.5**) take a position close to the center in the radial tree layout (**Figure 3.9**).

This is the case for most Buša breeds, Greek Brachyceros (GRB) and Turkish gray (TRG). Comparable to the above, nodes of four Anatolian breeds were placed on the top of very short branches closer to the representative of *Bos indicus* (GIR). Also, nodes of Greek island breeds (KAS, CRT, AGT) are placed near to GIR. Interestingly, the Cyprus cattle presented a prolongation of the branch with four Anatolian cattle breeds (ATER, ATSR, ATSY, and ATBC). Apart from GRB all remaining Greek cattle breeds were characterized by long phylogenetic branches, especially for the island breeds with high inbreeding (CRT, AGT, KAS, and NSY).

In a cluster that starts from the center of the radial tree layout, are placed Albanian and Kosovar Buša breeds (DBB, MAB, SKB, DGB, LKB) with Jersey influence (**Table 3.2**) together with the JSY and GNS. The same goes for the Italian podolic influence breeds (PODO, MPIS, CHI, MARE, RMG, and MCH), Corsican MOSA, and the local breeds from Sicily (CINI, MOSI, RSIC) which are placed together with podolian HRI.

As described in the introduction (**Chapter 1.6**), the KEA breed is the product of crossbreeding between indigenous Greek cattle and some Alpine cattle breeds at the begging of the previous century. As in **Figure 3.8**, KEA is the only Greek cattle breed which is clustering together with some yellow, brown, and gray cattle breeds from Alpine region. This cluster also includes some Italian breeds with Brown-Swiss influence (CABA, AGER, SBRU).

In addition, the allele counts of bi-allelic SNPs was used and reconstructed the phylogeny with the TreeMix program (**Figure 3.10**) by using Mongolian yak (YAK) to root the tree. Compared to other European cattle breeds, CYP, KAS and AGT were closer to the root of the tree and thus, closer to GIR, the *Bos indicus* representative. The aforementioned Greek breeds are gradually followed by the cattle breeds from Minor Asia, Greece, Bulgaria, North Macedonia, Kosovo and Serbia. The remaining Buša breeds sampled along the Ionian-Adriatic route, i.e., Albania, Montenegro, and Dalmatia, were placed after two clusters of Italian breeds. In agreement with the neighbor-net and Neighbor-joining tree, KEA is the only Greek breed that clusters in the Alpine cluster. Also, in the phylogenetic tree reconstructed based on SNP allele counts (TreeMix), for some breeds with a high inbreeding level (e.g., Crete and Bosnian Buša) long branches are observed whereas for less differentiated and highly diverse breeds (e.g., Turkish Grey and Red Metochian Buša) short or no branches are found.

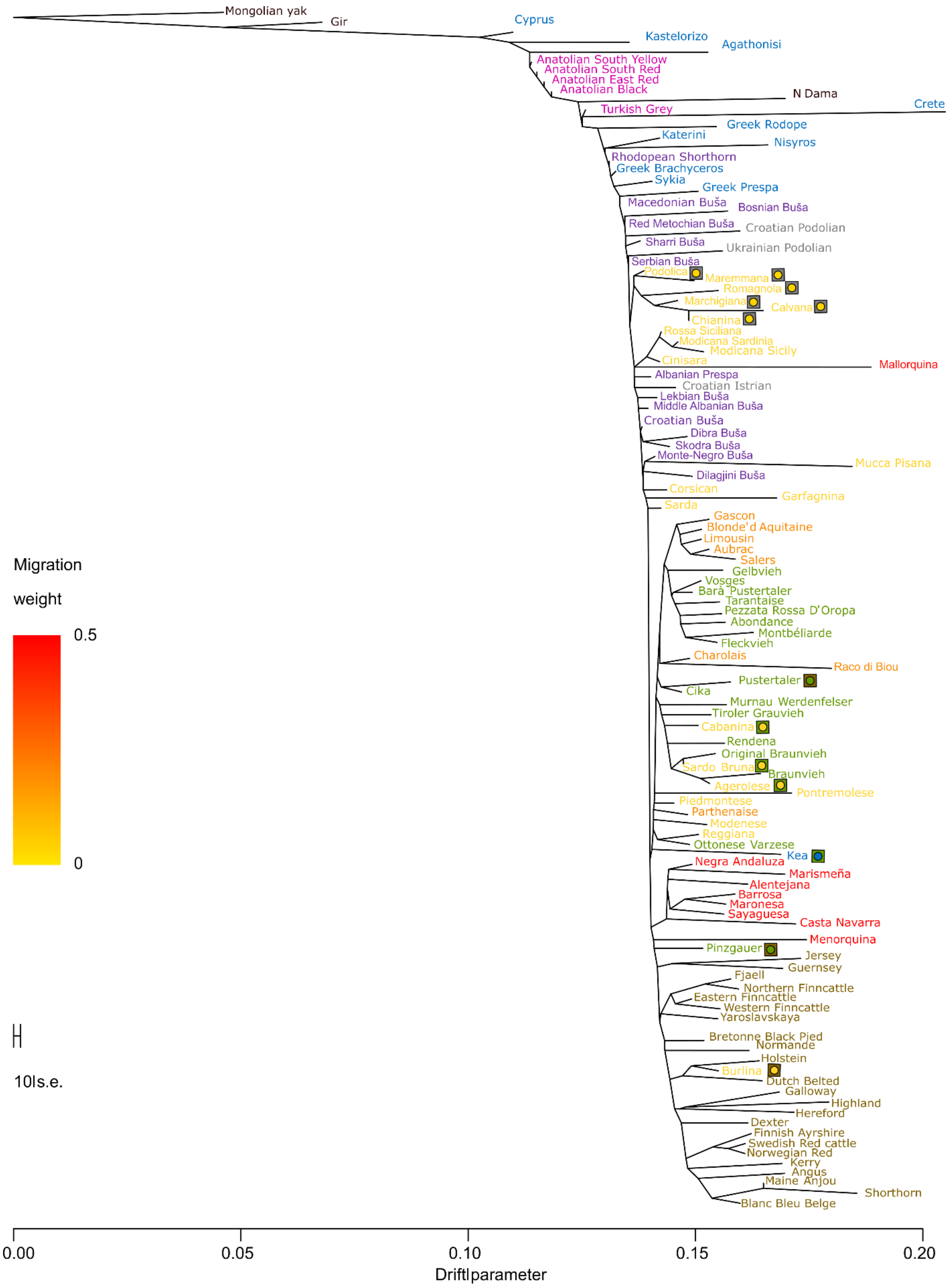


Figure 3.10. Maximum likelihood (ML) tree inferred from genome-wide allele frequency data by methods implemented in the *TREEMIX* program. The ML dendrogram of the relationships between the examined cattle populations was rooted with the Mongolian yak as an outgroup breed.

Our phylogenetic analyses, both with multi-allelic (**Figure 3.8** and **Figure 3.9**) and bi-allelic markers (**Figure 3.10**), do not aggregate the so-called podolian or gray steppe cattle breeds (TRG, KTR, SYK, HRI, HRP, and UKP) in a single separate cluster, instead they are scattered along the phylogenetic tree and are positioned closer to their geographic neighbor than to the hypothetical steppe cattle or Podolian group, that is the 'isolation-by-distance' model. On the one hand, TRG, KTR, and SYK are positioned between the Anatolian and some of the Greek and North Macedonian breeds. On the other hand, HRI, HRP and UKP do not form an own cluster but are placed among some of the Buša neighbor breeds (**Figure 3.10**). The Italian-podolian breeds form a separate cluster, which is placed between some of the Buša and other Italian breeds and the East podolian breeds.

3.6.2 Assessment of population structure using unsupervised heuristic and unsupervised model-based methods

Multi-allelic SNP blocks was used to estimate the allele sharing distance matrix ($\mathbf{D_{PS}}$) among 2,858 animals and projected these by multidimensional scaling (MDS) on the two-dimensional (2D) plane (**Figure 3.11**). The MDS projection of all animals is shown in the **Figure. 3.11(A)** while the **Figure. 3.11(B)** highlights Greek and Cyprus cattle breeds.

The Anatolian, Cyprus and many of Greek cattle took intermediate positions between *Bos indicus* and remaining European cattle breeds group along the first dimension of MDS. The second dimension of MDS clearly separated the Northwest Europe, Alpine, French, and Iberian cattle breeds from the Middle and South European breeds. The N'Dama as representative of African *Bos taurus* took a position closer to Mediterranean cattle breeds (**Fig. 3.10** and **Fig. 3.11(A)**).

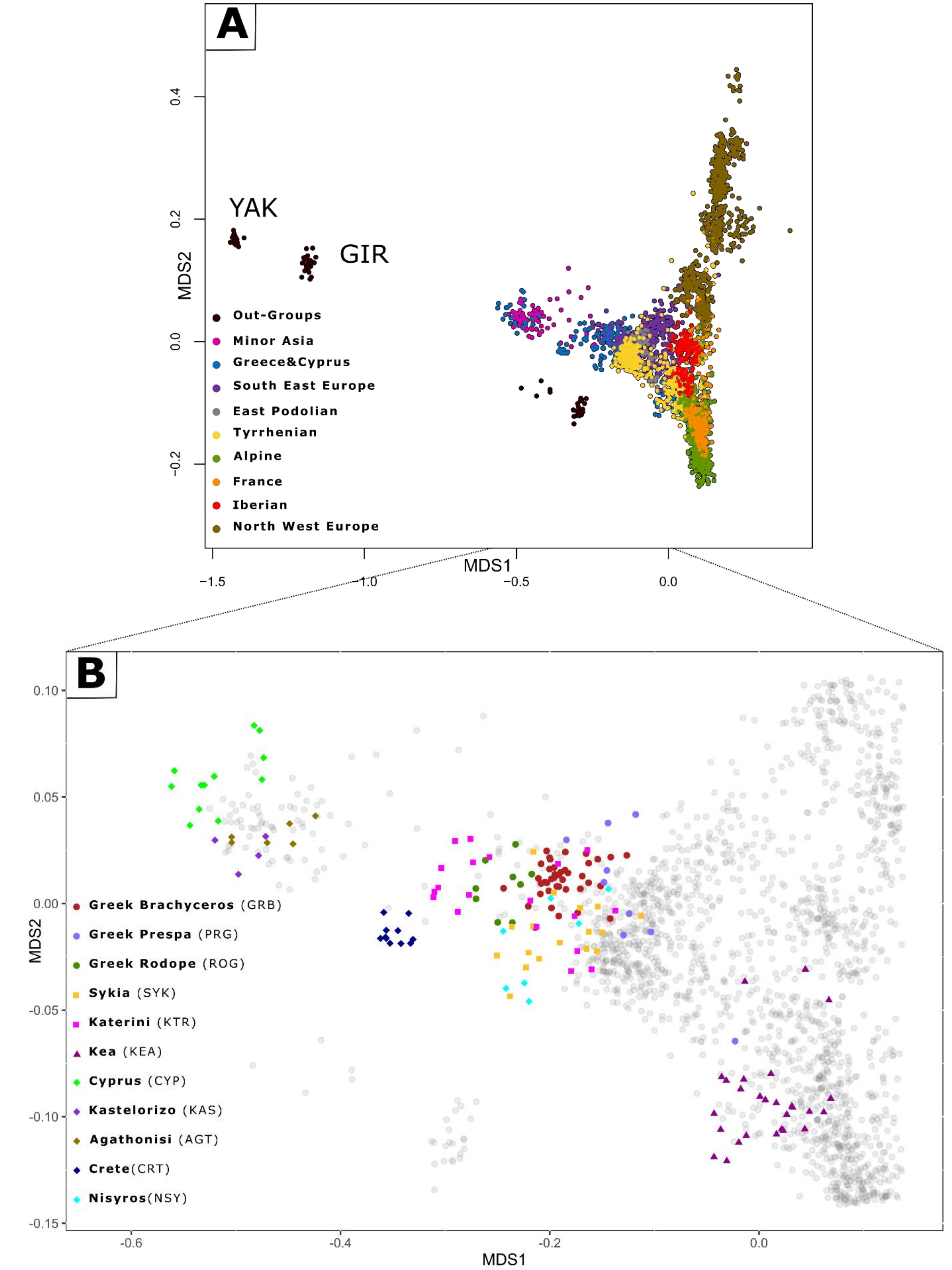


Figure 3.11. MDS projection of the estimated allele sharing distance matrix (DPS) at the individual level. in all (2858) animals (**A**) and Greek and Cyprus cattle samples separately (**B**), using multi-allelic SNP blocks.

The **Figure 3.11 (B)** shows that the breeds of the Greek and Cyprus geographic group form three clusters. In one, the cattle of Agathonisi, Kastelorizo, and Cyprus are gathered and in the other the cattle of Kea. The remaining Greek breeds occupy an intermediate space, quite far from the other two clusters, and in several cases, the individuals overlap. Only the Cretan cattle seem to group separately and somewhat distantly from the other breeds.

To better understand the placement of breeds, the MDS projection at the breed level was visualized. The MDS projection of 115 breeds is shown in **Figure 3.12**. Along the first dimension of MDS (MDS1), was observed that the Anatolian, Greek and Cyprus breeds have an intermediate position between *Bos indicus* and the remaining European cattle breeds. CYP, AGT and KAS cluster together with the Anatolian breeds, except TRG. Subsequently, the mainland Greek breeds (SYK, KTR, PRG, ROG, and GRB) and the Nisyros island breed (NSY) cluster in the geographic region corresponding to some of the Buša breeds (RHS, MKB, PRE, RMD, SHD, and BHB; South East geographic group), some of the breeds from the East Podolian geographic group (UKP, and HRP) and some Italian breeds (Tyrrhenian group) of South and Central Italy (CINI, MOSA, MOSI, and RSIC) including all Italian podolic breeds (PODO, MARE, RMG, CALV, CHI, and MCH).

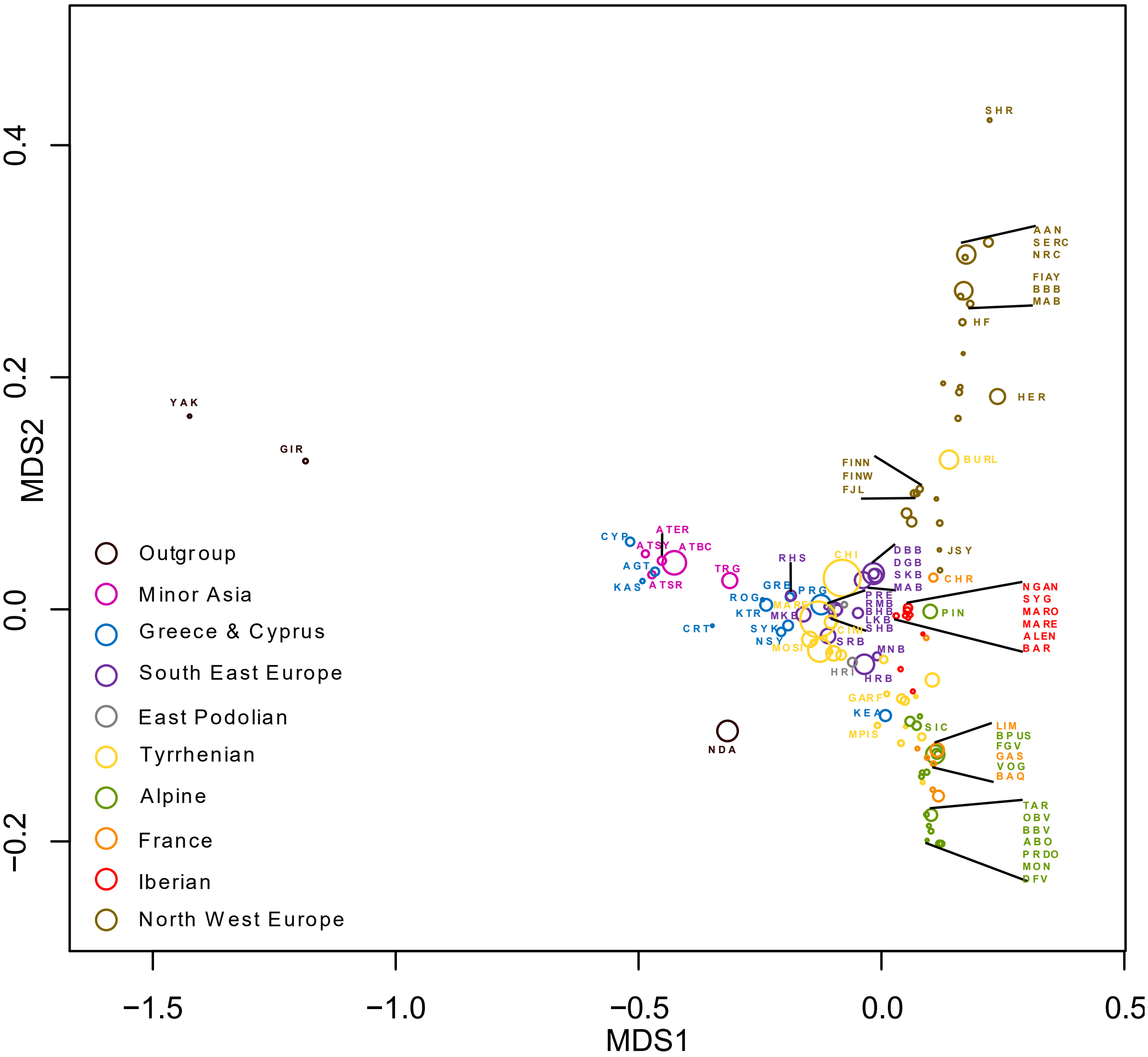


Figure 3.12. MDS projection of the estimated allele sharing distance matrix (D_{PS}) among 115 breeds using multi-allelic SNP blocks at breed level. The position of each breed is represented with a circle in which the Centre is the average position of all animals of the breed with a radius equal to the SD (standard deviation).

CRT is isolated from the other two main Greek breed clusters. KEA is positioned in the geographic region of some Alpine and Italian breeds, showing a closer relationship to these breeds than to its own (Greek) geographic cluster. The second dimension of MDS (MDS2) separates the breeds of North Europe and Alpine geographic area from the breeds of central, west, and southern Europe (**Figure 3.12**).

The Admixture analysis presents the second unsupervised clustering method using the genotypes of bi-allelic SNPs. Following the standard procedure of Admixture program which is very sensitive in the presence of highly related animals and not need outgroup, the family structure of Greek and Cyprus subpopulations was further reduced, and Mongolian Yak was excluded. Finally, from 2,858 animals the Admixture analysis was applied in 2,779 (26 Mongolian yak and 53 animals from Greek and Cyprus geographic group excluded) (**Table 2.1**). For K= 2 – 115 the lowest cross-validation error (*cv error* = 0.538) was determined at *K* = 76 (**Figure 3.13**).

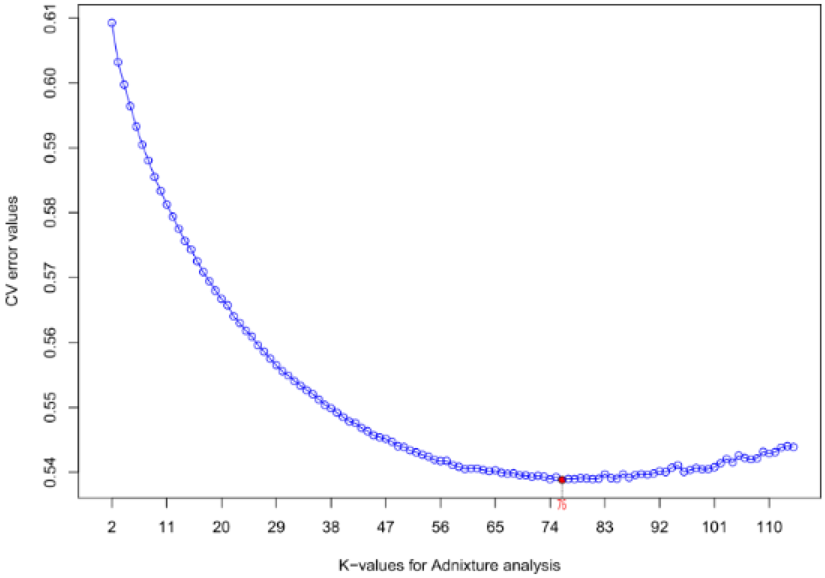
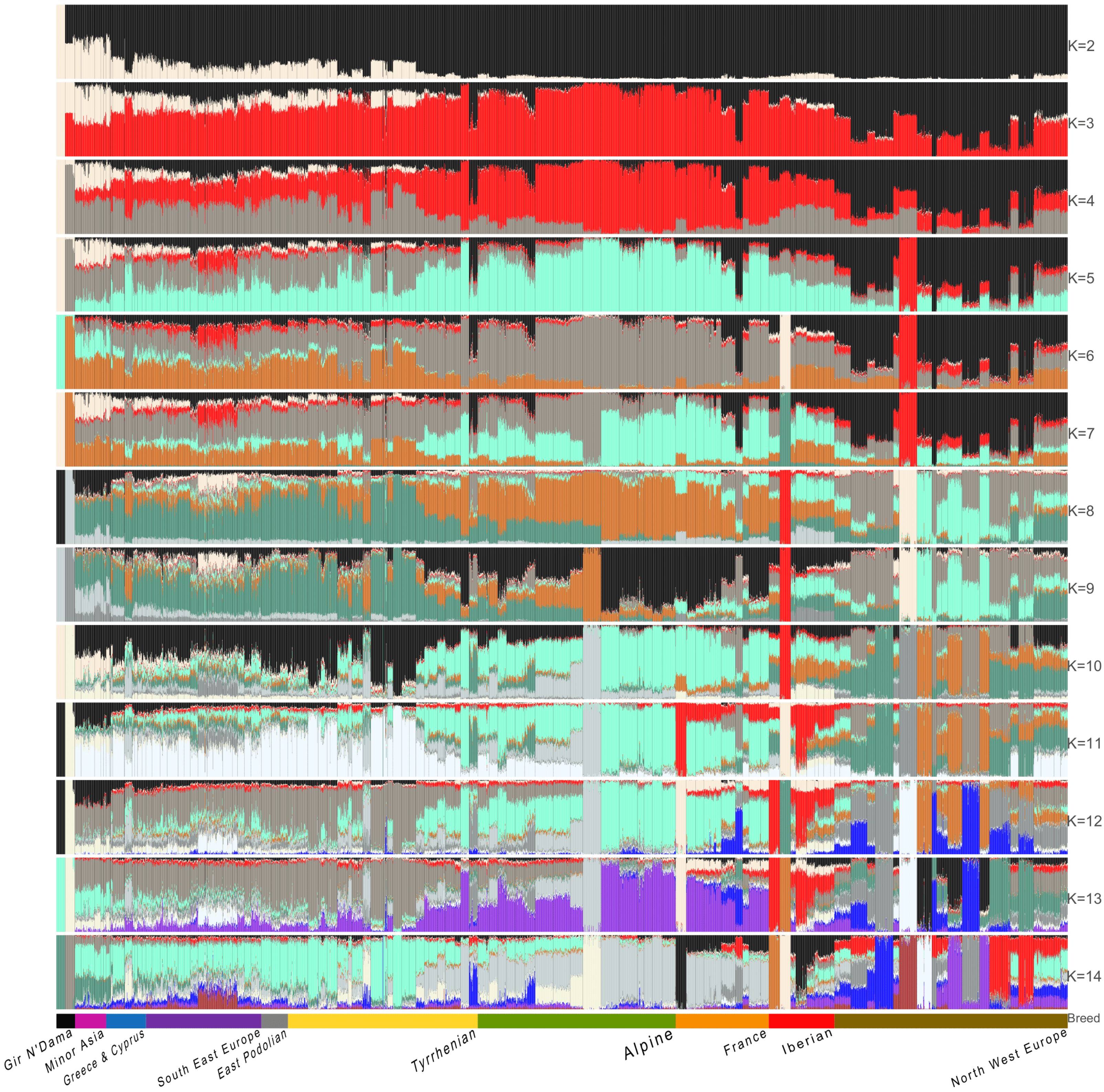
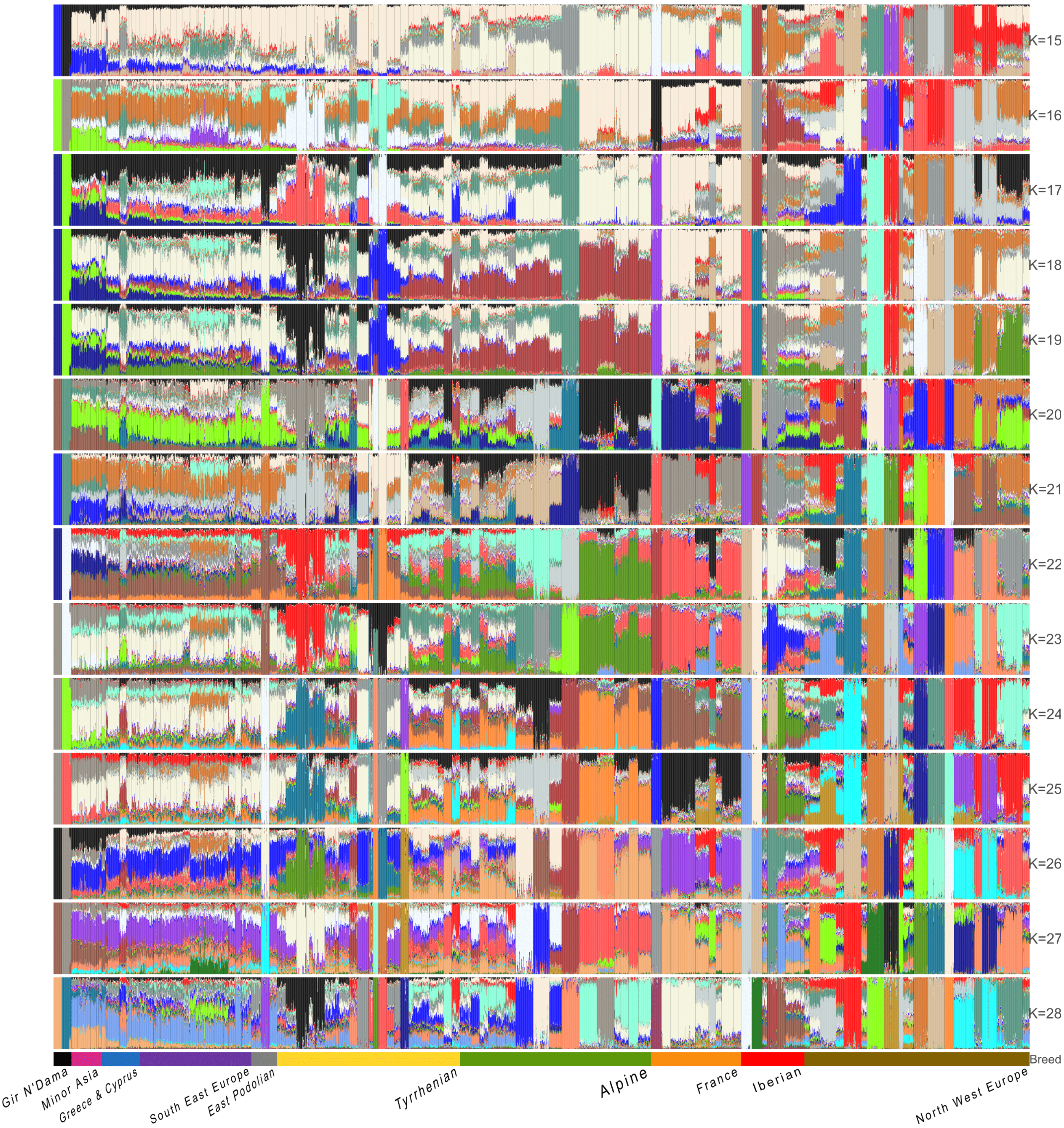


Fig 3.13. Cross-validation process to determination the appropriate number of ancestral (*K*) populations in the Admixture program. With red the number indicates the lowest cross-validation error (*cv error* = 0.538), at K=76.

The **Figure 3.14** presents the clustering at *K* = 2 - 55. The reason for choosing K = 2-55 for displaying is that until K = 55, the cross-validation decreases almost linearly, and after K = 56 stabilizes at more appropriate values from which the same procedure selects K=76 (**Figure 3.13**).


K=2
K=3
K=4
K=5
K=6
K=7
K=8
K=9
K=10
K=11
K=12
K=13
K=14
Breed
Gir N'Dama
Minor Asia
Greece & Cyprus
South East Europe
East Podolian
Tyrrhenian
Alpine
France
Iberian
North West Europe


K=15
K=16
K=17
K=18
K=19
K=20
K=21
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K=23
K=24
K=25
K=26
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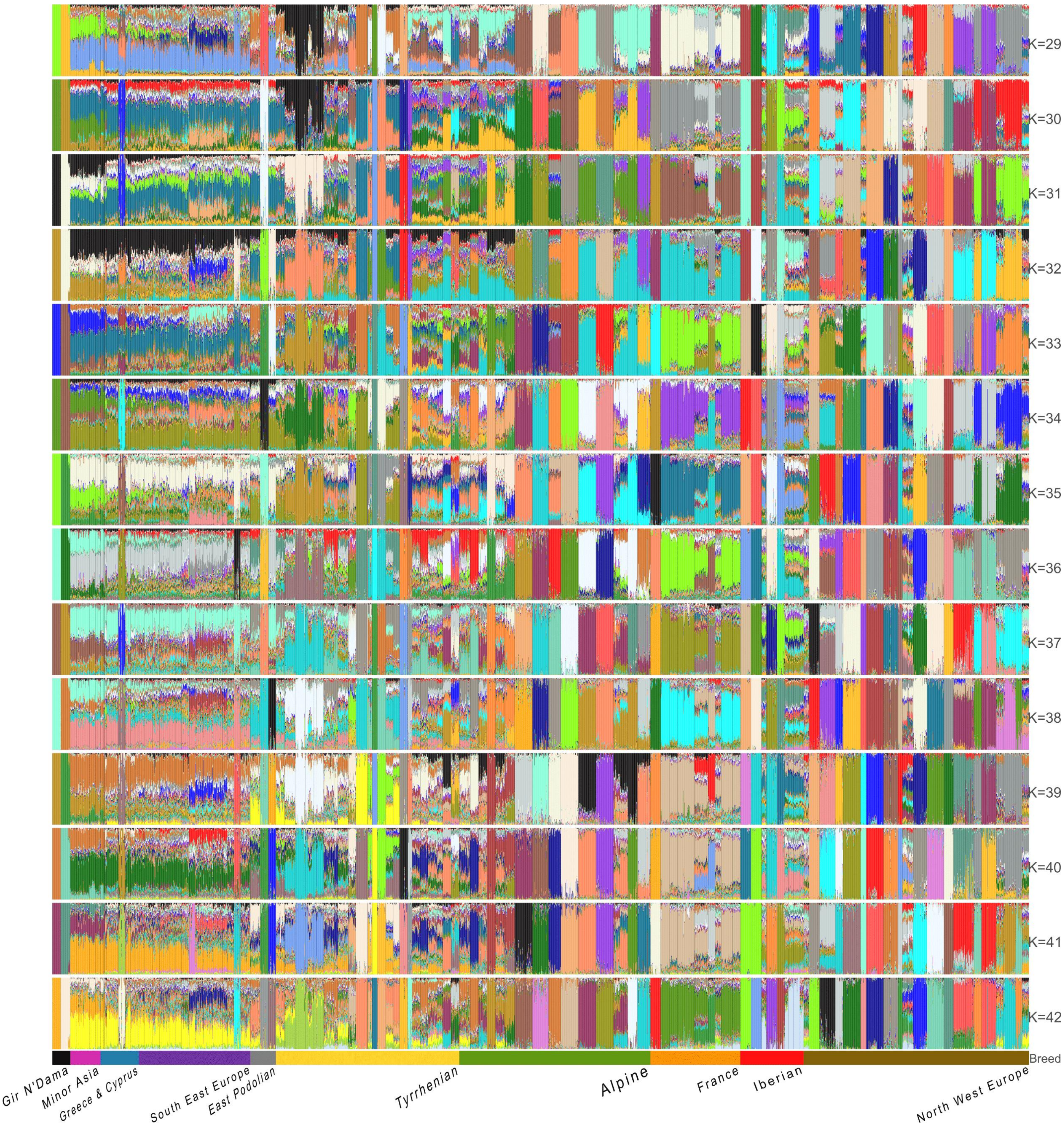
K=28
Breed
Gir N'Dama
Minor Asia
Greece & Cyprus
South East Europe
East Podolian
Tyrrhenian
Alpine
France
Iberian
North West Europe


K=29
K=30
K=31
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K=36
K=37
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K=39
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K=41
K=42
Breed
Gir N'Dama
Minor Asia
Greece & Cyprus
South East Europe
East Podolian
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France
Iberian
North West Europe

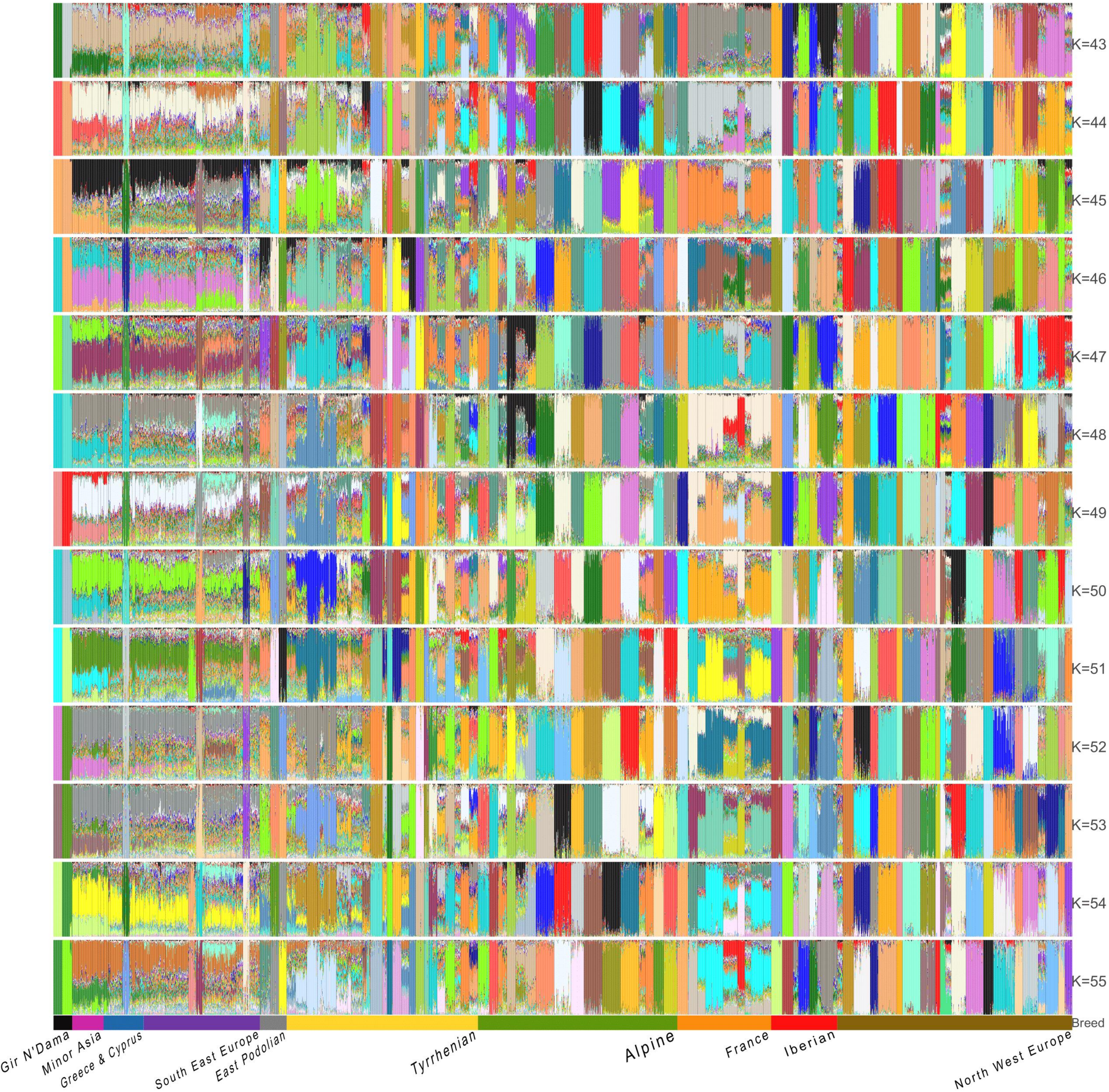

Figure 3.14. Model-based clustering of the estimated membership fractions of individuals from all examined breeds. Each cluster is indicated by a different color and each individual is shown as a vertical bar, at *K* = 2 - 55. The heights of the colored segments are proportional to genotype memberships. The names of the breed groups are given according to their geographical distributions as shown in Figure 2.2. For a full definition of the breed groups (Table 2.1). From left to right within each group are presented the following breeds: *Minor Asia*: ATER, ATBC, ATSR, ATSY, TRG; *Greece and Cyprus*: CYP, AGT, CRT, NSY, GRB, KAS, KEA, PRG, ROG, SYK, KTR; *South East Europe*: RHS, MKB, SRB, PRE, RMB, SHB, DGB, DBB, MAB,

LKB, SKB, MNB, BHB, HRB; *East Podolian*: HRI, HRP, UKP; *Tyrrhenian*: PODO, CINI, MOSI, RSIC, MOSA, SARD, SBRU, CORS, AGER, MARE, CHI, MPIS, CALV, MCH, RMG, GARF, PONT, MODE, CABA, REGG, PMT, BURL; Alpine: PRDO, OVAR, REND, BPUS, PUST, SIC, PIN, TGV, MWF, OBV, BBV, DFV, FGV, VOG, ABO, MON, TAR; *France*: RDBI, SAL, AUB, LIM, CHR, PAR, BAQ, GAS; *Iberian*: MNRQ, MALL, NGAN, CANA, MARI, ALEN, BAR, MARO, SYG; *North West Europe*: BPN, NOR, MAN, BBB, LKF, HF, GNS, JSY, HER, SHR, KRY, DXT, GLW, AAN, HGL, NRC, SERC, FJL, FIAY, FINE, FINW, FINN, YARO

In Admixture analysis, the main separation between *B. taurus* and *B. indicus* occurs at K = 2. In *K* larger than 4, animals from highly selected breeds start to separate into distinct clusters. From K=32, the geographic groups of Asia Minor, Greece and Cyprus, as well as the Southeast group, show a differentiation in their common origin ratio in relation to the rest of the geographic breed groups which they keep afterward.

A significant percentage of shared ancestry between KEA and alpine breeds (OBV, BBV) starts at K = 9 and remains until K = 33. From K = 9 the influence from the Alpine group begins to be seen in some Tyrrhenian (AGER) breeds, while the Jersey influence in some breeds of the South-Eastern European group begins very early at K = 5 and seems to remain even for K = 52 (**Figure 3.14**).

In **Figure 3.15**, the Greek, Anatolian breeds as well as most of the Southeast geographic group, mainly Buša from Bulgaria, North Macedonia, Kosovo, and Serbia showed a high level of complex admixture even at K = 76. All highly selected breeds and artificially isolated breeds formed their own cluster. The same was also observed for inbred small sized mainland population like Rhodope - ROG and BHB Buša breed (**Figure 3.16**).

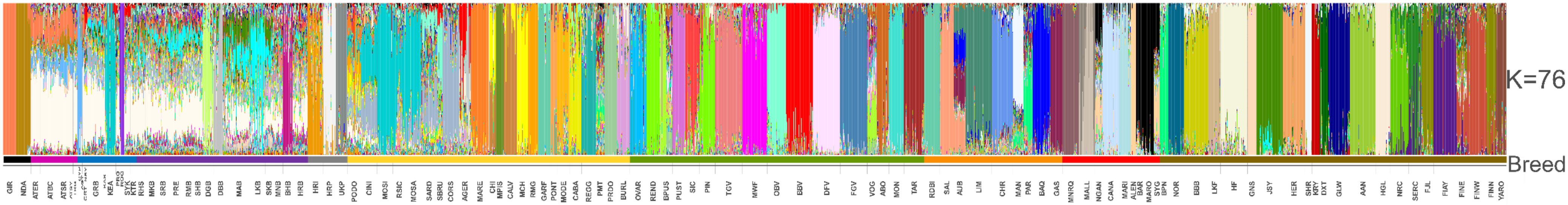

Figure 3.15. Model-based clustering of the estimated membership fractions of individuals from all examined breeds (N = 2,779). Each cluster is indicated by a different color and each individual is shown as a vertical bar, at K = 76. The K-value of 76 represents the lowest cross-validation error (cv = 0.538).

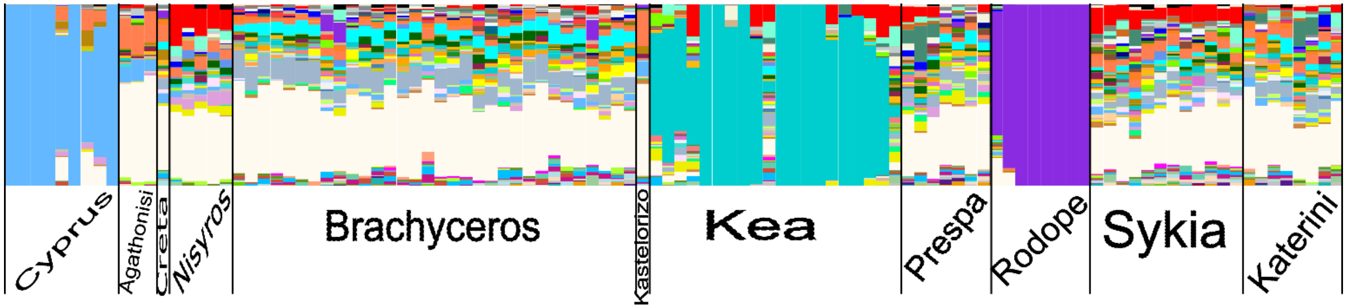

Figure 3.16. Model-based clustering of the estimated membership fractions of individuals from Greek and Cyprus geographic group (N=109), at K=76.

In **Figure 3.16**, apart from the Rhodope cattle, the island Kea, and the Cyprus breed, the rest of the Greek populations show common ancestry to a significant extent with evidence of shared ancestry between them and the Anatolian and neighbor Buša breeds. This is confirmed for the Brachyceros strains (GRB, PRE) and to the Greek Podolian breeds (KTR, SYK) as well as to the island populations (AGT, KAS, NSY). Also, the island populations of AGT, KAS, and CRT, share a common origin to a greater extent than the rest of the Greek populations with the Cyprus cattle and finally with the Anatolian breeds. Finally, an influence of the Alpine BBV is observed, in the individuals of podolic Sykia, Nisyros, and some animals of Prespa cattle (**Figure 3.15**, **Figure 3.16**).

3.7 Ancient Gene Flow Detection (D-Statistics analysis)

The historical admixture between taurine and indicine cattle was confirmed for most of the cattle breeds that originate from Anatolia, Cyprus, Greek and Southeast Europe and most of the Central and South Tyrrhenian breeds. The KEA, AGER and SBRU breeds, which are influenced by the Alpine group, as well as the MPIS and SARD breeds deviate from the above-described general geographic trend. In **Figure 3.17**, the values of all these significant D-statistics (Z > |3|) are shown with a red spot on a tessellated map to highlight the gradient of the *Bos indicus* introgression from the Southeast to the Northwest direction. The results provide strong evidence that support the influence of *Bos indicus* along the Balkan continental route and along the Mediterranean route up to North Italy. Among the breeds of the Greek and Cyprus geographic group, the Cyprus cattle - CYP, as well as the Eastern Aegean Island populations (KAS and AGT), show similar values to the eastern breeds, followed by the island CRT and the ROG population. The rest of the Greek breeds present values close to those of Neighbor Buša (RHS, MKB) and some local breeds from South Italy (e.g., PODO, MOSI, MOSA).

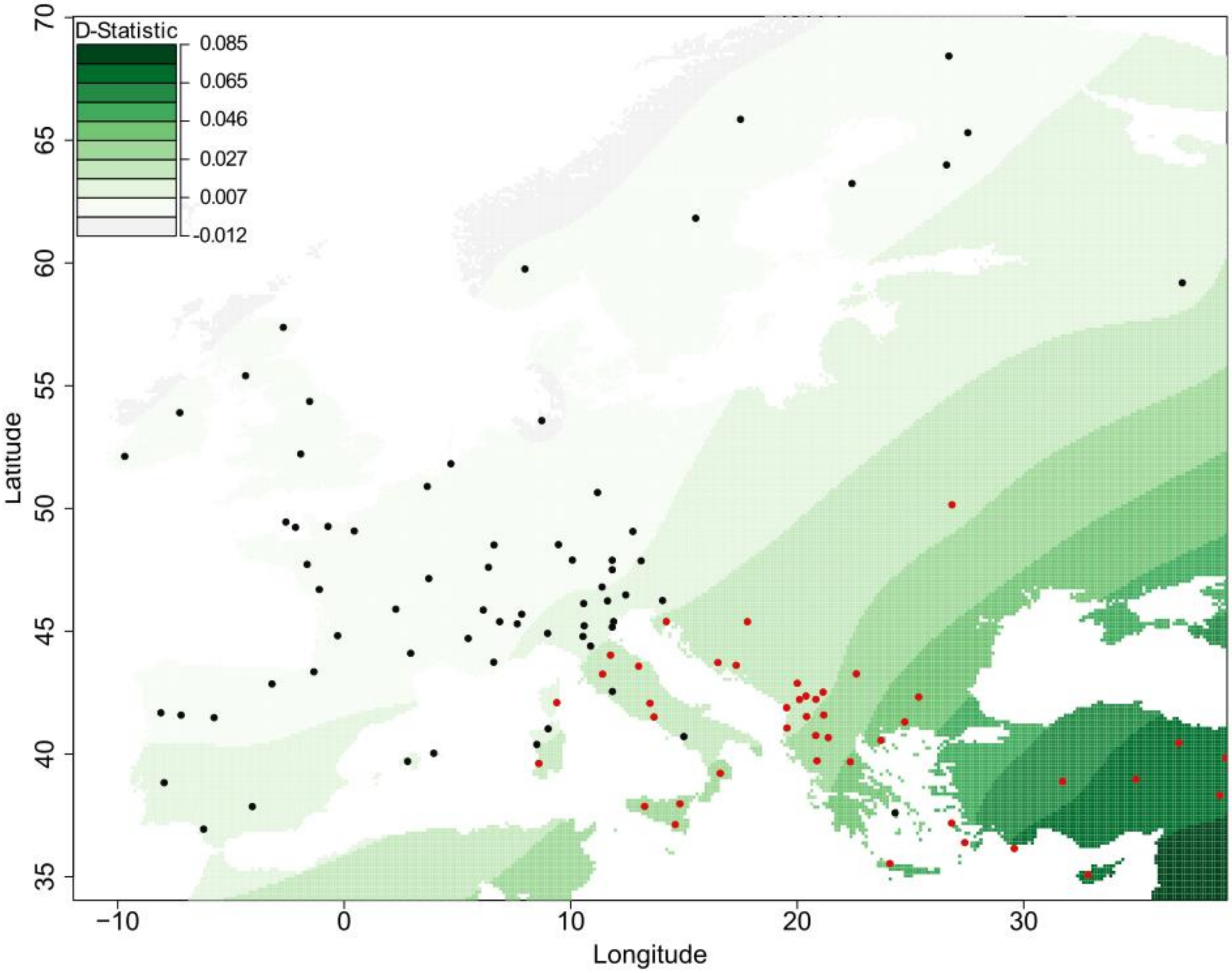

Figure 3.17. Tessellated projection of D-statistics values. Red dots represent a significant influence of *Bos indicus* (Z > |3|)

3.8 Identification of outliers within Greek cattle breeds

A matrix consisting of six different admixture signatures (Ramljak et al., 2018) for every member of the Greek cattle breeds was estimated and used for a multivariate outlier test (Filzmoser et al., 2005). The parameters were designed (**Chapter 2.3**) as input in "uni.plot" R function (Mvoutlier package) to estimate the outliers in multivariate test and visualize the results were with a custom R function (**Figure 3.18**, **Figure 3.19**). The custom function was built around the logic of finding out why an animal is " outlier ". Thus, the outliers were identified with a different color and symbol based on the number of variables in which they get values greater than the average. In red those that have 4 and/or 5 variables above the average, in black those that have 3, in blue those that have 2, and finally in green those that have 1 (**Figure 3.18**, **Figure 3.19**). In some breeds (KEA and

GRB) the *mvOutlier* was performed more than one times. After the first run, the most significant outliers were removed, then the parameters were recalculated, and the test started again. The conservation prioritization for the local breeds based on the second and additional rounds of outlier testing should be accompanied by information on the population size, the level of within-breed kinship as well as the level of heterozygosity at the individual level.

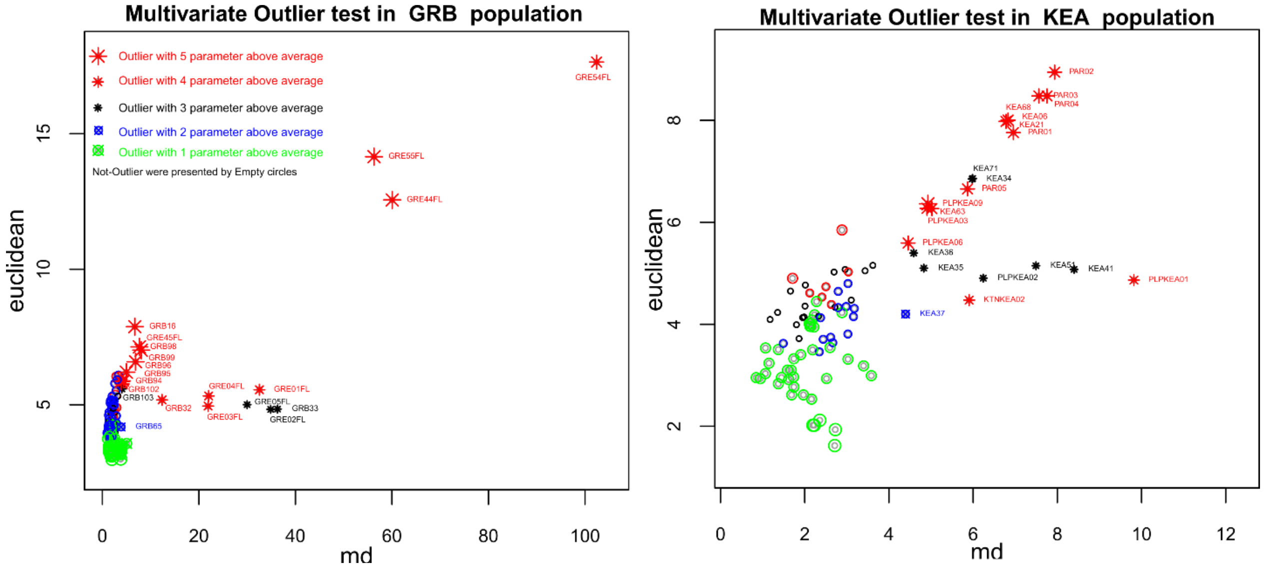

Figure 3.18. Multivariate analysis for GRB and KEA. Two-dimensional presentation of the multivariate outlier test. The y-axis presents the Euclidean and the x-axis the robust Mahalanobis distance of the multivariate data (Filzmoser et al., 2005). Each animal is presented by a single symbol.

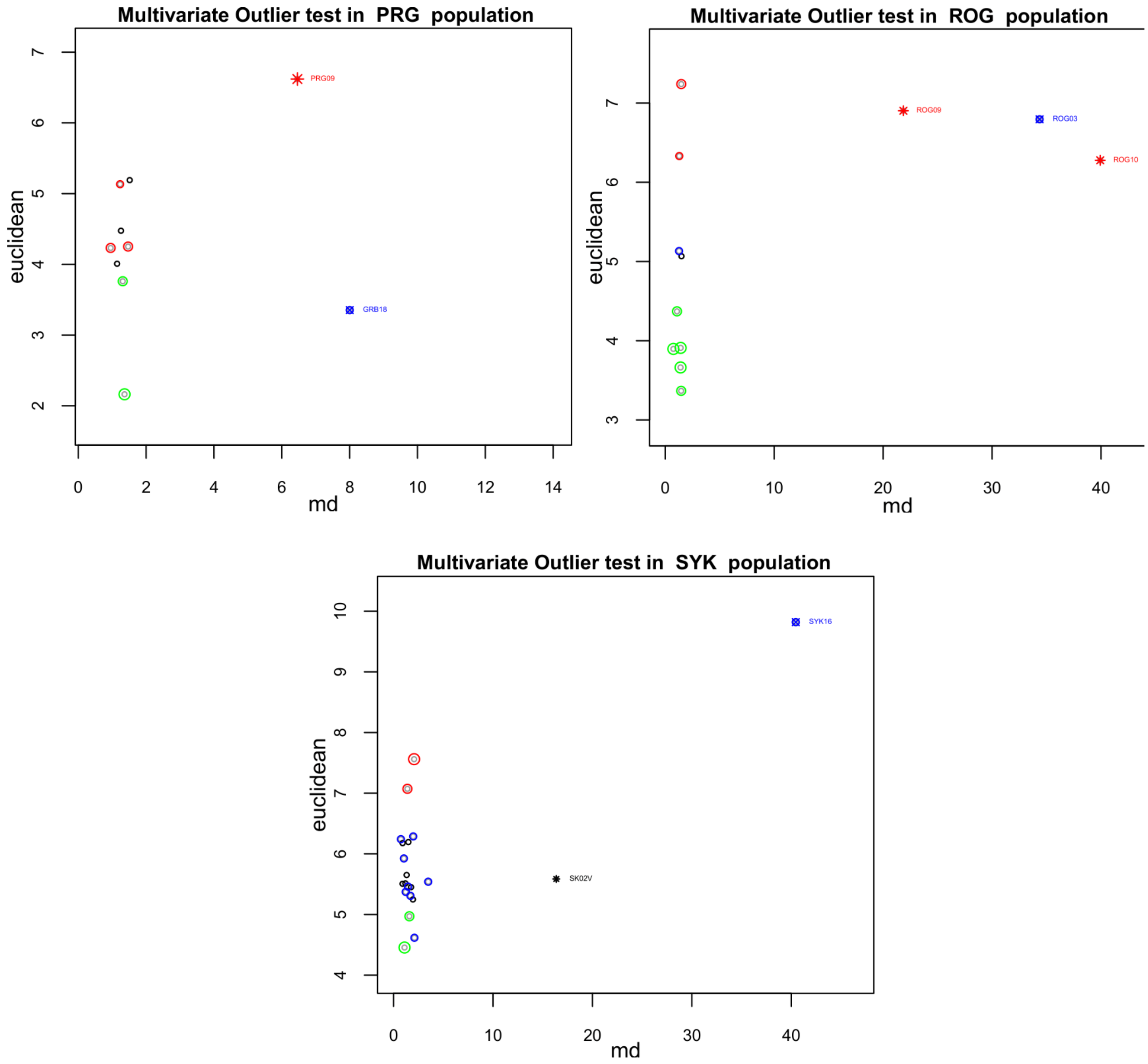

Figure 3.19. Multivariate analysis for PRG, ROG and SYK cattle breeds Two-dimensional presentation of the multivariate outlier test. The y-axis presents the Euclidean and the x-axis the robust Mahalanobis distance of the multivariate data (Filzmoser et al., 2005). Each animal is presented by a single symbol.

In the Brachyceros breed, for further conservation study, additional sampling of animals was done from different parts of Greece (Figure 3.20). Thus, out of the 145 total samples, 15 animals were found with a high degree of admixture with high-yielding foreign breeds. It's about mainly Alpine breeds (OBV, BBV, TGV), but donor breeds such as Limousin and Guernsey are also found in some cases. They were also found 13 individuals with significant admixture with the Greek Podolian breeds (Katerini and Sykia).

As it can be seen in the distribution image of these outliers (Figure 3.21), most purebred animals are found in the mountainous regions of Western Greece. Crossbred animals with influence from the Greek podolian type are found in the regions of Thessaly, Macedonia, and Thrace, areas with a large dispersion in the past of the podolian breeds of Katerini and Sykia. Finally, most of the admixed animals with influence from high-yielding breeds are found near the lowland areas of the country. This is probably because these areas have greater grazing capacity or are closer to the production of cereals and concentrates and thus can satisfy the higher requirements of the improved animals. In the mountainous regions of Western Greece where the local cattle are free-range, heavy high-yielding animals are more difficult to survive.

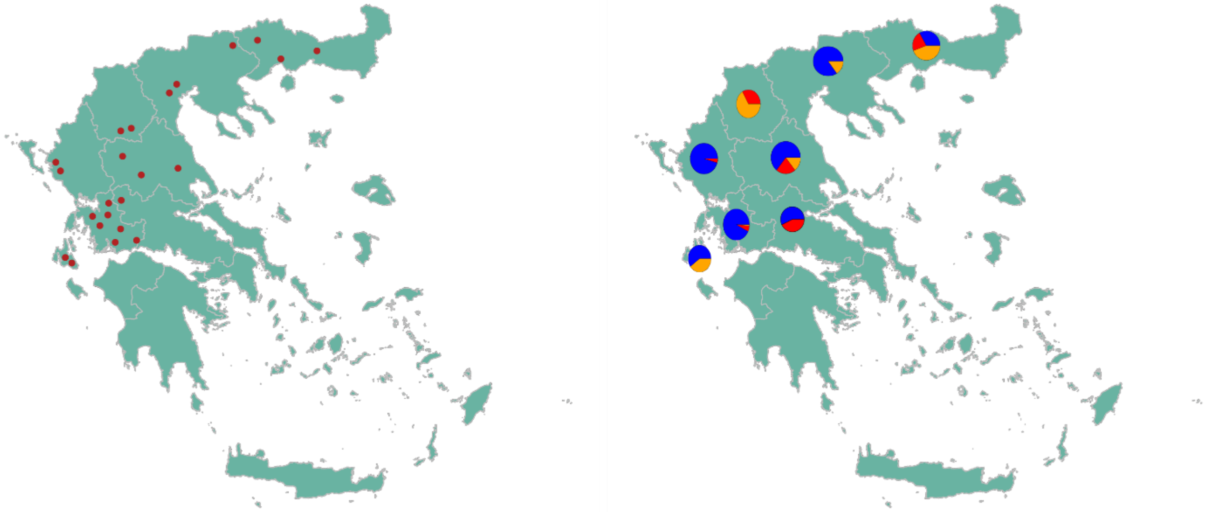

Figure 3.20. Sampling map (left) and distribution of outlier for GRB breed (right). the farms in the geographic areas with red points (left). Outliers due to influence from high-yielding breeds make up the red part. Outliers due to admixture with Greek breeds (Katerini, Sykia) make up the orange part of the pie and the blue part are purebred individuals (right).

After repeated runs of analyses in the Kea breed, 52 of the total 97 individuals were identified as outliers. Of the 52 animals, 45 were outliers due to their high affinity with individuals and breeds mainly from the Alpine (BBV) but also the Northwestern group of breeds (HF), while the remaining 7 are present due to their high affinity with individuals or breeds from Greece (NSY, GRB). More specifically, the Paros and Kythnos animals (23 in total) all turned out to be outliers due to their high affinity with the aforementioned high-yielding foreign breeds. The largest percentage of purebred individuals is found in Makronissos where out of 13 animals only two are characterized as outliers. On the homonymous island of Kea, out of 42 animals, 10 were found as crossbred, while most of the purebred individuals are descendants of a core of animals that were transferred from Makronissos in the early 2010s. In the Peloponnese, out of 19 animals, 13 were found admixed (**Figure 3.21).** The 7 from the above 13 animals are the outliers in which a significant level of mixing with Greek animals/breeds was found. The population of the Peloponnese (Triphylia) concerns descendants of animals of the Kea breed that were transferred there in the 1960s and to some extent crossed with local animals.

Figure 3.21. Distribution of outliers for KEA breed. Outliers are the red part of the pie and purebreds are the blue part. For sampling location see Figure 2.1.

Finally, the same procedure was followed for populations of Rhodope, Prespa, and Sykia. The three Rhodope animals were characterized as outliers due to the high genetic relationship with the Brachyceros, of the two animals of the Prespa cattle, one showed a high affinity with the Brachyceros and the other with the Limousin breed, while of the two animals of the Sykia, one showed a high relationship to Brachyceros while the other to Alpine BBV.

Chapter 4. Discussion

Based on the history from the initial domestication of cattle in the Neolithic to the creation of modern breeds, we try to answer the question of when and how the current diversity of cattle genetic resources has emerged (Felius M. et al., 2014).

Improving our knowledge of genetic diversity within and between local breeds is a crucial issue for the implementation of further conservation programs necessary for sustainable development and future livestock breeding in changing environments (Flori et al., 2019). This could be particularly important for traditional unselected breeds that cover a geographical area close to the center of domestication (Medugorac et al., 2009).

4.1 Sampling and Dataset

All known populations bred in Greece and systematically reported by Greek scholars since the middle of the previous century are represented in the study data set. These populations are the Brachyceros breed, the Greek steppe type (Katerini and Sykia breeds) as well as the island breed of Kea. As pointed out in the introduction (**Chapter 1**) purebred individuals of the Kea breed are found on some Aegean islands such as Kea, and Makronissos as well as in the mountainous Triphylia of mainland Greece. Thus, the sampling concerns representatives from all the above areas where such animals exist. The sampling was completed with specimens of the Brachyceros type from the Prespa and Rhodope region as well as from local populations of the Aegean islands such as Crete, Kastellorizo, Agathonisi, and Nisyros. Finally, the sampling was enriched with samples of the local Cypriot breed. Thus, the present study constitutes the first comprehensive sampling, recording, and processing of genetic data for the local breeds of cattle in Greece and Cyprus. The sampling included as many individuals as possible from the entire Greek territory (**Figure 2.1**). Some indigenous island populations (Crete, Agathonisi, Kastelorizo and Nisyros) were represented by very small numbers of specimens (**Table 2.1**). However, due to the aforementioned limited population sizes as well as herd limitations (i.e., many related individuals, wild and difficult to handle), it was unrealistic to assume that the number of samples could be increased. For the remaining study's breeds, a sufficient number of 10 or more of the most representative individuals of the local breeds were sampled. Therefore, in this study, the cattle phylogeny covers geographic origin ranging from the cattle near to domestication center to the westernmost and northernmost parts of Europe (**Figure 2.2**).

For a more complete understanding of the genetic structure of the breeds under study, the historical relationships between Greeks, Balkans, Romans, and Turks from antiquity to recent times was considered. Thus, the data set includes 112 breeds from these regions as well as from regions of Central, Western, and Northern Europe (Spain, Portugal, UK, Ireland, and Scandinavia). To put our analysis in the global contest N'Dama as purest representative of African *Bos taurus* and Gir as purest and most available representative of *Bos indicus* was included. Additionally, Mongolian Yak was included as outgroup for necessary genetic analyses like *TreeMix*, which is necessary for rooting the tree.

4.2 History and demographic evolution

The local cattle populations of the Greek and Cyprus geographic group show a similar demographic trend which is characterized mainly since the 1960s by their continuous substitution by famous high-yielding breeds. Imported high-yielding cattle existed in Greece from the beginning of the last century, but they were mainly raised around urban centers as well as near lowland areas. The main volume of cattle in mainland Greece concerned mainly unimproved Brachyceros and steppe-type cattle (**Figure 1.5**).

The need to replace indigenous cattle in Greece is due on the one hand to the transformation of the production model from an agricultural to a more industrial economy after the World War II, aided by social events such as the civil war, led to massive internal migration from the countryside to the city. Internal mobility led to increased demands for consumption of bovine products in the cities which could not be met by indigenous cattle populations who mainly served a small scale/local economy as no genetic improvement scheme had been supported in the past. On the other hand, the mechanization of agriculture at the same time intensified the decline of indigenous populations since of the approximately 1,000,000 cattle that existed in the 1930s, half were used as work animals and at that time they had no utilitarian value. The decrease in population size especially for the recognized mainland breeds (Greek Brachyceros breed, Katerini and Sykia breeds) reaches a few hundred animals in the late 1990s and early 2000s. (**Figure 1.6**).

As for the island populations, each island had a local cattle population, while until the 1940s there were 4 stable breeds (Tinos, Corfu, Kea, Andros) as a result of upgrade interbreeding, of which three are extinct, while the Kea breed has some dozens of people (Bizelis et al., 2019; Bizelis et al., 2021). In general, each Aegean Island represents a different entity with a variety of environmental and socioeconomic factors affecting animal populations (Spilanis & Kizos, 2015). However, the demographic trend is similar in island populations which have continuously suffered from population decline in recent decades. In the case of the CRT breed, even 20 years ago, statistical data on the number of indigenous cattle in Crete recorded only 149 out of 2,207 (6.8%) as indigenous or local, which is a rather small percentage (Hellenic Statistical Authority, 2001). This statistic also documents a very small number of animals for such a large island (one cow per 4.23 km^2). For the entire territory of the Dodecanese islands, where the Agathonisi, Kastelorizo and Nisyros cattle breeds are bred, the same source identified 2,434 of the 6,210 recorded animals (39%) as native. Today, the population size of all the above-mentioned cattle breeds is very small (**Table 1.2** & **Chapter: 1.6**). More generally, there is an opposite trend between the economic importance of cattle and small ruminants when comparing areas along the line from the Aegean islands to mainland Greece, the Balkans, and Central Europe.

4.3 Genetic diversity and Inbreeding

Despite the ascertainment bias of the BovineSNP50 chip data (Matukumalli et al., 2009; Simčič et al., 2015), it was highly informative for the analyzed bovine populations of Greece, as reflected by the notable values obtained for various parameters of genetic diversity levels.

Vilas et al (2015) reported that the higher adaptive potential of a population is better indicated by the allelic variety of neutral markers than by heterozygosity. Overall diversity estimators show higher allelic diversity in lineages from Southeast Europe and Asia Minor region. However, this general trend is interrupted by some highly fragmented and inbred Greek cattle breeds (**Figure 3.1** and **Figure 3.2**), especially by island Greek cattle breeds such as Crete-CRT, Agathonisi-AGT, Nisyros-NSY and Kastelorizo-KAS, which are represented by samples of the last remaining indigenous animals. These parameters indicated a deeper loss of genetic diversity as well as a higher inbreeding in the Greek island populations than in the Greek mainland breeds. For example, in the unbiased Allelic Richness index (*AR)* the Greek mainland subgroup gets the third highest mean value among the nine geographic groups after Minor Asia and Southeast Europe breeds while the Greek island breeds subgroup gets the lowest mean value among all groups. Also, in the indexes of private and semi-private alleles (*npA*, *nspA*) the Greek mainland subgroup gets the second highest value among the nine geographical groups after the Minor Asia breed group, while the Greek island breeds subgroup gets again the lowest value among. It is worth noting that the indices for measuring private and semi-private alleles are completely dependent on the rest of the breeds in the data set. Thus, donor breeds such as BBV, HF, and JSY take low values on both indices, as do recipient breeds (e.g., KEA, AGER, BURL) which also take low values for the same reason. By the same reasoning, it is worth thinking about some limitations on the already very high values in the neighboring breeds of the Greece, Balkan Peninsula, and Asia Minor regions (**Table 3.1**).

In all indicators of genetic diversity, the Kea breed shows significantly higher values than the geographical subgroup of Greek island breeds. This fact is related to parameters concerning the history but also the current state of the breed. First, it is a larger population estimated at around 100 animals while each of the others is estimated at around 20-25 animals. On the other hand, as a product of admixture of distant breeds (Papadopoulos 1946; Bizelis et al., 2021; **Chapter 1.6**). Finally, the dispersal of the breed to other islands as well as to the mainland as early as the 60s creates the impression of many and small subpopulations, with little or no gene flow (especially between the island and mainland Greece) among themselves but also different levels of admixture with other local or cosmopolitan animals (**Chapter 3.8**).

The low genetic variation observed in island populations, increases the risk of extinction more than population size. Moreover, empirical studies (Ørsted M. et al., 2019) provide evidence that rather than relatedness, genetic drift has led to a decrease in diversity in comparable scenarios. However, pruning in stressful environments (i.e., natural selection) can maintain a higher level of diversity than expected with inbreeding, because a higher level of nucleotide diversity is associated with a stronger selection response under stressful conditions. In addition to small population size, genetic drift is enhanced by geographic isolation that further impedes gene flow between breeds. Thus, in such cases, inbreeding and genetic drift are inevitable factors shaping the diversity of these populations. In full agreement with the strong effect of genetic drift, the corresponding mean frequency of private alleles (*fpA*, **Table 3.1**) reached the highest value in the Greek island breeds. Comparably high *fpA* values were observed only in genetically isolated island breeds such as MNRQ and MALL (**Table 3.1**). A general trend that characterizes the fragmented and endangered breeds was observed, namely that they consist of highly related individuals with a small number of private alleles with high frequency. This is reflected in the high positive correlation between *F* and *fpA* ($r_{(F,\, fpA)}$ = 0.72) and low negative correlation between *F* and *npA* ($r_{(F,\, npA)}$ = - 0.21). Finally, all the Greek cattle populations present in the long ROH category (> 16 Mb), the highest inbreeding values, which shows continuous consanguineous mating. This trend is more pronounced in island populations and leads to recessive deleterious genomic variants emerging at a population level.

The increased level of allelic and genetic diversity in breeds from Southeast Europe, and in Eastern and some Greek cattle breeds could also be the result of various demographic events, including the introgression of Bos indicus alleles. This is more likely due to ancestral relationships rather than contemporary gene flow. However, a genetic affinity between western Asian and Greek populations has also been reported in goats (Pogorevc et al., 2023). It should be noted that cannot done distinguish the proportion of diversity caused by introgression from that caused by other evolutionary forces, e.g., low artificial selection pressure. Still needs to be noted that some estimators of allelic diversity, such as the number of private and semi-private alleles, measure the proportion of private alleles that are not even present in GIR or other neighboring breeds. As discussed above, probably due to an abrupt climate change, farmers began crossbreeding taurine cattle with Bos indicus (Lenstra & Felius, 2014; Pitt et al., 2018) during the Early Bronze Age. Such crosses increased the already high level of diversity of the local soft-selected breeds. Subsequent long-term adaptation to local environments shaped mosaic cattle genomes with an unknown but low proportion of Indicine alleles in modern cattle breeds from Anatolia to the southern foothills of the Alps. Therefore, maintaining a high level of diversity in these partially fragmented breeds could provide valuable genetic resources for future human needs and future abrupt or gradual climate change events. In the process of finding or reconstituting genetic resources, the inclusion of wild ancestors in the reproduction of species that still exist is discussed (Taberlet et al., 2011). Also, as potential resources can be understood the four or five wild species of the genus Bos are still alive and can produce fertile hybrids with cattle. In any case, the local breeds of Southeast Europe should be understood as a global stock of important alleles, which could contribute to future breeding programs (Tapio et al.,2006). Although it has been argued (Decker et al., 2014; Medugorac et al., 2017) that introgression could help domesticated cattle to adapt to an extreme environment, could also be argued that interbreeding between breeds of temperate climates and breeds that have evolved in more extreme environments could also ensure the viability of high yielding breeds.

4.4 LD and effective population size

Estimating the effective population size based on LD for different time periods during cattle evolution provides an interesting insight into their demographic history (**Figure 3.6**). The pattern of *Ne* at the time of domestication (~2000 generations ago) suggests that geographic groups that placed near to the domestication center, had larger founder population sizes compared to Western European breeds (**Table 3.4**). For Ne_{2000}, Greek Brachyceros breed takes, after Minor Asia breeds the highest value. In this period, many Buša and local breeds of Southern Italy take equally high values among 112 breeds. From the time of the Industrial Revolution and the creation of modern breeds (~50 generations ago) until today the values of effective population size have drastically reduced in all populations **(Table 3.1)**. Southeastern European breeds such as the Buša and the Greek Brachyceros, as well as many local breeds of Southern Italy, are under the greatest pressure. The above local populations still get the highest values for Ne_{50}, but it is worth noting that many of today's famous breeds get in this period equally high values especially those of the French group of breeds such as Charolais, and Limousin. As we approach the present day (~5 generations ago), the dynamic of effective population size (Ne_5) reverses with the geographic groups of Central and Northern European breeds showing the highest values (**Figure 3.6**).

As Ne is inversely correlated with LD, the sharp decline in effective population size over the last 50 generations implies that such populations have accumulated a small number of common but long haplotypes, which contribute to high LD. The Industrial Revolution, modern breeding practices, and changes in consumer preferences led to the replacement of local cattle breeds with commercial cattle breeds, which originated mainly from Northwestern Europe. This factor, combined with uncontrolled inbreeding in the remaining fragmented populations due to the absence of modern breeding and management practices, contributed to the population decline of most of the previously highly diverse cattle breeds from Southeastern Europe.

4.5 Genetic distances and differentiation

Genetic distances based on allele frequencies and the proportion of shared alleles as well as model-based methods were used to cluster breeds (supervised) and individuals (unsupervised) as well as to estimate the differentiation within breeds. A heterozygosity-independent approach was chosen as a measure of genetic differentiation between breeds. In each case, the level of genetic differentiation within and between breeds is reflected in the clustering methods of breeds and individuals.

As underlined in the **chapter 1**, all indigenous breeds of Southeast Europe, Greece, Cyprus and Asia Minor are either subject to weak artificial selection or no coordinated artificial selection at all. However, there are substantial differences in the level of isolation. The estimated D_{EST} levels suggest a substantial genetic differentiation of the Greek island geographic subgroup compared to the other groups (**Table 3.5**). This high differentiation is attributed to genetic drift and relatedness in the highly fragmented and physically isolated island breeds (Crete, Agathonisi, Nisyros and Kastelorizo) and is confirmed by the low differentiation between animals within these breeds (**Figure 3.7**). In contrast to the island breeds, the lowest levels of genetic differentiation (D_{EST}) were observed for the breed groups from Asia Minor and Southeast Europe as well as for the Greek Brachyceros. This low differentiation is accompanied by a high level of alleles that includes a large number of private and semi-private alleles of low frequency (**Table 3.1**). Low differentiation and high diversity are probably the consequences of low artificial selection pressure combined with low genetic drift in effectively large and less isolated populations. The degree of differentiation within each breed (homogeneity index) is inversely proportional to its degree of differentiation with the rest of them ($r_{\text{(DPS (within Breed), DEST (among breeds))}}$ = - 0.82). Thus, the highly inbred - homogeneous island breeds (Low $D_{\text{PS (within breed)}}$) get the highest values of genetic differentiation, while on the contrary, the undifferentiated breeds of the geographical groups of Asia Minor and Southeast Europe (**Table 3.5**) as well as Greek Brachyceros breed (Low $D_{\text{EST (among breeds)}}$) show the highest $D_{\text{PS (within breed)}}$.

All supervised methods (phylogenetic trees and network) applied in this study clearly reveal the level of genetic differentiation. Highly differentiated breeds (genetic differentiation between each breed and all others) show long branches in neighbor network (**Figure 3.8**), neighbor joining tree (**Figure 3.9**) and maximum likelihood tree (**Figure 3.10**). This is independent of the evolutionary sources of differentiation. Thus, artificially selected and artificially isolated breeds such as those of UK beef (AAN, HER, SHR), as well as naturally isolated and naturally selected breeds (e.g., Greek island breeds) seem to show long branches. However, breeds under strong random sampling (small-sized Greek island breeds) show even larger branches, especially in the ML analysis (**Figure 3.10**). The Greek mainland population of Rhodope (ROG) also shows a significant amount of genetic drift that could reflect the long-term isolation of the breed and a limited number of founders after the bottleneck. In contrast, for local breeds with low differentiation and a high level of diversity (Anatolian, Buša, GRB), the three supervised methods reveal small branches or even no branches.

The MDS projection places almost all the unselected breeds from Minor Asia, Greece and Cyprus, and geographic breed groups from Southeast Europe and East Podolian, in the overlapping space (**Figure 3.11**, **Figure 3.12**). This mixed cluster between these neighboring breeds probably stems from frequent gene flow in the recent past. While the breeds of Anatolia, Cyprus, and the Greek island populations such as Kastelorizo and Agathonisi near the GIR, the rest Greek breeds were placed among the above-mentioned island breeds, the Southeast European Buša and some Italian local breeds. Therefore, breeds that are geographically adjacent to each other overlap in the MDS projection. Only the KEA breed, which is the historical product of a cross between indigenous animals like the modern Greek Brachyceros and some Alpine breeds (Original Braunvieh, Braunvieh, and Tiroler Grauvieh), is placed between the Alpine and Tyrrhenian breeds.

4.6 Relatedness, recent admixture and indicine introgression

To determine the genetic relatedness, values analogous to the IBDs resulting from the construction of the UAR matrix were calculated. The analysis of these values shows that all Anatolian breeds, Greek breeds, Cypriot breed as well as most Buša breeds (especially the neighboring ones from Bulgaria, North Macedonia, Kosovo, and Serbia) show the same ancestry pattern (**Table 3.3**). All these populations show the highest affinity with the representative of *Bos Indicus* (GIR) and then the highest values of affinity with breeds either from Asia Minor, Cyprus, or Greek KAS (**Table 3.2**). Also, from the same data, the strong influence of some cosmopolitan breeds on Balkan populations emerges. For example, the Kosovar DGB, the Albanian DBB, MAB, LKB, and SKB seem to have received a significant percentage of genes from the dairy breeds Jersey and Holstein, while the MNB from Montenegro from the Alpine group (**Table 3.2**). It is worth noting that the Greek KEA shows a completely different pattern of origin from the rest of the breeds of the geographical group to which it belongs, but similar to the breeds of the Alpine group. A similar picture to KEA is also presented by the Tyrrhenian breeds SARD, SBRU, AGER, and BURL (**Table 3.2**, **Table 3.3**). The above also explains the placement of the breeds in the distance-based phylogenetic analyses (**Figure 3.8**, **Figure 3.9**). In both figures, Buša breeds DGB, DBB, MAB, and SKB are placed between Jersey and other local breeds from Greece or Italy, while KEA breed as well as Tyrrhenian SARD, SBRU, AGER breeds are placed in the Alpine geographic group, while BURL in northwest geographic group.

Also of interest is the fact that most breeds of known podolian influence (PODO, CALV, MARE, RMG, MCH) of the Tyrrhenian group, as well as the local breeds of Sicily (CINI, MOSI, RSIC) and the MOSSA breed from Sardinia show significant levels of affinity with GIR (**Table 3.2**). These breeds show a similar pattern of descent to the breeds of Asia Minor, Southeast Europe and Greece and Cyprus geographical groups (**Table 3.3**). The genetic relatedness of the podolian breeds of Southern Italy with eastern breeds influenced by *Bos indicus* (Turkish Grey) is also confirmed by mtDNA research (Di Lorenzo et al., 2018), by High density BeadChip (Barbato M. et al., 2020) as well as by archaeological genomic data (Cubric-Curik et al., 2022).

The common ancestry pattern of the Balkan Peninsula and Asia Minor breeds is presented in the model-based admixture method as a complex mosaic of shared ancestry. Most animals from the artificially unselected breeds of Southeastern Europe, Greece, Cyprus, and Anatolia remain unclustered even at very high K values. In contrast, animals from the artificially selected breeds of Central and Northern Europe cluster separately from very low values K > 4 (**Figure 3.14**, **Figure 3.15**). Because breeds are typically reproductively isolated with little or no interbreeding, the cross-validation error continued to decrease, as the number of K ancestral populations modeled in the admixture analysis was increased. Up to K = 55, most of high selective and isolated breeds have been formed to their own clusters. This reflects the large differences in allele frequencies that exist between breeds resulting from separate geographic dispersal and isolation, breed formation, and the use of artificial insemination.

Among the undifferentiated unselected breeds from Balkans and Minor Asia, a clear grouping was observed of breeds characterized by a high affinity of the individuals that make it up, such as the BHB breed from the Buša group (from K > 28), as well as the Greek Rhodope and Cyprus Cattle (at high values of K > 55). The level of relatedness of the individuals that make up the breeds was calculated from the kinship table (UAR) and is depicted in **Figure 3.3**. As it can be seen within most Greek breeds, especially in island populations as well as in Cyprus Cattle, significant levels of relatedness are observed. Thus, to avoid a further grouping of individuals into the unselected breeds, which would prevent us from understanding the proportion of common ancestry and ultimately their history, several related individuals were removed from the analysis (**Table 2.1**). The reduction of individuals concerns all Greek populations and in extreme cases such as the island Agathonisi and Kastelorizo breeds, they are represented by just one individual, which certainly expresses in the admixture analysis the general picture of the above island populations.

A K value of 78 is associated with the lowest cross-validation error and it should represent the true number of groups in the used design. It was observed that unselected breeds and/or animals from Minor Asia, Greece, and Balkans remained an unresolved mixture even at the K value with the lowest cross-validation error. On the contrary, the influence of the Alpine group on the Kea breed as reflected in the kinship tables (**Table 3.2**, **Table 3.3**), is also confirmed by the admixture results (K = 9 to 33; see **Figure 3.14**).

Based on supervised phylogenetic network (**Figure 3.7**) and trees (**Figure 3.8**, **Figure 3.9**, **Figure 3.10**) as well as supervision MDS projection (**Figure 3.11**), and on the results of the previous studies (Upadhyay et al., 2019; Flori et al., 2019), it is assumed that introgression of indicine ancestry into Anatolian and some Mediterranean cattle has been occurred. To test this putative indicine introgression, which could have occurred along the migration route from Anatolia, Greece, and Southeast Europe to the southern foothills of the Alps and Northwest Europe, the D-statistics was calculated.

For all breeds from the regions of Asia Minor, Greece, Cyprus, Southeast Europe and East Podolian as well as for the Italian breeds of known podolian influence and some local breeds from Sicily and Corsica, they were obtained significant D-statistic values (**Figure 3.17**). Exception to this geographical distribution of the influence of *Bos Indicus* are the Greek breed KEA as well as the Tyrrhenian AGER, SBRU, SARD. In these populations, a significant influence of the Alpine breeds in their formation is observed (**Table 3.2**, **Table 3.3**). The first breed from the southern Alps for which no significant *Bos indicus* influence was found is SIC ($Z < |3|$), with a D value close to zero. D values clearly decrease as spatial distance from the origin of *Bos indicus* increases. Interestingly, three lineages from the East Pondolian group also show a significant introgression, among them the UKP sampled East of the Carpathian Mountains. As recently shown by Verdugo et al (2019), the indicative introgression began ~ 4000 years ago and may have been stimulated by the onset of a period of increased aridity known as the 4.2-thousand-year abrupt climate change event.

4.7 The value and conservation measures of Greek local cattle breeds

Local breeds are threatened by the success of highly selected breeds through two processes. Firstly, the high performance of highly selected breeds tends to force the replacement of traditional breeds. In many areas, farmers have strong financial pressure to switch to higher-yielding breeds. Such a phenomenon can be very fast, and a valuable local breed can be lost within a decade. Secondly, indigenous breeds are often crossed without any design or clear improvement objective with another more productive breed from elsewhere resulting, the distinctiveness of these breeds was lost to mongrels of all possible combinations. Thus, the adaptive traits of local breeds created over centuries by the action of natural selection can be quickly lost by anarchic interbreeding that eventually leads to genetic erosion of the adaptive loci of traditional breeds. Characteristics such as resistance to local infectious and parasitic diseases, the ability to adapt to moderate forage and rough stables are lost and difficult to rescue. In developing countries, many examples illustrate this invasive threat, where indiscriminate repeated interbreeding has rapidly interrupted generations of selection for adaptation to harsh environments (Taberlet et al., 2011; Cebeci et al., 2020). This replacement process has either already reached its final stage or is close to it in many regions (Bett et al., 2013). If animal diversity is considered a global common resource and a condition for sustainable development in changing environments, then the gradual depletion of neutral diversity existing in local breeds under soft selection pressure is a kind of "tragedy of the commons" (Hardin, 1968; Cebeci et al., 2020).

Conservation breeding programs must be viewed as a regulated long-term exploitation of common resources and the conservation of genetic diversity for sustainable development must be understood as a global long-term project. The repeatedly confirmed evolutionary trade-off hypothesis (Bennett & Lenski, 2007; Ørsted et al., 2019) suggests that increased ability in the selection environment is accompanied by decreased ability in other environments. This trade-off applies to high-yielding breeds adapted to benign (temperate) environments and to breeds adapted to produce in stressful environments.

Based on the natural geographic distribution, ecological conditions, genetic and phenotype characteristics, the Greek cattle breeds can be divided into three main groups: The mainland breeds (Greek Brachyceros breed, Prespa and Rhodope cattle), the island populations (Crete, Agathonisi, Kastelorizo, Nissyros) and the Greek Podolian breeds (Katerini and Sykia). The Kea Island breed, due to its different genetic structure and demographic history cannot be included in any of the above three main groups. It is a product of the upgrading of autochthonous island animals with the aim of forming a dual-purpose breed (**Chapter 1.6**), which presented stable phenotypic characteristics and became famous throughout the Cyclades region. Since the 1970s, farmers have continued to crossbreed their animals, with the result that the few purebred animals that exist today consist mainly of the descendants of a small, isolated population of the Makronissos island (**Chapter 3.8**). For the Greek island populations, the reduced gene flow due to long periods of isolation, the origin from small relict populations, and the reduction in population size due to a genetic bottleneck promoted genetic drift which led to a decrease in within-breed diversity and increased their differentiation.

Although the trend of extinction of local breeds and their replacement by high-yielding breeds is global, the fate of these extinct breeds as well as their importance is not always the same (Bett et al., 2013). In Greece, the officially extinct island breeds of Chios, Andros, Tinos, and Corfu were the product of upgrading the local brachyceros animals with foreign breeds of higher yields. Thus, the total loss of alleles of these populations is probably of minor importance. The situation is different in the rare breeds of the mainland. The endangered Rhodope breed is a strain of the Brachyceros type. Although the risk of its complete abandonment is visible, the population from which it arose (Balkan Brachyceros) still exists, albeit under constant pressure. The situation is similar with the Prespa cattle, which is also a strain of Brachyceros type. Proper conservation programs for these two endangered populations can ensure their existence. The situation is worse for the Greek podolian type (Katerini, Sykia), whose origin is partly unknown. These impressive cattle are now remnants of earlier, more numerous breeds, which still retain significant levels of allelic richness (**Table 3.1**). Because the replenishment of lost diversity from extinct breeds is often irreversible, the need for a conservation program for these small populations is imperative. Various methods for managing animal genetic resources can be proposed but each strategy must consider the different demographic history and genetic characteristics of local breeds. In a long-term conservation plan, breeds with distinct characteristics and heterozygosity of different alleles play an important role in heterosis because they offer a possibility to obtain a high degree of heterozygosity (Oldenbroek et al., 1999). Thus, low differentiated distinct populations, such as Greek Brachyceros, Minor Asia and some Buša breeds are important sources of variation because a large within-breed diversity is needed for better adaptation to unpredictable future needs (Simianer et al. 2003). In addition, future breeding strategies may rely on the improvement or introgression of particular traits through heterosis from highly differentiated distinct populations (e.g., island populations). In any case, the conservation programs are a compromise between what is feasible and what is desirable.

For the need to preserve Greek cattle populations, some simple guidelines could be suggested, considering their demographic history, their small actual population size as well as their level of inbreeding.

A. Regarding the mainland Brachyceros-type populations, the following conservation measures could be suggested.

1. Greek Brachyceros animals with a high inbreeding coefficient should be mated with unrelated (UAR-assisted choice) animals in different farms or subpopulations within Brachyceros breed or transferred into Prespa or Rhodope to serve as recipients or donors of gametes.

2. The Rhodope cattle as a highly inbred breed could receive sporadic gamete migrations from either Prespa or Greek Brachyceros breeds. The corresponding procedure could also be applied to the Prespa cattle.

3. Greek Brachyceros animals with a significant affinity to one or more animals outside of the Greek breed group are considered as admixed (outliers) and should not serve as donors of gametes in conservation program of Greek Brachyceros breed.

4. Prespa or Rodhope animals with a significant affinity to one or more animals outside of the Greek breed group which are considered as admixed (outliers) could remain in the Prespa or Rhodope populations and be crossed with purebred animals.

5. Prespa or Rhodope animals that are characterized by a high relationship with individuals or breeds within the Greek breed group and are considered admixed (outliers) could remain within the conservation programs of these breeds.

6. Greek Brachyceros animals that are characterized by high relatedness to individuals or breeds within the Greek breed group and are considered admixed (outliers) can be used as gamete recipients in the breed with which they show high genetic relatedness.

B. <u>In the podolian type breeds (Katerini and Sykia)</u>, due to their different phenotypic characteristics as well as their origin, an animal exchange program between farmers should be implemented within each breed to reduce inbreeding and not between them.

C. <u>Regarding the reconstitution and preservation of the Kea breed</u>:

1. Kea animals that are highly admixed with animals or breeds within Greek, Minor Asia, and Southeast geographic breed groups, such as the Peloponnese subpopulation, could be included in a KEA's breed conservation program and be mated with purebreds.

2. Kea animals that are highly admixed with animals or breeds of the Alpine group, such as island subpopulation cases, can remain separately and be mated with purebreds.

3. Kea animals that are highly admixed with animals of high-producing breeds outside the Alpine group, such as Holstein, must be excluded from reproduction.

D. <u>As far as island populations are concerned</u>, due to the high proportion of large continuous segments of homozygosity (ROHs) in island animals, the reduction of inbreeding could be done with mating with animals from mainland Greece (Greek Brachyceros animals) that are show a relatively high average relationship to their own breed. The case of merging of island populations through the transport of animals from one island to another can still be investigated.

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