Udder shape, milkability and genetic diversity of Istrian sheep

Šalamon, Dragica

Sveučilište u Zagrebu

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ABSTRACT

External udder shape, milkability, milk production and genetic variability were investigated in Istrian sheep, to evaluate the long-term perspective of the breed in milk production and the aptitude of Istrian sheep for machine milking. Heritabilities were estimated using single trait animal models. Generally, the heritabilities for daily milk yield, somatic cell score, fat, protein and lactose content were low. The udder shape heritabilities were 0.17, 0.15, 0.63, 0.50 for full udder height, maximum udder width, cisternal part below the teat orifice, and teat angle, respectively. The udder shape traits were influenced by number and stage of the lactation, and were more favorable in herds with applied machine milking. The milk flow traits' means were influenced by the stage of lactation. According to the estimated genetic parameters for udder shape traits, the cistern size is the most suitable target trait for selection that would benefit the proper machine milking. Based on the analysis of microsatellite markers, Istrian sheep is one of the three analysed breeds with the lowest observed heterozygosities (0.684), and with an inbreeding coefficient of intermediate value (0.061). When compared to neighbouring sheep breeds, it is one of the three most distinctive breeds with a large numbers of private alleles and relatively small level of introgression. In comparison with the Istrian sheep population from Slovenia, introgression is lower, inbreeding coefficient is smaller and diversity higher in Istrian sheep from Croatia. In summary, the results show that the external udder shape of the Istrian sheep is adequate for machine milking and that the breed has high variability in comparison to other sheep breeds.

Key words: genetic diversity; genetic parameters; genetic variability; milk content; sheep milkability

SAŽETAK

Kako bi ocijenili dugoročnu perspektivu istarske ovce u proizvodnji mlijeka i njenu podobnost za strojnu mužnju, istražena je muznost, vanjski oblik vimena, količina i sastav mlijeka te genetska varijabilnost ove pasmine.

Genetski parametri za dnevnu količinu mlijeka (MY), postotak masti (FC), proteina (PC) i laktoze (LC) te somatske stanice u mlijeku (SCS) procijenjeni su iz 23.396 kontrola mliječnosti, prikupljenih za 3172 ovce u razdoblju od 2005. do 2012. godine, regresijskim modelima koristeći REML algoritam. Genetski parameteri i uzgojne vrijednosti za vanjski oblik vimena izračunati su za 750 ovaca na 6 farmi na kojima se primjenjuje ručna i 5 farmi na kojima se primjenjuje strojna mužnja. Izmjere pune visine (Fh) i maksimalne širine vimena (Mw), cisternalnog dijela vimena ispod otvora sise (Cis) i kuta kojeg sisa zatvara s vertikalnom osi vimena (Alpha) prikupljene su digitalnim izmjerama fotografija posteriorne perspektive vimena u početku, sredini i krajem laktacije 2010. godine. Na farmama koje primjenjuju strojnu mužnju izmjerene su muzne karakteristike ovaca. Testirana je korelacija BLUP (najbolja linearna nepristrana procjena) vrijednosti Fh, Mw, Cis, Alpha, trajanja mužnje (Mt), količine strojno pomuzenog mlijeka (My), prosječne (Avgm) i maksimalne (Mmf) brzine protoka mlijeka. Varijabilnost i struktura istarske ovce procijenjene su kvantitativnim i molekularnim pristupom. Molekularna raznolikost, distinktivnost pasmine, te razina introgresije određeni su pomoću 27 mikrosatelitskih biljega u usporedbi s jedanaest pasmina pramenki iz Hrvatske i Bosne i Hercegovine. Dodatno je uspoređena populacija istarske ovce u Hrvatskoj s istarskom ovcom iz Slovenije. Kvantitativni pristup varijabilnosti istarske ovce uključuje procjenu plastične varijacije i plastičnosti temeljem modela razvijenih za procjenu genetskih parametara mliječnosti.

Procijenjeni heritabiliteti za MY, SCS, PC, FC i LC bili su niski. Heritabiliteti za oblik vimena iznosili su 0,17, 0,15, 0,63, 0,50 za Fh, Mw, Cis i Alpha, redom. Broj i stadij laktacije te farma, utjecali su na izmjere vimena. Prosjeci muznih karakteristika mijenjali su se tijekom laktacije. Značajne korelacije između BLUP vrijednosti oblika vimena i muznih karakteristika bile su visoke i pozitivne za Cis i Alpha. Razlike oblika vimena i uzgojnih vrijednosti za oblik vimena između farmi koje primjenjuju strojnu i onih koje primjenjuju ručnu mužnju bile su značajne. U usporedbi raznolikosti s

pasminama pramenki iz Hrvatske i Bosne i Hercegovine, istarska je ovca svrstana među tri pasmine s najnižim opaženim heterozigotnostima (0,684), srednjim koeficijentima inbridinga (0,061). U usporedbi s jedanaest pramenki uvrštena je među tri pasmine s velikim brojem privatnih alela i relativno malom razinom introgresije. U odnosu na populaciju istarske ovce u Sloveniji, istarska ovca u Hrvatskoj ima daleko manju introgresiju, povoljniji koeficijent inbridinga i veću raznolikost. Dakle, može se reći da je genetska varijabilnost proizvodnih i funkcionalnih svojstava istarske ovce u Hrvatskoj očuvana.

U usporedbi vanjskog oblika vimena i genetskih parametara za količinu i sastav mlijeka, istarska ovca nalikuje istočnoeuropskim i mediteranskim mliječnim autohtonim pasminama koje imaju relativno visok prinos mijeka, ali nisu visoko selektirane, te ima bolji oblik vimena za strojnu mužnju.

Ključne riječi: genetska raznolikost; genetski parametri; genetska varijabilnost; kemijski sastav mlijeka; muznost ovaca

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ABBREVIATIONS AND SYMBOLS

A - The additive genetic relationship matrix

Alpha – Angle that teat closes with the vertical axis of the udder

Alpha-l – Angle that teat closes with the vertical axis of the udder on the left udder half

Alpha-r - Angle that teat closes with the vertical axis of the udder on the right udder half

AMOVA - Analysis of molecular variance

Avgm - Average milk flow

BLUP – Best linear unbiased prediction

BPRC - Breeding plans for sheep in the Republic of Croatia (Mioč *et al.*, 2011)

CAA – Croatian Agriculture Agency

Cis – Height of the cisternal part below the teat orifice

Cl - Height of the cisternal part below the teat orifice of the left udder half

Cr - Height of the cisternal part below the teat orifice of the right udder half

CRE – Cres Island sheep

DAL – Dalmatian pramenka sheep

EU – European Union

FAO – Food and Agriculture Organisation

FC – Fat content (%)

Fh – Full udder height

F_{st} - Pair-wise genetic distances

F_{is} - Coefficients of inbreeding

h^2 – Heritability

He - Expected heterozygosity

Ho - Observed heterozygosity

HW - Hardy-Weinberg (equilibrium)

I - identity matrix

ICAR - International Committee for Animal Recording
ISAG – International society for animal genetics
IST - Istrian sheep
ISTc - Istrian sheep population in Croatia
ISTs - Istrian sheep population in Slovenia
IUCN - International Union for Conservation of Nature

K - Number of inferred clusters in structure analysis
KRK – Krk island sheep
KUP - Kupres pramenka sheep

LC – Lactose content (%)
LIK – Lika pramenka sheep

Mmf - Peak flow rate
mtDNA – Mitochondrial DNA
Mt – Machine milking time
Mw – Maximum udder width
My – Machine milking yield
MY – Daily milk yield

p^2 - Plasticity
PAG – Pag Island sheep
PC - Protein content (%)
PCR – Polymerase chain reaction
PIC - Polymorphic information content
PRI - Privor pramenka sheep

r^2 - Repeatability
RAB – Rab Island sheep
REML - Restricted maximum likelihood algorithm
RUD – Dubrovnik Ruda sheep

SCC – Somatic cell count

SCS – Somatic cell score (Ali and Shook, 1980)

STO - Hum/Stolac pramenka sheep

TD – Test Day (record)

V_a – Additive genetic variance component of population for a trait

V_d – Dominance variance component

Vi - Individual variance component

V_{ie} – Interaction or epistatic variance component

V_p - Phenotype variance component

V_{pe} – Permanent environment variance component, plastic variance

VLA- Vlasic/Travnik/Dubska pramenka sheep

WW2 - Second World War

1. INTRODUCTION

Istrian sheep is autochthonous and almost exclusive breed in the sheep dairy production of Istrian County, and essential for the identity and development of the County through high-quality products, primarily the hard artisanal sheep cheese. Profound knowledge about the genetic variability, milk production genetic parameters, as well as details on machine milking and udder traits of such a breed would benefit the future of the breed, but also of the County.

Loss of farm animal genetic diversity was on the rise during the last 50 years, as the spread of a few highly developed breeds started to threaten the existence of well adapted local breeds either by cross-breeding, which is allowed for endangered Istrian sheep according to Breeding plans for sheep in the Republic of Croatia (BPRC, Mioč *et al.*, 2011), or by substitution. For more than 30 percent of the livestock breeds in the world the situation is unknown, and 36% of known sheep breeds are now endangered or extinct. Marginal and transitional areas with harsh environment, often used for low-input sheep farming, are predominantly the ones affected by loss of farm animal genetic diversity. Knowledge of genetic variability of autochthonous Mediterranean sheep breeds is important for the sustainable use and development of native sheep populations. Protection and conservation of the sheep breeds, considered to be national cultural treasure in Croatia, should be easier for the breeds with high socio-cultural merit, especially if it is connected to economic value. Moreover, genetic variability is important for the future of sheep dairy production, as it enables adaptation to environment/market change. Because of the dynamic past of the Istrian sheep breed since the Second World War (WW2), it is important to estimate its genetic variability today.

Dairy sheep have been farmed traditionally in the Mediterranean and Middle Eastern countries, and the current farming systems vary from extensive to intensive depending on the economic relevance of their products, specific environment and breed. The milk is mainly used for cheese production, therefore milk content traits are very important, and increasing milk yield is still the most profitable breeding objective for several breeds. Furthermore, other traits related to more efficient milk production are gaining interest for selection: machine milking ability and udder morphology, resistance to mastitis, and the fatty acid composition reflecting the nutritional value of the milk. Currently implemented breeding programs in

different countries have achieved genetic gains for milk yield and somatic cell count, however implementing further selection goals such as stated above depends on recording cost and organisational effort, and which vary from breed to breed. According to BPRC, udder morphology is economically important in Istrian sheep. Nonetheless, guidelines or goals are not specified so far, nor genetic parameters estimated. Although the implementation of udder scoring techniques was considered, it would require certain amount of organisational effort in technician training for on-field implementation.

Therefore, this thesis was designed in order to help farmers towards time and cost efficient production of Istrian sheep by providing information on milkability and udder morphometry. Moreover, the goal was to provide detailed information required for more efficient breeding programs, as well as to assess the genetic variability of the breed required for adaptation of the animals to all the breeding demands. Additionally, a new method of udder morphometry appraisal which does not require skilled technicians on field and is time effective, was applied in production conditions.

2. LITERATURE OVERVIEW

2.1. Istrian sheep

Istrian sheep (IST) is an autochthonous protected breed (Ćinkulov *et al.*, 2008), with a registered population of 2 515 animals on 38 farms in Croatia, which makes it the second smallest autochthonous sheep population in Croatia (Mulc *et al.*, 2012). It makes 5% of the total number of sheep included in an approved selection program of the Croatian Agricultural Agency (CAA). In comparison, the most prominent autochthonous cheese production breed in Croatia, Pag island sheep (PAG), constitutes about 10% of the sheep registered population. Although it was formed as a multipurpose breed, Istrian sheep in Croatia is predominately used for dairy production, due to its relatively high yield of high quality milk. It was reported to have an average production of 193.82 kg/ewe in 179 days of lactation, including the 58 days of suckling. During the milking period it produced 1.04 kg/day of milk containing 7.15% of fat and 5.88% of proteins (Mulc *et al.*, 2012). Most of ewe milk is processed into hard artisanal cheese and crude on small family cheese dairies, and lesser amounts are sold for industrial cheese production.

The breed is classified as endangered according to FAO, EU and IUCN categorisation and potentially endangered according to the national classification (Barać *et al.*, 2011). Istrian County in Croatia with its recognizable Northern-Adriatic karstic landscape offers a habitat of high ecological value for the rearing of the autochthonous regional Istrian sheep. Since these sheep are reared in extensive and semi-extensive conditions on most of the farms, using predominately natural pasture (Mediterranean to sub-Mediterranean), they are important in prevention of succession of agricultural land due to vegetation overgrowth. Physiology and long-legged phenotype of Istrian sheep show good adaptation to the karstic habitat conditions.

Besides naturally occurring geography and isolation, important aspects of the history of the Istrian sheep breed include diverse political and economic changes, which influenced the borders, management practices, such as horizontal and vertical transhumance, and the controlled and uncontrolled crossbreeding (Böhm, 2004). Today, the initial breed population is fragmented in reproductively isolated sub-populations in Italy (1 000 animals), Slovenia (1 500 animals) and Croatia (2 515 animals).

Dairy sheep production is an important agricultural activity in Istria County, with 2 234 milking ewes on 34 farms, of exclusively Istrian sheep breed, under selection control

(Mulc *et al.*, 2012). This small number of animals is crucial for local production/income, but is clashing with the other socio-economic goals of the region such as tourism. Nevertheless, the hard artisanal sheep cheese is of high quality (Samaržija *et al*., 2003) and its limited production keeps a relative high price on local and tourist market even though the product at present does not yet have a protected denomination of origin. Industrial cheese production is not developed in the region and Istrian sheep cheese is not recognized as an export product.

Several more farms rearing this breed are present in three other Counties (Ličko-senjska, Varaždinska and Primorsko-goranska) showing that this breed is recognised as a valuable autochthonous dairy breed. Average milk production of Istrian sheep in year 2012 was 220.68 kg per lactation, which is the result of the development of applied management techniques through breeders association "Istrijanka". Selection is carried out on recorded milk yield and lactation yield estimates standardised to 180 days of lactation. Estimated breeding values for milk yield and protein and fat content are published yearly for ewes in Annual reports of the CAA. BPRC also publishes ram breading values where BLUP for protein content is valued twice as much as fat BLUP. The dissemination of the best animals is achieved through yearling sales between the breeders. Since there is no artificial insemination applied, herd connectivity can be assumed to be low. The production is extensive or semi-extensive, with most of the farms traditionally counting about 40 animals. Average herd size is 55 animals (Mulc *et al.*, 2012), only few of them have more than 200 animals. Herd size limitation is due to milking effort, and farms that apply hand milking tend to be smaller. Also, most of the breeders keep the Istrian dairy sheep only as an additional household income source.

In semi extensive systems where grazing represents an important portion of feeding, the increasing trend of milk production is lower and irregular because of annual variations in herbage availability to which this systems are very sensitive (Barillet *et al.*, 2001).

2.2. Istrian sheep milk production

Istrian sheep shows good potential for milk production. It has higher milk yield and longer lactation than Pag sheep, which is the most prominent autochthonous sheep in dairy production in Croatia (Mulc *et al.*, 2012). Comparisons of Istrian sheep in Slovenia with Slovenian autochthonous dairy sheep show that the potential of Istrian sheep should not be neglected because of their favourable protein and fat content (Komprej *et al.*, 2009)

throughout lactation and better persistence of lactation, but lower daily milk yields (Komprej *et al.*, 2003).

Genetic and environmental effects on milk quantity and production were investigated only recently for Istrian sheep in Croatia. General linear models showed that litter size influences daily milk yield and fat percentage, number of lactation showed significant influence on daily milk yield and protein percentage, and season of lambing showed significant influence on all three investigated parameters (Vrdoljak *et al.*, 2012). Heritabilities were reported for daily milk (0.15), fat (0.07) and protein (0.013) yields as well as fat (0.07) and protein (0.015) percentages using single-trait repeatability fixed regression models (Špehar *et al.*, 2012). Test day (TD) record collecting under the International Rules for Milk Sheep Recording (ICAR, 2003) is used in Istrian sheep, with records collected monthly under an alternate morning/evening system. Lactation is standardised to 180 days and the best producing ewes are announced in yearly public reports. Traditional approach of using lactation records is criticized because it does not balance out non-genetic effects on milk production, the goodness of the standardisation depends on the quality of milk recording with regard to temporal aspects. As TD measurements are frequently collected at highly variable time periods due to animal management, consequent inconsistencies are implied because standardisation of yields depends on the lactation stage at which samples were collected from each individual animal. Furthermore, substantial percentages of extensively collected and processed samples data are not usable due to lack of the minimal number of TD records for standardisation. Models using test day records attempt to account for systematic, environmental and genetic effects directly where they are expressed: on the day of recording. In this manner, removal of abnormal measures is enabled and more information can be used to assess the production of investigated trait. Numerous studies have dealt with the use of TD records as an alternative to standardised lactation yields in cows, goats and in most of the important dairy sheep breeds (Serrano *et al.*, 2001).

2.3. Machine milking and milkability

Unlike other countries, where machine milking of ewes started during 1960s (Kulinová *et al.*, 2010) and the physiological reactions of sheep breeds to machine milking were investigated from the 1970s (Tančin *et al.*, 2009), Croatia started to develop interest on machine milking only recently (Dzidic *et al.*, 2004). Machine milking in Istrian sheep is

present to some extent, unlike in other breeds of autochthonous dairy sheep in Croatia, which are milked almost exclusively by hand. In the last decades the number of sheep farms with machine milking is increasing, therefore it is important to know if the autochthonous Istrian sheep is suitable for machine milking. Benefits of machine milking of ewes are maximal milk yield of better hygienic properties than properties of hand-milked milk, and easier stripping (Dzidic, 2013).

Milkability can be evaluated by analysis of the milk flow curves and milk flow parameters that describe the physiological response of ewe to machine milking (Mayer *et al.*, 1989; Bruckmaier *et al.*, 1997), and by analysis of udder morphometry (Labuissier 1988; Fernandez *et al.*, 1995; Rovai *et al.*, 1999). Milk flow kinetics is related to milk production (Rovai *et al.*, 2002) especially in breeds that are not selected for high milk yields (Mačuhová *et al.*, 2008) because of importance of the milk ejection reflex for complete milk removal (Tančin and Bruckmaier, 2001).

Effective milkability depends on udder morphology (Labussiere, 1988) and is important for sustainable milk production because it affects functional life span of the animals (Casu *et al.*, 2006). Research shows that machine milking, when applied to the udder with appropriate morphology has positive effects on udder health and milk quality, namely reduction of subclinical and clinical mastitis in the animals (Fernandez *et al.*, 1997; Marie-Etancelin *et al.*, 2001; Bergonier *et al.*, 2003; Legarra and Ugarte 2005).

2.4. Udder morphology and milkability

The sheep mammary gland is an exocrine epithelial gland constituted of the tubulo-alveolar parenchyma with alveoli and well differentiated cisterns, and the stroma. While the parenchyma is a secretory part of the gland, the stroma is formed by other complementary tissues such as blood and lymph vessels and adipose, connective and nervous tissues. Milk secretion is described as transformation of the lactocyte into the product (milk), which develop during pregnancy and early lactation under neuro-endocrine and autocrine system control (Caja *et al.*, 2000).

Milk is stored in two anatomical compartments: alveolar (alveolar milk fraction) and cisternal (cisternal milk fraction). The cisternal fraction is the milk already transferred from alveoli to cistern, and is immediately obtainable for the milking. In dairy sheep it can represent more than 40% of total milk yield after 12-hour interval. Animals with larger cisterns are considered to be more efficient milk producers. Alveolar milk fraction is milk

stored in the alveoli. It is released gradually to cisterns during the interval between the milking. After the cisterns have been filled, milk remaining in the alveoli is the fraction that can only be obtained when milk ejection reflex (elimination of the alveolar milk due to oxytocin) occurs before or during the milking (Marnet and McCusic, 2001).

Relationship between udder shape characteristics and milking performance in dairy ewes was investigated since the 1970s (Sagi and Morag, 1974; Gootwine *et al.*, 1980) as an effort to adapt the ewe to machine milking. Labussière (1988) proposed that the use of morphology selection criteria benefit the milking ability of ewes. He declared the need of vertically implanted teats at the lowest point of the cistern, the need to introduce improved udder traits in the selection objectives of ovine breeding schemes, which was addressed in selection programs in the Mediterranean countries only a decade later (De La Fuente *et al.*, 1996; Casu *et al.*, 2006; Marie-Etancelin *et al.*, 2006). The reason for the increased interest on dairy sheep udder in the recent decade and new breeding schemes was "baggy udder", found in sheep selected for high milk yield. In those sheep, the cisternal part of the udder below the teat orifice is enlarged, as is the angle between the teat and the vertical axis of the udder (Fernandez *et al.*, 1997; Marie-Etancelin *et al.*, 2005). Milking of these "baggy udders" is not efficient because part of the cisternal milk remains below the teat orifice unless the milker applies manual manipulation of the udder during stripping (Bruckmaier *et al.*, 1997; Bruckmaier and Blum, 1998). Additionally, horizontally implanted teats cannot hold the weight of the milking unit, and it tends to fall off. That kind of additional manipulation during milking prolongs the total milking time of the herd, with milking already being one of the most time-demanding procedures on ewe milk farms. It can also lead to an inadequately milked udder that is undesired for udder health. Depending on the breed, incomplete milk removal during milking can be marked in the total daily yield of the herd. Therefore, the mammary gland morphology is an important factor in determining the aptitude for the machine milking of ewes. Recent findings in non-selected local dairy sheep breeds in Greece report udder morphology that is adequate for the machine milking, and worth conserving through selection plans (Gelasakis *et al.*, 2012).

Including udder traits of selective interest as selection goals is limited due to the recording cost in relation to the cost of the dairy ewe product. Nevertheless, an appraisal method for udder traits based on 9-point linear scales was first proposed for the Churra breed (De La Fuente *et al.*, 1996) and was later adapted for other dairy breeds (Fernandez *et al.*, 1997; Serrano *et al.*, 2002; Legarra and Ugarte 2005; Marie Etancelin *et al.*, 2005; Casu *et al.*, 2006). This evaluation method for teat placement, udder depth, udder cleft and udder

attachment requires high repeatabilities of classifiers, reliability and objectiveness. It also requires considerable organisational/financial effort with skilled appraisal technicians covering the farming area. In France and Italy only primiparous ewes are scored for udder shape. In addition to scoring, udder shape was measured in different countries, such as France, Italy, Spain, Germany, Slovakia and Czech Republic. Results for genetic evaluation based on measurements were for Lacaune and Sarda breeds until the year 2009. In order to lessen the cost of trained technicians, use of digital pictures of the posterior view of the udder for digital measuring of udder shape was proposed (Dzidic *et al.*, 2009).

Udder shape and milkability in the Istrian sheep are currently not well known. However, research on Istrian sheep crossbreeds shows that animals with high percentage of Istrian sheep genetic background shows udder shape that is suitable for the machine milking (Dzidic *et al.*, 2004; 2009).

2.5. Genetic variability and improvement of the breed

2.5.1. Molecular approach

Genetic variability has not been investigated in Istrian sheep so far. This knowledge is very important for the sustainable long-term production of this ovine breed because variability enables adaptation of the animals to the changes in the environment and in the market demand (Bozzi *et al.*, 2009).

The isolation of the three existing subpopulations of Istrian sheep started with the closing of the state borders during the WW2 when 14 000 individuals in total were recorded (Böhm, 2004). Another reduction of population occurred during the Croatian War of Independence, leaving the estimated population of about 1 000 animals. This kind of events could decrease genetic variability due to drift, population fragmentation, bottleneck effects and inbreeding (Halliburton, 2004). However, unlike in the other autochthonous breeds in Croatia with similar history, in the Croatian population of the Istrian sheep, two rams per 40 ewes are used and are replaced biannually (Mulc *et al.*, 2012). This kind of scheme results in growth of the effective number of the population in comparison to other sheep populations, and it is possible that the negative effects on variability are counteracted. To investigate the variability of the Istrian sheep, we chose to compare molecular genetic variability of Istrian sheep from Croatia with local populations from Croatia and from Bosnia and Herzegovina, as well as with Istrian sheep from Slovenia. The studied populations are of pramenka type,

which is the Bosnian, Croatian and Serbian name descriptive for open fleece of sheep breeds with mixed wool, included in the Zackel/Valachian phyletic sheep group (Draganescu and Grosu, 2010). Eight breeds representing almost completely the sheep production of the Mediterranean part of Croatia are compared in this thesis: Istrian sheep (IST), Krk island sheep (KRK), Rab island sheep (RAB), Cres island sheep (CRE), Lika pramenka sheep (LIK), Pag island sheep (PAG), Dalmatian pramenka (DAL) and Dubrovnik Ruda sheep population (RUD). Additional four multipurpose pramenka breeds included in this study graze more than 50% of the total agricultural areas in low-input highland systems of Bosnia and Herzegovina, and are currently stable at about one million sheep: Kupres pramenka (KUP), Vlasic/Travnik/Dubska pramenka (VLA), Privor pramenka (PRI), and Hum/Stolac pramenka (STO) sheep. The high level of phenotypic diversity within these populations (Böhm, 2004) and the large phenotypic differences among the different breeds studied here (Posavi *et al.*, 2003; Brka *et al.*, 2007) suggest high levels of genetic diversity within populations, as well as high levels of genetic differentiation among them. Differentiation and neutral nuclear diversity studies are scarce and have been reported only in a limited number of these pramenka populations (Bradic *et al.*, 2003; Lawson-Handley *et al.*, 2007; Ćinkulov *et al.*, 2008) using different sets of markers, which makes comparison of results within breeds and with other breeds difficult (Food and Agriculture Organization of the United Nations, 2011). Ancestral origin using mtDNA and Y chromosome data has also been previously investigated in different pramenka breeds (Bradic *et al.*, 2005; Ivanković *et al.*, 2005; Ferencakovic *et al.*, 2013). Assessment of genetic diversity using nuclear data for setting the conservation priorities is a standardised method for estimating the genetic diversity of different ruminant populations in many countries (Baumung *et al.*, 2004; Ligda *et al.*, 2009; Barreta *et al.*, 2012). Microsatellite markers are widely used in the study of genetic variability and population structure due to their high level of polymorphism, high mutation rates and wide presence in the eukaryote genome (Ligda *et al.*, 2009). A great number of European sheep breeds were assessed using the FAO recommended markers (FAO 2004, 2007; Ligda *et al.*, 2009; Tapio *et al.*, 2010). They enable reliable genetic evaluation of structure, differentiation and admixture (Tapio *et al.,* 2010). Quantified estimations using microsatellite markers can point out conservation priority populations, breeds and herds as well as help with formulation and implementation of the breeding, conservation and management policies (Arora *et al.,* 2011). A small number of published articles deal with genetic structure and variability of the Istrian sheep (Ivanković *et al.,* 2005; Lawson Handley *et al.*, 2007; Ćinkulov *et al.*, 2008).

2.5.2. Quantitative approach

Additionally, variability of the Istrian sheep in Croatia using a quantitative approach is explored. This approach enables understanding how genes influence phenotype, fitness and population dynamics, when the relatedness between individuals within a population is known. The genome and the environment are two interacting factors that act on the development of an animal and phenotype expression (Scheiner, 1993). Genetic variation for a fixed phenotype has been hypothesized to be favoured in stabile environments. Besides genetic variation for a selected phenotype (V_a) and phenotypic plasticity, an environmentally induced phenotypic change that occurs within an organism's lifetime is also likely to play an important role in the process of diversification (West-Eberhad, 1989). Recent evidence suggests that most of the environmentally induced phenotypic variation exhibited by organisms is selectively advantageous in wildlife species. Thus, phenotypic plasticity has recently come to be considered as a trait that can be subjected to selection (Scheiner, 1993). Such plasticity can often be an important adaptive strategy for coping with the changes of the environment (Scheiner, 1993).

A form of mixed model known as the animal model is used in many studies to decompose phenotypic variance into different genetic and environmental sources of variation and to estimate key parameters such as the heritability of the trait or the genetic correlations between traits in natural, laboratory and domestic populations (Wilson *et al.*, 2009). With repeated measures on individuals it is possible to partition the phenotypic variance into within and between individual components by fitting individual identity as a random effect with and without associating it to pedigree. The among-individual variance expressed as a proportion of the trait is repeatability (r) and it is in the extreme case the upper limit for heritability (h^2) (Falconer and Mackay, 1996).

Scheiner and Goodnight (1983), showed that the quantitative definition for plastic variation (Bradshaw, 1965) is the deviation of the mean phenotype of the genotype within an environment from the mean phenotype of that genotype across all environments. For a population plastic variation is then the environmental variance component including the variance component explaining the interaction of the genotype and the environment. By analogy with heritability Scheiner and Goodnight (1983) defined plasticity as the ratio of plastic variance to total phenotypic variance.

An animal model (Lynch and Walsh, 1998; Kruuk, 2004) is a statistical model used to estimate genetic contributions to trait variation using population pedigrees, and has been

applied successfully for several decades. Since the late 1990s, an increasing number of studies have applied animal models in wild populations because it simultaneously describes the resemblance among all individuals in a given data set, irrespectively of their level of relatedness, i.e. is not restricted to one level of relatedness (e.g. parent-offspring). It is thus optimal for use of the often complex and patchy pedigrees, and flexible enough to cope with variable amounts of missing data. Although, obviously, missing data will reduce the precision of estimates, and can in some cases cause bias. An animal model uses the information on the resemblance among individuals of known relatedness to estimate how genes influence phenotypes.

For a single trait we can estimate the amount of phenotypic variance (V_p) that is due to genetic differences among individuals (Falconer & Mackay 1996). Genotypic differences among individuals are composed of additive (V_a), dominance (V_d) and interaction or epistatic (V_{ie}) genetic sources of variance. However, V_d and V_{ie} are extremely difficult to estimate in non-experimental settings, and both animal breeders and field ecologists have tended to focus on measuring additive genetic variance by estimating the phenotypic similarity of relatives (Falconer & Mackay 1996; Kruuk 2004). In the simplest case, this involves statistically partitioning the phenotypic variance into two parts such that it includes additive variance component and the residual variance (V_r). V_r is normally interpreted as arising from environmental effects which entails the assumption that dominance and epistasis make negligible contributions to V_p. The narrow-sense heritability of a trait (h^2) is then defined as the proportion of phenotypic variance explained by additive genetic variance, and describes the degree of resemblance between relatives. Permanent environment variance component (V_{pe}) will include nonaditive genetic effects such as dominance variance, common environment (environment shared by members of family that affect individuals permanently, such as nest effect), maternal environment or maternal genetic variance.

Several different approaches are possible for genetic analyses using the animal model. Repeatability model is a method of choice in routine genetic evaluation due to its simplicity. It is based on the assumption that genetic correlations between all measurements are equal to one. Therefore, the examined trait in consequent measurements (e.g. milk yield on different days of lactation) is assumed to have a constant variance and a common correlation with each other. This assumption is not a valid in cases where individual variance changes according to the amount of time that has passed between measurements (e.g. growth or lactation curves). Multiple-trait approach and random regression models are more complex alternatives with their own advantages. Accuracy increase of the evaluations, especially for traits with low

heritability is the main reason for choosing the multiple-trait approach. However, computational complexity and increased number of parameters can sometimes cause problems with estimation. Namely, multiple-trait models are based on the assumption that the correlations between trait in successive measurements are lower than one. Thus, observations measured on individuals across time are treated as separate and unique traits that are genetically correlated to one another. Random regression models are used in the analysis of longitudinal data (i.e. function valued, or infinite-dimensional) with repeated measurements in different points during animal's life. Therefore, the model has fewer parameters necessary for the description of the data. Furthermore, (co)variance estimates are smoother along the trajectory, and it is possible to estimate them at any point along the trajectory.

Genetic correlations have not so far been provided for the sheep breeds in dairy production in Croatia, and estimates of heritability have only been published recently for the Istrian sheep daily milk yield, protein and fat contents and yields (Špehar *et al.*, 2012). During the recent two decades the practice in other European and Mediterranean sheep breeds shows the annual genetic gain of 0.8-2% of the average milk yield. Their experiences show the importance of taking into account the possibility of reduction of fat and protein content due to the genetic milk yield upgrade, as well as the negative effect on udder conformation with increasing milk production. Namely, lower fat and protein content negatively affect cheese-making value of milk, and undesirable udder conformation is unsuitable for machine milking.

The lactation approach is commonly used for the genetic evaluation of the milk yield in dairy ewes although the test-day approach may theoretically be of use (Carta *et al.*, 1995; El-Saied *et al.*, 1998; Serrano *et al*., 2001; Gutierrez *et al.*, 2007). In most of the breeds a repeatability BLUP-animal model with fixed environmental effects and random additive genetic and permanent environment effects is used (Astruc *et al.*, 1995).

3. AIM OF RESEARCH AND HYPOTHESES

The aim of the research was to investigate the potential of Istrian sheep milk production and possibilities of production intensification that would enable more of the required quality product through increased time effectiveness of production. Additional focus of interest are the possibilities of genetic improvement, taking into account the potential fragility of the population due to its limited size.

3.1. Hypotheses

1. External udder morphology of the Istrian sheep is adequate for machine milking because there was no significant selection for milk yield.

2. Based on the specific male to female ratio in the Istrian sheep population, and although there have been changes in the population size during the last decades, the Istrian sheep population appears to have genetic higher variability than neighboring sheep populations. Additionally, conserved genetic variability of milk yield and content is expected.

OBJECTIVES:

- to evaluate morphometry of the udder in the Istrian sheep in Croatia, the following list of traits will be measured from digital photographs of the posterior view of the udder: full udder height (Fh), maximal udder width (Mw), cisternal part below the teat orifice (Cis) and the angle the teat closes to the vertical line of the udder (Alpha)
- comparison with other dairy sheep breeds will be made, and variability of these traits on farms that apply machine milking and the farms with hand milking will be evaluated
- genetic parameters and breeding values for external udder shape will be evaluated in order to check possibility of selection/conservation of udder traits appropriate for machine milking

- to evaluate milk flow kinetics (milking time, average milk flow, peak flow rate and milking yield) in the Istrian sheep on the farms that apply machine milking
- to find correlation between BLUP values for milk flow kinetics and udder shape traits
- to estimate genetic parameters, variability and breeding values for milk yield and milk content (protein, fat and lactose percentage and somatic cell score) using the animal model
- to assess the current status of the genetic diversity and differentiation of the Istrian sheep population in comparison with similar sheep breeds of pramenka type, using microsatellite markers
- to compare genetic variability of the Istrian sheep population in Croatia with variability of the Istrian sheep population in Slovenia using microsatellite markers

4. MATERIAL, METHODS AND RESEARCH PLAN

4.1. DATA AND SAMPLING

4.1.1. Animals and the pedigree

Istrian sheep are reared in a large variability of farming conditions. They are mostly housed in closed barns during the winter or cold nights and hot afternoons, and in open pens from the beginning of the vegetation season (usually March), when they are offered natural pasture, until October/November. Their milk production of is seasonal due to seasonal fertility of the breed. Lambing is most often carried out during the second half of December, but some of the farmers prefer the beginning of vegetation season, in order to target the lamb production to period when there is traditionally a high requirement for lamb meat (Christmas and Easter). Records of lambing are ranging from late September to early May. Suckling period lasts for 30-60 days, rarely more, depending on the purpose of the lamb. Longer period of suckling is preferred by farmers for the animals that will be used for stock replacement. After weaning, ewes are milked twice a day by hand or by machine. The milking period lasts mostly until August, depending on the water availability during summer, when usually the larger farms first decrease milking to once a day before the end of the milking period. Depending on the farm, usually on the farms equipped with milking parlours, ewes are offered supplementary feed (barley, oat, and corn, sometimes with soy or sunflower concentrate) during milking.

Pedigree of Istrian sheep is recorded by Croatian Agricultural Agency. There were 24 219 records obtained for the period 1989 – 2012. After exclusion of the 9% of non-logical entries, 22 042 identities remained spanning over 9 generations. In the genetic models, all available relationships were used from the pedigree. More details on pedigree data are reported in Table 1.

Table 1. Pedigree characteristics of Istrian sheep from Croatia.

Pedigree records	22 042	Generations	9
Sires	353	Sires of Sire	148
		Dams of Sire	265
Dams	6 597	Sires of Dam	265
		Dams of Dam	2 841

4.1.2. Udder shape and milkability

Milk flow kinetics during machine milking of Istrian dairy sheep was measured in five commercial herds using Lactocorder© (WMB; Switzerland) in early (first 3 months), mid- (months 4 and 5) and late lactation (months 6 to 8) during year 2010. The animals were milked twice a day. Milk production lasted 8 hours during the day and 16 hours through the night. Milking units were used at a milking vacuum of 37 kPa, pulsation rate 120 cycles/min and pulsation ratio 50:50. The milk was collected in buckets. Teat cups were attached to the udder without previous touching of the udder. Milking routine was finalized with machine stripping: manual udder massage and lifting of the lowest part of the udder in order to position the teats as low as possible when the milk flow dropped below 100 g/min with teat cups still attached.

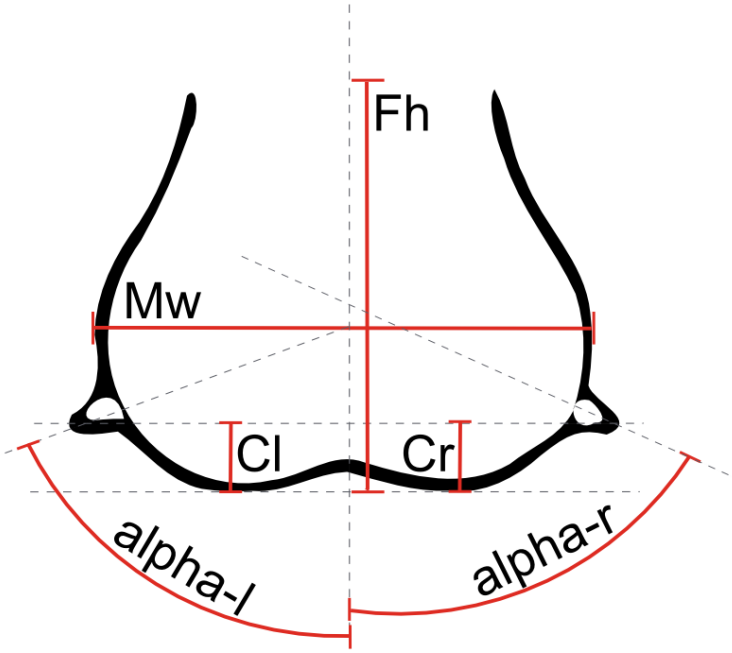
There were 611 records of milking time, milk yield, peak, and average flow rate obtained for 359 Istrian sheep using Lactocorder© (WMB; Switzerland) specially calibrated for milking of the ewes (Dzidic *et al.,* 2004). After removal of non-logical values, animals without ID information and animals with less than two records, 7.4% of data were eliminated, leaving 566 records of 336 sheep (148 morning and 418 evening) ranging from eight to 188 days in lactation. Lactation numbers of the measured ewes ranged from one to eight. Because of the small number of data in the higher lactation numbers, and since there were no pronounced differences of means between the higher lactations, ewes in their 5^{th} to 8^{th} lactation were grouped together. More details on milk flow kinetics data is reported in Table 2.

Table 2. Milk flow kinetics and udder morphometric data description.

	Animals	Records	Mean lactation number	Mean	SE
Milk yield (kg)	142	313	2.71	0.43	0.013
Average flow (kg/min)	97	214	2.53	0.55	0.014
Peak flow rate (kg/min)	120	265	2.63	0.66	0.014
Milking time (min)	141	311	2.71	1.20	0.038
Full height (cm)	258	621	3.05	13.46	0.095
Maximum width (cm)	250	596	2.98	10.69	0.086
Cistern height (cm)	243	1041	2.97	1.36	0.025
Teat angle (°)	243	1027	2.95	38.21	0.459

Digital photographs of 750 ewe's posterior view of the udders were taken prior to evening milking on 11 commercial farms in Istria three times during lactation. Early lactation measurement was performed during first 3 months of lactation, mid-lactation measurement during months four and five, and late lactation measurement was performed for months six to eight. Six of the farms performed milking by hand and five farms used machine milking. External udder shape was measured from the digital photographs using Image Tool software as shown in Dzidic *et al.* (2009). Figure 1 shows the measurements that were taken from the

photographs: full udder height (Fh); maximum udder width (Mw); part of the left (Cl) and right (Cr) udder cistern that is below the teat orifice; and the left (Alpha-l) and right (Alpha-r) teat angle, as the angle declines from the vertical axis of the udder (inter-mammary groove). Total of 1 397 records were edited by removing non-logical values, animals without ID information or information on the beginning of the lactation, as well as animals with less than two records. More details on udder shape data is reported in Table 2.



Full udder height (Fh); maximum udder width (Mw); part of the left (Cl) and right (Cr) udder cistern that is below the teat orifice; and the left (alpha-l) and right (alpha-r).

Figure 1. Udder shape measurements that were taken from the photographs of Istrian sheep.

4.1.3. Milk yield and content

Official test day (TD) records were used, gathered over the period 2005-2012 by Croatian Agricultural Agency for sheep breeding programme. Data for daily milk yield (MY), protein (PC), fat (FC) and lactose percentage (LC), and somatic cells count (SCC) were obtained from milk recording using ICAR regulations (ICAR, 2005) on the total Istrian sheep population in Croatia. Milk content was measured using standard infrared spectrophotometry

(HRN ISO 9622:2001). Method AT was used predominately, and the B4 method was used to obtain 17.4% of the initial TD records before data editing, and was applied only on limited number of farms since 2010. The milking period ended for each ewe when TD milk yield was less than 200 mL. Before editing there were 24 306 TD records with information on the date of beginning of lactation. There were 4 228 AT records (17.4% of the initial data before filtering), and 2 370 B4 records (9.7% of the initial data before filtering) obtained during evening milking. Data set for each variate was edited separately.

Only morning records were used in milk yield analysis, and the initial values were transformed to kilograms of daily milk yield using the factor 2 as an approximation for daily yield, and 1.036 kg/l as density factor. As somatic cell count (SCC) has a highly skewed distribution it was transformed to somatic score (SCS) in a classical way (Ali and Shook, 1980) using the formula:

$$SCS = \log_2(SCC/100000) + 3$$

Data were edited, and finally about 5% of the milk quality data and 30% of the milk yield data was removed from the set. Records were removed from analysis: 1) if first record occurred after 150 days post-partum; and 2) if ewes had less than 2 TD records. Milk yield data was additionally removed if the record was taken before 30 days post-partum or after 240 days post-partum. Final number of records and animals as well as raw means are presented in Table 3.

The first TD measurements were performed mostly from 16 days after lambing and the last were recorded for day 255. Abundance of milk yield and content data through lactation over 15 day intervals, starting with the first record day, are presented in Figure 2. The number of TD measurements over days in lactation decreased with the growth of lactation number. Average number of lactations in TD records for all of the traits after individual filtering was 3.3. Flocks with less than 10 ewes in the records were omitted. The number of flocks was 50 for milk content and 46 for milk yield. For all analysed traits, five levels of lactation number (first, second, third, fourth, and fifth-14^{th}), two levels of litter size (single and multiple birth), and three levels of month of lambing (January-March, April-June, October-December) were included in the models. As only small number of lambings occurred in July and September, these subclasses were merged with the previous or subsequent class of month of lambing.

Table 3. Raw means and basic statistics of data used in animal models.

	MY (kg)	PC (%)	FC (%)	LC (%)	SCS
Mean	1.03	5.95	7.21	4.37	4.43
Range	0.25 - 4.04	4.01 - 7.99	2.50 - 11.95	3.40 - 5.46	0.06 - 10.99
DoL range	32 - 239	16 - 250	16 - 250	16 - 250	16 - 239
DoL mean	124.4	120.5	120.9	120.2	120.1
Lactation number mean	3.27	3.26	3.26	3.25	3.26
Number of animals	3138	3172	3172	3165	3171
Number of records	16783	23396	23150	22561	23234

DoL – Day of lactation, MY – Daily milk yield, PC – Protein content, FC – Fat content, LC – Lactose content, SCS – Somatic cell score.

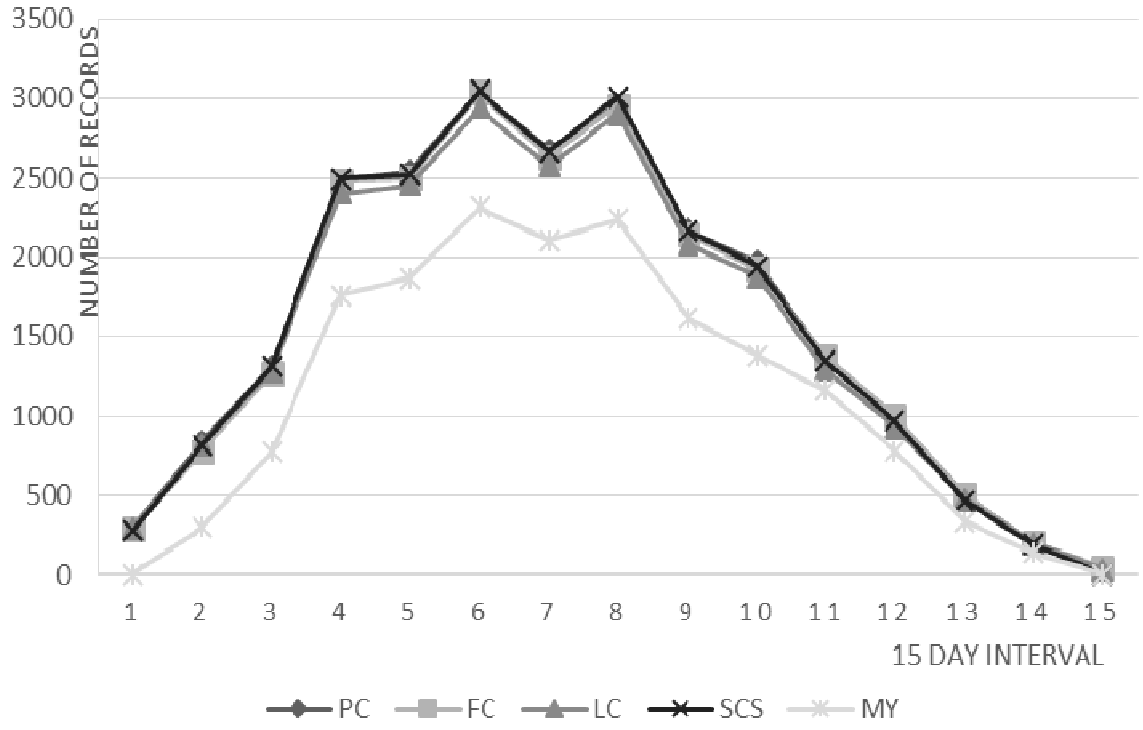
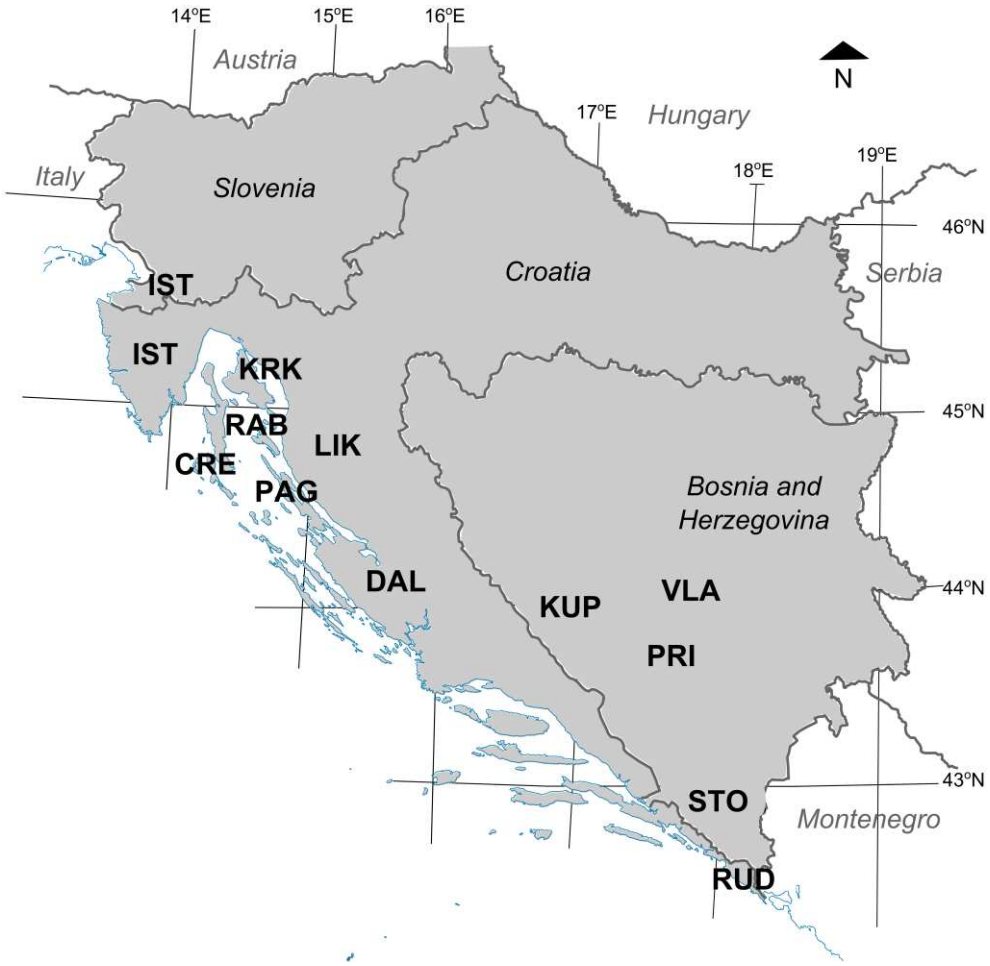


Figure 2. Structure of milk yield and content data in respect to the number of records through lactation over 15 day intervals.

4.1.4. DNA sampling and genotyping

A total of 341 blood samples from the jugular vein of sheep were collected for the 12 breeds under study (Figure 3), with 20 to 33 unrelated animals sampled from each population (Table 4). Additionally, one sample group was obtained from a reproductively isolated population of Istrian sheep in Slovenia.

Blood Genomic DNA Kit was used to extract DNA from the whole blood samples (GenEltue^{TM}, Sigma). For the initial selection of 30 markers, four different PCR-multiplex reactions were optimised using fluorescent-labelled primers and hot-start polymerase (JumpStart™ REDTaq® ReadyMix™, Sigma). Twenty of the markers had been selected from the sheep diversity list recommended by FAO (2011). The remaining 10 markers had previously shown good features for multiplexing. Diluted PCR products were processed in a 16-capillary electrophoresis ABI3130XL Genetic Analyser, with two of the PCR-multiplex reactions being combined into a single multi-loading mix (Table 5).



IST – Istrian sheep, KRK – Krk island sheep, RAB – Rab island sheep, CRE – Cres island sheep, LIK – Lika pramenka, PAG – Pag Island sheep, DAL – Dalmatian pramenka sheep, KUP – Kupres pramenka, VLA – Vlasic/Travnik/Dub pramenka, PRI – Privor pramenka, STO – Hum/Stolac pramenka, RUD – Dubrovnik Ruda.

Figure 3. Geographical locations of the eight autochthonous breeds of Croatia, and the four local populations of Bosnia and Herzegovina sampled in this study.

Table 4. Summarized description of the eight autochthonous breeds of Croatia and the four local populations of Bosnia and Herzegovina sampled in this study.

Population	Sample (n/flocks)	Sample location	Population size	Purpose	Phenotype
Istrian sheep	Slovenia 20M/4 Istria 49/18 (20F, 29M)	Coastal-Karst region-Slovenia; Istrian peninsula-Croatia[a]	≈ 4.600 [g]	milk, meat	70 kg (ram 100 kg), black legs, abdomen; spotty or black head; black, grey, brown fleece, (white preferred in Slovenia)
Krk island sheep	23/2 (23F)	Krk island[a]	< 19.000 [e]	meat, milk	33-38 kg (ram 50–55 kg), white, some black, grey or brown
Rab island sheep	25/1 (24F, 1M)	Rab island[a]	< 7.000[g]	meat, milk	35-40 kg (ram 55-60 kg), white, or with grey/black spots on legs and head
Cres island sheep	25/1 (23F, 2M)	Cres island[a]	< 16.000[e]	meat, milk	Small, white spotted with black
Lika pramenka	25/1 (21F, 4M)	Otočac[b]	< 38.000[e]	meat	44-55 kg (ram 65-75 kg)
Pag island sheep	25/2 (24F, 1M)	Pag island[a]	< 36.000[e]	milk, meat	30-40 kg (ram 40-50 kg) white, 2% black
Dalmatian pramenka	25/1 (24F, 1M)	Šibenik area[a]	≈ 209.000[e]	meat, milk	35-40 kg (ram 55–60 kg), white fleece, sometimes black, grey or brown
Dubrovnik Ruda	25/1 (20F, 5M)	Southward of Pelješac peninsula[a]	≈ 700[g]	meat	45 kg (ram 60 kg), fine wool
Kupres pramenka	25/4 (17F, 8M)	Rama, Zvirnjača, Tomislavgrad[b]	not estimated[f]	meat, milk	60 kg, white with black spots on the head, some without the ear-lobes
Vlasic pramenka	24/2 (10F, 14M)	Tomislavgrad[c]	≈ 100.000	milk, meat	70 kg sheep (ram 80 kg), black head
Privor pramenka	25/2 (7F, 18M)	Pridvorci, Uskoplje[c]	not estimated[f]	meat, milk	40 kg (ram 60 kg), black spots over the head
Stolac pramenka	25/3 (14F, 11M)	Nevesinje[d]	< 2.000[g]	meat	23 kg (ram 35kg), white with black spots over the head, finer fleece

For each sampled population, the corresponding geographical locations are indicated together with the number of samples, population size and status (national). The numbers of female (F) and male (M) samples are indicated in parentheses.

[a] Mediterranean; [b] Continental mountainous; [c] Continental; [d] Sub-Mediterranean; [e] Potentially endangered; [f] Highly endangered; [g] Endangered.

Table 5. Markers included in the three loading panels that were designed in this study.

Loading panel	Hybridization temperature	Fluorochrome	Marker	Allele range
1	55°C	6-FAM	*OarFCB128*	95-127
			INRA063	165-217
			HSC[a]	261-297
		VIC	*OarCP49*[a]	60-126
			MAF65	115-139
			INRA132[a]	144-174
			ETH10[a]	197-205
		NED	*SPS115*[a]	230-148
			SPS113[a]	125-155
			MCM527	160-180
			ILSTS005	184-208
			TCRVB6[a]	226-266
			TCRGC4B[a]	266-308
		PET	*MAF209*	103-133
			FCB304	148-188
			CSRD247[a]	207-251
			ILSTS011	268-284
2	56°C	6-FAM	*OarCP34*	107-119
			OarJMP58	124-170
		VIC	*JMP29*	110-158
			BM1824[b]	166-172
		NED	*BM8125*	103-123
			DYMS1	157-201
		PET	*OarVH72*	118-138
3	58°C	6-FAM	*MCM140*	160-190
			OarHH47	115-147
		VIC	*HUJ616*	88-168
		PET	*ILSTS28*[c]	131-177
	60°C	VIC	*MAF214*	170-230
		NED	*SRCRSP9*[c]	161-270

The loading panels included one or two groups of markers amplified in the same reaction by multiplex-PCR. The names of the markers analysed, the fluorochrome labelling and the PCR-hybridization temperature are given in the table together with the allele range observed for each marker in the sheep samples from 12 populations analysed.

[a] markers not included in the ISAG/FAO list of microsatellite markers for analysing sheep diversity; [b] marker with high frequency of null allele and the HWE deviation significant in 4 of 12 populations; [c] markers with 5% and more of genotypes missing, excluded from analysis.

4.2. METHODS AND ANALYSES

4.2.1. Genetic analysis

Descriptive statistics for data and development of the fixed part of the model were obtained using GLM procedure in statistical package SAS 9.3 (SAS, 2011). Genetic parameters and breeding values were estimated using univariate animal models and REML (Restricted Maximum Likelihood) in AS-Reml program release 3 (Gilmour *et al.*, 2009). Fixed environmental factors to be included in the models were additionally explored in AS-Reml program release 3, according to results of building successively univariate analysis of variance.

Stage of lactation was sub-modelled using Wilmink lactation model (Equation 1) (Wilmink, 1987) for all traits in milk yield and content analysis.

Equation (1)

$$Y = \beta_{0+} \beta_1 * x + \beta_2 * e^{kx}$$

Where:

- Y = MY, PC, LC, FC, SCS
- β_0 = coefficient describing peak production
- β_1 = coefficient describing lactation persistence inversely
- x = days in milk
- β_2 = coefficient describing beginning of lactation
- k = -0.05

Additional fixed effects were included in the milk yield and content models: farm, year and month of lambing, litter size, number and stage of lactation, as can be seen in repeated records animal model equation 2 for milk yield and SCS. Since we had repeated measurements within and between lactations for individual animals, random effects included additive genetic variance (V_a) and permanent effect of the animal (V_{pe}).

Equation (2)

$$y_{ijklmn} = \mu + \beta_{1i}DoL_{ijklmn} + \beta_{2i}e^{-0.05*DoL} + L_i + S_j + M_k + Y_l + F_m + Y_l*(M_k + L_i + F_m) + a_n + p_{ni} + e_{ijklmn}$$

Where:

- y_{ijklmn} = individual observation of MY, FC, PC, LC or SCS
- μ = intercept
- β_1 = coefficient describing lactation persistence inversely
- DoL_{ijklmn} = days in milk
- β_2 = coefficient describing beginning of lactation
- L_i = fixed effect of lactation number (i = 1, 2, 3, 4 and 5+)
- S_j = fixed effect of litter size (j = 1 and 2+)
- M_k = fixed effect of the month of lambing (k = 1, 2 and 3)
- Y_l = fixed effect of the year of measurement (l = 1, 2, 3, 4, 5, 6, 7 and 8)
- F_m = fixed effect of the farm (m = 1 to 50 for SCS, and 1 to 46 for MY)
- a_n = the random additive genetic effect of animal with complete relationship included (n values for different variates are shown in Table 3.)
- p_{ni} = the random permanent environmental effect within lactation
- e_{ijklmn} = the residual

Matrix form of the model is shown in Equation 3.

Equation (3)

$$y = X\beta + Z\alpha + Z\lambda + \varepsilon$$

- β = the vector of parameters for fixed effects
- α, λ = the vectors of parameters for additive genetic effect and permanent environment effect, respectively
- ε = the vector of residuals
- X = the incidence matrix for fixed effects
- Z = the incidence matrix for random additive genetic effect and permanent environment effect

Each animal has an additive genetic as well as a permanent environmental effect, hence both effects have the same design matrix. Permanent environmental effects for different animals are uncorrelated, and within an animal there is no correlation between its additive and

its permanent environmental effect. This distribution of the three random effects is shown in Equation 4.

Equation (4)

$$\text{var}\begin{bmatrix}\alpha\\ \lambda\\ \varepsilon\end{bmatrix}=\begin{bmatrix}A\sigma_\alpha^2 & 0 & 0\\ 0 & I\sigma_\lambda^2 & 0\\ 0 & 0 & I\sigma_\varepsilon^2\end{bmatrix}=\begin{bmatrix}G & 0\\ 0 & R\end{bmatrix}$$

Where:

- σ_α^2 is the direct additive genetic variance
- σ_λ^2 is the variance due to permanent environmental effects
- σ_ε^2 is the variance of the residuals

For any pair of individuals *i* and *j,* the expected additive genetic covariance between them is $2FI_{ij}V_a$ where FI_{ij} is the coefficient of coancestry, i.e. probability that an allele drawn at random from individual *i* is identical by descent to one drawn at random from individual *j*. When doubled, it yields values of "relatedness" (e.g. 0.5 for parent offspring and full sibs, or 0.25 for half-sibs). The higher the relatedness and the more V_a underlying the trait, the greater the expected covariance between two individuals. Among all the *n* individuals in the pedigree, the matrix of additive genetic covariance for a trait is given with AV_a, where *A* is the additive genetic relationship matrix (size *n*n* with all the pairwise values of relatedness).

In random regression animal models for PC, FC and LC, stage of lactation was treated as a covariate. Random additive genetic effect was modelled as a function of time and was fitted as random regression on days in lactation. Orthogonal Legendre polynomials were used to standardize time scale into values between -1 and +1 using AS-Reml 3 (Gilmour *et al.*, 2009). Legendre polynomials of the first order were fitted as lactation covariate in FC model, and second order Legendre polynomials were fitted in PC and LC models (Equation 5 and 6). Hence, with second order Legendre polynomials we fit a three order regression, and estimate genetic variance of the intercept (i.e. of the average variate values of the animals), variance of the slope (i.e. of the growth/decrease of the variate of the animals during lactation) and quadratic term of the variate. Values for particular days of lactation were calculated back from the standardised time scale using coefficients provided by AS-Reml (Gilmour *et al.*, 2009) in SAS/IML module.

Equation (5)

$$y_{ijklmn} = \mu + \beta_{1i}DoL_{ijklmn} + \beta_{2i}e^{-0.05*DoL} + L_i + S_j + M_k + Y_l + F_m + Y_l*(M_k + L_i + F_m) + a_n*\varphi_o(DoL) + p_{ni} + e_{ijklmn}$$

Where:

- y_{ijklmn} = individual observation of MY, FC, PC, LC or SCS
- μ = intercept
- β_1 = coefficient describing lactation persistence inversely
- DoL_{ijklmn} = days in milk
- β_2 = coefficient describing beginning of lactation
- L_i = fixed effect of lactation number (i = 1, 2, 3, 4 and 5+)
- S_j = fixed effect of litter size (j = 1 and 2+)
- M_k = fixed effect of the month of lambing (k = 1, 2 and 3)
- Y_l = fixed effect of the year of measurement (l = 1, 2, 3, 4, 5, 6, 7 and 8)
- F_m = fixed effect of the farm (m = 1 to 50 for SCS, and 1 to 46 for MY)
- a_n = the random additive genetic effect of animal with complete relationship included (n values for different traits are shown in Table 3.)
- φ_o = polynomial of the o^{th} order for days in lactation (o = 1 in FC an 2 for PC an LC models)
- p_{ni} = the random permanent environmental effect within lactation
- e_{ijklmn} = the residual

Equation (6)

$$y = X\beta + \Omega(Z_\alpha\alpha, DOL, o) + Z_\lambda\lambda + \varepsilon$$

Where:

- y = the vectors of observations for PC, LC, FC
- β = the vector of parameters for fixed effects
- Ω ( ) = Legendre polynomial function
- α, λ = the vectors of random regression coefficients for additive genetic effect and permanent environment effect, respectively
- DoL = days in milk
- o = order of the polynomial
- ε = the vector of residuals
- X = the incidence matrix for fixed effects

- Z_α = the matrix of the regression coefficients for the oth polynomial of random additive genetic effects
- Z_λ = the incidence matrix for permanent environment effects

Expected values of observations E(y) were product of the incidence matrix for fixed effects and parameters for fixed effects (Equation 7), and expected values for all random effects were equal to zero. (Co)variances for random effects (G_α, G_λ) and residuals compose phenotypic (co)variances as shown in Equation 8.

Equation (7)

$$E(y) = X\beta$$

Equation (8)

$$V = var(y) = Z_\alpha G_\alpha Z_\alpha' + Z_\lambda G_\lambda Z_\lambda' + R$$

Where: $var(\alpha) = G_\alpha$

$var(\lambda) = G_\lambda$

$var(\varepsilon) = R$

Equations 9 and 10 describe (co)variance structure for the individual random effect. Sign $\otimes$ indicates Kroneckers product that denotes an operation on two matrices of arbitrary sizes resulting in a block matrix. Matrix *I* is identity matrix for permanent environment effect. Levels are assumed to be uncorrelated for trivial random effects, while for additive genetic effect the relationship among levels is shown in the matrix *A*. Measurements are correlated within levels for the individual random effects, as shown by (co)variance structure in matrix $G_{0\alpha}$ for additive genetic effect (Equation 10). Matrix R_{0i} is diagonal matrix where index *i* indicates matrices for residuals. The matrix for residuals is a direct sum (indicated by sign $\Sigma\otimes$) of R_{0i} matrices. The residuals from different animals are additionally assumed to be independent and normally distributed. Likelihood of the models was obtained under general-positive constraint for the matrix elements of the *G* structure.

Equation (9)

$$\text{var}\begin{bmatrix}\alpha\\ \lambda\\ \varepsilon\end{bmatrix}=\begin{bmatrix}G_\alpha & 0 & 0\\ 0 & G_\lambda & 0\\ 0 & 0 & R\end{bmatrix}=\begin{bmatrix}A\otimes G_{0\alpha} & 0 & 0\\ 0 & I\otimes G_{0\lambda} & 0\\ 0 & 0 & \Sigma\otimes R_{0i}\end{bmatrix}$$

Equation (10)

$$G_{0\alpha}=\text{var}\begin{bmatrix}\alpha_{k0}\\ \alpha_{k1}\\ \alpha_{k_A-1}\end{bmatrix}=\begin{bmatrix}\sigma^2_{\alpha 0} & \sigma_{\alpha 0\alpha 1} & \dots & \dots & \sigma_{\alpha 0\alpha(k_A-1)}\\ & \sigma^2_{\alpha 1} & \dots & \dots & \sigma_{\alpha 1\alpha(k_A-1)}\\ & & & & \sigma^2_{\alpha k_A-1}\end{bmatrix}$$

Main sources of variance that were estimated in these models are:

- additive genetic V_a
- permanent environment V_{pe} ,
- residual variance V_r
- individual variance $V_i = V_a + V_{pe}$
- phenotypic variance $V_p = V_a + V_{pe} + V_r$

Repeatability (r) is a proportion of V_i and V_p, and heritability (h^2) is a proportion of V_a and V_p.

4.2.2. Analysis of udder shape and milkability

Breeding values for full udder height (Fh), maximal udder width (Mw), angle that teat closes with the vertical axis of the udder (Alpha), height of cisternal part of the udder below the teat orifice (Cis), machine milking time (Mt), machine milking yield (My), average milk flow (Avgm) and peak flow rate (Mmf) during machine milking were estimated using univariate mixed models (Equations 11 and 12) and REML algorithm in AS-Reml program release 3 (Gilmour *et al.*, 2009).

Farm, litter size, number of lactation and day of measurement are defined as fixed influences in udder shape models. Cis and Alpha models included additional fixed effect of the udder half with two levels: additive genetic value of the individual and permanent environmental effect within the day of measuring as the random effect.

Equation (11)

$$y_{ijkln} = \mu + D_i + S_j + L_k + F_l + F_l*L_k + a_{ni} + p_{ni}*D_i + e_{ijkln}$$

Where:

- y_{ijkln} = individual observation of Fh, Mw, Alpha, Cis
- μ = intercept
- D_i = fixed effect of measuring day (i = 1, 2 and 3)
- S_j = fixed effect of litter size (j = 1 and 2+)
- L_k = fixed effect of the lactation number (k = 1, 2, 3, 4 and 5+)
- F_l = fixed effect of the farm (l = 1 to 11)
- a_n = the random additive genetic effect of animal
- p_{ni} = the random permanent environmental effect within day of measurement (for Alpha and Cis)

  e_{ijkln} = the residual

Farm, number of lactation, milking interval and day of measurement are defined as fixed effects in milk flow kinetics models. Additive genetic value of the individual was the random effect. Mmf model included additional random effect of permanent environment.

Equation (12)

$$y_{ijkln} = \mu + D_i + S_j + L_k + F_l + a_{ni} + e_{ijkln}$$

Where:

- y_{ijkln} = individual observation of Mt, My, Avgm, Mmf
- μ = intercept
- D_i = fixed effect of measuring day (i = 1, 2 and 3)
- S_j = fixed effect of milking interval (j = 1 and 2)
- L_k = fixed effect of the lactation number (k = 1, 2, 3, 4 and 5+)
- F_l = fixed effect of the farm (l = 1, 2, 3, 4, 5)
- a_n = the random additive genetic effect of animal with complete relationship included (n values for different traits are shown in Table 2.)
- e_{ijkln} = the residual

Repeatability within lactation is calculated as a ratio of the covariance between the measurement day and the total variability. Pearson correlations of BLUP between Mmf, Avgm, Mt, My, Fh, Mw, Cis and Alpha were calculated using CORR procedure (SAS 9.3).

4.2.3. Molecular variability analyses

The Istrian sheep sampled in Croatia and Slovenia was compared to eleven indigenous pramenka breeds from Croatia, and from Bosnia and Herzegovina. Additionally, variability and structure of Istrian sheep breed was analysed by comparing the population from Croatia to population from Slovenia using Lika pramenka and Krk island sheep as out-groups.

Allele frequency, the number of alleles (A), observed heterozygosity (Ho) and heterozygosity expected (He) under the Hardy-Weinberg (HW) equilibrium assumption across the populations and the markers, were calculated using the GENETIX 4.04 software (Belkhir *et al.*, 2002). Locus-wise deviations of the markers from HW equilibrium across the populations were tested by means of the GENEPOP 4.1.3 software package (Raymond and Rousset, 1995) and the method of Guo and Thompson (1992). The same software was used to determine the possibility of null-alleles and gametic disequilibrium test. Statistical significance of the values obtained in all the cases was estimated by bootstrapping, using 1 000 replications. Markers showing deviation from the HW equilibrium were excluded from further analysis if the deviation was significant in more than half of the populations studied.

Private alleles were accounted for utilizing the GDA software (Lewis and Zaykin, 2001). Polymorphic information content (PIC) and the rarefacted allelic richness were estimated in MOLKIN 3.0 (Gutierrez *et al.*, 2005), using bootstrapping to standardize among different sample size populations. Hulbert's rarefaction correction and sample size correction were based on 50 diploid individuals. Pair-wise genetic distances (Fst), coefficients of inbreeding (Fis) and gene flow estimates were obtained using ARLEQUIN 3.1 (Excoffier *et al.*, 2005) and GENETIX 4.04 (Belkhir *et al.*, 2002). GENETIX 4.04 was also used to evaluate the significance of the Fis by permuting the alleles within populations over all loci in each breed, and under the assumption of heterozygosis deficit, as well as for the factorial correspondence analysis.

Genetic variation and the distribution of genetic diversity among and within the groups were determined through the analysis of molecular variance (AMOVA) using the ARLEQUIN 3.1 software. Several groupings of populations in nested AMOVA were tested in order to find the grouping that best explains the variance in the genotype data. Individual multi-locus genotypes were used in clustering methods to study the population differentiation. Individual assignment in the populations was investigated using the STRUCTURE 2.3.1 software (Pritchard *et al.*, 2000). Ten runs were performed to choose the appropriate number of inferred clusters (K), fitting K from 2 to 20 for the 12 breeds. For the two populations of

Istrian sheep 10 runs fitting K from 1 to 8 were performed. Burn-in period for all runs was 20 000 iterations, and data was collected during the period of 10 000 iterations. To choose the optimal K, the posterior probability L(K) was calculated using the mean log-likelihood of K for each value of Evanos' ΔK. L"(K) in respect to K was also calculated. Graphic representations of these statistics were obtained from Structure Harvester 0.6.8 (Dent and VonHoldt, 2012).

5. RESULTS

5.1. GENETIC ANALYSIS OF MILK YIELD AND QUALITY

5.1.1. Averages and trends

The average daily milk yield (MY) of the total population was 1.68 ± 0.07 kg with 7.04 ± 0.30% of fat, 5.56 ± 0.07% of protein, 4.94 ± 0.05% of lactose and the SCS of 5.31 ± 0.33.

All of the fixed effects were significant ($P<0.01$) in the MY and LC model, except for litter size. In the PC model day of lactation, lactation, month, year, farm, interaction of year with month and with farm were significant ($P<0.05$). In the FC model day of lactation, month, year, farm, and interaction of year with farm, as well as interaction of year with month were significant ($P<0.05$). In the SCS model Wilmink curve coefficient (β_1) and month as well as all other fixed effects, except for litter size and interaction of year and month, were significant ($P<0.05$).

The lowest mean of daily MY were predicted for years 2006, 2009 and 2010 and for the ewes with winter lambing, while the ewes with autumn lambing had the highest means of daily milk yield. The MY means trend was positive until the third lactation, after which the means of daily milk yields decreased for ewes in lactations 4 and 5+. Ewes in the first lactation showed the highest LC mean. Ewes in the second lactation had the lowest mean, and the LC means were higher with every lactation. The means of daily milk yield and LC were decreasing through lactation. The lowest LC mean was predicted for 2012 and the highest was in 2005.

Depending on the year, and with the exception of the years 2008 and 2009, FC means were predicted to be higher for ewes with spring lambing (months 4, 5 and 6) and lower for ewes with winter lambing (months 1, 2 and 3). Ewes with autumn lambing showed intermediary FC means, with exception of the years 2007 and 2009, when it was the highest, and 2010 when autumn FC mean was the lowest. The means of ewe's FC and PC were increasing through lactation. The largest PC means were predicted for years 2007, 2009 and 2011, while in 2012 ewes showed the lowest PC mean. Similar as for FC and LC, ewes with spring lambing showed the highest PC mean, while the lowest was in ewes with winter lambing. Among lactations the PC means trend is not clear since the ewes in second lactation

showed the lowest mean. However, in the fourth lactation ewes showed the highest mean, and ewes with higher lactation numbers showed lower PC mean.

Means of SCS had a decreasing trend through lactation. The first lactation ewes showed the highest SCS mean, after which it was decreasing with every further lactation number, having a substantial drop for the ewes in the fourth lactation. Ewes with fall lambing had the highest, and those with spring lambing the lowest SCS mean. For the year 2010, the lowest mean SCS was estimated, while the highest was in 2007. The mean for 2012 shows slight increase in comparison to the previous four years.

5.1.2. Genetic parameters

The variance components, heritability, and repeatability for the milk yield and content traits are presented in Table 6. The repeatabilities ranged from 8.1% in SCS to 33.5% in PC records. The permanent environment explained from 2% in FC to 41.7% of phenotypic variation in MY. The heritabilities were low.

The unexplained variance in milk yield and content traits is accumulated in the residual variance. The highest residual to phenotype variance ratio was found in MY and SCS (1.9, 1.1). The ratio was the lowest in PC (0.66).

The (co)variance component estimates of random regression coefficients for FC, PC and LC are presented in Table 7.

The changes of additive genetic variances, phenotypic variances and heritabilities for PC, LC and FC are shown in Figures 4 to 6, respectively. Heritability estimates were relatively low in all three traits. The highest values for heritabilities were estimated in early (0.34, 0.28, 0.15 on day 16 of lactation for PC, LC and FC respectively) and late in lactation (0.25, 0.11 and 0.17 on day 225 of lactation, respectively), while the lowest values were estimated in the middle of lactation (0.01, 0.04 and 0.01 respectively).

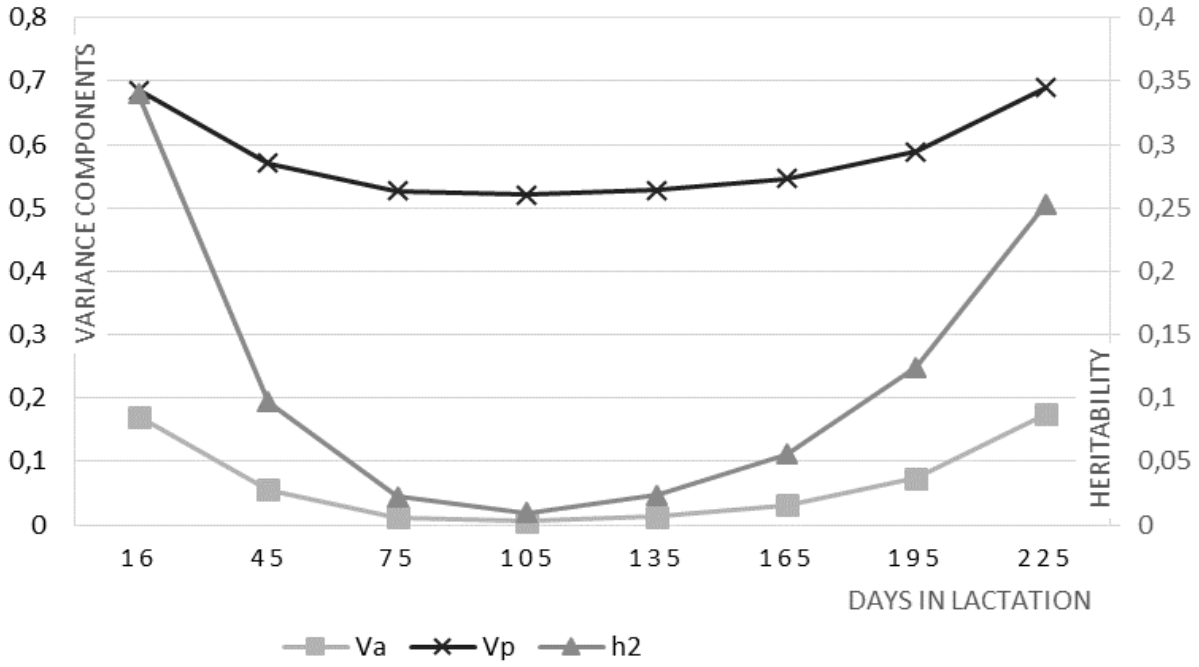
Table 6. Variance components, heritability and repeatability for the milk yield and content traits analysed in the present study.

	Residual variance	Additive variance	Permanent environment	Individual variance	Phenotype variance	Heritability	Repeatability
MY	0.12	0.004	0.03	0.8E-02 ± 0.5E-03	0.06 ± 0.001	0.02 ± 0.009	0.13 ± 0.0078
PC	0.31 ± 0.003	0.022 ± 0.006 0.084 ± 0.009 0.032 ± 0.006	0.02 ± 0.002	0.16 ± 0.013	0.47 ± 0.013	0.05 ± 0.012 0.18 ± 0.015 0.07 ± 0.012	0.34 ± 0.020
FC	1.77 ± 0.018	0.047 ± 0.019 0.280 ± 0.033	0.04 ± 0.010	0.37 ± 0.038	2.14 ± 0.038	0.02 ± 0.009 0.13 ± 0.014	0.17 ± 0.015
LC	0.06 ± 0.001	0.004 ± 0.001 0.008 ± 0.001 0.006 ± 0.001	0.003 ± 0.001	0.02 ± 0.002	0.09 ± 0.002	0.04 ± 0.015 0.10 ± 0.014 0.07 ± 0.014	0.25 ± 0.021
SCS	4.86 ± 0.040	0.04 ± 0.024	0.32 ± 0.031	0.36 ± 0.025	4.42 ± 0.043	0.01 ± 0.005	0.08 ± 0.005

Table 7. Estimated PC, LC and FC additive variances (on the diagonals), covariances (below the diagonals) and correlations (above the diagonals) between random regression coefficients.

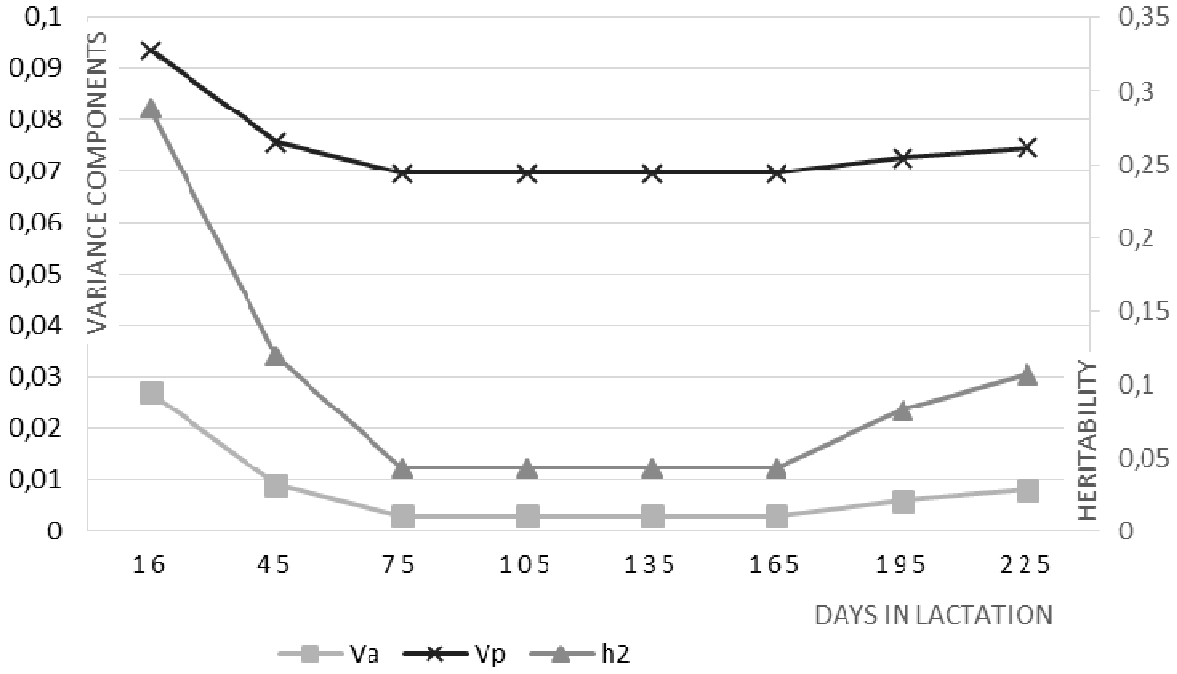
		Intercept	Slope	Quadratic term
PC	0^{th}	0.02	0.77	0.65
	1^{st}	0.03	0.08	0.13
	2^{nd}	0.02	0.01	0.03
LC	0^{th}	0.004	0.304	0.54
	1^{st}	0.002	0.008	0.18
	2^{nd}	0.003	0.001	0.01
FC	0^{th}	0.047	0.608	/
	1^{st}	0.069	0.280	/

Intercept, slope and quadratic term correspond to the power of Legendre polynomials used in the models.



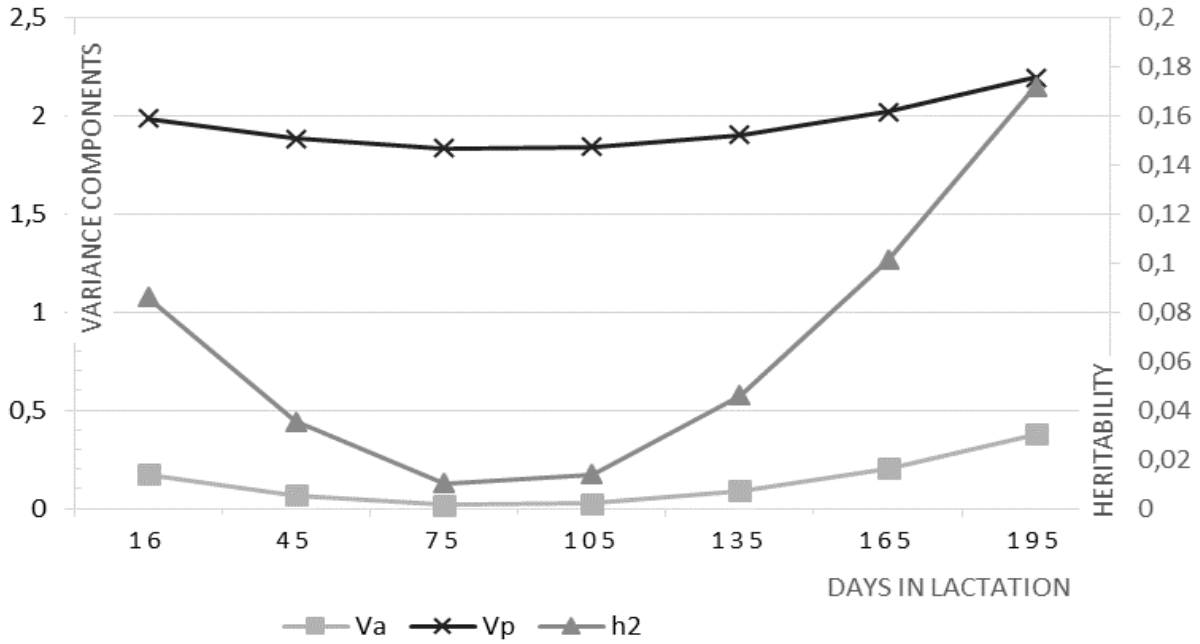
Va – additive variance; h2 – heritability; Vp – phenotypic variance

Figure 4. Changes of protein content additive and phenotypic variance, and heritability through days of lactation.



Va – additive variance; h2 – heritability; Vp – phenotypic variance

Figure 5. Changes of lactose content additive and phenotypic variance, and heritability through days of lactation.



Va – additive variance; h2 – heritability; Vp – phenotypic variance

Figure 6. Changes of fat content additive and phenotypic variance, and heritability through days of lactation.

5.2. MILKABILITY OF ISTRIAN SHEEP

5.2.1. Genetic analysis of udder shape traits

The mean values for Fh, Mw, Cis and Alpha were 14.13 ± 1.84, 13.53 ± 0.45, 1.36 ± 0.24 and 29.42 ± 3.88 respectively. The Fh mean increased in mid-lactation and decreased at the lactation end. It was the highest in third lactation ewes. Mw and Cis means were decreasing towards the end of lactation. Alpha did not change within or among lactations. Cis mean was the lowest in the first lactation and was increasing for every following lactation, and it was highest for ewes in the 5th and later lactations.

The genetic parameters for udder morphometry traits are shown in Table 8. Repeatabilities ranged from 0.42 in Mw, to 0.81 in Cis. The heritabilities ranged from 0.17 in Fh to 0.63 in Cis.

Table 8. Variance components, heritability and repeatability for the udder morphometry related traits in the present study of Istrian sheep in Croatia.

	Residual variance	V_a within measure day	V_{pe}	V_i	V_p	h^2	r^2
Fh	1.94	0.74	1.760	2.50	4.44	0.17	0.56
Mw	1.72	0.44	0.82	1.26	2.97	0.15	0.42
Cis	0.14	0.45	0.13	0.58	0.71	0.63	0.81
Alpha	102.27	112.27	11.69	123.97	226.23	0.50	0.55

Fh – Full udder height (cm); Mw – Maximum udder width (cm); Cis - Height of the cisternal part below the teat orifice (cm); Alpha - Angle that teat closes with the vertical axis of the udder (°); V_a - Additive genetic variance component; V_{pe} – Permanent environment variance component, plastic variance; V_i – Individual variance component; V_p - phenotype variance component; h^2 – Heritability; r^2 – Repeatability.

5.2.2. Correlation of BLUP estimates for udder shape and milkability traits

Mean average milk flow (Avgm) was 0.48 ± 0.047 kg/min and a mean peak flow rate (Mmf) 0.52 ± 0.071 kg/min. Mean milking time (Mt) was 1.23 ± 0.139 min and the mean milk quantity per milking (My) was 0.47 ± 0.055 kg. Milking interval and the number of lactations did not affect these means. Farm and stage of lactation were significant effects ($P < 0.01$) in all of the analyses. Average and peak milk flow were the lowest in mid- lactation. Unlike Avgf, that was the highest in late lactation, Mmf was the highest at the beginning of lactation. Milking time was the shortest in late lactation and the longest in mid- lactation. Milk yield per milking was the highest at the beginning of the lactation. It decreased in mid-lactation and even more at the end of lactation. As in Avgm, Mmf and Mt, it differed more among the farms than through lactation.

The additive genetic correlations were estimated using the udder shape and milkability BLUP values of additive genetic effects. The correlation coefficients are shown in Table 9. The milking time was positively correlated with milk yield and udder height in late lactation, as well as with udder width in mid- and late lactation. Milk yield was positively correlated with peak and average milk flow rate, as well as with udder height in mid-lactation, and width in mid- and late lactation. The peak flow rate was positively correlated to average milk flow. The teat angle was positively correlated with cistern height and udder height at the beginning of lactation, while it was negatively correlated with udder height and width at mid- lactation. The cistern height was positively correlated with udder height and width at the beginning of lactation, while it was negatively correlated with udder height at mid- lactation. The udder height at the beginning of lactation was negatively correlated with height and width at mid- and end lactation, but positively correlated with udder width at the beginning of lactation. The udder width at the beginning of lactation was negatively correlated with width at mid- and late lactation.

The highest positive coefficients of correlation were noted between milking time and yield (0.70), average and peak milk flow (0.88), and teat angle and cistern height (0.74). The highest negative correlation coefficients were found between udder height (-0.46) and width (-0.46) in the beginning of lactation, and height and width in mid – lactation.

5.2.3. Udder shape traits differences of ewe means and BLUP estimates - hand and machine milking

When examining the means of udder shape traits measurements and BLUPs, we found differences between udder shape of ewes from farms that milk by hand and the farms that apply machine milking. Differences of the means are reported in Table 10. Significant differences of means between ewes milked by machine and by hand were found in teat angle and cistern height averages, but not in udder height and width averages across lactation. All BLUP values showed differences, except for teat angle. The BLUP values for Fh and Mw were predicted separately for beginning, mid-, and late lactation. Teat angle averages across lactation, and range, were smaller in ewes on farms that apply machine milking. Cistern height was smaller in machine milked ewes as well, however, the range did not differ remarkably. BLUP values for Cis were negative (-0.02) for machine milked ewes, and positive in hand milked ewes (0.12), showing the same pattern as the measurements: smaller cisternal part below the teat orifice in machine milked ewes. BLUP values for full udder height in the beginning of lactation were negative in machine milked ewes (-0.11), opposed to hand milked ewes (0.26). Mid- and late lactation Fh BLUP values showed the opposite pattern, and were better in machine milked ewes (-0.10 and 0.03 respectively). Udder width BLUPs across whole lactation were better in machine milked ewes as well.

Table 9. Correlation coefficients among morphometry BLUPs and milk flow kinetics' BLUPS studied in Istrian sheep.

	Mt	My	Mmf	Avgm	Alpha	Cis	Fh-1	Fh-2	Fh-3	Mw-1	Mw-2	Mw-3
Mt	1	**0.704** ***	0.100	-0.168	-0.065	-0.010	-0.028	0.172	0.190 *	-0.131	0.256 **	0.239 **
My		1	0.496 ***	0.288 **	0.021	0.033	-0.083	0.289 **	0.112	-0.063	0.367 ***	0.203 *
Mmf			1	**0.875** ***	-0.035	-0.081	-0.166	0.057	-0.026	0.088	0.156	-0.037
Avgm				1	0.005	-0.007	-0.142	-0.024	-0.079	0.079	0.056	-0.108
Alpha					1	**0.743** ***	0.152 **	-0.191 **	0.024	0.028	-0.165 **	-0.030
Cis						1	0.357 ***	-0.135 **	0.032	0.137 **	-0.091	0.021
Fh-1							1	**-0.463** ***	-0.340 ***	0.484 ***	-0.247 ***	-0.207 ***
Fh-2								1	-0.027	-0.288 ***	0.617 ***	-0.073
Fh-3									1	-0.158 **	0.030	0.551 ***
Mw-1										1	**-0.457** ***	-0.273 ***
Mw-2											1	0.047

*** P < 0.0001, ** P < 0.001, * P < 0.05

BLUP values: Mt – Machine milking time (min); My – Machine milking yield (kg); Mmf - Peak flow rate (kg/min); Avgm - Average milk flow (kg/min); Alpha – The angle that teat closes with the vertical axis of the udder (°); Cis – Height of the cisternal part below the teat orifice (cm); Fh-1 – Full udder height during the first measuring day (cm); Fh-2 – Full udder height during the second measuring day (cm); Fh-3 – Full udder height during the third measuring day (cm); Mw-1 – Maximum udder width during the first measuring day (cm); Mw-2 – Maximum udder width during the second measuring day (cm); Mw-3 – Maximum udder width during the third measuring day (cm).

Table 10. Mean differences of ewe mean measurements, and BLUPs of udder shape traits regarding type of milking applied on farm of Istrian sheep.

	Machine milking			Hand milking		
	Mean	Min	Max	Mean	Min	Max
Mw	10.71 ± 0.12	7.56	15.84	11.27 ± 0.21	8.30	14.15
Fh	13.65 ± 0.14	8.83	21.41	13.02 ± 0.29	9.21	17.21
Alpha	38.17^{a} ± 0.77	7.31	74.29	42.62^{b} ± 1.17	13.00	82.01
Cis	1.33^{c} ± 0.04	0	4.16	1.76^{d} ± 0.07	0	4.40
B-Fh1	-0.11^{c} ± 0.024	-1.15	1.17	0.26^{d} ± 0.034	-0.46	1.60
B-Fh2	-0.10^{a} ± 0.022	-1.54	1.05	-0.21^{b} ± 0.031	-1.43	0.60
B-Fh3	0.03^{c} ± 0.021	-0.82	1.10	-0.07^{d} ± 0.014	-0.57	0.44
B-Mw1	-0.01^{c} ± 0.020	-0.74	0.94	0.14^{d} ± 0.022	-0.80	0.97
B-Mw2	-0.03^{c} ± 0.018	-0.60	0.79	-0.12^{d} ± 0.019	-0.75	0.39
B-Mw3	0.03^{a} ± 0.013	-0.56	0.80	-0.02^{b} ± 0.011	-0.40	0.53
B-Alpha	1.06 ± 0.444	-19.16	24.47	1.46 ± 0.402	-15.27	16.88
B-Cis	-0.02^{a} ± 0.030	-1.18	2.04	0.12^{b} ± 0.034	-1.17	2.00

Means in the rows with superscript differ regarding the type of milking applied: ^{a,b} $P < 0.001$; ^{c}, ^{d} $P < 0.01$. Mw - Maximum udder width (cm); Fh - Full udder height (Fh); Alpha - angle that teat closes with the vertical axis of the udder (°); Cis - Height of the cisternal part below the teat orifice (cm); B-Fh1- Full udder height BLUP during the 1st measuring day (cm); B-Fh2- Full udder height BLUP during the 2nd measuring day (cm); B-Fh3- Full udder height BLUP during the 3rd measuring day (cm); B-Mw1- Maximum udder width BLUP during the 1st measuring day (cm); B-Mw2- Maximum udder width BLUP during the 2nd measuring day (cm); B-Mw3 - Maximum udder width BLUP during the 3rd measuring day (cm) ; B-Alpha – BLUP value of the teat angle (°) ; B-Cis – BLUP value of the height of the cisternal part below the teat orifice (cm).

5.3. MOLECULAR DIVERSITY

5.3.1. Diversity and variability in comparison with eleven pramenka breeds

We identified a high level of genetic diversity based on the analysis of the 28 loci (Table 11). A total of 392 different alleles were identified in the 341 genotyped individuals. The average number of alleles per locus was 14. The highest number of detected alleles recorded was 26 for marker *HUJ616*, whereas *ETH10* showed only three alleles (Table 11). The PIC values per marker varied from 0.142 (for *ETH10*) to 0.943 (for *OarCP49*). The highest Ho was recorded for locus *HSC* (0.854). The highest He was estimated for locus *INRA132* (0.889). In the global population, accounting for multiple tests (28 loci, 12 populations), 13 loci were found to be in HW disequilibrium, with the average number of 2.5 populations in disequilibrium per marker. The maximum of six populations in HW disequilibrium was recorded for marker *OarFCB128*. Non-amplifying null alleles showed frequency estimates ranging from 0.0030 (*BM8125*) to 0.3634 (*BM1824*) (Table 11). Marker *BM1824* was excluded from subsequent analysis of genetic differentiation due to the high estimated frequency of null allele. Hence, the results of genetic variability for the 12 studied populations are given based on the remaining 27 microsatellite markers analysed.

Table 11. Genetic diversity parameters estimated for the 28 microsatellite loci analysed in the 12 sheep populations.

Marker	A	Ho	He	HWE	F(null)	Fis	PIC
HUJ616	26	0.678	0.727	**	0.033	0.047	0.706
MAF214	10	0.458	0.603	***	0.122	0.188	0.559
MCM140	12	0.801	0.825	n.s.	0.049	-0.001	0.804
OarHH47	16	0.752	0.857	***	0.058	0.079	0.842
TCRVB6	16	0.824	0.821	n.s.	0.035	-0.047	0.804
TCRGC4B	20	0.702	0.836	***	0.070	0.132	0.823
SPS115	11	0.632	0.749	**	0.064	0.107	0.711
SPS113	13	0.759	0.748	n.s.	0.009	-0.038	0.713
FCB304	15	0.691	0.681	n.s.	0.010	-0.050	0.642
OarFCB128	12	0.615	0.828	***	0.121	0.227	0.838
OarCP49	25	0.767	0.876	***	0.060	0.073	0.943
MCM527	12	0.695	0.756	n.s.	0.033	0.029	0.726
MAF65	12	0.761	0.799	n.s.	0.012	-0.00019	0.770
MAF209	13	0.684	0.818	***	0.071	0.114	0.797
INRA132	16	0.831	0.889	*	0.035	0.036	0.878
INRA063	20	0.729	0.789	n.s.	0.037	-0.00048	0.765
ILSTS011	8	0.712	0.792	*	0.052	0.060	0.761
ILSTS005	11	0.542	0.672	***	0.091	0.148	0.630
HSC	17	0.855	0.887	n.s.	0.010	-0.015	0.876
ETH10	3	0.158	0.152	n.s.	0.051	-0.099	0.142
CSRD247	20	0.710	0.821	***	0.053	0.075	0.801
BM1824	4	0.589	0.684	**	0.363	0.096	0.626
BM8125	9	0.706	0.711	n.s.	0.003	-0.049	0.677
DYMS1	16	0.709	0.730	n.s.	0.011	0.002	0.711
JMP29	22	0.836	0.844	n.s.	0.022	-0.040	0.827
OarCP34	7	0.694	0.755	n.s.	0.044	0.069	0.719
OarJMP58	17	0.750	0.800	n.s.	0.027	-0.010	0.780
OarVH72	9	0.732	0.796	n.s.	0.103	0.036	0.774
Overall	392	0.692	0.759				

A = Number of alleles per locus, Ho = Average observed heterozygosity, He = Average expected heterozygosity, HWE = Deviation from the HW equilibrium (* P<0.05, ** P<0.01, *** P<0.001, n.s. non-significant), F(null) = Frequency of null alleles estimated for each locus, Fis = Coefficient of inbreeding, PIC = polymorphic information content.

Considering detected alleles per population per locus, one marker was found to be fixed in three populations (*ETH10* in RUD, PAG, CRE), whereas the highest number of alleles was 15 (*TCRGC4B* in STO). In total, 61 private alleles were sampled (total N of alleles for 27 marker was 387), and were distributed across all populations. The highest numbers of private alleles were noted in PAG (12) and IST (10) breeds (Table 12). The highest frequencies of private alleles were observed in CRE for *TB6* (0.18), RAB for *HSC* (0.16), and CRE for *ILST5* (0.14). The largest rarefacted mean number of alleles per locus (MNA), when all of the markers are considered jointly, was found in STO (8.63). Similar MNA values were estimated for VLA, KUP and DAL (Table 12). Average Ho values among all of the populations were high and resembling; ranging from 0.643 ± 0.145 (LIK) to 0.743 ± 0.129 (VLA). Likewise, the average He values varied from 0.643 ± 0.142 (LIK) to 0.757 ± 0.120 (DAL) (Table 12). Possible artefacts due to the different sample sizes can be ruled out, since the values obtained after the sample size correction did not show remarkable differences when compared to the diversity estimates reported above. Fis was estimated for each locus in the global population and for each population across loci. Estimated Fis values for the markers ranged from -0.099 (*ETH10*) to 0.227 (*OarFCB128*) (Table 12), and were positive and significant ($P<0.05$) for 13 markers, while for six of them the values were high. High Fis value for *OarFCB128* was evident in 10 of the breeds, ranging from 0.026 (DAL) to 0.484 (KRK). Considering the individual populations, half of them showed significant ($P<0.05$), positive and low Fis values (Table 12). The highest significant Fis values were estimated for RAB and KUP (Fis= 0.091, $P<0.001$).

Table 12. Genetic variability parameters estimated for the 12 populations of sheep studied, based on the analysis of the 27 microsatellite markers.

Group	n	Ho	He	MNA	pA	Fis
CRE	25	0.699 ± 0.225	0.664 ± 0.192	6.51	2	-0.033
DAL	25	0.718 ± 0.146	0.757 ± 0.120	8.53	8	0.072***
IST	69	0.684 ± 0.149	0.722 ± 0.148	7.18	10	0.061***
KRK	23	0.699 ± 0.166	0.726 ± 0.130	7.93	2	0.060**
KUP	25	0.700 ± 0.169	0.752 ± 0.125	8.61	3	0.091***
LIK	25	0.643 ± 0.145	0.643 ± 0.142	6.03	4	0.021
PAG	25	0.693 ± 0.188	0.707 ± 0.166	8.06	12	0.040*
PRI	25	0.720 ± 0.184	0.711 ± 0.158	7.77	2	0.008
RAB	25	0.652 ± 0.187	0.701 ± 0.155	7.04	2	0.091***
RUD	25	0.698 ± 0.218	0.691 ± 0.187	7.71	5	0.010
STO	25	0.723 ± 0.157	0.730 ± 0.149	8.63	6	0.031
VLA	24	0.743 ± 0.129	0.749 ± 0.121	8.62	5	0.030
Overall	341	0.707	0.765	8.92	61	

n = Sample size, Ho = Average observed heterozygosity (± SD), He = Average expected heterozygosity (± SD), MNA = Mean number of alleles (rarefacted), pA = Number of private alleles, Fis = coefficient of inbreeding. Fis estimates and significance of the deviation of HW equilibrium per population across the 27 loci (* P<0.05, ** P<0.01, *** P<0.001).

CRE – Cres island sheep, DAL – Dalmatian pramenka sheep, IST – Istrian sheep, KRK – Krk island sheep, KUP – Kupres pramenka sheep, LIK – Lika pramenka, PAG – Pag island sheep, PRI – Privor pramenka, RAB – Rab island sheep, RUD – Dubrovnik Ruda, STO – Hum/Stolac pramenka, VLA – Vlasic/Travnik/Dub pramenka.

Table 13. Genetic differentiation parameters estimated for the 12 populations of sheep in the present study based on analysis of 27 microsatellite markers.

group	CRE	DAL	IST	KRK	KUP	LIK	PAG	PRI	RAB	RUD	STO	VLA
CRE	-	0.074***	0.066***	0.058***	0.063***	0.149***	0.047***	0.072***	0.091***	0.078***	0.063***	0.063***
DAL	3.13	-	0.039***	0.033***	0.015***	0.082***	0.033***	0.025***	0.043***	0.054***	0.023***	0.018***
IST	3.54	6.09	-	0.026***	0.028***	0.104***	0.029***	0.042***	0.055***	0.056***	0.038***	0.028***
KRK	4.04	7.28	9.35	-	0.022***	0.106***	0.020***	0.035***	0.040***	0.062***	0.032***	0.027***
KUP	3.75	15.99	8.52	11.11	-	0.071***	0.030***	0.024***	0.047***	0.040***	0.015***	0.007*
LIK	1.43	2.79	2.16	2.12	3.27	-	0.106***	0.108***	0.114***	0.120***	0.097***	0.074***
PAG	5.04	7.32	8.27	12.22	8.04	2.10	-	0.035***	0.034***	0.058***	0.032***	0.028***
PRI	3.23	9.91	5.68	6.98	10.33	2.05	6.90	-	0.055***	0.058***	0.034***	0.013**
RAB	2.51	5.52	4.29	6.06	5.11	1.94	7.15	4.28	-	0.088***	0.054***	0.050***
RUD	2.94	4.40	4.20	3.77	6.05	1.84	4.02	4.10	2.59	-	0.040***	0.042***
STO	3.69	10.60	6.30	7.50	16.46	2.33	7.57	7.12	4.37	5.97	-	0.021***
VLA	3.75	13.63	8.71	8.90	33.84	3.14	8.74	18.68	4.72	5.64	11.42	-

Pair-wise genetic distances (Fst) with their significance levels (* P<0.05, ** P<0.01, *** P<0.001), and number of effective migrants per generation (Nm) are presented above and below the diagonal, respectively.

CRE – Cres island sheep, DAL – Dalmatian pramenka sheep, IST – Istrian sheep, KRK – Krk island sheep, KUP – Kupres pramenka sheep, LIK – Lika pramenka, PAG – Pag island sheep, PRI – Privor pramenka, RAB – Rab island sheep, RUD – Dubrovnik Ruda, STO – Hum/Stolac pramenka, VLA – Vlasic/Travnik/Dub pramenka.

For the 12 considered groups, the genetic differentiation estimates of pair-wise Wright's fixation index (Fst) were low (0.007 for VLA-KUP pair) to considerable (0.149 for LIK-CRE pair) (Table 13). Largest genetic differentiation was found for the LIK population and the estimated Fst coefficients ranged between 0.071 and 0.149. Even for the substantial genetic differentiation identified for LIK, the estimates for the number of effective migrants (Nm) were very low (1.43 to 3.27). In contrast, KUP showed a low level of differentiation, with Fst coefficients reaching maximally 0.063 (KUP-CRE). The highest gene flow was estimated for the KUP-VLA pair (33.84), and both of these groups had the highest estimates for the gene flow compared with other populations (Table 13). AMOVA showed a significant ($P<0.001$) and higher source of variation within (94.79%) than among (5.21%) populations (Table 14). The Fst value (0.052) obtained by this analysis suggested a moderate genetic differentiation for the global population. Variance components among populations were highly significant ($P<0.001$) for all of the studied loci, and markers *OarJMP58* and *INRA063* contributed to explain 8.05% and 8.57% of the variability, respectively. Utility-wise and geography-wise nested AMOVA showed similar results (Table 14), with more variability among populations than between geographical or utility groups. In the factorial correspondence analysis, the first three components together accounted for 43.42% of the variation, and explained 16.73%, 13.78% and 12.91% of the total variation, respectively. The first component separates the LIK and CRE groups from the rest of the populations (Figure 7). Addition of the second component confirms the differentiation of CRE and IST, while the third component separated IST and demonstrated the isolation of LIK from all of the other populations.

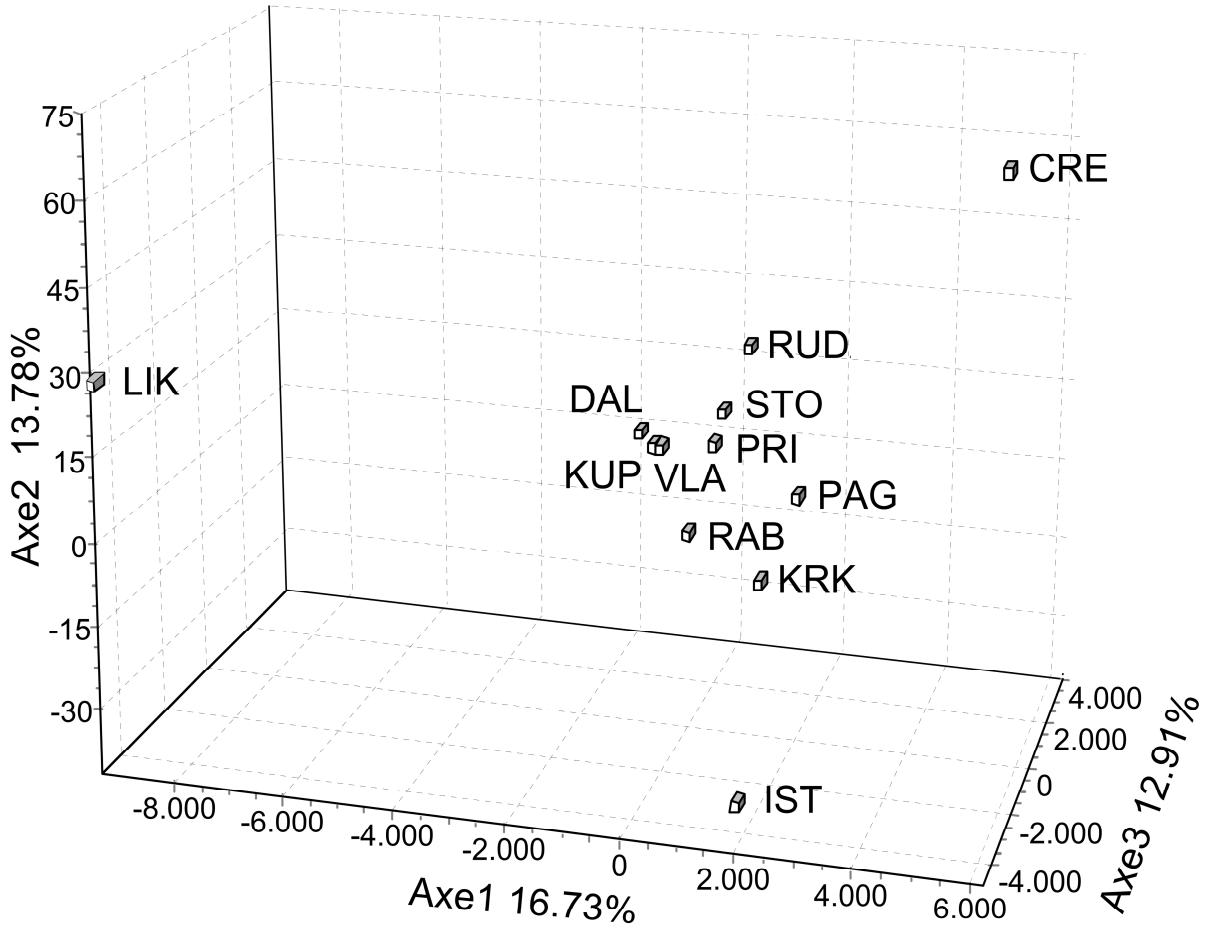
Table 14. Global AMOVA results for the 12 populations under study and results of the nested AMOVA performed by grouping the sheep geographically[a] and utilitywise[b].

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	Fst estimate
Among populations	11	417.335	0.514	5.29	
Within populations	670	6 172.428	0.213	94.71	0.053***
Among groups geographically[a]	1	64.16	0.074	0.76	0.008**
Among populations within groups	10	352.17	0.474	4.85	0.050***
Within populations	670	6 172.43	9.213	94.39	0.056***
Among groups utilitywise[b]	1	60.00	0.060	0.61	0.006*
Among populations within groups	10	357.33	0.481	4.94	0.050***
Within populations	670	6 172.43	9.213	94.45	0.056***

Genetic distances (Fst) with their significance levels are indicated (* P<0.05, ** P<0.01, *** P<0.001).

[a] Geographically: Islands and peninsula (IST, KRK, CRE, PAG, RAB, RUD); mainland (DAL, LIK, KUP, VLA, PRI, STO).

[b] Utilitywise: Group used predominately for milk (IST, KRK, CRE, PAG, RAB, VLA); group used predominately for meat (DAL, LIK, KUP, PRI, STO, RUD).

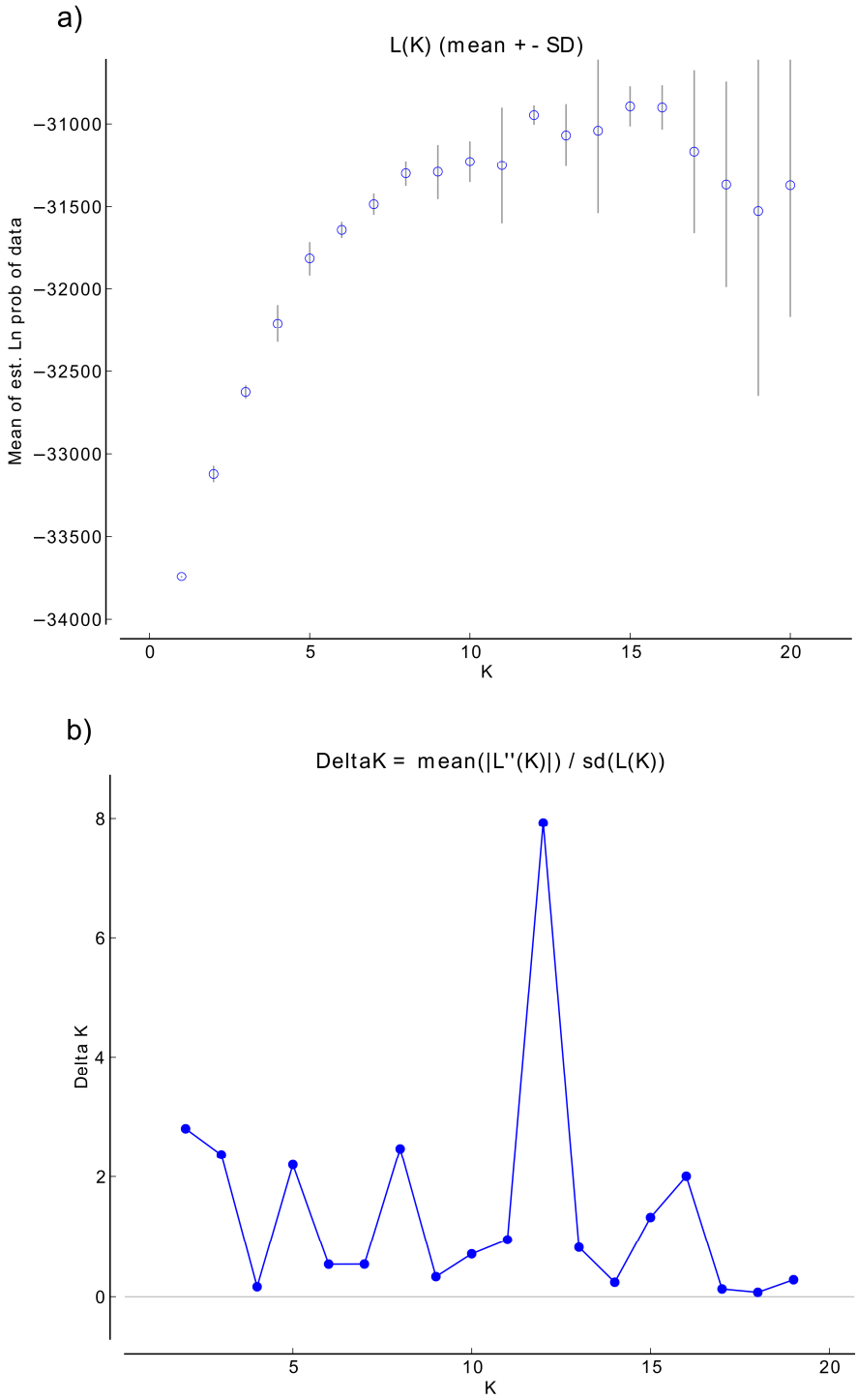


The percentage of inertia explained by each component is indicated next to the axes names. CRE – Cres island sheep, DAL – Dalmatian pramenka sheep, IST – Istrian sheep, KRK – Krk island sheep, KUP – Kupres pramenka sheep, LIK – Lika pramenka, PAG – Pag island sheep, PRI – Privor pramenka, RAB – Rab island sheep, RUD – Dubrovnik Ruda, STO – Hum/Stolac pramenka, VLA – Vlasic/Travnik/Dub pramenka.

Figure 7. Spatial representation of the 12 populations of sheep analysed, based on the results of the factorial correspondence analysis of 341 individual and 27-locus genotypes.

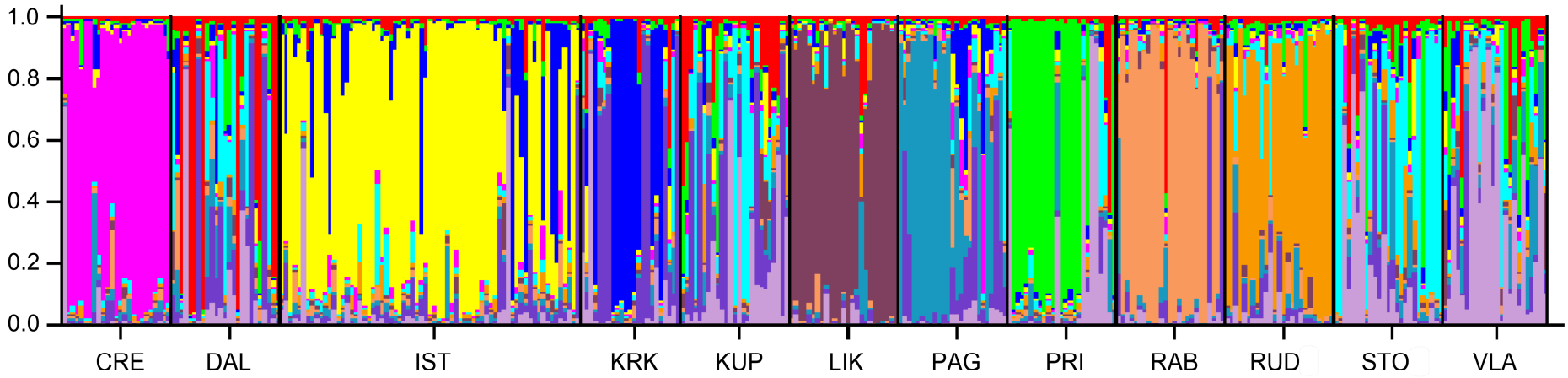
The most appropriate number of clusters for the 12 populations according to Delta *K* (7.92) was ascertained to be 12, and the best value of lnPr(*X*|*K*) for *K* = 12 was -30 947.2 (Figure 8).

Graphical representation of the clustering outcomes suggested for *K* = 12 is shown in Figure 9, and the proportion of membership for the identified clusters is provided in Table 15. Populations CRE, LIK and RAB were each associated to their own cluster, for which the corresponding estimated membership coefficient (Q) was higher than 0.725. RUD, IST, PRI and PAG populations were also assigned to their own clusters, but due to a higher admixture level, their highest estimated membership was moderate and higher than 0.539. The admixtures were low and homogeneously distributed, except in PRI, which was influenced by VLA related cluster 12, and PAG, which showed influence of cluster 11. For populations VLA, DAL, KRK, STO, and KUP, the higher proportion of membership was lower than 0.417 and showed influence of many of the identified clusters. Although there are 12 clusters for 12 populations, cluster 11 does not seem to correspond to any of the sampled populations in particular. It influences most of the populations to some extent, except for the CRE, LIK, and PRI populations. The second most heterogeneous cluster is the VLA-related Cluster 12, influenced by the STO, PRI and KUP populations. All of the 12 analysed sheep breeds showed, to some degree, a "background" influence from the two rustic populations, DAL and VLA.



a) Mean likelihood L (K) (±SD) over 20 runs for each K value tested; b) Delta K curve estimated according to Evano et al. (2005). Graphics obtained with the Structure Harvester software 0.6.92 (Earl and vonHoldt, 2011).

Figure 8. Graphical representation of the results of the structure population analysis used to determine the true number of clusters (K) of the sheep populations analysed in this work.



Each colour represents one cluster, and the length of the vertical coloured bar represents the individuals' estimated proportion of membership in that cluster. Black lines separate the individuals of the 12 studied populations. CRE – Cres island sheep, DAL – Dalmatian pramenka sheep, IST – Istrian sheep, KRK – Krk island sheep, KUP – Kupres pramenka sheep, LIK – Lika pramenka, PAG – Pag island sheep, PRI – Privor pramenka, RAB – Rab island sheep, RUD – Dubrovnik Ruda, STO – Hum/Stolac pramenka, VLA – Vlasic/Travnik/Dub pramenka.

Figure 9. Graphical presentation of the clustering outcome suggested by the Bayesian analysis performed to assess the structure of the studied populations at K=12.

Table 15. Proportion of membership for the 12 sheep populations across the clusters identified in the assignment analysis.

Group	Cluster											
	1	2	3	4	5	6	7	8	9	10	11	12
CRE	0.009	0.017	0.022	0.012	**0.807**	0.012	0.010	0.007	0.017	0.029	0.016	0.042
DAL	**0.338**	0.053	0.046	0.015	0.016	0.160	0.013	0.021	0.032	0.053	0.163	0.091
IST	0.019	0.013	0.133	**0.650**	0.017	0.024	0.014	0.011	0.016	0.018	0.043	0.039
KRK	0.048	0.021	**0.417**	0.030	0.014	0.053	0.009	0.010	0.016	0.026	0.279	0.077
KUP	0.120	0.073	0.029	0.033	0.015	0.224	0.030	0.032	0.013	0.032	**0.236**	0.163
LIK	0.033	0.008	0.010	0.013	0.007	0.018	0.020	**0.822**	0.011	0.028	0.012	0.018
PAG	0.014	0.011	0.078	0.027	0.047	0.060	0.014	0.012	0.043	**0.539**	0.128	0.026
PRI	0.045	**0.604**	0.017	0.010	0.007	0.053	0.014	0.006	0.009	0.040	0.018	0.178
RAB	0.038	0.009	0.013	0.012	0.012	0.017	0.010	0.021	**0.725**	0.038	0.081	0.022
RUD	0.019	0.037	0.012	0.016	0.013	0.061	**0.698**	0.011	0.018	0.034	0.046	0.035
STO	0.064	0.087	0.031	0.023	0.022	**0.404**	0.046	0.013	0.010	0.048	0.090	0.161
VLA	0.079	0.125	0.042	0.024	0.017	0.109	0.028	0.043	0.014	0.094	0.075	**0.350**

The highest contribution is shown in bold. CRE – Cres island sheep, DAL – Dalmatian pramenka sheep, IST – Istrian sheep, KRK – Krk island sheep, KUP – Kupres pramenka sheep, LIK – Lika pramenka, PAG – Pag island sheep, PRI – Privor pramenka, RAB – Rab island sheep, RUD – Dubrovnik Ruda, STO – Hum/Stolac pramenka, VLA – Vlasic/Travnik/Dub pramenka.

5.3.2. Comparison of Istrian sheep populations in Croatia and Slovenia

An additional analysis was performed to compare Istrian sheep population from Croatia (ISTc) and Slovenia (ISTs), using the well differentiated LIK and less differentiated KRK as groups for comparison. A total of 291 different alleles were found in 103 genotyped individuals. The average number of alleles per locus was 10.39. The highest number of detected alleles recorded was 18 for marker *HUJ616*. The PIC values per marker varied from 0.142 (for *ETH10*), to 0.943 (for OarCP49) (Table 16). In the global population, and accounting for the multiple tests performed (28 loci, 4 populations), 11 loci were found to be in Hardy-Weinberg (HW) disequilibrium (Table 16). Markers *MAF214* and *OarFCB128* were excluded from further analysis since the HWE deviation was recorded in more than half of the populations. Frequencies of non-amplifying null alleles inferred from the heterozygote deficiency for the complete set of makers analysed showed estimates ranging from 0.000 (*ETH10* and *FCB304*) to 0.365 (for *ILSTS011*), and 0.372 (for *BM1824*) (Table 16). The last two markers were excluded from subsequent analyses of genetic diversity and differentiation.

As in previous analysis of the 12 breeds, with the exception of LIK, the local sheep populations (ISTc, ISTs and KRK) revealed a high level of genetic diversity and variability, based on the analysis of the 24 loci (Table 17). Significant ($P < 0.05$) inbreeding coefficients were found in all four of the populations except LIK (Table 17). The AMOVA analysis showed a significant and higher source of variation within (93.75%) than among (6.25%) populations. The Fst value (0.062, $P < 0.001$) suggested a moderate genetic differentiation for the global population, and was higher than in the previous analysis of the 12 breeds.

For the ISTc, ISTs, KRK and LIK groups, the genetic differentiation estimates of pair-wise Wright's fixation index (Fst) were low (0.015 for ISTc-ISTs pair) to considerable (0.111 for LIK-ISTc pair) (Table 18). The largest genetic differentiation was found for the LIK group and was associated with restricted gene flows with other populations. On the contrary, ISTs showed little differentiation paired with IST and KRK populations. The highest gene flow was estimated for the ISTc-ISTs pair (16.96), and both of these groups showed a considerable estimate for the gene flow with the KRK sheep population (Table 18).

Table 16. Genetic diversity parameters estimated for the 28 microsatellite loci (more than 95% genotyping success) analysed in the ISTc, ISTs, LIK and KRK.

Marker	Multiplex[a]	A[b]	Ho[c]	He[d]	HWE[e]	F(null)[f]	Fis[g]	PIC[h]
OarVH72[i]	PET, 56ºC	8	0.775	0.797	n.s.	0.083	0.011	0.771
OarJMP58[i]	6-FAM, 56ºC	13	0.706	0.771	n.s.	0.068	0.026	0.747
OarCP34[i]	6-FAM, 56ºC	6	0.677	0.751	n.s.	0.074	0.097	0.713
JMP29[i]	VIC, 56ºC	14	0.825	0.820	n.s.	0.022	-0.049	0.797
DYMS1[i]	NED, 56ºC	12	0.689	0.687	n.s.	0.004	-0.033	0.665
BM8125[i]	NED, 56ºC	8	0.673	0.716	n.s.	0.032	-0.003	0.678
BM1824[i]	VIC, 56ºC	4	0.427	0.648	**	0.372	0.286	0.594
CSRD247	PET, 55ºC	14	0.743	0.814	n.s.	0.039	0.052	0.791
ETH10	VIC, 55ºC	3	0.214	0.192	n.s.	0.000	-0.119	0.175
HSC	6-FAM, 55ºC	10	0.842	0.848	n.s.	0.041	-0.072	0.832
ILSTS005[i]	NED, 55ºC	8	0.549	0.655	***	0.081	0.155	0.604
ILSTS011[i]	PET, 55ºC	7	0.696	0.787	*	0.365	0.097	0.756
INRA063[i]	6-FAM, 55ºC	12	0.657	0.713	n.s.	0.009	-0.014	0.670
INRA132	VIC, 55ºC	14	0.804	0.900	*	0.093	0.081	0.892
MAF209[i]	PET, 55ºC	11	0.677	0.808	**	0.074	0.096	0.784
MAF65[i]	VIC, 55ºC	11	0.657	0.758	n.s.	0.048	0.081	0.727
McM527[i]	NED, 55ºC	6	0.608	0.630	n.s.	0.026	-0.013	0.595
OarCP49	VIC, 55ºC	15	0.711	0.873	**	0.095	0.129	0.935
OarFCB128[i]	6-FAM, 55ºC	9	0.505	0.802	***	0.289	0.347	0.791
FCB304[i]	PET, 55ºC	11	0.784	0.742	n.s.	0.000	-0.094	0.708
SPS113	NED, 55ºC	10	0.777	0.758	n.s.	0.004	-0.033	0.728
SPS115	VIC, 55ºC	8	0.598	0.725	*	0.084	0.078	0.678
TCRGC4B	NED, 55ºC	15	0.695	0.807	**	0.068	0.107	0.790
TCRVB6	NED, 55ºC	11	0.767	0.762	n.s.	0.035	-0.030	0.737
OarHH47[i]	6-FAM, 58ºC	15	0.778	0.858	n.s.	0.052	0.018	0.842
MCM140[i]	6-FAM, 58ºC	10	0.753	0.767	n.s.	0.058	-0.022	0.734
MAF214[i]	VIC, 58ºC	8	0.485	0.644	***	0.143	0.203	0.596
HUJ616[i]	VIC, 58ºC	18	0.592	0.741	**	0.087	0.192	0.713
Overall		291	0.667	0.742	***			

[a] The three multiplexes are indicated by the fluorochorme used for the marker and the annealing temperature of the PCR. [b] A - number of alleles per locus. [c] Ho - average observed heterozygosity. [d] He - average expected heterozygosity. [e] HWE - significant deviation from the Hardy-Weinberg

equilibrium (* P<0.05, ** P<0.01, *** P<0.001, n.s. – not significant). [f] F(null) - frequency of null alleles estimated for each locus. [g] Fis - coefficient of inbreeding. [h] PIC - polymorphic information content. [i] FAO recommended marker for sheep diversity. ISTc – Istrian sheep population from Croatia, ISTs – Istrian sheep population from Slovenia, KRK – Krk Island sheep, LIK – Lika pramenka sheep.

Table 17. Genetic variability parameters estimated for ISTc, ISTs, KRK and LIK populations, based on the analysis of the 24 microsatellite markers.

Group	n[a]	Ho[b]	He[c]	MNA[d]	pA[e]	Fis[f]
ISTc	35	0.695 ± 0.163	0.714 ± 0.148	5.88	20	0.042*
ISTs	20	0.694 ± 0.160	0.710 ± 0.148	6.08	12	0.052*
KRK	23	0.723 ± 0.153	0.732 ± 0.133	6.73	24	0.035*
LIK	25	0.648 ± 0.150	0.634 ± 0.147	5.22	11	-0.001
Overall	103	0.668	0.745	6.71	67	

[a] n - sample size. [b] Ho - average observed heterozygosity (± SD). [c] He average expected heterozygosity (± SD). [d] MNA - mean number of alleles (rarefacted). [e] pA - number of private alleles. [f] Fis estimates and significance of the deviation of HWE per population across the 24 loci analysed (* P<0.05). ISTc – Istrian sheep population from Croatia, ISTs – Istrian sheep population from Slovenia, KRK – Krk Island sheep, LIK – Lika pramenka sheep.

Table 18. Genetic differentiation parameters estimated for ISTc, ISTs, KRK and LIK, on the basis of the 24 microsatellite markers.

Group	ISTc	KRK	LIK	ISTs
ISTc	-	0.027	0.111	0.015
KRK	8.99	-	0.108	0.025
LIK	2.01	2.08	-	0.102
ISTs	16.96	9.86	2.21	-

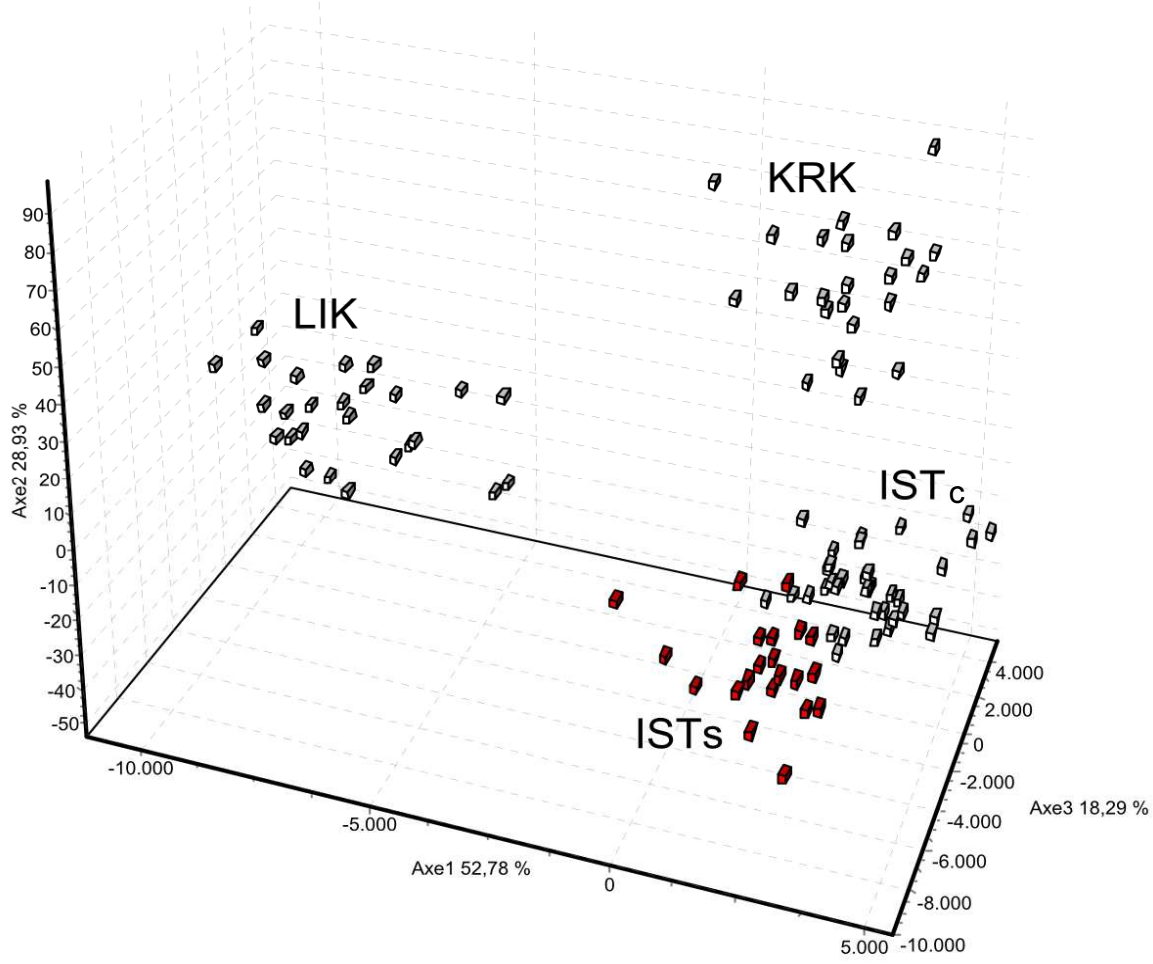
Significant (P<0.001) pair-wise genetic distances (Fst) (above diagonal), and number of effective migrants per generation (Nm) (below the diagonal). ISTc – Istrian sheep population from Croatia, ISTs – Istrian sheep population from Slovenia, KRK – Krk Island sheep, LIK – Lika pramenka sheep.

In the factorial correspondence analysis, the first three components together accounted for 100% of the variation (Figure 10). As visible from the scatter plot, the first component, which explained 52.78% of the variation separates the mountain LIK breed from Adriatic

sheep (ISTc, ISTs and KRK). The second component, explaining 28.93% of the variation, separates KRK from both ISTc and ISTs. Finally, the third component, which explained 18.29%, showed a certain separation of the two Istrian sheep populations under study, although they showed a close genetic relationship.

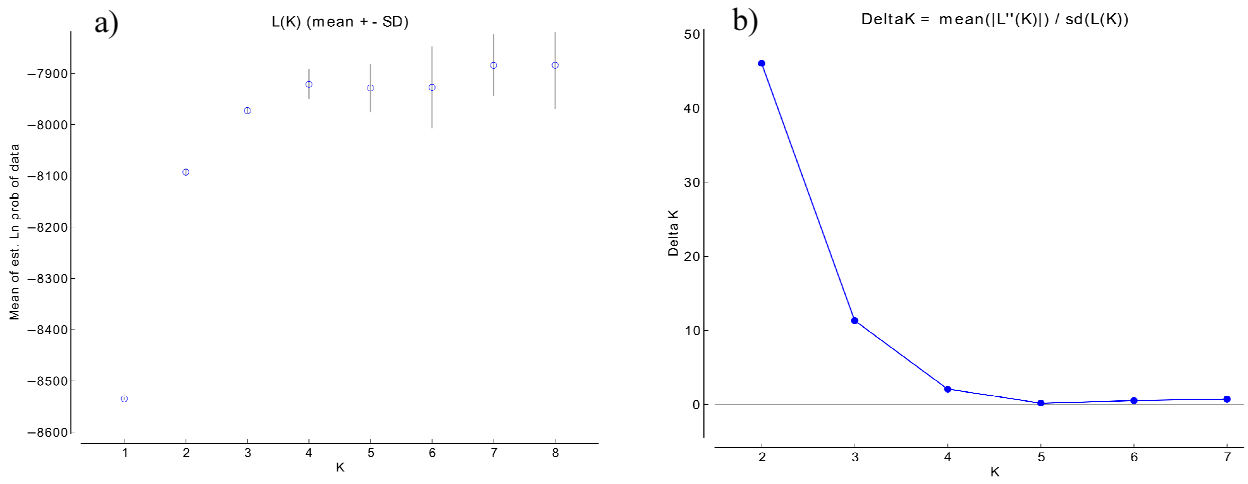
Values of mean log-likelihood and estimates of ΔK are represented in Figure 11. The Evannos' method implemented in Structure Harvester software 0.6.8 (Dent and vonHoldt, 2012) showed that the highest mean log-likelihood was reached when K was set to seven. However, the plateau on the graphic was reached at K=3 as can be seen in Figure 11a). Delta K curve (Figure 11 b) shows the largest ΔK when K=2 with the second largest value for K= 3. Other authors identifying a similar discrepancy have reported maximal ΔK at K = 2 to be an artefact resulting from markedly low likelihoods for K = 1 (Vigouroux *et al.,* 2008). Based on this and the biological significance of the results, K = 3 was chosen as the final estimated number of groups.

The proportion of membership for the identified three clusters is provided in Table 19. Estimated membership coefficients were high (Q > 0.87) for LIK, KRK and ISTc. Cluster 3 was found to be LIK related (0.899), and Cluster 1 was KRK related (0.889). Cluster 2 was found to be Istrian sheep related, although it showed a stronger influence on ISTc (0.870) than on ISTs samples (0.501). At a similar level the ISTs regional group was influenced by Cluster 1 (0.464). KRK also showed sub structuring with 8% of the samples grouping to Cluster 2 (0.095). However, this admixture was lower than in ISTs, where 50% of the sample was assigned to the KRK related Cluster 1. Although there was some influence of the KRK related Cluster 1 in ISTc samples, it was mostly due to low admixtures at the individual samples, and only 14% of the ISTc sample was assigned to the KRK related Cluster 1 (Figure 12).



The percentage of inertia explained by each component is indicated next to the axes names. ISTc – Istrian sheep population from Croatia, ISTs – Istrian sheep population from Slovenia, KRK – Krk Island sheep, LIK – Lika pramenka sheep.

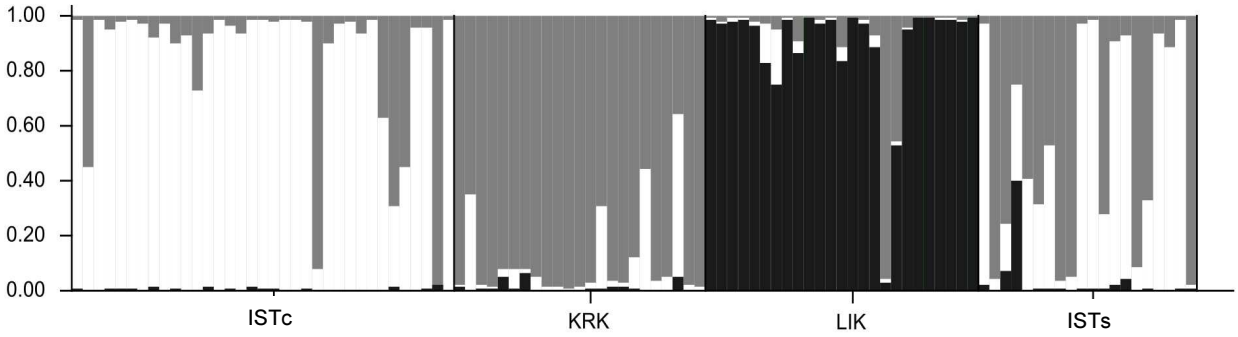
Figure 10. Spatial representation of the 103 individuals of the four populations of sheep analysed based on the results of the factorial correspondence analysis for 24-locus genotypes.



a) Mean likelihood L (K) (±SD) over 8 runs for each K value tested;

b) Delta K curve estimated according to Evano et al. (2005). Graphics obtained with the Structure Harvester software 0.6.92 (Earl and vonHoldt, 2011).

Figure 11. Graphical representation of the results of the structure population analysis used to determine the true number of clusters (K) of the sheep populations analysed in this work.



Each colour represents one cluster, and the length of the vertical coloured bar represents the individuals' estimated proportion of membership in that cluster. Black lines separate the individuals of the four studied populations. ISTc – Istrian sheep population from Croatia, ISTs – Istrian sheep population from Slovenia, KRK – Krk Island sheep, LIK – Lika pramenka sheep.

Figure 12. Graphical presentation of the clustering outcome suggested by the Bayesian analysis performed to assess the structure of the four studied populations at K=3.

Table 19. Proportion of membership for the four sheep populations across the three clusters identified in the assignment analysis.

Group	Cluster		
	1	2	3
ISTc	0.122	**0.870**	0.008
KRK	**0.889**	0.095	0.015
LIK	0.077	0.025	**0.899**
ISTs	0.464	**0.501**	0.035

The highest cluster contribution for each population is shown in bold font. ISTc – Istrian sheep population from Croatia, ISTs – Istrian sheep population from Slovenia, KRK – Krk Island sheep, LIK – Lika pramenka sheep.

6. DISCUSSION

6.1. Environmental and genetic effects: milk yield and quality

6.1.1. Means ad fixed effects

The estimated average daily milk yield (MY) of the total population was 1.68 ± 0.069 kg, which is higher than the highest corrected daily milk yield between the 20th and the 30th day of lactation (1.37 kg) reported by Vrdoljak et al. (2012) for Istrian sheep in Croatia, and is also higher than the average reported for dual purpose Istrian sheep in Slovenia, dairy Bovec and improved Bovec in Slovenia (Komprej *et al.*, 2009; 2012), dual purpose Lacaune in Slovakia (1.05 kg, Oravcova *et al.*, 2006) and similar to the dairy Lacaune breed (1.6 l, Berger, 2004), but lower than Assaf (1.93 l, Pollot and Gootwine, 2004) or East Friesian breed (2.33 kg, Hamann *et al.*, 2004). The differences reported for MY mean of Istrian sheep in Slovenia could be due to the fact that this breed is reared in Croatia predominately for milk, while the duality of purpose may be more pronounced in Slovenia. Difference of MY means reported for animals reared in Croatia could be because of different records analysed and different models used (the years 2005-2009 analysed using general linear model by Vrdoljak *et al.*, 2013). Wilmink curve tends to overestimate daily milk yield in early and late lactation comparison with other models (Cadavez *et al.*, 2008).

The average fat percentage of 7.04 ± 0.303 was similar to that of the Istrian sheep in Slovenia (7.20%; Komprej et al., 2009; 2012), improved Walachian, lower than the one of Tsigai and higher than the fat percentage in the Lacaune (Oravcova *et al.*, 2007) and was in accordance with the values obtained by Vrdoljak et al. (2012) who observed 6.15% - 7.79% depending on the stage of lactation. It was higher than Bovec and improved Bovec in Slovenia (Komprej *et al.*, 2009; 2012). The average protein percentage (5.56 ± 0.066) was similar to that of the improved Walachian, and was lower than in Tsigai and Lacaune (Oravcova *et al.*, 2007), and was in accordance with values obtained by Vrdoljak *et al.* (2012) who observed 5.55 – 6.14%, depending on the stage of lactation. The PC average was lower than in Istrian sheep in Slovenia (5.63%; Komprej *et al.,* 2012; 2009). Both PC and FC were similar to that of Sarda and Chios breeds (Ligda *et al.,* 2002; Sanna *et al.,* 1997), higher than in Valle del Belice (Cappio-Borlino *et al.*, 1997), Assaf (de La Fuente 2006) and East Friesian (Hamann *et al.* 2004) and lower than in Greek breeds (Boyazoglu and Morand-Fehr, 2001).

This results show a valuable milk yield and composition in Istrian sheep corresponding to other European indigenous breeds used for cheese production. Istrian sheep shows more favorable milk content traits than European ewes that have been strongly selected for milk yield.

Somatic cell score and lactose percentage are not investigated as often as protein and fat in ewe milk, and estimates and predictions using genetic models were not reported for Istrian sheep so far. Lactose percentage is one of the variables directly associated with milk yield and inversely related to SCC in cows (Schultz, 1977). Mean lactose percentage (4.943 ± 0.045) was lower than reported in Churra breed (5.35; Fuertes *et al.*, 1998) and in the range reported for Comisana (3.83-5.21 depending on the stage of lactation and lambing season; Sevi *et al.*, 2004). Interestingly, even though LC and MY are directly associated, in Istrian sheep LC is lower than in Churra, but MY is higher. In this regard, Istrian sheep appears to be more similar to Comisana sheep breed. Mean SCS (5.31 ± 0.3340) was similar to that of Churra population (4.95±0.01) analysed by Fuertes *et al.* (1998) as well as of intensively reared Comisana ewes (5.60 - 627 Sevi *et al.*, 2004); but higher than reported for French dairy Lacaune mean SCS (2.92 - 3.36, depending on the stage of lactation; Rupp *et al.*, 2003) and Manchega sheep (3.76 – 4.44, depending on the stage and number of lactation; Serrano *et al.*, 2003). While in Churra, Manchega and Comisana sheep there was an increase of SCS at the end of lactation explained by lower milk yield at that time, we found light decrease of the mean SCS through lactation, implying either better health conditions towards the end of lactation, or better functional coping of the mammary gland tissue and epithelial cell survival at the end of lactation and milking period (Sevi *et al.,* 1999). Better hygiene conditions at the end of lactation in Istrian sheep could be due to extensive conditions with open karstic pasture and high daily UV index. As in Rupp *et al.* (2003), there was no increase of SCS with parity that was found in Manchega ewes (Serrano *et al.*, 2003). In fact, we noted the highest value for the first lactation, and the lowest in the 5th and further lactations, similar to Fuertes *et al.* (1998), who found lower SCS means in ewes of 4.5 and 5.5 parity age.

Important sources of environmental variation in dairy sheep milk yield, fat and protein content affected Istrian sheep mostly as in other studies. Litter size was exception in this regard, and did not affect any studied trait which was reports also for Valle del Belice breed (Cappio-Borlino *et al.*, 1997). On the contrary, Vrdoljak *et al.* (2013) found that Istrian sheep ewes with multiple births had higher milk yield and lower fat percentage. Other authors, that found strong and significant effect of lambs, tested the effect of suckled lambs rather than litter size (Fuertes *et al.*, 1998). Effect of litter size was found less evident in dairy ewe breeds

in Slovakia (Oravcova *et al.*, 2007), probably due to the fact that the effect of the number of weaned lambs is evident only in breeds of low production level, while in high-producing breeds a positive effect exists only on the first test-day records of 2-lamb ewes (Carta *et al.*, 2009).

LC and MY followed the lactation curve decreasing towards the end of lactation (Fuertes *et al.*, 1998; Vrdoljak *et al.*, 2013; Komprej *et al.,* 2012), as expected since lactose is the main osmotic component in milk and is substantially constant during lactation in healthy animals (Sevi *et al.*, 2004). Almost linear increase towards the end of lactation in PC and FC was expected according to PC and FC trends reported in other studies of dairy sheep milk content (Vrdoljak *et al.*, 2013; Komprej *et al.*, 2012; Oravcova *et al.*, 2007).

As reported for balanced means in Istrian sheep (Vrdoljak *et al.*, 2013), parity did not affect FC in our model. Limited effect was found for LC and PC in Valle del Belice (Cappio-Borlino *et al.*, 1997). Higher FC and PC means through parities have been reported for Churra breed (Fuertes *et al.*, 1998) indicating that the rate of fat and protein content decrease over parities was lower than decrease of milk yield resulting in higher percentages. Contrary to our result for FC, PC showed low values in second parity and increase in the 4^{th} parity, coinciding with the decrease of MY. Difference in response of FC and PC to the course of parities may lead to an increase of the ratio between fat and protein in ewes of advanced parities, as reported by Cappio-Borlino *et al.* (1997).

Month of lambing is an important source of variation in ewe milk production, especially in farming systems with large variability in management techniques and environmental conditions (Carta *et al.*, 2009), like the one of Istrian sheep in Croatia. Ewes with spring lambing had the highest PC, and higher FC because of herbage feed rich in fibre. Ewes with winter lambing had the lowest PC, and FC low as in Sevi *et al.* (2004) reported for Comisana, because of feed diet based majorly on hay during those months. In lactating cows, seasonal variations for milk protein and, in a lesser extent, for milk fat were found (Coulon and Remond, 1991), which can be ascribed to a lower prolactin release with shorter photo-periods resulting in less milk with higher content concentration (Marcek and Swanson, 1984). Season of lambing affected milk yield in accordance as reported by Cappio-Borlino *et al.* (1997), who noted that milk yield depends on feed available to ewe during months of pregnancy. Ewes with fall lambing had the highest, and those with spring lambing the lowest SCS mean which can be connected to worse general hygiene conditions during fall and winter months when the ewes are housed and milked in closed barns.

Other fixed effects were included in the models since they are expected to have a remarkable effect in extensive systems based on grazing because of the strong relationship with milk yield as well as the seasonal and annual variations of herbage availability. Farm effect was significant in all traits explaining environment (geography and climate), feed and management practices. Farm in the year effect accounts for random changes in that environment regarding different years and response changes due to different environment (vegetation, financial). Lactation within the year accounts for diverse responses of ewes in different lactations to different year environment. This effect was not significant in PC nor FC. Month within a year effect accounts for differences of the seasons between the years. Even though this effect could be expected to influence SCS, effect was not observed in this study. For the year 2010, the lowest mean SCS was estimated, while the highest was in 2007. The mean for 2012 shows slight increase in comparison to the previous four years.

6.1.2. Genetic parameters

Daily milk yield heritability estimate (0.017±0.009) in this study was lower than the values reported to be low in Portuguese Churra, (0.03-0.08; Cadavez *et al.*, 2012), or in Slovak sheep (0.15; Oravcova, 2007), or in Istrian sheep (0.15; Špehar *et al.*, 2012). Milk yield is a trait with a moderate heritability (next to 0.3, Park and Haenlein, 2006), however, exceptionally low heritability values are suggested to be predicted due to extensive production systems (Cadavez *et al.,* 2012). Heritability predicted for milk yield in Slovenian dairy sheep (0.08-0.13) reported to be lower than in other literature (Ligda *et al.*, 2000; Komprej *et al.,* 2003) was a result of differences in the random part of the model (Komprej *et al.,* 2013). Furthermore, our results may also reflect enormous variability in milking practices and management (i. e. stripping, nutrition) in Istrian sheep, as well as a possible effect of the manner of sample collection, as was reported by Othmane *et al.* (2002). Such records would contain variation, but in a way that is difficult for the model to recognize and partition correctly.

Heritability of PC reported from single trait repeatability fixed regression test-day model by Špehar *et.al.* (2012) was in range with our estimated heritability of protein (0.05 in intercept, to 0.18 in slope). Heritability of FC (0.02 to 0.13) content is also comparable to the estimate reported by Špehar *et al.* (2012). Both estimates were low, since expected values generally range from 0.1 to 0.61 for FC, and 0.31 to 0.69 for PC (Carta *et al.*, 2009). However, similar low values for FC (0.1) were obtained by Oravcova (2007) and by Komprej

et al. (2013) for ISTs. Lower heritability estimate for FC was reported by Othmane *et al.* (0.06; 2002), while Oravcova *et al.* (2005) found lower heritabilities for both PC and FC. Heritability for protein was similar to the one reported for Churra (El-Saied *et al.*, 1998), and lower than those found by previously mentioned authors, which fall in the range of 0.20 to 0.25 (Serrano *et al.*, 2001). The same as in other breeds, higher heritability was found for PC than FC (Oravcova *et al.*, 2007; Komprej *et al.,* 2013). The reason behind that fact is that fat content shows more non-genetic variability due to measurement specifics, but also because milk fat content variability is influenced remarkably, and more than PC, by feed.

As in PC and FC analyses, data allowed for expansion of the LC model by modelling the change of additive variance through lactation. Reported additive variances and heritabilities for PC, FC and LC obtained by using Legendre polynomials of first and second order represent the heritability of the trait variability intercept, and the trait variability slope which were the highest in PC. Trend of the PC heritability estimates using Legendre polynomials of third order through lactation in ISTs (Komprej *et al.*, 2013) was sine shaped with the peak around day 79 and the lowest value at 137, after which it increased reaching the maximum value at the end of lactation. Our estimates for both PC and FC heritability had lower minimal values and higher maximum values than those found in ISTs. Also, we found the highest heritability at the beginning of lactation, and the minimum around day 75, after which there was an increasing trend towards the end of lactation. Similar to the FC estimates using Legendre polynomials of third order through lactation in ISTs (Komprej *et al.*, 2013), our estimates for FC heritability were the highest at the end of lactation. However, the trend of the heritability change was different. While in ISTs increasing trend was found over lactation, we observed decrease reaching the minimum around day 75, and growth after that. Opposite trends to the ones we observed were reported for dairy cows (Druet *et al.*, 2005) with the highest FC and PC heritability estimates in mid-lactation.

Relatively small number of authors report on SCS genetic estimation in ewes. However, Rupp *et al.* (2009) found that selection for reducing milk SCS leads to improved resistance to clinical and subclinical intramammary infections in 2 lines of Lacaune sheep. Heritability found for SCS was lower than for MY, and lower than reported for other breeds, where it reached 0.1 to 0.2 (Rupp *et al.*, 2003; Legarra and Ugarte, 2005; Carta *et al.*, 2009), but similar to Serrano *et al.* (2003) for univariate repeatability animal models. This is a first report of SCS genetic parameters estimation in Istrian pramenka.

Lactose content genetic parameters are an interesting trait, because of direct association of LC with MY, and inverse relationship with SCS due to observed drop in LC in

milk of clinically and sub clinically affected ewes (Kalantzopolous, 1994; Sevi *et al.*, 2004). This is the first report of such research in Istrian sheep, performed to obtain preliminary results that could be of further use in developing multivariate models with MY and SCS data. Obtained LC heritability was higher than SCS and MY estimates, with permanent environment by far lower, and repeatability higher than in MY and SCS estimation. This result indicates a marked possibility to use LC with other traits in multivariate genetic models, which are more accurate than repeatability model.

Repeatabilities, except for MY and SCS which were lower than in other studies, were within the range of the reported values. PC repeatability was higher than FC, as was also expected due to differences of the two components that arise during sampling.

Except for LC residual, which was by far the lowest, the pattern of the residual variance corresponds to all previously mentioned univariate and multivariate studies, where the largest residuals are found for FC and the lowest for PC.

6.2. Environmental and genetic effects: udder shape traits

6.2.1. Means and trends

Most of authors used scoring scale for evaluation of udder morphometry (De La Fuente *et al.*, 1996; Fernandez *et al.*, 1997; Casu *et al.*, 2002; Serrano *et al.*, 2002; Legarra and Ugarte, 2005; Marie-Etancelin *et al.*, 2005; Kukovics, 2006; Casu *et al.*, 2010). Direct measurements are performed on site, and often include different definition of morphometry traits that coincide with our measurements from digital photographs only in one part, or not at all in the case that the measuring was performed after milking when the udder is empty (Altincekic and Koyuncu, 2011; Iniguez *et al.*, 2009, McKusick *et al.*, 1999; Martinez *et al.*, 2011). Both scoring and on-site measuring were performed by several authors in search of non-subjective and time-effective method (Fernandez *et al.*, 1995; Margetin *et al.,* (2011); Milerski *et al.*, 2006; Mačuhová *et al.*, 2008; Gelasakis *et al.*, 2012). Udder measurements obtained from digital photographs were used by Dzidic *et al.* (2004; 2009), and showed that udder height might be underestimated from digital pictures, while there were no differences between measurements of udder width or cistern height using different methods.

Mean value of full height (Fh) of the udder was smaller than reported for Chios ewes (19.3-22.9 cm depending on the parity), larger than mid – lactation values for Turkish breeds (7.3-7.7 cm, depending on the breed; Altincekic and Koyuncu, 2011), and similar to means of Slovak dairy ewes, especially improved Walachian (13.4 – 18.4 cm, depending on the breed; Milerski *et al.*, 2006). Istrian x Awassi crossbreeds had higher udder (15.9 cm) that did not differ between days 60, 90 and 120 of lactation (Dzidic *et al.,* 2009). Udder depth is a score variable corresponding to Fh (correlation 0.768 - 0.802; Gelasakis *et al.*, 2012; Milerski *et al.*, 2006). Fh was affected by parity the same as in Gelasakis *et al.* (2012), who found the largest Fh mean in the third lactation. Additionally, we found lower means for fourth and for higher parities. Even though in Chios ewes effect of day in lactation was not significant, we found test-day affecting the Fh, which showed the lowest mean at the end of lactation, the same as found for udder depth in Churra (De La Fuente *et al.*, 1996) and length in Chilota and Suffolk Down (Martinez *et al.*, 2011).

Maximum udder width (Mw) was somewhat smaller than in Chios or Istrian x Awassi crossbreeds (14.8-15.3 cm; Gelasakis *et al.*, 2012), but bigger than in Slovak dairy ewes, more similar to Slovak Lacaune (10.6-13.12 cm). As in Chios ewes, the width did not differ with parity. Even though we noted decrease towards late lactation, in Chios days in milk did not

affect the width, and in Churra the difference through lactation was more pronounced (Fernandez *et al.*, 1995). The size of the udder is smaller than in most of the compared breeds, which could indicate smaller cisterns.

In the present study, cistern height below the teat orifice (Cis) was lower than in Churra (1.5 cm), much lower than in Istrian crossbreeds (2.5 - 2.9 cm) or Awassi (3.4 cm; Iniguez *et al.*, 2009), and by far lower than in Chios (4.6 - 5.4 cm), but similar to Slovak Tsigai (1.3 cm). While Fernandez *et al.* (1995) found effect of flock as significant, we did not. While Dzidic *et al.* (2009) did not find the effect of lactation in Istrian crossbreeds, and Gelasakis *et al.* (2013) of days in milk, we found that Cis was lower at the end of lactation, the same as reported for Churra (Fernadez *et al.,* 1995). Gelasakis found that the cistern mean was larger for third parity ewes, and we found an increasing trend through all parities, as did Fernandez *et al.* (1995). This result shows that the udder shape in Istrian sheep is favorable for machine milking, and that the ewe can be milked without additional manipulation of the udder required to empty the udder when the cisternal part below the teat orifice is high.

Teat angle was better than in Churra (50.39^{o}, Fernandez *et al.,* 1995), Istrian crossbreeds (44-49^{o}) and Slovak sheep (38-47^{o}; Milerski *et al.*, 2006). While in Churra it was variable within and among lactations, we did not observe that. Moreover, we did not observe worsening of the teat placement at the end of lactation as was reported for Chios sheep. This kind of teat placement enables proper milking with teat cups attached firmly, and with no air flow entrance that would disturb the vacuum and the milk flow.

6.2.2. Genetic parameters

The repeatabilities we obtained for Fh, Mw and Cis were higher than repeatabilities for udder measurements in Chios or Churra ewes (Gelasakis *et al.*, 2012; Fernandez *et al.*, 1996; 1997; Legarra and Ugarte, 2005; Martinez *et al.*, 2011). Generally, the cistern height and teat angle repeatabilities, as well as repeatabilities of corresponding score variables, tend to be higher than repeatabilities of udder size measurements or scores. The values we obtained were similar to measurement repeatabilities in Istrian crossbreeds (Dzidic *et al.*, 2009), with the exception of Cis repeatability. Repeatability in Cis was considerably higher than any score or measurement repeatability in Chios, including the very high corresponding teat placement scoring trait in Chios (0.75; Gelasakis *et al.*, 2012). Correlation of angle measurement with teat position score was high in Tsigai, improved Walachian and Lacaune (0.69 – 0.76; Milerski *et al.*, 2006). Additionally, the teat position score was correlated

strongly to cistern height measurement in the above mentioned research (0.61 - 0.76). Cistern height showed by far the highest values in comparison with other traits in Churra as well (0.77; Fernandez *et al.*, 1996). Unlike in other studies mentioned above, we found teat angle repeatability lower than full udder height, which might be due to the more complex nature of the angle measurement trait measured through digital photographs. Also, repeatability of the Fh might be higher than in Alpha because Fh measurement includes Cis measurement. As was suggested by Fernandez *et al.* (1997), for udder traits with repeatabilities above 0.5, single measurement per lactation would be sufficient for the purpose of addressing the basic selective objectives and criteria.

While linear scoring has been evaluated as a good approach for the purpose of udder shape genetic evaluation (Margetin *et al.*, 2011), we have obtained higher heritability predictions using the measurements from digital photographs. Values were higher than those reported in studies based on genetic parameters of direct udder measurements or different scoring systems (Casu *et al.*, 2002; Legarra and Ugarte, 2005). Full udder height had low heritability prediction similar to the value reported in Churra, Manchega or French Lacaune (Fernandez *et al.,* 1997; Serrano *et al.*, 2002; Marie-Etancelin *et al.*, 2005), but lower than moderate prediction for black-face Latxa reported by Legarra and Ugarte (2005) or Margetin *et al.* (2011). Interestingly, Cis and Alpha show high heritabilities as well as repeatabilities. Teat angle heritability was higher than reported in French Lacaune or Manchega for teat placement score (0.33 and 0.20, respectively; Marie-Etancelin *et al.*, 2005; Serrano *et al.*, 2009) or teat angle (0.3; Margetin et al., 2011). In all of the above mentioned studies and breeds (French Lacaune, Manchega, Latxa, and in Churra) teat angle was the trait with the highest heritability. However, those studies did not include analysis of cisternal part below the teat orifice. Trait related to Cis that was used in this study would be udder cleft score. Marie-Etancelin *et al.* (2005) reported heritability for that trait (0.26), which was lower than the teat placement score heritability prediction, or the Cis heritability prediction. Results of the thesis provide the basic parameters for discussion of selective objectives and criteria in the Istrian sheep considering udder shape. Genetic parameters obtained for udder shape show that cistern size would be a more logical target for selection than teat angle, with expectance to account for more appropriate teat position due to the high correlation between the traits.

Intermediate to high heritabilities for udder shape traits measured through digital photographs indicate that there is potential for selection. Moreover, udder shape evaluation using digital photography gives higher heritability estimates than scoring-based systems or direct measurements. It can also be presumed that it provides more comparable results between

different countries, considering standardized protocols, in comparison to subjective scoring systems or diverse protocols for direct measurements.

6.3. Milkability of Istrian sheep in Croatia: udder shape, milkability

6.3.1. Means and fixed effects

Mean average milk flow was similar as reported by Casu *et al.* (2008), and in the range reported for Lacaune and East Friesian (0.37- 0.67 kg/min, depending on stage of lactation, Bruckmaier *et al.*, 1997), or Istrian crossbreeds (0.36-0.43 kg/min; Dzidic *et al.*, 2004, 0.44 - 0.64, kg/min depending on the stage of lactation; Dzidic *et al.*, 2009), but lower than in Sardinian ewes (Carta *et al.*, 2000). Mean peak flow rate was lower than all the reported values: remarkably lower than in Casu *et al.*, 2008 (19.7 ml/s), lower than in French dairy ewes (12.9 ml/s; Marie Etancelin *et al.*, 2006), or the range reported for Lacaune and East Friesian (0.54 - 1.02 depending on stage of lactation, Bruckmaier *et al.*, 1997), lower than that found for Slovak dairy ewes (0.88-1.07: Tančin *et al.*, 2011; 1.23: Kulinova *et al.*, 2010; 0.74 - 0.83: Mačuhova *et al.*, 2011) and in Istrian crossbreeds (0.53-0.80 and 0.57-0.92: Dzidic *et al.*, 2004; 2009). Peak flow rate mean was most similar to the Mmf of 75% Istrian crossbreeds that had the lowest Mmf in comparison with crossbreeds with lower percentage of Istrian genetic background as reported by Dzidic *et al.* (2004). Intrinsic factors influencing the peak flow rate, such as teat sphincter opening characteristics, can be improved through selection. However, environmental sources constant through lactation affecting the peak flow rate could be symptomatic of insufficient adaptation of milking setting or machine characteristics to the breed (type and shape of liners, diameters of milk lines and tubes, air entry flow), especially as the lactation stage advances and milk production declines. Therefore, further study accounting for milk machine setting, routine, and physiological response of the ewe is suggested to improve the milking, prior to culling or selection.

Mean milking time (Mt) was lower than the range reported for Lacaune and East Friesian (1.78 - 3.52 min depending on stage of lactation: Bruckmaier *et al.*, 1997), higher than reported for Slovak dairy ewes (0.78 - 1 min : Tančin *et al.,* 2011, Kulinova *et al.*, 2010; Mačuhova *et al.*, 2011) and higher than reported by Casu *et al.* (1.44 min: 2008). Mean milk

quantity per milking was lower than the range reported for Lacaune and East Friesian (0.67 - 1.34 kg depending on stage of lactation: Bruckmaier *et al.*, 1997), and by Casu *et al.* (0.676 l: 2008), or Istrian crossbreeds (Dzidic *et al.,* 2004; 2009) but similar to total milking yield found for Slovak dairy ewes (0.32-0.55 kg : Tančin *et al.*, 2011; 0.41: Kulinova *et al.*, 2010; Mačuhova *et al.*, 2011).

The number of lactations did not affect Avgm, Mmf, Mt nor My means, which is contrary to results of Casu *et al.* (2008) who found Mmf and Avgf to decrease with parity. Additionally, both Casu *et al.* (2008) and Bruckmaier *et al.* (1997) found Mmf and Avgf to decrease towards the end of lactation, while we found Avgf and Mmf to be the lowest in mid-lactation. Unlike Avgf, which was the highest in late lactation, Mmf was the highest at the beginning of lactation. Milking interval did not affect means in our study, while Dzidic *et al.* (2009) found higher peak flow rate, milking time and milk yield in the morning milkings for Istrian crossbreeds. Machine milk yield had decreasing trend through lactation, as expected and reported in other research (Marie Etancelin *et al.*, 2006). Similar decreasing trend is observed in Mt, with the exception of the longest milkings that we observed in mid- lactation.

The highest negative correlation coefficients were found between udder height (-0.463) and width (-0.457) in the beginning of lactation, and height and width in mid – lactation indicating two types of udder that can be observed when the udder is adequately full: high ones; or wide ones. Teat angle and udder height were correlated weakly at the beginning of lactation, indicating coinciding of long udders with unfavourable teat angles. Moreover, negative correlation of teat angle with udder width at mid- lactation indicates cases in which wide udders tend to have smaller teat angles better for machine milking.

High positive coefficients of correlation were noted between milking time and yield (0.704), contrary to report of Casu *et al.* (2008), who found this correlation to be low, or Dzidic *et al.* (2004), who found them to be intermediary. Carta *et al.* (2000) found stronger individual correlation between these two traits. Machine milk yield was strongly affected by average and peak flow rate, analogous to result of Casu *et al.* (2008). Milk emission traits (peak and average milk flow) were favourably correlated among each other and with milk yield as in Casu *et al.* (2008), Marie-Etancelin *et al.* (2006), Dzidic *et al.* (2004), since physically a high milk quantity in the udder increases the level of intra-mammary pressure. Teat angle was positively correlated (0.743) with cistern height as was in Slovak dairy sheep, in Manchega and East Friesian (Mačuhova *et al.*, 2008; Rovai *et al.,* 1999; McKusick *et al.*, 1999). While Mačuhova *et al.* (2008) reported on correlation between teat angle and of

cistern height with milk yield, we did not observe it. Negative correlation of Alpha with Mt that was observed in Dzidic *et al.* (2004) was not found at all in Slovak sheep (Mačuhova *et al.* (2008) nor in our research. Additionally, correlation of Mt with Cis was not found in this research, which was also the case in Slovak dairy sheep.

Although there is no official selection of udder traits in Istrian sheep, differences between udder shape of ewes from farms that milk by hand and the farms that apply machine milking were found, indicating that there are different preferences of the owners. Herds that are machine milked have ewes with higher udder, teats that are more vertically implanted, and lower cisternal part below the teat orifice. There are no other studies of the similar comparison. Teat angle average and cistern height were smaller in machine milked ewes. BLUP value differences indicated that machine milked herds tend to have ewes with smaller cisternal part below the teat orifice that are of less udder height in the beginning of lactation and wider at the end of lactation, possibly due to selection of ewes that are milked more efficiently and easier.

6.4. Variability and structure of the Istrian sheep

A large number of markers was covered in this study and, with the exception of *ETH10*, all were highly polymorphic. We observed a clear deficit of heterozygotes, as reported in other sheep breeds (Lawson-Handley *et al.*, 2007). In the overall population, 13 of the 28 markers analysed had a significant deviation from HW equilibrium (Table 1). Except for *OarFCB128*, these markers showed no correlation to the occurrence of null-alleles, and can be explained by a reduced effective size of the flocks in the studied populations.

Although a comparison of the genetic diversity parameters described here with those reported in other populations is difficult because of different markers and sample sizes used, our results indicate values similar to those reported for the Balkan pramenka breeds (Ćinkulov *et al*., 2008). In general, our analyses revealed higher within-breed variability than reported for selected breeds such as Sarda sheep, as expected for sheep populations which have not been highly pressured by selection (Arranz *et al.*, 2001; Pariset *et al.*, 2003). Moreover, we found that sheep genetic diversity in this south European region was not lost, with values fitting the pattern of radiation of genetic variation from the Near East hot-spot (Tapio *et al.*, 2010). For example, we found the MNA (range 6.03 – 8.6) to be in the range reported for Balkan pramenka breeds (Ćinkulov *et al.*, 2008) and alpine sheep breeds (Dalvit *et al.*, 2008).

However, it was lower than that reported for Greek (Ligda *et al.*, 2009) and Turkish sheep breeds (Gutierrez-Gil *et al.*, 2006).

Moderate genetic variation component estimated between the studied populations (5.29%) was similar to values reported for other Balkan sheep (Ćinkulov *et al.,* 2008), and somewhat higher than those reported for Greek breeds (Ligda *et al.*, 2009). The low percentages of the variance explained by utility-wise (0.008, $P<0.01$) and geography-wise (0.006, $P<0.05$) grouping, compared to results reported in Indian sheep (Arora *et al.*, 2011), suggest a low influence of specific selection strategies, but also a poor influence of the geographical isolation in the overall sampling area. The genetic differentiation estimates of pair-wise Fst index, complemented with the gene flow estimates, are in accordance with the results of the factorial correspondence and structure analyses, and consistent with the geographical distribution of the breeds, their history, and their breeding practices. The most recent threats to genetic variability of sheep populations include the Croatian War of Independence and the War in Bosnia and Herzegovina. The total number of sheep was almost halved. However, the island and peninsula breeds were not affected directly by war activities, as was the case with LIK, RUD, DAL, and the breeds from Bosnia and Herzegovina, which could have been exposed also to isolation breakage. Re-establishing of the studbooks after the war was performed by assigning the breed membership according to phenotypic appearance, similar as with other indigenous breeds in this area (Galov *et al.*, 2013).

As reported in Ćinkulov *et al.* (2008), IST group can be considered as a distinct breed, but VLA is clustering with other Balkan pramenka populations. Factorial correspondence and structure analysis showed that LIK, CRE and IST are the most distinct groups. From the genetic point of view, the rest of the populations are closer together, with RUD, KRK and DAL showing certain differences. In Croatia, most of the breeds are isolated on the Mediterranean islands, and the one showing the largest genetic distance (LIK) is located in the mountain region. Although the factorial correspondence analysis shows IST as the third most distinct population, it is close to KRK, and its distinction is only identified when considering the third component. The number of migrant individuals estimated for the KRK-IST pair (9.35) is also visible in the structure results. However, we found that the KRK admixture was not widely spread, but limited to some of the IST samples and was explained more in the separate analysis of ISTs and ISTc samples. Additionally, IST had one of the higher numbers of private alleles, indicating that, due to samples with a lot of KRK admixture, distinctiveness of IST was probably underestimated. RAB and RUD showed firm clusters and distinction from the other studied sheep populations because of their isolation.

Namely, RAB is an island population, while RUD population is considered endangered (712 animals), isolated in the area on the furthest south of Croatia and bred for a different purpose (wool). The most poorly defined population among the eight groups from Croatia was DAL, and was also the only population in Croatia that showed connections to all four populations from Bosnia and Herzegovina. This is by far the largest population, covers areal along the Adriatic coast with some of the islands, and is sympatric with the other studied groups. The difference of results regarding the distinctiveness between populations sampled in Bosnia and Herzegovina and in Croatia could be the result of systematic recording and selection program implemented by the Croatian Agriculture Agency during several decades, complemented with clear geographical boundaries between the populations/breeds in Croatia. As reported for Baltic sheep (Tapio *et al.,* 2005), four populations, sampled as traditional breeds in Bosnia and Herzegovina, do not equate to genetically distinct populations. According to the structure analysis and the Nm and Fst estimates reported here, the largest influence on other Bosnian populations comes from VLA, with status and distribution similar to DAL in Croatia. KUP was influenced by the largest number of the identified clusters and it is questionable how much of the initial diversity and specific adaptations to mountain environment remains in the KUP population proposed for "status nascendi" conservation efforts. Although, in order to have better milk and wool production, "hybridization programs" were established with PRI rams in KUP flocks, a higher influence is recorded from VLA (Palian *et al.*, 1960). The most distinct among the Bosnia and Herzegovina populations was PRI, with a well-defined cluster showing minor admixtures and obvious influence from the sympatric VLA population.

According to Lawson Handley *et al.* (2007), the Weitzman's conservation approach, which favours the groups that clearly stand out in the factorial correspondence analysis (LIK, CRE and IST), or are differentiated in clusters showing unique genotypes (LIK, CRE and RAB), should be treated with caution. Likewise, it can be noted that this approach does not account for the within-group genetic diversity levels and the geographical structure that can be found in some breeds. Namely, LIK had the lowest diversity indicators, as was also reported by Ferencakovic *et al.* (2013) for mtDNA and chromosome Y diversity. The second most distinct population, CRE, had Ho somewhat higher than that observed for LIK, but other diversity indicators were quite low as well. Additionally, the same as in RUD, Ho being higher than He indicates suspicion regarding an isolate braking effect. Interestingly, Pavić *et al.* (2006) found lower He (0.6575) and higher Fis (0.094) in RUD, using 10 microsatellite markers on 44 animals, indicating a possible recovery of the population size in the recent five years. Contrary to LIK and CRE, the IST population showed reasonable distinctiveness and

favourable levels of diversity parameters. Nonetheless, the significant and relatively high estimated Fis, which is higher than reported for IST by Ćinkulov *et al.* (2008) (0.011), indicates heterozygote deficiency, which might be caused by a population subdivision effect due to the sampling of animals in different locations. When the sampled IST subpopulations are analysed separately (Salamon *et al.,* 2012), Fis values are lower. The Fis values for other sheep are similar to ones reported for Greek breeds (Ligda *et al.,* 2009), and lower than those reported for Portuguese sheep (Santos-Silva *et al.,* 2008). Unlike in IST, estimated Fis values in other populations are most likely caused by breeding practices carried out without knowledge regarding the genetic variants available in flocks of these populations. Artificial insemination is not used and, depending on the population, one ram is used per 17 to 33 ewes. The results of avoidance of mating the rams with their offspring are questionable, since there is no parentage assessment in any of the investigated breeds. As Pariset *et al.* (2003) noted, ram exchange policy provides gain of a very few genetic variants when the rams are exchanged between flocks with a similar genetic pool. In the Table 4, we presented small estimated sizes for the studied geographical groups, especially for RAB, IST and KRK. While Ćinkulov *et al.* (2008) estimated the highest Fis value among the analysed western Balkan pramenka breeds in Dubska sheep (aka VLA), we did not estimate significant Fis in this population.

Additionally, we report herein the first detailed analysis about the genetic structure of Istrian sheep populations, which has never been subject to this kind of analysis.

When ISTc and ISTs are compared, the range of mean number of rarefacted alleles was in the low levels of the range reported for Balkan pramenka type populations (Ćinkulov *et al.,* 2008) and lower than in Alpine (Dalvit *et al.,* 2008), Spanish (Rendo *et al.,* 2004), and Greek sheep (Ligda *et al.*, 2009), but higher than in Italian sheep (Bozzi *et al.*, 2009). Significant ($P<0.05$) inbreeding coefficients were found in all the populations except LIK. Estimated inbreeding coefficients (Fis) for populations across loci are within literature ranges, with the estimates being similar to values found in Greek breeds (Ligda *et al.,* 2009), and lower than in Portuguese sheep (Santos-Silva *et al.,* 2008).

The AMOVA analysis showed a significant and higher source of variation within (93.75%) than among (6.25%) populations. The Fst value (0.062, $P<0.001$) suggested a moderate genetic differentiation for the global population, similar to that reported in west Balkan sheep (Ćinkulov *et al.,* 2008) and somewhat higher than in Greek sheep breeds (Ligda *et al.,* 2009). On the contrary, ISTs showed little differentiation paired with IST and KRK populations. The highest gene flow was estimated for the ISTc-ISTs pair (16.96), and both of

these groups showed a considerably high estimate for the gene flow with the KRK sheep population.

Both the factorial correspondence analysis and the clustering-based structure analyses suggested the same results, with the identification of two genetically distinct populations within the Istrian sheep sampling groups analysed: one including the regional groups of ISTc and ISTs and the other one involving the LIK regional group. The substructure detected in ISTs and the clustering the great proportion of this sample associated to the KRK-related Cluster 3 requires explanation, while the two populations are not geographical neighbours. The number of individuals and the number of loci are similar to those in other studies reporting population structure analyses in sheep (Pariset *et al.,* 2003). Therefore, the amount of admixture and the extent of allele frequency differences among populations are the factors upon which the accuracy of probabilistic assignment of individuals to populations depends (Pritchard *et al.,* 2000). There is a possibility that the allele frequencies are similar in Krk island sheep and Istrian sheep, as is inferred by the factorial correspondence result, because of their closer relatedness. The breeds' history shows that these northern-Adriatic breeds developed under similar circumstances in sub Mediterranean climate, unlike Lika pramenka sheep. However, since the admixture is present in a greater extent only in the *ISTs* population, this cannot be considered the only explanation. The studbooks for local breeds were re-established after the Croatian War of Independence assigning breed membership according to phenotypic appearance, as was the case with other indigenous breeds in this area (Galov *et al.,* 2013). Therefore, gene flow between the breeds as recent as the separation of the two subpopulations is possible. Since the preferred colour variety of Istrian sheep in Croatia is black, the phenotypic membership assignment could have been more successful than in Slovenia where the white variety of Istrian sheep was preferred. White colour is the only variety in Krk island sheep as well as in other local pramenka breeds in the area.

In this analyses, the LIK population was found to be the most distinct population at the genetic level. The factorial correspondence analysis also showed a clear separation of the KRK population from both populations of Istrian sheep. This observation confirms the previously reported results on pair-wise genetic distances, which showed that the distance of ISTc and ISTs from KRK were similar and greater than the distance between the two populations of Istrian sheep (Salamon *et al.*, 2012).

Although the present study has shown that there is not a marked genetic divergence between *ISTc* and *ISTs* populations, genetic distinctiveness is not the only criterion that should be used for conservation decisions. According to Rege and Gibson (2003), socio-

cultural contexts in which the breed exists and future economic goals rooted in functional diversity are important considerations as well.

The results provided in this thesis can be a start point for the future development of conservation program and policies focusing on decreasing identified admixture, eliminating estimated coefficients of inbreeding and preserving high allele numbers and private alleles.

7. CONCLUSIONS

Istrian sheep has favourable milk production traits, excellent udder shape and good milkability.

Istrian sheep breed in Croatia has excellent udder shape for machine milking: desirable angle that teat closes with the vertical axis of the udder, and cisternal height below the teat orifice is small.

Average milk flow in Istrian sheep is appropriate, comparable to European dairy sheep, and supported by the conclusions on excellent udder shape. Peak flow rate in Istrian sheep is lower than in European dairy breeds.

Intrinsic factors influencing the peak flow rate, such as teat sphincter opening characteristics, can be improved through selection. However, environmental sources constant through lactation affecting the peak flow rate could be symptomatic of insufficient adaptation of milking setting or machine characteristics to the breed (type and shape of liners, diameters of milk lines and tubes, air entry flow), especially as the lactation stage advances and milk production declines.

The milking speed is expected to increase because of the correlated response of this trait with milk yield selection. Because better udder morphology at the farms applying machine milking was observed, it could be expected that those herds would also have improved milking kinetic traits.

Quantitative analysis of Istrian sheep production and life traits discovered marked potential in protein content trait and cisternal part below the teat orifice.

Istrian sheep breed is genetically distinct population of sheep with favourable diversity indicators, when compared to eastern Adriatic and western Dinaric sheep breeds. Additionally, predicted plasticity and heritability imply conserved genetic variability of udder shape traits.

Based on the study of genetic diversity among the 12 breeds studied, the Istrian sheep and Pag Island sheep breeds showed the highest level of genetic diversity, whereas the Lika pramenka and Rab Island sheep breed showed the lowest level of genetic diversity. The most differentiated populations were Cres Island sheep, Lika pramenka and Istrian sheep, whereas Dalmatian, Vlasic, Stolac pramenka and Krk Island sheep showed a large level of admixture.

The study of the Istrian sheep populations from Croatia and the one from Slovenia, did not show a marked genetic divergence between them. Minor structure differences were observed between populations of Istrian sheep in Croatia and Slovenia.

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9. SUPPLEMENTARY MATERIAL

SUPPLEMENTARY 1

Dzidic A, Šalamon, D, Kaić, A, Salajpal, K, Kapš, M 2009. Relationship between udder and milking traits during lactation in Istrain dairy crossbreed ewes. Italian Journal of Animal Science 8, Supplement 3; 154-156. (IF=0.139)

SUPPLEMENTARY 2

Salamon D, Gutierrez-Gil B, Kostelic A, Gorjanc G, Kompan D and Dzidic A 2012. Preliminary study on the genetic diversity of the Istrian sheep, Lika and Krk pramenka sheep populations using microsatellite markers. Acta Agriculturae Slovenica 100 (3), 125-129.

SUPPLEMENTARY 3

Salamon D, Gutierrez-Gil B, Arranz JJ, Barreta J, Batinic V, Dzidic A 2013. Genetic diversity and differentiation of 12 eastern Adriatic and western Dinaric native sheep breeds using microsatellites. Animal, doi:10.1017/S1751731113002243 (In press) (IF=1.648)

10. CURICULUM VITAE

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1988- 1996 - Elementary school Veliki Bukovec

WORK EXPIRIENCE

2008 - Current - junior researcher at University of Zagreb, Faculty of Agriculture, Department of animal science

- Main project: Genotypic and environmental effects on milkability and udder morphology in sheep, by Alen Dzidic, financed by Ministry of Science, Education and Sports, ID: 178-1780460-0407

2007 - 2008 - Expert biologist-herpetologist at Croatian herpetological society-Hyla, Zagreb

2006 - 2007 - Aquaristics at Pet centar d.o.o., Zagreb

2005 - 2006 - Zoology collection guide at Croatian natural history museum, Zagreb

BIBLIOGRAPHY AND ACTIVE PARTICIPATION IN CONFERENCES

Scientific publications

a1)

Dzidic A, Salamon D, Kaić A, Salajpal K, Kapš M 2009. Relationship between udder and milking traits during lactation in Istrian dairy crossbreed ewes. Italian Journal of Animal Science 8(3):154-156. (IF=0.139)

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