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Bolivian Creole cattle

Population structure, genetic diversity and management practices

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Bolivian Creole cattle – Population structure, genetic diversity and management practices

Abstract

Characterization studies of local livestock breeds are important to identify their potential and value for the Global Animal Genetic Resources. Important traits have been attributed to local breeds including adaptability and resilience to harsh environments and other traits with potential use in breeding schemes oriented towards the challenges of climate change. The aim of this thesis was to generate baseline information for the development of future conservation and breeding programs for the Creole cattle in Bolivia. For this purpose, three Creole cattle populations were included in this study: the Chaqueño (CHA), the Saavedreño (CEASIP) and a population from Pasorapa (PASO).

In the first part of this thesis, the main characteristics of the production and management system in Pasorapa were described. Further, the identification and evaluation of elements affecting the sustainability of the production system and the development of breeding and conservation programs was done. Open-ended and closed-ended questionnaires and interviews with 81 smallholders from 11 communities from Pasorapa were performed. Poor infrastructure and deficient health management practices were found. The rearing system based on two stages with animals released in the mountains for about 7 months of the year and feeding mainly of native plant species, showed a strong interaction between the cattle and the environment. Farmer perceptions about diseases, mortality causes and selection criteria were described, showing that additionally to body conformation, farmers considered coat color for selection of breeding and replacement animals. Productivity, income and ecosystem services were identified as the main factors affecting the sustainability of the system.

In the second part of this study, the population structure, genetic diversity, linkage disequilibrium (LD) and ROH based inbreeding levels of the three cattle populations were assessed using a 50K SNP array. Pairwise FST values indicated low to moderate differentiation between populations. Admixture analysis showed clear signs of admixture among and within populations. CEASIP had the highest proportion of ROHs for the length classes 4-8 Mb, 8-16 Mb and >16 Mb. FROH coefficient values were higher for CHA and CEASIP populations for the >4 Mb and >16 Mb length classes indicating past and recent inbreeding. PASO had the lowest inbreeding coefficients for all ROH length classes. LD and effective population size of PASO might have been influenced by recurrent bottleneck episodes. LD pattern in CEASIP indicated that a 50K SNP array could be applied for association studies in this population.

Keywords: Creole, management, farmer perceptions, genetic diversity, linkage disequilibrium, inbreeding, Runs of Homozygosity

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Abstract

Karaktärisering av lokala husdjursraser är viktigt för att klarlägga deras potential och värde för den globala genetiska mångfalden. Värdet ligger ofta i att de bär på många gynnsamma egenskaper såsom anpassningsförmåga till svåra miljöförhållanden och andra egenskaper som kan vara av nytta i avelsprogram som fokuseras på att svara mot de problem som klimatförändringarna leder till. Syftet med denna avhandling var att få fram grundläggande information som kan ligga till grund för utvecklingen av framtida bevarande- och avelsprogram för kreolnötkreatur i Bolivia. För detta syfte studerades tre kreolpopulationer: Chaqueño (CHA), Saavedreño (CEASIP) och en population från Pasorapa (PASO).

I den första delen av avhandlingen beskrivs huvuddragen i skötseln och produktionssystemet i Pasorapa. Dessutom analyseras de faktorer som kan påverka hållbarheten i produktionssystemet och utvecklingen av bevarande- och avelsprogram. Enkäter (såväl s k öppna som slutna) och intervjuer gjordes med 81 småbrukare från 11 samhällen i Pasorapa. Dålig infrastruktur och ofullständig hälsovård var vanliga problem. Produktionssystemet som bygger på två säsonger, där djuren under ranchsäsongen släpps lösa i bergen under ca 7 månader och då huvudsakligen livnär sig på den naturliga växtligheten, bygger på ett starkt samspel mellan djuren och den omgivande miljön. Böndernas uppfattningar om sjukdomar, orsaker till dödlighet och vilka kriterier man använde för att välja djur till avel beskrevs. Förutom olika kroppsmått var även färg en viktig egenskap man tog hänsyn till vid urval av djur. Produktivitet, inkomstnivå och ekosystemtjänster identifierades som de viktigaste faktorerna som påverkar hållbarheten i produktionssystemet.

I den andra delen av avhandlingen beskrivs populationsstrukturen, den genetiska variationen, kopplingsojämvikt (LD) och inavelsnivån i de tre populationerna med hjälp av ca 50 000 s k enbaspolymorfismer (SNP). De parvisa FST-värdena visade en tämligen liten differentiering mellan populationerna. Det fanns en klar uppblandning mellan och inom populationerna. CEASIP hade högst andel av homozygota segment (ROH) med längderna 4-8 Mb, 8-16 Mb och >16 Mb. Inavelsgraden baserad på ROH var högre för CHA och CEASIP för klasserna >4 Mb och >16 Mb, vilket tyder på såväl gammal som ny inavel. PASO hade lägst inavelsgrad för alla ROH-klasser. LD och effektiv populationsstorlek för PASO kan ha påverkats av nyligen inträffade flaskhalsar. LD-mönstret för CEASIP visade att det bör vara tillräckligt med ca 50 000 DNA-markörer för att kunna göra genetiska associationsanalyser i den populationen.

Nyckelord: kreolboskap, skötsel, lantbrukaruppfattningar, genetisk mångfald, kopplingsojämvikt, inavel, sekvenser av homozygoti

Dedication

To Kiara…
The music of my heart, and the strength of my soul
I could not have had a better partner in this journey

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This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Bottani Claros G*, Jonas E, Rojas Beltrán J A and Strandberg E (2019). Description of the production and management system of the Creole cattle from Pasorapa, Bolivia, a well-adapted population to harsh environments. *Livestock Research for Rural Development. Volume 31, Article #156.* Retrieved January 29, 2020, from http://www.lrrd.org/lrrd31/10/gabri31156.html

II Bottani Claros G, Jonas E, & Strandberg E. Relevance of the production system for conservation and breeding programs for the Creole cattle in Pasorapa, Bolivia. *Manuscript*

III Bottani Claros G, Jonas E, Lopez Maria E., Johansson A.M & Strandberg E. Genetic diversity and population structure of the Bolivian Creole cattle using a 50K SNP array. *Manuscript*

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Abbreviations

AnGR	Animal Genetic Resources
CBBP	Community-based breeding program
CEASIP	Centro de Ecología Aplicada Simón I. Patiño
DAD-IS	Domestic Animal Diversity Information System
FAO	Food and Agriculture Organization
FIS	Inbreeding coefficient
FMD	Foot and mouth disease
FROH	ROH based inbreeding coefficient
HE	Expected heterozygosity
HO	Observed heterozygosity
IBS	Identical by State
kb	Kilobase
LD	Linkage Disequilibrium
LTA	Livestock technical assistants
Mb	Megabase
Ne	Effective population size
PCA	Principal Components Analysis
ROH	Runs of homozygosity
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
SWOT	Strengths weaknesses opportunities Threats
UAGRM	Universidad Gabriel Rene Moreno
UAJMS	Universidad Autónoma Juan Misael Saracho

1 Background

1.1 Creole cattle in The Americas

Livestock species arrived to The Americas for the first time when Cristopher Columbus reached the continent for the second time in 1493. The arrival was to the Hispaniola, an island in the Caribbean archipelago, part of what is known today as the Greater Antilles. The stock consisted of a few hundred to one thousand animals derived mainly from Cadiz and the Canary Islands (Gautier and Naves, 2011; Martínez et al., 2012; McTavish et al., 2013). These animals did not belong to any specific breed and showed a great variability of phenotypic traits. It is possible that this observed variability facilitated the evolution of the different ecotypes of Creole cattle observed nowadays on the continent (De Alba Martinez, 2011). Importation of livestock continued with the following trips until around 1512, when the successful survival and multiplication of animals allowed the self-sufficiency of cattle in the colonies. Spanish conquerors introduced cattle through the Hispaniola, whereas the Portuguese route was mainly from Brazil (Ginja et al., 2010; McTavish et al., 2013).

The initial multiplication of cattle was done in The Hispaniola and Puerto Rico for around four generations after their arrival. Animals multiplied almost in complete freedom and only under pressure of natural selection. Animals with short hair, pigmented skin and tolerant to high temperatures were favored. Animals taken to environments with higher altitudes like Pico Duarte (3,098 m.a.s.l), showed instead predominately long hair and black coat color (De Alba Martinez, 1987, 2011). In the following years cattle moved to the south with conquerors and settlers searching for new lands to be claimed in the name of the Spanish Crown arriving then to Cuba, Panama, Mexico, Nicaragua, Venezuela, Colombia, Ecuador, Peru, Bolivia, Argentina and finally to Paraguay.

Among the main Creole cattle breeds described in South America nowadays there is a great variation of exterior and production characteristics. Some of these populations are at risk of extinction. Creole cattle are found in different regions of South America and some examples are given here. For the Colombian Creole cattle, for which 7 breeds have been well described: Costeño con Cuernos, Romosinuano, Blanco Ojinegro, Harton del Valle, Casanareño, Sanmartinero and Caqueteño (De Alba Martinez, 1987; Asocriollo, 2003). In the Dominican Republic the Romana Red breed (Romana Rojo) is derived from a crossbreeding between the Creole cattle from the country and zebu breeds. The Cuban Creole cattle reared for milk and beef production are predominately dun colored with short hair similar to the Romosinuano breed (De Alba Martinez, 1987).

The Creole cattle in Ecuador, which are used for milk and beef production, have not been selected for coat color, and a great variability for this trait is observed (Ceballos F., 2012). Such color variability is also observed in the Creole cattle populations of the Patagonia in Argentina, being adapted to a cold wet environment with average temperatures between -3°C and 12°C, and descendant from the bovines introduced from the end of the 18th century to the beginning of the 20th century in the Pampa region (Martinez, 2008). The Argentinean Creole breed from the northwest part of the country shows the same variability of coat color, the most frequent being the red color. Both ecotypes from Argentina are used mainly for beef production (Lirón et al., 2006; Martinez, 2008).

The Creole breed in Venezuela is named Limonero and it is composed by animals adapted to a tropical environment, but a recent study indicated that this population is at risk of extinction (Florio-Luis and Pineda-Graterol, 2018). Mexican Creole cattle have as main characteristics the variability of coat colors and the presence of long horns (Quiroz, 2007).

The Creole cattle population in Brazil, is a result of a fusion between the first animals brought from Spain and Portugal during the Colonial Period (Camargo, 1990). The most important Creole breed in this country is the Caracú. Animals are considered as dual purpose, dun or red colored with males exhibiting darker brown color on head and shoulders. The “Mocho Nacional” breed is another Creole breed of Brazil, characterized by polled animals with red or bay coat color. In early 1990s only a dozen individuals were reported (Primo, 1992).

1.2 Creole cattle in Bolivia

It was not until 1549 that cattle arrived to Tarija, Bolivia, with the settlement of Juan Nunez Del Prado in the south part of the country (De Alba Martinez, 2011). Documentation from the Ministry of Rural Development and Land from Bolivia states that in 1548, cattle were also introduced to what is nowadays the Department of Beni with the first Jesuit mission. Since then, four different cattle populations have been described the Yacumeño, the Saavedreño, the Chaqueño and the Chusco Creole cattle (Vargas, 2013).

1.2.1 The Yacumeño Creole cattle

This population is mainly found in the North East part of the country (Department of Beni). They are adapted to a seasonal flood plain with an average of 2000 mm of annual precipitation. It was raised for beef production. The most representative group of animals was conserved in the Estancia Espiritu farm from 1964 until 2005, when only 120 females and 5 bulls were conserved by the Gabriel Rene Moreno Agrarian University (UAGRM), with the purpose of starting a breeding and conservation program of the Yacumeño Cattle (Vargas, 2013).

Animals are mainly red or yellow coated with black spot around the eyes. The cowboys of the area used to describe these animals as “Caracu Colorado ojinegro” (Vargas, 2013).

1.2.2 The Saavedreño Creole cattle and the CEASIP herd

This population is adapted to a tropical plain and is raised for beef. It was mainly found in the Experimental Station of Saavedra in the Department of Santa Cruz. In 1978 the Research Center for Tropical Agriculture (Centro de Investigacion Agricola Tropical-CIAT) selected animals from different farms in the Department of Santa Cruz and started a project for genetic improvement of this population. The purpose was to obtain a dual purpose breed. According to the available information artificial insemination using semen from Creole bulls from Bolivia, Costa Rica, Nicaragua, Cuba and Brazil was used (Rojas T., 2015).

In 2007 the Center of Applied Ecology Simon I. Patiño CEASIP (Centro de Ecologia Aplicada Simon I. Patiño) established a herd of 16 females and 2 males from an initial stock of 90 animals. Selected animals to be part of the herd came from the Experimental center of El Salvador (1 animal), the Farm “Las Colinas” (3 animals), the Tatayti farm (1 animal), the farm “Alta-Vista” (1 animal) and the CIAT (12 animals). The CEASIP herd has been raised as a dual purpose cattle. Males are sold to be sires or for beef production once they are 24 months old. Their live body weight is on average 520 Kg at that age. Milk produced is used for the production of yogurt, cheese and butter (CEASIP information).

1.2.3 The Chaqueño Creole cattle

This population is found in the south-eastern part of Bolivia (Departments of Tarija, Chuquisaca, and South of Santa Cruz) and it is adapted to a dry forest environment. Animals have been raised mainly for beef production. The Experimental Center of El Salvador in the Department of Chuquisaca has carried out a conservation program of the Chaqueño cattle population. Unfortunately, pedigree information and data of productive and reproductive traits was lost because of the lack of continuity of government authorities.

During the field work in this study a small research center for Chaqueño Creole cattle was identified in the Municipality of Villamontes, in the Department of Tarija. The "Kilometer 20" research center had a small herd of around 100 individuals. Unfortunately it was not possible to get pedigree information or additional data from the animals.

The University Juan Misael Saracho (Universidad Autónoma Juan Misael Saracho- UAJMS) from the Department of Tarija is in charge of the conservation of one herd of Chaqueño cattle in the Experimental Center in the community of Puerto Margarita, located in the south of Bolivia.

1.2.4 The Chusco Creole cattle

The Chusco population is adapted to the highland plain of Bolivia with an average altitude of 4000 m.a.s.l. and average maximum temperatures up to 2 to 14°C and average minimum temperatures between -1.5 and -6°C. Little information is available about this population. Farmers from the highland plain have an average herd sizes of 9 heads of cattle. Animals are raised for milk, beef production and draught power. Long, thick and dark colored coat is predominant in the herds. Feeding is based on native pastures and barley and animals are exposed to the harsh climate conditions because the infrastructure of farms is very poor. Animals are frequently found held in a trunk outside overnight (Rojas P, 1988).

1.3 Genetic diversity

Genetic diversity is defined as the set of differences between species, breeds within species and individuals within breeds. These differences are present in their DNA or observed in the animals as a consequence or expression of it (Oldenbroek, 2017). Availability of affordable methods to analyse the DNA, for example via genomic markers, allows to measure the genetic diversity directly in the genome.

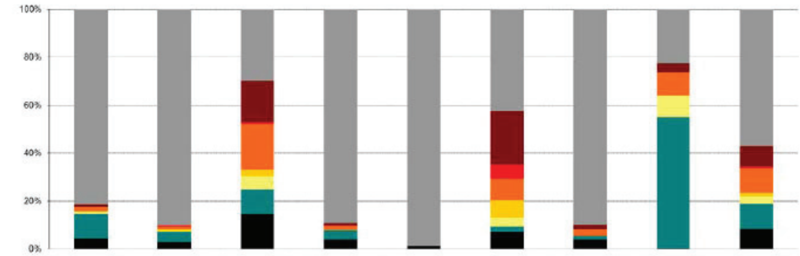
In natural populations, genetic variation is important for the process of adaptation. If a population is large enough it has better chances to gradually become adapted to its environment (e.g. altitude, climate, pathogen pressure). However, it is important that the genetic diversity in such population is large as well. More diversity implies better chances that the population will contain individuals carrying alleles that contribute to the adaptation to new environmental conditions, and those individuals will transmit such favorable alleles to the next generations. The process of natural selection of adaptive traits depends in general on the genetic diversity present in the population.

Additionally, the capacity of a livestock population to respond to artificial selection depends on the level of genetic variation for the trait undergoing selection (Kristensen et al., 2015). In other words, the greater the genetic diversity within the breed resulting in more phenotypic variation for the trait, the greater the potential selection response. Diversity among breeds is also important. Crossbreeding programs with specialized breeds are very efficiently applied in commercial breeding programmes for pigs and poultry, as well as in beef and mutton production systems (Oldenbroek, 2017).

But with natural and artificial selection of populations, it is also important to conserve genetic variation for future breeding strategies in order to match individuals to a variety of husbandry systems and to allow adaptation to environmental changes (Lenstra et al., 2012). Adaptation is necessary as a mechanism to respond to climate change and meet future challenges, for example new or resurgent disease threats, increasing temperatures, modified precipitation patterns or increased frequency of extreme events (Hoffmann, 2013; Leroy et al., 2018).

1.3.1 Livestock genetic diversity status

Genetic diversity in livestock is declining rapidly as specialization in plant and animal breeding and the harmonization effects of globalization advance. According to the Global Databank for Animal Genetic Resources which currently contains data from 182 countries and for 38 species, a total of 7,745 local breeds have been reported (FAO, 2019). The most up-to-date data available in DAD-IS (Domestic Animal Diversity Information System of FAO) shows that among mammalian species, sheep, cattle and horses have the largest number of breeds at risk. The same report shows a large number of breeds for which no risk-status data are available. This lack of data is a serious constraint to effectively prioritize and plan breed conservation measures. Latin America and the Caribbean Region have one of the highest proportions of mammalian breeds with unknown risk status (Figure 1). In the particular case of Bolivia, around the 70% of the number of breeds recorded in the DAD-IS have an unknown status and lack population data.



	Africa	Asia	Europe & the Caucasus	Latin America & the Caribbean	Near & Middle East	North America	Southwest Pacific	International transboundary breeds	World
Unknown	578	1238	841	452	188	59	132	88	3 576
Critical	7	3	479	4	0	31	3	14	541
Critical-maintained	0	6	25	2	0	8	0	0	41
Endangered	13	17	539	9	0	13	4	38	633
Endangered-maintained	1	10	70	1	0	10	0	0	92
Vulnerable	7	3	160	0	0	5	0	34	209
Not at risk	71	56	287	18	0	3	2	212	649
Extinct	33	43	410	21	2	10	6	1*	526
Total	**710**	**1 376**	**2 811**	**507**	**190**	**139**	**147**	**387**	**6 267**

*African Aurochs, which once lived in parts of both the Africa and the Near and Middle East regions.

Figure 1. Risk status of mammalian breeds in the world until March 2018. The table expresses absolute values and the chart percentage figures by region. Source: Commission on Genetic Resources for Food and Agriculture 2019.

Among mammals, 183 cattle breeds have been reported as extinct in the world, 20 of which belong to Latin America and the Caribbean region. Since 2007, few updates on the population size of local breeds were provided by countries. The result is a still high proportion (61%) of local breeds with unknown risk status in 2018 (FAO, 2019). Figure 2 illustrate the percentage of local breeds not at risk, at risk and with unknown risk status by region in the world.

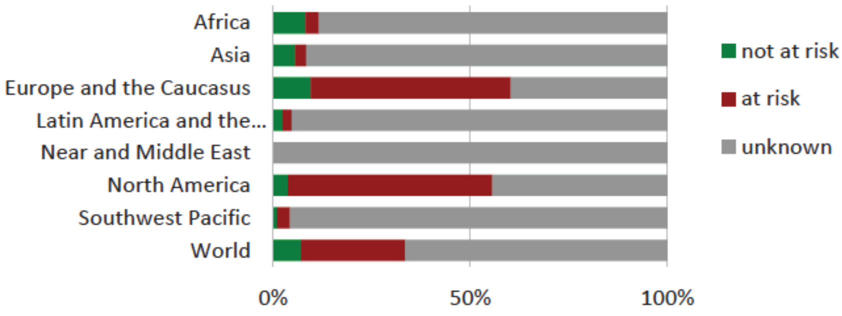


Figure 2. Proportion of local breeds classified as being at risk, not at risk or unknown level of risk of extinction. Source: Commission on Genetic Resources for Food and Agriculture 2019.

The target 2.5 of the 2030 Agenda for Sustainable Development refers to the maintenance of diversity of farmed and domesticated animal populations. One of the indicators to evaluate this target is the proportion of local breeds and their classification on the level of risk of extinction (Transforming our world: the 2030 Agenda for Sustainable Development, 2015). In this sense, generation and updating of information about local breeds is important to evaluate the achievements of the 2030 Agenda.

1.4 Local adapted livestock breeds

In rural areas of developing countries, local livestock breeds play a crucial role in food security, nutrition and health. These animal genetic resources have socio-economic, cultural, historical and ecological value. From a genetic point of view, they contribute across-breed genetic diversity to the global animal genetic resources (AnGR), which is as important as the within-breed diversity in terms of conservation genetics (Ollivier and Foulley, 2005; Biscarini et al., 2015).

Important traits that have been attributed to local breeds are disease resistance, low quality feed tolerance, relatively high reproductive performance especially under poor environmental conditions, extreme temperature tolerance and low maintenance requirements (Thornton, 2010; Hoving et al., 2014; Shabtay, 2015). Thus, locally adapted breeds are expected to cope better with the effects of climate change than exotic temperate breeds (Hoffmann, 2013). Moreover, a local breed can be likened to cultural property, because they might be a point of reference in ancient local traditions, playing a role in the agriculture tenures and social life of rural populations (Gandini and Villa, 2003).

Unfortunately and their importance notwithstanding, many of the local breeds are at risk of extinction or their status of risk is unknown because of lack of information (FAO, 2019). One main factor driving the loss of these well adapted local breeds, is that they are less productive compared to exotic breeds (Biscarini et al., 2015; Shabtay, 2015). With the purpose of improving their productivity, local breeds have been crossed with high productive exotic breeds. Usually, the crossbred animals show better performance in productive traits in a short term, but over time, they tend to fail as many of these crossbred animals are not well adapted to the harsh climate conditions and management practices, including the use of low quality feeding (Philipsson et al., 2017). Uncontrolled crossbreeding and the loss of interest among farmers when positive results cannot be maintained have led to a reduction of the population size in many local populations.

The global marketing of exotic breeding material and the gradual substitution of local breeds with such stock poses a threat to the conservation of Animal Genetic Resources. Unfortunately, there is a decrease of the number of breeds adapted to low inputs and a replacement with breeds that respond to high inputs. In many countries and areas of the world local breeds guarantee a livelihood in harsh areas where high-input high-output livestock systems are not feasible. The common complete absence of breeding programs for these breeds, is also a direct threat for the survival of the breed. In other cases, unsuitable breeding programs have been implemented in the production systems of local breeds. Such selection schemes and management requirements depend on high outputs in response to high inputs in less developed countries, where the livestock typically have low outputs but are still able producing from low inputs and survive in more severe environments (Oldenbroek, 2007).

Even though local breeds are well adapted to the harsh environments where they are reared, there is a limited or complete lack of their use in structured breeding programs. The main reason is, that they are often not fully characterized (Frankham et al., 2014). Nowadays very little is known about the performance and fitness of local breeds in many developing countries. There is a need to increase that knowledge in order to define their importance and utilize them efficiently.

Conservation of these local breeds is important from a genetic but also a socio-economic and ecological point of view. Local breeds play and will play an important role for farmers in the future. In order to improve livelihoods of smallholders in developing countries, suitable breeding and conservation programs must be implemented. Development and implementation of programs adapted to the local environments and specific management practices must be seen as long term investments. A first and key step to achieve this is the complete characterization of the locally adapted breeds in terms of their genetics and the production systems to which they are adapted.

1.5 Classification of livestock rearing systems

Systems of livestock production vary with climate conditions, availability of production resources, the economic ability and market orientation of producers, and consumer demands (Thornton, 2010; Ojango et al., 2016). Different studies have found that also factors such disease risks and impacts, livelihood benefits and environmental risk of crops vary considerably according to the type of production system (Robinson et al., 2011).

Robinson et al. (2011) reviewed the main classification of production systems that have been proposed. Here we describe some of them, starting with the classification proposed by Rothenberg in 1980, which classifies the cultivation according to the type of rotation, the intensity of rotation and the type of water supply (irrigated or rainfed). According to this classification the grassland utilization has the following levels and characteristics:

- Total nomadism: With no permanent place of residence and no regular cultivation performed.
- Semi-nomadism: With a permanent place of residence and use of supplementary cultivation but for long periods of time animal owners travel to distant grazing areas.
- Transhumance: With a permanent residence place, but herds are sent to distant grazing areas usually on seasonal cycles.
- Partial nomadism: With farmers living continuously in permanent settlements and have herds at their disposal that graze in the vicinity.
- Stationary animal husbandry: With animals remaining on the holding or in the village throughout the year.

Dixon et.al (2001), proposed a system based only on whether production was rainfed or irrigated. Detailed levels of this classification system is as follows:

- Irrigated farming systems
- Wetland rice based farming systems
- Rainfed farming systems in humid high potential areas with systems with predominant crop activity and mixed crop-livestock systems.
- Rainfed farming systems in steep and highland areas, often mixed crop-livestock systems.
- Rainfed farming systems in dry or cold low potential areas with mixed-crop livestock and pastoral systems.
- Dualistic mixed large-scale commercial and smallholder farming system
- Coastal artisanal farming systems, with mixed farming.
- Urban-based farming systems, focused on horticulture and animal production.

A more complex classification was proposed by Sere and Steinfeld in 1996 (Robinson et al., 2011) implying different levels for two main types of production systems: Solely livestock systems and Mixed farming systems. A brief description of both systems and the levels to be found in them is as follows:

- Solely livestock systems: In which more than 90 percent of the dry matter fed to animals comes from rangelands, pastures, annual forages and purchased feeds, and less than 10 percent of the total value of production comes from non-livestock farming activities.
    - Grassland based system, in which more than 10 percent of the dry matter fed to the animals is produced on the farm and the annual average stocking rate is less than 10 temperate livestock units per hectare. In other words, the crop-based agriculture is minimal.
    - Landless livestock production system, in which less than 10 percent of the dry matter fed to the animals is produced on the farm and the annual average stocking rate is more than 10 temperate livestock units per hectare.
- Mixed farming systems: In which more than 10 percent of the dry matter fed to animals comes from crop by-products or stubble, or more than 10 percent of the total value of production comes from non-livestock farming activities. Mixed farming can be classified then as:
    - Rainfed mixed farming
    - Irrigated mixed farming

With focus on the vulnerable poor livestock systems of Asia and sub-Saharan Africa, Thornton et al. (2007), characterized three types of livestock systems in a simplified way:

- Agro-pastoral and pastoral systems: in which natural resources are constrained and people and their animals adopt strategies to meet these constraints.
- Smallholder crop-livestock systems in which natural resources can be managed to intensify the production of the system.
- Industrial livestock systems, which are highly intensive and tend not to be so tied to the local natural resource base, as are the agro-pastoral and smallholder mixed systems.

A similar simplified classification of farming systems is used by “The High level Panel of Experts for Food Security and Nutrition of the Committee on World

Food Security” (HLPE, 2016; Magnusson, 2016). A summary of that classification is shown in Table 1.

Table 1. *Typology of farming systems in the HLPE report.*

Livestock system	Livestock species	Social features
Smallholder mixed farming	Mostly pigs, poultry and dairy	Family labor. Most common type of farm in the world.
Pastoral systems	Cattle, sheep, goats, camels and camelids	Mobile, with common use of pool of resources, often weak land right. Family based system
Commercial grazing systems	Beef, dairy and sheep	Secure access to land and strong land rights. Use of rural labor for hire.
Intensive livestock systems	Mainly pigs and poultry, but also feedlots for beef	High level of productivity. Hired labor at a significant level.

Adapted from Magnusson (2016)

2 Aims of the thesis

The importance of local breeds for the Global Animal Genetic Resources and the livelihoods of smallholders in developing countries has been well established. Nevertheless, many of these cattle populations and the valuable adaptive traits they carry are threaten, owing to unplanned crossbreeding or the unsustainability of their production systems. Lack of information about pedigree, genetic diversity and inbreeding status are a common limitation for the development of suitable breeding and conservation programs in these production systems. Information about current management practices and farmer perceptions may also contribute to establishing such programs.

The general aim of this thesis was to generate base information for the development of future conservation and breeding programs for the Creole cattle populations in Bolivia.

The specific aims in this thesis were:

- Describe the most important elements related to the management practices for Creole cattle in Pasorapa, Bolivia.
- Identify and evaluate elements affecting the sustainability of the production system in Pasorapa and the development of breeding and conservation programs.
- Study the population structure, genetic diversity and inbreeding level of three Creole cattle populations using a medium density SNP platform (50K)

3 Summary of studies

This thesis comprises three papers (I-III) with the aim of generating baseline information for breeding and conservation programs for three populations of Creole cattle in Bolivia: the Chaqueño, the Saavedreño (CEASIP), and a third newly described population from Pasorapa. In paper I, main elements related to the management practices for one of the populations, the Creole Cattle from Pasorapa, Bolivia are described. In paper II, an analysis of the rearing system, its sustainability and the farmer perceptions about health, breeding strategies and selection criteria in Pasorapa is performed. In paper III, the genetic diversity and population structure of the three listed populations was studied using a 50K SNP platform.

3.1 Materials and methods

The work in this study was divided in two main parts. Part 1 focused on the collection and analysis of data used in papers I and II. Part 2, comprised the analysis of the genotyping data used for paper III.

3.1.1 Study area

This study was carried out in four of the nine Departments of Bolivia: Cochabamba, Santa Cruz, Chuquisaca and Tarija. Seventeen communities and 97 farms were included in the study. Table 1 summarizes the information about the sampling area, number of communities, number of farms and the number of sampled animals.

Table 2. *Summary of the Departments, communities, farms and sampled cattle individuals included in the study.*

Cattle Population	Study area	Departments	Communities	Number of farms	Sampled Individuals
Pasorapeño (PASO)	Pasorapa	Cochabamba	11	81	378
Chaqueño (CHA)	Chaco	Santa Cruz Chuquisaca Tarija	6	13	305
Saavedreño (CEASIP)	CEASIP	Santa Cruz (Experimental Center)	1	1	100

Pasorapa is located in the Second Section of the Province Narciso Campero in the south-easternmost part of the Department of Cochabamba in Bolivia. Its altitude ranges between 1200 and 2068 m.a.s.l. The average temperature is 15.5°C, with temperatures ranging from below 0°C to 33.9°C. Communities included in the study were: La Aguada, Buena Vista, La Abra, Pasorapa (OTB Norte), Conchu Laguna, Robles, Ruda Pampa, Seibas, Tabacal, Toyota Baja and Zapallar. A total of 985 farms keeping cattle in the 33 communities of the Municipality were registered in the database of the Municipal Government of Pasorapa until 2017. In the 11 communities included in the study, 375 farms are registered, out of which we were able to include 81 (22%). Information about the purity of the cattle herd is not included in the database. For this reason, farms included in the study were previously inspected in order to determine if the herds were composed by pure Creole or crossbred animals.

Around 13% of the Chaco Area in South America belongs to Bolivia. This xerophytic forest is located in the southeast part of the country and has an area of around 15,3000 Km2, covering part of the Departments of Santa Cruz, Chuquisaca and Tarija. The altitude ranges between 800 and 2000 m.a.s.l. Temperatures are between -15°C and 35°C and the average annual precipitation is 300-500 mm. Sample collection in this study was performed in the municipalities of Yacuiba, Villamontes, Puerto Margarita, Machareti and Boyuibe including samples from the Experimental Centers of El Salvador and Kilometer 20, established for conservation and breeding purposes.

The Center of Applied Ecology Simon I. Patiño (CEASIP) is located in the Department of Santa Cruz and has as a main purpose the development, validation and transfer of methodologies to implement sustainable agriculture in tropical soils. This center owns an important genetic reserve of the Bolivian Saavedreño Creole cattle. To improve the genetic quality of the herd insemination of animals using the semen of sires from other herds is performed. In turn, semen of bulls from the center is sold to farms with Creole herds. Figure 3 illustrates the distribution of the Bolivian Creole cattle populations and the sampling points in this study.

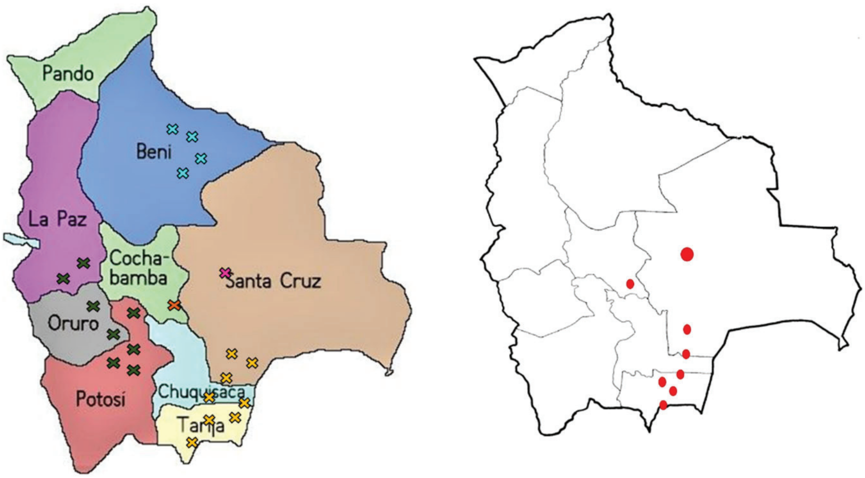


Figure 3. Map of Bolivia with the distribution of Bolivian Creole cattle populations (left) and sampling points (right).

3.1.2 Collection and analysis of data for Papers I and II

The field work consisted in the following activities: reconnaissance trips and selection of participants, execution of questionnaires and interviews, field observations, and sample and data collection from animals. Questionnaires, interviews and field observations were performed only in the Community of Pasorapa.

Reconnaissance trips and selection of participants

At the beginning of the study there were no reliable records available about the proportion of pure Creole and crossbred animals in the study area. For this reason a series of reconnaissance trips was performed in Pasorapa and the Chaco area in order to identify the farms with a larger number of Creole type animals, and farmers willing to participate in the study.

A list of typical morphological traits of Creole individuals was elaborated, based on interviews performed with the veterinarian of Pasorapa and the older farmers from Chaco and Pasorapa. Once criteria to discriminate between herds of Creole and crossbreed cattle were established, identification of farms rearing mainly Creole cattle was done.

Criteria for selection of participants were: ownership of Creole cattle herds, accessibility to the community and to the farm, and willingness of farmers to participate in the study. Snowball sampling was followed to identify more participants. Because more distant communities were accessible only by motorbike or by horse, and the conditions of roads were not always good, these factors were the most predominant criteria for selecting the communities for the study.

Questionnaires and interviews

Questionnaires and interviews were performed in Pasorapa in two stages. The first stage consisted of open and closed-ended questionnaires (Q1) with 81 farmers distributed over the 11 communities included in the study. In Q1, qualitative and quantitative data related to householders, location of the farms (including a map and road access (horse, motorbike, or car)), herd structure, health management, feeding strategies, water sources mortality causes of cattle, and information on the production system were collected.

A second stage with open-ended questionnaires (Q2) and face to face in-depth interviews (Int-A) following a semi-structured approach was carried out with a subset of 16 farmers belonging to 8 of the 11 communities, identified as the most traditional Creole cattle breeders. Data saturation was reached before interviewing all 16 farmers. This second stage was performed to obtain additional qualitative data to understand the reasons behind rearing and management practices. Variables covered in Q2 were related to calf management, selection criteria of sires and replacements, improvements of infrastructure, workforce and association between coat color and temperament or beef and milk production. During the interviews in the second stage (Int-B), the main topics covered were: main concerns about the production systems, perceptions about the value of the breed, diseases and treatments, and difference between crossbred and pure Creole individuals.

In January 2018, 10 of the subset of 16 farmers included during the second stage were interviewed once more to identify possible changes in perceptions obtained during Int-B. Because of the reduced availability of time on this last field work, it was not possible to contact and set a meeting with all members of the subset of 16 farmers from the second stage.

Questionnaires and interviews were prepared in Spanish. Interviews were transcribed to text documents and analyzed with the QSR International's NVivo 10 software, to identify the most frequent expressions and terms given by the farmers related to a specific topic or variable. Basic descriptive statistics were calculated for quantitative data using Excel and R version 3.5.1 (R Core Team, 2018).

Field observations

During the farm visits in Pasorapa, direct observations about infrastructure of the paddocks and the main characteristics of the management practices were documented as field notes and pictures. If visits were scheduled between June and October (Paddock stage), tours in the farms surroundings were done to observe the cattle behavior and feeding options. If the interviews were scheduled between November to June, and depending of the availability of a guide, tours across the rangeland were done to observe behavior, feeding options, eating behavior, places to sleep (dormideras), water sources in the rangelands, and walking ability of the Creole cattle in rugged terrains. Information from the rangelands was documented in field notes, pictures and video recordings. With the help of a guide, who was always a farmer from Pasorapa, native plant species consumed by the cattle were collected and identified by their common name with the support of at least three different farmers. Taxonomic identification was done at San Simon University with the support of the appropriate keys. Information of age and sex of cattle was collected for a general overview of the herd structures. Additional data collected but not used in the three studies are described in section 4.1.4 below.

3.1.3 Collection and analysis of data for paper III

Animal sampling and DNA extraction

Hair samples were collected from 829 individuals. A total of 424 individuals were sampled in Pasorapa during both the Paddock and the Ranch stages, 305 samples were collected in the Chaco area and the complete herd of 100 individuals were sampled in the CEASIP Center at the Department of Santa Cruz.

For the genetic analysis samples were grouped into three main populations, Pasorapa (PASO), Chaco (CHA) and CEASIP. Because of distance between sampling areas, animals from Chaco were split into 7 subgroups: Boyuibe (ChBo), Villamontes (ChKm), Puerto Margarita and Palos Blancos (ChMar), Macharejti (ChacoSal) and Yacuiba (ChYac).

DNA was extracted from hair samples using Chelex 5%. After discarding possibly related individuals, a total of 672 samples were genotyped using the GeneSeek Genomic Profiler (GGP) Bovine 50K SNP array. An identical by state (IBS) analysis was performed using PLINK software v1.90b6.9 (Purcell et al., 2007) before the quality control (QC). After QC 273 samples from Pasorapa, 281 samples from the Chaco area and 100 samples from the CEASIP herd were included in following steps of the analysis.

Genetic diversity parameters and Inbreeding

Inbreeding coefficients, expected (H_E) and observed (H_O) heterozygosities were computed using PLINK (Purcell et al., 2007). Inbreeding coefficients (F) for the populations and the whole set of them were calculated based on Wright's definition (Wright, 1951) as the difference between the expected and the observed heterozygosity over the expected heterozygosity. F_{ST} values between populations were calculated using StAMPP (Pembleton et al., 2013). Molecular diversity indexes such nucleotide diversity (π) and polymorphic loci were computed using the ARLEQUIN software V 3.5.1.2 (Excoffier and Lischer, 2010).

Population Structure analysis

A principal component analysis (PCA) was performed after linkage disequilibrium (LD) pruning of data, eliminating markers with an LD higher than 0.5. As a result, the PCA analysis was performed with 33,106 variants and 654 individuals. Global genetic ancestry was analyzed with a model based clustering approach using STRUCTURE (Pritchard et al., 2000). The software was run using the R package ParallelStructure (Besnier and Glover, 2013). The length of the burn-in period was 1000, with 10000 iterations for the Markov chain Monte Carlo sampling and 3 replicates were performed. The best K was determined with the Evanno method (Evanno et al., 2005) using the Structure Harvester program (Earl and VonHoldt, 2012).

LD decay and Effective population size (N_e)

Linkage Disequilibrium (LD) estimation was done with the squared Pearson's correlation coefficient (r^2), computed as pairwise comparisons limited to a window of 1000 kb. LD decay was plotted using the average values of r^2 for each bin of 10 kb. Historic effective population size was calculated based on the relationship between the squared Person's correlation coefficient (r^2), the effective population size (N_e) and the recombination rate (c) using SNeP software (Barbato et al., 2015). The recombination rate between pairs of SNPs was inferred applying the Haldane's model (Haldane, 1919). Contemporary N_e was computed using the LD based method with the software NeEstimator V2.1 (Do et al., 2014). Critical values of Pcrit (0.05, 0.02 and 0.01) were applied.

Runs of homozygosity and F_{ROH}

Runs of homozygosity (ROH) were identified using the detectRuns R package Version 0.9.6 (Biscarini et al., 2019) using the consecutive method (Marras et al., 2015). The criteria followed for the identification of runs was: (i) The minimum length of ROH was set to 1Mb, (ii) the minimum number of SNPs in a run was fixed to 15, (iii) the maximum distance between adjacent SNPs was 1Mb, (iv) the number of allowed heterozygotes was set to zero and (v) a maximum of two missing genotypes were allowed. The dataset analysed was not pruned for LD, since the minimum length of the run was set to 1Mb.

The genomic ROH based inbreeding coefficient (F_{ROH}) for Creole cattle populations was estimated for three ROH length classes: 4-8Mb, 8-16Mb and >16Mb, to avoid an overestimation of small ROH segments (Ferenčaković et al., 2013b, 2013a).

3.1.4 Additional data collected for the phenotypic characterization across populations

Morphometric measurements and phenotypic data were collected for the three populations. The number of measurements taken was limited by the behavior of the animals and lack of appropriate infrastructure. Data collected was related to: coat color, horn shape, sex, age, and three, five and seven morphometric measurements for the PASO, CHA and CEASIP samples respectively. Because of lack of records in all farms in the CHA and PASO populations, information about age was given from the farmers at the moment of collecting data from each animal. Data related to coat color and horn shape was documented in pictures for all sampled individuals.

Body measurements were obtained using a measuring tape. For animals from farms sampled in Pasorapa the three body measurements taken were: Withers height (WH), Oblique Length (OL), and Hip height (HH). The Five measurements taken in animals sampled in the Chaco population were: Withers height (WH), Oblique Length (OL), Hip height (HH), Chest girth (CG) and the Bicostal Diameter (BD). In the CEASIP, since animals had a more calm behavior and they were used to be handled by humans it was possible to take the following measurements: Withers height (WH), Oblique Length (OL), Hip height (HH), Horizontal length (HL), Chest Depth (CD), Bicostal diameter (BD) and Hip length (HG). Figure 4 illustrates each body measurement location in the body of the animal.

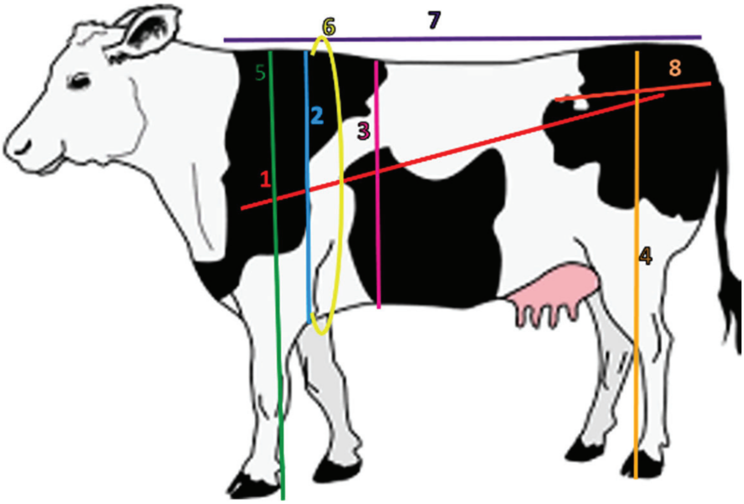


Figure 4. Scheme of body measurements parts taken. 1) Oblique length (OL) 2) Chest depth (CD) 3)Bicostal diameter (BD) 4) Hip height (HH) 5) Wither height (WH) 6) Chest girth (CG) 7) Horizontal length (HL) 8) Hip length (HG).

3.2 Main findings

3.2.1 Description of the production and the management system of the Creole cattle from Pasorapa, Bolivia, a well-adapted population to harsh environments

The rearing system for Creole cattle in Pasorapa is divided into two seasons per year. A Paddock stage (dry season) between June and October and a Ranch stage (rainy season) between November and June. Animals are not herded during the Ranch stage spending most time browsing in the hills (Figure 5). The main crops used for farmers for their own consumption and as feed for cattle in all communities were: maize (93%), oat (38%), sorghum (14%), and barley (5%). In the communities of Conchu Laguna and Ruda Pampa wheat was used by 22% of the farmers Silage was produced on small scale. Figure 6 illustrates Creole cattle feeding on crop residues during the Paddock stage in the community of Seibas.

Figure 5. Creole cow found during the Ranch stage.

Figure 6. Creole cattle feeding from crop residues during the Paddock stage.

Most farms had poor small no-grazing pens made of wood boards, trunks and bushes (Figure 7). Lack of crush pens, neck clamps or squeeze chutes makes handling of animals difficult and dangerous. No feed bunks and few water troughs were observed in the paddocks. Water ponds called "atajados" are built in the ground of the farm surroundings (Figure 8) and across the ranches to provide water for animals. Some farmers stored maize green forage to feed animals on surfaces built on the top of willows or a cactus species called Carapari, these structures are called "Calcheros".

Figure 7. Paddock for Creole cattle made of trunks and bushes.

Figure 8. Creole cows going to drink water in an "Atajado" during the Paddock stage.

Herd sizes varied between the years due to mortality and high sale frequencies during drought periods. Weaning was done when calves were 4 to 8 months old.

Male calves selected by the farmers as future sires suckle up to one year. Calf/cow ratios varied substantially between farms, with no apparent effect of community. 12% of farms showed a ratio of at least 1 calf per cow, 46% a ratio of 1 calf for every 2 cows, 26% of farms had a ratio of 1 calf for every 3 cows, and 14% showed a ratio of 1 calf for more than 5 cows. The lowest ratio was 1:16 found in one farm. Two landholders with herd sizes of 11 and 12 heads of cattle reported no calves. Ratios between young bulls and breeding bulls varied also between farms. 19% of farms kept only breeding bulls (from 1 to 7) in the herd, 14% had no male, 23% had only one young male possibly to become the breeding bull, and 19% of the farms declared to have a ratio of 2 or more young bulls per breeding bull.

Identification of animals was mainly based on coat color, shape and presence or absence of horns, age and dam recognition. Performance recording is not done, body weight is not measured at any stage of development and no pedigree information was available.

Only one veterinarian offered health care services in Pasorapa. In each community one farmer is trained by the veterinarian to attend the most common illnesses in animals. These farmers are called livestock technical assistants (LTAs). Vaccinations and deworming are performed by either the local veterinarian, LTAs or the farmers According to the questionnaires only 9% of farmers followed the vaccination schedule. Remaining interviewees (91%) vaccinated animals only against either rabies or anthrax, or immunize animals against both diseases but only once per year. Immunization against Foot and Mouth Disease (FMD) is still scheduled in the calendar, but none of the farmers vaccinated against this disease. Immunization against Brucellosis was not included in the calendar. Figure 9 illustrates the handling of animals during a vaccination campaign.

Figure 9. Handling of cattle during a vaccination campaign in Pasorapa.

The most frequent disease-related causes of cattle mortality disclosed by the farmers were in order of importance and frequency: Piroplasmosis, macurca, diarrhea, anthrax, rabies, arthritis and intoxication with stick insect (Pajuela). Among the environmental causes predator attacks, accidents and feed shortage were reported in higher frequencies. Water scarcity had the lowest frequency, possibly because animals were sold before they die, therefore farmers do not identify this factor as a cause of mortality. However, the death of calves at birth during a drought period during the Ranch stage was mentioned during the interviews.

Piroplasmosis, Anaplasmosis, foot rot during rainy season, posterior paralysis due to piroplasmosis, miscarriages due to trauma, retained placenta, weight loss due to parasites, and abdominal pain due to either gastrointestinal diseases or intoxication, and plant poisoning were the most frequently diseases reported and treated by the local veterinarian.

3.2.2 Relevance of the production system for conservation and breeding programs for the Creole cattle in Pasorapa, Bolivia

All Creole cattle breeders were smallholders operating mixed low input crop-livestock production systems and in some cases solely livestock landless systems. Most respondents (39%) were in the age range between 60 and 70 years old, followed by the group between 50 and 59 years (35%) and a group between 40 and 49 years (22%). Only 4% of farmers were below 30 years old. No respondents between 30 and 39 years old were identified. Most households were male-headed (86%) and only 14% were female-headed.

Women participated in milking, carrying water, cheese processing, herding, vaccination and separating calves. One third of householders had only help of their wives to manage cattle; 24% expressed that they sometimes had help from a neighbor, friend or relative. Only 14% hired 1 cowboy and 5% hired 2 cowboys during the Paddock stage.

The management system

Availability of feed was the main determining factor to extend or decrease the time of keeping animals in the paddock (Paddock stage) (Figure 10). According to interviews (Int-A) and observations during visits to the farms lactating, old or sick cows and calves were kept in the paddocks the longest. If there is no feed left they are sold or released to the ranch land, where they often become easy prey for predators. Most frequently used crops to feed animals were listed in paper 1, in paper 2 we present a list of native plant species consumed by the Creole cattle during both the Ranch and the Paddock stage (Table 2). This has a potential impact on the on these native plant populations.

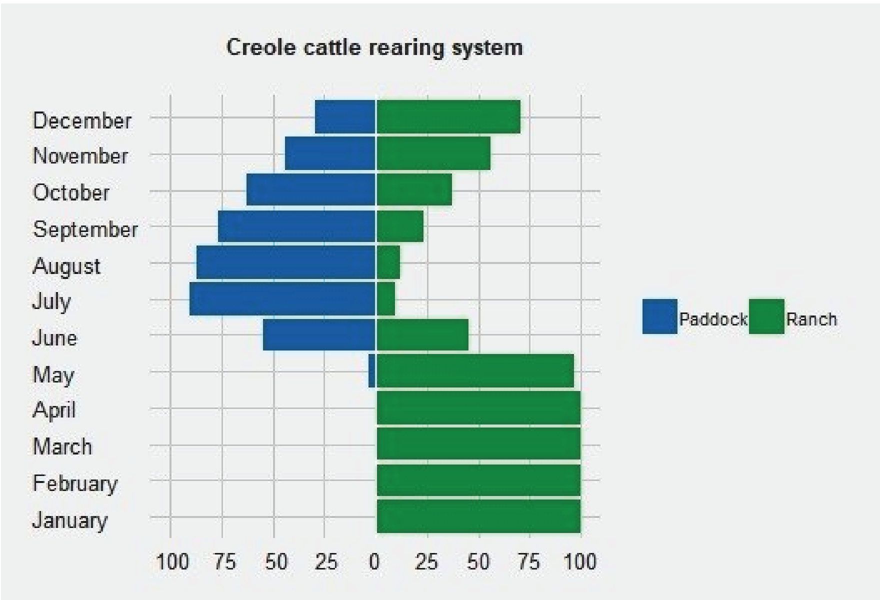


Figure 10. Percentage of Creole cattle located in the paddocks or in the rangelands along the year.

Table 3. *List of native plant species consumed by cattle in Pasorapa, Bolivia*

Common Name	Scientific Name
Achuma	*Trichocerus bridgesii*
Achupalla	*Eryngium paniculatu*
Algarrobo	*Prosopis alba* Griseb
Carapari	*Neoraimondia herzogiana* Backeb
Cegadera	*Heterophyllaea lycioides* (Rusby) Sandwith
Chacatea	*Dodonea viscosa* (L.) Jacq.
Jarka/Arca	*Senegalia visco* (Lorentz ex Griseb.) Seigler & Ebinger
Khayara/K'jayara	*Bromelia sera*
Khari (Cari cari)	*Senegalia bonariensis* (Gillies ex Hook. & Arn.) Seigler & Ebinger
Kiñi	*Vachellia aroma* (Gillies ex Hook. & Arn.) Seigler & Ebinger
Melendre	*Gochnatia palosanto* Cabrera
Melendrillo	*Gochnatia boliviana* S.F. Blake
Munchuelo	*Senegalia gilliesii* (Steud.) Seigler & Ebinger
Paja brava	*Stipa ichu* (Ruiz & Pav.) Kunth
Pasto	*Digitaria californica*
Quina quina	*Myroxylon peruiferum L.*
Soto	*Schinopsis haenkeana*
Tipa	*Tipuana tipu* (Benth.) Kuntze
Villca	*Anadenanthera colubrina* (Vell.) Brenan
Yareta	*Azorella compacta*

Optional feeding strategies adopted for some farmers to prevent food shortages during drought periods were: prickly pear cactus (Opuntia ficus-indica), Gatton panic and a blend called T'aqo. This blend was made by ground corn grains, ground pods and seeds of Algarrobo (Prosopis alba).

Purpose of rearing Creole cattle

Adaptability, resistance and walking ability of Creole cattle, as well as tradition, the reduced labor and "less care" needed, were presented as main reasons to conserve and rear Creole cattle by most interviewees. Farmers describe this cattle as a dual purpose breed. In the most remote communities, oxen or even breeding bulls are still used for draught power. Cattle are occasionally sold to generate income. Male calves are sold before they are 1.5 years old. Females are used for calf production and stock replacement and are kept in the herd as long as possible

Milk was produced only for self-consumption and production of cheese, occasionally sold at a winter festival. According to interviews, meat and cheese were not produced in larger quantities owing to the lack of infrastructure and equipment. Creole cows produced between 3 and 5 liters of milk per day according to the information provided during the interviews. No fattening practice was identified. Farmers and workers from the only slaughterhouse in Pasorapa explained that the quantity of beef produced from each animal ranges between 120 and 180 kg.

Breeding strategies and selection criteria

Mating of animals is poorly controlled during the paddock stage and basically at random during the Ranch stage. For this reason, 1.5 years heifers are commonly found being pregnant. These animals are usually smaller than the average cow.

Criteria followed by farmers to select breeding bulls in order of importance were: body size, body conformation and milk yield of dams, body size and conformation of the male, thickness and length of the neck, not born from a first calving, small navel, big and well-shaped horns, and aggressive temperament of the animal. Sires are selected either from the same herd or purchased from other farms, or even from Chaco Creole herds. Contacts between farmers to purchase a bull are a very common practice. Bulls can also be on loan between related farmers. Questionnaires revealed that breeding bulls were pure Creole males in two-thirds of visited farms, while in one-third either a crossbred or an exotic breeding bull were used. Replacement females and calves came from the same herd. Young females with good milk yield, good calving rate (one calf per year), docile, and “feminine appearance” remained in the herd. Old, sick and sterile animals as well as those with poor udder and teat conformation were sold.

It is important to point out the importance of coat color for the selection of Creole cattle. Color was mentioned as a criterion to select breeding bulls (Q2) by 44% of farmers: Black, “Hosco” and dark red coat colors were the preferred colors. White and brindle coated animals were usually not selected as breeding bulls. More than half of farmers (52%) mentioned preferences for black, red and “Hosco” coat colors when selecting replacement heifers. As well as with bulls, brindle and white coat colored females were more frequently discarded. White animals are considered to be more susceptible to the “cegadera” disease, and brindle animals are rejected by traders. Half of interviewed farmers described an association between the temperament and the coat color of animals. In this way, coat color can influence the selection of replacement animals and therefore genetic diversity indirectly. Genetic studies to clarify the genetic relationship between coat color and temperament or productive traits should be carried out, to avoid that a potential misconception becomes a risk factor for genetic diversity.

Farmer perceptions of mortality causes, common diseases and animal welfare

Most relevant farmer perceptions about frequent diseases and causes of mortality of cattle are listed in Table 3. Interviewees associated common diseases such as mastitis, diarrhea, anthrax and macurca to environmental factors but lack of vaccination or proper health management practices. These farmer perceptions might explain the percentage of farmers not following strictly the vaccination schedule (91%) showed in paper 1.

Farmer perceptions related to the strength of Creole type cattle and lack of awareness of the pain of animals is reflected in the execution of painful surgical procedures such as male castration and dehorning without anesthetics.

Table 4. *Most relevant farmer perceptions about common diseases and animal treatments*

Disease or treatment	Farmer perception
Mastitis	"We know it as the "Chupado de vibora" (Snake suckling)
Anthrax	"Fat animals get anthrax, when vegetation in the ranches is green"
Macurca	"After the rain the heat from the ground evaporates and make cows sick"
Brucellosis	"Brucellosis does not kill the animals fast, that is why we don't vaccinate against it.
Diarrhea in calves	"It is because they drink too much milk"
Vitamins as supplementary feeding	"I don't give vitamins to the animals because I don't have money"
Castration and dehorning	"We do it by ourselves. Creole animals are strong, they recover quickly". "We use Llanten (Plantago major L.), Verbena (Verbena officinalis L.) or Achuma (cactus)." (Pain relief)

Although interviewed farmers expressed their understanding about the importance of colostrum intake by calves, no farmer reported monitoring the intake during the first hours after birth neither during the Paddock nor the Ranch stage. We suggest that reports of 1-month-old dead animals with similar symptoms to Anthrax might be related to an inadequate colostrum intake.

Improving the sustainability of the system

In this study three main factors were identified affecting the sustainability of the Creole cattle production system in Pasorapa: productivity, income and ecosystem services. The productivity is affected by the management practices, but at the same time productivity has its impact on the incomes of farmers.

Forage production on-farm is considered a driving factor for more sustainable agricultural systems and has a positive effect on productivity. It is also an important strategy to be adopted in order to deal with the effects of climate change. Some farmers have established cultivated fields with Gatton panic and prickly pear cactus. This has contributed to reduce mortality of cattle during the drought period in 2018 (F. Cabrera, personal communication, October 2, 2018).

The adoption of new technologies and strategies to guarantee feed and water availability, and improvements on the health management practices is required. In the list of effects of climate change the quality and quantity of feed, water availability, water demand on livestock, the distribution and abundance of disease vectors and diseases as itself have been listed (Thornton et al., 2009). New strategies and technologies with a positive impact on the management practices of Pasorapa are then required. Since the impacts of climate change are expected to affect the productivity of rangeland and mixed-crop systems (Thornton et al., 2009, 2018), the cattle population from Pasorapa will be affected in both the Ranch and the Paddock stages. The vulnerability of the production system to the impact of climate change, is increased because of the strong interaction between it and the environment.

Climate change is also associated with the increased occurrence diseases (Nardone et al., 2010; Thornton et al., 2009). Training of farmers about the causes, prevention and treatment of the most common diseases should be performed. An example of this need was seen in farmer perceptions about diarrhea in calves. Most of interviewees believed this disease to be a consequence of excess milk consumption. Rather, the focus should be on monitoring the effective ingestion of sufficient quantities of colostrum soon after birth, as this is a key issue in improving calf health and survival (Trotz-Williams et al., 2008). However, improved surveillance and medical assistance for animals require the availability of more workers for tracking and handling animals. These issues highlight the importance of training programs to help animal caretakers to recognize main diseases, apply an adequate treatment and recognize the importance of a proper health management and its impact on productivity.

There is a complex interaction between this cattle population and the ecosystem. Cattle from Pasorapa feed on native plant species during the Ranch and the Paddock stages. Therefore, pressure on the environment is present the whole year. Under these conditions, appropriate conservation programs for native plant species and plans for grazing and browsing areas are required. Implementation of these programs might have a positive impact on the productivity of the system. Nevertheless, positive effects of the cattle on the ecosystem must also be considered: competition with other herbivores, the role for the survival of carnivores, nutrient cycling (manure) and its effects on the diversity and distribution of plant species in the rangelands.

Implementation of a multi-paddock grazing system and the extension of the Paddock stage are suggested in this study. Although both strategies might have a positive impact on the productivity, the conservation of plant native species and on the ecosystem, they require improvements in the infrastructure, and the availability of workforce, water and feed in the paddocks.

Considerations for breeding and conservation programs

Conservation and breeding programs must consider the importance of keeping favourable alleles related to adaptive traits and behavior in the population. This should allow Creole cattle to survive and have a good fitness under prevailing management and feeding strategies. Breeding goals should therefore include traits related to reduced sensitivity to environmental variation and resilience to high temperatures, low quality feed, and diseases to ensure proper animal response to climate change consequences. Uncontrolled crossbreeding using temperate breeds might risk the conservation of the mentioned traits in this cattle population.

Because of the close interaction between this population and its ecosystem, fluctuating environmental conditions such drought and feed shortages, have a strong impact on the population structure and consequently might lead to changes in the effective population size. Severe drought episodes lead to population bottlenecks that could result in loss of genetic variation. A main factor to be covered in a conservation program must be then related to improvements addressed to reduce the impact of environmental conditions during both the Ranch and Paddock stages.

Keeping records related to important traits and pedigree information is an important factor for carrying out breeding and conservation programs. In the case of Pasorapa, the development of methods (routines) to collect reliable data must be compatible with the management practices in both the Paddock and Ranch stages.

SWOT analysis of the system

The following strengths were identified in the SWOT analysis: The system as such is productive, at a low level, but enough to be the main or in many cases the only income source of smallholders in Pasorapa. Additionally, Creole populations has been described as a genetic pool of adaptive genes for heat stress, disease resistance and tolerance to parasites. Our field observations suggest that the Creole cattle from Pasorapa might be well adapted to the challenging environment and the current rearing practices. Finally, the high cultural value of this cattle population in the family farm system was included in the list of strengths.

The weaknesses we identified were the following: Low standards of management, suboptimal infrastructure, low possibilities to use hired workforce because of low income levels, low access to markets, high dependence on environment, variation of productivity and nutritive value of products and lack of records.

As opportunities, extending the Paddock stage, the availability of genotyping data and the development of a conservation plan for native plant species were identified. In contrast, climate change, the variability of environmental conditions, the constant risk of water and feed shortages, and the loss of adaptive traits due to the ongoing uncontrolled crossbreeding and the introduction of temperate breeds were the main threats identified in this study.

3.2.3 Genetic diversity and population structure of the Bolivia Creole cattle using a 50K SNP chip

Genetic diversity and population structure

The Chaco population (CHA) showed the highest values for the computed Expected and Observed heterozygosity, Nucleotide diversity (0.409) and Number of polymorphic sites (32,390 from 33,106 variants). Expected heterozygosity (H_E) values were 0.40 for Pasorapa (PASO), 0.41 for CHA and 0.38 for the CEASIP herd (0.38). These were higher values in comparison with studies performed with Italian, Spanish and Korean native breeds, European and African taurine breeds, Indicine breeds and indigenous cattle from Ethiopia (Edea et al., 2013; Pertoldi et al., 2014; Sorbolini et al., 2015; Sharma et al., 2016; Kim et al., 2018; Mastrangelo et al., 2018)

The nucleotide diversity (π) was 0.396 for PASO and 0.376 for the CEASIP population. The lowest value for FIS was obtained for the CEASIP group (-0.053), a negative value denoting a slight excess of heterozygotes. The highest value was found for PASO (0.032), indicating that this population has the highest loss of heterozygosity.

The overall F_{ST} value was 0.039. The pairwise F_{ST} values between groups (Figure 11) ranged from 0.018 (Pasorapa-Boyuibe) to 0.084 (Yacuiba-Kilometro20) indicating low to moderate differentiation between groups. Pairwise F_{ST} values between main populations were 0.052 (CHA-CEASIP), 0.068 (PASO-CEASIP) and 0.016 (PASO-CHACO).

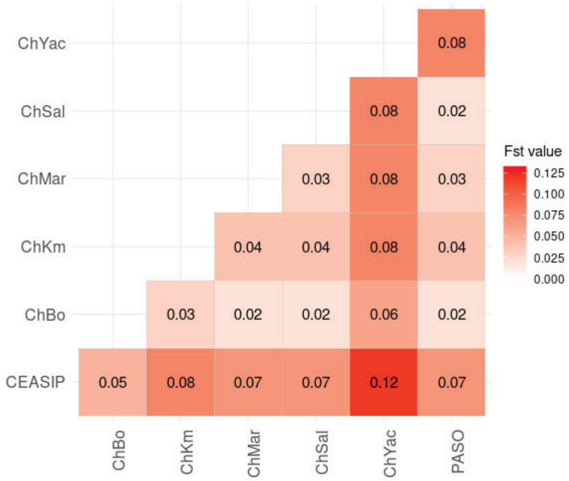


Figure 11. Heatmap of F_{ST} values for three Bolivian Creole cattle populations: Pasorapa (PASO), CEASIP and Chaco (CHA), the latter split in groups according to geographic location (ChBo, ChKm, ChMar, ChSal and ChaYac). Genetic distances were computed based on 33,106 autosomal SNP markers.

Principal Components Analysis (PCA) showed a moderate differentiation between the PASO and CHA populations, while the CEASIP herd is clearly separated from the other two populations (Figure 12). Some substructure was observed for CHA, samples from the research center of "El Salvador" were clearly congregated in a small closed group within the whole CHA population. Outliers corresponding to samples from the community of Yacuiba (ChYac) and three from Boyuibe (ChBo) possibly correspond to crossbred animals.

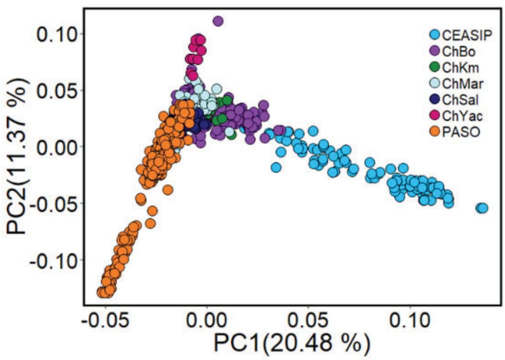


Figure 12. Principal components analysis of the Bolivian Creole cattle. Two main subpopulations: Pasorapa (PASO and Chaco divided in 5 groups (ChBo, ChKm, ChMar, ChSal and ChaYac). The first two principal components explain around 20.7% of the total variation.

Adaptation to different kinds of environments and different selection pressures might explain the level of differentiation among populations. The three populations included in this study have undergone different selection processes. CEASIP individuals have been selected for conservation and milk production purposes. The CHA population has been selected predominately for beef production, while PASO individuals have been mainly under the pressure of natural selection and very low artificial selection pressure.

Admixture analysis

Analysis with the STRUCTURE software showed clear signs of admixture among populations. A high degree of heterogeneity was observed among and within populations for all values of K. The highest Delta K was obtained for K=8. PASO and CHA showed similar patterns of ancestry with K=3, which might indicate that they derived from one whole population. The shared ancestry between CHA and PASO was congruent with the low F_{ST} obtained for this populations (0.016). We suggest that geographical distance between the Pasorapa and the Chaco Area might have played a role in a slight reproductive isolation between CHA and PASO leading to a slight differentiation between these two populations.

When K=7, differences in the color palettes for the three main populations and between groups inside the CHA population were observed (Figure 13). ChBo and ChKm20 groups showed a similar ancestral pattern with a lot of individual variation. Different proportions of ancestry can be observed in the ChMar, ChSal and ChYac groups.

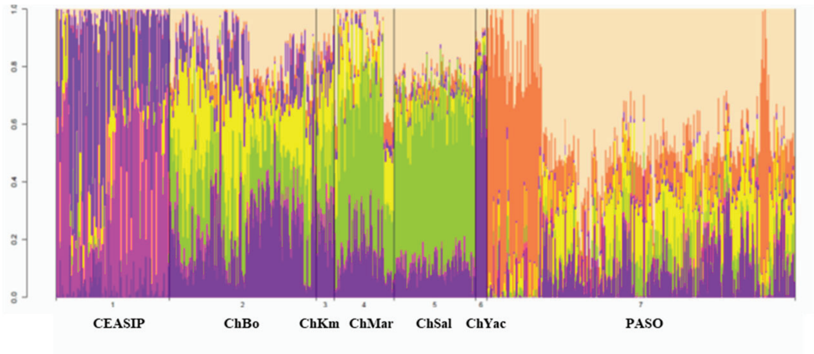


Figure 13. Global ancestry analysis performed with STRUCTURE software for CEASIP (Group 1), CHA (Groups 2 to 6) and PASO (Group 7) populations. The Chaco subpopulation is divided into the 5 sampling groups ChBo (2), ChKm (3), ChMar (4), ChSal (5) and ChaYac (6). Result for K=7 is displayed.

Linkage Disequilibrium analysis

The LD started decaying at lower distances in the three Creole cattle populations studied here, comparing with other taurine and indicine breeds using a 50K platform and bins of 10 kb, average r^2 values started at 0.46 for the CEASIP group, 0.40 and 0.43 for the CHA and PASO populations respectively. The LD values in the CEASIP decayed rapidly up to around 375 kb showing a smoother decay after this distance reaching r^2 values around 0.1. The LD decay for CHA and PASO had a steeper slope over short distances, decreased slowly after that and reached values around 0.03. An average r^2 value of 0.2 was reached using the data from CEASIP group at 60 kb, while the corresponding value was reached at 40 kb for PASO and 30 kb for samples from CHA. Low LD levels are congruent with less intensively selected populations such as PASO and CHA (Pérez O 'Brien et al., 2014).

Effective population size

Estimated values at *t* generations ago (from 13 up to 107) were higher for PASO than for the other two populations. CEASIP displayed clearly the lowest values. LD-based current effective population size (N_e) was substantially higher for CHA (169.7) and for PASO (144.8), than for CEASIP (16.7).

The CEASIP Ne size has been lower than 100 since 18 generations ago, which would mean that the capability of this population to adapt to changes in the environment might be at risk. The smaller contemporary Ne of PASO might be a consequence of recent bottlenecks.

Runs of homozygosity and ROH based inbreeding coefficients

F_{ROH} coefficient values were higher for CHA and CEASIP populations for the 4-8Mb and >16Mb length classes. In each ROH length category, PASO had the lowest inbreeding coefficients. The lowest values of F_{ROH} are in the 8-16Mb length category, for all three groups reaching values of 0.023 for PASO, 0.025 for CHA and 0.023 for CEASIP. CEASIP had the highest proportion of ROHs for the length classes 4-8 Mb, 8-16 Mb and >16Mb. Figure 14 illustrates the ROH based inbreeding coefficients frequency per chromosome in each population.

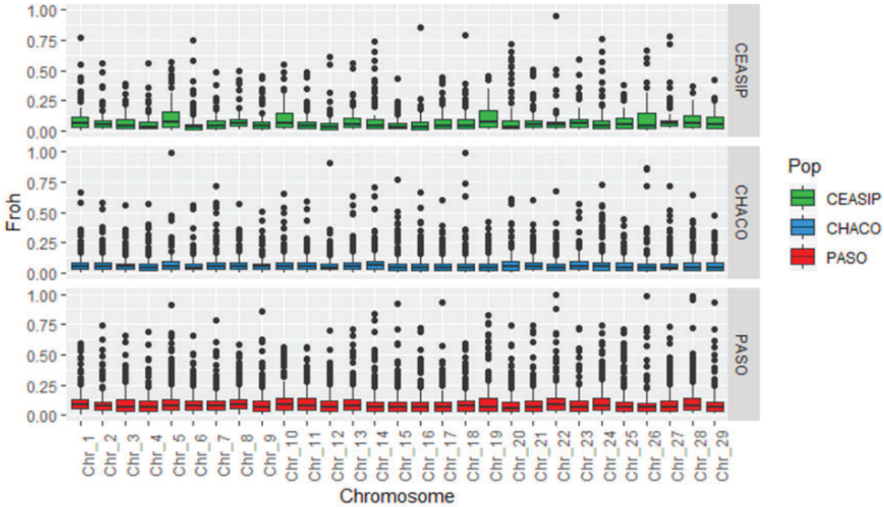


Figure 14. Box plots of ROH based inbreeding coefficients frequency per chromosome in each subpopulation.

3.2.4 Summary of results for the phenotypic characterization

Animals included in the collection of data used for the phenotypic characterization were between 2 and 4 years old. The age of animals from these populations was based on information collected from the farmers at the moment of sampling and information obtained from the pictures such the number of rings on the horns. Because the information was not accurate or completely reliable the class of age chosen had a wide range (2-4 years). Table 5 summarizes the main phenotypic data collected in the three populations.

The Creole type from Pasorapa

The dewlap in the Creole cow was in general small, if not completely absent. Males have a more pronounced but still small dewlap. Horns are upward-forward oriented, white in the base and black or brown from the middle or from the two-third part of the horn to the tip. Among all sampled animals only 1 polled animal was found. Ears are laterally oriented and humps are absent. The head is small and thin. Body hair was straight and of medium length in all cases. The backline was not straight, in all animals it sloped down from withers and slope up towards the rump. Animals had the tail of medium size going down to the hooks. Legs were in general thin and long. In general Creole cattle was smaller in size than crossbred animals. If comparing among populations included in this study the smallest cattle belonged to Pasorapa, but this can be explained by the characteristics of the management practices described in papers I and II and the feed scarcity during drought periods. The main characteristic of the Creole type from Pasorapa was the diversity of coat colors. The frequency of coat with solid color, white patterning, diluted and white splashed was similar among herds and communities included in the sampling. The less frequently found color was the brindle and the white, still present in the population but in low frequency. The reason for this was explained in the section dedicated to describe the selection criteria followed by the farmers. Figures 15 and 16 illustrates the phenotypic characteristics of a Creole type cow from the Pasorapa population. Table 5 lists the main coat colors found in the population.

Figure 15. The Creole cattle type from Pasorapa.

Figure 16. Detail of the head of Creole cows from Pasorapa. Horns are white with black color and upward oriented. Ears laterally oriented. Dewlap is small sized (left image).

Crossbred animals with zebuine breeds were identified by the presence of a large dewlap, thicker and shorter legs, bigger heads and faces with a straight profile, horns with tips pointing almost laterally and bigger ears still not completely dropped. Bodies of crossbred animals were clearly more robust with a straight backline. During the interviews (Int-A and Int-B) the farmers from Pasorapa described the Creole type using the following expression "Creole cows have a more feminine body conformation".

The Creole type from the Chaco

Cattle from the Chaco had in general the same characteristics of body conformation than those from Pasorapa, with the following main differences. Animals were clearly bigger compared with the cattle from PASO, which might be explained by the difference in management practices. Use of supplementary feeding including vitamins and minerals is a common practice of farmers from the Chaco area. Health practices were also more efficiently carried out. Different strategies were applied to deal with drought episodes and guarantee feed availability for animals such as silage, cultivation of exotic pastures and rotation of paddocks in the rangelands.

We found cattle with both upward-forward and outward oriented horns. The first ones had the same pattern of color as in cattle from Pasorapa, whereas the outward-oriented horns had solid colors (white, grey or black). Some horns with outward orientation had black tips but also some pigmentation on the base of the horn. The backline was straighter, but some animals also had slightly sloped spines. In the Research center of El Salvador (Macharejti) some bulls with straight facial profile were found. This characteristic was not found in other sampled farms. As additional information, farmers from this region still used the terms "feminine appearance" to describe Creole cows.

Less variety of coat colors was found in this population. Red and yellow coat colors were more frequent. Few animals with solid black, "Hosco" patterns, white splashed, diluted and brindle color were found. It was usual to observe red cattle with black spots on the face or only around the eyes and the ears. These animals are classified by the farmers in the "Hosco" color category. Figures 17 and 18 illustrate the main characteristics of the cattle from the Chaco population.

Figure 17. The Creole type from the Chaco.

Figure 18. Coat colors and horn shapes found in the Chaco population.

The Creole type in the CEASIP

The CEASIP population was composed for animals with bigger body size than cattle from CHACO. As in the previous population this might be explained by the differences in the management. Horns were absent in most animals. If present, horns were white with black tips and upward-forward oriented. Legs were shorter and thicker than in the cattle from PASO or the CHA populations. All the animals had a straight backline. The coat color of these cattle was mainly red, although some cows had yellow color (Figure 19).

Figure 19. Coat colors and horn shapes found in the CEASIP cattle.

Table 5. *Summary of phenotypic variables and morphometric measurements for cattle in Pasorapa, Chaco and the CEASIP.*

Phenotype trait	Pasorapa	Chaco	CEASIP
Horn presence	Present	Present	Present and polled
Horn orientation	Upward-Forward	Upward-forward Outward	Upward-forward
Horn color	White and Black White and brown	White and Black White and brown Solid color (White, grey, black)	White with black tips
Hair type	Straight,medium length	Straight,medium length	Straight,medium length
Ear orientation	Lateral	Lateral	Lateral
Dewlap size	Small sized	Small sized	Small sized
Backline profile	Sloped	Sloped Straight	Straight
Tail length	Medium size	Medium size	Medium size
Coat colors in The Population	Black, white, Red/ Yellow “Hosco”, Color sided in black, Color sided in red, White splashed Overo in black Overo in red Berrendo in black Berrendo in red Diluted brown Brindle	Red/Yellow, “Hosco” White splashed Diluted black Brindle	Red/Yellow
Morphometric measurements	Mean±SD	Mean±SD	Mean±SD
OL (m)	1.12±0.09	1.12±0.16	0.84±0.21
CD (m)			0.63±0.11
BD (m)		0.60±0.11	0.77±0.12
HH (m)	1.29±0.06	1.27±0.11	1.37±0.12
WH (m)	1.22±0.06	1.22±0.12	1.27±0.12
CG (m)		1.67±0.26	
HL (m)			1.07±0.13
HG (m)			0.37±0.11

Summary of main coat color found in the three populations

Table 5 summarizes the main coat color found in each of the three cattle populations included in this study. Following we include some pictures to illustrate the characteristics of each coat color (Figures 20 to 28).

Figure 20. Cattle showing the black (left) and white (right) coat colour.

Figure 21. Cattle showing the yellow (left) and red (right) coat colour.

Figure 22. Cattle showing the colour sided in black (left) and in red (right) coat colour.

Figure 23. Cattle showing examples of white splashed coat colour patterns found.

Figure 24. Cattle showing the overo in red (left) and the overo in black (right) coat colour.

Figure 25. Cattle showing the berrendo in red (left) and the berrendo in black (right) coat colour.

Figure 26. Cattle showing the diluted coat colour.

Figure 27. Cattle showing the "Hosco" coat colour. The jaws, neck and legs are black, whereas the spine has a red color. The main characteristic is the presence of a red cowlick on the head.

Figure 28. Cattle showing the brindle coat colour (left). The brindle colour was also found as a colour sided pattern (right).

4 General discussion

4.1 Importance and potential use of Creole breeds

Local breeds have been defined as those that being in a location or country for sufficient time became genetically adapted to one or more traditional production systems or environments in the country (FAO, 2012). The value of these local breeds for the Global Animal Genetic Resources relies in part on important traits that have been attributed to them such as: resilience to harsh environments, tolerance to high temperatures, resistance to endemic diseases, tolerance to parasites, high levels of fertility and the capability to produce even with low-quality feed (Hoffmann, 2013; Leroy et al., 2015b; Shabtay, 2015; Burrow et al., 2019). These traits have been also reported for Creole cattle breeds from different countries in Latin America including the Yacumeño Creole population from Bolivia (Wilkins, 1984; Wilkins et al., 1984; De Alba Martinez, 1986, 2011; Lirón et al., 2002; Vargas, 2013).

The interest in the genetics of such adaptive traits has increased in recent years in view of the climate change and its potential effects on livestock production systems. An example of this is the slick hair coat trait which has been associated with tropically adapted breeds including Creole cattle, such as the Limonero breed from Venezuela and the Romosinuano from Colombia. This trait has been studied at the molecular level, allowing the identification of two different mutations responsible for the trait in the SLICK locus, one of them present in the Romosinuano and the other in the Limonero Creole breeds (Landaeta-Hernández et al., 2011; Huson et al., 2014; Porto-Neto et al., 2018). The mutation present in the Romosinuano breed was initially discovered in the Senepol cattle. This mutation has been introgressed in Holstein cows, which showed superior thermoregulatory ability and less drastic depression in milk yield under heat stress conditions, compared with non-slick animals (Dikmen et al., 2014).

Similar studies to evaluate, describe and reveal the genetics involved in adaptive traits in other Creole populations should be carried out, opening the possibilities of using these local breeds in breeding programs oriented towards combining productive and adaptive traits to face the impacts of climate change. In order to achieve this purpose, detailed information about the genetic diversity and the population structure of these local cattle populations is required, as well as information about the characteristics of the production systems to which they are adapted.

The important role of local breeds in the livelihoods of smallholders has been well established, as well as their sociocultural value (Gandini and Villa, 2003; Hoffmann, 2010, 2013; Marsoner et al., 2018). In paper II we established the importance of Creole cattle in the livelihoods of farmers from Pasorapa. Operating a mixed crop-livestock and solely livestock landless systems, smallholders from Pasorapa have as main income source the occasional sale of their cattle, since milk production was only for self-consumption. Among the main reasons given by the farmers for rearing Creole cattle the adaptability, disease resistance, walking ability, reduced labor and less care were mentioned. These characteristics acquire more importance under the current management practices in this community described in paper I. In paper II, the availability of feed and water was identified as a main concern of the farmers and a threat to the production system. Under this scenario the walking ability on the rugged terrains of Pasorapa is an essential trait in the animals, given that they have to search for feed and water in the surroundings both during the Paddock and the Ranch stage. This search becomes more extensive and hard during a drought, when the animals need to walk longer distances to find feed and water sources. Despite the deficient health management practices that were reported in paper I, the most frequent mortality causes of cattle identified by the farmers and the veterinarian of the community were of environmental origin. Under this scenario, and given that the animals are exposed to often harsh environmental conditions over the whole the year, conserve in the population the disease resistance is recommendable.

4.2 The risks of crossbreeding for the Creole cattle in Pasorapa

After describing the characteristics of the production system in Pasorapa, one of the strengths we identified was that despite the low standards of the management practices and feeding strategies the system was productive enough to be the main income source of smallholders in the community. Cattle was exposed to challenging environmental conditions and nutritional stress during recurrent drought periods. It has been stated that nutritional stress affects reproduction growth and milk production and causes delay in puberty reducing the conception rate (Qureshi, 2012; Abdul Niyas et al., 2015). There were cases of females becoming pregnant before they were 2 years old, were reported by the farmers and the veterinarian of Pasorapa (Paper II), even after feed shortages. This might be an indicator of the resilience of the Creole population from Pasorapa. Nevertheless, record keeping is required to evaluate this.

Consequences of heat stress on reproduction and production traits have been widely studied (Thornton et al., 2009; Nardone et al., 2010). Two of the effects of climate change are the increase of temperatures and water scarcity (IPCC, 2014). The negative effects of heat stress on dairy cattle, most of them described as temperate breeds have been reported (Ravagnolo and Misztal, 2002; Bohmanova et al., 2007; Hansen, 2007; Dikmen and Hansen, 2009). Replacement of the Creole cattle with more productive temperate commercial breeds might be unsustainable under the characteristics of the management system in Pasorapa, with lack of housing, deficient health practices, feed and water scarcity and lack of supplementary feeding. Shabtay (2015), discussed the use of crossbreeding as strategy to increase the productivity of indigenous breeds. According to Shabtay (2015), crossbreds tend to be more productive than the local breeds but to achieve this, it is necessary to maintain favorable conditions, which most smallholders are not able to do in the long run. It has been claimed that there is a need of long term sustainable crossbreeding programs in developing countries in order to respond to the increasing demands of the market and to deal with the effects of climate change (Leroy et al., 2015a). Crossbred animals tend to be more sensitive to environmental conditions than local breeds are, therefore appropriate housing and health management practices are required. These factors were precisely identified as weaknesses of the production system in Pasorapa. Therefore, the implementation of crossbreeding as a strategy to increase the incomes of farmers must be carefully considered and well planned, in light of that improvements in the management, infrastructure, and access to markets must be done first. In addition, it has been suggested that lack of pedigree records compromises the long-term stability of crossbreeding programs (Roschinsky et al., 2014), another weakness identified in Pasorapa (Paper II).

On the other hand, unplanned crossbreeding might endanger the maintenance of the genes involved on the adaptive traits and resilience capabilities of the Creole animals. Results in papers I and II, support that the survival of the production system in Pasorapa is also thanks to the cultural value of the breed and the farmer perceptions about the robustness or hardiness of the animals. Nevertheless, under this scenario the factor mainly responsible for the survival of the system might be the natural capabilities of the Creole cattle to survive under such conditions.

4.3 Conservation and breeding programs

Throughout this study the importance of a conservation program for the Creole cattle populations in Bolivia has been justified. However, such programs must be accompanied by breeding programs focused on increased productivity of these populations, since they are the basis for the livelihood of their owners. If there is an interest of using the genetic background of Creole populations to increase the resilience capabilities of more productive breeds, the performance of this locally conserved population should not be far behind the commercial breed (Toro et al., 2009). In the case of the cattle from Pasorapa, the natural selection pressure is higher than the artificial, therefore, an equilibrium between fitness and production must be the main goal of any implemented program.

In this work we have generated information that may help to guide conservation and breeding decisions. It has been stated that populations with low within genetic diversity have also a low genetic contribution to the total genetic diversity (Lyimo et al., 2014). Expected heterozygosity estimates for the three populations included in this study showed a high genetic diversity level within each population, demonstrating that they might have a large contribution to the total genetic diversity. FST estimates for Creole cattle from Pasorapa and Chaco indicated low to moderate differentiation between populations, and there was little overlap between these two populations in the PCA analysis (Paper III). Although both populations are adapted to similar environments, there were clear differences in the management practices and the relevance of natural and artificial selection pressure for these populations. Conservation and breeding goals then should be designed considering all these differences and treating them as two different Creole populations. The CEASIP population showed high levels of genetic diversity according to the Expected heterozygosity estimate (0.38), although the Ne size was low (16.7). Conservation programs then might be oriented towards maintaining diversity levels in each population, increasing the effective population size of the CEASIP and evaluating the benefits and disadvantages of gene flow among populations, considering that the Saavedreño cattle was adapted to a tropical and not to a xerophytic ecosystem.

Given the characteristics of the production system in Pasorapa we suggest that a community-based breeding program (CBBP) would be suitable in this case. The CBBPs require knowledge about the circumstances of the farmers (Nandolo et al., 2016), knowledge that was generated in this work. Although CBBPs have been applied more in small ruminants systems, they may be a valuable alternative in systems of local cattle breeds where farmers have limited or no access to improved breeding stock and depend mainly on their own traditional breeding practices (Mueller et al., 2015). Nevertheless, starting record keeping is still a prioritized change in the system in order to have positive results. Infrastructure to make animal handling easier and safer might be built in strategic locations in some communities. Weighting of animals might be done during the Paddock stage and in parallel to vaccination and deworming activities.

Breeding goals might be focused on achieving a breed well-adapted to local environmental conditions in addition to being productive. The establishment of a nucleus herd for recording traits and selecting breeding animals (FAO, 2013) might be a proper option in Pasorapa, in order to avoid costly improvements in the infrastructure of all the community. Indeed, when availability of pedigree data and records of performance is limited or nonexistent, this strategy might be the most suitable. The genetic improvement process might be carried out in a small portion of the whole population and disseminated after among farmers in the community (Biscarini et al., 2015). Nevertheless, proper governmental regulations, incentives and support are needed to assist implemented programs.

The main differences in physical characteristics among individuals from the three populations were described in the additional information collected in this study related to the phenotypic characterization of the cattle populations. The body size of animals might be strongly influenced by the differences in the management systems to which animals belong, especially as regards feed availability. Nevertheless, the potential relevance of traits such the length and appearance of legs, the diversity of coat colors and the shape of the spine might be studied and analysed. These traits and the body size might have an effect on the walking ability of cattle in Pasorapa, an important requirement for searching feed and water especially during the Ranch stage (Paper I). In the description of the feeding strategies of animals in paper II, we listed the native plant species consumed by the cattle. Creole cattle from Pasorapa are more browsing than grazing animals. During the field trips animals standing on their hind legs leaning on trees with their four legs were observed trying to consume green leaves, parasitic plants growing on the top branches or the forage stored by the farmers in the "Calcheros" (Paper II). The importance of the external characteristics like the spine shape and the length of legs in this kind of behavior might be studied. Morphological differences between grazer and browser animals have been discussed previously, but these differences were more focused on the characteristics of the gastro-intestinal tract, mouth and teeth (Robbins et al., 1995; Gordon, 2003).

4.4 Genetic diversity and genotyping data

Although the availability of molecular data provide important baseline information, there is still a gap to be filled with proper phenotypic information related to traits potentially involved in adaption of the local Bolivian Creole cattle populations. If this gap is filled, the availability of reliable data would make it possible to include markers in SNP arrays related to disease resistance, drought tolerance or other local adaptive traits (Bruford et al., 2015).

During the development of this study previous works in Creole cattle populations from other countries in Latin America were found. However, these studies were performed using microsatellite and mitochondrial markers (Lirón et al., 2002; Carvajal-Carmona et al., 2003; Martinez, 2008; Ulloa-Arvizu et al., 2008; Martínez et al., 2012). How to handle the transition between microsatellite (SSRs) and single nucleotide polymorphism (SNP) genotypes has been discussed (Bruford et al., 2015). Although microsatellites have demonstrated to be adequate markers to describe the genetic diversity and demographic relationships, they might not be as efficient for the identification of genomic regions under selection (Herrero-Medrano et al., 2012). In addition, SNP data has demonstrated to be more repeatable than SSR data (Lenstra et al., 2012).

We found high levels of within-population genetic diversity in the three Creole cattle populations included in this study (Paper III). The PCA results also showed individuals spread over a great area in the plot as an indicator of between-population genetic diversity. In the case of Pasorapa population, the management practices and a rearing system allowing a random mating among animals during the Ranch stage, might have contributed to maintaining high levels of genetic diversity within the population. This fact is important, considering the frequent bottlenecks the population is exposed to, during the strong and recurrent drought episodes. The CEASIP population, its small N_e notwithstanding (16.7), showed a high heterozygosity level, which might be an indicator of well conducted management and conservation practices in the CEASIP center. In the case of the Chaco population, a moderate differentiation was identified among the groups corresponding to different communities sampled in the area (ChBo, ChSal, ChMar, ChYac, ChKm). The gene flow among these groups might have contributed to maintain the diversity levels. The great diversity within and between Creole populations in this study, might facilitate or increase the chance to unravel the genetics and selection process under their local adaptations.

4.5 Future studies

The ability of local breeds to use low-quality forage and exploit the natural vegetation of their environment has been established before (Köhler-Rollefson et al., 2009; Shabtay, 2015). Studies focused on monitoring and revealing the genetics underlying the efficiency of feed utilization of Creole cattle would be of interest. The organoleptic properties of the meat and milk produced from the Creole cattle are also in part consequence of the feeding management. Studies describing the quality and specific properties of these products might also be interesting, and would contribute to identify future markets and promote such products, resulting in a positive effect on farmer income levels.

Studies oriented towards determining the suitability and the effects of a multi-paddock system on the environment during the Ranch Stage should be conducted. As consequence of the strong interaction between the cattle population and the ecosystem in Pasorapa, we suggested a parallel developing of a conservation program for native plant species. A multi-paddock system would probably benefit the sustainable use of these plant species, by distributing the pressure more homogeneously. The location of the "dormideras", the vegetation density and the status of the native plant species should be considered when organizing the paddocks in the ranches. It has been stated that decreasing the distance between grazing areas and resting places, decreases the daily walking distances of grazing cattle approximately 5 km (Schlecht et al., 2006). This has as a consequence that less energy is invested in walking activities and more energy is available for productive traits. In addition, herding animals and decreasing the distances they walk will reduce their water needs. This is important under the expectation of an increase of water scarcity as an effect of climate change. Rearing animals with lower demands of water means saving water (Nardone et al., 2010).

Studies focused on selection signatures in the Creole cattle populations in Bolivia, and the identification of polymorphisms in genomic regions involved in potential adaptive traits should be performed. Medium and high-density SNP arrays are a powerful tool to achieve this goal (Huang et al., 2010; Bruford et al., 2015). Genotyping data generated in this study will allow us to continue with such studies.

5 Conclusions

In this thesis we have generated baseline information for the development of future conservation and breeding programs for three Creole cattle populations: Chaqueño, Saavedreño and a newly described population from Pasorapa.

The Creole cattle from Pasorapa is a well-adapted population to a xerophytic environment and challenging management practices. The rearing system was based in two stages with animals being herded during a Paddock stage between June and October, and released to the mountains during the Ranch stage between November and June. Although most farmers were classified into a mixed-crop production system, the feeding strategies were mainly based on native plant species during both stages. The system was characterized by poor infrastructure in the farms and deficient health management practices. Despite this, main mortality causes of cattle were of environmental origin. Among the disease-related causes of mortality the "macurca" was the second most frequent cause. The causative agent of this disease is still unknown. Future studies to discover the causative agent and treatments for this disease might be of great benefit.

Three main factors were identified affecting the sustainability of the Creole cattle production system in Pasorapa: productivity, income and ecosystem services. The current management practices have a direct impact on productivity. Therefore, the adoption of new technologies and strategies related to infrastructure, hired workforce, access to markets, training of farmers, and to ensure water and feed availability must be considered, in order to improve the current management system. Training of farmers and improvements in the infrastructure might encourage farmers to start keeping performance records and pedigree information.

Because of the characteristics of the production system in Pasorapa, there is a strong interaction between the cattle and the ecosystem. This means that the pressure on the environment and the native vegetation occurs all year round. Therefore, the development and implementation of breeding and conservation programs for cattle must be accompanied by conservation programs for the native plant species consumed by cattle. Implementation of a multi-paddock grazing system and the extension of the Paddock stage are suggested in this study.

The genetic diversity and population structure study revealed low to moderate differentiation among populations as shown by F_{ST} estimates and PCA analysis. The STRUCTURE analysis showed clear signs of admixture among populations. A high degree of heterogeneity was observed among and within populations for all values of K. The shared ancestry between CHA and PASO was congruent with the low F_{ST} obtained for these populations. Geographical distance between the Pasorapa and the Chaco area might have played a role in a slight reproductive isolation between CHA and PASO leading to a small differentiation between these two populations. However, it will be interesting to compare the information from the populations analyzed here, with data from other Creole cattle populations. Also, a more detailed analysis including collections of data from cattle world-wide might assist to get a deeper insight into the history of these three Creole cattle populations in Bolivia.

Regarding the aim to establish more structured breeding programs and to build a basis for the future conservation of the populations, it is important to gain in-depth knowledge on the population structure including current levels of inbreeding. CEASIP had the highest proportion of ROHs for the length classes 4-8 Mb, 8-16 Mb and >16 Mb. F_{ROH} coefficient values were higher for CHA and CEASIP populations for the 4-8 Mb and >16 Mb length classes indicating past and recent inbreeding. In each ROH length category, PASO had the lowest inbreeding coefficients. We can conclude from these results, that levels of inbreeding vary among the populations and that it will be important to take relationships into account to avoid further losses of genetic diversity within the populations.

Estimates for nucleotide diversity, expected and observed heterozygosity were not significantly different among populations. The current N_e was substantially higher for CHA and PASO, than for CEASIP, which might be explained by the small number of founder animals to establish the CEASIP population. But despite the smaller N_e size of CEASIP, the estimates of genetic diversity were similar to the ones for PASO and CHA. LD decay pattern was very similar in PASO and CHA while higher LD was found in CEASIP. This is consistent with a smaller N_e and is likely due to the fact that CEASIP is a population under a well-controlled breeding program. Lower LD values in CHA and PASO are on the other hand consistent with less intensive selection. Nevertheless, LD and N_e of PASO might be influenced by recurrent bottleneck episodes due to drought periods. Considering the r^2 values, we conclude that a 50K SNP platform could be applied for association studies in this population.

The information in this thesis in general shows the relevance and the potential of the three Creole cattle populations from Bolivia. The challenges of the management of cattle in the regions was evidenced clearly in the first studies and some of the genetic analysis evidenced these practices, such as the lack of structured mating and breeding programs. Further studies in the future, including the comparison with other cattle populations and the in-depth study of the genetic background of some of the characteristics of the individuals (such as coat color, exterior traits, production and traits relevant for local breeds) will offer a valuable basis for structured breeding programs. Such programs will assist the farmers in the region to ensure their livelihood and will assist the conservation of these valuable populations in the future.

6 Thesis summary

The value of livestock local breeds for the Global Animal Genetic Resources relies in part on the important traits that have been attributed to them such as: resilience to harsh environments, tolerance to high temperatures resistance to endemic diseases, tolerance to parasites, high levels of fertility and their capability to survive and produce on low-quality feed. These attributes are the product of the adaptation of these breeds to the environment and the production systems to which they belong. In addition, local breeds have a sociocultural value and play an important role for the food security of smallholders in developing countries.

The Creole cattle populations are an example of these valuable local breeds. They are descendants from the first cattle populations that arrived to The Americas with Christopher Columbus after 1493. These first cattle populations underwent a long process of expansion and adaptation to different environments. Four of these populations were previously identified in Bolivia: the Chusco Creole cattle, adapted to the highland plain; the Saavedreño cattle, adapted to a tropical plain; the Yacumeño cattle adapted to a seasonal flood plain; and the Chaqueño Creole cattle, adapted to the dry forest, xerophytic, environment in the Chaco region of Bolivia.

Livestock production systems in developing countries are already experiencing the effects of climate change. The consequences of heat on reproduction and production traits, the quality of feed crop and forage, water availability, animal growth and milk production have been widely discussed. In this scenario, the value of genes related to resilience and adaptive traits is increased, which is why the conservation of the local breeds is even more important. Given the role of these cattle populations for the livelihoods of smallholders, not only conservation is important, but also the improvement of the productivity and sustainability of their production systems. Consequently, conservation programs must be accompanied by suitable breeding programs adapted to the characteristics of the production systems. A baseline information about the production system, the genetic diversity and inbreeding levels is required for the development of such programs.

In this thesis we study three Bolivian Creole cattle populations, two of them previously identified: the Chaqueño and the Saavedreño cattle, the latter one conserved in the CEASIP center (Centro de Ecologia Aplicada Simon I. Patiño). We also include a new population not previously described, located in the community of Pasorapa and apparently adapted to a xerophytic environment. The aims of the study were focused on the description of the management practices in Pasorapa and the identification and evaluation of elements affecting the sustainability of the production system and the development of breeding and conservation programs. We complement this information with a study of the population structure, genetic diversity and inbreeding levels of the three cattle populations using medium density single nucleotide polymorphism (SNP) platform (50,000 SNPs).

In Paper I, we describe the main characteristics of the production and the management system in Pasorapa. Open-ended and closed-ended questionnaires and interviews were performed with 81 smallholders from 11 communities of Pasorapa. Before sampling, five field trips were done to identify farms with Creole cattle and farmers willing to participate in the study. The rearing system was based on two stages, with cattle being released in the mountains for about 7 months of the year, and for the remaining time herded in the paddocks. Feeding strategies were based on crop residues and consumption of native plant species. High mortality rates were explained mainly by environmental factors, such as predator attacks. Poor infrastructure, insufficient governmental support and deficient health management practices were found.

In paper II, factors to be considered before implementing breeding and conservation programs were analyzed. Farmer perceptions about diseases, mortality causes and selection criteria were described. Results showed that additionally to body conformation traits, farmers from Pasorapa consider coat color as an important trait at the moment of selecting breeding and replacement animals. Half of interviewees perceived an association between coat color on one hand and temperament, milk and beef production on the other. We performed a SWOT analysis to summarizing the strengths, weaknesses, opportunities and threats of the current production system. Productivity, income and ecosystem services are identified as the main factors affecting the sustainability of the system. In order to improve the productivity, new strategies and technologies focused on increasing the forage production, ensuring water availability, improving the infrastructure, creating access to markets and training of farmers is recommended. Increasing the productivity of the system is expected to have a positive impact on the income level of the farmers, which will improve the sustainability of the system. A complex interaction of this cattle population and the ecosystem is discussed. Because of the current rearing practice of animals feeding on native plant species most part of the year, this means that the pressure on the environment is constant throughout the year. In this view, a conservation of native plant species must be also developed for the benefit of the environment and the sustainability of the system.

In paper III, the population structure, genetic diversity and inbreeding level of the three Creole cattle populations is described. Individuals were genotyped with a 50K SNP chip. The genetic analysis was performed with hair samples collected from 672 individuals from the three populations: Pasorapa (PASO), Chaco (CHA) and CEASIP. Because of distance between sampling areas, animals from Chaco were split into 5 subgroups: Boyuibe, Villamontes, Puerto Margarita and Palos Blancos, Macharejti and Yacuiba.

The Chaco population (CHA) showed the highest values for Expected and Observed heterozygosity, Nucleotide diversity (0.409) and Number of polymorphic sites (32,390 from 33,106 variants). Expected heterozygosity (H_E) values were 0.40 for PASO, 0.41 for CHA and 0.38 for the CEASIP. These were higher values than in studies performed in other cattle populations. The coefficient of inbreeding within subpopulation (F_{IS}) had the lowest value for CEASIP (-0.053), a negative value denoting a slight excess of heterozygotes. The highest value of F_{IS} was found for PASO (0.032), indicating that this population has the highest loss of heterozygosity. Runs of homozygosity-based inbreeding coefficients (F_{ROH}) were higher for CHA and CEASIP populations for the 4-8 Mb and >16Mb length classes. In each ROH length category, PASO had the lowest inbreeding coefficients. Current effective population size (N_e) based on linkage disequilibrium was substantially higher for CHA (169.7) and for PASO (144.8), than for CEASIP (16.7). The CEASIP N_e size has been lower than 100 since 18 generations ago, which would mean that the capability of this population to adapt to changes in the environment might be at risk. The smaller contemporary N_e of PASO might be a consequence of recent bottlenecks.

The overall measure of subpopulation structure, F_{ST}, was 0.039. The pairwise F_{ST} values between groups ranged from 0.018 (Pasorapa-Boyuibe) to 0.084 (Yacuiba-Kilometro20) indicating low to moderate differentiation between groups. Pairwise F_{ST} values between main populations were 0.052 (CHA-CEASIP), 0.068 (PASO-CEASIP) and 0.016 (PASO-CHA). Supporting these results, the principal components analysis (PCA) showed a moderate differentiation between the PASO and CHA populations, while the CEASIP herd was clearly separated from the other two populations. Analysis with the STRUCTURE software showed clear signs of admixture among populations. A high degree of heterogeneity was observed among and within populations for all values of K. The shared ancestry between CHA and PASO was congruent with the low F_{ST} obtained for this populations (0.016).

Arguably, the geographical distance between the Pasorapa and the Chaco areas might have played a role in a slight reproductive isolation between CHA and PASO leading to some differentiation between these two populations. Also, the adaptation to different kinds of environments and different selection pressures might explain the level of differentiation among populations. The three populations included in this study have undergone different selection processes, CEASIP individuals have been selected for conservation and milk production purposes; the CHA population has been selected predominately for beef production, while PASO individuals have been mainly under the pressure of natural selection and very low artificial selection pressure.

7 Sammanfattning av avhandlingen

Lokala husdjursraser i utvecklingsländer är värdefulla för den globala genetiska mångfalden framför allt genom att de bär på många gynnsamma egenskaper: de kan klara sig under svåra miljöförhållanden med höga temperaturer, högt sjukdomstryck, dålig tillgång till både vatten och foder, som dessutom ofta är av låg kvalitet, och ändå överleva, ge avkomma och producera mjölk och kött. Detta beror på att de under lång tid anpassat sig och selekterats för att passa för dessa produktionssystem. Dessa raser har dessutom ett stort kulturellt värde och är viktiga för tillgången till mat för lokalbefolkningen i dessa länder.

Kreolnötkreatur i Sydamerika är ett exempel på sådana värdefulla lokala raser. De härstammar från de första nötkreatur som kom till Amerika med Christofer Columbus efter 1493. Dessa första djur genomgick en lång process av både expansion och anpassning till olika miljöer. Fyra populationer i Bolivia har redan beskrivits: Chusco, anpassad till ett liv på höglandsplatån; Saavedreño, anpassad till ett tropiskt slättlandskap; Yacumeño, anpassad till en miljö med säsongsmässiga översvämningar samt Chaqueño, anpassad till ett torrt skogslandskap in Chacoregionen.

Husdjursproduktionen i utvecklingsländer känner redan av effekterna av klimatförändringarna. Konsekvenserna av detta på reproduktions- och produktionsegenskaper, foderkvalitet, tillgång till vatten samt på djurens tillväxt och mjölkproduktion har diskuterats mycket. I detta sammanhang blir värdet av gener kopplade till anpassnings- och återhämtningsförmåga ännu större, vilket också gör att bevarande av lokala raser blir än viktigare. Men eftersom dessa raser också är viktiga för livsmedelsförsörjningen för landsbygdsbefolkningen, är inte bara ett bevarande av rasen viktigt utan även att man på olika sätt förbättrar produktiviteten och hållbarheten av produktionssystemen. Därför måste bevarandeprogram också kombineras med avelsprogram som är anpassade till just dessa produktionssystem. För att utveckla sådana program behövs grundläggande kunskap om produktionssystemet men även om den genetiska sammansättningen hos rasen ifråga.

I denna avhandling studeras tre bolivianska kreolnötkreaturspopulationer, varav två har beskrivits tidigare: Chaqueño och Saavedreño, där den senare bevaras vid en besättning som heter CEASIP (Centro de Ecologia Aplicada Simon I. Patiño). Den tredje populationen, som återfinns i ett område som heter Pasorapa och som är anpassad till ett torrt klimat, har inte beskrivits ingående tidigare. Syftet med denna avhandling var att beskriva skötseln och produktionssystemet i Pasorapa och identifiera och utvärdera olika faktorer som kan påverka hållbarheten i produktionssystemet och utarbetandet av bevarande- och avelprogram. Som underlag för detta studerades också populationsstruktur, genetisk variation och inavelsnivåer i dessa tre populationer med hjälp av molekylärgenetiska markörer, s k enbaspolymorfismer (SNP), d v s variation i de ”bokstäver”, nukleotider, som ligger till grund för bildandet av proteiner i cellerna; ca 50 000 sådana markörer användes.

I artikel I beskrivs huvuddragen i skötseln och produktionssystemet i Pasorapa. Enkäter och uppföljande intervjuer genomfördes med 81 småbönder från 11 samhällen. Innan dessa valdes ut, genomfördes fem resor i området för att identifiera besättningar med huvudsakligen kreolboskap och bönder som var villiga att delta i studien. Produktionssystemet bygger på två säsonger: ranchsäsongen då djuren släpps lösa i bergen under ca 7 månader och hägnsäsongen då djuren befinner sig närmare gården, ofta i någon form av inhägnad. Utfodringen baserades på naturlig växtlighet men under hägnsäsongen även på skörderester. Den höga dödligheten bland djuren kunde framför allt förklaras av miljömässiga faktorer, t ex rovdjur. Dålig infrastruktur, otillräckligt stöd från myndigheter och ofullständig hälsovård var vanliga problem.

I artikel II analyseras olika faktorer som bör beaktas innan man startar ett bevarande- eller avelsprogram. Böndernas uppfattningar om sjukdomar, orsaker till dödlighet och vilka kriterier man använde för att välja djur till avel beskrevs. Förutom olika kroppsmått var även färg en viktig egenskap man tog hänsyn till vid urval av djur. Hälften av intervjupersonerna menade att det fanns en koppling mellan pälsfärg å ena sidan och temperament, mjölkavkastning och köttproduktionsförmåga å andra sidan. En s k SWOT-analys, där styrkor, svagheter, möjligheter och hot sammanfattas, utfördes också.

Produktivitet, inkomstnivå och ekosystemtjänster identifierades som de viktigaste faktorerna som påverkar hållbarheten i produktionssystemet. För att förbättra produktiviteten behövs nya strategier och tekniker för att öka foderproduktionen, säkerställa vattentillgången, förbättra infrastrukturen, skapa tillgång till marknader samt öka möjligheten till utbildning av bönderna. Ökad produktivitet förväntas ge en ökad inkomstnivå för bönderna, vilket kommer att förbättra hållbarheten hos produktionssystemet. Det komplexa samspelet mellan boskapen och ekosystemet diskuterades också. Eftersom man i nuvarande djurhållning är beroende av naturlig växtlighet under hela året, finns det också en påfrestning på ekosystemet hela året. Därför bör också ett bevarandeprogram för de inhemska växterna utarbetas för att öka hållbarheten.

I artikel III beskrivs populationsstrukturen, den genetiska variationen och inavelsnivån i de tre populationerna. Den genetiska analysen gjordes för 672 individer från Pasorapa (PASO), Chaco (CHA) and CEASIP, som genotypats för ca 50 000 enbaspolymorfismer (SNP). På grund av de långa avstånden mellan olika områden inom Chaco, delades dessa djur upp i sju delområden: Boyuibe, Villamontes, Puerto Margarita and Palos Blancos, Macharejti and Yacuiba.

Chaco hade högst förväntad och faktisk heterozygotigrad, nukleotidmångfald (0,409) och antal markörer som hade mer än en variant (32 390 av 33 106 möjliga). Förväntad heterozygotigrad (H_E) var 0,40 för PASO, 0,41 för CHA and 0,38 för CEASIP. Dessa värden var högre än i studier av andra nötkreaturspopulationer. Inavelsgraden inom område (F_{IS}) var lägst för CEASIP (-0,053), där det negative värdet tyder på en högre andel heterozygoter än förväntat. PASO hade det högsta värdet för F_{IS}, vilket tyder på att man där har den högsta förlusten av heterozygoti. Inavelsgrader baserade på sekvenser av homozygoti (ROH) var högre för CHA och CEASIP för sekvenser 4-8 Mb och >16Mb (Mb= 1000 baspar). I alla grupper av sekvenslängder hade PASO lägst inavelsgrad. Den nuvarande effektiva populationsstorleken (N_e), baserad på analys av kopplingsojämvikt, var betydligt högre för CHA (169,7) och PASO (144,8), än för CEASIP (16,7). N_e för CEASIP har legat under 100 i över 18 generationer, vilket kan betyda att populationen nu kan ha svårt att anpassa sig till miljöförändringar. Den lägre nuvarande N_e för PASO kan bero på att populationen utsatts för flaskhalsar i populationsstorlek tämligen nyligen.

Det övergripande måttet på om det finns subpopulationer inom en större population, det s k fixeringsindexet, F_{ST}, var 0,039. De parvisa F_{ST}-värdena gick från 0,018 (Pasorapa-Boyuibe) till 0,084 (Yacuiba-Kilometro20), vilka alla tyder på en liten skillnad mellan grupperna. De parvisa F_{ST}-värdena mellan de tre populationerna var 0,052 (CHA-CEASIP), 0,068 (PASO-CEASIP) och 0,016 (PASO-CHA). En principalkomponentanalys (PCA) visade också på en liten skillnad mellan PASO och CHA, medan CEASIP var klart skild från övriga. Man kunde också se en hög grad av uppblandning mellan populationerna, oavsett om man antog att det fanns 3, 7 eller 8 grupper. Att CHA och PASO delar samma gener visades också genom det låga F_{ST}-värdet.

Det geografiska avståndet mellan Pasorapa och Chaco kan ha lett till en viss reproduktiv isolering mellan dessa två populationer. Även anpassningen till olika miljöförhållanden och olika selektionstryck kan ha bidragit: inom CEASIP har fokus legat på bevarande och selektion för mjölkproduktion; CHA har mestadels selekterats för köttproduktion och PASO har mestadels varit utsatt för naturlig selektion.

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The aim of this thesis was to generate baseline information for the development of future breeding and conservation programs for Creole cattle in Bolivia. For this purpose, the production system from Pasorapa was described, identifying the main factors affecting the sustainability of the system. The genetic diversity, population structure, linkage disequilibrium and ROH-based inbreeding of three populations from Chaco, CEASIP and Pasorapa were estimated. Low to moderate differentiation among populations and high levels of diversity and heterogeneity were found.

Gabriela Bottani Claros received her doctoral education at the Department of Animal Breeding and Genetics (SLU). She obtained her MSc degree from Universidad Mayor de San Simón – CIUF (Consejo Interuniversitario de la Comunidad Francesa de Bélgica).

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