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Estimation of breed admixture levels in Sri Lanka cattle and its relation to performance traits

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Statutory declaration

I hereby declare that I am the sole author of this work. No assistance other than that which is permitted has been used. Ideas and quotes taken directly or indirectly from other sources are identified as such. This written work has not yet been submitted in any part.

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10/06/2021, Vienna

Time and place

Abstract

The dairy sector in Sri Lanka received high priority to increase local milk production and reduce the import of milk products. To reach this objective, exotic cattle breeds were imported and used for the implementation of crossbreeding schemes to increase performance while pertaining favourable characteristics of the indigenous cattle, such as heat resistance, tick resistance and the ability to perform on low-quality feedstuffs. These favourable characteristics are mainly induced though the introduction of favorable parental genes and heterosis effects such as decreased inbreeding depression and beneficial gene interactions, both at locus level (dominance) and across different loci (epistasis). Measuring ancestry levels of local admixed cattle in Sri Lanka could provide more insight on which exotic breeds are best used to produce well-performing dairy cattle for the different local climates. Currently, little research has been performed on breed composition of dairy cattle in Sri Lanka. To model the performance of dairy cattle using breed admixture levels and dominance effects, phenotypes and blood samples for genotyping were collected between 2005 and 2019 for admixed dairy cows (n=197) from two governmental farms in the Kurunegala District of Sri Lanka. Performance was measured as 305-day milk yield and number of services per conception. For 305-day milk yield, recordings with less than 210 lactation days or less than 250kg milk yield were excluded from our analysis. Additional genotypic information was collected for several indigenous cattle breeds (n=4) and for reference populations including Pakistan Sahiwal as well as Jersey and Holstein Friesian under US, Sri Lankan and Indian conditions. Locus specific ancestry was estimated by phasing the chromosomes and subsequently creating genetic blocks for admixture events up to ten generations in the past using three reference populations (Jersey, Holstein Friesian and Sahiwal). Admixture levels were calculated as proportional abundance of the Jersey, Holstein Friesian, and Sahiwal ancestry for all the generated genetic blocks per animal. Based on these ancestral haplotypes the dominance component of heterosis could be determined by calculating the abundance of non-homozygous genetic blocks. Admixture levels for locus-specific ancestry and the dominance component of heterosis were included in a mixed linear model to find how performance and admixture levels were associated. For 305-day milk yield, significant predictors were found for Jersev ancestral breed proportion (p=0.013) and the dominance component of heterosis (p=8.045e-06) for p<0.05. We estimated an average 305-day milk yield of 1801-2089 kg for first parity crossbred Sahiwal x Jersey, compared to 1076-1477kg for local Sahiwal. Although the feasibility of a crossbreeding program for smallholder farmers needs to be considered, our results demonstrate that performance of local Indicine cattle can be improved substantially through crossbreeding with Taurine cattle, mainly through the dominance effects of heterosis.

Keywords: Admixture, Crossbreeding, Ancestry, Genomics, Cattle, Sri Lanka, Heterosis

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Introduction

Although Sri Lanka is self-sufficient for most livestock products, the production of dairy products currently only reaches 35% of the current demand for national consumption (Vernooij et al., 2015). The local government of Sri Lanka has therefore given high priority to dairy development, aiming to double the local milk production, and significantly reducing the proportion of imported milk (Perera & Jayasuriya, 2008), ultimately aiming for self-sufficiency in milk production (Vernooij et al., 2015). To reach these objectives, the government has invested heavily in the development of the sector, by means of local incentives and the implementation of breeding schemes that include the import of exotic cattle breeds (Vernooij et al., 2015). In the past decades, many live cattle and semen have been imported into Sri Lanka from time to time to improve breeding programs, in addition to several foreign and local funded projects to support livestock breeding activities in Sri Lanka (Ministry of Livestock and Rural Community Development, 2010). The majority (70%) of dairy cattle in Sri Lanka are crosses between the Indigenous Zebu cattle (Bos Indicus) and European/American temperate breeds (Bos taurus) or improved Zebu breeds from the Indian sub-continent (Bos indicus) in addition to 5% purebred European/American dairy breeds and 25% local cattle breeds (Zebu type cattle) (Perera & Javasuriya, 2008; Vernooij et al., 2015).

The management systems for cattle differ substantially throughout the country, being influenced by climate, type of crops grown and cropping pattern, availability of grazing land, genetic make-up of the animals and the main production objectives (Abeygunawardena et al., 1998). The National Breeding Policy for Dairy Cattle in Sri Lanka (Ministry of Livestock and Rural Community Development, 2010) has adapted to these different needs, considering the prevailing environment (based on altitude and rainfall, Sri Lanka is divided into three main agro-ecological zones) and the management systems adopted by farmers (roughly classified as intensive, semiintensive and extensive). Nevertheless, to a large extent these breeding policies have stimulated a continuous grading up of existing cattle using purebred exotic animals (Ministry of Livestock and Rural Community Development, 2010). Crossbreeding is a widely used implementation in (sub)tropical countries to increase performance while pertaining favourable characteristics of the indigenous cattle, such as heat resistance, tick resistance and the ability to perform on low-quality feedstuffs (Galukande et al., 2013). Continuous upgrading, however, has been reported to decrease performance (Bhat et al., 1978; Rao & Taneja, 1982; Syrstad, 1989; Taneja & Bhat, 1972) due to a decrease in heterosis as well as the loss of beneficial and necessary key traits, mainly relating to the adaptation of indicine cattle breeds to the local environmental conditions.

Crossbreeding programs should therefore consider the fine line between a highly productive crossbreed and an animal originating from several rounds of upgrading, possessing a too high degree of exotic genetic make-up to make it functional in the local environment. The favourable characteristics achieved through crossbreeding are mainly derived through the introduction of favourable parental genes, and a phenomenon known as heterosis, mostly displayed through decreased inbreeding depression and beneficial gene interactions (VanRaden & Sanders, 2003). Heterosis is a beneficial effect that can be measured as the additional increase in performance

exceeding the average of both parental populations (Falconer & Mackay, 1996; Shull, 1948). Although we do not know the exact genetic background of heterosis, several components have been identified, such as dominance, indicated as the favourable combinations of alleles at heterozygous loci, and epistasis: resulting from interactions of genes among different loci (Amuzu-Aweh et al., 2013; Khayatzadeh et al., 2018; Lynch & Walsh, 2002).

Measuring ancestry levels of local admixed cattle in Sri Lanka could provide more insight on which exotic breeds are best suited for producing crossbreeds for milk production in the different local climates. Additionally, ancestral breed proportions can also give an indication of the optimal breed proportion for exotic breeds. Traditionally, individual levels of admixture can be derived from pedigree information (Sölkner et al., 2010). However, in many developing countries, among which Sri Lanka, this information is often not reliably recorded or simply unavailable (Ouédraogo et al., 2021; Rege et al., 2001). With the application of genotyping becoming more readily available and affordable in the form of single nucleotide polymorphisms (SNPs), we can now estimate individual breed composition as well as possible heterosis effects for livestock in developing countries. Combining knowledge on breed composition with phenotypic data such as milk records and reproduction data makes it possible to identify the best fit in terms of breed composition for high performance in the different agro-ecological zones and management systems (VanRaden & Sanders, 2003). Ideally, performance and admixture levels would be analysed for the different local climates of Sri Lanka, as different levels of exotic genes may be beneficial for the different climates and production systems. Currently, little research has been performed on breed composition of dairy cattle in Sri Lanka, although identifying admixture levels that correlate with high performance under the different local climates of Sri Lanka could substantially improve dairy production. We hypothesized that crosses between local Indicine cattle and high-producing Taurine breeds such as the Holstein-Friesian or Jersey could significantly improve dairy production but also anticipated that too high levels of exotic genes originating from temperate breeds would probably negatively affect the cow and its production through reduced adaptation to the prevailing environment in Sri Lanka.

Literature review

Overview of livestock production in Sri Lanka

Livestock plays an important role in food systems and livelihoods of rural households in Sri Lanka. More specifically, the livestock sector contributes to food and nutritional security as well as human health and plays a big role in the transition towards sustainable consumption, production, and development (Slycan Trust et al., 2020). The agricultural industry in Sri Lanka covers nearly 30 percent of the total land surface, with the majority of this industry made up of smallholdings. These smallholdings are predominantly focused on crop production, whereas nearly 30% of these farms produce a mixture of crops and livestock and only a few farms are known for sole livestock production (Kumari et al., 2019). Nonetheless, the total area of farms with livestock takes up around 0.56 million ha in Sri Lanka (Perera & Jayasuriya, 2008), boosting almost 600.000 livestock farms, the majority consisting of poultry and dairy farms (Table 1, Department of Animal Production and Health, 2019). The dairy sector is considered to be the most important subsector of livestock production (Kumari et al., 2019), currently representing 592,260 dairy cattle and 161,250 dairy buffaloes (Slycan Trust et al., 2020). The importance of the dairy sector can partly be explained by the fact that dairy farming is accepted by all ethnic cultures, in contrast to some other livestock subsectors (e.g. swine) despite religious objections of cattle culling (FAO & Chandrasiri, 2002). Dairy products provide a crucial source of high-quality protein and both cattle and buffalo are still important for low-cost draught power. Additionally, the dairy cow can provide subsidiary products such as hides, skin and manure and serve as a financial reserve in means of a "living bank", buffering the risks associated with crop production (Perera & Jayasuriya, 2008).

	Cattle	Duffele	Cost	Chaon	Curin e	Pou	ltry
	Cattle	Buffalo	Goat	Sneep	Swine	Commercial Bacl	Backyard
Sri Lanka	307,180	25,338	60,537	483	6,975	32,099	151,459

Table 1. Number of Registered Livestock Farms (DAPH Sri Lanka, 2019)

Climate and agro-ecological zones

Although Sri Lanka is a relatively small island (total land area of 65,610 km²: FAO, 2011), it hosts an impressive variety of climatic conditions being mainly displayed through temperature, rainfall, humidity and evaporation. These climatic parameters are the reason for the wide range in regional climates and agricultural productivity of the country (Panabokke, 1996). The climate in Sri Lanka can typically be described as tropical monsoonal. The different regions of Sri Lanka differ substantially in terms of annual rainfall, soil types, altitude, monsoon rains, land use and vegetation characteristics (Leal et al., 2018). Based on rainfall, Sri Lanka can be divided in three major climatic zones: the dry zone; intermediate zone; and wet zone (World Bank; CIAT, 2015). The dry zone has been classified as receiving less than 1750 mm annual rainfall on average, predominantly covering the northern and eastern part of the country with a distinct dry season from May to September. The wet zone receives a mean annual rainfall over 2500 mm without pronounced dry periods, mainly covering the south-western region including the central hill country. In the intermediate zone,

mean annual rainfall ranges between 1750-2500 mm with a short and less prominent dry season (World Bank; CIAT, 2015).



Figure 1. Agro-climatic zones of Sri Lanka (Punyawardena, 2020)

Further differentiations can be found by local temperature variations due to altitude, impacting the type of agricultural product that can be produced and the range of species that are found in terms of animals, crops, and forests (Leal et al., 2018). Especially in the Wet and Intermediate zones, temperature (and thus elevation) is an important climatic factor for plant growth (and thus agricultural productivity). The main climatic zones are therefore further subdivided based on temperature limitations due to elevation, creating the Low country (land below 300 m in elevation), Mid country

(elevation between 300 and 900 m) and Up country (above 900 m elevation). Although the three different elevation levels can be found in both Wet and Intermediate zones, the Dry zone is confined to elevation levels below 300 m, hosting only the Low country and resulting in seven agro-climatic zones (Punyawardena, 2020). These seven agroclimatic zones are further subdivided into 24 agro-ecological regions (AER) based on rainfall, land use, vegetation and soil type. The availability of more spatial and temporal data, also due to advancement of GIS technology has led to a further subdivision, resulting in a total of 46 AER. Each of these 46 agro-ecological regions represent a uniform agro-climate, representing a fairly even agro-climate, type of soil and terrain conditions supporting a particular farming system with a certain range of crops and farming practices, including forage cultivation and livestock farming (Leal et al., 2018).

Dairy production in the different climatic zones

The management systems for dairy production in Sri Lanka are influenced by many external factors such as climate, crop type grown, cropping pattern as well as the availability of grazing land, the breeding stock, and the production objectives (FAO & New Zealand Agricultural Greenhouse Gas Research Centre, 2017). The main production systems can be differentiated into four groups based on the different climatic zones, where in addition to Up-, Mid-, and Low-country an additional zone known as the Coconut Triangle (the main production area for coconut the Wet and Intermediate zone of the Low country) has been added (Table 2). Semi-intensive or intensive dairy smallholding farms can be found in the wet zone of Up- and Midcountry. The primary objective in this area is milk production, characterized by the use of pure- or crossbred European cattle breeds in combination with high inputs of feed stuffs and zero-grazing. In these areas, meat is a by-product of the milk production system. In the Coconut Triangle small dairy herds are found, which are managed under semi-intensive settings with the inclusion of tethering or free grazing on pastures or used for the production of coconut or other perennial crops. Primary production objectives are milk and draught power, where surplus animals are being sold for meat. The breeding stock in the coconut triangle mainly consist of exotic breeds, Zebu types, indigenous animals, and some crossbreeds. The Low-country Wet and Intermediate zone is characterised as the most intensively exploited zone with over half of the area used permanently for agriculture. Cattle breeds used in this area are mostly crosses of indicine cattle, being fed on local fodder from roadsides with limited grazing options. The Low-country Dry zone covers the largest part of the island and therefore hosts a variety of agricultural settings. Most cattle are also found in this area, where herd size is significantly larger than in the other production areas. Typically, cattle here enjoy free grazing, and the breeding stock consists of a variety of indigenous cattle, Zebu cattle and crossbreeds. While the largest populations of cattle are found in the Dry and Intermediate zones under (semi-)extensive production systems, the main milk production areas can be found in Mid- and Up-country where semi-intensive production systems are run with the inclusion of feeding high inputs of concentrate feed stuffs and the use of (pure or crossbred) exotic animals (FAO & New Zealand Agricultural Greenhouse Gas Research Centre, 2017). These production systems are summarized in Table 1.2.

Table 2. Summary of dairy production systems in Sri Lanka (FAO & New ZealandAgricultural Greenhouse Gas Research Centre, 2017)

Production systems				Characterization
			Diet	Local fodder, high inputs of concentrate feeds, small herds tethering
0			Genotype	Pure exotic/crossbred, some Zebu crosses
intensive	Up- and Mid- Country	Wet zone	Productivity	Exotic: 12-18 litres milk/cow per day Crossbred: 5-8 litres/cow per day
			Herd size	
			Reproductive practice	Breeding by artificial insemination, average calving interval 12-18 months
			Diet	Mainly grazing, some local fodder, small amounts of concentrate feeds
			Genotype	Crosses of exotic breeds, improved indigenous
Semi- extensive	Low-Country	Intermediate and Wet zone	Productivity	Crossbred: 3-5 litres/cow/day Indigenous: 1-2 litres/cow/day
			Herd size	On average five cattle
			Reproductive practice	Natural breeding with some artificial insemination
	Coconut- Triangle (Low- country)	Intermediate	Diet	Mixed grazing on pastures/ coconut; tethering on roadside and fallow land, rice straw freely available, limited concentrates
Extensive (free			Genotype	Crosses of exotic breeds, Zebu, Indigenous animals, and crosses
grazing and tethering)		and Wet zone	Productivity	Crossbred: 5-8 litres/cow/day Indigenous: 2-3 litres/cow/day
•			Herd size	On average two cattle
			Reproductive practice	Artificial insemination and natural breeding
Extensive (grazing)			Diet	Free grazing with rice straw; sedentary small herds in irrigation schemes
			Genotype	Mainly indigenous, very few crossbreds
	Low- country	Dry zone	Productivity	Crossbred: 5-8 litres/cow/day Indigenous: 1-2 litres/cow/day
			Reproductive practice	Natural breeding, average age at first calving 30 months

Productive efficiency of dairy cows

The productivity of milking cows in Sri Lanka has a wide range, including low productive indigenous cows in extensive production systems, producing only 1-2 litres of milk per day, as well as high productive cows in semi-intensive systems, producing up to 18 litres of milk per cow per day (Table 2). Although it might seem like the productivity of dairy cows is mostly influenced by the management system and choice of cow breed, it is important to note that both are under the influence of the local climate. The climate, however, does not only determine the type of management system and cow breed that are best suited – and thus most adapted by local farmers, but the local climate also influences the average maintenance energy requirements of cows, directly influencing the available energy for milk production (FAO & New Zealand Agricultural Greenhouse Gas Research Centre, 2017). There are two main components for which the dairy cow needs her nutrients - maintenance and production. Once the requirements for maintenance - vital activities such as breathing, digesting food, and regulating body heat - have been fulfilled, the surplus of nutrients can be invested in productive activities such as milk production, reproduction, and growth (FAO & New Zealand Agricultural Greenhouse Gas Research Centre, 2017). The level of daily nutrients a dairy cow requires for maintenance does not change for different levels of milk production and can therefore be viewed as a fixed energy requirement. The average nutrient maintenance requirements for dairy cows in Up- and Mid country, Coconut triangle, Wet lowland, and Dry lowland systems are 27.8, 25.8, 20.5 and 19.9 MJ per day, respectively (Figure 2). Important to note that a high-producing cow needs more nutrients as input but is also more efficient, reducing the total nutrient requirement per kg of milk output. Based on average nutrient maintenance requirements, Up- and Midcountry seem to the most promising areas for increasing milk production levels in dairy cows, as cows in this area need the smaller part of their nutrient input for maintenance activities – and can thus appoint a significant amount of their energy to milk production (or reproduction).



Figure 2. Energy requirements for milking cows in dairy systems in Sri Lanka (FAO & New Zealand Agricultural Greenhouse Gas Research Centre, 2017)

Constraints on development in the dairy industry

Despite the importance of dairy cattle, the dairy industry is still far below expectations: in 2015, the production of dairy products only reached 35% of the demand for national consumption (Vernooij et al., 2015). Although the Ministry of Livestock and Rural Community Development (2012) noted that the domestic dairy industry has the capacity and capability of producing substantial amount of quality milk and milk products, the trend of milk production over the past decade has been stagnating; both annual milk production as well as the number of dairy cows has only shown moderate improvement (Table 3: Perera & Jayasuriya, 2008). The local government of Sri Lanka has therefore given high priority to dairy development, aiming to double the local milk production, and significantly reducing the proportion of imported milk (Perera & Jayasuriya, 2008), ultimately aiming for self-sufficiency in milk production (Vernooij et al., 2015). Although efforts have been dedicated to this strategy, imports of milk products still took up 61% of the national consumption in 2019 (Department of Animal Production and Health, 2019).

Item	2015	2016	2017	2018	2019
Cattle population	1,227,379	1,366,195	1,399,815	1,462,988	1,527,649
Annual Milk Production (L)	331,197,597	377,740,000	404,600,001	414,748,924	356,590,117
Human population (mio) Per-capita availability	20.97	20.97	21.44	21.67	21.80
Milk and Milk Products (Liquid Milk Equivalent)	48.56	51.94	55.99	58.03	52.16

Table 3. Key statistic of the dairy industry 2015-2019 (DAPH Sri Lanka, 2019)

There are many constraints to increasing milk production in Sri Lanka, which according to the Animal Production and Health Commission for Asia and the Pacific (APHCA) of the FAO can be assigned into five main categories: stock availability; animal health management; land availability; farmer knowledge and skills; and extension system limitations (Perera & Jayasuriya, 2008). In terms of stock availability, limitations include a lack of good quality dairy animals, limited outreach of AI services, and misuse of genetically improved animals (Perera & Jayasuriya, 2008). Further limitations at the primary production level include insufficient feed resources and limited profits due to the high production costs and relatively low farm gate price. The limited efforts admitted to research and development focused on improving the production chain is also one of the major limitations at primary level (Ministry of Livestock Development, 2008). The main entry-point for improving the productivity of dairy cattle starts with upgrading the native herd, as the shortage of quality breeding stock has been pointed out to be one of the fundamental issues to the low development of the dairy sector. Upgrading the native herd envisages preserving traits of technical importance that have allowed the native herd to endure the challenging environment and low-quality feed stuffs while improving productivity (Perera & Jayasuriya, 2008). A national breeding policy for dairy cattle was first formulated in 1989 and modified in 2010 by the Department of Animal Production and Health of the Ministry of Livestock and Rural Community Development. The breeding objectives and strategy are adapted to the prevailing environment (agroclimatic zones) and management systems (intensive/extensive) that are adapted locally by farmers. The general strategy includes upgrading the native population through crossbreeding with the use of genetically superior cattle breeds known for their high production levels (Ministry of Livestock and Rural Community Development, 2010).

Overview of dairy cattle breeds in Sri Lanka

A diversity of cattle breeds can be found across Sri Lanka, these are classified into three main categories: cattle indigenous to Sri Lanka, imported cattle breeds either belonging to the group Bos Indicus, originating from the Indian subcontinent, or to the group Bos Taurus, cattle of European origin.

Indigenous cattle breeds of Sri Lanka

The department of animal production and health of Sri Lanka defined the Lanka Cattle and White cattle as the indigenous cattle breeds of Sri Lanka. The Lanka cattle that are nowadays found in Sri Lanka are probably not purebreds anymore since they have been crossbred with other local breeds. White cattle are also described as a crossbreed between local Lankan cattle with imported Indian breeds (FAO, 2005). Indigenous cattle have long been identified as a separate category of cattle and have been used for several genetic improvement programmes in the past (Tilakaratne et al., 1974; Wijeratne, 1970). Information on indigenous cattle of Sri Lanka unfortunately is scarce (Silva et al., 2010).

Lanka Cattle (synonyms: Sinhala; Batu Haraka)

The Lanka cattle are described as compact cattle breeds that are found in isolated pockets of Sri Lanka. Their arrival dates back to the arrival of the first Sinhala Indians to the island of Sri Lanka. Physical characteristics include predominantly dark tan and black skin colours with a shiny short coat. Although the Lanka cattle are poor producers in terms of milk or meat production, they are highly fertile and survive well in Sri Lanka's climate. Lanka cattle do not have a prominent hump, a dewlap, or an umbilical fold (Ministry of Livestock and Rural Community Development, 2010). Although the Lanka cattle are not differentiated into breeds, there are reports of two locally distinguishable varieties, the Lena-iri and Thawalam. The Lena-iri is a draught type kept in the west and south of Sri Lanka whereas the Thawalam is a pack type kept in the Central and Uva provinces. Thawalam bulls are taller than other Sinhala cattle but shorter than the Tamankaduwa (Porter et al., 2016).

White Cattle (synonyms: Tamankaduwa; Kinniya)

White cattle are believed to be a crossbreed between Lanka cattle and several Bos Indicus breeds kept for either dairy production or for draught purposes. Nowadays, the white cattle are mainly used for draught and meat production. White cattle can be easily differentiated from other cattle breeds in Sri Lanka because of their distinctive features, which include a white coat and tall thin body shape. The main population of white cattle can be found in the Eastern province of Sri Lanka (Ministry of Livestock and Rural Community Development, 2010).





Bos Indicus (zebu cattle)

The Bos indicus cattle, originating from the Indian subcontinent, are indigenous to the tropics. Logically, these cattle have developed traits that are well adapted to the tropical surroundings, which can be grouped into three main important traits. First, Indicus cattle show a high degree of heat tolerance, which is due to low heat production and accumulation as well as from the well-developed ability to dissipate heat, also known as the cooling capability (Cunningham & Syrstad, 1987). Second, Bos Indicus cattle show a significant degree of resistance to ticks as well as to many other tropical diseases. Third, due to their relatively small size, low metabolic rate and efficient digestion, the Bos Indicus cattle have very low nutritional requirements. Although the Bos Indicus is well adapted to the tropical climate, milk production is poorly developed for most of the breeds or non-classified types within this group. Additionally, these cattle are also known to exhibit late maturation, both physiologically and sexually. Conformational traits that define the Bos Indicus are the well-known hump over the shoulders, a narrow body, slowing rump and rather long legs. The hump's function is likely for the storage of energy, but the increased surface area also aids in heat dissipation (Buchanan, 2016). The hide of the Bos Indicus cattle is thin and loosely attached, brisket and dewlap are usually well-developed, especially in males. Average weight ranges from below 200 kg to over 400 kg for mature cows due to the wide range of sizes found in cattle of Bos Indicus origin (Cunningham & Syrstad, 1987).

Sahiwal

The Sahiwal is a Bos Indicus cattle breed originating from the Punjab region of Pakistan or the present-day Sahiwal district. Sahiwal cattle are known as a good dairy producer compared to other Bos Indicus breeds. Sahiwal cattle predominantly have a reddish dun colour although sometimes pale red. Male animals have a big hump and height at withers differs between 136 and 120 cm. Females have udders that are large compared to other Bos Indicus breeds. The calves of Sahiwal cattle are relatively small, Sahiwal cattle calf easily without birthing difficulties. Behaviour-wise they are generally docile and lethargic. Sahiwal cattle have also been shown to show resistance to tick-borne diseases, which makes them a good fit for dairy production in tropical conditions (FAO, 2005; Trail & Gregory, 1982).

Tharparkar

The Tharparkar is an indicine breed originating from the western arid region of India, kept for its milk production, and well known for its ability to cope with harsh desert climatic conditions. The breed is compact of size and has a white or light grey coat. Apart from its heat tolerance, it also has characteristics including disease and tick resistance, therefore the Tharparkar is of great importance to farmers in arid regions. The Tharparkar has also been used in cross breeding programs with both dairy and beef cattle to improve their production performances. Unfortunately, very little information on the Tharparkar is currently reported (Devadasan et al., 2020).



Figure 4. Images of (a) the Sahiwal, (b) Red Sindhi and (c) Tharparkar breed (The Cow Wall®, 2020)

Red Sindhi

The Red Sindhi originates from the North part of the Sindh province of Pakistan and is also well known for its high tolerance against heat and dehydration. The Red Sindhi has been imported to many other arid countries where it has demonstrated good adaptation to a variety of environmental conditions (Mello et al., 2020). The breed is mainly kept for milk production, in terms of physical characteristics it has a mediumsized dewlap, and its coat colour is deep, rich red colour but this can vary from a yellowish brown to dark brown (FAO, 2005).

Bos Taurus

Cattle belonging to Bos Taurus are humpless animals, originating from Northern areas of Asia and from Europe. Nevertheless, some Bos Taurus also migrated along Western Africa and America when the Spaniards explored these areas. Generally, Bos Taurus are more adapted to temperate regions, although migrations of Bos Taurus to Africa has also resulted in some tropically adapted Bos Taurus breeds. Generally, Bos Taurus cattle are known for their ability to produce high quantities of milk (Buchanan, 2016)

Holstein Friesian

The Holstein Friesian is one of the most important and well-known dairy breeds, best fitted for temperate climates. The breed originates from the Northern provinces of the Netherlands (Friesland and Noord-Holland) where the European migrant tribes herding these cattle spread far into Germany, as far as the province Holstein, resulting in the breed's name. The Holstein Friesian is well-known for its black-and-white colour pattern, and its large body size (600-800kg), although there are also some Holstein Friesians that have a red-and-white coat colour The breeds popularity is due to the high level of milk production, around 25-30 kg of milk per day (Buchanan, 2016).



(c)

Figure 5. Images of (a) the Holstein Friesian, (b) Jersey and (c) Ayrshire breed (The Cow Wall®, 2020)

Jersey

The Jersey breed finds it origin in the Channel Islands. In the early nineteenth century Jersey cattle were introduced into America. Already in the eighteenth century the Jersey breed was known for its ability to produce high quality milk with a high percentage of fat. The import of other cattle breeds from Europe to the island of Jersey was therefore prevented to maintain the purity of the Jersey breed. Coat colour varies from light grey to light brown. The milk composition of Jersey cattle is high in fat and other solids. The Jersey's stature is rather small (350-550 kg) and milk yield is rather low (16-20 kg per day) compared to other temperate breeds such as the Holstein Friesian (Buchanan, 2016).

Ayrshire

Originating from the Ayr Country of Scotland, contributions to this breed likely originate from several breeds with progenitors belonging to the Shorthorn and Holstein breeds. Ayrshire's coat colour ranges from any shade of red, to mahogany or brown including well-defined areas of white. Their size is rather moderate (550-700 kg) and their average milk production is between 19-25 kg per day (Buchanan, 2016). The breed is widespread in Finland, where it was first imported in 1845 and crossed with a variety of other local breeds to produce the Finnish Ayrshire. The Finnish Ayrshire is now a well-established breed with high export numbers in terms of frozen semen and breeding stock to more than 20 countries all over the world (Felius, 1995).

Imported crossbreeds

Australian Friesian Sahiwal (AFS)

The Australian Friesian Sahiwal was developed as a tropical dairy breed with high milk yield and tick resistance. The breed was developed by the Queensland Department of Primary Industries from a closed population of half-bred Friesian x Sahiwal, which was initiated in 1961 (Madalena, 2002; Moran, 2012). Milk performance was indicated at an average of 3250 kg of milk per lactation at the research station of Queensland.

Materials and methods

Site of data collection

Phenotype data was collected from two governmental (NLDB) farms in Sri Lanka, both located in the North-western Province, in the Kurunegala District of Sri Lanka (Figure 6). The Andigama Farm is situated in Dambadeniya and the Maradawila farm is located in Bingiriya, both belonging to the agro-ecological intermediate zone in the low country with an expected annual rainfall over 1600 mm (IL2 see Figure S1 in appendix). Animals were maintained under the semi-intensive and intensive system, which included grazing on the pastureland in the morning and in a paddock grazing system in the evening.



Figure 6. Map of Sri Lanka showing the locations of Andigama (purple pin) and Maradawila (red pin) farm.

Collection of genotypes

Genotypes were collected at Andigama (n=86) and Maradawila farm (n=111), blood samples were also collected from cattle belonging to an additional NLDB farm in Sri Lanka which included mostly Jersey or Jersey crossbreds (n=79). Samples were also taken from pure Holstein Friesian (n=35) and Jersey (n=71) kept in Sri Lanka. Additionally, samples for genotyping were collected from local Sri Lankan cattle (n=17) and of several indigenous cattle breeds including Batu Haraka (n=40), Thawalam (n=25) and White cattle (n=40). Since ancestry of Sri Lanka cattle was explored in global context, genotypes were also collected for reference populations including purebred Sahiwal from Pakistan (n=40), Indian Holstein Friesian (n=25) and Indian

Jersey (n=31). These animals were all genotyped by the International Atomic Energy Agency (IAEA) in Seibersdorf (Austria) using an Affymetrix Axiom Bovine chip with a density of 52518 SNPs. Genotypes originating from the International Bovine HapMap Project, genotyped using the Illumina BovineHD chip with a density of 777,962 SNPs (Bovine HapMap Consortium et al., 2009), genotypic information from purebred Holstein Friesian (n=71) and purebred Jersey (n=46) individuals was also added for overlapping SNPs. Some individuals of the HapMap dataset classified as Jersey contained high values of other breed components and were therefore deleted (n=9), leaving a reference population of 37 purebred Jersey. The data was merged and manipulated in PLINK1.9 (Chang et al., 2015), resulting in a total of 35284 overlapping SNPs. Prior to merging the data, the SNP positions from the HAPMAP data were updated to the Affymetrix Axiom Bovine settings. Data merging errors for SNPs with similar positions were corrected using the -exclude flag. Quality control (QC) parameters were already applied during genotyping with a QC call rate of >93%. Additional quality control was performed when processing the data with Plink, excluding individuals with more than 5% missing genotypes (--mind 0.05), excluding SNPs below 95% genotyping rate (--geno 0.05) or with a minor allele frequency below 0.05 (--maf 0.05) resulting in a total of 28981 SNP variants for 619 cattle. The merged data (Table 4) was explored using multidimensional scaling in the form of a PCA plot and ADMIXTURE analysis (Alexander et al., 2009).







(C)

(b)



(d)

Figure 7. Photographs of crossbred cattle in Sri Lanka at Andigama (a and b) and Maradawila (c and d) farm.

Table 4. Breeds and populations used in exploration of the ancestry of Sri Lankan cattle (after quality control).

Breed/population	Country	n
Jersey ²	USA and New Zealand	37
Holstein Friesian ²	USA and New Zealand	71
Indian Jersey ¹	India	29
Indian Holstein Friesian ¹	India	25
Sri Lankan Holstein Friesian ¹	Sri Lanka	33
Sri Lankan Jersey ¹	Sri Lanka	71
Sri Lankan Local Cattle ¹	Sri Lanka	17
Batu Haraka ¹	Sri Lanka	37
Thawalam ¹	Sri Lanka	25
White Cattle ¹	Sri Lanka	38
Sahiwal ¹	Pakistan	39
Admixed cattle ¹	Sri Lanka	197

¹ Affymetrix Axiom Bovine chip (52,518 SNPs)

² Illumina BovineHD chip (777,962 SNPs)

Performance data

Phenotypes and blood samples were collected from dairy cattle of the Andigama farm (n=86) and Maradawila farm (n=111), which were the samples used to estimate the link between performance and admixture level. Cow service records, birth register, individual cow record cards and other records from the NLDB farms dating between 2008 to 2018 were used to obtain data pertaining to each cow's parameters for reproduction such as age at first service, number of services per conception, calving interval, pregnancy period, days open and calf birth weight. The cow's parameters for production included actual lactation yield, full lactation length, average daily yield and 305-day yield per parity. An overview of phenotypes for cattle passing the quality control for genotyping (Andigama n=85, Maradawila n=105) are described in Table 5, further selection for cows to include in the model was performed in the next step.

Table 5. Summary of cow parameters (means and ranges, in brackets) collected at

 Andigama and Maradawila farm

Farm	Cows (n)	Birth year	Birth weight (kg)	Herd life
Andigama	85	2005-2015	19.03 (12-28)	5.11 (1-11)
Maradawila	105	2005-2019	19.89 (12-28)	4.82 (1-10)
Farm	Age first Al (months)	305-day milk yield (kg)	I Services per conception	_
Andigama	33.28 (22-48)	1450 (361-2998)	2.17 (1-5)	
Maradawila	40.12 (26-66)	1986 (288-4757)	1.95 (1-4)	

Data editing and selection

For further analysis two parameters were chosen to model the relationship between admixture level and performance: number of services per conception, ranging between 1-5 services per parity, and 305-day yield per parity, ranging between 288-4757 kg/milk/cow/parity (Table 5). Data was first analysed per parity, where parity 1 was subdivided into 5 quintiles (1A-1E) based on the age of first insemination in the cow's life.



Figure 4. 305-day milk yield per parity for each individual sampled cow (show as dots) as well as for the average of each farm (dotted line) for Andigama farm (red) and Maradawila farm (blue).

Cows who were relatively the youngest when receiving their first insemination were grouped as 1A, and cows who were relatively the oldest were grouped as 1E and the groups in between were classified as 1B-1D according to their respective quantile. No significant difference was found for the different subgroups in terms of 305-day milk yield or number of inseminations per conception, where after these groups were merged together again as parity number 1. Parity's 6-11 were grouped together as parity 6+ due to the small amount of data available for the separate parities and the overall declining trend for milk production and conception rates. Furthermore, parity data was excluded when the full lactation length was less than 210 days, or the 305-day milk yield was below 250 kg, which reduced the total number of performance records per parity from 702 to 482. An overview of performance data of the admixed cattle in this dataset is displayed in Table 6.

Multidimensional scaling, principal component-, and exploratory ADMIXTURE analysis

Data was merged and manipulated using PLINK1.9 (Chang et al., 2015) and explored using the Multidimensional scaling (MDS) analysis on the merged dataset using the -- distance-matrix flag of PLINK1.9 and the MDS function, and subsequently plotted

using a principal component analysis (PCA) in R (R Core Team, 2020). We also ran unsupervised ADMIXTURE analysis (Alexander et al., 2009) for the number of ancestral populations (K) ranging from two to ten. Results were visualized using POPHELPER (Francis, 2017).

Table 6. Performance data of admixed cattle per parity from Andigama and Maradawila farm.

Breed/cross	305 day yield (kg)			number of services per						
	Records (n)	mean	std	min	max	n	mean	std	min	max
Sri Lanka Jersey x AFS	2	2422	35.36	2397	2447	2	2	0	2	2
Sri Lanka Jersey	3	2512	372.92	2216.5	2931	3	2.33	0.58	2	3
Sahiwal x AFS	82	1647.5	605.15	435	3239.5	81	2.15	0.88	1	4
Sahiwal x Friesian-										
Holstein	7	2966.79	414.61	2417	3526	7	1.86	0.69	1	3
Sahiwal x Jersey	384	1689.42	671.71	288	4756.6	376	2.05	0.87	1	5
Sahiwal x Jersey x AFS	4	1682.42	486.39	1044	2102.7	4	2.25	0.5	2	3

Phasing, haplotyping and locus-specific ancestry analysis

To estimate the locus-specific ancestry in the autosomal chromosomes (n=29), data was phased with SHAPEIT v2 (Delaneau et al., 2012) using a cattle recombination map from the Dryad (Ma et al., 2016). SHAPEIT uses haplotype frequencies and identical by descent (IBD) segments probabilities to model linkage disequilibrium (LD). Subsequently, locus-specific ancestry was calculated using the Ghap package (Utsunomiya et al., 2020) where the reference population (k=3) used to test the admixed Sri Lankan cattle consisted of Friesian-Holstein (n=30), Jersey (n=30) and Sahiwal cattle (n=29). Genetic blocks were generated for admixture events up to 10 generations in the past based on the mean density of the markers, which resulted in a window-size of 56 markers with steps of 14 markers, as proposed in the Ghap software by (Utsunomiya et al., 2020). Admixture levels were calculated as proportional abundance of the Jersey, Holstein and Indicus ancestry for all the generated genetic blocks per animal. Based on the ancestral haplotypes the dominance component of heterosis could also be determined by calculating the abundance of non-homozygous genetic blocks. This meant counting the number of genetic blocks that had one haplotype originating from Jersey, Holstein or Indicus ancestry, and the second haplotype not being equal to that, and taking this number as a proportion of the total amount of genetic blocks evaluated per animal.

Mixed linear model – relationship between performance and breed composition

Admixture levels for locus-specific ancestry and the dominance component of heterosis were included in a mixed linear model to find how performance and admixture levels were associated. The ancestral populations for K=3 were identified to represent breed composition percentage for Jersey, Holstein Friesian, and the collective of Bos Indicus breeds. A mixed linear model was run for performance (milk yield and number of services per conception) using the Ime4 package (Bates et al., 2015) in R (R Core Team, 2020) to investigate the breed effect:

1) $y_{ijklmnop} = \mu + \beta_1 HF_i + \beta_2 JR_j + \beta_3 Dominance_k + Parity_l + Farm_m + Cow_n + Year_o + e_{ijklmnop}$

Where:

Y_{ijklmnop} is the dependent variable (305-day milk production, number of inseminations),

 μ is the overall intercept of the model,

 $\beta_1 HF_i$ is the fixed effect of the proportion Holstein Friesian admixture level (*i* = 0 to 1),

 $\beta_2 J R_j$ is the fixed effect proportion Jersey admixture level (*j* = 0 to 1),

 β_3 Dominance_k is the fixed effect of the dominance component of heterosis defined as the percentage homozygous blocks (k = 0 to 1)

Parity_{*l*} is the fixed effect of the *k*th number of parity (l = 1 to 6, where 6 encompasses the parities 6-10),

Farm_m is the fixed effect of the mth farm (m = Andigama or Maradawila),

 Cow_n is the random effect of the *n*th cow (n = 1 to 168),

Year_o is the random effect of the birth year of the cow (0 = 2005 to 2019)

 $e_{ijklmnop}$ is the random residual effect with mean 0 and homogenous variance σ^2 .

An additional model was run for crossbred cattle of Sahiwal and Jersey (LSJ), as this was the main crossbreed represented in our dataset. Additionally, the number of animals originating from crosses including Holstein Friesian in our dataset was also rather limited (see Table 5). For the locus-specific ancestry the number of ancestral populations was set to 2 (K=2), resulting in admixture levels presenting the proportion of Bos Taurus and Bos indicus in these cattle. The dominance component of heterosis stayed equal to the previous model, which was based on three ancestral populations.

2) $y_{ijklmno} = \mu + \beta_1 Taurus_i + \beta_2 Dominance_j + Parity_k + Farm_l + Cow_m + Year_n + e_{ijklmno}$

Where:

 $Y_{ijklmno}$ is the dependent variable (305-day milk production, number of inseminations),

 μ is the overall intercept of the model,

 β_1 Taurus_i is the fixed effect of the proportion Taurus admixture level (*i* = 0 to 1),

 β_2 Dominance_j is the fixed effect of the dominance component of heterosis defined as the percentage homozygous blocks (k = 0 to 1),

Parity_k is the fixed effect of the kth number of parity (I = 1 to 6, where 6 encompasses the parities 6-10),

Farm, is the fixed effect of the mth farm (m = Andigama or Maradawila),

 Cow_m is the random effect of the *n*th cow (n = 1 to 168),

Year_n is the random effect of the birth year of the cow (0 = 2005 to 2019)

 $e_{ijklmno}$ is the random residual effect with mean 0 and homogenous variance σ^2 .

Both models were tested for significant predictors using the lme4 summary statistics and a type III ANOVA test (Wald chi-square test).

Results

Multidimensional scaling (MDS) and Principal component (PCA) analysis

The dataset with all genotyped animals used for the MDS and PCA analysis contained 28981 SNPs and 619 animals representing 17 (cross)breeds. The first eigenvector of the MDS analysis separated the pure Bos Indicus breeds from the rest of the populations. The second eigenvector separated the two dominant Bos Taurus breeds: splitting the Jersey population from the Holstein Friesian population. About 36.5% of the variation was explained by both eigenvectors (Figure 8). The samples taken from the Sri Lankan cattle of the Andigama and Maradawila farm are visualized over a wide range, located between the cluster representing pure Indicine breeds (e.g., Pakistan Sahiwal, Sri Lankan Local) and the Jersey cluster (Indian Jersey, Sri Lanka Jersey, pure Jersey). Some of the samples for Indian Holstein Friesian seem to have been misclassified as they seem to have a high degree of Jersey breed proportion, being located near the Jersey cluster.



Figure 8. Principal Component Analysis of Batu Haraka (LBH), purebred Holstein Friesian (HF); Indian Holstein Friesian (IHF); Indian Jersey (JR); pakistan Sahiwal (PSH); crossbred Sahiwal x Jersey x Australian Friesian Sahiwal (SJA); Sri Lankan Holstein Friesian (LHF); crossbred Sri Lankan Holstein Friesian x Jersey (LFJ); Sri Lankan Jersey (LJR); crossbred Sri Lankan Jersey x Australian Friesian Sahiwal (LJA); Sri Lankan Local (LKL); crossbred Sri Lankan Sahiwal x Australian Friesian Sahiwal (LSA); crossbred Sri Lankan Sahiwal x Holstein Friesian (LSF); crossbred Sri Lankan Sahiwal x Jersey (LSJ); Thawalam (LTM); White Cattle (LWC). The first eigenvector separates the Bos Indicus breeds from the Bos Taurus breeds. The second eigenvector separated the two dominant Bos Taurus breeds: splitting the Jersey from the Holstein Friesian cattle.

ADMIXTURE analysis

The unsupervised ADMIXTURE analysis (Alexander et al., 2009) was used to infer ancestral populations (K) using meaningful clusters. We selected results at K=3 as clusters for any subsequent K did not represent ancestral populations properly. A clear distinction was seen between proportion Holstein Friesian (light blue), Jersey (red) and Bos Indicus (dark blue), represented by several indigenous breeds as well as Pakistan Sahiwal in our dataset (Figure 9).



Figure 9. Ancestry cow populations for K=3 including Batu Haraka (LBH), purebred Holstein Friesian (HF); Indian Holstein Friesian (IHF); Indian Jersey (IJR); purebred Jersey (JR); Pakistan Sahiwal (PSH); crossbred Sahiwal x Jersey x Australian Friesian Sahiwal (SJA); Sri Lankan Holstein Friesian (LHF); crossbred Sri Lankan Holstein Friesian x Jersey (LFJ); Sri Lankan Jersey (LJR); crossbred Sri Lankan Jersey x Australian Friesian Sahiwal (LJA); Sri Lankan Local (LKL); crossbred Sri Lankan Sahiwal x Australian Friesian Sahiwal (LSA); crossbred Sri Lankan Sahiwal x Holstein Friesian (LSF); crossbred Sri Lankan Sahiwal x Jersey (LSJ); Thawalam (LTM); White Cattle (LWC). The main temperate breeds, Holstein Friesian (light blue) and Jersey (red) are separated from the Indicine breeds (PSH, LKL and LWC; dark blue) and indicated as the three ancestral populations for K=3.

The crossbred cattle analysed in this study predominantly show ancestries belonging to the group Bos Indicus and the Jersey breed. Based on this admixture plot we can conclude that the main temperate breed used in crossbreeding programs at these farms has been Jersey, also because there are only few animals with a high degree of Holstein Friesian breed proportion. Surprisingly enough, there are quite a few animals classified as Sri Lankan Jersey (LJR) which have a significant Holstein Friesian breed proportion.

Locus-specific ancestry analysis & dominance effect heterosis

The supervised locus-specific ancestry analysis used to infer ancestral populations on haplotype level of the admixed cattle on Andigama and Maradawila farm was visualized using Ghap (Utsunomiya et al., 2020). A similar trend was identified as in the unsupervised admixture analysis where the main breed components consist of Bos Indicus (red) and Jersey (green). The Holstein Friesian breed component, however, was more visible in this analysis, whereas at SNP level, the Holstein Friesian breed component is less pronounced in for instance the Sri Lankan Jersey population (LSJ), see Figure 10.



Figure 10. Locus specific ancestry predicted for admixed cattle population at Andigama and Maradawila farm, based on a reference panel including Jersey (JR: green), Holstein Friesian (HF: blue), and Pakistan Sahiwal (PSH: red). Admixed cattle include crossbred Sahiwal x Jersey x Australian Friesian Sahiwal (SJA); crossbred Sri Lankan Holstein Friesian x Jersey (LFJ); crossbred Sri Lankan Jersey x Australian Friesian Sahiwal (LJA); crossbred Sri Lankan Sahiwal x Holstein Friesian Sahiwal (LSA); crossbred Sri Lankan Sahiwal x Holstein Friesian Sahiwal X Jersey (LSJ).

Although heterosis consist of several components, in this study we only estimated the dominance component to get some general insight on the possibility of heterosis having an impact on performance, additional to breed proportion. Overall dominance ranged between 0.021 and 0.993 based on the abundance of non-homozygous genetic blocks (Table 7). The highest levels of dominance effects were found in the crossbreeds of Jersey and AFS, and Sahiwal and Holstein Friesian although these classes were highly underrepresented (n=1 and n=2, respectively).

Crossbreed	count	mean_dom	min_dom	max_dom
Jersey x AFS	1	0.920	0.920	0.920
Sahiwal x AFS	37	0.656	0.248	0.965
Sahiwal x Holstein Friesian	2	0.910	0.880	0.939
Sahiwal x Jersey	126	0.714	0.021	0.993
Sahiwal x Jersey x AFS	3	0.524	0.344	0.704

Table 7. Estimated dominance effect of heterosis based on the abundance of non-homozygous genetic blocks.

Mixed linear models estimating performance

Using genomic data, we predicted ancestral breed proportions at global level in admixed Sri Lankan cattle kept at two governmental farms. These breed proportions were linked to performance (305-day milk yield and number of inseminations) to identify the best performing breed composition for both performance traits.

Mixed linear model for 305-day milk yield

Based on the summary statistics of the model for the response of 305-day milk yield, significant predictors were found for parity (p=0.0199), Jersey ancestral breed proportion (p=0.0131) and the dominance component of heterosis (p=8.045e-06) for p<0.05 (Table 8). The Holstein Friesian breed component was showing a high standard error and was indicated as insignificant (p=0.1425) for p<0.05. Both breed components had a negative effect on milk yield, whereas the dominance component of heterosis increased milk yield substantially. When combining these two components we can estimate milk yield response for full heterosis based on dominance effects at haplotype level, which could be approached when crossing a pure Sahiwal with either a purebred Jersey or Holstein Friesian. Based on the results of this analysis, a pure Sahiwal shows 1076 kg milk at first parity. A first cross Sahiwal x Jersey would result in an estimated milk yield of 1839 kg for the first parity on Andigama farm (1076.47 - (789.22*0.5) +1156.85) and 2076 kg for Maradawila farm (1076.47 - (789.22*0.5) + 1156.85 + 237.63). A crossbred Sahiwal x Holstein Friesian (not-significant) would result in an estimated milk yield of 1871 kg for the first parity on Andigama farm (1076.47 -(724.61*0.5) + 1156.85) and 2109 kg for Maradawila farm (1076.47 - (724.61*0.5) + 1156.85 + 237.63

Table 8. Summary of linear mixed effects model for 305-day milk yield based including the fixed effects: Holstein Friesian proportion; Jersey proportion; parity; farm; and the dominance effect of heterosis (model 1). REML criterion at convergence: 7316.4. Included in the model are 479 observations for 168 unique cows from two farms.

a. Scaled	l residuals			
Min	1Q	Median	3Q	Max
-2.9112	-0.5699	-0.0775	0.5786	3.3456

h	Random	effects:
υ.	Random	enecis.

Groups	Name	Variance
Cow	(Intercept)	117602
Birth year	(Intercept)	6102
Residual		242956

c. Fixed effects:

	Estimate	Std. Error
(Intercept)	1076.47	143.15
Holstein Friesian	-724.61	494.04
Jersey	-789.22	317.93
Parity 2	131.35	62.01
Parity 3	95.04	69.86
Parity 4	10.01	87.27
Parity 5	-106.10	107.54
Parity 6+	-178.19	102.00
Dominance	1156.85	259.15
Maradawila farm	237.63	91.15

d. ANOVA type III for response

	Chisq	df	<i>p</i> value	
(Intercept)	56.55	1	5.491e-14	***

Holstein Friesian	2.15	1	0.1425	
Jersey	6.16	1	0.0131	*
Parity	13.41	5	0.0199	*
Dominance	19.93	1	8.045e-06	***
Farm	6.797	1	0.0091	**
0: :0	*** 0 004 ** 0 04 * 0 05			

Significance codes: *** 0.001 ** 0.01 * 0.05

Mixed linear model for number of services per conception

For the response in number of services per conception, no significant predictors were found for p<0.05 (Table 9). The Holstein Friesian breed component was close to being significant (p=0.082) for p<0.05 with a negative (increasing) effect on the number of services per conception.

Table 9. Summary of linear mixed effects model for number of services per conception. REML criterion at convergence: 1196.1. Included in the model are 470 observations for 168 unique cows from two farms.

e. Scaled	l residuals			
Min	1Q	Median	3Q	Max
-1.5608	-0.9683	-0.0490	0.8611	3.3147

f. Random effects:

Groups	Name	Variance
Cow	(Intercept)	0.03113
Birth year	(Intercept)	-
Residual		0.70192

g. Fixed effects:

	Estimate	Std. Error
(Intercept)	2.203	0.165
Holstein Friesian	0.999	0.566
Jersey	0.120	0.364
Parity 2	0.121	0.102
Parity 3	0.218	0.114
Parity 4	0.123	0.140
Parity 5	-0.172	0.181
Parity 6+	0.033	0.166
Dominance	-0.504	0.311
Maradawila farm	-0.107	0.107

h. ANOVA type III for response

	Chisq	df	<i>p</i> value	
(Intercept)	169.986	1	< 2e-16	***
Holstein Friesian	3.016	1	0.082	
Jersey	0.105	1	0.745	
Parity	6.241	5	0.283	
Heterozygosity	2.619	1	0.106	
Farm	0.991	1	0.320	

Significance codes: *** 0.001 ** 0.01 * 0.05

An overview of predicted performance for 305-day milk yield of various types of populations can be found in table 10, although it is important to note that our samples

did not include pure Jersey or pure Holstein Friesian, yielding unreliable results for these populations (Sölkner & James, 1990).

Table 10: Predicted 305-day milk yield for various types of populations on both farms, including purebreds, F1, F2 and backcrosses (BC) in both directions, based on effects from mixed model results

		305-day milk yield	
Population	Effects	Andigama	Maradawila
Pure Sahiwal (PSH)	Intercept	1076	1314
Pure Jersey (JR)	Intercept+JR	287	525
Pure Holstein Friesian (HF)	Intercept+HF	352	589
PSH x JR F1	Intercept+0.5*JR+Dominance	1839	2076
PSH x HF F1	Intercept+0.5*HF+Dominance	1871	2109
PSH x JR F2	Intercept+0.5*JR+0.5*Dominance	1260	1498
PSH x HF F2	Intercept+0.5*HF+0.5*Dominance	1293	1530
PSH x JR BC (25% PSH)	Intercept+0.75*JR+0.5*Dominance	1063	1301
PSH x HF BC (25% PSH)	Intercept+0.75*HF+0.5*Dominance	1111	1349
PSH x JR BC (75% PSH)	Intercept+0.25*JR+0.5*Dominance	1458	1695
PSH x HF BC (75% PSH)	Intercept+0.25*HF+0.5*Dominance	1474	1711

Mixed linear model for milk yield for population LSJ

This model only included the subpopulation LSJ (Sri Lankan Sahiwal x Jersey), where the model used Taurus breed proportion as fixed effect (relative to Indicus breed proportion) based on the admixture levels of the haplotypes. Both Taurus breed proportion (p=0.0439) and dominance effect of heterosis (p=0.0012) were significant predictors in this model for p<0.05 (Table 11). A crossbred Sahiwal x Jersey would result in an estimated milkyield of 1801 kg for first parity at Andigama farm (1189.22 – (626.92*0.5) + 925.49) and 2089 kg at Maradawila farm (1189.22 – (626.92*0.5) + 925.49 + 288.04) similar to the predicted milk yield in the model that included all subpopulations (1839 kg and 2076 for Andigama and Maradawila farm, respectively). Purebred Sahiwal yielded a slightly higher milk yield in this model (1189 & 1477 kg, compared to 1076 and 1314 kg milk yield in the previous model, for Andigama and Maradawila farm, respectively). The estimation of milk yield for purebred Sahiwal might be more prone to variation in the different models due to the lack of phenotype data for purebred Sahiwal cattle.

Table 11. Summary of linear mixed effects model for 305-day milk yield forsubpopulation LSJ. REML criterion at convergence: 5860.6. Included in the model are384 observations for 126 unique cows from two farms.

a. Scaled	residuals			
Min	1Q	Median	3Q	Max
-2.9073	-0.5960	-0.0468	0.5770	3.3766

b. Random effects:

Groups	Name	Variance
Cow	(Intercept)	117609

Birth year	(Intercept)	9725
Residual		248313

c. Fixed effects:

	Estimate	Std. Error
(Intercept)	1189.22	157.60
Taurus	-626.92	311.15
Parity 2	59.70	70.56
Parity 3	70.75	78.33
Parity 4	-99.85	98.86
Parity 5	-81.43	121.87
Parity 6+	-204.27	106.60
Dominance	925.49	285.31
Maradawila farm	288.04	106.48

d. ANOVA type III for response

	Chisq	df	p value	
(Intercept)	56.938	1	4.498e-14	***
Taurus	4.056	1	0.0439	*
Parity	8.929	5	0.1119	
Dominance	10.522	1	0.0012	**
Farm	7.318	1	0.0068	**

Significance codes: *** 0.001 ** 0.01 * 0.05

An overview of predicted performance for 305-day milk yield and number of services of various types of populations based on the LSJ subpopulation can be found in table 12, although it is important to note that our samples did not include pure Jersey, possibly yielding unreliable results for the pure Jersey population.

Table 12: Predicted 305-day milk yield of LSJ subpopulation on both farms, including purebreds, F1, F2 and backcrosses (BC) in both directions, based on effects from mixed model results

		305-day milk yield	
Population	Effects	Andigama	Maradawila
Pure Sahiwal (PSH)	Intercept	1189	1477
Pure Jersey (JR)	Intercept+Taurus	562	850
PSH x JR F1	Intercept+0.5*Taurus+Dominance	1801	2089
PSH x JR F2	Intercept+0.5*Taurus+0.5*Dominance	1339	1627
PSH x JR BC (25% PSH)	Intercept+0.75*Taurus+0.5*Dominance	1182	1470
PSH x JR BC (75% PSH)	Intercept+0.25*Taurus+0.5*Dominance	1495	1783

Discussion

The current study investigated breed admixture levels of Sri Lankan cattle at two governmental farms in the North-western Province (the Kurunegala District) of Sri Lanka. The aim was to link breed proportion to performance, where 305-day milk yield was chosen as indicator for production, and number of services per conception as indicator for fertility. The breed admixture levels were modelled using breed ancestry information of SNPs, therefore not considering the actual genotypes. It is important to note that this study did not include performance of locally kept purebred Holstein Friesian and Jersey cattle. Sölkner & James (1990) indicated that the loss in efficiency of crossbreeding designs without all parental pure breeds was substantial due to a reduced range of coefficients for parameters, and collinearity of effects. The design of our model might therefore have been improved with the use of purebreds, but as Sölkner & James (1990) also indicated, with the unavailability of purebred data, the best approach would be to include backcrosses, as performed in this study. Nevertheless, these findings suggest that the lack of parental phenotypes may have significantly impacted the results, especially for the estimation of performance of pure Jersey and Holstein Friesian.

The dominance component of heterosis was estimated using ancestral haplotypes and included in the model as a measure of heterosis. For heterozygosity however, we did not include epistatic effects, also known as recombination loss (Dickerson, 1973) as modelling these would require knowledge of parental phase at each locus and across chromosomes (Khavatzadeh et al., 2018) which was beyond the scope of this study. Experimental studies involving cross-bred dairy cattle showed that when using purebred sires, epistatic effects were negligible, having only little or no importance (Robison et al., 1981). Nevertheless, epistatic effects may be more pronounced in F2 populations. An overview of several studies on performance of crossbred cattle in the tropics indicated that the observed deterioration in milk yield of F1 to F2 dairy cattle is too large to be explained by dominance effects alone (Syrstad, 1989). Although several explanations were proposed, such as lack of selection among F1 bulls, or deterioration in management (Syrstad, 1989), a more plausible explanation was given by Sheridan (1981) who proposed that reduced performance may actually be due to the breakdown of epistatic combinations in F2, which is in line with the epistasis model suggested by Dickerson (1974). The loss of favourable gene combinations was also seen to reduce the level of heterosis in a more recent study by Cassell and McAllister (2009). These results clearly indicate the need for more research on the possible effects of recombination loss in crossbreeding.

Our findings suggest that an increased response in performance was mainly caused by the dominance effects of heterosis at haplotype level. This was indicated by the high correlation between the dominance effect of heterosis and 305-day milk yield. F1 crossbred dairy cows benefit from optimal levels of heterosis, achieved by crossing cattle from Taurine and Indicine ancestry. Heterosis effects are generally higher in functional traits with low heritability, such as those accounting for reproduction, survival, and fitness, whereas production traits with moderate heritability affecting milk yield and growth are less affected by heterosis (Hansen, 2006). Interestingly, our results showed a higher influence of heterosis on milk yield than on reproduction (number of services per conception). Since the (timing of) insemination of cows is nevertheless under the influence of the farm management there might be additional influences that we did not account for. The influence of heterosis on a trait such as milk yield is difficult to predict as it differs on the type and number of breeds included in the population (Sørensen et al., 2008). Additionally, Wakchaure et al. (2015) indicated that heterosis effects are much larger in crosses between temperate and tropical breeds, due to the large genetic distance between them, partly explaining the high influence of heterosis on milk yield in our results.

The superiority of F1 crossbred cattle in our study is in line with research performed in similar circumstances mainly due to the fact that heterosis is a well-known concept that is exploited in many breeding programs (Madalena et al., 1990a; Madalena et al., 1990b; Cunningham & Syrstad, 1987). These studies also indicated that, similar to our study, further upgrading by repeated backcrossing often resulted in declined performance. A study performed by McDowell (1985), compared performance of Sinhala crossbred cattle in Sri Lanka, where average milk yield of Sinhala cattle (570 kg) was substantially improved in F1 crossbreds with either Holstein Friesian (1573 kg) or Jersey (1215 kg) but decreased significantly in F2 crossbreds (987 and 809 kg respectively). Ahmad et al. (2001) analysed crossbreeding effects using Holstein Friesian, Jersey, and Sahiwal cattle in Pakistan. Similar to our study, their results showed that heterosis effects were significant for traits of these Sahiwal crosses (p<0.01) and were substantially larger than breed additive effects. Recombination effects in this study were included and also indicated as significant for most crosses. Tablott et al. (1994) analysed additive and heterosis effects in the same populations but excluded recombination effects. Their study showed lower estimates for additive effects but higher estimates for heterosis effects, more in line with our results. Although our study could not properly estimate the breed difference between Jersey and Holstein Friesian in crossbreeding with Sahiwal, mainly due to the low sampling number of HF crosses (n=7), these samples did indicate a high average 305-day milk yield for HF x Sahiwal crosses (2966.79 kg). The estimates for number of services per conception displayed a clear difference between the Jersey and Holstein Friesian breed component, although not significant, where crosses containing a high level of Holstein Friesian displayed an increasing number of services for conception. Similar studies on reproductive performance of crossbred cattle also indicated an increase in number of services per conception for HF crosses (Rokonuzzaman et al., 2009), and anoestrus was recorded at 37.8% in a study on reproductive performance of crossbred Holstein Friesian x Zebu in Ethiopia (Mekonnin et al., 2015). These findings clearly suggest the need for more sampling data to estimate the performance of crosses with Holstein Friesian properly.

Our study aimed to investigate the effect of climate on performance, also in relation to breed composition. Our aim therefore was to include the agro-ecological climate in our model in addition to the adapted management system to account for differences in the animal's environment. Unfortunately, due to the limited number of records available and the interference of the Corona pandemic, we were unable to collect more phenotypes of different dairy populations in Sri Lanka. The findings in this study therefore estimate the effect of breed proportion for the Kurunegala district located in the agro-ecological zone IL2 and for cattle kept under (semi)intensive conditions. Because Sri Lanka boosts a variety of climates as well as management practices (Abeygunawardena et al., 1998), a deeper analysis on the effect of local climate and management practices could provide more insight on the cattle breed composition best adapted to Sri Lankas different prevailing conditions. It is worthwhile to note that these conditions were already taken into consideration in the national breeding policy for dairy cattle in Sri Lanka which was adapted by the Ministry of Livestock and Rural Community Development in 2010. This breeding policy however also suggested a continuous upgrading of local cattle in some areas, which might not necessarily result in high performance due to segregation and recombination loss (Sendros, 2002) as also indicated in this study.

Although our findings strongly suggest the implementation of crossbreeding programs with Indicine and Taurine cattle for improved performance, it is important to consider whether the local infrastructure, management and breeding practices allow for a continues production of F1-crosses, as backcrossing to either Taurine or Indicine might significantly reduce performance (Yadav et al., 2018). The effectiveness of crossbreeding systems is determined by factors such as herd size, grazing systems, management expertise and the application of artificial insemination (Yadav et al., 2018) which will differ substantially across dairy herds in Sri Lanka. More data is therefore needed to improve our understanding of the use of exotic cross breeds in local small-scale farming systems in Sri Lanka.

Conclusion

In this study we analysed ancestral breed proportion and heterosis effects of crossbred cattle on two governmental farms in Sri Lanka using locus-specific ancestry. We demonstrated that performance of local Indicine cattle could be improved substantially through crossbreeding with Taurine cattle, mainly through the dominance effects of heterosis. Our study estimated an average 305-day milk yield of 1801 kg up to 2089 kg for first parity crossbred Sahiwal x Jersey, compared to 1076 kg up to 1477 kg for local Sahiwal, based on the results of two comparable linear mixed models. No significant influences were found for number of services per conception in crosses between Jersey and Sahiwal. Unfortunately, we could also not identify significant differences between the two Taurine breeds (Jersey and Holstein Friesian) used for crossbreeding on these farms, mainly due to a small sample size of Holstein Friesian crossbreds. Our results suggest however that using Holstein Friesian instead of Jersey for crossbreeding might result in higher milk yield, but a decreased fertility, measured as an increase in the number of services per conception. Since the cattle used for this study originated from the same agro-ecological zone under semi-intensive management systems, more research is needed to include environmental effects, as results may differentiate in the different local climates and livestock management systems of Sri Lanka. The findings of this study strongly suggest implementing a crossbreeding program that aims to optimize heterosis effects. More research is however needed to indicate the feasibility of such a program, especially for smallholder farmers as these make up the bulk of dairy farmers and are the main entry-point to increasing milk production in Sri Lanka.

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Appendix



Figure S1. Agro-ecological regions of Sri Lanka (Punyawardena, 2020).