Genetic Resources

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ORIGINAL ARTICLE

Carcass characteristics of Nordic native cattle breeds

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Abstract: Native livestock breeds are part of the history of the Nordic people and comprise a resource for future food production. In this study, net gain and carcass characteristics of two Danish, three Finnish, one Icelandic, six Norwegian and five Swedish native cattle breeds were retrieved and compared to commercial breeds: two beef breeds and two dairy breeds.

Breed data were collected from national databases and sorted into six animal categories: young bull, bull, steer, heifer, young cow and cow, for which means and standard deviations were calculated within each country. The native breeds ranged from small-sized milking type breeds with low net gain, carcass weights and EUROP classification to larger multipurpose breeds with high net gains, carcass weights and EUROP classification.

All Finnish and most of the Norwegian and Swedish native breeds had lower net gain and carcass weight than the dairy breeds in the same category and country, but with similar carcass conformation and fatness scores. The two Danish native breeds had higher net gain, carcass weight and conformation class than the reference dairy breed, but lower than the reference beef breeds. The net gain and carcass traits of the Icelandic native breed were similar to the smallest-sized native breeds from the other countries. The carcass traits of the native breeds indicate that they have comparative advantages in an extensive production system based on forage and marginal grasslands. They may also succeed better in the value-added markets than in mainstream beef production.

Keywords: Native cattle breeds, genetic resources, weight gain, carcass conformation, carcass fatness, carcass weight, meat production, cattle production, slaughter categories

Introduction

Genetic diversity of farm animals is a key factor in addressing some of the challenges posed by climate change. The variability of genetic resources underpins sustainable agriculture and food security and provides a broad spectrum of farm product quality characteristics (FAO, 2007). Moreover, diversity contributes to the achievement of several UN Sustainable Development Goals (SDGs), such as SDG2 (Zero hunger) and SDG15 (Life on land) (https://sdgs.un.org/goals). Finding profitable ways to utilize native livestock breeds is one of the biggest challenges in conserving rare livestock breeds. Detailed characterizations of beef production carcass and meat quality traits of several European beef and dairy breeds have been reported (Albertí *et al*, 2008; Christensen *et al*, 2011; Sevane *et al*, 2014). However, none of the native Nordic cattle breeds were included in those studies. There is a severe shortage of characterization studies for many Nordic native breeds (Kierkegaard *et al*, 2020). In addition, Nordic native cattle breeds are often low in numbers and are mostly kept as a hobby, thus little information has been gathered on their specific characteristics or the marketing of their products. This may suggest that these rare breeds are underutilized (Tienhaara, 2020). Traditionally, native Nordic cattle were raised as dual-purpose breeds giving both milk and meat. The combined production of milk and meat can reduce the total climate impact compared to the specialized separate production of milk and meat (De Vries *et al*, 2015). Today, however, part of the native cattle breed population is kept in suckler-based beef production systems, not selling milk for the dairy, and used for obtaining beef only (Hessle, 2009; Holene and Sæther, 2021).

During evolution, native animals have adapted to their environment and the available resources have been optimally utilized for different biological processes to maximize the animal's fitness in that environment (Beilharz *et al*, 1993). In the Nordic countries, cattle foraging historically took place mainly in the marginal outlands, i.e. forests and mountains (Dahlström, 2006; Emanuelsson, 2009; Bläuer, 2015). Adaptation to a harsh environment with low-energy diets is the reason for native breeds being small-sized with low maintenance requirements and high feed-intake capacity, categorizing them as early maturing breeds. Today's native breeds show more active foraging behaviour when roaming freely on outlands compared with modern commercial breeds (Sæther *et al*, 2006; Hessle *et al*, 2014), whereas breed differences in limited pasture areas are less pronounced (Hessle *et al*, 2008).

When it comes to the commercial beef breeds in the Nordic countries, results from a Norwegian study with Angus, Hereford and Charolais kept under various feeding levels revealed that Angus had higher production than Hereford in the extensive feeding system while Charolais needed the most intensive feeding system to reach their production potential (Wetlesen *et al*, 2018). In a Danish study, Hereford, Limousine, Simmental and Holstein suckler cows were compared at two feeding intensities in the suckling period (Olesen *et al*, 2004). The beef breed suckler cows had an extensive but breed-specific ability to adapt to various feeding levels. At the high feeding level, the response in Simmental was a large increase in milk yield and thus a large increase in calf growth, while the response in Hereford and Limousine was an increased live weight and body condition in the cows. At the low feeding level, the cows lost weight and body condition, and reduced milk yield but the response was much lower for Limousine than for Simmental (Olesen *et al*, 2004). Based on this, genotype x environment effects exist, but no data are available from experiments comparing native breeds and commercial breeds at various feeding intensities. However, it is hypothesized that commercial breeds are more efficient than native breeds in intensive production systems, whereas native breeds are more efficient in extensive systems with minor breed differences when fed medium-quality diets.

Beef producers in the Nordic countries are paid from slaughterhouses based on the European carcass classification system EUROP (Council of the European Union, 2013; Commission of the European Union, 2017). Most of the slaughterhouses favour carcasses of a specific weight range with high EUROP conformation class and medium fatness. However, the payment models are adjusted weekly due to changes in the market, and there is a variation between countries due to policies protecting national products. In general, rare native breeds have difficulties fulfilling the requirement for optimal pricing. Often, the small-sized and early-maturing native breeds do not reach the minimum carcass weight and carcass conformation for obtaining the best payment before the animal has deposited too much fat and has become inefficient. In early life, a growing animal will deposit a high proportion of muscles and later a higher proportion of fat. To avoid a too-high fat deposition before a desirable carcass weight is reached, the feeding intensity should be kept at a moderate level, especially for females (Robelin and Daenicke, 1980). Thus, the breeders of rare native breeds are challenged and often left with low prices unless they sell the beef on other markets.

This study aimed to characterize weight gain and carcass traits of Nordic native cattle breeds according to sex and age categories, and further categorize them in relation to commercial breeds found in four of the five countries. The study represents the most comprehensive comparison of variation among Nordic breeds and may as such be helpful to find opportunities for keeping them *in situ* for a later utilization of their inherent resources.

Materials and methods

Based on a Nordic network of animal and meat scientists, nine years of data describing the net gain (i.e. carcass gain) and carcass characteristics of Nordic native cattle breeds from Denmark, Finland, Iceland, Norway and Sweden were collected and compared with similar

data from commercial beef and dairy breeds in each country.

Data collection

Data included in the survey cover animals slaughtered from 2010 to 2018, except for Iceland where data were collected from 2018–2020, based on the national databases on cattle recordings and information obtained at slaughter. In Denmark, data was collected from the Danish Cattle Database (https://www.seges.dk/da-dk/fagomraader/kvaeg/raadgivning/kvaeg-it), in Finland from the official livestock register held by Mtech Digital Solutions (https://www.mtech.fi/en/), in Iceland from Huppa (https://www.rml.is/is/forrit-og-skyrsluhald/nautgriparaekt), in Norway from Animalia (https://www.animalia.no/no/kjott--egg/klassifisering/klassifisering-av-storfe/) and in Sweden from Kokontrollen (Växa Sverige https://www.vxa.se/kokontrollen). Due to variations in data ownership in the different countries and a need to keep data anonymous, this work used simple means of traits within each animal category and breed. Thus, a statistical comparison across countries, breeds and categories was not possible.

Definition of categories and traits

The data collection included six categories of cattle, based on their sex and age as shown in Table 1.

The traits included in the data collection are presented in Table 2 and were selected based on similar types of information available from the national databases and records in all five countries.

Description of breeds included in the study

The characterization was conducted for native breeds selected in each country for which the requested data were available. From Denmark: Jysk Kvæg and RDM-1970; from Finland: Länsisuomenkarja (Western Finncattle), Itäsuomenkarja (Eastern Finncattle) and Pohjoissuomenkarja (Northern Finncattle); from Iceland: Íslenska kúakynið; from Norway: Sidet Trønderfe og Nordlandsfe (STN), Telemarksfe, Dølafe, Østlandsk rødkolle, Vestlandsk Raudkolle and Vestlandsk Fjordfe; and from Sweden: Fjällko, Rödkulla, Väneko, Bohuskulla and Ringamålako. The characterization also included reference beef and dairy breeds: sub-populations of Charolais, Hereford, Holstein and Red dairy cattle (NRF and SRB) raised in the Nordic countries. A description of the breeds can be found in Supplemental Data.

Data handling and statistics

Within each country and breed, simple means and standard deviations were calculated for the traits: daily net gain, carcass weight, carcass conformation class and carcass fatness class (Table 2) for the six animal categories: young bull, bull, steer, heifer, young cow and cow (Table 1). A comparison between the native breeds and the reference breeds within a country

was undertaken on the basic data. For across country comparison of the reference and native breeds, a standardization was made for representatives of full-grown animals for slaughter, i.e. bulls and young cows. The commercial beef breeds, Hereford and Charolais, were used for the between-country adjustment of data. The average slaughter age of bulls of Hereford and Charolais across countries was 21.5 months calculated as the mean slaughter age of Charolais and Hereford bulls in Denmark (20.7 and 23.8 months, respectively), Finland (21.72 and 22.89 months, respectively), Norway (19.8 and 20.4 months, respectively) and Sweden (19.62 and 23.05 months, respectively). Thus, the carcass weight of the category bulls of all breeds was adjusted to a live weight at 21.5 months of age by multiplying the net gain per day by the days of difference in slaughter age deviating from 21.5 months, e.g. Danish Charolais bull had a net gain of 696g/d and a carcass weight of 384.5kg at slaughter thus the adjusted carcass weight at 21.5 months was 401.2kg (384.5kg + (0.8 months*30 days*696g/day/1,000)). In the same way, the carcass weight of the category young cows of all breeds was adjusted to the live weight at 34.3 months based on the average age at slaughter of young cows of Hereford and Charolais across countries. The figures were produced using R (R Core Team, 2020) with the R-package 'ggplot2' (Wickham, 2016).

Results

The number of animals and the average age of each category of the reference cattle used from Denmark, Finland, Norway, and Sweden are presented in Supplemental Table 1 and the same numbers of the native breeds from Denmark, Finland, Iceland, Norway and Sweden are presented in Supplemental Tables 2, 3, 4, 5 and 6, respectively.

Daily net gain and carcass characteristics

Danish breeds

The daily net gain of young bulls of Jysk Kvæg and RDM-1970 was high and only 5% lower than Holstein's (Figure 1a). For steers and heifers, this difference was 17% and 13%, respectively. A comparison for bulls is not relevant due to marked age differences in slaughter age between Holstein (14 months) and Jysk Kvæg and RDM-1970 (23 months). For all categories except young cows and cows, Jysk Kvæg, RDM-1970 and Holstein had numerically lower net gain than the two beef breeds. On the other hand, the variation in net gain specifically for the young bulls and bulls was numerically larger for the beef breeds compared with Holstein, and with the native breeds in between.

The average carcass weight of young bulls of Jysk Kvæg, RDM-1970 and Holstein was approximately 200 kg, with RDM-1970 being the highest and Jysk Kvæg being the lowest of the three (Figure 1b). Likewise, for the other categories, the native breeds were similar to Holstein but had lower carcass weights than the beef

Table 1. Definition of categories of cattle and national deviations used in the study

Category of cattle	Definition	Deviation
Young bull	Bull slaughtered younger than 12 months	
Bull	Bull slaughtered older than 12 months	In Iceland: 12–30 months
Steer	Steer slaughtered older than 12 months	
Heifer	Heifer slaughtered older than 12 months	In Norway: 12–24 months; in Iceland 12–30 months
Young cow	Cow slaughtered younger than 48 months	In Norway: Female 24–48 months
Cow	Cow slaughtered older than 48 months	

Table 2. Traits, definitions and national deviations used in the study

Trait	Definition	Deviation
Age, months	Age at slaughter	
Carcass weight, kg	Carcass weight at slaughter	
Daily net gain, g/day	(Cold carcass weight $- \frac{1}{2}$ birth weight)/age at slaughter	In Finland and Sweden, breed-specific birth weights[a] were used, in other cases, 20kg was used
Carcass conformation	EUROP classification, from class 1 (poor) to 15 (excellent)[b]	In Iceland E, U, R+, R, R-, O+, O, O-, P+, P, P. Converted to the 1–15 scale[c]
Carcass fatness	EUROP classification from class 1 (lean) to 15 (fat)[d]	In Denmark and Finland, the scale is 1–5, thus these numbers were multiplied by three, for comparison

[a] Växa Sverige (2021)
[b] Council of the European Union (2013); Commission of the European Union (2017) where original scale E, U, R, O, P has been extended with a –, 0, or + after each letter
[c] Iceland Regulation 500 (2017)
[d] Council of the European Union (2013); Commission of the European Union (2017), where original scale 1, 2, 3, 4, 5 has been extended with a –, 0 or + after each figure

breeds for young cows, cows, young bulls and bulls. For heifers and steers, the average carcass weights were quite similar between breeds, only the Charolais steers were heavier compared to the other breeds. In addition, for carcass weight, there was a large variation among the young bulls and bulls of the beef breeds whereas the variation was smaller among dairy and intermediate among native breeds.

The breeds differed more in carcass conformation, where the Holstein cattle of all categories had the numerically lowest carcass conformation, and with little variation, whereas the native breeds had carcass conformation above Holstein and close to Hereford, but below Charolais (Figure 1c). For the bull category, the mean carcass conformation was 6.2, 5.2 and 3.5 for Jysk Kvæg, RDM-1970 and Holstein, respectively. In comparison, Hereford bulls had 7.7 and Charolais 10.6. Despite cows being not specifically intended for beef production, the same ranking was seen for heifers, young cows and cows. No matter the category of animal, the Holstein cattle had the numerically lowest variation in carcass conformation, whereas the variation for beef breeds and native breeds were similar except for the young bulls, where the beef breeds again showed a larger variation compared with the native breeds.

The variation in carcass fatness was large, however, mean carcass fatness was similar for Jysk Kvæg, RDM-1970, Holstein and Charolais in the young bull, bull and steer category, and somewhat lower than Hereford (Figure 1d). For heifers, Jysk Kvæg was similar to Hereford. For heifer and cow categories, Jysk Kvæg had

higher carcass fatness than Holstein with RDM-1970 being in between.

Finnish breeds

In general, the mean daily net gain was higher in beef breeds and dairy breeds than in native breeds in all animal categories except for the cows (Figure 2a). The within-breed variation was numerically higher in beef breeds than in native breeds, especially in the categories of young bulls and bulls. Among native breeds, growing animals of Länsisuomenkarja had the highest daily net gain and largest variation within the group.

The mean carcass weight was lowest in native breeds of all animal categories (Figure 2b). In the native breed bulls, which was the category with the highest number of carcasses (Supplemental Table 3), Länsisuomenkarja had the heaviest carcasses, which was at the same level as the carcasses of beef breed heifers. The second heaviest native breed bull carcasses were Pohjoissuomenkarja and third Itäsuomenkarja. The within-breed variation was numerically higher in beef breeds than in other breeds in all animal categories except for heifers where the variation was equal in all studied breeds.

The two beef breeds had the numerically highest mean carcass conformation and within-breed variation in all studied animal categories (Figure 2c). The carcass conformation was at the same level in native breeds and the dairy breed.

The mean carcass fatness was in general numerically highest for Hereford, lowest for the dairy breed and

Figure 1. Daily net gain (a), carcass weight (b), conformation score (c) and fatness score (d) in six animal categories of three reference (Charolais, Hereford, Holstein) and two native (Jysk Kvæg, RDM-1970) breeds of Danish cattle, where the dots are means and bars are standard deviations.

Charolais with the native breeds in between (Figure 2d). Among the native breeds, carcass fatness was similar among heifers, young cows and bulls. In cows, young bulls and steers of native breeds, it was highest in Itäsuomenkarja and lowest in Länsisuomenkarja, but the differences were small. The numerical within-breed variation for carcass fatness was similar between breeds and larger among females than in intact males.

Icelandic breed

The daily net gain in the Íslenska kúakyniðbreed followed a similar pattern between categories as the other Nordic native breeds (Figure 3a). The daily net gain decreased with the age of the animal in females, where the daily net gain of heifers was approximately 200g, while it was around 150g for cows. The daily net gain of young bulls and bulls was around 300g.

The carcass weight of young bulls of the Íslenska kúakynið breed was lower (100kg) compared to the other categories (Figure 3b). The average carcass weight for heifers, young cows and cows was around 200kg, whereas bulls obtained an average carcass weight of 250kg.

All the female animals had similar carcass conformation around 3 (Figure 3c), while bulls showed a numerically higher carcass conformation (approximately 5) compared to young bulls (around 2).

Similar trends were observed for carcass fatness (Figure 3d), where no differences were observed among the female categories. However, large standard deviations indicate a high variation in carcass fatness of these animals. Bulls had carcass fatness around 5 whereas young bulls scored around 3, showing that carcass fatness of the male animals increased with maturity.

Norwegian breeds

All four reference breeds had higher daily net gain than the native breeds in all animal categories with the highest net gain in Charolais, which also had the largest variation in net gain (Figure 4a). Østlandsk Rødkolle was the native breed with the highest net gain in all categories (Figure 4a). Their mean net gain was close to the gains of Hereford and the dairy breeds Holstein and NRF for young bulls (441g). All the other native breeds had lower net gains. Telemarkfe had the lowest daily net gain among young bulls, whereas the lowest net gain for steers was found in Dølafe and Vestlandsk Raukolle.

The carcass weights in Norwegian young bulls are in general low compared to the other countries, probably due to a lower average slaughter age (Supplemental Tables 1 and 5). The carcass weights of the native breeds were lower than all the reference breeds (Figure 4b). Østlandsk Rødkolle was the native breed with the highest carcass weights (112kg) similar to the reference breeds Hereford, Holstein and NRF for young bulls (Figure 4b). Females of Vestlandsk Fjordfe had the lowest carcass weights. Variations in carcass weights were similar among all breeds, but with large variations

in the small groups of steers in Dølafe and Vestlandsk Raukolle.

Between the reference breeds, the two beef breeds had the numerically highest carcass conformation and the largest variation among all breeds, whereas Holstein had the numerically lowest and NRF intermediate carcass conformation (Figure 4c). The carcass conformation of the native breeds was similar to NRF for young cows and cows, similar to Holstein for bulls, and varying for young bulls, steers and heifers. Østlandsk Rødkolle had the numerically highest carcass conformation in all categories among the native breeds, except for the cow category where Dølafe had the numerically highest carcass conformation (Figure 4c). The variation in carcass conformation within the native breeds was largest in the small groups of steers in Dølafe and Vestlandsk Raukolle.

Hereford had the highest carcass fatness in all animal categories with more than 100 animals. Furthermore, Hereford was one of the breeds with the largest variation in carcass fatness for females and steers. Holstein had the lowest carcass fatness in the two cow categories and young bulls (Figure 4d). Among the native breeds, there was no general trend for one breed being either fatter or leaner across all animal categories.

Swedish breeds

The daily net gain was lowest in the native breeds, intermediate in the dairy breeds and highest in the beef breeds (Figure 5a). The breed difference was more pronounced in growing cattle than in adult cows, where also varying life span influenced the results. The within-breed variation in net gain was highest in the two bull categories, especially for beef breeds, followed by the native breeds Fjällko and Rödkulla.

The carcass weights were lowest in the native breeds, followed by dairy breeds and highest in the beef breeds (Figure 5b). For some of the categories, however, the native breed Väneko had carcass weights similar to dairy breeds. The variation of carcass weight within breed x category was extensive with a standard deviation of 55kg across breeds and categories (Figure 5b). The within-breed variation in carcass weight was larger for the native breeds than for the reference beef and dairy breeds for the heifer, bull and steer categories. For young bulls, the within-breed variation was highest for beef breeds followed by the native breeds Fjällko and Rödkulla.

The native breeds Väneko, Ringamålako and Rödkulla had similar carcass conformation as the dairy breed SRB, whereas the native breeds Fjällko and Bohuskulla had numerically lower carcass conformation, similar to the dairy breed Holstein (Figure 5c). Carcass conformation was numerically highest for the beef breeds.

There was no pattern related to breeds regarding carcass fatness (Figure 5d). Four breed x category with numerically extreme high or low carcass fatness were due to very few (n = 2 − 7) animals per group. Carcass fatness was more related to sex than breed, with
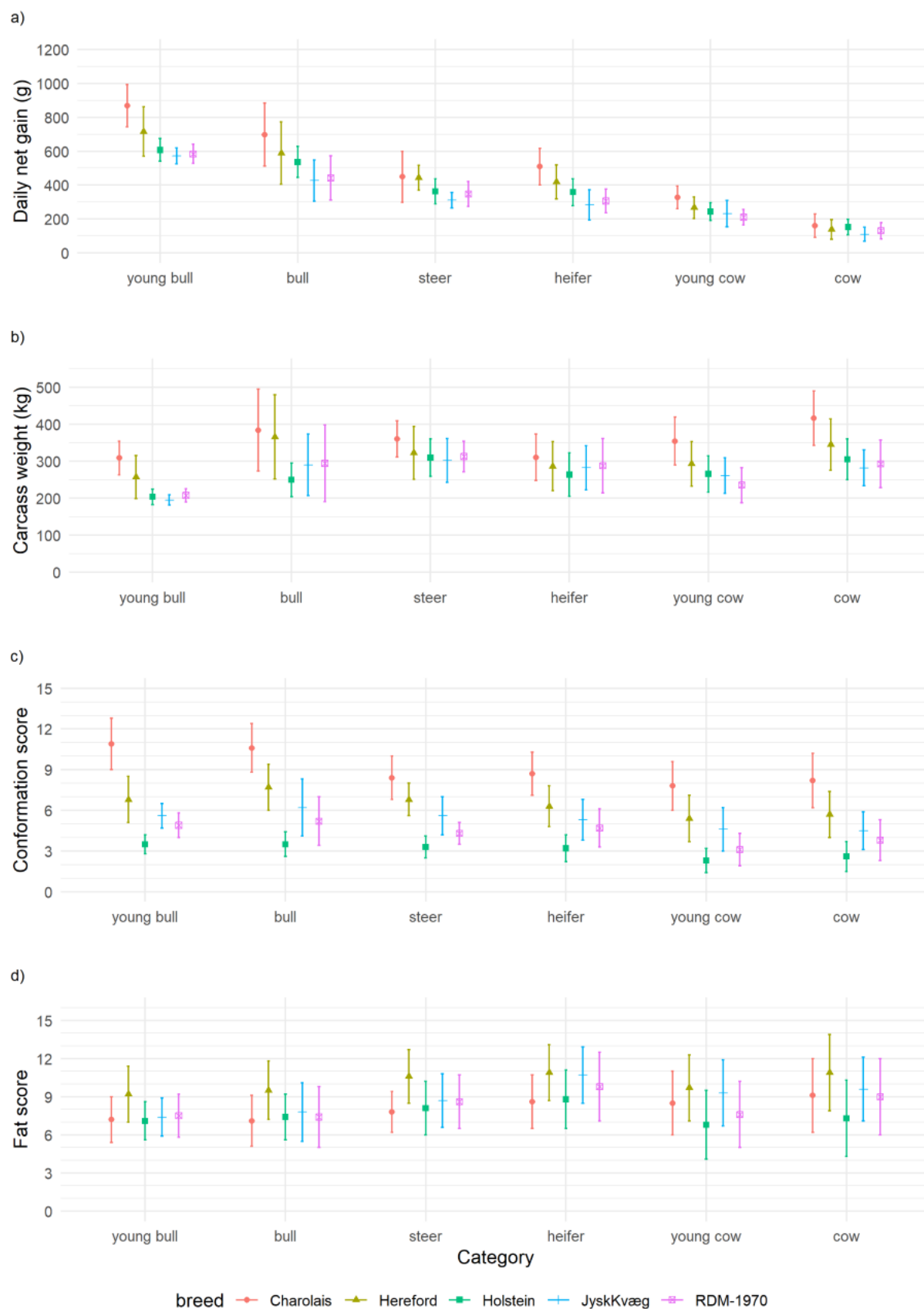
Figure 2. Daily net gain (a), carcass weight (b), conformation score (c) and fatness score (d) in six animal categories of three reference (Charolais, Hereford, Holstein) and three native (Länsisuomenkarja, Itäsuomenkarja, Pohjoissuomenkarja) breeds of Finnish cattle, where the dots are means and bars are standard deviations.

Figure 3. Daily net gain (a), carcass weight (b), conformation score (c) and fatness score (d) in five animal categories of the native (Íslenska kúakynið) Icelandic cattle breed, where the dots are means and bars are standard deviations. There is no steer production in Iceland.
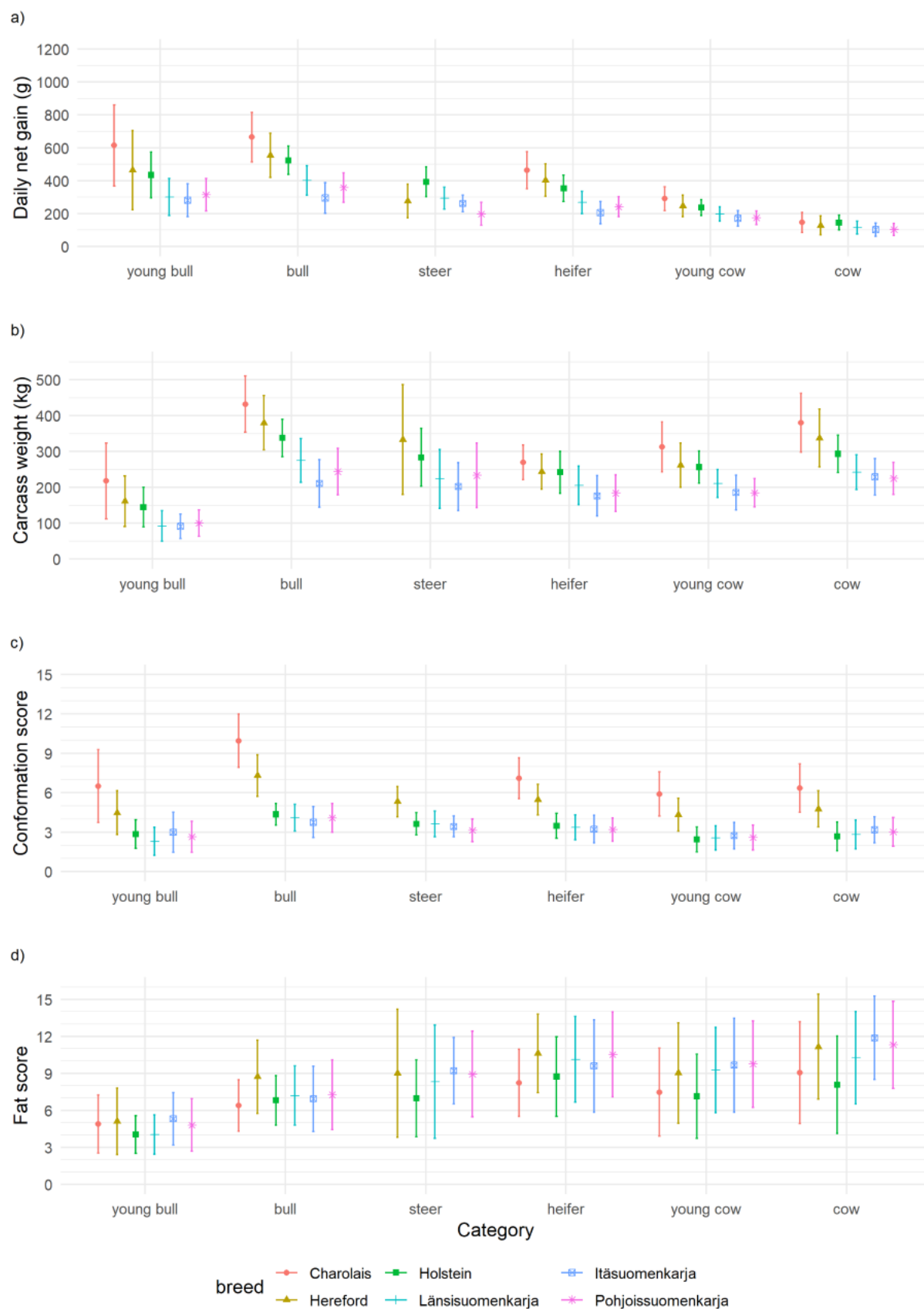
Figure 4. Daily net gain (a), carcass weight (b), conformation score (c) and fatness score (d) in six animal categories of four reference (Charolais, Hereford, Holstein, NRF) and six native (STN, Telemarksfe, Dølafe, Østlandsk Rødkolle, Vestlandsk Raukolle, Vestlandsk Fjordfe) breeds of Norwegian cattle, where the dots are means and bars are standard deviations.

females having higher carcass fatness than males and also showing a higher within-breed variation.

It should be noted that the numbers of Väneko, Ringamålako and Bohuskulla animals were low with fewer than 10 heads per breed x category for 9 out of 14 groups (Supplemental Table 6).

Comparison between countries

The adjusted carcass weights of the beef bulls showed a similar pattern for Charolais and Hereford, as both were highest in Finland and lowest in Norway, with a difference of 33kg and 46kg for Charolais and Hereford, respectively (Figure 6). For Holstein bulls, it was a bit different, as the Danish Holstein bull turned out as being the heaviest. However, the average age of the Danish Holstein bulls was only 14.4 months, thus using the daily net gain at that age to linearly predict the carcass weight at 21.5 months would probably overestimate the carcass weight at the adjusted 21.5 months used for the comparison. The weight of Holstein bulls from Finland, Norway and Sweden is more realistic, as they did not differ more than one to two months from the adjusted age of 21.5 months. Among those, the Finnish bulls were the heaviest and the Norwegian the lightest, with a difference of 20kg. For the young cows of the reference breeds, the Norwegian Charolais, Hereford and Holstein were always the lightest relative to Danish (+64, +44 and +23kg, respectively), Swedish (+46, +62 and +25kg, respectively) and Finnish cows (+26, +20 and +10kg, respectively) (Figure 7).

The adjusted carcass weights of the native breeds were close to 250kg for one group of bulls (Danish Jysk kvæg and RDM-1970, Finnish Länsisuomenkarja and Pohjoissuomenkarja, Norwegian Østlandsk Rødkolle, and Swedish Rödkulla, Väneko, Bohuskulla and Ringamålako) and closer to 200kg for a group of smaller native breeds (Finnish Itäsuomenkarja, Icelandic Íslenska kúakyniŏ, Norwegian STN, Telemarkfe, Dølafe, Vestlandsk Raukolle, and Vestlands Fjordfe, and Swedish Fjällko) (Figure 6). The carcass weights of bulls were lowest for all native breeds, followed by Hereford and dairy breeds, whereas Charolais from all countries had the highest carcass weights.

For young cows from native breeds, the two Danish native breeds and the Swedish Väneko had the highest carcass weights, whereas the lowest carcass weights were seen among the Finnish Itäsuomenkarja and Pohjoissuomenkarja, Icelandic Íslenska kúakyniŏ, the six Norwegian breeds and the Swedish Fjällko (Figure 7). The adjusted carcass weights of young cows showed a similar pattern as bulls, with the heaviest carcass weights being the Charolais from Denmark, Finland and Sweden, followed by Charolais from Norway and Hereford from Denmark, Finland and Sweden. The dairy breeds from all countries were similar to the Norwegian Hereford as well as the two Danish native breeds and Väneko from Sweden.

The adjusted carcass weights of bulls of the native breeds ranged across countries from 196 to 275kg with an average of 233kg, corresponding to 57, 71 and 67% of the average carcass weight of Charolais, Hereford and Holstein, respectively. The adjusted carcass weight of young cows of the native breeds across the countries was 172–253kg, with an average of 203kg, corresponding to 62, 72 and 78% of the average carcass weight of Charolais, Hereford and Holstein, respectively.

Regarding carcass conformation, bulls of the Danish breeds Jysk Kvæg and RDM-1970 and Swedish breeds Rödkulla, Väneko and Ringamålako scored 5 or more, similar to the red reference dairy breeds NRF and SRB. Bulls of the remaining native breeds had carcass conformation around 4, similar to the reference dairy breed Holstein. In addition, young cows of the native breeds Jysk Kvæg, Rödkulla and Väneko had numerically higher carcass conformation (4) compared to the other native breeds, which had about 3 in carcass conformation. Generally, young cows of native breeds were in the same range as the dairy breeds (2.5–4.5).

Carcass fatness for bulls within country differed by less than one unit for all native breeds, dairy breeds and Charolais. The Icelandic Íslenska kúakyniŏ and Norwegian Telemark, Dølafe and Østlandsk Rødkolle were the numerically leanest breeds with a carcass fatness close to 5 followed by Charolais, dairy breeds and the other native breeds with a carcass fatness close to 6. Hereford was numerically the fattest breed in all countries. For young cows, the variation in carcass fatness between breeds within country was larger than for bulls. The young cows of Danish Jysk Kvæg, the three Finnish native breeds and the Swedish Väneko were similar in carcass fatness to Hereford, whereas the other native breeds were numerically fatter or similar to Charolais and dairy breeds, but leaner than Hereford.

Discussion

Preservation of native breeds

Preserving native breeds can occur in two main directions: preserving by conservation or preserving by development (FAO, 2007). Preserving by conservation implies aiming at keeping the characteristics and their variation within the population as they were at the start of the conservation work. Preserving by development means selecting specific characteristics within the population, such as higher milk yield or more developed muscle conformation (Statistics Iceland, 2022). Different stakeholders can have different opinions on which of these two directions is desirable, and this is the case for the Nordic native cattle breeds. These breeds are preserved in different ways aiming at either conservation or development for specific production traits, or something in between. The way of preserving a specific breed impacts what means are available to influence traits, e.g. carcass characteristics. For breeds being developed, genetic selection is a measure to improve this trait, whereas only environmental factors, such as production systems
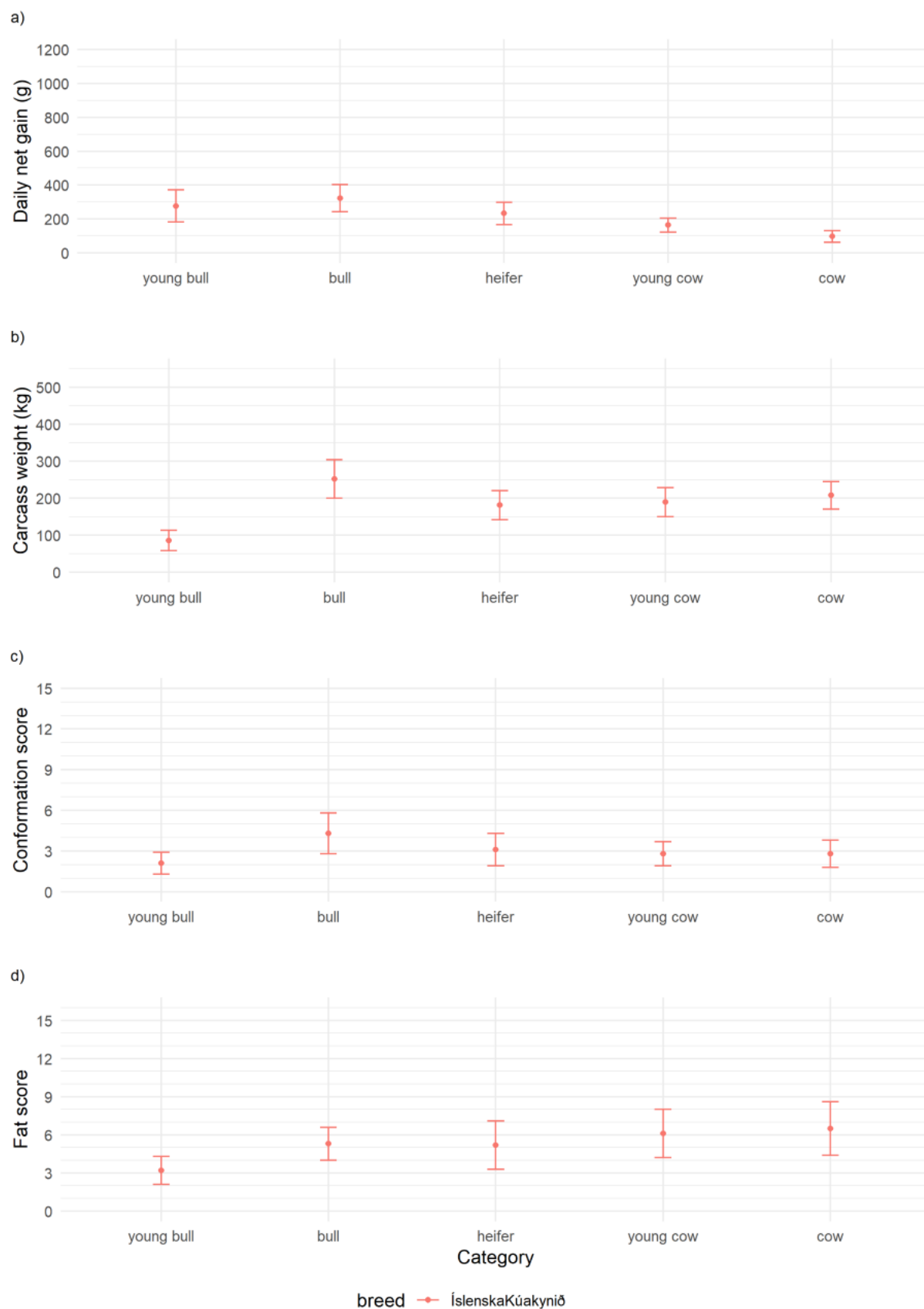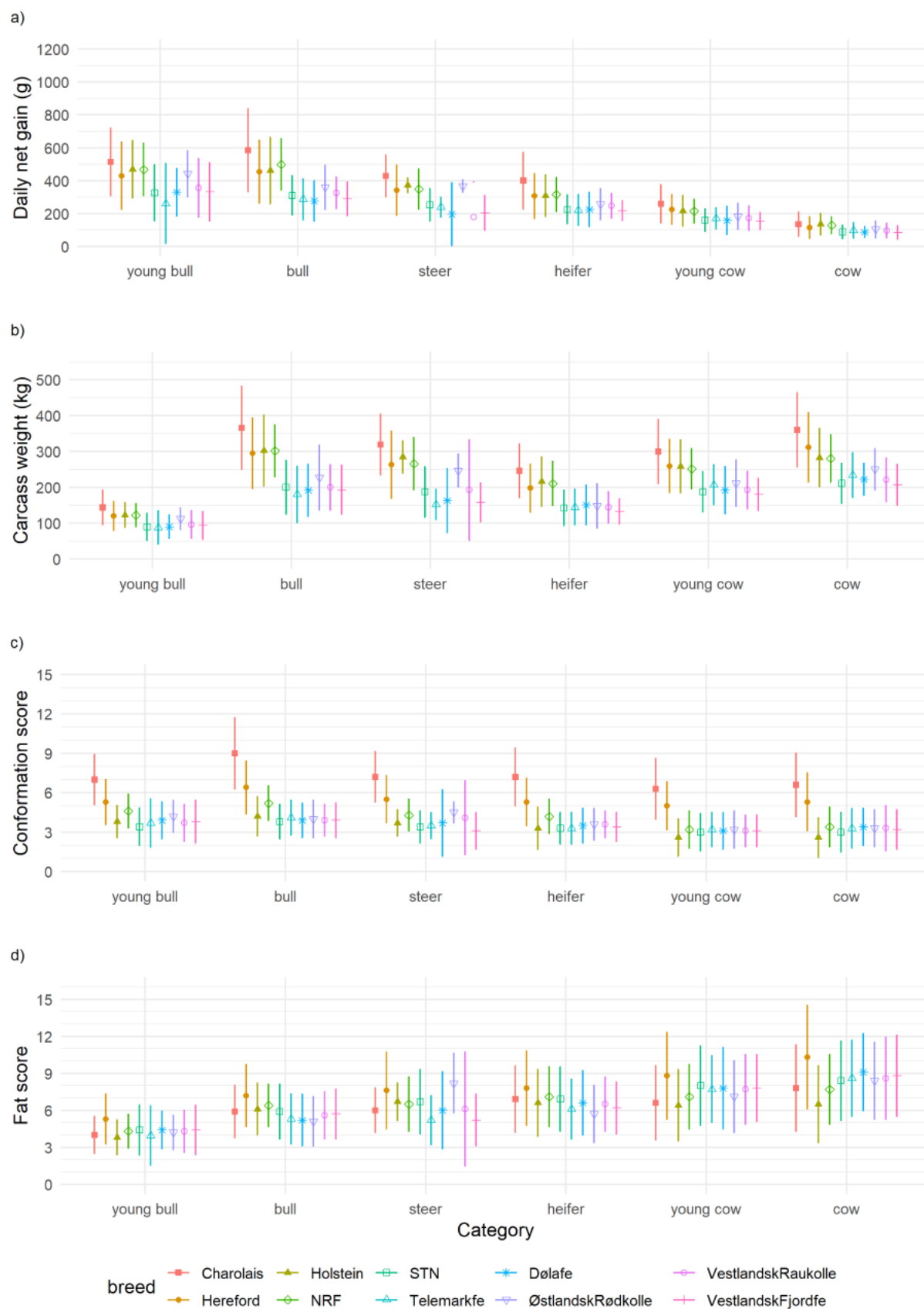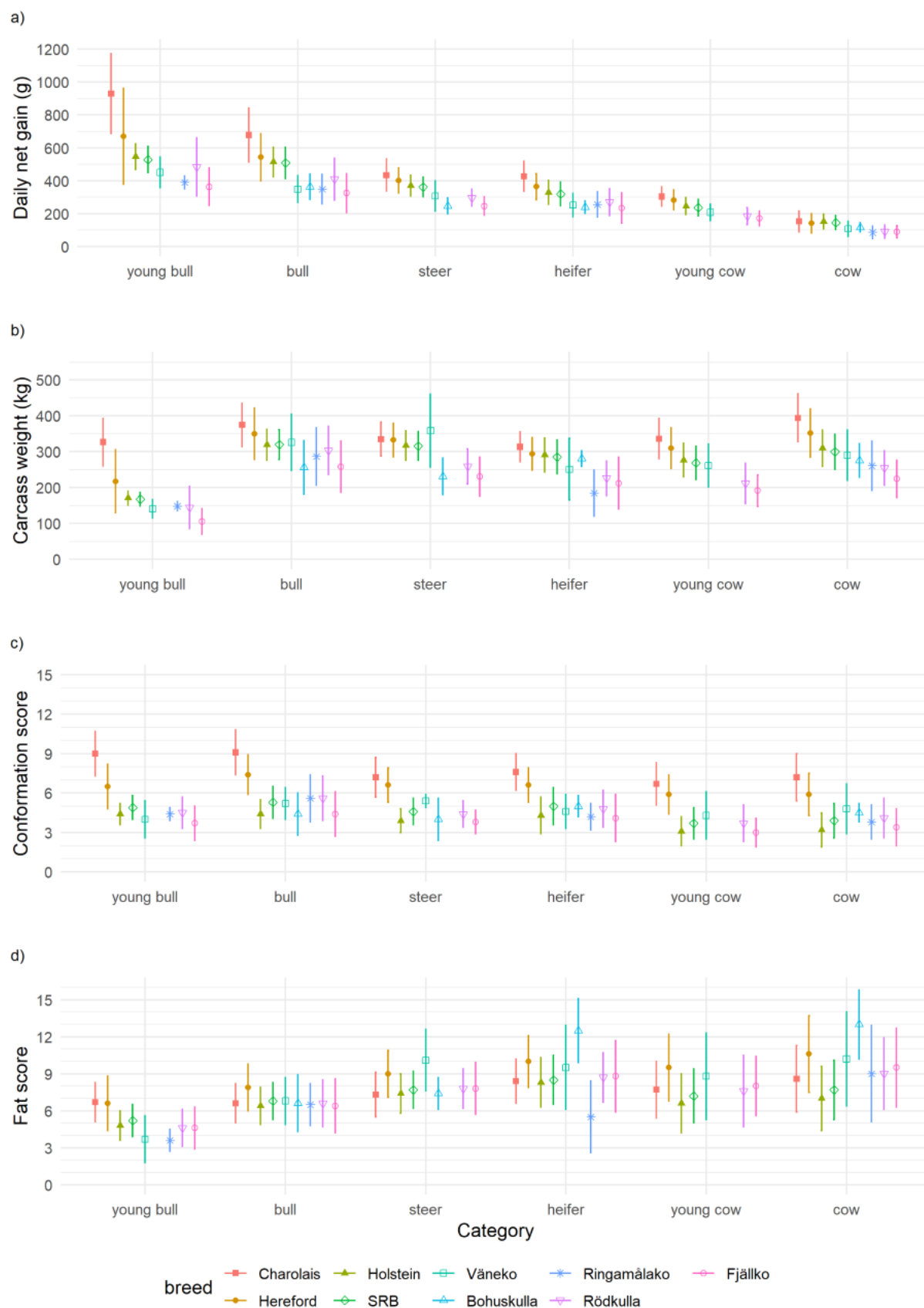
Figure 5. Daily net gain (a), carcass weight (b), conformation score (c) and fatness score (d) in six animal categories of four reference (Charolais, Hereford, Holstein, SRB) and five native (Väneko, Bohuskulla, Ringamålako, Rödkulla, Fjällko) breeds of Swedish cattle, where the dots are means and bars are standard deviations.

Figure 6. Adjusted carcass weights of bulls of reference (Charolais, Hereford, Holstein, NRF, SRB) and native breeds in Denmark (DK) (Jysk Kvæg, RDM-1970), Finland (FI) (Länsisuomenkarja, Itäsuomenkarja, Pohjoissuomenkarja), Iceland (IS) (Íslenska kúakynið), Norway (NO) (STN, Telemarksfe, Dølafe, Østlandsk Rødkolle, Vestlandsk Raukolle, Vestlandsk Fjordfe) and Sweden (SE) (Väneko, Bohuskulla, Ringamålako, Rödkulla, Fjällko), where slaughter age was adjusted to 21.5 months, which was the average age of Charolais and Hereford across countries.

and market opportunities, can be used to exploit the characteristics of conservation breeds.

The most obvious example of preserving by development in the present study is the Icelandic Íslenska kúakynið, which is a native but commercial dairy breed considered large enough for active breeding purposes, without severe risks of inbreeding (Gautason *et al*, 2020). The Íslenska kúakynið is one of the few native cattle breeds remaining in northern Europe that still comprises a large population size and a well-defined purpose (Ásbjarnardóttir *et al*, 2010). Icelandic agriculture is now in the dilemma of maintaining the breed to preserve biodiversity by development or relying on more efficient imported continental and British meat breeds to compete with imported meat and other food products on the market. Today, the market share of meat from Íslenska kúakynið, crossbred bulls and imported meat is about 70%, 15% and 15%, respectively (Statistics Iceland, 2022).

Like the Íslenska kúakynið, there is an ongoing, albeit weak, genetic selection in the Swedish Fjällko focused on milk production, having today, despite its small body size, an average milk yield of 60% of the dominating commercial breeds Holstein and SRB (Växa Sverige, 2021). However, there is a subgroup of Fjällko focused on conservation (Föreningen Äldre Boskap,

2022). In Finnish Länsisuomenkarja, an improvement in milk yield was undertaken between 1950 and 1970 by crossbreeding it with Friesian (currently Holstein) before the actual conservation work started with the purebred animals (Luke, 2015). The yield is today 68 to 74% of the commercial breeds of Holstein and Ayrshire used in Finland, respectively (Nokka, 2021). Breeding for increased milk production has also led to a rise in body size. The change in height varies from 7 to 14% in all Finncattle breeds. Correspondingly, the alteration in live weight is on average 18%. This is partly explained by improved conservation status, but more likely due to pleiotropic effects of genes controlling both milk production and body size (Viitala *et al*, 2006; Xiang *et al*, 2017).

Preserving by conservation is the most common direction for a majority of the Nordic native breeds. It is applied to the Danish breeds Jysk Kvæg and RDM-1970, Finnish breeds Itäsuomenkarja, Pohjoissuomenkarja and Länsisuomenkarja since the beginning of 1980s, Norwegian breeds Dølafe, Telemarkfe, Vestlandsk Raudkolle and Vestlandsk Fjordfe, and Swedish breeds Rödkulla, Väneko, Bohuskulla and Ringamålako. There are, however, some breeds where the stakeholders are aiming at simultaneously conserving and developing the breed,
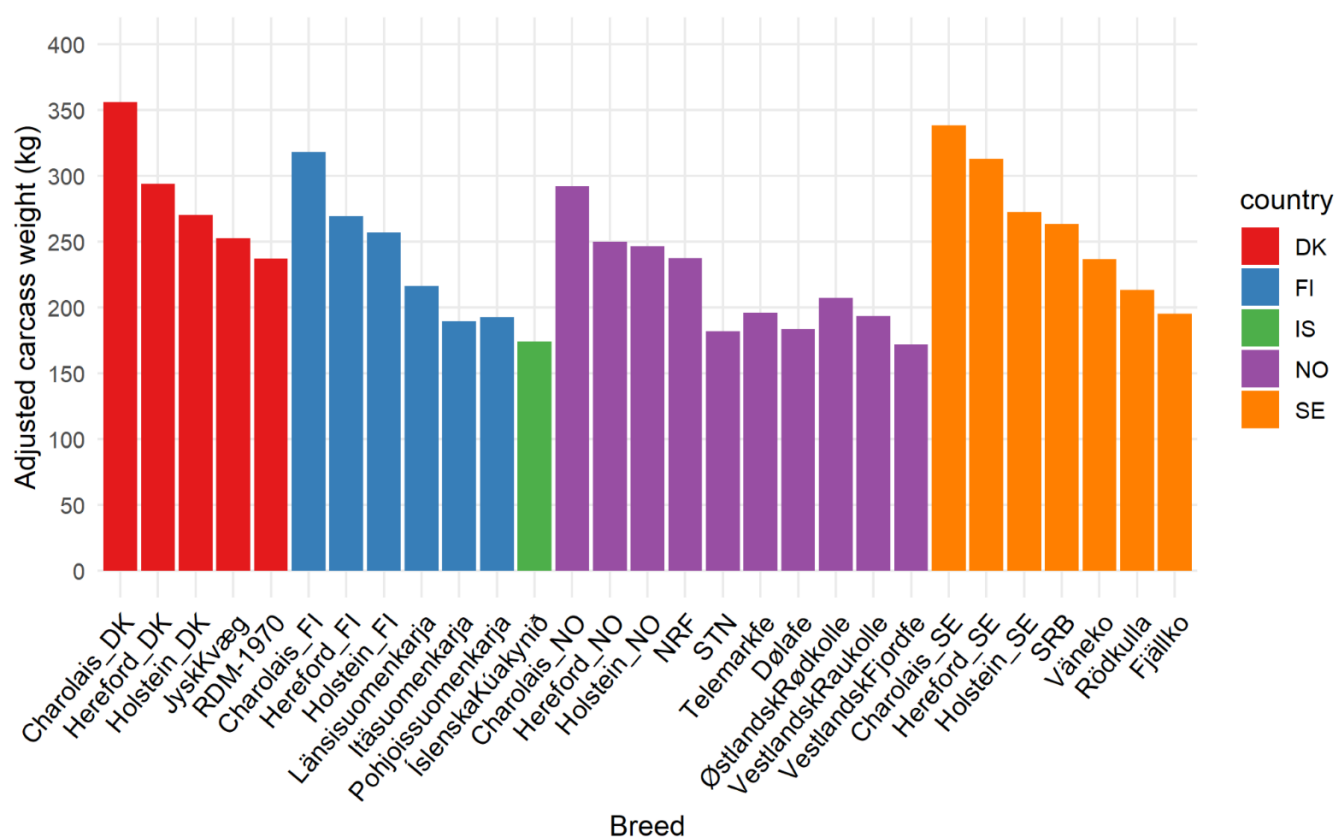
Figure 7. Adjusted carcass weights of young cows of reference breeds (Charolais, Hereford, Holstein, NRF, SRB) and native breeds in Denmark (DK) (Jysk Kvæg, RDM-1970), Finland (FI) (Länsisuomenkarja, Itäsuomenkarja, Pohjoissuomenkarja), Iceland (IS) (Íslenska kúakyniõ), Norway (NO) (STN, Telemarksfe, Dølafe, Østlandsk Rødkolle, Vestlandsk Ra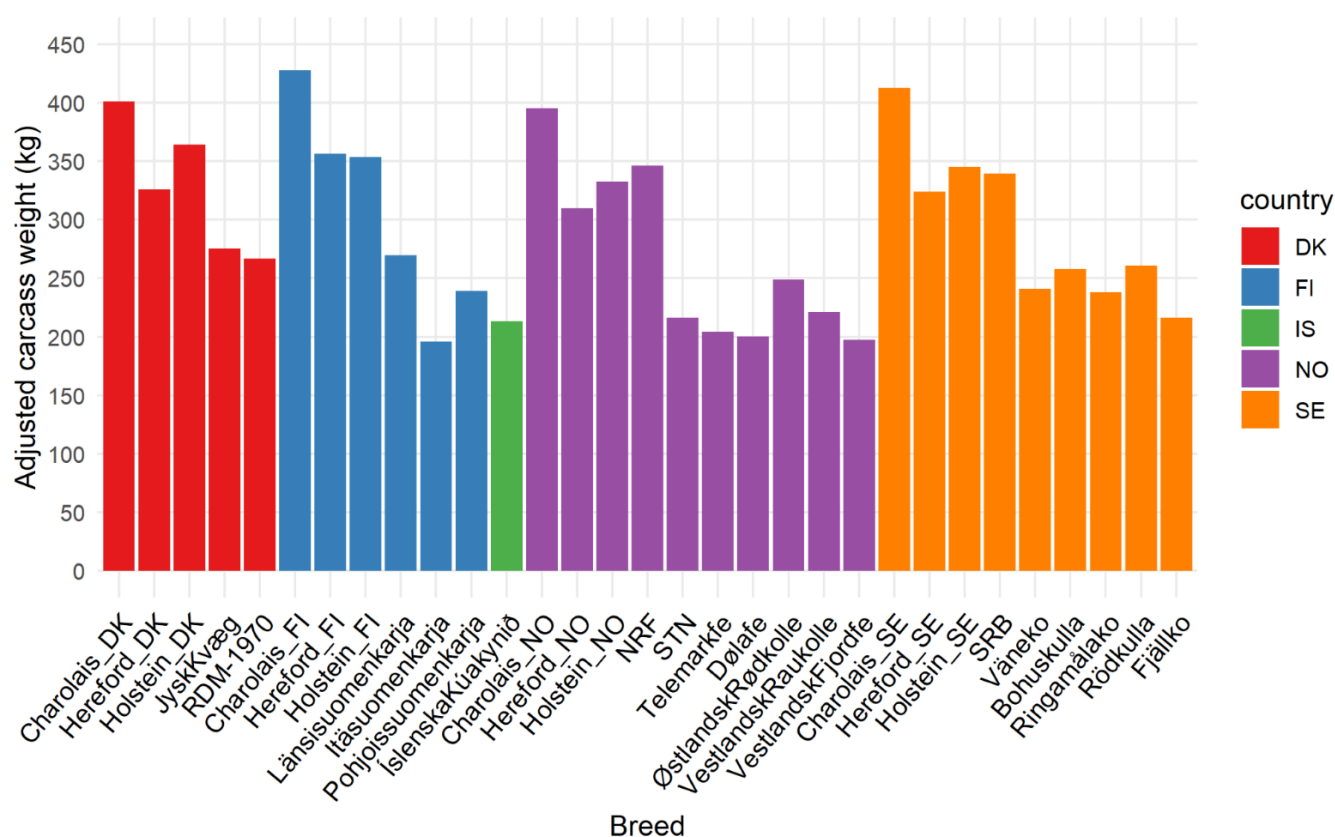ukolle, Vestlandsk Fjordfe) and Sweden (SE) (Väneko, Rödkulla, Fjällko), where the slaughter age was adjusted to 34.3 months, which was the average age of Charolais and Hereford across countries.

namely, the Finnish breed Länsiuomenkarja, and the Norwegian breed Sidet Trønderfe og Nordlandsfe (STN).

Variation between breeds

The differences in carcass characteristics among the native breeds could be either due to genetics, developed in varying historical production systems or due to various environmental factors such as general feeding levels and national market preferences. The two Danish native breeds Jysk Kvæg and RDM-1970 had a higher net gain, carcass weight and carcass conformation than the reference dairy breed, although the reference beef breeds had even higher values. Contrary, all Finnish and most of the Norwegian and Swedish native breeds had lower net gain and carcass weights than the dairy breeds in the same category and country, but with similar carcass conformation and carcass fatness. The net gain of the bulls of the native breeds from these countries was at the same level as the heifers of beef breeds. There were no reference breeds in Iceland, but previous studies have found bulls of pure native breeds to be inferior to native x beef crossbred bulls in weight gain and carcass weight (Rikhardsson *et al*, 1996; Hilmarsson *et al*, 2000).

Breed means of carcass characteristics indicate that there is a range of native breeds where one end in the spectrum contains small-sized breeds of distinctive milk type with low net gain, carcass weights and carcass conformation, and the other end consists of big-sized dual-purpose breeds with high net gains, carcass weights and carcass conformation. The comparison between breeds and specifically the Nordic native breeds compared with the reference breeds, show that it is the carcass weight that deviates most from the reference breeds, whereas the conformation and the fatness of the Nordic native breeds are close to the Holstein and the red dairy breeds bulls and young cows. Bigger-sized breeds such as Danish Jysk Kvæg and RDM-1970, the four southern-most Swedish breeds Rödkulla, Väneko, Bohuskulla and Ringamålako originate from regions with both lusher conditions and a higher influence of foreign genetics. Finnish Länsisuomenkarja originates also from more lush conditions than the other two Finnish native breeds. However, at the time of establishing the herd book, the true representatives of each Finnish native breed were thought to be found in the small, isolated villages where cattle most likely suffered from lack of feed. Therefore, the breeds of Finnish Itäsuomenkarja and Pohjoissuomenkarja were considered smaller than Finnish Länsisuomenkarja (Juvani,

2014). Smaller-sized breeds, such as the six Norwegian breeds, especially Telemarksfe, and the Swedish Fjällko originate from harsher environments. The small-sized Íslenska kúakynið from Iceland has not been influenced by foreign genetics, whereas the biggest-sized Norwegian native breed Østlandsk Rødkolle historically has been crossed with NRF and has previously shown high weight gains (Rundlöf, 2014).

All Nordic native breeds were originally kept mainly for milk production, but today a majority of all the Norwegian and Swedish native breeds, except Fjällko, are kept as suckler cows (Hessle, 2009; Belin, 2017; Holene and Sæther, 2021; Sæther *et al*, 2021). However, according to the data analyzed here, more Finnish native breeds are still kept for milk production (45, 26 and 36% for Länsisuomenkarja, Itäsuomenkarja and Pohjoissuomenkarja, respectively). Weight gain is most likely promoted by the suckling system, especially for breeds with high milk-producing potential such as Länsisuomenkarja, Østlandsk Rødkolle and Rödkulla. Thus, comparing net gain in suckling vs. manually fed calves can be biased. The variation in net gain within the heifer, young bull and bull categories of the Swedish Rödkulla and Fjällko might be an effect of mixed groups with both manually milk-fed and suckling calves. Also, comparison among countries might be biased as the introduction of native breeds in suckling systems did not start simultaneously in all Nordic countries. This might be a further explanation for the higher net gains for growing cattle in the Danish and four southern-most Swedish breeds compared to the other native Nordic breeds. On the other hand, even if the native breeds are kept as dairy cows, the practice may also vary between breeds and countries – in Denmark the native cows would typically be part of a conventional system, which then challenges the native breeds on fatness and health, due to the high-quality diet offered to the cows (Munk *et al*, 2020).

Comparisons over time

In small populations, there is a significant risk of genetic drift, also in the Nordic native cattle breeds (Upadhyay *et al*, 2019). Such unintended changes in the breed are not desirable in conservation.

The net gain of the Danish native breeds RDM-1970 and Jysk Kvæg in the present study was only 5% lower than the net gain seen under the current commercial praxis of slaughter calves (Myhlendorph-Jarlfoft, 2022). Furthermore, it was only slightly lower than the level obtained for young bulls of RDM and Sortbroget Dansk Malkekvæg, in studies from the 1970s (Andersen, 1975; Andersen *et al*, 1977). Also, the growth potential, as evaluated using net gain, of the Danish reference dairy breed Holstein seems to be only marginally improved during 40 years of selection for primarily milk yield, illustrated by the stable genetic level for growth traits in the last 20 year in all the Nordic dairy breeds (https://nordic.mloy.fi/NAVTrends). However, the lower carcass conformation of Holstein in the present study, compared

to that of Jysk Kvæg and RDM-1970 breeds, suggests a lower dressing percentage in the former, which in turn may have led to a smaller increase in average daily live weight gain for the Holstein. In accordance with studies from the 1970s (Andersen, 1975; Andersen *et al*, 1977), the Jysk Kvæg in the present study had 11% higher carcass weight compared with RDM in the 1970s.

While Holstein has developed from a dual-purpose type to a milk type with low carcass conformation during the last decades, the two Danish native breeds in the present study had similar carcass conformation as in the studies from the 1970s (Andersen *et al*, 1977). Then as now, Jysk Kvæg had a considerable higher carcass conformation than RDM-1970 (Andersen *et al*, 1977). Carcass fatness had not changed much for any of the Danish breeds.

Fulfilling market's demand for carcass characteristics

Carcass weight, carcass conformation and carcass fatness aim at giving an estimate of the relative value of the carcass for the industry. Lower prices paid to the farmer for small-sized cattle with less developed muscle conformation, compared to mainstream carcasses, are due to a similar cost of processing but obtaining less saleable meat. The highest prices for a specific weight range are also due to the end-consumer expecting specific retail cuts to be of the same size and shape. Mainstream selection in beef production is towards bigger carcasses (Pesonen, 2020), leaving the small populations of conserved native breeds behind.

Along with increasing live weight, growing cattle deposit a lower proportion of muscles and an increasing proportion of fat. For small-sized and early maturing breeds, such as the native breeds, the challenge is to plan for a time of slaughter when the body has become heavy enough but not too fat. Consumers' desire for fatness varies among markets. Therefore, the beef market differs among the studied countries, and thus affects the carcass composition. In Norway, lean bovine carcasses with carcass fatness of 3–6 are preferred (Nortura SA, 2021), whereas fatter carcasses are asked for in Denmark (7–12, Danish Crown, 2022), Finland (9, Kiviranta, 2022a,b), Iceland (6–8, SS Meat Company Iceland, 2022) and Sweden (6–9, HkScan Agri, 2021). Hence, in the present study, the average carcass fatness and carcass weights were lower in Norway than in the other countries. National individuality in the rosé veal market affects the results of the heifer and young bull categories, with varying target weights and proportions of cattle within the groups reared for rosé veal versus beef.

The heavier native breeds, i.e. Danish Jysk Kvæg and RDM-1970, the Finnish Länsisuomenkarja, the Norwegian Østlandsk Rødkolle and the Swedish Väneko and Rödkulla, had carcass characteristics best fitting into the present market preferences, with moderately high carcass weight and net gain. The other native breeds, i.e. the Finnish Itäsuomenkarja and Pohjoissuomenkarja,

the Icelandic Íslenska kúakyniŏ, the Norwegian STN, Telemarkfe, Dølafe, Vestlandsk Raudkolle and Vestlandsk Fjordfe, and the Swedish Fjällko, Bohuskulla and Ringamålako, had carcasses which could be more problematic to fit into the mainstream market.

Recommendations for future beef production on the endangered native breeds

As stated above, there is a risk for cattle of native breeds and other small-sized breeds to become too fat before the lower limit of optimal carcass weight range has been reached. The best way to avoid excessive fat deposition at too low live weights is an extensive forage-based production system with only moderate weight gain (Robelin and Daenicke, 1980; Webster, 1989).

In the present study, the rearing generally seems to have been less intensive for the native breeds than for the beef breeds, also when the difference in mature live weights is considered. The carcass weights of the native breeds were about 71% of the beef breeds, whereas their net gain was only 65% of the beef breeds' net gain. Despite this low feeding intensity, a vast majority of the male cattle of the native breeds were raised as intact bulls (70–100%), which generally are more suited to intensive feeding. It is known that the growth potential of, for example, the Icelandic Íslenska kúakyniŏ is not fully exploited (Sveinsson, 2017). In accordance with previous research (Sveinsson, 2016), the present study confirmed a 25-month slaughter age for indoor bulls of this breed, which, together with the Swedish native breeds, was the oldest at slaughter. The long rearing time for the Íslenska kúakyniŏ bulls has a negative economic impact on the production output of facilities, feed and labour (Sveinsson, 2017). Previous studies have shown that at least under Swedish conditions, male calves for the highest profitability should either be reared very intensively as indoor bulls or very extensively as grazing steers, whereas semi-intensive rearing results in a poor economy (Hessle and Kumm, 2011).

Castrating a higher proportion of the males of the native breeds, and rearing them as steers, would be a means to keep them in even more extensive production systems where their carcass characteristics would be less deficient compared to commercial breeds. An increasing proportion of cattle of the Nordic native breeds are kept in suckler-based beef production systems, often in small integrated herds, where castration would enable a rational common keeping of males and females (Hessle, 2009; Claesson and Ekberg, 2015; Belin, 2017; Holene and Sæther, 2021; Sæther *et al*, 2021).

Animals' growth potential and carcass characteristics influence the economy on the farm. Other factors than carcass revenues, such as agri-environmental payment and support and costs for winter housing, influenced the profitability more than genotype, especially in extensive pasture-based systems where the higher genetic growth potential in the crossbreds could not be

fully expressed (Holmström *et al*, 2021). The differences between the genotypes in the most extensive system studied by Holmström *et al* (2021) was 50kg in carcass weight and 2.6 in carcass conformation, which is in the same range as breed differences for steers, heifers and young cows in the present study. Hence, although production conditions vary among the Nordic countries, the economical drawback of not fully market-oriented carcasses in the native breeds, compared to commercial breeds, should not be exaggerated, especially not in extensive production systems. Hence, the results of this study support the multi-use potential of Nordic indigenous cattle as beef production in extensive production systems on marginal grasslands, forests and mountains, while providing ecosystem services for the revitalization of traditional biotopes. The Finnish native breed Itäsuomenkarja is an example of a breed that has found its role in providing ecosystem services grazing traditional landscapes (Lilja *et al*, 2009). Dairy farmers in Iceland have chosen development over conservation of the native Íslenska kúakyniŏ. The farmer will continue to use it as the main production breed in the country, despite economic arguments against it. The multicoloured breed is what makes cattle farming in Iceland unique. It is considered the best way to meet the growing competition for dairy and meat products (Agrogen Is, 2021). Most of the Icelandic population supports it for traditional and cultural reasons. Growing tourism also plays a role. One reason for Iceland´s popularity as a destination is its unique landscape and scenery (Ferðamálastofa-Icelandic Tourist Board, 2016). Maintenance of the native grazing livestock both perpetuates the scenery and constitutes a part of the uniqueness. Changing or replacing native livestock with commercial breeds would most likely decrease the attractiveness of Iceland as a tourist destination.

The eating quality of meat is not estimated in the EUROP carcass classification system (Guzek *et al*, 2016; Bonny *et al*, 2016). Meat traits in Nordic native breeds have previously been compared with meat from commercial breeds with varying results. Their meat has been found to have more intramuscular fat with a healthier fatty acid composition and to be tastier, juicier and more tender than meat from commercial breeds (Aass and Fristedt, 2003; Suleimenova, 2016) and is appreciated by chefs in restaurants (Exceptionell Råvara, 2022; Soini *et al*, 2019). On the other hand, other studies show bulls of purebred native breeds being inferior to native x beef breed bulls in juiciness, tenderness and overall sensory quality (Rikhardsson *et al*, 1996; Hilmarsson *et al*, 2000). The divergence in results of these studies is most likely due to varying experimental designs. However, the present favouring of increased carcass weight and carcass conformation in the price setting does not support a focus on valuable meat quality traits, which could be used to increase the market share of native beef products. In addition to eating quality traits, there might be other characteristics

of the meat from native breeds, similar to specific traits of their milk, which could be used as benefits when selling the product. The specific milk protein composition giving a high cheese yield in the Swedish Fjällko has been known for a long time (Hallander, 1989). Likewise, a recent milk oligosaccharide profiling study (Sunds *et al*, 2021) demonstrated that milk of Finnish Länsisuomenkarja has a suitable quality for special food ingredients such as infant formula or healthy ingredients. To support similar development for meat from native breeds, the characterization of meat quality traits and composition is required.

Possible shortcomings of the study

Sample sizes was the smallest for the Swedish breeds Väneko, Bohuskulla and Ringamålako with 22–80 animals, indicating results from these breeds are more uncertain than the others. Across countries, the number of samples in the steer category was relatively low for all native breeds, especially in some Norwegian and Swedish breeds, indicating results from the steer category should be interpreted with reservation. Besides the small populations, the low number of animals of native breeds could be due to absent carcass data from some small abattoirs. Finally, a source of error in the used data could be incorrectly reported breed codes in the databases.

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Author contributions

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Declaration of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplemental Data

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Phenotypic variations among okra (*Abelmoschus esculentus* (L.) Moench) genetic resources in Sudan

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Abstract: A total of 366 accessions of okra (*Abelmoschus esculentus* (L.) Moench) obtained from the Agricultural Plant Genetic Resources Conservation and Research Centre (APGRC) in Sudan were characterized for a number of morphological characters using a descriptor list derived from the list published by the International Board for Plant Genetic Resources in 1984. Those accessions, which were collected from different regions of Sudan, were grown in the APGRC research farm in Wad Medani town in central Sudan during ten different seasons within the period between 2000 and 2019. Phenotypic variations were observed among and within the different accessions for plant, stem, leaf, inflorescence and fruit characters. Up to 59% of accessions were found to be heterogeneous for different traits. The descriptor states observed ranged from very rare in 5% or less of the accessions, to abundant in more than 90% of the accessions. Substantial phenotypic variation was observed for okra fresh fruits, the main organs used for food, in terms of shape, colour, pubescence and number of ridges. Accessions carrying fruits preferred in local or foreign markets were identified making them good candidates for further breeding to produce new cultivars for both markets. The cluster analysis resulted in 13 subclusters at a similarity level of 60%. When comparing the subclusters with collection sites, no direct relation was detected indicating that okra germplasm has been spreading all over the country resulting in diversified materials across different regions.

Keywords: Okra, genetic resources, characterization, *Abelmoschus*, Sudan

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Introduction

Okra (*Abelmoschus* spp.) is widely cultivated for its edible green fruits, which are harvested when immature and are famous for their slimy mucilage. It is widespread in tropical, subtropical and warm temperate regions. Common okra (*Abelmoschus esculentus* (L.) Moench) has been reported in the whole of tropical Africa, whereas West African okra (*Abelmoschus caillei* (A.Chev.) Stevels) is restricted to the humid and perhumid climates of Africa ([Siemonsma and Kouamé, 2004](#)).

Abelmoschus esculentus (usually 2n = 130) is probably an amphidiploid (allotetraploid), derived from *Abelmoschus tuberculatus* Pal & H.B.Singh (2n = 58), a wild species from India, and a species with 2n = 72 chromosomes (possibly *Abelmoschus ficulneus* (L.) Wight & Arn. ex Wight) ([Siemonsma and Kouamé, 2004](#)). The exact origin of okra is unknown, however, one putative ancestor (*A. tuberculatus*) is native to Uttar Pradesh in North India, suggesting that *A. esculentus* originated in India. Another evidence is based on the plant's cultivation in ancient times and the presence of another putative ancestor (*A. ficulneus*) in East Africa, suggesting also northern Egypt or Ethiopia as the geographical origin of *A. esculentus* ([Kumar *et al*, 2011](#)).

Okra (*Abelmoschus* spp.) is the most popular traditional vegetable in Sudan, where both wild and cultivated types of okra are known. It is used in almost all parts of the country. It is cooked either after being dehydrated or as fresh pods ([Mohamed, 1991](#)). Some of the wild types seem to belong to the cultivated species *A. esculentus* while others belong to other species such as *A. ficulneus*. In Sudan, there is a late 19th-century record of its occurrence in the wild along the White Nile ([Singh *et al*, 1975](#)). [Schippers](#) ([2002](#)) reported that *A. ficulneus*

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L. was found in Sudan and other regions of the Sahel (Mali, Chad) and East Africa, but more so in South and South East Asia. This wild species, of which both fruit and leaves are eaten in Sudan is one of the two ancestors of the common okra. Schippers (2002) also indicated that a range of primitive landraces could still be found in Sudan, where people collect fruit from wild plants for drying purposes. For example, the dehydrated okra locally called 'Waika', is collected from the wild from rain-lands in the central region and the southern Blue Nile, Kordofan and Darfur (Geneif, 1984).

Okra is cultivated in different parts of Sudan, where farmers depend almost completely on the use and production of traditional farmers' varieties. Apart from some introduced varieties such as Clemson Spineless and Pusa Swani, there are a number of farmers' varieties obtained from local types, which in many cases have names relevant to the localities, where they might have evolved and are usually produced. They include a spiny type called Khartoumia and other local varieties such as Karrari, Kassala, Medani, Sinnar and others (Ahmed and Mohamed, 1995).

Such variation in cultivated and wild okra varieties constitutes a wealth of indigenous genetic resources in Sudan that should be given the necessary attention to ensure their conservation against any possible threats, as well as to enhance their sustainable use. Therefore, germplasm collection efforts by the Plant Genetic Resources (PGR) Programme of the Agricultural Research Corporation (ARC) since the 1980s have resulted in the collection of more than 600 accessions of okra from different regions in Sudan with remarkable variation in plant and fruit characters. Seeds of such accessions are conserved in the genebank of the Agricultural Plant Genetic Resources Conservation and Research Centre (APGRC) of the ARC, which is located in Wad Medani town in Gezira state in central Sudan. Batches of seed samples are preserved under long-term seed storage conditions, which are realized in deep freezers adjusted at around -20°C, with seed moisture content within a 4–7% range. They are packaged in laminated aluminium foil packets, which are hermetically sealed before being deposited in the freezers.

This paper reports on efforts made and results obtained in Sudan under the APGRC for the enhancement of the use of okra genetic resources through morphological characterization from 2000 to 2019.

Materials and methods

Germplasm materials

Materials used and reported in this paper were genetic resources of okra (*Abelmoschus esculentus*) of which Sudan is a country of origin. 366 *A. esculentus* accessions, which were obtained by either direct collecting from different regions of the country or acquiring from other sources, such as okra breeders, were used in this work (Table 1, Supplemental Table 1). Passport data on the accessions are documented in the genebank documentation system of the APGRC, and partially in the Genesys database, including georeferenced data of origin for some of them (Figure 1). All accessions were conserved in the APGRC seed genebank under long-term seed storage conditions. These accessions were grown and characterized in batches across ten different cultivation seasons during the period from 2000 up to 2019 as shown in Table 2.

Germplasm characterization

Samples of the 366 okra accessions were grown under full irrigation in the APGRC field for morphological traits characterization. The APGRC field is located within the research farm of the Gezira Agricultural Research Station at Wad Medani town, at latitude 14° 24' N and longitude 33° 29' E and altitude of 406.9m a.s.l. The climate of the area is hot semi-arid, and the soil is vertisol with clay content of 40–65%, and pH value ranging from 8 to 9.6, with less than 1% organic carbon, 300ppm total nitrogen and 406 to 700ppm total phosphorus (Ishag and Said, 1985).

Seed samples were obtained from the APGRC genebank and directly sown in the field in different seasons with sowing dates varied across seasons between the last week of June and the second week of July. Each accession was grown in a one-line plot on a ridge that was 80cm apart from others with in-row spacing of 20cm between plants. Each entry was represented by ten plants, at maximum, grown as one plant per hole. Routine cultural practices including manual removal of weeds, irrigation and raising of ridge sides were practised when required at intervals of around 7–15 days. Plants were nitrogen-fertilized using urea at a rate of 238kg of urea per hectare containing a total of 109.5kg of pure nitrogen. This total amount of urea was split into two doses; the first dose (119kg of urea per ha) was applied one month after sowing, while the second equal dose was applied one month later. Plants of all accessions were phenotypically characterized using 17 qualitative descriptors on plant, stem, leaf, inflorescence and fruit traits derived from the International Board for Plant Genetic Resources descriptor list for *Abelmoschus* (Charrier, 1984) (Supplemental Table 2). All traits were fully described in the field by recording all descriptor states that were observed within each accession.

Statistical analysis

The frequency level of different descriptor states was calculated as the percentage of accessions showing homogeneously only the same specific descriptor state within the 366 accessions characterized. Accessions showing different states for a descriptor were considered heterogeneous for that specific descriptor and their frequency percentages were calculated as well. Accordingly, the descriptor states were considered to be occurring at different levels including very rare, rare, moderate, common and abundant. They were categorized as very rare when occurring with a frequency level of 5% or less,

rarely when occurring with a frequency level > 5% and ≤ 30%, moderately when occurring with a frequency level > 30% and ≤ 60%, commonly when occurring with frequency levels > 60% and ≤ 90% and abundantly when occurring with frequency levels > 90%.

Multivariate analysis was run on the data obtained through the hierarchical cluster analysis with complete linkage using the software GenStat Twelfth Edition (GenStat Release 12.1).

Table 1. Number of accessions collected from different geographical regions in Sudan and used in this study.

Administrative state	Total accessions
South Kordofan (including the present South Kordofan and West Kordofan states)	73
West Darfur	50
Northern State	38
West Kordofan	31
South Darfur	27
Blue Nile	25
Red Sea	24
Gedarif	21
North Kordofan	17
Sinnar	17
Kassala	13
River Nile	11
Central Darfur	6
Khartoum	5
White Nile	5
Gezira	4
Unknown	29
Total	**366**

Table 2. Number of okra accessions characterized in different cultivation seasons

Season of cultivation	Total number of accessions characterized
2000–2001	43
2006–2007	86
2007–2008	35
2008–2009	39
2009–2010	33
2011–2012	16
2013–2014	15
2015–2016	12
2017–2018	33
2018–2019	54
Total	**366**

Results

A total of 60 descriptor states were observed for the 17 descriptors covering plant, stem, leaf, inflorescence and fruit characters across the 366 okra accessions studied (Supplemental Table 3). A total of 150 accessions (41%) were observed to be homogeneous for all descriptors, while the remaining 59% (216 accessions) were heterogeneous for one or more descriptors. However, the level of heterogeneity for each descriptor, calculated as the frequency of accessions showing different descriptor states for the same descriptor within the same accession, ranged between 3% being the lowest level of heterogeneity for the descriptor of fruit peduncle length, and 34% being the highest level of heterogeneity for the leaf shape descriptor (Table 3).

The frequency level of different descriptor states ranged between less than 1% and 100% as shown in Table 3. Some of the descriptor states were observed to be abundant in more than 90% of the accessions characterized such as the linear shape of epicalyx segments (91%), or the yellow flower colour (100%). On the other hand, some descriptor states were very rarely observed in only 5% or less of the accessions such as the procumbent general aspect of plant (2%), purple stem colour (3%), pendulous fruits on main stem (< 1%) or fruits that were red or green with red patches (< 1%, and 4% respectively). Otherwise, phenotypic variations were observed at different levels across the different plant organs of the okra accessions.

Phenotypic variations related to plant vegetative characters

Variations were observed in the vegetative characters that included general plant aspect, branching, leaf and stem characters, as shown in Table 3.

General plant aspect

Erect, medium and procumbent plant aspects were observed. Half of the characterized accessions had medium aspect, while 23% had erect plants, and the procumbent aspect was observed very rarely in only 2% of the accessions. The rest of the accessions (25%) were heterogeneous for this descriptor.

Branching

Different descriptor states of branching were observed among the okra accessions studied including orthotropic, medium and strong branching. While medium branching was observed in the majority of the accessions with a frequency of 51%, orthotropic and strong branching were rarely observed (19% of the accessions for either). The rest of the accessions were heterogeneous for this descriptor.
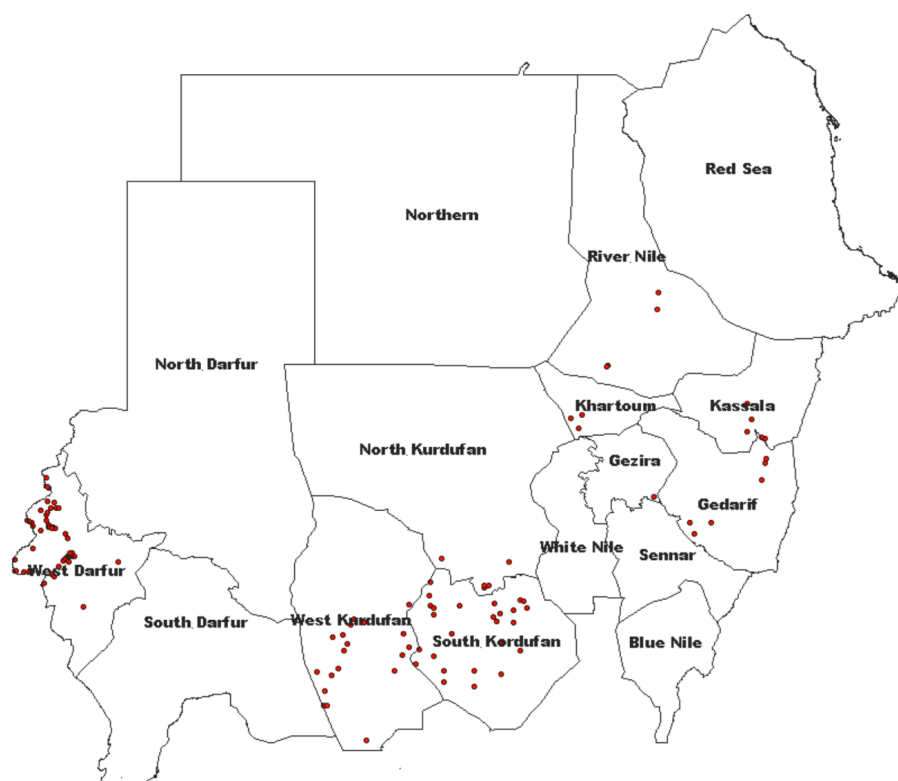
Figure 1. Collection sites in Sudan of some okra accessions described in this paper, for which georeferenced data was available from APGRC.
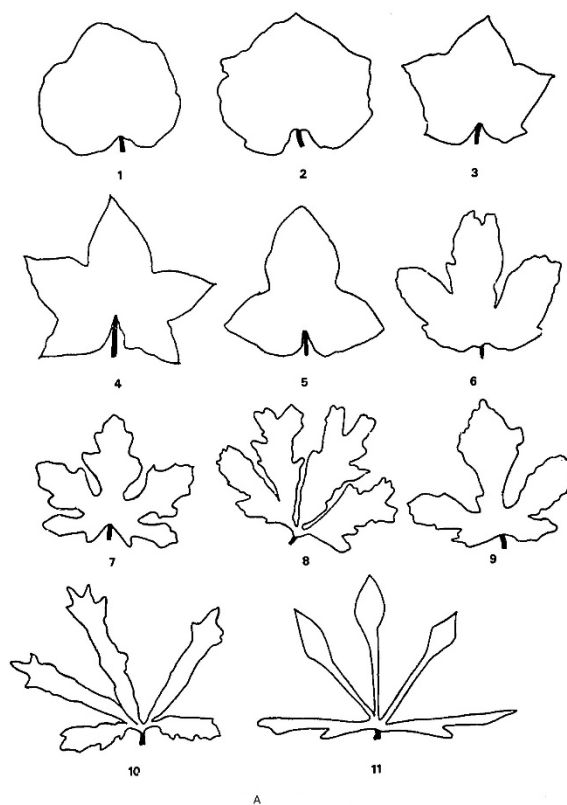
Figure 2. Descriptors for leaf shapes used for characterization purposes (Charrier, 1984)

Table 3. Phenotypic variations observed in the 366 characterized okra accessions. Occurrence level: Very rare ($\leq 5\%$), rare ($> 5\%$, $\leq 30\%$), moderate ($> 30\%$, $\leq 60\%$), common ($> 60\%$, $\leq 90\%$), and abundant ($> 90\%$).[1]Leaf shape and some fruit characters are depicted in Figure 2 and Figure 5.

Descriptor	Occurrence level of each descriptor state and its frequency percentage					
	Abundant	**Common**	**Moderate**	**Rare**	**Very rare**	**Heterogeneous**
General aspect			Erect (50%)	Medium (23%)	Procumbent (2%)	25%
Branching			Medium (51%)	Orthotropic (19%)		11%
				Strong (19%)		
Stem pubescence			Slight (45%)	Glabrous (22%)		26%
				Conspicuous (7%)		
Stem colour			Green (49%)	Green with red patches (22%)	Purple (3%)	26%
Leaf shape[1]				3 (7%)	5 (< 1%)	34%
				4 (24%)	6 (< 1%)	
				7 (24%)	8 (1%)	
				10 (7%)	9 (2%)	
Leaf colour			Green with red veins (48%)	Green (24%)		28%
Number of epicalyx segments			8–10 (42%)		5–7 (2%)	16%
			> 10 (40%)			
Shape of epicalyx segments	Linear (91%)				Lanceolate (9%)	
Petal colour	Yellow (100%)					
Red colouration of petal base		Both sides (76%)		Inside only (17%)		7%
Position of fruit on main stem		Erect (85%)		Horizontal (6%)	Pendulous (< 1%)	9%
Fruit colour			Green (47%)	Yellowish green (23%)	Green with red patches (4%)	26%
					Red (< 1%)	

Continued on next page

Table 3 continued

Descriptor	Occurrence level of each descriptor state and its frequency percentage					
	Abundant	**Common**	**Moderate**	**Rare**	**Very rare**	**Heterogeneous**
Fruit length at maturity			8–15 cm (40%)	< 8cm (11%)		26%
				> 15cm (23%)		
Length of peduncle		1–3 cm (84%)		> 3cm (13%)		3%
Fruit shape[1]				1 (21%)	2 (< 1%)	25%
				3 (23%)	5 (< 1%)	
				4 (16%)	8 (< 1%)	
				6 (6%)	10 (< 1%)	
					11 (< 1%)	
					12 (< 1%)	
					14 (5%)	
					15 (< 1%)	
Number of ridges per fruit		5–7 (70%)		< 5 (9%)	> 10 (1%)	10%
				8–10 (10%)		
Fruit pubescence				Downy (29%)		27%
				Slightly rough (17%)		
				Prickly (27%)		

Table 4. Number of subclusters at similarity level of 60% among which accessions from each state are distributed.

State	Total accessions	No. of subclusters
Northern State	38	10
South Darfur	27	10
Gedarif	22	9
South Kordofan	73	9
Red Sea	24	7
North Kordofan	17	6
Sinnar	17	6
Blue Nile	25	5
West Darfur	50	5
White Nile	5	4
Gezira	4	3
Kassala	13	3
River Nile	11	3
Central Darfur	6	1
Khartoum	5	1
Unknown	29	9
Total	**366**	

Leaf characters

Leaf shape was among the characters with maximum variations recorded in the okra accessions characterized. Eight descriptor states were observed in terms of leaf shapes. Consequently, all descriptor states observed for shapes of leaf were found to be either very rarely or rarely occurring with frequency levels of less than 1% and up to 24%. However, the most common leaf shapes were shape 4, with a five-lobed leaf with smooth margins (24%); and shape 7, with a five-lobed leaf with non-smooth undulating margins (Figure 2) (24%). On the other hand, the least occurring leaf shapes were shapes 5, 6, 8 and 9, which were very rarely occurring at frequencies of less than 1% to 2%. The rest of the accessions (34%) were heterogeneous for this descriptor.

Leaf colours were green (24%) and green with red veins (48%), in the accessions that were homogeneous for this character. The rest of the accessions were heterogeneous for this descriptor.

Stem characters

Stems of most accessions were either green or green with red patches with frequencies of 49% and 22%, respectively, among the homogeneous accessions characterized. On the other hand, purple stems were very rarely observed in only 3% of the accessions.

Stems were either glabrous, slight or with conspicuous pubescence. Most of the accessions were either glabrous (22%) or had slight hairs on their stems (45%). Otherwise, only 7% of the accessions had conspicuous hairs, while the rest of the accessions were heterogeneous for this character.

Variations in inflorescence characters

The only characters that were abundantly dominating in the studied germplasm were the linear shape of epicalyx segments, and the yellow flower colour, which were occurring in 91% and 100% of the accessions, respectively.

Red colouration was commonly found at both sides of the petal base in the majority of the accessions (76%), while only 17% of the accessions produced flowers with red colouration only inside the petal base. The rest of the accessions were heterogeneous for this character.

Forty two percent of accessions had between 8 and 10 epicalyx segments, and 40% had more than 10 segments with a moderate level of occurrence. On the other hand, 5–7 segments were observed very rarely, in only 2% of the accessions. The rest of the accessions were heterogeneous.

Variations in fruit characters

Variations were observed among the okra accessions on different fruit-related characters (Table 3).

Position of fruit on main stem

Erect position of fruits on main stem was a common character observed in the majority of the accessions at a frequency of 85%. The horizontal and pendulous positions of fruits on main stems were rarely or very rarely observed, with frequencies of 6% and less than 1%, respectively. The rest of the accessions were heterogeneous for this character.

Fruit colour

The majority of fruits produced were green (47% of the accessions) or yellowish green (23%). Fruits that were red or green with red patches were very rarely produced by fewer than 1% and 4% of the accessions, respectively. The rest of the accessions were heterogeneous for this character. Exceptionally, it was observed that remarkable greener ridges were observed on green or yellowish green fruits in some accessions (Figure 3).

Fruit length at maturity

Forty percent of the characterized accessions produced fruits that were 8–15 cm long when mature. Fruits shorter than 8cm or longer than 15cm were produced by 11% and 23% of the accessions, respectively (Figure 4). The rest of the accessions were heterogeneous for this character.

Length of peduncle

The majority of the accessions had fruits with peduncles that ranged between 1 and 3cm long in 84% of the accessions, while 13% of the accessions were producing fruits with peduncles longer than 3cm or were heterogeneous for this character (3%).

Fruit shape

Fruit shape was among the characters with maximum variation in terms of the number of descriptor states observed within the germplasm collection studied,
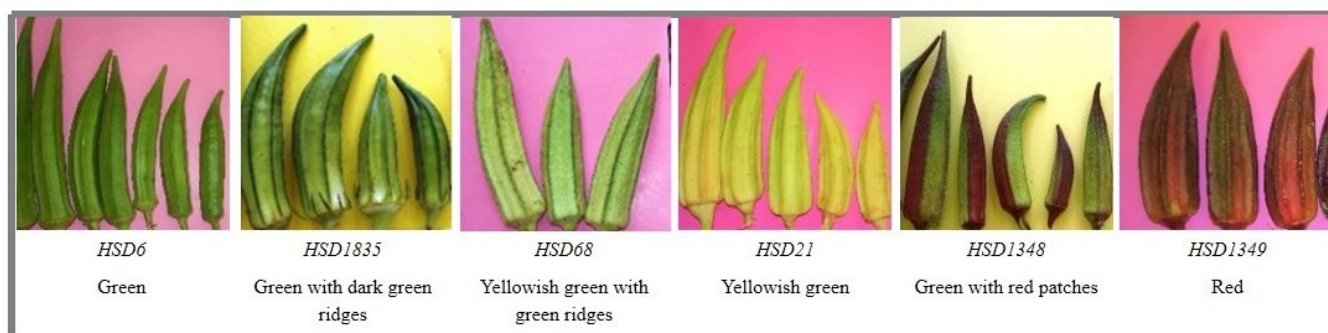
Figure 3. Variations in fruit colour as observed on some accessions.

Figure 4. Variations and frequency percentages of mature fruit lengths among the characterized accessions.

recording 12 descriptor states at different frequencies that ranged between less than 1% and 23%. The most common fruit shapes observed in 16–23% of the accessions were shapes 1, 3 and 4, which are characterized by elongated ridged capsules that were slender and moderately curved in shape 1, but straight or very slightly curved for shapes 3 and 4, with a wider base in shape 4 (Figure 5). Nine fruit shapes were observed very rarely, occurring in less than 1% to 6% of the accessions, while 25% of the accessions were heterogeneous for this descriptor (Figure 5).

Number of ridges per fruit

The number of ridges per fruit ranged between five and seven in the majority (70%) of the accessions characterized. Fruits with fewer than five ridges or with eight to ten ridges were rarely observed, in 9% and 10% of accessions, respectively. Fruits with more than ten ridges were very rarely observed (less than 1%). Ten percent of accessions were heterogeneous.

Fruit pubescence

Most of the accessions characterized were homogeneous for fruit pubescence producing either downy, slightly rough or prickly pubescent fruits occurring at rare or

moderate levels with frequencies ranging between 17% and 29%.

Results of cluster analysis

Cluster analysis resulted in a clustering pattern composed of two major clusters at similarity level of 30%. These major clusters were further grouped into 13 subclusters at a level of similarity of 60% including 4 subclusters under the first major cluster, and 9 subclusters under the second (Figure 6). When comparing the clustering pattern with the collection sites of the accessions, no direct relation was detected (Table 4). The only exceptions were the accessions from Central Darfur and Khartoum states as they clustered in only one group indicating a limited variation among them. However, this could be attributed to the limited number of accessions obtained and studied from these two states (six from Central Darfur and five from Khartoum).

Discussion

The okra accessions described in this study were collected and obtained from different regions and sources in Sudan. The majority of the accessions were heterogeneous, which is normal in a crop for which farmers' varieties are mainly used, as is the situation in
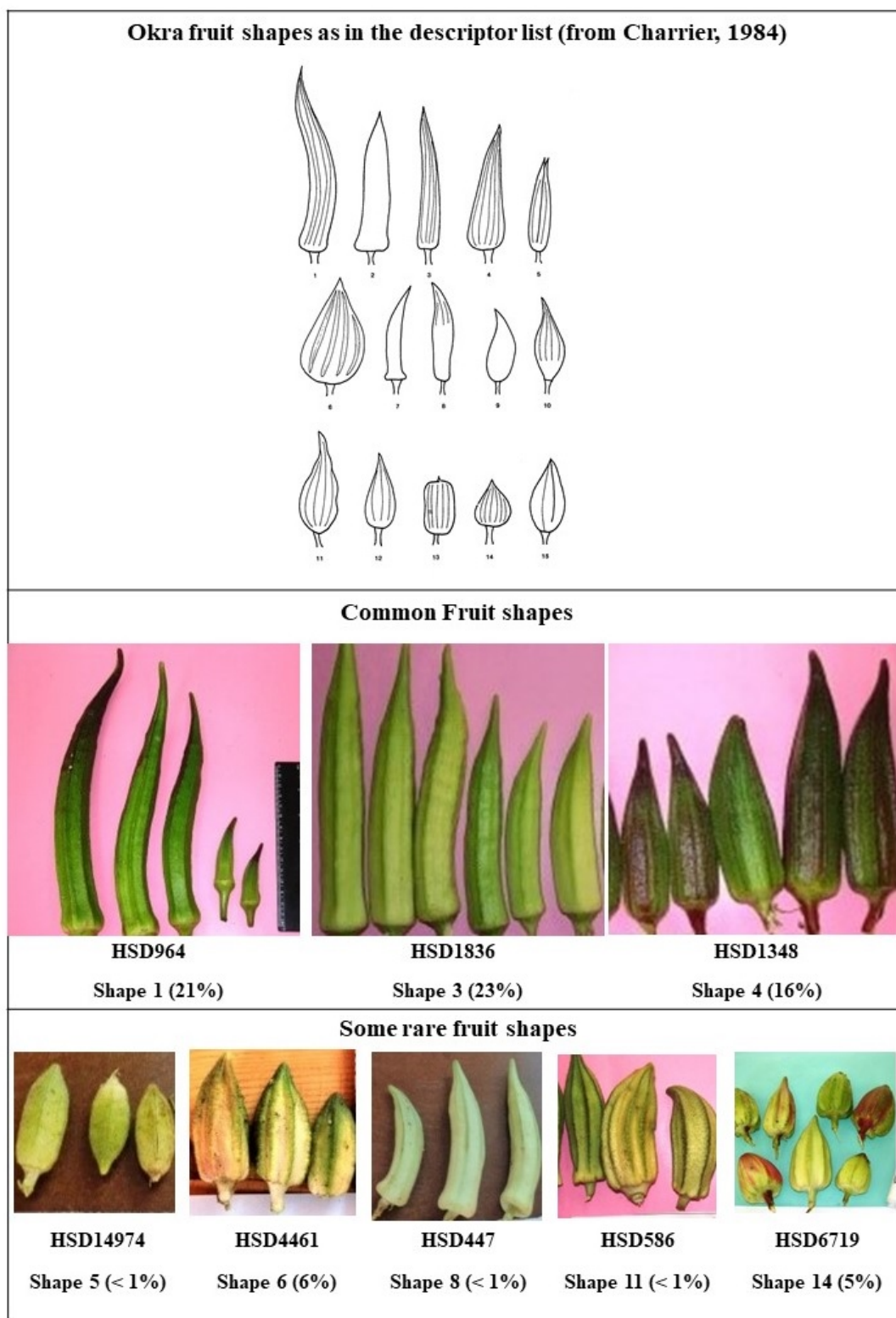
Figure 5. Descriptor states of fruit shapes (Charrier, 1984), and some of the observed fruit shapes and their frequency percentages as shown by some accessions.
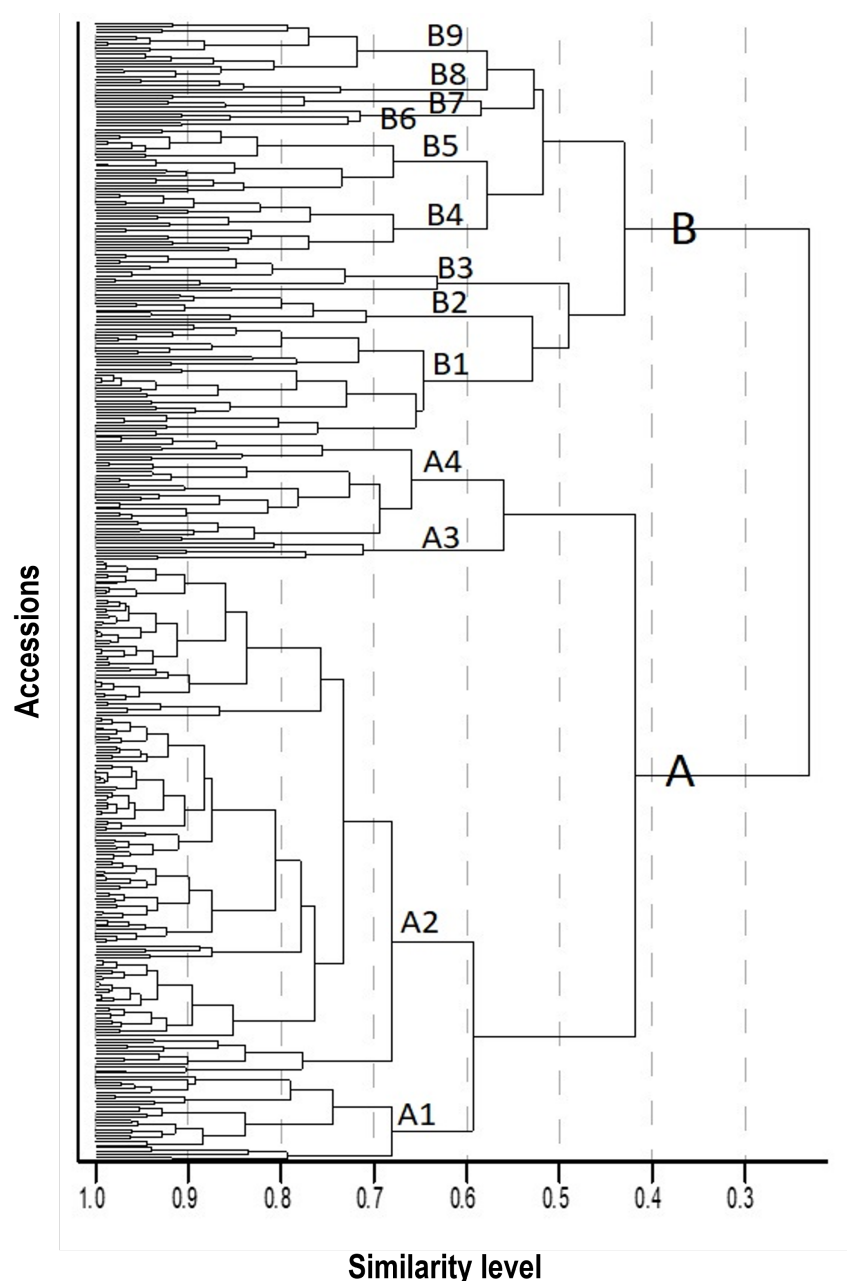
Figure 6. Dendogram of clustering patterns of 366 characterized okra accessions showing the two major clusters (A and B) and the four subclusters under A (A1-A4) and the nine subclusters under B (B1-B9).

Sudan. Hamon and Van Sloten (1989) indicated that a homogeneous accession is a rare occurrence in okra as it is reproduced by seeds and is not strictly autogamous.

This study has shown that the Sudanese okra germplasm is rich in terms of variation for a number of morphological traits. Among them are traits significant for yield and quality of the okra fruits, which are consumed by people. A considerable number of the okra accessions in this study had erect growth habit and medium to strong branching, which could be indication for high yielding potential, as described in a similar study that recorded strong erect stems and dense branching for *Abelmoschus* species at production sites with extended harvest throughout the year (Omonhinmin and Osawaru, 2005). It is interesting to note from

this study that fruit shape was among the characters with the highest level of observed variation in Sudan with 12 descriptor states out of the 15 recommended in the IBPGR descriptors for *Abelmoschus* (Charrier, 1984). This was similar to the results reported by Oppong-Sekyere *et al* (2011) on okra germplasm from Ghana, where fruit shape showed the greatest diversity among the okra accessions, from short and triangular to long straight or long curved. However, only three shapes were the most frequently observed in this collection, which were actually closely similar to each other as all of them were of elongate fruit type with variation in thickness and curvature. In fact, this is the type of okra fruits that are most commonly consumed and sold in the vegetable markets in the country. Such phenomenon indicates the

trend among the okra farmers in Sudan to select for this type of elongate fruits to meet the market demand and the consumers' preference.

A number of characters observed seem to be very rarely or rarely present in the described collection indicating a higher risk to lose them. Among those are characters such as procument aspect of plant, purple stems, pendulous fruits on main stems, fruit that were red or green with red patches, and a number of fruit shapes other than the elongate ones. More attention should be paid to conserve accessions carrying such characters in order to preserve, multiply and evaluate them for traits other than those related to the preferred fruit quality and yield potential such as resistance to biotic and abiotic factors. However, some of these rare characteristics were also observed with similar frequency in a recent study conducted in neighboring Ethiopia where only 19.44% of accessions had red or purple stem colour (Temam *et al*, 2021).

The majority of the accessions produced fruits that were either green or yellowish green, while red fruits or green fruits with red patches were rarely produced in about 5% of the accessions. This also refers to the tendency of the okra farmers in Sudan to select for greenish fruits that are usually preferred by the consumers. On the other hand the results of the study in Ethiopia by Temam *et al* (2021) showed that fruits that were green with red patches were the most highly represented in their collection at a frequency of 55.56%. While Sudanese consumers do not tend to use green fruits with red patches, it seems this fruit colour is preferred by the consumers of okra in at least some regions of Ethiopia. On the opposite the Ethiopian consumers may not like fruits that were yellowish green as only 2.78% of the characterized Ethiopian accessions produced such type of fruits, while 23% of the Sudanese accessions in the present study had yellowish green fruits.

Okra consumers in Sudan also tend to prefer the spiny okra fruits, as they are locally believed to be more slimy and tasty than others. The results obtained from this study showed that a considerable number of characterized accessions (44%) had spiny texture by being either prickly or slightly rough. However, downy fruits with smooth texture were also recorded for a considerable number of accessions (29%) indicating the potential of the okra germplasm in Sudan for use to produce varieties that are more likely to be suitable for exportation outside the country where downy green fruits seem to be more preferred.

The abundant occurrence of linear epicalyx segments in the collection described with very rare cases of lanceolate segments and the full absence of triangular segments confirm that the accessions characterized belong to the species of the common okra *Abelmoschus esculentus* that is common in east Africa. In contrast to this the species *A. caillei*, which is known as the West African okra, is more common in West Africa. As mentioned by Siemonsma and Hamon (2004) *A.*

esculentus differs in several respects from *A. caillei*, but the epicalyx offers the best discriminating characteristic: the width of the epicalyx segments is 4–13mm in *A. caillei* and 0.5–3mm in *A. esculentus*.

Generally, the okra genetic resources from Sudan have considerable phenotypic variations among them as shown by the clustering pattern of the accessions described in this study reaching to 13 sub-clusters at 60% level of similarity. The variation is also detected among the okra accessions collected from each of the geographical states as they were mostly clustering in more than one group reaching to 10 sub-clusters in some states. Such trend of variations in okra genetic resources means that okra germplasm has been spreading in Sudan from different sources resulting in diversified phenotypes all over the country and in each of the different regions.

Conclusion

This study has shown that the germplasm collection of okra from Sudan held by the APGRC, which is mostly composed of farmers' varieties, is diverse in a number of characters including those important for yield and quality for both local and foreign markets. Such diversity in this collection makes it highly promising for breeding for improved okra cultivars through purification, selection and evaluation for superior lines. However, more germplasm collection of okra genetic resources is needed with emphasis on regions and geographical pockets that are poorly represented in the materials collected and studied so far. Further characterization for morpho-agronomic traits is also necessary as well as evaluation of such germplasm for desirable traits including resistance and adaptability to biotic and abiotic stresses.

Acknowledgements

Conflict of interest statement

The author declares that there is no conflict of interest.

Supplemental Data

- Supplemental Table 1: List of all okra accessions characterized, their sites of collection and characterization seasons
- Supplemental Table 2: Okra descriptors and descriptor states used in the study
- Supplemental Table 3: Characterization results recorded on the different okra accessions

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ORIGINAL ARTICLE

Trends, challenges and opportunities in the *in situ* conservation of cereal landraces in Scottish islands

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Abstract: Landraces are traditional crop varieties that often have special adaptations to the farming environment in which they have evolved and are therefore a valuable source of useful traits for plant breeders. In most agriculturally advanced countries, landraces of the main crops have generally been superseded by modern varieties. An exception to this in the United Kingdom is the cultivation on the Scottish archipelagos of Orkney, Shetland and the Outer Hebrides of three cereal landraces: bere, a 6-row barley (*Hordeum vulgare*), small oat (*Avena strigosa*) and Hebridean rye (*Secale cereale*). Our study focused on trends in their cultivation and use over the past 20 years. In the Outer Hebrides, a mixture of all three has continued to be grown on more than 200ha for feed because of its tolerance of nutrient-deficient sandy soils. Future cultivation is threatened, however, by damage from geese and deer, especially to fields used for seed production. In Orkney and Shetland, only bere and small oat are grown, and always as sole crops. The area of bere has increased in Orkney, from about 10ha in 2004 to almost 75ha in 2020 and has been driven by two supply chains producing bere for milling and malting. However, small oat in Orkney, and both small oat and, especially bere, in Shetland have been grown by very few farmers since 2018 and are at serious risk of being lost from cultivation. We discuss these results in the context of measures to support greater on-farm cultivation of these landraces.

Keywords: *In situ*, on farm, conservation, bere, small oat, Hebridean rye

Introduction

Background

Food security is of growing global concern due to the rapidly increasing human population and the adverse impact of climate change on food production (Godfray *et al*, 2010; FAO, IFAD, UNICEF, WFP and WHO, 2017). Research into food security should focus on all aspects of the human food chain but logically should start with ensuring stable production of highly nutritious and diverse crops (Dhankher and Foyer, 2018). However, in recent years industrial agriculture has focused on the cultivation of fewer genetically homogeneous crop varieties leading to a reduction in crop diversity (FAO, 2012) caused by fewer crops in cultivation, fewer cultivars of each crop and reduced genetic diversity within each cultivar (Negri *et al*, 2009). There are about 31,128 plant species which currently have documented uses, of which 5,538 are associated with direct food production and a further 5,338 have potential for adaptive allele donation (RBG Kew, 2016). However, basic global human nutrition comes from just 30 species, and three major crops – rice (*Oryza sativa*), wheat (*Triticum aestivum*) and maize (*Zea mays*) – provide 50% of calories consumed globally. Food production is increasingly focused on these few crops while the bulk of semi-domesticated and wild edible species are underutilized (FAO, IFAD, UNICEF, WFP and WHO, 2017; Padulosi *et al*, 2013). Furthermore, industrial

production and consumer demand are often focused on uniformity and achieving maximum production and consumer shelf appeal (Kilcast and Subramamiam, 2011). To achieve this, modern cultivars are genetically homogeneous, lacking the geographically localized infra-varietal diversity found in traditional varieties. These traditional varieties are produced via generational cycles of farmer seed selection, which results in adaptations to local environment and higher levels of genetic diversity than occur in modern varieties.

Cultivar uniformity increased production overall but the switch from traditional genetically variable crop landraces significantly depleted the gene pool of diversity available to breeders, which is required to sustain crop improvement in the future (Hawkes *et al*, 2000; Van De Wouw *et al*, 2009; Maxted *et al*, 2020). The price paid for crop uniformity is that these homogeneous cultivars are vulnerable to new strains of diseases or pests, or extreme changes in the growing environment (Committee on Managing Global Resources, 1993; FAO, IFAD, UNICEF, WFP and WHO, 2017). The threat to overall crop diversity by the replacement of landraces by higher-yielding but genetically uniform cultivars is well established at a global level (Negri *et al*, 2009; FAO, 2010). While modern varieties usually outperform landraces under optimal conditions, landraces may still be competitive in marginal environments where modern cultivars lose their advantage or where there is an associated niche market to sustain production (Yahiaoui *et al*, 2014; Marone *et al*, 2021). The intrinsic genetic diversity present in landraces means they will often produce some yield even in the face of new strains of diseases or pests, or sudden extreme changes in the growing environment. Conserving the maximum pool of diversity in landraces is a priority to sustain future agricultural production (FAO, 2012), and landraces grown in regions subject to very variable conditions are likely to have evolved traits that allow them to tolerate such changes. These traits are likely to be particularly valuable to breeders to mitigate the impact of conditions like climate change (Hawtin *et al*, 1997; Maxted *et al*, 2020).

Within the United Kingdom (UK), the agricultural regions where landrace cultivation and resulting diversity are greatest, are the Scottish archipelagos of Orkney and Shetland (the Northern Isles) and the Outer Hebrides (Kell *et al*, 2009) (Figure 1). The main landraces grown are three cereals: bere,[1] an ancient type

of 6-row barley (*Hordeum vulgare* L.), small oat[2] (*Avena strigosa* Schreb.) and Hebridean rye[3] (*Secale cereale* L.). While bere and small oat are grown in all archipelagos, Hebridean rye is only grown in the Outer Hebrides. There are fundamental differences in the manner of cultivation of the landraces between the archipelagos. In the Outer Hebrides, the most common practice is to grow all three together as a mixture (Scholten *et al*, 2009) for animal forage. In contrast, in Orkney and Shetland, bere and small oat are grown as sole crops, with bere being used in Orkney for milling and malting. In Shetland, small oat is usually grown for straw used in basketry, making traditional straw-backed chairs or for thatching. In the Outer Hebrides and Shetland, and to a lesser extent in Orkney, cultivation of the cereal landraces is usually associated with crofting (see Supplemental Note 2), a small-scale, low-input traditional type of farming. Two other landraces that survive in Shetland are Shetland cabbage or kale (*Brassica oleracea* L.) and Shetland black potato (*Solanum tuberosum* L.). Livestock landraces have also been preserved in the Northern Isles, with the multipurpose Shetland cow in Shetland and seaweed-eating North Ronaldsay sheep in Orkney.

Locational and environmental context

Undoubtedly, the location and history of these archipelagos are important factors contributing to the survival of landraces in these areas. They are all peripheral areas, geographically isolated from mainland Scotland, which have always experienced difficulties, or high costs, in importing goods, resulting in a requirement for self-sufficiency. Climatically, they are hyper-oceanic with high rainfall, frequent gales and cropping seasons that are cooler and shorter than in other parts of Britain (Chappell *et al*, 2017). Other challenges for cropping include relatively poor soils and limited availability of machinery and equipment. The three archipelagos also have unique cultural identities and traditions. Although all were settled by Viking invaders during the 9th century, Norse control of the Outer Hebrides ended earlier, in the 13th century, and was superseded by a Gaelic culture which includes its own language still

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[1] Under current terminology the name 'bere' is used to describe a distinct landrace population of lax-eared, 6-row, hulled spring barley that has been grown in Scotland and the north of Britain for over 1,000 years (Drosou *et al*, 2022). In the past, several synonyms appear to have been applied to bere including 'beare', 'bygg' or 'bigg' (Jarman, 1996; Wallace *et al*, 2019). In most cases, these names appear to refer to a similar type of barley to today's bere, but sometimes they were used to refer to any 6-row barley (Jarman, 1996). Phenotypically, bere from the different Scottish archipelagos as well as most of 35 accessions in a germplasm collection held by the James Hutton Institute, are similar although differences occur in some characteristics like days to heading and tolerance to manganese-

deficient soils. Genotypically, these beres are also closely related to each other although cluster analysis has shown that the accessions separate into three distinct groups according to their archipelago of origin (Schmidt *et al*, 2019). In spite of these phenotypic and genotypic differences, different types of bere are not currently recognized by growers in Scotland although in the 19th century named selections were available (Martin *et al*, 2009).

[2] We have used the name small oat for the Scottish *A. strigosa* landrace population which is the terminology used by SASA's (Science and Advice for Scottish Agriculture) Scottish Landrace Protection Scheme (Scottish Landrace Protection Scheme (SLPS) — SASA (Science & Advice for Scottish Agriculture)). On Orkney it is referred to as black oats and on Shetland as Shetland aets (Supplemental Table 1). Although there are phenotypic and genetic differences amongst accessions of small oat, growers in the island groups do not identify different types (Scholten, 2012).

[3] We again follow the terminology of the SLPS for the Scottish *S. cereale* landrace population. This is represented by relatively few accessions in the SLPS collection and has been much less studied than bere or small oat.

Figure 1. Map of northern Scotland with red arrows showing the location from north to south of the Shetland Isles, Orkney Isles and Outer Hebrides. The image was sourced from Scotland_topographic_map-en.jpg (2400×3450) (wikimedia.org) and originally produced by Eric Gaba; Supplemental Note 1 provides the complete attribution.

spoken by most islanders. Orkney and Shetland only became part of Scotland in the late 15th century and many traditions and aspects of culture in the Northern Isles reflect the earlier Scandinavian links, especially in Shetland.

In all the archipelagos, but particularly the Outer Hebrides, a significant proportion of arable land is on sandy, coastal soils which have a high pH and low availability of trace elements, especially manganese and copper, forming a special habitat called 'machair' (Scottish Natural Heritage, 2003). Locally adapted types of bere have a remarkable ability to tolerate these deficient soils and produce reasonable

grain yields while modern barley varieties lack this trait and yield very poorly or not at all (Schmidt *et al*, 2019). The Hebridean rye and small oat landraces are also tolerant of these soil conditions and this trait is likely to have been an important factor explaining the survival of all three landraces in cultivation down to current times. The machair is a particularly harsh agricultural environment because of its soil properties but also because of its exposure to gales, salt spray and periodic droughts; drifting of sand and soil erosion also occur (Scottish Natural Heritage, 2003). Over centuries, landraces have adapted to these conditions and on the Outer Hebrides the

bere, small oat and Hebridean rye mix is able to produce a reasonable yield with minimal inputs, apart from fertilizer, for which local stranded seaweed and animal manure are often used (Brown *et al*, 2020). Traditional cropping of the cereal landraces involves leaving land fallow as part of the rotation, resulting in the development of a unique coastal grassland with a rich and distinctive flora and fauna. Its importance is recognized by its inclusion in Annex 1 of the EU Habitats Directive (Walton and Mackenzie, 2009), and several machair areas are included amongst Natura 2000 sites (Scottish Natural Heritage, 2018). Continued low-input cultivation of these cereal landraces in combination with the application of organic fertilizers and fallow years is key to machair conservation (Scottish Natural Heritage, 2003), and so local biodiversity and cultural interests in this rare habitat both favour continued landrace use. In Orkney and Shetland, cropping of the machair is less formalized and, especially in Orkney, there are larger areas of better quality agricultural land for cropping. Some bere accessions collected from Orkney, which do not originate from machair areas, lack a marked ability to tolerate its manganese-deficient soils (Schmidt *et al*, 2019).

Although the three cereal landraces are well-adapted to the Scottish island environment, compared with modern varieties, they have several agronomic shortcomings (Martin *et al*, 2010), which limit their attraction to farmers. In particular, their grain yields are low and their straw is long, making crops very susceptible to lodging close to harvest. Lodging is aggravated on fertile land or with high levels of nitrogen and is sometimes accompanied by premature grain germination because the heads of lodged crops remain wet for long periods after rain. This is of concern where crops are grown for malting, milling or seed. Bere has relatively long awns and in some years these do not completely break off the grain during combine harvesting and this can result in time-consuming blockages in machinery.

Historical context

Bere was probably the earliest of the three cereal landraces to be introduced to Scotland, most likely during the Bronze Age or possibly in the Neolithic (Drosou *et al*, 2022). Small oat and Hebridean rye were probably more recent introductions, with oats first appearing at archaeological sites during the Iron Age (Bond, 1998) and rye during Viking times when there was an intensification of cereal production in the Scottish Isles (Smith, 2005). There may also have been more recent seed introductions of both Hebridean rye and small oat to the Outer Hebrides (Scholten *et al*, 2009).

Bere was widely grown throughout Scotland until the 19th and 20th centuries. It was mostly cultivated as a sole crop, even in the Outer Hebrides, and was important for in-kind and land rental payments and for milling into beremeal and making into malt, which was used for brewing and distilling. The latter allowed

significant value to be added to the crop (Devine, 1994). Given the short cool growing season of all the archipelagos, a major advantage of bere was its ability to produce a crop of grain in a short time. Historically, this allowed bere to be sown after oats (both *A. sativa* L. and *A. strigosa*), which had a longer period to maturity, but to be still harvested before them in late August or September (Dodgshon, 2004), usually before the weather deteriorated and spoilt the crop or prevented harvesting. Consequently, bere was more likely to be harvested successfully than oats and was therefore important for food security. In all the islands, the importance of local cereals for food and malt declined as transportation links improved in the 19th century, allowing the import of cheap wheat flour to the area and providing distilleries with access to barley with better malting characteristics than bere (Martin *et al*, 2009). The introduction of potatoes during the 18th century and their widespread adoption for food also reduced the importance of cereal landraces as food staples. During the 20th century, mechanization effectively lengthened the cropping season in the study area because it reduced the time required for cultivation and harvesting. This helped growers to adopt later-maturing, higher-yielding varieties than bere.

In Orkney, the conservation of bere during the latter part of the 20th century can largely be attributed to the presence of a functioning water mill (Barony Mill) producing beremeal for local bakeries which provided a small market for locally grown bere. Since 1998, the mill has been run by Birsay Heritage Trust (BHT), which maintains its own line of bere and organizes its growing in the Birsay area with a local agricultural contractor, mostly leasing land from farmers. From 2004, the Agronomy Institute (AI) started to develop new brewing and distilling markets for bere and established its own supply chain with a small number of local growers (3-4 per year).

Cereal cultivation in Shetland has always been more difficult than in Orkney because of its more challenging climate and much smaller area of suitable arable land (Martin, 2015). During the 20th century, the area of bere grown on Shetland declined from about 419ha in 1912 (Board of Agriculture for Scotland, 1913) to a few hectares by the end of the century and there were estimated to be only two to three growers of bere in 2002 (Shetland Organic Producers Group, 2003). Crucially, unlike Orkney, no large water mill survived in operation on Shetland into the 21st century and there was therefore no milling market for bere.

It is not known when the practice of growing bere, small oat and Hebridean rye in a mixture started in the Outer Hebrides, but it may be relatively recent as historical accounts from the early 20th century do not mention this practice (MacDonald, 1919). It likely became possible as the importance of local cereals for food and malt declined, as Hance (1952) reported that the decline in barley cultivation in the Uists from about 1,797ha in 1925 to about 335ha in 1946 had been

offset by an increase in rye and mixed grains. It has been suggested that a major advantage of the landrace mixture, apart from its tolerance to low levels of soil trace elements, is that it provides production resilience under the very variable soil moisture conditions of coastal, sandy soils (Smith, 1994). This can vary from drought to waterlogging depending on rainfall and the drainage characteristics and topography of individual fields. Within the mixture, Hebridean rye is the most drought-tolerant species while bere and small oat perform better on wetter land and bere is also valued for its nutritional contribution (Smith, 1994). Bere matures earlier than the other two species, however, and often starts to shed seed before harvest; the other two species, therefore, tend to dominate the mixture.

Study aims

An initial survey in 2003 of UK landraces (Scholten *et al*, 2011) identified the three Scottish island archipelagos as regions where the three cereal landraces as well as Shetland cabbage (Scholten *et al*, 2008) were still prized and maintained. The aim of the present paper is to update the results of the first assessment with more recent information on the landraces grown, grower numbers, area under cultivation and to identify interventions which could encourage wider growing of these landraces. These results are presented within the context of other factors which have affected the cultivation of the cereal landraces on these archipelagos. This update includes data collected during a survey undertaken in 2018 as part of the EC-funded Farmer's Pride project (Farmer's Pride, 2019), one element of which was to study the economic feasibility of landrace cultivation. Although the scope was inclusive, the main focus was on cereal landraces and the results reported are mainly for these.

Materials and methods

Area of study

The area of study included the three Scottish archipelagos of Orkney, Shetland and the southern part of the Outer Hebrides (the 'Uists') comprising North Uist, Benbecula, South Uist and Barra (Figure 1). The latitudinal range of these archipelagos is from approximately 57^oN to 61^oN.

Assessing area of landraces and grower numbers 2002–2020

For Orkney and Shetland, the number of landrace growers and area grown was small and we provide estimates of this based on our knowledge and information from key informants (for example, Shetland Organic Producers Group, the Agronomy Institute (AI) at Orkney College UHI and Birsay Heritage Trust).

With the much larger number of growers in the Outer Hebrides, we can only provide an estimate of grower numbers and the area grown and consider this to be most reliably based upon Scottish Government

annual census data from 2002 to 2019 for the area of crops grown by region. For most years, data for the Outer Hebrides (Na h-Eileanan Siar) include the area of oats and mixed grains grown on agricultural holdings. Several local key informants, as well as extensive field surveying in 2006 (Scholten *et al*, 2008) indicated that most oat fields were small oats, and the mixed grain crops were the mixture of landraces – mainly small oat and Hebridean rye, but often also included some bere. Another strong reason for assuming that the census data for these crops reflect landraces is that the arable areas are mostly on the machair, and landraces are much better suited to these soil conditions than modern varieties. In some years the census provides the same data for the barley crop and, for the same reasons, this is most likely to be bere. However, we stress that for crofting communities, where crofts are often extremely small and holdings can contain several crofts, the use of the census data is likely to underestimate the area and, especially, grower numbers. Given the uncertainties in the data, we provide both the agricultural census data and estimates derived from consulting key informants.

Design of the 2018 survey and questionnaire

In 2018, the Farmer's Pride project provided an opportunity to reassess the landrace situation in the Scottish islands. A landrace grower questionnaire (Supplemental Note 3) was developed to update and further our understanding of the diversity of landrace cultivation in the UK and continental Europe (Austria, Hungary and Turkey). The questionnaire was used in face-to-face interviews with current or former landrace growers on the three island archipelagos. Interviews started with an introduction to the Farmers Pride project and the reasons for the interview. During the interview, background information was requested on the farmer's household and land use (Sections A, B and F) and participation in agri-environmental schemes (Section D). Specific information on landraces was requested in the following Sections: Section B (landraces grown and the reasons for growing them or stopping this); Section C (the interviewee's intentions about future landrace cultivation); Section E (the interviewee's response to a hypothetical support scheme for growing landraces); Section G (the extent of interviewee's agreement/disagreement with various statements about the situation of landrace conservation and support measures).

Crofters and farmers cultivating landraces were interviewed in July and August 2018. Interviewees were each questioned face-to-face, and answers were recorded in a Microsoft Excel table directly by the interviewer.

Crofter and farmer selection in 2018

In each of the island groups, key informants were used to identify an initial target group of farmers known to have recently grown landraces. Additional farmers were

added to the survey based on the recommendations of interviewees. A short public awareness article was published in *The Crofter* (Maxted *et al*, 2018) and local radio interviews were undertaken in Orkney and Shetland to increase visibility among the target community. By the end of the 2-month surveying period, a total of 42 farmers and crofters had been interviewed.

Travelling was time-consuming in all the archipelagos in the study and it was not possible to reach the remoter islands. In the Outer Hebrides, the study focused on growers in the Uists. Due to the scale of cultivation on the Uists, data were also collected from local Scottish Government offices and local observers on North Uist who had been consulted during the first field survey in 2006.

Results

Landrace diversity, area grown and grower numbers

Except for bere on Shetland, the 2018 landrace survey suggested relative stability in crop diversity (the number of landrace species cultivated) and also in their uses compared with the 2003 study (Table 1).

On Shetland from 2004 to 2006, an attempt was made to revive the growing of bere and small oat by Shetland Organic Producers Group. Although this resulted in a brief increase in growers (Table 2) to about ten of bere and five of small oat (3.3ha of bere and 0.7ha of small oat in 2004), several growers only planted extremely small areas and most failed to maintain their own supply of seed. Crucially, no new markets were developed for either crop. We estimate that from at least 2018, there has only been one grower of bere and just a few growers of small oat on Shetland.

Between 2003 and 2006 on Orkney, the area of bere grown by the AI and BHT supply chains was about 9–12ha and most of the growers included in the 2003 desktop survey were associated with these supply chains (Table 2). Subsequently, BHT successfully expanded the market for beremeal, exporting it to other parts of Scotland, and there was a considerable increase in interest in bere for both brewing and distilling following the release in 2012 of single malt whiskies made with bere (Martin and Wishart, 2015). As a result, the combined area of bere grown by the AI and BHT supply chains increased to about 51ha in 2018 and 69ha in 2020 when about 200t of bere grain were supplied for producing specialist whiskies.

For the Outer Hebrides, Scottish Government census data indicate a very slight decline in the area of oats and mixed grains grown between 2005 and 2018 (Table 2) and a more marked decline in the number of holdings growing these crops. It is possible that this reflects a consolidation of growing on the larger holdings. Census data for the Outer Hebrides only include barley from 2002 to 2008 and over this period the average area was 28ha on eight holdings; it is likely that most of this was bere. A later study (Scholten *et al*, 2009) estimated

about 12 growers of sole crop bere on the Uists in 2008.

Landrace cultivation by growers in the 2018 survey

In 2018, amongst the 42 landrace growers interviewed, 29 were still growing at least one landrace (Table 3). The number of interviewees growing landraces in each archipelago (Table 3) broadly reflected the estimated grower numbers (Table 2) and was highest on the Uists (15), followed by Orkney (9) and Shetland (5). Amongst the growers of cereal landraces, most on the Uists (12 out of 14) were growing two or three species in mixtures while in Shetland there were only two growers of small oat and in Orkney, there were two growers of small oat and five of bere. Average arable land use per croft or farm varied between the island archipelagos (Table 3), with Orkney growers having the highest proportion of their land in arable cultivation (70% arable, compared with 30% pasture) and the highest average area of arable land per farm (49.7ha). Growers in the Uists had the second-highest average area of arable land per farm (13.5ha), but this was only 8% of their land, with 92% being pasture. Shetland had the lowest average area of arable land per farm (1.2ha). These figures broadly reflect more comprehensive agricultural census data for these areas (Supplemental Note 2).

Reasons for growing, or ceasing to grow, landraces

Growers were asked their reasons for growing landraces and given the option of multiple responses (Table 4). The most consistent replies came from Uist growers who unanimously listed the adaptation of the landraces to local soil conditions as the main reason for growing them (15 out of 15), followed by tradition (12) and low management requirements (9), with market demand figuring quite low (3). Tradition was also the most common reason given by respondents in Shetland (5 out of 5) and Orkney (5 out of 9).

The number of interviewees who had ceased growing landraces was largest on Orkney (7 out of 16) and the reasons mentioned included: lodging, weak straw, inability to apply fertilizer, lack of a market for bere, problems during combine harvesting, disease, loss of traditional knowledge, unfavourable weather and a high workload. On Shetland, 3 out of 8 respondents had stopped, and the reasons provided were: damage from sparrows, low yield, difficult to grow and problems with machinery. On the Outer Hebrides, 3 out of 18 had stopped and mentioned damage from geese, machinery problems and costs and adverse weather as the main reasons.

Participation in financial support schemes

Although several growers, especially on the Uists, preferred not to say what percentage of annual household income was derived from their croft/farm,

Table 1. Landrace species and their uses documented between 2003 and 2020 in the different Scottish archipelagos.

Archipelago	Landrace	2003 (Scholten *et al*, 2011)	2018 (Source: Farmer's Pride survey)	2019-2020 (Sources: Agronomy Institute; CDP! Project[1])
Shetland	Cabbage	Winter fodder	Winter fodder	Winter fodder
	Bere	Potential food and drink	None grown	Maintaining a local seed supply
	Small oat	Animal feed; straw for basketry, chairs and thatch	Animal feed; straw for basketry, chairs and thatch	Animal feed; straw for basketry, chairs and thatch
Orkney	Bere	Food and drink	Food, drink and animal feed	Food, drink and animal feed
	Small oat	Heritage crafts	Heritage crafts	Heritage crafts
Uists	Small oat mixtures with Hebridean rye and /or bere	Winter animal feed	Winter animal feed	Winter animal feed; exploring use of bere and rye for whisky

[1]CDP! Project (Crofters' Diversity Pays!, 2020)

Table 2. Approximate area of landraces grown and estimated grower numbers in the Scottish archipelagos.

Archipelago	Landrace	2002–2005	2018 Survey	2018–2020
Shetland[1]	Shetland cabbage	Data not available	Data not available	Data not available
	Bere	4ha; 10 growers	None grown	< 0.1ha; 1 grower
	Small oat	1–2ha; 5 growers	3ha; 2–5 growers	1–2 ha; 2–5 growers
Orkney[1]	Bere	9–12ha; 3–7 growers	59ha; 5–8 growers	73ha; 5–8 growers
	Small oat	1ha; 2 growers	< 1ha; 2 growers	< 1ha; 2 growers
Outer Hebrides[2]	Small oat mixtures with Hebridean rye and/or bere	i) 275–317ha of oats and mixed grains; 215–232 holdings; (Scottish Executive, 2005) ii) 300–600ha; 200–400 growers estimated by Scholten *et al* (2008)	i) 269 ha of oats and mixed grains; 147 holdings; (Scottish Government, 2018) ii) Key informants estimated the areas to be similar to those given by Scholten *et al* (2008)	i) 260ha of oats and mixed grains; 139 holdings; (Scottish Government, 2020) ii) Key informants estimated the areas to be similar to those given by Scholten *et al* (2008)

[1] The areas and numbers of growers of landraces in Orkney and Shetland are based upon our own knowledge and information provided by key informants (for example, Shetland Organic Producers Group, the Agronomy Institute (AI) at Orkney College UHI and Birsay Heritage Trust)
[2] Two estimates are provided for the areas and numbers of holdings of small oat and small oat mixtures in the Outer Hebrides: i) derived from the areas for oats and mixed grains given in Scottish Government census data and ii) derived from local key informants.

Table 3. Summary of respondent characteristics in the 2018 survey by Scottish Isle archipelago.

Archipelago	Number of interviewees; number growing landraces in brackets	Range of arable area (ha); averages in brackets	Number of landrace growers in agri-environment schemes	Number of landrace growers with more than 50% of income from croft/farm; number of responses in brackets
Shetland	8 (5)	0–3 (1.2)	4	1 (5)
Orkney	16 (9)	1–324 (49.7)	5	4 (8)
Uists	18 (15)	1–30 (13.5)	9	3 (4)

Table 4. Summary, by archipelago, of reasons for farmers growing landraces.

Archipelago	Number of respondents	Reasons for growing landraces (number of respondents who gave each answer in brackets)
Shetland	5	Tradition (5); not available elsewhere (2); market demand (3); adaptation to soils (3); disease resistance (2); quality of products (1); low management requirements (1)
Orkney	9	Low management requirements (5); tradition (5); market demand (3); adaptation to soils (3); disease resistance (2); quality of products (2)
Uists	15	Adaptation to soils (15); tradition (12); low management requirements (9); market demand (3); good feed for cows (2); yield (2); tolerance to extreme temperatures (1); quality of product (1).

this appeared to be greater on Orkney and the Uists than on Shetland with 50%, 75% and 20% of those replying to this question on Orkney, the Uists and Shetland, respectively, reporting that it contributed 50% or more of household income (Table 3). Half of those interviewed mentioned that they were currently, or had in recent years, taken part in an agri-environment scheme (Table 3). Amongst these, 67% were currently taking part in one, and 33% had done so previously. Accurate collection of data on scheme participation was not possible as not all growers had kept or had access to the relevant paperwork, and not all could recall which schemes they had taken part in or when. Amongst those who could recall, the following schemes were mentioned: the Agri-Environment Climate Scheme (9 growers), the Scottish Rural Development Programme (SRDP; 4), the Basic Payment Scheme (4), the Environmentally Sensitive Area Scheme (3), the Habitat Scheme (1), the Organic Maintenance Scheme (1), the Rural Stewardship Scheme (1).

Hypothetical bere barley support measures

None of the growers interviewed were currently involved in any specific support programme for bere. Nevertheless, 48% said that they would be interested in a support scheme which rewarded growers for each hectare of bere grown as a pure stand with the intention of saving seed each year. When asked how much monetary support they would require for this, responses ranged from £110 to £1,000 per ha with a mean of £338 per ha, which compares with previous support schemes such as SRDP payments which had a maximum of £500 per ha and the agri-environment scheme for machair soils of £230 per ha.

Planned croft or farm future

Growers were asked what they intended to do with the croft or farm once they stopped working it. The majority (61% of those asked) planned to hand them on to younger family members, while just over a quarter (27%) planned to sell them outside of the family. Those who were tenants (7%) would return the crofts to the owner upon retirement and the remaining 5% were not sure what they would do with the croft. On both Orkney and Shetland, the majority of growers planned to sell them outside the family. The Uists had the highest number of younger crofters and all of those

interviewed intended the croft to continue to be worked upon retirement, with the majority planning to pass it on to a relative.

Discussion

Landrace maintenance in Orkney

Since 2002, there has been a very significant increase in the area of bere grown in Orkney from about 10 to 73ha, although this has not been matched by a large increase in growers (Table 2). This is explained by the role of BHT and the AI in managing and expanding supply chains for bere in which growing tends to be carried out by just a few growers and on leased land. To put the area of bere grown in Orkney into perspective, over the 20th century this declined from about 1,600ha in 1912 (Board of Agriculture for Scotland, 1913) to about 5ha at the end of the century. Despite the recent increase in the area of bere, there are essentially only two maintainers of bere seed – the AI and BHT – and these organizations provide seed to their own growers.

The focus in Orkney on grain production for higher value off-farm use is facilitated by the larger arable area on Orkney farms (see Table 3 and Supplemental Table 2), a high level of mechanization and the availability of several batch grain dryers. In spite of the success of the AI and BHT supply chains, the 2018 survey showed that several farmers had stopped growing landraces (mostly bere) because of their agronomic shortcomings. These would be very obvious to Orkney farmers because of the large area (over 4,000ha) of modern 2-row barley grown on the island; this is higher yielding and less susceptible to lodging than bere. Nevertheless, other factors like tradition and bere's low management requirement are also important to some growers, as found by both the 2018 survey and an earlier study (Mahon *et al*, 2016). Here, management includes aspects like the use of inputs (fertilizer, herbicide, growth regulator and fungicide) and the quality of land required by the crop. Although not mentioned during the survey, straw is an important byproduct of bere grown for grain and is readily sold if farmers do not require it themselves. Since bere is usually harvested before the weather deteriorates too badly, the quality of its straw is normally very good and is used for feeding as well as bedding.

On Orkney, the main driver for continued growing of bere has been the demand for grain processed

locally into beremeal or sent to mainland Scotland for malting. These are then used to produce niche market, high-provenance food and drink products for both the local and export market and the tourism sector, including traditional bere bannocks (a type of flat bread), biscuits and bread made from beremeal and beers, whiskies and a craft vinegar made from bere malt. Long-term collaborations between the bere supply chains and distilleries have been important in building up a consistent market for bere, and several growers in the 2018 survey commented that they would be unlikely to continue cultivating bere without an assured buyer. Apart from being the only mill producing beremeal in Scotland, Barony Mill is also a visitor attraction which promotes bere, beremeal and other bere products. Although the conservation status of Orkney bere appears strong at present, it is dependent on demand from a relatively small number of end-users and would benefit from further market diversification and on-farm use. Increased interest in using bere for both brewing and distilling has resulted in small areas of the crop being grown for malting in parts of mainland Scotland and the north of England since about 2020. The success of bere has also encouraged farmers, craft maltsters and distilleries to start experimenting with other Scottish barley landraces like Scotch Common and Scotch Annat.

In contrast to bere, the growing of small oat in Orkney has virtually ceased. Its main potential value would likely be as a feed crop on Orkney's coastal sandy land where it was once commonly grown because of its tolerance to manganese deficiency. Its straw might also be of value for straw products and was once used for making traditional Orkney straw-back chairs. A major constraint on the wider growing of small oat in Orkney is a lack of seed. There is also a reluctance to grow it because of its ability to contaminate other crops and fields through volunteer plants or seed remaining in machinery.

Landrace maintenance in Shetland

The study showed that the situation of cereal landraces is most precarious in Shetland, both in terms of the number of growers and the areas of crops grown and the most cited constraint there was the lack of market demand for landrace products. By 2020 it is thought that there was only one grower of bere on Shetland who grew it on a very small scale in a net tunnel to protect it from damage by sparrows and about three growers of small oat who were growing this for straw. The difficulties of commercializing bere in Shetland have included a scarcity of machinery and equipment for growing the crop and drying grain on a larger scale, a lack of processing equipment for local milling or malting and high transportation costs, which make it expensive to ship elsewhere for processing.

Potentially, in Shetland, the growing of small oat for straw for basketry, straw-backed chair or thatching could be expanded, but these markets are very small. Additionally, farmers growing it for straw cannot use combines for harvesting as these crush the straw and they must either harvest using scythes or old reaper binders, which often have maintenance issues.

Landrace maintenance in the Uists

In the Uists, our study showed that the cereal landrace mixture is still a very valuable component of the machair crofting system and is economically important as a well-adapted, low-input crop for winter feed which allows growers to avoid the expense of importing feed (estimated to be £32–38 per tonne in 2018 (Jones, 2018)) and its associated carbon footprint. The continued use of seaweed and animal manure as a fertilizer for the mixture by some growers also contributes to its sustainability. A crucial trait underpinning the value of the mixture is the tolerance of all its component species to high-pH soils deficient in manganese and copper. This was reflected by replies in the 2018 survey where all respondents included the ability of the landrace mixture to grow on poor soil as a reason for growing the crop.

For continuity of cultivation, it is vital to attract a new generation of stakeholders to adopt and use landraces (Raggi *et al*, 2021). At the initial assessment in 2003 most of the Uist crofters were in their fifties or sixties, but during the 2018 field work it was apparent that there are now many younger crofters in North Uist involved in growing landraces and these showed a keen awareness of the uniqueness of these crops and, specifically, of the potential of bere. One example of this is the interest from a new distillery in the Baleshare crofting community, in sourcing both Hebridean rye (Blackley *et al*, 2022) and bere from local growers.

Local seed production is crucial for growing the landrace mixture and while some growers obtain seed from others, many maintain their own seed (Scholten *et al*, 2009). The composition of species and proportion of each is dynamic and varies with the grower and how weather and field conditions affect the crop. A major threat to the island seed system is damage from geese and deer. Data for greylag (*Anser anser* L.) and barnacle (*Branta leucopsis* Bechstein) geese numbers (Mitchell and Hall, 2020; WWT, 2022) indicate increases for the two species on the Uists from about 7,400 in 2003 to around 11,800 in 2018. At the time of the 2018 survey, there were at least four areas, one on North Uist and three on South Uist, where seed production had ceased because of the geese threat. This potentially threatens local landrace maintenance. Almost two out of three crofters in the survey mentioned geese as the main constraint on growing landraces, and this was included as a reason for stopping, or for not resuming, growing landraces. While some Government funding has been available for the management of geese numbers, local crofting stakeholders consider this inadequate for the scale of the problem (Scottish Crofting Federation, 2022). It has also been reported that some growers are

using farmer contacts in other parts of Scotland to grow the landrace mix to provide seed for them.

Tradition

Across all archipelagos, tradition was an important factor cited by many growers (22 out of 29) driving their current use of landraces. On Orkney, this may be due to the rise in demand for traditional products made from bere, but is not the case on the other archipelagos, indicating that growers enjoy the tradition for the connection that growing the crops provides with their heritage. Many commented to this effect during the survey and it was also identified as an important consideration in an earlier study (Mahon *et al*, 2016). Another possible explanation is that tradition is linked to the less intensive approach to agriculture which crofters and small farmers often adopt, including aspects like fewer inputs, small-scale production on more marginal land and use of less costly or technically advanced machinery and equipment. Despite the importance of tradition, crofters and farmers have not restricted themselves to traditional methods of cultivating them. Thus, over the last 40 years on the Uists, most crofts and farms have transitioned from harvesting the landrace mix with reaper binders and putting sheaves into stacks for later threshing, to using combine harvesters and, most recently, to harvesting the crop earlier and preserving it in wrapped bales. The latter, however, reduces the biodiversity value of the crop as it is harvested before wildflowers within it can set seed. As well as tradition, adaptation to local soils and low management requirements were also important reasons for continuing to grow landrace crops on all archipelagos. While all Uist crofters gave this reason, many also commented that if they could grow modern higher-yielding varieties, they would.

Grower satisfaction with current support measures

Half of the growers interviewed had taken part either currently or in the past in agri-environmental schemes (Table 3), of which seven different schemes were mentioned. This diverse range of grower support indicates an awareness that growers need support but also suggests that some rationalization could simplify the system for the growers and aid implementation. A major criticism of past schemes was that some, which were designed to protect wildlife (primarily birds), did not allow early harvesting of cereals. Consequently, harvesting was later than desirable and often resulted in crop lodging because of unfavourable weather. Another major complaint was that migrating geese arrived at harvest time and ate or damaged the crop before it could be harvested, compounding the problem of the specified harvesting date and adding to the uncertainty of a successful harvest. The consensus amongst crofters was that if there was more flexibility, they would be more likely to join agri-environment schemes. Most of those interviewed considered that resolving

the problem of the geese outweighed the importance of financial support. Another issue raised was the lack of a market for landrace products, suggesting that stimulating this market would increase landrace conservation. Therefore, the solution is not simple and possibly a mix of financial support and appropriate policy actions would best support landrace maintenance long-term.

Recently a new post-Brexit UK Agriculture Bill has received royal assent. Part of the bill includes a clause allowing the Secretary of State to: "give financial assistance for or in connection with any one or more of the following purposes . . . (i) conserving plants grown or used in carrying on an agricultural, horticultural or forestry activity, their wild relatives or genetic resources relating to any such plant" (UK Parliament, 2020). Through the implementation of this clause, the Secretary of State can provide long-term funding for *in situ*/on-farm conservation of UK crop wild relatives and landrace genetic diversity conservation which could significantly support landrace maintenance and help underpin future UK food security. The practical application of this new law in England has yet to be formulated but such consideration as the flexibility of harvest time could be accommodated to ensure a desirable outcome for landrace maintenance. Within the UK, agriculture is a devolved policy area, however, and this clause has not yet been included in Scottish law.

Heightened profile of Scottish cereal landraces since 2002

The development and marketing of a diverse range of new commercial products from bere have helped considerably to raise its profile and that of other Scottish cereal landraces and have demonstrated the potential for realizing significant economic benefits from their conservation. Notable amongst bere products have been several single malt whiskies. The use of bere in such a high-value, iconic Scottish product has introduced a new clientele and audience to both the crop and discussions about the importance of landraces through the marketing and social media activities of distilleries with a global reach. This demonstrates the potential for commercialization to assist the promotion of landraces as well as add value to them (Raggi *et al*, 2021).

Commercialization of bere has also helped lever funds for scientific research on Scottish landraces which in turn has stimulated further interest in these crops. For example, research on bere has encompassed a diverse range of topics including: the nutritional properties of its grain and beremeal (Chappell *et al*, 2017; Theobald *et al*, 2006); agronomy (Martin *et al*, 2010; Brown *et al*, 2020); sustainability traits (Schmidt *et al*, 2019; Cope *et al*, 2021); and the origins of bere (Drosou *et al*, 2022; Wallace *et al*, 2019). Much of this research has been possible because of the collection of bere accessions that has been built up at the James Hutton Institute (JHI), and progress with genotyping the collection will allow genetic diversity to be taken into account as *in*

situ conservation strategies are developed. A spin-off of this research has been the multiplication of bere seed and the distribution of Scottish island landraces to the mainland by the JHI working with the Seed Sovereignty Programme in 2020 and 2021.

The need for an *in situ* on-farm conservation strategy

Given the threats to continued *in situ* growing of cereal landraces in the Scottish islands and the relatively good knowledge about their cultivation, utilization and genetic variation, it is an appropriate time to develop an *in situ* on-farm conservation strategy for them. Ideally, this would identify desirable conservation targets in terms of numbers of growers and seed maintainers and the area of crops required for this on each archipelago. It should also include measures for conserving both their genetic diversity and the underpinning traditional knowledge about these crops and support ways of passing this on to new, younger growers. Results from recent and ongoing genotyping and phenotyping (Schmidt *et al*, 2019; Scholten, 2012; Hagenblad *et al*, 2016) will be important for ensuring efficient conservation of genetic variation and providing these crops with the greatest possibility of being able to adapt to future changes in growing conditions. The strategy could be included in Scotland's evolving post-Brexit agricultural policy.

Conclusions

Implications for future *in situ* conservation of Scottish landraces

This study has shown that both the cereal landrace mix grown in the Outer Hebrides and bere grown in Orkney continue to contribute value to their respective farming communities and, for bere, there are considerable added value benefits to others in the food and drink value chains. While the conservation status of these crops in these locations seems promising, and for bere in Orkney, is much better than it was in 2003, there are still causes for concern. In particular, seed production of the landrace mix is threatened by geese in the Outer Hebrides and, with most Orkney bere being grown for distilling, it is vulnerable to changes in market trends. Also, there are only two organizations maintaining bere seed in Orkney and they both grow very similar lines which probably do not include the range of genetic variation found amongst Orkney bere within germplasm collections (Schmidt *et al*, 2019). Therefore, a review of the regeneration strategy to ensure a broader coverage of the diversity that exists would be beneficial.

There are now very few growers of small oat and, particularly, bere in Shetland and of small oat in Orkney and these crops are under serious threat of being abandoned and lost in these locations. It is important that accessions of this material continue to be incorporated into *ex situ* collections at the JHI and SASA's Scottish Landrace Protection Scheme (Green *et al*, 2009) to pro-

vide a foundation for any potential future rejuvenation schemes. The situation demonstrates the difficulty of *in situ* conservation of crops when they do not generate value (not necessarily financial) for growers. In both Orkney and Shetland, there is potential for wider use of both small oat and bere, alone or in mixtures, for animal feed on coastal, sandy soils as they are used in the Outer Hebrides. A key requirement for this, however, would be the availability of seed and this could become a commercialization opportunity for a grower or group of growers.

The food and drink value chains developed for Orkney bere might be replicated in the Outer Hebrides and Shetland to promote greater growing of bere and Hebridean rye but this would require suitable processing facilities, collaboration between growers and commercial end-users and probably support to growers to acquire appropriate machinery. These value chains also provide successful examples of landrace maintenance of much wider, even global, relevance.

Within the UK, the development, post-Brexit, of new devolved agricultural policies provides the Scottish Government with an important opportunity to develop an *in situ* on-farm conservation strategy for cereal landraces on the Scottish islands.

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Author contributions

OS carried out the farmer survey with assistance from the other authors. All authors contributed to writing the manuscript, which was finalized by PM.

Conflict of interest statement

The authors declare that they have no financial or competing interests.

Supplemental data

- Supplemental Note 1. Figure 1
- Supplemental Note 2. Crofting
- Supplemental Table 1. Linguistic diversity in Scottish cereal landraces
- Supplemental Table 2. Main differences and similarities in cropping land use and crofting between the archipelagos in this study
- Supplemental Note 3. Survey questionnaire for landraces on Northern and Western Scottish islands 2018

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SHORT COMMUNICATION

Characterization of microsatellite markers for the duckweed *Spirodela polyrhiza* and *Lemna minor* tested on samples from Europe or the United States of America

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Abstract: Microsatellite primers are a valuable tool to use for both observational and experimental studies in numerous taxa. Here, we develop 18 and 16 microsatellite markers for the widespread duckweeds *Lemna minor* L. and *Spirodela polyrhiza* (L.) Schleid, respectively. All 18 *L. minor* primers and 12 of the 16 *S. polyrhiza* primers amplified polymorphic loci when tested on samples from Europe or Western Pennsylvania, USA.

Keywords: Lemnaceae, simple sequence repeats, genotyping, genetic identification, molecular markers

Introduction

The globally distributed duckweed family (Lemnaceae) or subfamily (Lemnoideae) is composed of 36 species (Bog *et al*, 2020) of very small floating or submerged aquatic plants (Landolt, 1986; Sree *et al*, 2016). Duckweeds have a long history of scientific study given their highly specialized morphology, widespread distribution, high abundance and production of the world's smallest flowers (Jacobs, 1947; Hillman, 1961; Landolt, 1986, 1992). More recently, there has been an explosion in research interest given their potential applied uses including for agricultural feed (Cheng and Stomp, 2009), bioremediation (Gupta and Prakash, 2013; Ekperusi *et al*, 2019) and biofuel production (Cui and Cheng, 2015). Furthermore, their use as a model

system to experimentally study numerous topics in ecology and evolutionary biology is quickly expanding (Laird and Barks, 2018). This growing basic and applied interest stems from their ability to reproduce clonally very quickly with population doubling times in as little as 1.5 days (Ziegler *et al*, 2015). In addition, they are amenable to large-scale manipulative experiments in both the lab and field mesocosms (Armitage and Jones, 2019; Hart *et al*, 2019; Tan *et al*, 2021; O'Brien *et al*, 2022), and have growing genomic data and tools (Wang *et al*, 2014; Ho *et al*, 2019; Xu *et al*, 2019; Cao *et al*, 2020) and characterization of their microbiome and herbivore communities (Acosta *et al*, 2020; Subramanian and Turcotte, 2020). Finally, duckweed express variation in numerous traits across species and among genotypes (clonal lineages) within species (Van Steveninck *et al*, 1992; Hart *et al*, 2019; Chen *et al*, 2020; Hitsman and Simons, 2020; Anneberg *et al*, 2023). Therefore, being able to identify genotypes may also be beneficial in many

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ecological studies to assess differences in traits among genotypes and to determine how these genotypes may respond to different environmental conditions.

Genetic markers, such as microsatellite markers, are important tools to study population genetics. Microsatellites, also known as simple sequence repeats (SSR), are tandem repeats two to ten base pairs in length, that are flanked by conserved sequences and occur ubiquitously throughout eukaryotic genomes (Tautz and Renz, 1984). They are highly informative as locus-specific genetic markers due to their high abundance, high reproducibility, co-dominance, and polymorphic nature (Morgante and Olivieri, 1993; Powell *et al*, 1996). The length of the sequence repeats can be determined through PCR amplification using primers specific to their flanking regions; variation in PCR product length is a function of the number of repeated sequences. The high levels of polymorphisms observed in SSR markers (Tautz, 1989; Schlötterer and Tautz, 1992) and the relative ease of detection of these polymorphisms by PCR amplification have led to the wide applications of microsatellites as genetic markers (Vieira *et al*, 2016). Such within-species markers have numerous applications including quantifying biogeographic distributions, population genetic structure, evolutionary history, and mating systems.

Moreover, a growing number of experimental evolution studies use SSR markers to track changes in genotypic composition of asexually reproducing populations over multiple generations (Turcotte *et al*, 2011; Hart *et al*, 2019; Agrawal *et al*, 2013) in large replicated experiments for which genotyping-by-sequencing remains too costly. These cost savings are magnified when several loci can be multiplexed and genotyped in the same reaction (Markoulatos *et al*, 2002).

With the growing interest in duckweed, microsatellite markers have been developed for a few duckweed species. Wani *et al* (2014) developed nine polymorphic and 24 monomorphic haplotype chloroplast DNA-based microsatellite primers for L. minor. Xu *et al* (2018) developed 60 microsatellite primers for *Spirodela polyrhiza*, 19 of which were polymorphic within three populations of *S. polyrhiza* from China. Feng *et al* (2017) developed three microsatellite primers for the identification of *S. polyrhiza* and *Landoltia punctata* (G. Mey.) Les & D. J. Crawford haplotypes. More recently, Fu *et al* (2020) developed 70 microsatellite primers within coding regions for *L. gibba* L. It is important to continue developing and reporting new microsatellite markers as populations can differ in which markers function (e.g. due to null alleles) and are polymorphic Chapuis and Estoup (2007).

Here, we report on the successful development of 18 and 16 new microsatellite markers, respectively, for two commonly studied and widespread duckweed species: the common duckweed *Lemna minor* L. and the greater duckweed *Spirodela polyrhiza* (L.) Schleid. A small subset of these microsatellite primers was used to differentiate genotypes in our experimental studies on evolutionary coexistence (Hart *et al*, 2019). In addition,

we report genotyping results using these markers on individuals sampled in Europe and the United States of America (USA). We thus provide new tools and evidence that they function, which can be utilized by the growing community of duckweed researchers (Laird and Barks, 2018).

Materials and methods

Sample collection

Our objective when sampling was not to genetically characterize duckweed populations, but instead find genotypes that differ in ecologically relevant traits to use in various experiments. Thus, we genotyped few individuals from numerous bodies of water in various locations. Primers were developed at ETH Zurich (Europe) and the University of Pittsburgh (USA), and thus were tested on different collections of duckweeds. We collected duckweeds from numerous still bodies of water (e.g. ponds, lakes, wetlands) primarily in Switzerland and Western Pennsylvania (USA); however, a few samples were also collected from the Netherlands and Germany. In addition, some European duckweed samples included in the set were obtained from the Landolt Duckweed Collection (formerly in Zurich, Switzerland, see Supplemental Tables S1 and S2 for collection locations). Given the two-part development of the primers, some duckweed samples were only tested on the primers developed in that country (as noted in Supplemental Tables S1 and S2).

Duckweeds mostly reproduce clonally via meristematic pockets from which clonal daughters emerge, creating clonal clusters of one to eight individuals that eventually split into smaller clusters (Landolt, 1986). We sampled single duckweed clusters and established isofemale laboratory colonies from these clusters. We then sterilized each colony using sodium hypochlorite following a method adapted from Barks *et al* (2018). From each colony, we put single individuals into individual sterile petri dishes (one individual per dish) containing sterile 0.5 strength Schenk and Hildebrandt growth medium (Schenk and Hildebrandt, 1972) supplemented with sucrose (6.7g/L), yeast extract (0.067g/L), and tryptone (0.34g/L) for 24 hours to encourage algal and bacterial spore germination. Then each individual was exposed to one of an array of concentrations of sodium hypochlorite (0.3% or 0.5%) for varying amounts of time (3 or 6 minutes for *L. minor*, 4 or 7 minutes for *S. polyrhiza* respectively), then rinsed with autoclaved distilled water and allowed to grow (Barks *et al*, 2018). Sterile colonies were maintained in sterile 0.25 strength Schenk and Hildebrandt media (Schenk and Hildebrandt, 1972) without the additional supplements in room temperature laboratories or growth chambers under plant grow lights. These collections do not reproduce sexually under lab conditions.

Table 1. *Lemna minor* microsatellite markers and motifs including the location of initial primer development (USA or Europe), optimized $MgCl_2$ concentrations, and annealing temperatures (T_A). In addition, we report marker success rate, which is the number of samples successfully genotyped divided by those attempted, the number of unique alleles, and number of unique genotypes for each primer. Average heterozygosity (H) is the fraction of individuals that are heterozygotic for each primer. See Supplemental Table S1 for specific allele values. Allele lengths with an * denote that these lengths include the M13 tail sequence.

Primers	Forward Primer (5'-3') Reverse Primer (5'-3')	Motif	Location of Development	$MgCl_2$ (mM)	T_A (°C)	Observed Product Length (bp)	Marker Success Rate	Unique Alleles	Unique Genotypes	Average H
LmR.1.A	F: GTTCCTAAGGATTCATCACC R: TACGAGGAGGGACACGAG	AAG	Europe	2.0	60	178–185*	75/81	2	2	0
LmR.4.A	F: AGTGGCTACGAACGGAAGAG R: AGAGGAACGTTGTGTCTGGG	AAG	Europe	0.9	63	219–234	28/28	5	5	0.036
LmR.4.B	F: CTTATTGGATCTTCGCGCCG R: AAGATATCTGACGGCGTTGG	AG	Europe	1.2	63	366–392	28/28	6	6	0.071
LmR.5.C	F: GATGCCAGTAGATCCGGC R: ACGCCTGAACACGATTGATG	AGAT	Europe	2.0	60	320–444	104/109	25	41	0.846
LmR.8.B	F: TGTACTCATCTGTGGGCGAG R: AACAATTTGGCCACCGTCAG	AGAT	USA	1.2	63	306–376	28/28	10	9	0.036
LmR.8.C	F: GACAACTTAGGGTGCACGC R: GGAGTGAGAGCTGAGGACTG	AGG	USA	1.2	60	435–450	28/28	3	3	0
LmR.10.A	F: TCCTTTCTCGTGTCTCCCAG R: ATGCCCGACCTAGTCC	AG	Europe	2.0	60	222–254*	31/81	4	5	0.032
LmR.10.C	F: CTCTCCTTTCTCCTCCACGG R: ATCGCAACCCTCTAGCCG	AGAT	Europe	2.0	60	179–254*	79/81	4	4	0.278
LmR.12.B	F: TCTCTGCTGACCGACTCAAG R: GCCGTTGGATCTTTCTCACG	AT	USA	1.2	60	274–320	27/28	8	9	0.111

Table 1 continued

Primers	Forward Primer (5'-3') Reverse Primer (5'-3')	Motif	Location of Development	MgCl$_2$ (mM)	T$_A$ (°C)	Observed Product Length (bp)	Marker Success Rate	Unique Alleles	Unique Genotypes	Average H
LmR.14.A	F: TCGCACTAGAGAGATGGGTG R: TCCCATTACCAGGATGCGAG	AAT	USA	1.2	60	261–270	24/28	3	3	0.042
LmR.14.B	F: CATGCCAGGTAAATGCCCTC R: TCGAGCTCCTTCTCCAAACC	ATC	USA	0.9	63	430–440	28/28	3	3	0
LmR.14.C	F: TTCGTCGAGGGTATGAGCTG R: TCTCTTATTTGACACGCGCG	AG	USA	0.9	63	162–178	28/28	7	7	0.036
LmR.15.A	F: GTGACAGCGTATCCTTGTGC R: CAGCGGCAAGATCATCAAG	ATC	Europe	1.2	60	222–285	109/109	13	15	0.578
LmR.15.B	F: TCGAGCTAATCAGTGGAGCC R: GAGTGCTCGGCTTGACTTTC	AG	Europe	1.2	60	170–210	104/109	13	25	0.692
LmR.15.C	F: CATGTTCCCACCCACTTGAC R: AAGGAAGAGGGAGCAAGGG	AT	Europe	1.2	60	368–400	109/109	14	26	0.743
LmR.26.B	F: GTGTCTCCGAGAGCCTACAG R: TTTAAAGCTCGGTGGGTCCC	AG	USA	1.2	63	283–329	28/28	10	7	0.964
LmR.31.A	F: GGTGATCTCAGGTAGCCGAG R: TGAGATCACCACTGTCTGCC	AAG	USA	0.9	63	402–432	26/28	5	6	0.077
LmR.31.B	F: AGTCGGCATAGTACTTCCCG R: CTTCTTCAAGACCGTTCCGC	AAG	USA	1.2	63	155–239	28/28	7	9	0.071

Table 2. *Spirodela polyrhiza* microsatellite markers and sampling results as described in Table 1 with allele calls in Supplemental Table S2.

Primers	Forward Primer (5'-3') Reverse Primer (5'-3')	Repeat Motif	Location of development	MgCl$_2$ (mM)	T$_A$ (°C)	Observed Product Length (bp)	Marker Success Rate	Unique Alleles	Unique Genotypes	Average H
Sp.1035	F: TGCTTGGTCACTCTTGTCTG R: CGATTCCTAGCTCCTCTGC	AT	Europe	1.2	60	361–369	42/42	4	5	0.381
Sp.1467	F: AGTTGAGGAAGCTTCATGG R: ATTACCTCCAGCACCTCTCC	AG	Europe	2.0	58	386–411*	9/20	5	4	0.444
Sp.2597	F: TCCCATTCACCACAGTCTCC R: TCATTCCACCACGTCCCAC	AT	Europe	2.0	58	397–399*	14/20	2	2	0.071
Sp.5050	F: ATTAACCTTGGGCGCAGAG R: TAGCAGCAGAGTGTGAGGG	AAT	Europe	2.0	58	287*	14/20	1	1	0
Sp.5250	F: AAACGAGACCTCCTACGCC R: GCCTGCGAGTAATATGTGC	ATGCCC	Europe	2.0	58	385*	19/20	1	1	0
Sp.7286	F: CGAATATGCCGAGGAATGC R: TCCTCGATCTGCCGCTTTAG	CG	Europe	1.2	60	386–394	42/42	5	7	0.310
Sp.7688	F: AATGGTTGACTCGACGCTG R: TCACACCGCCATAATTTCGC	AGC	Europe	2.0	58	199–211*	19/20	2	2	0.158
Sp.7814	F: AGTGTAGGGTGCAGCTGTG R: TTCGTGAAAGGCCTAGCAC	AG	Europe	1.2	60	220–228	42/42	5	6	0.095
Sp.7908	F: GAGACACATCATTGCCAGC R: TAATGCAGGCCACACAACC	AG	Europe	2.0	58	234–236	20/20	2	2	0.850
Sp.8563	F: GTATTGGGTGGGCAAATCG R: AAGGGATAGGGTCGTGTCC	AG	Europe	2.0	58	350–354*	14/20	3	4	0.071

Continued on next page

Table 2 continued

Primers	Forward Primer (5'-3') Reverse Primer (5'-3')	Repeat Motif	Location of development	MgCl$_2$ (mM)	T$_A$ (°C)	Observed Product Length (bp)	Marker Success Rate	Unique Alleles	Unique Genotypes	Average H
Sp.8910	F: CCTTCCCTACGTTGACTCCC R: GCGTTTCTCTGATCAGCACC	ACG –> CGT	Europe	2.0	58	358	20/20	1	1	0
Sp.9307	F: GGGAGCGAGCTGTATGAAG R: TTTCAACACCCTCACCATGC	AG	Europe	2.0	58	450–452*	9/20	2	3	0.444
Sp.9311	F: GTGAGAAAGGAAAGGTGGC R: TGCTCAGGATTCTATGGGCC	AG	Europe	2.0	58	253–255*	10/20	2	3	0.400
Sp.Pso27	F: AAGGGTTTCAGTGCGGACG R: CTCGCCTTCTCGTACATCATC	AAG	Europe	2.0	58	133*	9/20	1	1	0
Sp.Pso31	F: TCCACCGTCTCCCTGTAATG R: CCACTCCCTCGTCGTGAAG	AAG	Europe	1.2	60	240–270	32/42	7	7	0.406
Sp.Pso32	F: TGCTGGCGATGTCAATGTTG R: CTTCAGCACCAAGAGAGCTC	ATC	Europe	2.0	58	377–380*	19/20	2	3	0.895

Microsatellite marker development

A total of 18 *L. minor* and 16 *S. polyrhiza* microsatellite markers were developed across Europe or the USA. We downloaded the whole genome shotgun sequence data for *S. polyrhiza* strain 7498 from the National Center for Biotechnology Information's GenBank database (accession ATDW01000001.1) deposited by Wang *et al* (2014). For *L. minor*, a draft genome (strain 8627) was downloaded from www.lemna.org on 16 October 2015 (genome draft lm8627.ASMv0.1). A recent study using tubulin-based polymorphism suggests that this lineage is in fact an interspecific hybrid of *L. japonica* Landolt and *L. turionifera* Landolt both closely related to *L. minor* (Braglia *et al*, 2021). The species identity for most samples on which we report below has been confirmed using morphology and/or barcoding (Fazekas *et al*, 2012; Barks *et al*, 2018). While some microsatellite markers are known to amplify across more than one duckweed species (Xu *et al*, 2018), we have not yet explicitly tested these markers against other species. Using msatcommander (version 1.0.8, Faircloth 2008), we identified microsatellite loci using the default settings, avoiding mononucleotide repeat motifs. We then selected loci that would produce products of different lengths, had different motif lengths and were found on different contigs. The 5' end of forward primers were labelled with one of several fluorescent dyes from various suppliers.

Primers developed at ETH Zurich were M13-tailed to reduce cost during development (Boutin-Ganache *et al*, 2001). This entailed adding the full or a partial M13 sequence of TGTAAAACGACGGCCAGT for the *S. polyrhiza* primers and GGAAACAGCTATGACCAT for *L. minor* primers to the 5' end of the forward primer. The M13-labelled forward primers were used in combination with an M13 primer that had the same sequence but was fluorescently dye-labelled at its 5' end. Some primers amplified loci that were fully or mostly monomorphic or did not amplify as consistently as others. For these primers, we only have fragment lengths that include the M13 tail (see Table 1 and Table 2), and we estimate that this lengthens the PCR product by 12–19 base pairs. For most primers, however, following initial testing with M13, we ordered new labelled primers that did not include the M13 tail.

At least 20 duckweed samples were tested using each primer. European duckweed samples were tested across 7 *L. minor* and 16 *S. polyrhiza* primers, and USA duckweed samples were tested across 15 *L. minor* and 4 *S. polyrhiza* primers (see Supplemental Tables S1 and S2 for details). Each duckweed sample was tested with each primer using at least two independently extracted DNA samples. We only report allele lengths that were consistent in both samples.

Microsatellite amplification and optimization

All duckweed collections were extracted and genotyped at least twice by first sampling four to ten individuals from each monoclonal collection and lyophilizing them for 24 hours. We then extracted DNA using a modified CTAB-based method by Healey *et al* (2014). For primers developed in Europe, the conditions were the following: PCR amplification was conducted in 15μL volume reactions containing 3μL of template DNA, 3μL of 5X Colorless GoTaq Flexi buffer (Promega, USA), 2.0mM $MgCl_2$, 0.2mM dNTP mix, 0.05μM of forward primer, 0.2μM of reverse primer, 0.2μM of M13 primer tagged with a fluorescent probe (e.g. 5' 6-FAM or 5' HEX), and 1 unit of GoTaq G2 Flexi DNA Polymerase (Promega, USA). DNA concentrations were rarely quantified as amplification was successful across a range of values (e.g. 2–40ng/μL). Thermocycling conditions for both *S. polyrhiza* and *L. minor* from Europe that were M13 tagged were: initial denaturing at 94°C for 5 min, followed by 30 cycles of 1 min at 94°C, 1 min at 60°C, 1 min at 72°C, followed by eight M13 cycles consisting of 1 min at 94°C, 1 min at 53°C, 1 min at 72°C, followed by a final extension at 72°C for 10 min. For all primers developed in the USA, the conditions were the following: PCR amplification was conducted in 15μL volume reactions containing 3μL of template DNA, 3μL of 5X Colorless GoTaq Flexi buffer (Promega, USA), 1.2mM $MgCl_2$, 0.2mM dNTP mix, 0.08μg/μL of Bovine Serum Albumin (BSA), 0.2μM of each forward and reverse primer, and 1 unit of GoTaq G2 Flexi DNA Polymerase (Promega, USA). Thermocycling conditions for *S. polyrhiza* were: initial denaturing at 94°C for 5 min, followed by 34 cycles of: 1 min of denaturing at 94°C, 1 min of annealing at 60°C, and 1 min of extension at 72°C, followed by a final extension at 72°C for 10 min. For *L. minor*, touchdown PCR was employed with an initial denaturation of 94°C for 5 min, followed by five cycles of denaturation (94°C, 1 min), annealing (67°C, 1 min; decreasing by 1°C per cycle), and extension (72°C, 1 min). Then 25 cycles of 1 min at 94°C, 1 min at 63°C, and 2 min at 72°C, followed by a final extension at 72°C for 15 minutes. Primers were then optimized for annealing temperatures and $MgCl_2$ concentration (Table 1 and Table 2).

Fragment length analyses for all primers were conducted on ABI 3730 Genetic Analyzers (Applied Biosystems) at either the ETH Zurich Genetic Diversity Center (Switzerland), Keck DNA Sequencing Lab at Yale University (USA), or the University of Pittsburgh Genomics Research Core (USA), using either GeneScan™ 500 or 600 LIZ™ Dye Size Standards (Applied Biosystems). Allele calls were made using either Geneious (version 9.1.6, Kearse *et al* (2012)) or GeneMarker software (version 3.0.0, SoftGenetics, State College, Pennsylvania).

Results and discussion

We successfully developed 18 *L. minor* and 16 *S. polyrhiza* microsatellite primers (Table 1 and Table 2) which were tested on samples of duckweeds from Europe or Western Pennsylvania (USA). Some markers were more successful than others (Table 1 and Table 2). All markers amplified in some samples; of these, all

18 *L. minor* primers and 12 of the 16 *S. polyrhiza* primers amplified polymorphic loci, having more than one allele. Moreover, these polymorphic loci differed in product length and can be used in multiplex reactions to increase efficiency and lower genotyping costs. We also found that some loci were much more polymorphic than others. For *L. minor*, these included loci amplified by primers LmR.5.C, LmR.8.B, LmR.15.A, LmR.15.B, LmR.15.C and LmR.26.B, some of which showed high allele richness even when tested on only 28 samples (Table 1). For *S. polyrhiza* these included loci amplified by primers Sp.1467, Sp.7286, Sp.7814, and Sp.Pso31 (Table 2). Monomorphic loci may still be useful in different duckweed populations (Chapuis and Estoup, 2007). Many microsatellite loci also showed heterogeneity (Supplemental Tables S1 and S2), which helps make the primers more informative to distinguish genotypes. We note that some primers developed in one continent were not tested on samples from the other continent (see caption in Supplemental Tables S1 and S2); we suspect these primers will work across continents given patterns observed in the others, but this remains to be tested.

Comparing between species, we saw that *S. polyrhiza* has lower allelic and genotypic richness across most primers, although we also tested fewer samples of this species. This is consistent with our own recent large-scale sampling (Hobble et al. In preparation) as well as other studies using different genotyping methods, that similarly found low genetic diversity in *S. polyrhiza* (Bog *et al*, 2015; Xu *et al*, 2015; Feng *et al*, 2017). It has been hypothesized that this low genetic variation in *S. polyrhiza* is due to its low mutation rate (Xu *et al*, 2019). In addition, primers differed greatly in average observed heterozygosity, but species had similar mean heterozygosities (0.256 for *L. minor* and 0.283 for *S. polyrhiza*). Given that our sampling was designed to find unique genotypes (shallow and widespread) and not characterize populations, we limit our discussion of population genetic indices. The primers we developed can help researchers address various ecological and evolutionary questions as well as better identify and catalogue genotypes for the expanding applied uses of duckweed in bioremediation, biofuel production and as a forage crop.

Supplemental data

Supplemental Table S1: *Lemna minor* sample collection sites and allele lengths.
Supplemental Table S2: *Spirodela polyrhiza* sample collection sites and allele lengths.

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Author contributions

All authors contributed to testing, optimizing, and evaluating marker data, and contributed to reviewing the manuscript. JEK and MMT wrote the initial draft of the manuscript.

Conflict of interest statement

The authors have no conflicts of interest to report.

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ORIGINAL ARTICLE

Phenotypic diversity between and within Harar and Ogaden cattle breeds in eastern Ethiopia: The first step for conservation

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Abstract: Fourteen morphological and eight morphometric traits on a total of 456 adult cattle (354 cows and 102 oxen) from 5 purposively selected districts were recorded to characterize the cattle populations in eastern Ethiopia. Frequency, general linear model and multivariate analysis procedures of Statistical Analysis Software (SAS 9.0) were used to analyze the data. The morphological similarities among the cattle populations from Tulo, Jarso and Fedis districts confirmed their uniqueness and belonging to Harar cattle. Morphological similarities were also observed between the cattle populations from Jigjiga and Kebri Beyah districts, which categorize them as Ogaden cattle. The Harar cattle were characterized by their forward-oriented, widely spaced, and medium-length horns while the Ogaden cattle possessed upright-oriented, narrowly spaced, short horns. The majority of the Ogaden cattle had grey body colour while the Harar cattle had multiple body colours with red combined with white and black observed frequently. Most of the morphometric measurement values were higher for the oxen, while the cows had longer horns. Moreover, Ogaden cattle had higher morphometric parameters than Harar cattle. According to the multivariate analysis results, the cattle populations in the study area were separated into two breeds – Harar and Ogaden. However, these results only showed phenotypic differences, which might not necessarily be due to genetic differences. Therefore, further molecular characterization is recommended to understand their level of relationships which will help to design appropriate conservation and breeding programmes.

Keywords: Breeds, Characterization, Indigenous, Morphology, Morphometric, Multivariate

Introduction

The cattle population size of Ethiopia, 70.3 million head ([Central Statistical Agency](#), [2021](#)), is the largest in Africa and more than double the cattle population registered by Chad (32.2 million) which is the second country with high cattle population ([Statista](#), [2020](#)). Cattle genetic resources, the major contributor to the livestock sector, serve as sources of milk, meat, draught power, hide, manure, nutrient recycling and foreign exchanges for Ethiopia ([Central Statistical Agency](#),

[2021](#)). Due to the very important role cattle genetic resources play in the economy of the country, various diversity and genetic improvement studies have been made so far to ensure their sustainable utilization. Diversity studies in animal genetic resources are important to better understand the breed and design appropriate breeding programmes for current and future research and development works ([FAO](#), [2012](#)). Variation within and among breeds is among the key inputs in genetic improvement and conservation programmes; the more diverse the population the more likely it leads to bring genetic improvement ([Falconer](#), [1989](#)).

Identification, phenotypic and genetic characterization, and advanced performance evaluation can help

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us understand the diversity of a given population. The first diversity assessment of Ethiopian cattle genetic resources was made by the International Livestock Centre for Africa (ILCA), now the International Livestock Research Institute (ILRI), in 1992 (Rege, 1999; Rege and Tawa, 1999). It sought to determine the status and compile information on the characteristics of cattle. It does not constitute the level of assessment required to make decisions on use and conservation, as the information was incomplete due to its very broad brush survey. However, it provides a basis for additional, targeted surveys (Rege, 1999; Rege and Tawa, 1999; Mustefa *et al*, 2021).

According to Ethiopia's focal point for genetic resources, the Ethiopian Biodiversity Institute, Ethiopia possesses 28 indigenous cattle breeds; Abergelle, Anuak, Adwa, Afar, Ambo, Arado, Arsi, Bale, Begait, Begaria, Borena, Fogera, Gofa, Gojam Highland Zebu, Guraghe, Hammer, Harar, Horro, Irob, Jem-Jem, Jijiga, Kereyu, Medenes, Mursi, Ogaden, Raya, Sheko, and Smada (Rege and Tawa, 1999; EBI, 2016). Among the listed cattle breeds, some (Adwa, Ambo, Bale, Guraghe, Hammer, Harar, Jem-Jem, Jijiga, and Smada) do not have a detailed description of their physical appearance. Therefore, it is important to consider filling the missing information to capture a country-wide picture.

According to Getachew *et al* (2014), Ogaden cattle are thought to be a variety of the Borena cattle found in the Ogaden area of the Somali region of Ethiopia and bordering East Hararghe. They have a well-developed hump, large dewlap and short horns. They are mainly kept for milk production but are good beef animals. The Ogaden cattle were also reported to be distributed up to Jigjiga and parts of East Hararghe (Rege and Tawa, 1999; Mengesha, 2019). On the other hand, according to the reports of Rege and Tawa (1999), the Harar cattle are found in the East and West Hararghe plateau. They have short, thick horns and well-developed dewlaps. The common coat colours are black, roan and red and are used primarily for draught. These two cattle breeds are found adjacent to one another. To quantify the level of relationship between them as well as to know the available cattle diversity in that area, phenotypic characterization is required. Moreover, phenotypic characterization is a crucial first step for *in situ* conservation. The current study aims to phenotypically characterize these two cattle breeds and to quantify the level of relationship between them.

Materials and methods

Study areas

This study was conducted in five districts of three zones in two regions: Tulo district of West Hararghe zone, Jarso and Fedis districts of East Hararghe zone of Oromia region and Jigjiga and Kebri Beyah districts of Fafen zone of Somali region (Figure 1). The weather conditions, altitude, ethnicity, and human and cattle

population sizes of the sampled districts are presented in Table 1.

Site selection

According to Rege and Tawa (1999), Harar cattle were found in East and West Hararghe zones of the Oromia region. Similarly, Getachew *et al* (2014) reported the distribution of Ogaden cattle to be the Somali region and adjacent areas of eastern Oromia. Therefore, the current study took representative samples from the two breeds of cattle. Accordingly, for the Harar cattle, Tulo district was randomly selected from West Hararghe zone while Jarso and Fedis districts were randomly selected from East Hararghe zone. On the other hand, Jigjiga and Kebri Beyah districts were selected randomly from Fafen zone to represent Ogaden cattle. The kebeles (sampling sites) within each district were categorized into highland and lowland areas. One kebele from the highland and one kebele from the lowland areas were selected randomly. Households were also randomly selected from each kebeles (sampling sites) and 2–3 adult animals (four years old and above) were sampled randomly from each household.

Data collection

Data collection procedures were adapted from the FAO guidelines for the *Phenotypic Characterization of Animal Genetic Resources* (FAO, 2012). Sampled cattle were carefully handled by trained labourers and their owners. To avoid measurement error, the cattle were made to stand properly on flat grounds with parallel legs. Four researchers were involved in the data collection: two for the quantitative and two for the qualitative data recording. Measurements were carried out by the same researcher throughout the study to minimize subjectivity errors. Data recording was carried out early in the morning before the animals were fed and watered. Textile measurement tape in a centimetre unit was used to record quantitative data. Eight morphometric/quantitative (Table 2) and fourteen morphological/qualitative traits (Table 3, Figure 2) were recorded on a total of 456 adult cattle (354 females and 102 males).

Data analysis

Univariate analysis

Data entry and management were performed using Microsoft Excel© worksheet (Microsoft Office 2016). UNIVARIATE procedure of Statistical Analysis Software (SAS) 9.0 (SAS Institute, 2002) was used to test the normality of the morphometric data. Data on morphological/qualitative traits were subjected to chi-square (χ^2) tests of the frequency (FREQ) procedure of SAS 9.0 software (SAS Institute, 2002) . Qualitative data analysis was performed using the following model by fitting sex, breed and location as class variables. $Y_{ijk} = \mu + S_i + B_j + L_k + e_{ijk}$ where Y_{ijk} is an observation, μ is the overall mean, S_i is the fixed effect of ith sex, B_j is
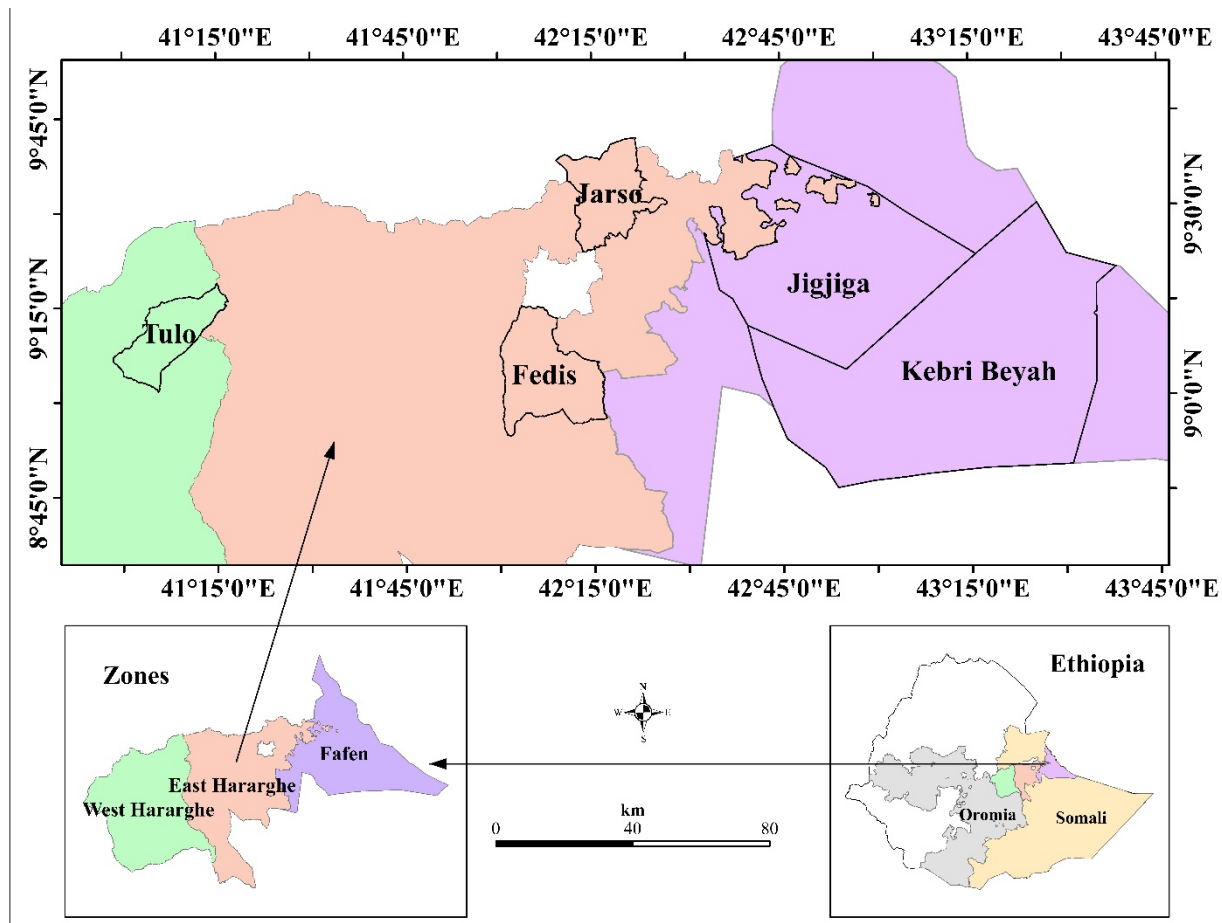
Figure 1. Map of the study areas

the fixed effect of jth breed, L_k is the fixed effect of kth location, and e_{ijk} is the random error attributed to the nth observation.

On the other hand, morphometric data analysis was performed separately for males and females by fitting location and breed as fixed variables. Morphometric data were analyzed using the general linear model (GLM) procedure of SAS 9.0 software, with adjusted Tukey-Kramer test to separate the least square means (LSM). Morphometric data analysis was performed using the following model: $Y_i = \mu + L_i + e_i$ where Y_i is an observation, μ is the overall mean, L_i is the fixed effect of ith location, and e_i is the random error attributed to the nth observation. $Y_{ij} = \mu + B_i + L_j +$

e_{ij} where Y_{ij} is an observation, μ is the overall mean, B_i is the fixed effect of ith breed, L_j is the fixed effect of jth location, and e_{ij} is the random error attributed to the nth observation.

Multivariate analysis

Quantitative measurements that better discriminate the cattle populations from different locations were identified using the forward selection method of the stepwise discriminant function analysis (STEPDISC) procedure of SAS 9.0. The discriminant function analysis (DISCRIM) procedure of SAS 9.0. was also used to assign observations to locations and evaluate probabilities of misclassifications. A linear combination

Table 1. Weather and population-related information of the selected districts (City Population, 2007; Keskes *et al*, 2013; EHZLFDO, 2018; WHZLFDO, 2018; Ayana, 2019; UNHCR, 2020; Kebede and Utta, 2021; Abdi and Shiferaw, 2022)

Agroecology	Tulo	Jarso	Fedis	Jigjiga	Kebri Beyah
Human population projection 2022	215,337	165,712	161,214	417,688	242,880
Area (km^2)	430.6	515.1	720.8	2,859	4,839
Temperature (^{o}C)	17–32	12–25	14–28	16–29	16–27
Rainfall (mm)	600–900	400–900	516.3	300–500	582.4
Altitude (m.a.s.l.)	1,600–2,400	1,500–3,060	1,702	500–1,600	1,530
Cattle population	131,643	76,873	-	-	-
Ethnicity	Oromo	Oromo	Oromo	Somali	Somali

Table 2. Description of the collected morphometric traits. Adapted from FAO (2012). Measurement was performed using a centimetre (cm) unit.

No.	Morphometric traits	Definitions
1	Body length	Horizontal length from the point of the shoulder to the pin bone
2	Heart girth	Measurement around the animal right behind its front legs
3	Height at withers	Height from the bottom of the front foot to the highest point of the withers
4	Pelvic width	Horizontal distance between the extreme lateral points of the hook bone (tuber coxae) of the pelvis
5	Muzzle circumference	Circumference of the mouth a little above the nostrils and around the point where the dewlap meets the chin
6	Ear length	Length of the back side of the ear from the root to the tip
7	Horn length	The longest distance from the root of the horn to its tip along the outer curvature
8	Cannon bone length	Distance from the lateral tuberculum of the os metacarpale IV to the fetlock joint

of morphometric measurements that provide maximal separations between locations was performed using the canonical discriminant function analysis (CANDISC) procedure of SAS 9.0. The scored canonical variables were used to plot pairs of canonical variables to get a visual interpretation of location differences. Pairwise squared Mahalanobis distances between locations were computed as: $D^2(i|j) = (x_i - x_j)' cov^{-1} (x_i - x_j)$. Where $D^2(i|j)$ is the distances between locations i and j, cov^{-1} is the inverse of the covariance matrix of measured variables, x_i and x_j are the means of variables in the ith and j^{th} populations.

Results

Morphological traits

The qualitative characteristics of both sexes (male and female) and both breeds (Harar and Ogaden) along with their chi-square values and levels of significance are presented in Table 3. Accordingly, sex and breed significantly ($p < 0.05$) affected the qualitative characteristics of the cattle populations. The majority of the males had straight-shaped lateral and upright horn orientation, shaded body colour pattern, large hump size located at thoracic position, and large dewlap width. On the other hand, the majority of the females had curved and forward-oriented horn, uniform body colour pattern, small hump size located at cervico-thoracic position, and medium dewlap width.

Moreover, the studied cattle breeds showed significantly different qualitative characteristics. The majority of Harar cattle had wide, curved and forward-oriented horn. They also possessed a sloppy rump with a small to medium hump located at the cervivo-thoracic position (Figure 3A). On the other hand, the Ogaden cattle possessed both narrow and wide horn spacing, straight and curved horn shapes, as well as flat and sloppy back profiles at an equivalent ratio. They also possessed an upright-oriented horn and a small hump placed at thoracic position. The majority of the Ogaden cattle also had a long tail with no naval flap (Figure 3B).

The qualitative characteristics of the cattle populations from the different locations along with their chi-square values and levels of significance are presented in Table 4. All the studied qualitative characteristics of the cattle populations were significantly ($p < 0.05$) affected by location differences. Accordingly, almost all the cattle populations from Tulo and Jarso districts possessed wide horn spacing, while this was comparably narrow in cattle from Jigjiga and Kebri Beyah districts. The horn shape of the majority of the cattle populations was curved while it was straight in cattle from Kebri Beyah district. Upright horn orientation was dominantly observed in cattle from Jigjiga and Kebri Beyah districts while it was oriented forward in cattle from Tulo and Jarso districts. The hump of the cattle populations from Jigjiga and Kebri Beyah districts was located at the thoracic position while the hump of most of the cattle populations from the other locations was located at the cervico-thoracic position. Flat-rump profile was observed in the majority of the cattle populations from Kebri Beyah district while all the cattle from Tulo, Jarso and Fedis districts had a sloppy rump. The majority of the cattle populations possessed a uniform body colour pattern, erected small hump, and straight back profile.

The body colour of the cattle populations disaggregated into male and female is presented in Figure 2A. Higher proportion of grey, red, white, and red + white body colours were observed in females than the males. On the other hand, black body colour and its mix with other colours (i.e. white + black and red + black) were observed frequently in the males.

Body colour disaggregated by cattle breeds (Harar and Ogaden) is presented in Figure 2B and representative examples shown in Figure 3. Grey body colour was observed predominantly in Ogaden cattle while several body colours were observed frequently in Harar cattle.

The body colour of the cattle populations from different locations is presented in Figure 2C. Location affected the body colour of the cattle populations significantly ($p < 0.0001$). Grey body colour was dominantly observed in Jigjiga and Kebri Beyah districts while cattle in Tulo and Jarso districts were predominantly red and the cattle population from Fedis district had white body colour followed by grey.
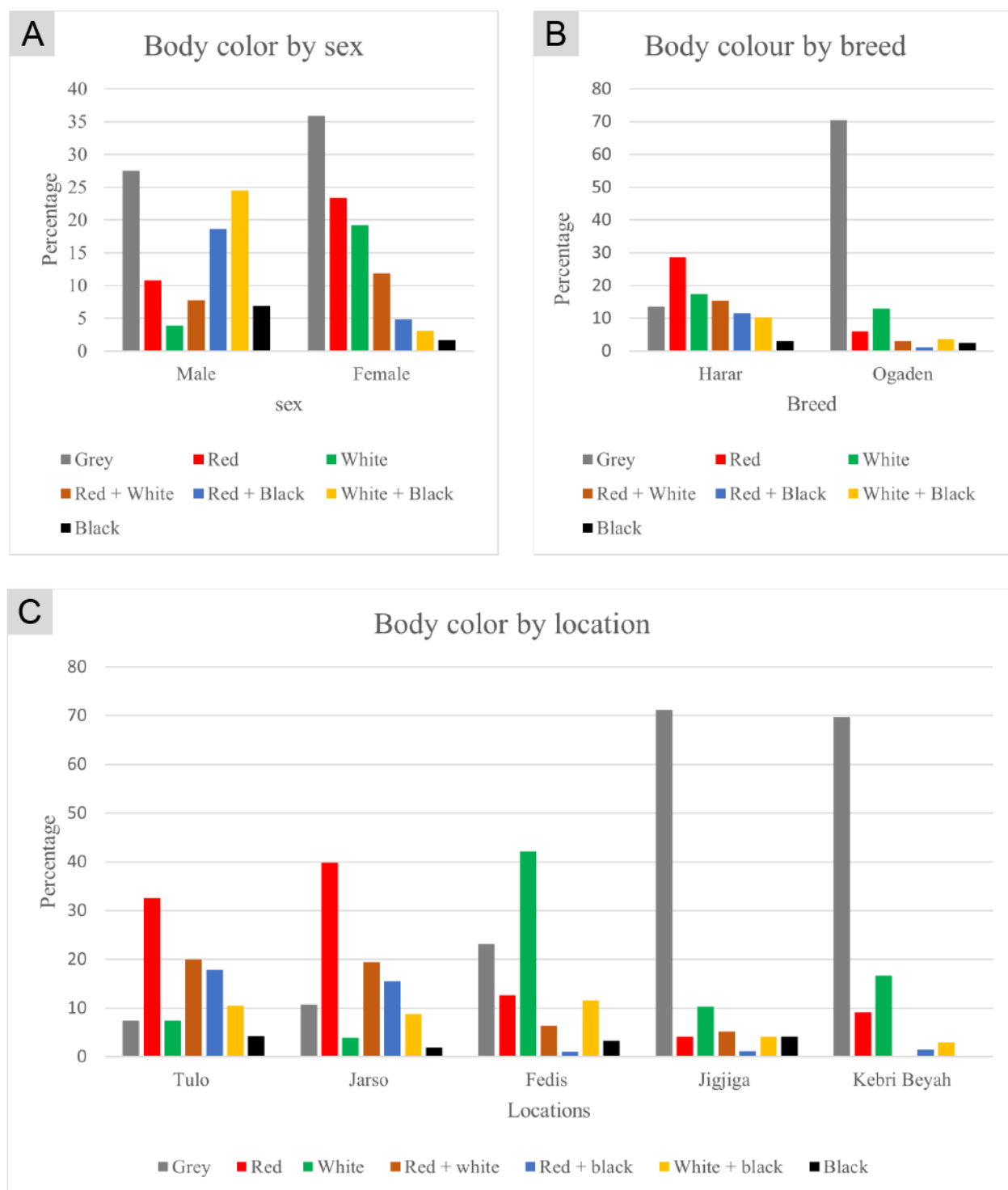
Figure 2. A: Effect of sex on body colour (chi-square value 93.2, p < 0.0001); B: Effect of breed on body colour (chi-square value 161.5, p < 0.0001); C: Effect of location on body colour (chi-square value 275.8, p < 0.0001).

Table 3. Percentages of qualitative characteristics of cattle populations by sex and breed. N, number of animals sampled; *, p < 0.05; **, p < 0.01; ***, p < 0.0001; NS: not significant.

Qualitative traits		Sex				Breed			
		Male	Female	X^2 value	P	Harar	Ogaden	X^2 value	P
N		102	354			293	163		
Horn spacing	Narrow	38.2	22.0	10.9	**	11.6	50.9	84.9	***
	Wide	61.8	78.0			88.4	49.1		
Horn shape	Straight	65.3	24.0	61.9	***	26.6	45.4	16.6	***
	Curved	34.3	76.0			73.4	54.6		
Horn orientation	Lateral	42.1	12.2	56.5	***	25.2	7.4	126.3	***
	Upright	36.3	33.6			15.7	67.5		
	Forward	19.6	44.9			50.2	19.6		
	Dropping	2.0	9.3			8.9	5.5		
Colour pattern	Uniform	26.5	71.7	78.3	**	55.0	73.6	20.3	**
	Spotty	2.0	3.7			5.1	0		
	Pied	9.8	4.8			6.1	5.5		
	Shaded	61.7	19.8			33.8	20.9		
Hump shape	Erect	86.3	100	50.1	***	95.2	100	8.0	**
	Dropping	13.7	0.0			4.8	0.0		
Hump size	Small	12.7	78.0	232.7	***	55.6	77.3	50.7	***
	Medium	35.3	21.5			35.2	5.5		
	Large	52.0	0.5			9.2	17.2		
Hump position	Thoracic	85.3	44.1	54.1	***	30.0	95.1	178.1	***
	Cervico-thoracic	14.7	55.9			70.0	4.9		
Back profile	Straight	96.1	88.1	5.5	*	89.1	91.4	0.6	NS
	Curved	3.9	11.9			10.9	8.6		
Rump profile	Flat	29.4	12.7	16.1	***	0.0	46.0	161.4	***
	Sloppy	70.6	87.3			100	54.0		
Tail length	Short	6.9	7.9	5.3	NS	10.2	3.1	31.4	***
	Medium	24.5	35.9			40.3	20.9		
	Long	68.6	56.2			49.5	76.0		
Naval flap width	Absent	-	41.0	NA	NA	30.0	61.3	44.4	***
	Small	-	42.4			45.7	36.3		
	Medium	-	12.4			17.8	2.4		
	Large	-	4.2			6.5	0.0		
Perpetual sheath	Absent	7.8	-	NA	NA	0.0	20.5	50.1	***
	Small	27.5	-			11.1	53.9		
	Medium	62.7	-			88.9	20.5		
	Large	2.0	-			0.0	5.1		
Dewlap width	Small	2.0	22.9	65.7	***	2.4	46.6	143.6	***
	Medium	43.1	59.6			63.1	43.0		
	Large	54.9	17.5			34.5	104		

Morphometric measurements

Least square means with their respective standard errors (LSM ± SE) and pairwise comparison of the morphometric measurements of both cattle breeds disaggregated by sex are presented in Table 5. Overall, males of each breed and location had higher size-related linear body measurements than their female counterparts. Both the Ogaden male and female cattle had higher measurements for most of the morphometric parameters (i.e. heart girth, height at withers, pelvic width, muzzle circumference, and canon bone length).

Some traits (horn, ear and body length) of the two breeds were sex dependent. Within the females, Harar cows had longer horns while in Ogaden cattle, males possessed longer horns. Similarly, Harar cows' body length was significantly higher than their counterparts from Ogaden while males' body length did not differ significantly. Moreover, Ogaden oxen's ear length was significantly higher than their counterparts from Harar while the value was not significantly different between the cows.

Table 4. Percentages of qualitative characteristics of cattle populations from different locations. N, number of animals sampled; *, p < 0.05; **, p < 0.01; ***, p < 0.0001; NS, not significant.

Qualitative traits		Tulo	Jarso	Fedis	Jigjiga	Kebri Beyah	X^2 value	P
N		95	103	95	97	66		
Horn spacing	Narrow	2.1	5.8	27.4	46.4	57.6	106.1	***
	Wide	97.9	94.2	72.6	53.6	42.4		
Horn shape	Straight	21.0	20.0	39.0	38.1	56.1	31.9	***
	Curved	79.0	80.0	61.0	61.9	43.9		
Horn orientation	Lateral	25.3	20.4	30.5	10.3	3.0	152.5	***
	Upright	10.5	13.6	23.2	62.9	74.2		
	Forward	61.0	56.3	32.6	20.6	18.2		
	Dropping	3.2	9.7	13.7	6.2	4.6		
Colour pattern	Uniform	50.5	46.6	68.4	74.2	72.7	33.8	**
	Spotty	5.3	5.8	4.2	0	0		
	Pied	8.4	6.8	3.2	7.2	3.0		
	Shaded	35.8	40.8	24.2	15.6	24.3		
Hump shape	Erect	89.5	97.1	99.0	100	100	24.2	***
	Dropping	10.5	2.9	1.0	0	0		
Hump size	Small	56.9	66.0	43.1	82.5	69.7	74.5	***
	Medium	34.7	22.3	49.5	4.1	7.6		
	Large	8.4	11.7	7.4	13.4	22.7		
Hump position	Thoracic	27.4	26.2	36.8	91.8	100	181.8	***
	Cervico-thoracic	72.6	73.8	63.2	8.2	0		
Back profile	Straight	93.7	97.1	75.8	86.6	98.5	34.7	***
	Curved	6.3	2.9	24.2	13.4	1.5		
Rump profile	Flat	0	0	0	38.1	57.6	172.1	***
	Sloppy	100	100	100	61.9	42.4		
Tail length	Short	12.6	4.9	13.7	3.1	3.0	40.4	***
	Medium	45.3	39.8	35.8	19.6	22.7		
	Long	42.1	55.3	50.5	77.3	74.3		
Naval flap width	Absent	23.4	32.4	34.2	70.5	45.6	62.3	***
	Small	57.1	43.3	36.7	28.2	50.0		
	Medium	16.9	16.2	20.2	1.3	4.4		
	Large	2.6	8.1	8.9	0	0		
Perpetual sheath	Absent	0	0	0	31.6	10.0	70.0	***
	Small	33.3	3.5	0	42.1	65.0		
	Medium	66.7	96.5	100	15.8	25.0		
	Large	0	0	0	10.5	0		
Dewlap width	Small	2.1	2.9	2.1	57.7	30.3	207.8	***
	Medium	76.8	70.9	41.1	29.9	62.1		
	Large	21.1	26.2	56.8	12.4	7.6		

Least square means with their respective standard errors (LSM ± SE) and pairwise comparison of the morphometric measurements disaggregated by the five locations for both sexes are presented in Table 6. Most of the morphometric measurements were significantly affected by the location of the cattle populations. Significantly higher heart girth, pelvic width, muzzle circumference and cannon bone length values were observed for the populations from Jigjiga and Kebri Beyah districts while the horn length of Tulo and Jarso cows was significantly higher than the others. The shortest horn was registered in oxen from Fedis district.

Multivariate analysis for discrimination of cattle populations

Cannon bone length, horn length and pelvic width were the three most important morphometric variables used in discriminating the cattle populations from different locations (Table 7). These results were confirmed by Wilk's lambda test where the selected variables made a highly significant (p < 0.0001) contribution in discriminating the cattle populations (Table 7).

Results of a location-wise discriminant function analysis (Table 8) show the overall classification of individual animals into their location (population). The

Table 5. Least square means (LSM ± SE) and pairwise comparisons of the morphometric measurements of both cattle breeds under both sexes. N, number of animals sampled; BL, Body length; HG, Heart girth; HW, Height at withers; PW, Pelvic width; MC, Muzzle circumference; EL, Ear length; HL, Horn length; CBL, Cannon bone length. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.0001$; NS, not significant.

Traits	Females			Males		
	Harar	Ogaden	P	Harar	Ogaden	P
N	230	124		63	39	
BL	106.1 ± 0.38	104.2 ± 0.52	**	108.6 ± 0.91	110.6 ± 1.16	NS
HG	140.0 ± 0.53	149.1 ± 0.72	***	143.8 ± 1.17	165.6 ± 1.49	***
HW	112.3 ± 0.30	113.6 ± 0.40	*	115.2 ± 0.73	120.9 ± 0.93	***
PW	35.3 ± 0.16	38.5 ± 0.22	***	33.5 ± 0.38	40.6 ± 0.48	***
MC	38.6 ± 0.13	40.0 ± 0.17	***	40.1 ± 0.30	44.6 ± 0.38	***
EL	17.9 ± 0.11	17.8 ± 0.16	NS	18.0 ± 0.19	17.4 ± 0.24	*
HL	20.8 ± 0.54	17.2 ± 0.73	***	13.1 ± 0.72	15.9 ± 0.92	*
CBL	20.6 ± 0.12	27.7 ± 0.16	***	21.0 ± 0.22	27.6 ± 0.28	***

Table 6. Least square means (LSM ± SE) in centimetre units and pairwise comparisons of the morphometric measurements of the cattle populations from different locations by sex. N, number of animals sampled; BL, Body length; HG, Heart girth; HW, Height at withers; PW, Pelvic width; MC, Muzzle circumference; EL, Ear length; HL, Horn length; CBL, Cannon bone length. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.0001$; NS, not significant.

Traits	Location					P
	Tulo	Jarso	Fedis	Jigjiga	Kebri Beyah	
Females						
N	77	74	79	78	46	
BL	106.7 ± 0.66^a	106.1 ± 0.67^{ab}	105.5 ± 0.65^{ab}	104.0 ± 0.65^b	104.5 ± 0.85^{ab}	*
HG	142.8 ± 0.90^b	139.0 ± 0.92^c	138.3 ± 0.89^c	147.9 ± 0.89^a	151.2 ± 1.16^a	***
HW	112.9 ± 0.51^{ab}	112.4 ± 0.52^{ab}	111.6 ± 0.50^b	113.4 ± 0.51^{ab}	113.9 ± 0.66^a	*
PW	35.5 ± 0.27^c	35.6 ± 0.28^c	34.7 ± 0.27^c	37.7 ± 0.69^b	39.7 ± 0.35^a	***
MC	38.9 ± 0.22^b	38.49 ± 0.22^b	38.3 ± 0.22^b	39.9 ± 0.22^a	40.1 ± 0.28^a	***
EL	18.3 ± 0.19^a	17.5 ± 0.20^{bc}	18.0 ± 0.19^{ab}	18.1 ± 0.19^{ab}	17.1 ± 0.25^c	**
HL	25.0 ± 0.86^a	21.9 ± 0.88^a	15.8 ± 0.85^b	17.8 ± 0.86^b	16.0 ± 1.12^b	***
CBL	21.1 ± 0.20^c	20.7 ± 0.20^{cd}	20.1 ± 0.20^d	28.1 ± 0.20^a	27.0 ± 0.26^b	***
Males						
N	18	29	16	19	20	
BL	107.9 ± 1.69	110.4 ± 1.33	106.1 ± 1.79	109.16 ± 1.64	111.95	NS
HG	146.9 ± 2.10^b	145.2 ± 1.66^{bc}	138.0 ± 2.23^c	163.6 ± 2.05^a	167.5 ± 1.99^a	***
HW	115.2 ± 1.34^b	116.9 ± 1.05^{ab}	112.3 ± 1.42^b	120.5 ± 1.3^a	121.3 ± 1.27^a	***
PW	33.6 ± 0.70^b	34.1 ± 0.55^b	32.3 ± 0.74^b	40.0 ± 0.68^a	41.2 ± 0.66^a	***
MC	40.8 ± 0.55^b	40.5 ± 0.43^b	38.8 ± 0.58^b	44.6 ± 0.53^a	44.6 ± 0.52^a	***
EL	18.6 ± 0.35^a	17.8 ± 0.28^{ab}	17.8 ± 0.37^{ab}	17.8 ± 0.34^{ab}	16.9 ± 0.33^b	*
HL	16.1 ± 1.19^a	14.6 ± 0.93^a	6.9 ± 1.26^b	16.2 ± 1.15^a	15.7 ± 1.12^a	***
CBL	21.7 ± 0.39^c	20.8 ± 0.30^c	20.4 ± 0.41^c	28.6 ± 0.38^a	26.7 ± 0.37^b	***

Table 7. Order of traits used in discriminating the cattle populations from different locations.

Step	Variables entered	Partial R-Square	F value	Pr > F	Wilks' Lambda	Pr < Lambda
1	Cannon bone length	0.7927	431.02	< 0.0001	0.2073	< 0.0001
2	Horn length	0.1445	19.00	< 0.0001	0.1773	< 0.0001
3	Pelvic width	0.1495	19.73	< 0.0001	0.1508	< 0.0001
4	Body length	0.1080	13.56	< 0.0001	0.1345	< 0.0001
5	Ear length	0.0884	10.83	< 0.0001	0.1226	< 0.0001
6	Heart girth	0.0436	5.08	0.0005	0.1173	< 0.0001
7	Height at withers	0.0362	4.18	0.0025	0.1130	< 0.0001
8	Muzzle circumference	0.0159	1.79	0.1290	0.1112	< 0.0001

Figure 3. A: Representative Harar cattle bull; B: Representative Ogaden cattle cows. Photos: Amine Mustefa

Figure 4. Location-wise (A) and breed-wise (B) plot of canonical discriminant analysis. Districts in A) are indicated by numbers: 1, Tulo; 2, Jarso; 3, Fedis; 4, Jigjiga; 5, Kebri Beyah. Breeds in B) are indicated by Ha, Harar and Og, Ogaden.

overall analysis shows medium (61%) classification of individuals into their corresponding location with an error rate of 39%. Furthermore, the highest (69.7%) classification of individuals into their location was observed in Kebri Beyah district while the lowest (45.6%) classifications were recorded in Jarso district.

Results of a breed-wise discriminant function analysis (Table 9) show the overall classification of individual animals into their breed. The overall analysis shows high (99%) classification of individuals into their corresponding breed with a small error rate of 1%. Furthermore, a higher (99.39%) classification of individuals into their breed was observed in Ogaden cattle than Harar cattle (98.63%).

Location-wise pairwise squared Mahalanobis distances are presented in Table 10. The distances were highly significant (p < 0.0001). The shortest distance (0.77) was obtained between Tulo and Jarso populations, while Fedis and Jigjiga populations were most distantly related (27.34). The breed-wise analysis also showed long Mahalanobis distance between the two breeds (22.15, p < 0.0001).

Location wise, multivariate statistics outputs showed the significance of Can 1 due to its high eigenvalue (5.3) and proportion (93%) to discriminate the cattle populations from the different locations. Can 2–Can 4 combined had only 7% proportion in discriminating the cattle populations with significantly low eigenvalue (0.37 combined). Similarly, Can 1 significantly discriminated the breeds during the breed-wise analysis with high eigenvalue (5.1) and 100% proportion. Therefore, the outputs of Can 1 are indicative and significant. Location-wise and breed-wise plot of the first two canonical vari-
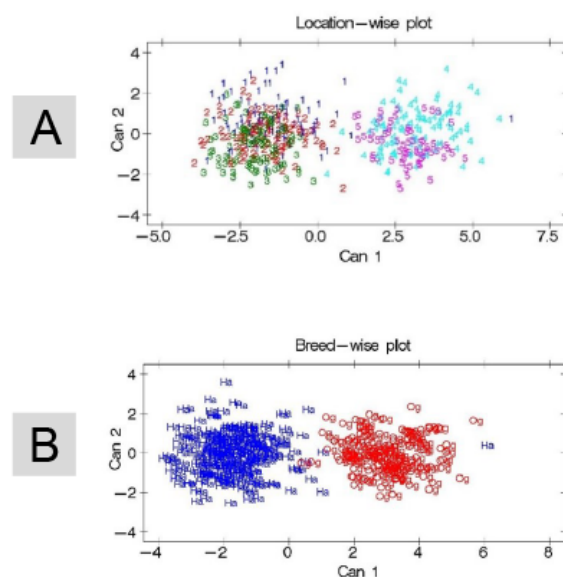
ables to discriminate the cattle populations is presented in Figure 4. The cattle populations from Tulo, Jarso and Fedis districts were inseparable and in the same group; similarly, the populations from Jigjiga and Kebri Beyah districts were also inseparable and placed in the same group. However, those two groups were clearly separated from each other.

Discussion

Qualitative morphological traits can help to easily differentiate breeds. The observed qualitative characteristic similarities among the cattle populations from Tulo, Jarso and Fedis districts support the presence of a unique cattle breed (the Harar cattle) in East and West Hararghe zones of Oromia region. This is also backed by the report of Rege and Tawa (1999), which stated the East and West Hararghe zones of Oromia region as the breeding tract of Harar cattle. Similarly, morphological similarities shared between the cattle populations from Jigjiga and Kebri Beyah districts and their variation from the previous group were also reported by Getachew et al (2014) who characterized them as Ogaden cattle. These qualitative results confirm the presence of two cattle breeds (Harar and Ogaden) in the eastern part of the country. In line with different publications including Mustefa et al (2021) on Raya cattle and Terefe et al (2015) on Mursi cattle, the results of the current study also confirm the existence of within-breed variations besides the between-breed differences.

The Ogaden cattle possess a relatively unique body colour compared to the Harar cattle, which has multiple body colours. The presence of multicolour (red, red +

Table 8. Number (and percent) of observations classified into locations.

From district	Tulo	Jarso	Fedis	Jigjiga	Kebri Beyah	Total
Tulo	**54 (56.84)**	27 (28.42)	12 (12.63)	1 (1.05)	1 (1.05)	95 (100)
Jarso	28 (27.18)	**47 (45.63)**	27 (26.21)	0	1 (0.97)	103 (100)
Fedis	15 (15.79)	17 (17.89)	**63 (66.32)**	0	0	95 (100)
Jigjiga	0	0	1 (1.03)	**64 (65.98)**	32 (32.99)	97 (100)
Kebri Beyah	0	0	0	20 (30.30)	**46 (69.70)**	66 (100)
Error rate	0.4316	0.5437	0.3368	0.3402	0.3030	0.3911
Priors	0.2	0.2	0.2	0.2	0.2	

Table 9. Number (and percent) of observations classified bybreed.

From breed	Harar	Ogaden	Total
Harar	289 (98.63)	4 (1.37)	293 (100)
Ogaden	1 (0.61)	162 (99.39)	163 (100)
Error rate	0.0137	0.0061	0.0099
Priors	0.5	0.5	

Table 10. Pairwise squared Mahalanobis distances between locations. ***, p < 0.0001

From District	Tulo	Jarso	Fedis	Jigjiga	Kebri Beyah
Tulo	0	-	-	-	-
Jarso	0.77 ***	0	-	-	-
Fedis	2.09 ***	0.87 ***	0	-	-
Jigjiga	22.17 ***	24.13 ***	27.34 ***	0	-
Kebri Beyah	20.23 ***	21.20 ***	24.47 ***	1.80 ***	0

white, red + black, white + black, white, and grey) cattle in Harar might be due to the relatively highland-dominated areas of its distribution, especially the Tulo and Jarso districts. The frequently observed grey body in Ogaden cattle was due to the agropastoralists' preferences and selection of criteria for that particular coat colour (Getachew *et al*, 2014). Getachew *et al* (2014) also related the uniformly patterned grey body colour with their adaptation mechanism to the arid and semi-arid agroecologies of the Ogaden rangelands. This was in line with the report of Titto *et al* (2016), who reported animals with light coat colouring absorb less heat than those with darker coats.

Alongside the most observable qualitative characteristics, morphometric measurements can also produce more reliable information in characterizing and differentiating cattle breeds. The above grouping made by the qualitative characteristics of the cattle populations was also supported by the results of the quantitative measurements. Significantly higher values of heart girth, pelvic width, muzzle circumference and cannon bone length with shorter horns were observed in the Ogaden cattle (Jigjiga and Kebri Beyah districts) than in the Harar cattle.

Like for the qualitative results, within-breed variations were also observed in the morphometric measurements. Within Ogaden cattle, values of heart girth and pelvic width measurements were significantly higher for the population from Kebri Beyah district than Jigjiga district. These results made the population from Jigjiga dis-

trict relatively closer to the Harar cattle. This might be due to the presence of mid- and high-altitude areas of Jigjiga district compared to the Kebri Beyah district, as well as the closeness in ground distance of the Jigjiga district to the distribution areas of Harar cattle.

In comparison to other Ethiopian indigenous cattle breeds, the morphometric measurements of both Harar and Ogaden breeds were found to be significantly lower than some lowland cattle breed like Begait cattle (Mulugeta, 2015). Similarly, body length, height at withers, ear length and horn length measurements of both breeds from the current study were lower than those of Raya cattle, while the reverse was true for heart girth and pelvic width measurements (Mustefa *et al*, 2021). Compared to the adjacent Afar cattle, the Ogaden cattle had higher values for height at withers and hearth girth while the Afar cattle had a longer body than both Harar and Ogaden cattle breeds (Tadesse *et al*, 2008).

The observed higher size-related linear body measurements of the males in each breed and location follow Rensch's rule (Rensch, 1950), which states that males are usually larger than females. Such differences between males and females might be due to the testosterone secreted in males which causes the growth of muscle mass and skeletal development (Baneh and Hafezian, 2009). Estrogen secreted in females has a limited effect on growth (Chriha and Ghadri, 2001; Baneh and Hafezian, 2009). The current results were comparable with the reports of Mustefa *et al* (2021) on Raya cattle, Terefe *et al* (2015) on Mursi cattle, and Genzebu

et al (2012) on Arado cattle. Some size-linked morphological parameters (i.e. hump size and dewlap width) were also larger for males than females, as these traits are associated with the overall size of the cattle.

The morphometric variables, which discriminated the cattle populations, were ranked according to their importance. The inclusion of horn length within the top-three discriminatory variables is comparable with the reports of Mustefa *et al* (2021), who also classified it among the top-three variables to discriminate Raya cattle from other highland cattle breeds.

The high error rate of the discriminant function analysis among the different districts shows a lack of uniqueness within each location. On the contrary, some similarities were shared among locations. The cattle populations from Tulo, Jarso and Fedis districts shared similarities justifying their belonging to the same group (the Harar cattle group). Similarly, the cattle populations from Jigjiga and Kebri Beyah districts shared some similarities, which support their categorization into the same group (the Ogaden cattle group). This confirms the idea of previous studies which state the presence of Harar and Ogaden cattle breeds in the eastern part of Ethiopia (Rege and Tawa, 1999; Getachew *et al*, 2014; Mengesha, 2019). This grouping was also supported by the morphometric and morphological results of the current study.

The pairwise squared distance results between locations confirmed the already known differences between Harar and Ogaden cattle breeds, supporting the morphological, morphometric and multivariate results. However, these distances showed only the relative size differences between each population. Such differences might not necessarily be due to genetic differences (Zechner *et al*, 2001; Mustefa *et al*, 2021, 2022). Therefore, further diversity studies using molecular techniques are recommended to understand the level of genetic diversity within and between each breed.

In conclusion, two cattle breeds in Eastern Ethiopia listed under the FAO Domestic Animal Diversity Information System (DAD-IS), were phenotypically characterized to obtain and quantify the within- and among-breed diversity. Strong within-breed similarities and large between-breed differences (distance) were observed. Thus, the current study confirmed the presence of two cattle breeds (the Harar cattle and the Ogaden cattle) in Eastern Ethiopia. Besides breed differentiation, this study will be used to design conservation and genetic improvement programmes for each breed.

Data availability

The data that has been used is confidential.

Acknowledgments

Conflict of interest statement

The authors declare that they have no conflict of interest.

Author contributions

All authors contributed to the study conception and design. Material preparation and data collection were performed by Amine Mustefa, Tesfalem Aseged, Seble Sinkie, Fasil Getachew, Tesfu Fekensa, and Manaye Misganaw. Amine Mustefa performed the data analysis and wrote the first draft of the manuscript. All authors commented on previous versions of the manuscript, and read and approved the final manuscript.

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Genetic Resources

SHORT COMMUNICATIONS

Wild relatives of fruit trees in Syria: Genetic resources threatened by conflict

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Abstract: Wild relatives of fruit trees (WRFT) are highly valued for food and tradable products by rural communities, especially in low-income countries and as such are a vital resource for tree improvement. During periods of conflict, a lack of support and protection by national authorities may make WRFT vulnerable. In Syria, WRFT are at risk of extinction due to the ongoing crisis, which has limited efforts to conserve and propagate these unique genetic resources. We collected information about the current status and key threats to WRFT in northwest Syria from 50 agricultural experts using structured interviews. Our results show that many sites have experienced erosion, overgrazing and drought. To initiate *in situ* and *ex situ* conservation, the locations of WRFT exposed to deterioration were identified and mapped using GPS, and seeds from five genotypes per species were collected from each of ten species to be used for the establishment of an *in situ* and *ex situ* WRFT collection.

Keywords: Conservation of wild fruit plants, In situ conservation, Plant Genetic Resources, Genetic Erosion, Crop wild relatives

Introduction

Ensuring future global food security is one of the biggest challenges facing humans today. One in nine people worldwide already suffers from poor access to food resources (FAO, IFAD and WFP, 2015), and with predicted human population growth, pressure on food resources is likely to increase dramatically (Godfray *et al*, 2010; UN DESA, 2015). New high-yielding varieties of crops that can withstand adverse climatic conditions and new pests and diseases are urgently needed (McCouch *et al*, 2013). All cultivated crops derive from historic human domestication of wild genotypes, which have been gradually selected to produce commercial varieties (Engels and Thormann, 2020). Crop wild relatives are the wild original sources of these modern cultivars and remain the primary resource of genetic diversity for breeding new, climate-change tolerant, and higher-yielding cultivars (Maxted and Kell, 2009; Vincent *et al*, 2019). As such, it is vital to ensure wild relatives are identified, protected and propagated to help secure the future of the species we depend on.

Globally, fruit trees are of enormous economic importance. In many places (Erçişli *et al*, 2009; Otieno, 2017), the wild relatives of fruit trees (WRFT) remain essential sources of food and medicines (Symphorien *et al*, 2016) but also provide feed for domestic animals and food additives. They also play a crucial

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role in maintaining ecological diversity (Musayev and Huseynova, 2016). Notably, some of these wild relatives are known to possess traits that could greatly benefit contemporary agriculture through the breeding of new, improved cultivars. For example, wild genotypes of species in the genus Prunus are characterized by adaptation to extreme climatic conditions (Uğur and Gündeşli, 2020). Moreover, wild fruit tree genotypes are an essential source of resilient rootstock, as grafting is one of the key methods for making new fruit varieties available to growers (Zhebentyayeva *et al*, 2019). Several wild edible fruits are used by rural and tribal populations and significantly contribute to their livelihood. The use of non-cultivated foods, of which wild fruits form a part, as a dietary supplement or as a coping mechanism in times of food shortage, provides an important safety net for the rural poor, especially in low-income countries (Ercişli and Sagbas, 2017).

The term underutilized and neglected species refers to a category of wild and cultivated plant species characterized by a low level of investment in research and development, and a recognized but untapped livelihood potential (Eyzaguirre *et al*, 1998). Widespread genetic erosion of these species has been recorded worldwide: for example, in Nepal, more than half of the traditional landraces have become absent from the farmer's fields (Paudel *et al*, 2016). Generally, despite several initiatives in recent years aimed at the conservation of such genetic resources, these wild relatives are still at risk of erosion or extinction (Pilling *et al*, 2020). They have been almost entirely neglected in *ex situ* genebanks (Castañeda-Álvarez *et al*, 2016), although some efforts to change this have begun (Dempewolf *et al*, 2013). In addition, current *in situ* reserves often do not meet the required management standards to maintain the wild relatives and their genetic diversity for long-term use (Iriondo *et al*, 2012; Weibull and Phillips, 2020).

Syria lies within a region of substantial topographic and climatic diversity in the Fertile Crescent, which is the centre of origin and domestication of many globally important crops. Syria is rich in agrobiodiversity (food, forage crops and fruit trees) like wheat, barley, lentils, chickpeas, vetch, olives, almonds, pear, plums, medic, clover, as well as other ornamental, medicinal and aromatic plants. Such plants are the main stocks and heritage for farming in Syria and are widely used elsewhere in the world. According to the *Fourth National Report on Biodiversity in Syria* (MSEA, 2009), the reduction in plant genetic resources was caused by urbanization, climate change, decreasing rainfall and increasing temperatures in dry seasons, which the region has experienced in the last seven years. An increasing incidence of wildfires also poses a particular threat.

During the conflict period from 2012 to 2019, Syria lost 20.4% of its tree cover (Gaafar, 2021), much of it in Idlib and Lattakia governorates. The depletion of wild tree populations has been associated with several factors related to the conflict, including

intense reliance on trees for heating and shelter, frequent fires in wild forests, charcoal production, illegal logging, agricultural expansion, and the weakness of state institutions in protecting natural resources and in managing environmental development. The losses of wild tree species include the cherry *Prunus mahaleb*, oak trees, wild pines and hundreds of other plant (tree and crop) species are also threatened.

Unfortunately, there is no data available about the current situation of the WRFT, the only available data were published by FAO (1996), which clearly mentioned the deterioration of plant genetic resources in Syria started before the crisis. It can be assumed that the deterioration of WRFT has increased following the ongoing crisis in Syria resulting from the absence of any control for the protection of these genotypes against the deterioration. Moreover, there was clear evidence of plant genetic resources decline in northwest (NW) Syria based on the finding of previous research about crop landraces in the same locations in NW Syria (AlDarvish *et al*, 2022) The primary causes were the spread of cultivation to new areas, the need to grow economic crops, particularly cereals, forages and food legumes, and accidental forest fires (FAO, 1996; AlDarvish *et al*, 2022). Hence the WRFT were already facing extinction before the Syrian crisis began. Since then, the absence of research authorities responsible for the conservation and propagation of genetic resources has severely exacerbated the problem. Currently, no conservation activity is occurring as the conflict is preventing any access to the field by Syrian botanists, in particular in mountain areas where the threat from mines and bombs is severe (Valderrabano *et al*, 2018).

As a first step towards addressing the lack of activity on these vital global resources, this study aimed to: i) collect information about the current status and critical threats to ten species of WRFT in NW Syria, ii) characterize and map the locations of WRFT exposed to deterioration, and iii) collect seed from multiple genotypes of each species to be used for the establishment of an *in situ* and *ex situ* WRFT collection.

Materials and methods

Data collection and analysis

The National Strategy for Conservation and Management of Plant Genetic Resources for Food and Agriculture 2015–2035 (FAO, 2015) directs national actions for WRFT in Syria, and our methods were designed to address these goals using guidelines recommended by Valderrabano *et al* (2018). The study was conducted between September 2021 and February 2022 in Idleb Governorate (Ehsem, Mhambal, Jisr-Ash-Shugur, Badama, Darkosh, Janudiyeh, Harim, Dana, Kafr Takharim, Qourqeena, Armanaz subdistricts). This region is located within Syria's second and third agroecological zones, incorporating mountains and plains. Agroecological zone 2 covers 2,473,000 ha (13.4% of the country) with an annual rainfall of 250 to 350mm

and no less than 250mm across two-thirds of the monitored years. Agroecological zone 3 comprises 1,306,000 ha (7.1% of the country) with an annual rainfall of 250 to 350mm and no less than 250mm over half of the monitored years (FAO, 2003). These agroecological zones are key factors affecting the spread of the WRFT, which is key information when it comes to the planning for subsequent studies on their propagation and preservation. It is worth noting that this region is currently exposed to climatic changes, especially drought, based on the amount of seasonal rain mentioned in the description of this region. To identify the location of WRFT exposed to deterioration after the conflict, the researchers conducted focus group discussions with community representatives in each subdistrict to explain the aim of the study, its methods and objectives (one in each of 11 subdistricts under study during the period from 25 September 2021 to 25 October 2021). These representatives nominated 50 key informant candidates, such as agricultural engineers, senior farmers and agricultural researchers, which had considerable knowledge of the agriculture sector in their region and were targeted for data collection via interviews and a multiple-choice questionnaire (Supplemental data). The questionnaire was designed to gather local knowledge on the location, usage, reasons for decline and appropriate conservation strategies for WRFT.

Due to the outbreak of COVID-19 in NW Syria during data collection, 38 of the targeted informants elected to participate via online interviews, while the remaining 12 agreed to in-person interviews. It's worth mentioning that the quality of online interviews was the same as in-person data collection.

Data collection started on 25 September 2021 and was completed by 25 October 2021. The data collected from the questionnaires were transferred to MS Excel (Microsoft Office 2020) prior to analysis. Data cleaning was undertaken to ensure the accuracy and integrity of the dataset. This involved identifying and rectifying any inconsistencies, outliers or missing values that could potentially skew the results. Variability and dispersion within the dataset were calculated with Excel formulas, including but not limited to measures such as standard deviation, to extract valuable insights.

Seed collection and storage

The main criterion to decide whether the collected seeds were WRFT or not, was their natural spread sites. More specifically, the collected WRFT were collected from wild and forest locations. For species with similar phenotypes and for which phenotype is not sufficiently reliable to distinguish between genotypes, for example, *Ficus carica*, *Olea europaea* and *Vitis vinifera*, the seeds were collected from wild mountainous sites far away from agricultural areas, and according to the interviewees and field team observations. The collected WRFT existed in these wild sites for a long time, and their morphological growth differs from the cultivated species and landraces

of the same species (especially the shape of leaves, fruits and trees).

WRFT fully ripe fruits were collected during the harvesting season and the fruit flesh (pulp) was removed. The extracted seeds were then spread out on paper sheets and dried in the open air for 30 days, with the seeds flipped daily to make sure they were completely dry. Then they were disinfected using thiram fungicide, placed in a paper bag labelled with the species name, location, date and storage data, and stored in airtight plastic barrels with dry silica gel for future studies associated with the propagation and establishment of local WRFT *in situ* conservation sites and *ex situ* collections. This conservation method was selected due to the ongoing war in Syria, where electricity is not available for preserving seeds under refrigerated conditions, based on the recommendation of the Millennium Seed Bank staff provided to the research staff through their visit to the Millennium Seed Bank in July 2019 as an alternative method for seed conservation. Moreover, Ashok *et al* (2017) showed that this method of conservation could help to conserve seeds for several years as recommended by Yoshinaga (2010).

For each of the collected accessions, 100 seeds were weighed for each genotype, and the value was multiplied by 10 and expressed as g/1,000 seeds; average values per species, with standard deviation, were calculated.

Results

Focus group discussions conducted with community representatives and experts in the agricultural sector in the targeted subdistricts showed that several WRFT locations had been subject to neglect after the Syrian crisis and had experienced significant deterioration (Figure 1).

Data from the 50 interviews showed that WRFT were found in the following four types of locations within the study area: forests, verges along agricultural roads, rocky and neglected parts of farms, and working farms. The percentage of responses among the 50 participants for the distribution of WRFT in study locations is illustrated in Figure 2.

The interviews revealed that the following ten WRFT species are experiencing erosion: *Prunus mahaleb*, *Prunus orientalis*, *Vitis vinifera*, *Rhus coriaria*, *Olea europaea*, *Crataegus azarolus*, *Ficus carica*, *Pistacia atlantica*, *Prunus spinosa*, and *Pyrus syriaca*.

A range of reasons was reported for WRFT deterioration (Figure 3). The primary causes were overgrazing, frequent droughts and climate change. The predominant conflict-related factors reported were neglect by local authorities, lack of awareness of the importance and value of WRFT and deforestation resulting from the collection of wood for fuel and conversion to arable lands. Several respondents mentioned the lack of interest by the younger generation in making use of local wild fruit landraces, overuse (massive unmanaged fruit gathering), desertification and expansion of building into for-
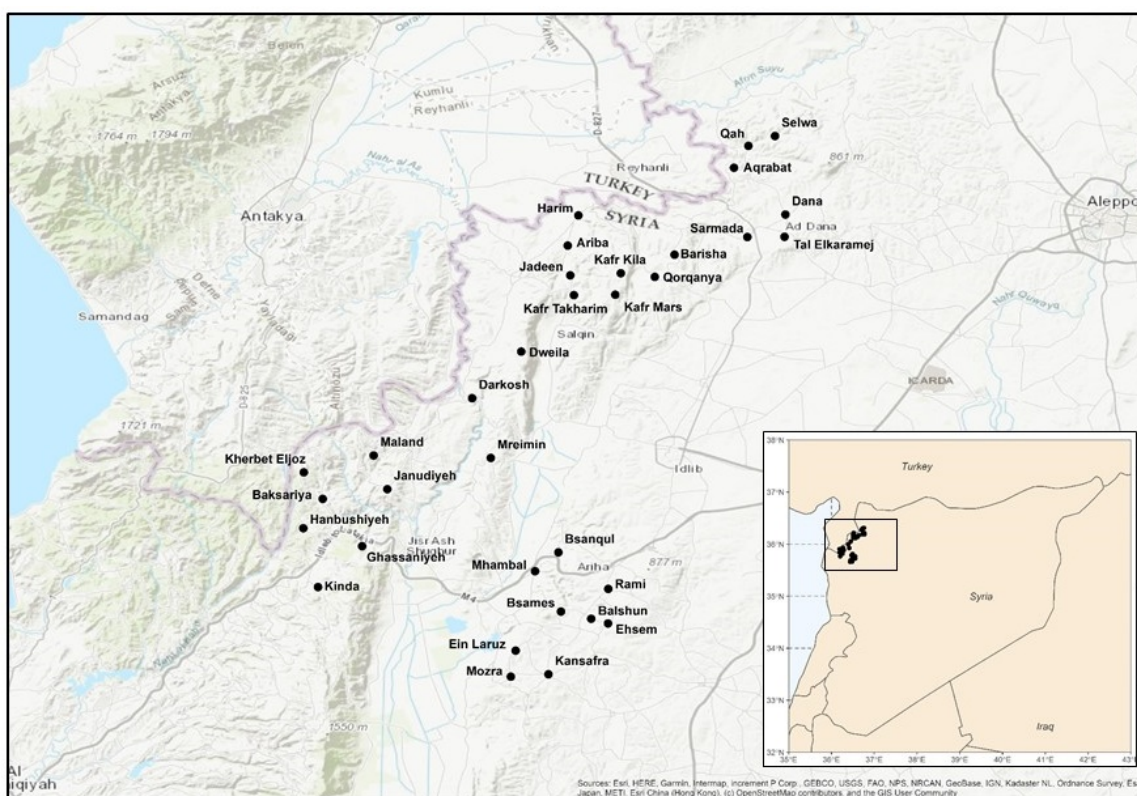
Figure 1. Study area for identification of wild relatives of fruit trees (WRFT) exposed to deterioration. Source: Humanitarian Data Exchange
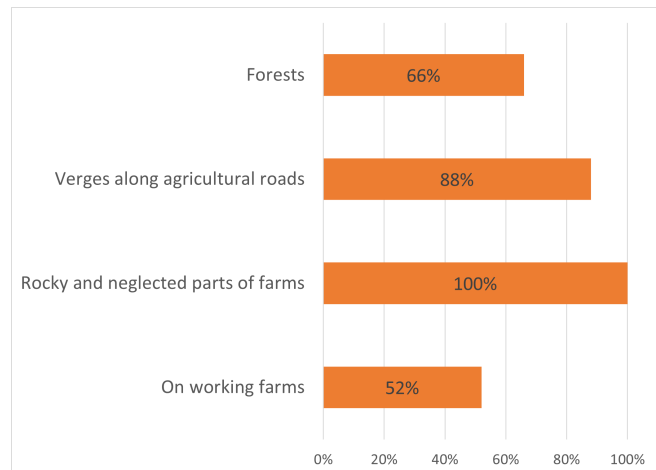

Figure 2. Proportion of respondents reporting types of locations in which wild relatives of fruit trees (WRFT) are found.

est locations as contributing factors. Floods were not considered a threat by any of the respondents. Based on the assessment result with the interviewees, the reasons for erosions of WRFT are equal within all areas of the study.

With respect to the impact of the ongoing conflict in NW Syria on WRFT, all participants stated that the Syrian armed conflict had intensified the deterioration due to the lack of government control leading to overgrazing and excessive cutting of trees in these locations.

When questioned about conservation actions that were considered to be the most important for the protection of WRFT against the current deterioration, participants responses showed the following answers: 36% suggested exchanging seeds of WRFT with other farmers, 26% supported the establishment of local collections for multiplication of these species, 22% considered public awareness campaigns and 14% favoured seed collection and conservation of these species in local genebanks. Only 2% considered awareness campaigns to be the most important action for the protection and preservation of these species.

Regarding the availability of seeds or cuttings of the WRFT species, 56% of the participants stated that seeds or cuttings of these species were not available, whereas 44% stated that they have access to seeds and cuttings. This response suggests that some WRFT are currently at high risk of decline and may even suffer extinction over time.

Collection of WRFT genotypes

The results of the interviews and questionnaire allowed the identification of locations where WRFT had been exposed to deterioration and needed conservation. Based on available resources, the researchers collected mature seeds of each WRFT species from these locations as a first research and conservation step.

For each of the priority WRFT species identified, five genotypes were collected from areas facing particularly severe deterioration There were slight variations in seed
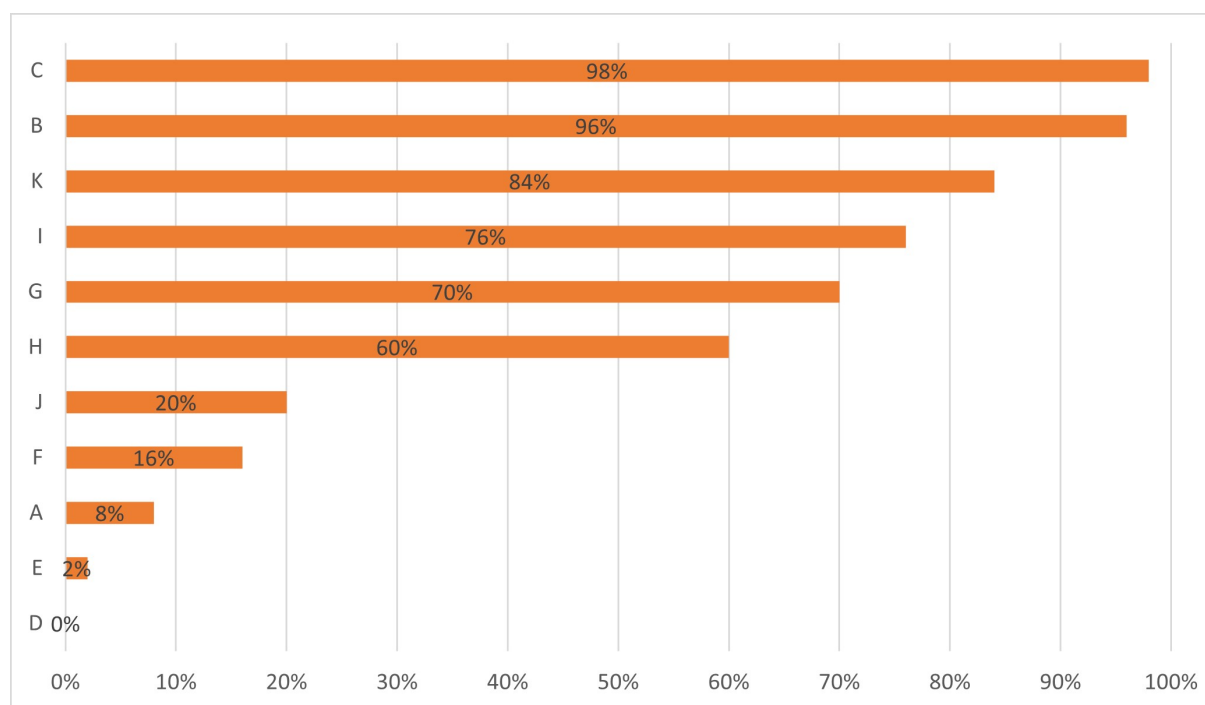
Figure 3. Reasons given, and percentage of respondents reporting, for deterioration of wild relatives of fruit trees (WRFT). From top to bottom – C: Overgrazing; B: Frequent droughts and climatic changes; K: Consequences of Syrian crisis (lack of law enforcement); I: Lack of knowledge about the importance and value of these species; G: Deforestation, including collection of wood for fuel; H: Neglecting by local authorities; J: Interest of the younger generation for local wild fruit landraces is very low; F: Overuse (massive unmanaged gathering); A: Spread of cultivation to new areas and need to grow economic crops; E: Desertification and expanding of buildings towards forest locations and arable lands; D: Floods.

weight among the genotypes within species, and the species averages are given in Table 1.

As seed weight can influence the speed and percentage of germination and can be particularly important in challenging environments (Upadhaya *et al*, 2007), and since maternal effects should be accounted for in any subsequent comparative testing of plants derived from the collection, seed weights for each genotype were taken. The primary purpose of presenting the weight of seeds was to know the standard deviation for the seed weight within different genotypes collected from the same species. This can be investigated in future studies (genetic fingerprinting for these genotypes) to distinguish between phenotype and genotype within the same species and to identify if these variations in seed weight result from environmental factors or are related to genetic features

Discussion

Future food security for humankind is likely to rely heavily on the sustainable availability of a wide range of plant genetic resources for food, agriculture and nutrition, amongst other needs. WRFT are highly valued genetic resources for food production and to support the livelihoods of rural populations, especially in low-income countries. WRFT in Syria are threatened by extinction due to conflict, which has led to increased direct pressure on the tree populations themselves, and to an absence of research authorities working in the field

Table 1. Average seed weights (g/1,000 seeds) for each wild relative of fruit trees (WRFT) species collected.

WRFT Species	Average seed weight (g/1,000 seed)	Standard deviation
Ficus carica	14.82	0.38
Olea europaea	720.00	144.05
Prunus spinosa	309.00	20.74
Vitis vinifera	100.00	7.91
Prunus orientalis	506.00	20.74
Pistacia atlantica	141.00	20.43
Rhus coriaria	92.00	9.08
Prunus mahaleb	180.00	16.96
Crataegus azarolus	400.00	15.81
Pyrus syriaca	165.20	2.83

of conservation and propagation of genetic resources. Consequently, there is a high risk that this vital resource may be lost to future research, and its fruits to future generations.

Our results parallel those of previous studies, which have identified that plant genetic resources in Syria are particularly exposed to deterioration arising from the ongoing conflict (Gaafar, 2021; AlDarvish *et al*, 2022). However, similar threats to wild plant genetic diversity have also been reported worldwide (Iriondo *et al*, 2012; Castañeda-Álvarez *et al*, 2016; Paudel *et al*, 2016).

Prior to this study, the most recent available information on the current status of WRFT dates from 11

years ago (FAO, 1996) before the start of the conflict. At that time in the study area, the key locations for WRFT were rich in wild genotypes because they were protected by government authorities such as the Ministry of Agriculture and the Directorates of Environmental Protection. As reported by our interviewees, the absence of state protection has resulted in increased deterioration of WRFT populations in these locations, as exploitation such as grazing or cutting of fuelwood for heating is now uncontrolled (Gaafar, 2021). This neglect and lack of protection for WRFT by state institutions has and continues to exacerbate ongoing pressure from climate change and is resulting in severe genetic erosion of these species. As government bodies have not been present in the study area for over a decade, the condition of WRFT has continued to deteriorate, as reported by Valderrabano *et al* (2018). Moreover, all interviewees stated that the weakness of current state institutions, which demonstrate little interest in wild fruit trees, has led to increased genetic erosion and deterioration of these genotypes in NW Syria.

WRFT are highly valued for use directly as food resources and as commodities that contribute to livelihoods, especially in rural populations in low-income countries. They are also an essential source of genetic diversity for breeding new, higher-yielding, climate change-tolerant varieties. Despite their high value for food and agriculture (Maxted and Kell, 2009; Musayev and Huseynova, 2016; Symphorien *et al*, 2016; Erçişli and Sagbas, 2017; Vincent *et al*, 2019; Zhebentyayeva *et al*, 2019; Engels and Thormann, 2020; Uğur and Gündeşli, 2020), WRFT have been almost entirely neglected in both *ex situ* and *in situ* conservation programmes. As they are likely to be locally adapted and genetically distinctive, WRFT can provide vital novel variation for domesticated fruit species to help meet the challenge of increasingly stressful environments and climatic changes. For example, they have been used by plant breeders to develop more efficient nutrient uptake and utilization, and to integrate genes for adaptation to stressful environments such as water stress, salinity and high temperatures (Ayenan *et al*, 2019; Mishra *et al*, 2022). As for WRFT, Spiegel-Roy (1986) stated that wild relatives of fruit trees are one of the essential resources for fruit tree breeding, especially for disease tolerance, rootstocks and genetic engineering.

To secure the future of these vital resources, conservation protection efforts are urgently needed for the degraded areas they occupy. The priority species we have identified need to be included in plantation programmes to secure the genetic diversity that will be needed for the improvement of commercially cultivated crops. Countries of the Near East and the Mediterranean basin have perhaps the longest tradition in fruit cultivation for historical, environmental and evolutionary reasons (Barone and Caruso, 1998; Wolf *et al*, 2000; Khoury *et al*, 2017; Migicovsky and Myles, 2017; Bissessur *et al*, 2019).

This study represents the first step in highlighting the extremely threatened status of these genotypes. Our initiative to obtain accurately mapped locations for these genetic resources can underpin future research efforts towards *ex situ* and *in situ* conservation programmes. Furthermore, the seed we have collected will form the start of a local genebank collection for future studies of genetic variation and propagation, which can help to ensure resilient and sustainable agricultural development for future generations in Syria and worldwide.

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Supplemental Data

Interview questionnaire

Author contributions

Munzer Aldarvish coordinated the research and contributed to the research design and manuscript; Anas Al Kaddour, Akram Bourgol, and Yasser Ramazan contributed to the research design and manuscript and undertook the data analyses, Yousef Hallak contributed to the research design and carried out the field data collection; Stephen Cavers and Joan Cottrell provided academic guidance and support throughout the research process and contributed to the manuscript.

Conflict of interest statement

The authors of this manuscript have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript, and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

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