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Chevalier barley: The influence of a world-leading malting variety

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Abstract

During the 19th century, ‘Chevalier’, said to have been developed from a single plant found in 1820, was the world-leading malting barley (*Hordeum vulgare*). The superior malting quality of Chevalier led to its world-wide spread at the time of the development of the malting industry. In this study, we investigate how this cultivar was spread and adopted to Nordic seed systems of the time. Single nucleotide polymorphism genotyping of up to 155-yr-old museum specimens of historical grains labelled “Chevalier” and of Chevalier accessions preserved in genebanks, in total 282 individuals representing 47 accessions, allowed us to divide the accessions into four categories: True Chevalier, seed mixtures, crosses, and non-Chevaliers. Comparisons with previously genotyped Nordic landraces showed how, in the 19th century, Chevalier seed was mixed with locally produced landrace seed and cultivated together. We suggest that spontaneous outbreeding events gave rise to hybrids which were subsequently selected and propagated when resulting in superior genetic combinations. Such farmer-driven breeding activities would have preceded modern plant breeding but resembled the breeding principles that were later used, even though the scientific understanding of inheritance was not yet known.

1 | INTRODUCTION

The breakthrough that is modern plant breeding is often said to have followed the rediscovery of the Mendelian laws of inheritance in 1900 and Wilhelm Johannsens pure line theory a few years later (Roll-Hansen, 2000). The extent that this was actually a turning point or only a small step in a longer, continuous process of empirically derived breeding knowl-

edge has been debated (e.g., Harwood, 2015; Palladino, 1994; Wieland, 2006). It is clear that efforts to produce better seed stock for the improvement of agricultural production had been pursued for most of the 19th century. These activities were usually based on farm owners’ observations of off-types in their cereal fields and the subsequent multiplication and distribution of these plants (Reitemeier, 1905). One of the best-known examples of such mass selection is the development of ‘Chevalier’ (or ‘Chevallier’) barley (*Hordeum vulgare*) in Great Britain, which spread from a single farm and reached world-wide dominance.

The origin of Chevalier barley is surrounded by myth. The most reliable records tell how a few grains of barley were planted in a cottage garden in Debenham, Suffolk, England by John Andrews in 1820. On an inspection tour of the cottage, his landlord, Dr. Charles Chevallier, noticed the particularly fine ears and started multiplying the grain (Beaven, 1947). The origin of the first plants is unresolved but was probably an

Abbreviations: B, barley accession from John Innes Center, UK; CIho, barley accession from the U.S. National Plant Germplasm System; GE, barley accessions taken from the 19th-century seed collections of Gamle Estrup, Denmark; HOR, barley accession from IPK Gatersleben, Germany; KASP, kompetitive allele specific polymerase chain reaction; NGB, barley accession from Nordic Genetic Resource Center; NM, barely accessions taken from the 19th-century seed collections of the Nordic Museum; PCA, principal component analysis; PCR, polymerase chain reaction; PI, barley accession from the U.S. National Plant Germplasm System; SNP, single nucleotide polymorphism.

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off-type of the landrace ‘Archer’ (Fischbeck, 2003), but distinguished from it by short rachilla hairs and an earlier maturity. After 7 yr of multiplication, distribution to other areas began, and in the 1830s, Chevalier was cultivated in multiple counties in both England and Scotland (Walton, 1999). In the decades that followed, Chevalier completely dominated British barley production and is said to have accounted for 80 to 90% of the barley grown in England at its peak popularity (Beaven, 1947). In fact, Chevalier was so dominating that after 1860 descriptors for barley cultivars disappeared from British agricultural magazines, as malting barley per definition was Chevalier barley (Walton, 1999).

From the very beginning, Chevalier was considered a premium malting barley, selling at higher prices than other cultivars. The malting properties of Chevalier with uniform grain size and shape, even germination, and high extract perfectly matched the demands of the growing commercial brewing industry. As late as 1936, the barley breeder E.S. Beaven remarked that no cultivar had yet outmatched the malting properties of Chevalier (Beaven, 1936). Its less favourable traits, such as being prone to lodging and having poor fodder quality were of lesser importance (Fischbeck, 2003; Walton, 1999). During the last decades of the 19th century, cultivation of Chevalier in Britain declined. Recurrent poor harvests due to weather conditions and re-instatement of the malt tax turned the brewing industry towards imported barley instead (Beaven, 1936; Walton, 1999). However, recent studies show that Chevalier still meets current malting quality standards (Goddard et al., 2019) and Crisp Malt, Great Ryburgh, UK, currently produces a Chevallier heritage malt.

The success of Chevalier barley and other mass-selections (e. g. ‘Kolben’, ‘Shireff’ and ‘Squarehead’ wheat; *Triticum aestivum* and ‘Ligowo’ and ‘Probsteijer’ oats; *Avena sativa*) encouraged farmers all over Europe to initiate similar ventures. Superior seeds, not least Chevalier barley, being as it was the standard for malting barley, were exchanged and compared (Fischbeck, 2003). By the end of the 19th century, Chevalier had reached most European countries as well as America and Australia (Peterson & Foster, 1974; Sparrow & Doolette, 1975). In addition, further selections were made, giving rise to multiple cultivars, essentially derived from Chevalier. The most renowned ones were ‘Kinver’s Chevalier’, ‘Chevalier Hallet’s pedigree’ and ‘Kings Prize Chevalier’, whereas other strains were known as “French Chevalier”, “Californian Chevalier” and “Chilean Chevalier”. The origin and identity of these different strains are unclear as is the fate of Chevalier barely once it reached the farmers’ fields and system of self-recruitment of seed (farmer-saved seed). Some lines of evidence also suggest that the term “Chevalier”, at least among brewers, eventually became synonymous to two-row barley, to distinguish the grain from six-row barley (Beaven, 1936).

Until the late 19th century, landraces, adapted to local condition and maintained through farmers’ self-recruitment of

Core Ideas

- Twenty 150-yr-old and 27 extant accessions of ‘Chevalier’ barley were genotyped.
- Within-accession variation revealed breeding history also with a limited number of markers.
- The 150-yr-old ‘Chevalier’ samples consisted of pure Chevaliers, seed mixtures and landraces.
- Extant ‘Chevalier’ consisted of pure Chevalier and material derived from crosses.
- Spontaneous outbreeding contributed to pre-Mendelian farmer-driven plant improvement.

seed, dominated barley cultivation in the Nordic countries (Leino, 2021). Landraces of barley are generally very heterogeneous, but the variation comes from mixtures of homozygous plants, rather than heterozygous individuals (e.g., Bellucci et al., 2013; Forsberg et al., 2015), a consequence of the predominantly self-fertilizing habit of barley. Chevalier barley reached the Nordic countries in the mid-19th century and can be found in the large seed collections preserved from this time period (Leino et al., 2009). The Royal Academy of Agriculture first grew Chevalier at its Experimental field in Stockholm in the 1860s and harvest samples from multiple farms in Finland and Sweden from the 1880s and 1890s suggest widespread cultivation of Chevalier by the late 19th century (Leino et al., 2009). The breeding company Swedish Seed Association in Svalöv, founded in 1886, took up Chevalier multiplication and improvement as one of its first tasks (Persson & Hansson, 1986). Once more, the driving force was the emerging brewing industry in Sweden, which was particularly interested in new cultivars that suited large-scale malting much better than the six-row barleys previously used (Neergaard, 1889).

In this study we investigate whether different materials described as “Chevalier” indeed descend from an original source of Chevalier developed as described by Beaven (1947). We investigate this in two different types of material: first, preserved 19th-century harvest samples (Leino et al., 2009) collected at leading plant improvement institutes, large estates, and smaller farms across southern Sweden and second, a world-spanning collection of extant Chevalier accessions preserved in genebanks.

2 | MATERIALS AND METHODS

2.1 | Plant material

Both historical harvest samples of Chevalier barley and extant accessions, preserved in gene banks, were included in the study. We will henceforth use the word accession to refer to

seeds collected at a specific site on a specific occasion. From the historical seed collection of the Royal Swedish Academy of Agriculture (housed at the Nordic Museum, Sweden) 17 accessions, with “Chevalier” included in the labelling, were chosen (prefix NM). Two of these had been cultivated at the Academy’s experimental field in 1865 and 1889, respectively, one accession in Finland in 1882 and 14 accessions had been harvested on estates and at farms in different parts of southern Sweden in 1896. From the seed collection of Gamle Estrup, Denmark (prefix GE, available at the Nordic Genetic Resource Center, NordGen, Sweden) three accessions were chosen, one of which was known to have been harvested in 1893, and two believed to date to the same year. The historical harvest samples are no longer viable and have not been regenerated since the initial harvest. The grains are thus bona fide representatives of Chevalier barley cultivated in the late 19th century. From the gene banks NordGen, Sweden (prefix NGB), John Innes Center, UK (prefix B), IPK Gatersleben, Germany (prefix HOR) and the U.S. National Plant Germplasm System (prefix PI and CIho) a total of 27 extant accessions, with an accession name including either “Chevalier” or the alternative spelling “Chevallier”, were chosen. In addition, three accessions, that according to genebank passport data, were selections from Chevalier barley (‘Brage’ [PI266127], ‘Stensgard’ [PI361631] and ‘Scotch Common’ [PI467671]) were included. In total 47 accessions were studied (Table 1).

2.2 | DNA extraction and genotyping

DNA was extracted from six randomly chosen individual grains from each accession. The grains of the historical accessions are no longer viable and hence DNA had to be extracted directly from the grains. For the historical accessions, DNA was extracted using the FastPrep kit (MP Biomedicals, Solon) according to the manufacturer’s instructions, but with the following modifications. Prior to DNA extraction, grains were crushed using sterilized pliers and after rupturing in the Fast-Prep instrument a 1-h incubation at room temperature was added. To minimize the risk of contamination, DNA extractions from the historical samples were carried out in a sterile bench where work with extant DNA had never been performed. Extant accessions were allowed to germinate, and leaves were dried on silica gel whereafter DNA was extracted using the DNeasy Plant Mini kit by Qiagen, following the manufacturer’s instructions.

Genotyping was carried out by LGC genomics using the kompetitive allele-specific polymerase chain reaction (KASP) method (He et al., 2014). Due to the DNA quality of the historical samples, only a limited number of genetic markers could be used. A total of 100 markers, primarily sin-

gle nucleotide polymorphisms (SNPs), were genotyped, some of which were associated with known traits (Supplemental Table S1). The remaining SNPs were chosen from the 384 C-SNP set developed for European landraces (Moragues et al., 2010) from the BOPA1 array (Kota et al., 2008). The SNPs chosen were distributed across all seven barley chromosomes and had previously be verified to be polymorphic in Nordic barley (Denmark, Sweden, Norway and Finland) (Forsberg et al., 2015; Lempiäinen-Avci et al., 2020). Following genotyping, two markers, 11_11059 and 11_20897, were removed due to poor success rate (24.5 and 14.2% success rate, respectively). No individual had a success rate lower than 85% and all genotyped individuals were kept in the data set for further analysis (Supplemental Table S2).

2.3 | Genetic analyses

Purpose-written Perl scripts (available upon request) were used to calculate within-accession diversity, measured as Nei’s h (Nei, 1973), and pairwise F_{ST} , calculated according to Wright (1951). The software STRUCTURE (v 2.3.4; Falush et al., 2003; Pritchard et al., 2000) was used to identify genetic clustering among the genotyped accessions. STRUCTURE was run using the haploid setting, as suggested for predominantly self-fertilizing species by Nordborg et al. (2005), and allowing for admixture. STRUCTURE was run with a burn-in length of 20,000 iterations followed by 50,000 iterations for estimating the parameters, with 10 repeated runs at each level of predetermined clusters (K) ranging from 1 to 10. The software CLUMPP (v 1.1.2; Jakobsson & Rosenberg, 2007) was used to compare the outcome of individual runs with the Greedy algorithm for $4 < K < 6$ and with the LargeK-Greedy algorithm for $K \geq 6$. The number of clusters best describing the data was evaluated from the CLUMPP H' values and ΔK calculated according to (Evanno et al., 2005). Results were visualized using DISTRUCT (v 1.1; Rosenberg, 2004). In addition, Principal Component Analysis (PCA) was also used to visualize genetic clustering. PCA was carried out in R (v. 3.1.2; R Core Team, 2018) using the *prcomp* command. In the analyses, the frequencies of each allele at each locus within an accession were used as independent variables for the accession-level PCA and the number of alleles at each locus of each individual were used for the individual-level PCA.

The majority of the markers had previously been genotyped in historical two-row landrace barley from the Nordic area (Norway, Sweden, and Finland; Lempiäinen-Avci et al., 2020; Hagenblad & Leino, unpublished data, 2011; Supplemental Table S3). Data from these accessions were used to compare the Chevalier barley genotyped in this study with landrace barley of the two-row type.

TABLE 1 List of studied accessions, within-accession diversity and designation based on STRUCTURE clustering and principal component analysis

Accession No.	Description	Year ^a	Nei's h	Designation
NM.0406664	Chevalierkorn Experimental Field, Stockholm	1865	0.092	Seed mixture
NM.0405769	Chevalier from Rödorp, Pojo, Finland	1882	0.238	Seed mixture
NM.0405663	Skånskt Chevalier. Experimental Field, Stockholm	1889	0.108	Seed mixture
NM.0406081	Chevalier Hyllie Malmöhus län	1896	0	True Chevalier
NM.0406091	Chevalier Barsebäck Malmöhus län	1896	0.215	Seed mixture
NM.0406133	Chevalier Lerchenborg's Säbyholm Malmöhus län	1896	0	True Chevalier
NM.0406184	Chevalier, "Hallet" Säbyholm. Malmöhus län	1896	0	True Chevalier
NM.0406189	Chevalier "Kinver" Säbyholm Malmöhus län	1896	0.159	Seed mixture
NM.0406219	Chevalier "Melon" Säbyholm. Malmöhus län	1896	0	True Chevalier
NM.0406053	Chevalier Hanaskog Christianstads län	1896	0.163	Seed mixture
NM.0406056	Chevalier Kastlösa Södra Kalmar län	1896	0.064	Seed mixture
NM.0406186	Chevalier Röen Göteborgs & Bohuslän	1896	0	True Chevalier
NM.0406242	Chevalier Wisingsö Jönköpings län	1896	0	True Chevalier
NM.0406145	Chevalier Idingstad Östergötlands län	1896	0.176	Seed mixture
NM.0406164	Chevalier Nybble Östergötlands län	1896	0.206	Seed mixture
NM.0406181	Chevalier Blästad Östergötlands län	1896	0	True Chevalier
NM.0406146	Chevalier Ekeby Södermanlands län	1896	0.257	Non-Chevalier
GE1	Californisk Chevalier Byg	unknown	0	True Chevalier
GE26	Engelsk Byg Norfolk Chevalier	unknown	0.191	Non-Chevalier
GE34	Chili Chevalier Byg	1893	0.029	True Chevalier
NGB4612	Chevallier Race Francaise	1984	0	True Chevalier
NGB4693	Chevalier II	1984	0	True Chevalier
NGB9443	Chevallier Tystofte	1991	0.061	Cross
NGB9444	Chevallier	1991	0.087	Non-Chevalier
B4838	Chevallier Australia	1972	0	Cross
B3431	Chevallier Chile	1960	0.035	True Chevalier
B7742	Chevalier	1966	0.136	Non-Chevalier
B3432	Chevallier 1	1960	0	Non-Chevalier
B3437	Chevalier French	1960	0	Non-Chevalier

(Continues)

TABLE 1 (Continued)

Accession No.	Description	Year ^a	Nei's h	Designation
HOR3003	Neuhaus Selchower Chevallier	unknown	0	True Chevalier
HOR3639	Chevallier von Neuhaus	1964	0	True Chevalier
HOR20821	Mettes Chevalier	2003	0	True Chevalier
HOR12097	Chevallier Tyst	1994	0.080	Cross
HOR20516	Neuhaus Selchower Chevalier	2003	0.097	True Chevalier
HOR3009	Rokkaku Chevallier	unknown	0	Non-Chevalier
HOR11127	Hokkaido Chevalier	1992	0	Non-Chevalier
HOR11520	French Chevalier	unknown	0	Non-Chevalier
HOR14092	Sholley Chevalier	2003	0	Non-Chevalier
Clho1142	Chevalier	1916	0	True Chevalier
Clho156	Chevalier (King's Prize Chevalier)	1901	0	Non-Chevalier
Clho1245	Chevalier	1917	0	Non-Chevalier
PI5853	Chevalier	1901	0	True Chevalier
PI190202	Chevalier	1950	0	True Chevalier
PI266127	Brage Selection from not pure Chevalier	1960	0	Cross
PI361614	Chevallier Tyst	1971	0	Cross
PI361631	Stensgard	1971	0.097	Seed mixture
PI467671	Scotch Common	1982	0.087	Non-Chevalier

^aFor extant accessions, year indicates year of inclusion in the genebank, and for historical accessions, harvest year.

3 | RESULTS

A set of historical and extant accessions of Chevalier barley was genotyped with SNP markers for six grains from each accession. In total, 282 individual seeds from 47 accessions were genotyped using 100 KASP markers of which 98 were retained for analysis. A single individual (the historical NM.0406081.2) had a success rate of 85%, and the remaining individuals all had success rates above 90%. All markers retained for analysis had a success rate above 90%. The six SNPs that were heterozygous were removed from further analysis. A single individual was heterozygous for two loci and four individuals for a single locus each.

3.1 | Homogeneous and variable accessions

Homogeneous accessions (in total 27 out of 47 or 57.4%), completely lacking within-accession diversity for the genotyped markers, were found both amongst the historical and extant accessions (Table 1). Variable accessions were also found amongst both the historical ($n = 12$ out of 20 or 60%) and extant accessions ($n = 8$ out of 27 or 29.6%). The highest within-accession diversity was found in the historical accession NM.0405769, from Rödörp in Finland, harvested in 1882. The extant accession with the highest within-accession

diversity was B7742, included in the gene bank in 1966. The within-accession diversity of extant accessions was lower than that of historical accessions both when including (two-sided t-test, $p < .01$) and excluding (two-sided t-test, $p < .05$) homogenous accessions. The extant set of accessions contained a higher proportion of homogenous accessions (19 out of 27) compared with the historical set (8 out of 20).

3.2 | Genetic structure analyses suggest pure lines, crosses, and seed mixtures of Chevalier

After analysis with STRUCTURE both ΔK and the CLUMPP H' values suggested that two clusters best described the data. At $K = 2$, both the identified clusters contained both historical and extant accessions (Figure 1). Both the historical and the extant material included accessions where all individuals belonged entirely to either one or the other of the two clusters. In the remaining accessions both clusters were found, either in different individuals or in the same individual.

Among the extant accessions all individuals of several accessions (primarily B4838, HOR12097, NGB9443, and PI361614) were partially assigned to both of the two clusters (Figure 1), indicative of a hybrid origin and therefore forthwith designated crosses. This was also the case for 'Brage'

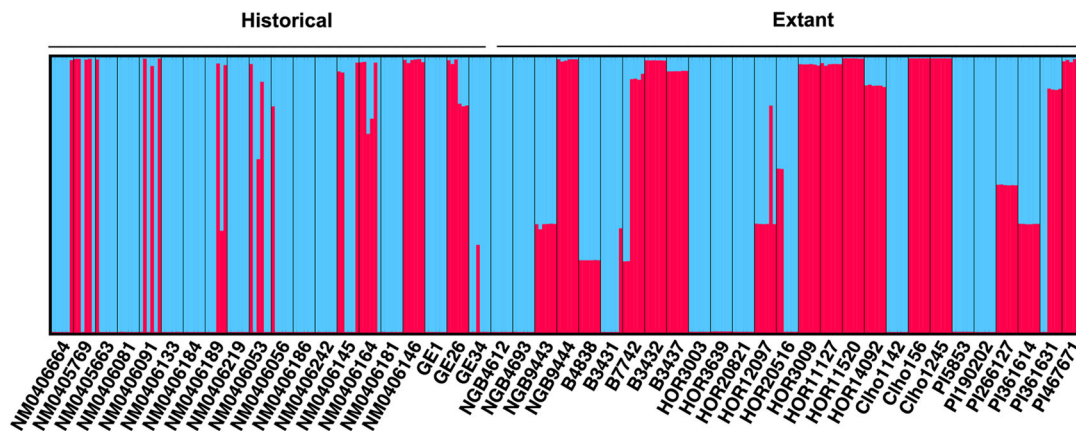


FIGURE 1 Result of STRUCTURE analysis for $K = 2$. Each vertical line represent data from a single individual where the two colors decode the proportion of identity of that individual to each of the two clusters explained by the investigated model

(PI266127), said to be a selection made in Chevalier. Of the other two Chevalier-derived cultivars, ‘Stensgard’ (PI361631) had five individuals belonging completely to one cluster (red in Figure 1.) and one to the other and ‘Scotch Common’ (PI467671), clustered entirely with one of the clusters (red in Figure 1).

Among the historical accessions, some accessions (e.g., NM.0405769, NM.0406053, NM.0406091, NM.0406145, and NM.0406189) were made up of individuals that were either completely assigned to one or completely to the other of the two clusters (Figure 1), indicative of seed mixture. In four of the historical accessions (NM.0405663, NM.0406056, NM.0406164, and NM.0406664) one of the six genotyped seeds was identified as deviant.

In PCA of all extant and historical accessions PC1 and PC2 initially explained 23.88 and 13.01% of the variation, respectively. Principal Component 2 primarily separated HOR3009 from all other accessions. Our genotyping of functional SNPs in *Vrs1* and *Int-c* suggest that this is a six-row barley. After removing this accession, PC1 and PC2 explained 24.61 and 10.22% of the variation, respectively (Figure 2). Both extant and historical accessions were distributed along PC1 without forming clearly separated groups. At the negative end of PC1, however, twelve accessions (NM.0406081, NM.0406133, NM.0406181, NM.0406184, NM.0406186, NM.0406219, NM.0406242, GE1, Clho1142, PI190202, NGB4612, and NGB4693) were located on identical PC values. The same accessions were all completely in the same STRUCTURE cluster (blue in Figure 1). Another four accessions (HOR3639, PI5853, HOR20821, and HOR3003) were located nearby in the PCA. Pairwise F_{ST} -values (Supplemental Table S4) confirmed that this group of 16 accessions were identical or very similar. We hypothesize that these accessions are most likely Chevalier barley, and they will henceforth be referred to as “True Chevaliers” (blue in Figure 2).

3.3 | Nordic landraces contribute to the non-Chevalier component

To investigate whether the second cluster (red in Figure 1 and Figure 2) detected in the STRUCTURE analysis was of landrace origin, genotyping data obtained in this study for the NM, GE, and NGB accessions, with origin in the Nordic countries, was merged with data from previously genotyped historical and extant Finnish and Swedish two-row landrace barley. In PCA of the Nordic accessions, the first two principal components explained 37.12 and 14.21% of the variation respectively. Principal Component 2 separated the landrace accessions NM.0405781 and NM.0406095 from the remaining accessions (Figure 3). Accessions labelled “Chevalier” but clustering primarily in the red cluster in the STRUCTURE analysis (NM.0406146, NGB9444, GE26, NM.0405769, and NM0406164) fell among the two-row landrace accessions along PC1 (Figure 3). The PCA of individual genotypes showed that individuals from accessions identified as seed mixtures in the STRUCTURE analysis fell either among the True Chevaliers or among the landraces (Supplemental Figure S1). In the accession-level PCA, the historical landrace accession NM.0406147 clustered among the seed-mixture accessions, whereas the extant landrace NGB9472 was located close to the True Chevaliers (Figure 3). Both these accessions have a provenance in the county Östergötland in Sweden.

4 | DISCUSSION

It has been said that from the first single plant of Chevalier “no race of any species of any farm plant ever before, or since, have spread so extensively as this” (Beaven, 1936). Our genetic analyses of 47 accessions of Chevalier, from different countries and time periods, show that this is a truth

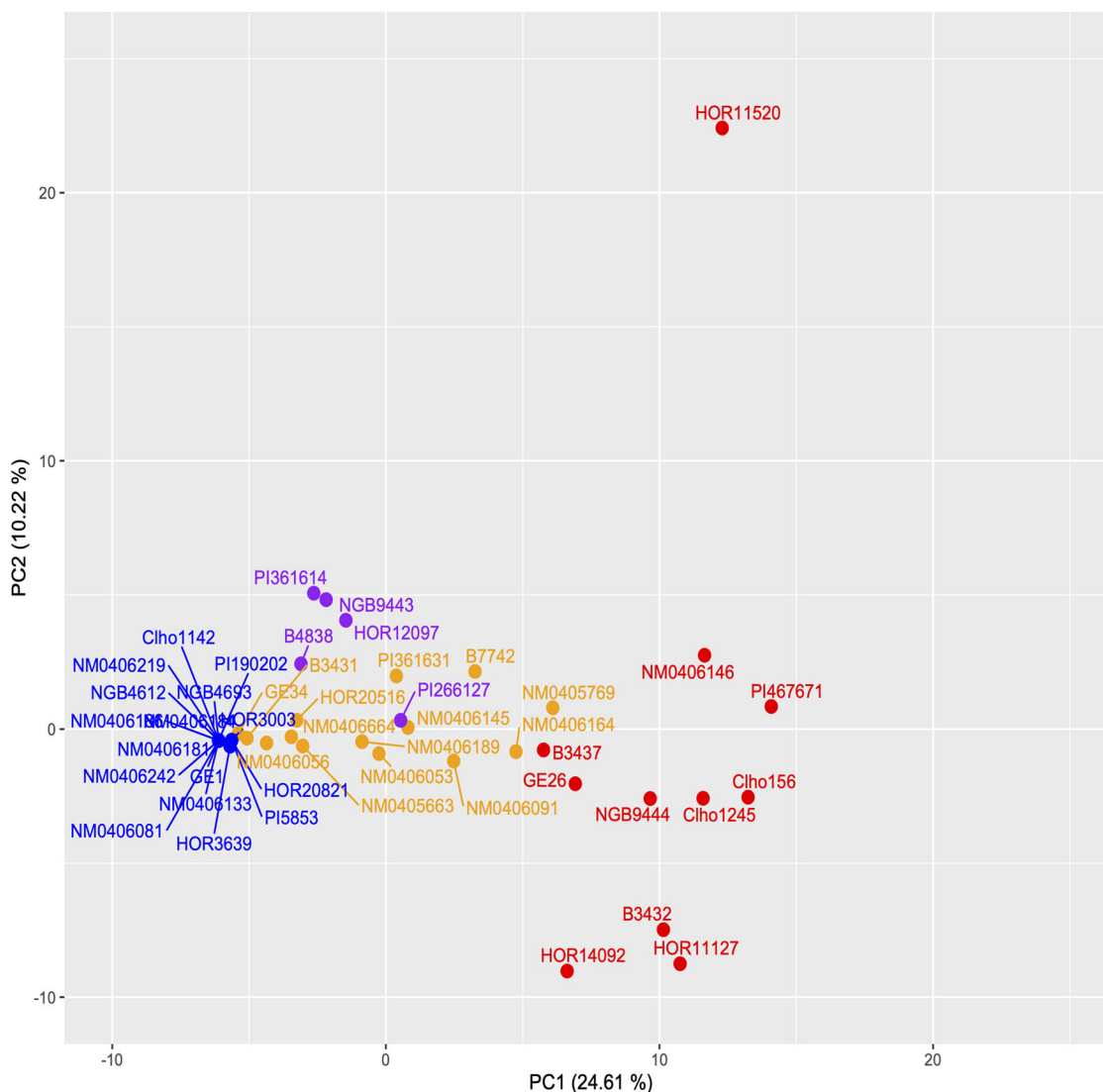


FIGURE 2 Principal component analysis (PCA) of all Chevalier accessions. Coloring indicate designation based on clustering. Blue: true Chevalier; orange: seed mixtures; purple: crosses; red: non-Chevalier

with modifications. In our sample we could find accessions that we classify as True Chevalier, but also seed mixtures, crosses, and even samples totally unrelated to Chevalier in spite of being labelled as Chevalier barley.

4.1 | True Chevaliers

Today's crop cultivars are genetically more or less uniform as a result of selection and the genetic drift caused by multiplication of single individuals. This pure line theory, established by Johansen (1903), is still fundamental in all breeding programs in self-fertilizing crops. If the narrative surrounding Chevalier barley is true, genetic homogeneity, at least to a certain extent, is expected. Indeed, 40 and 70.3% of the historical and the extant Chevaliers included in this study, respectively, had no within-accession diversity. Furthermore, several of the

Chevalier accessions were also near isogenic, with an average of 98.9% commonality for the markers used when using the accession Clho1142 as a reference accession.

Although the number of markers used in this study was limited, it seems likely that the development of Chevalier barley has occurred much as described with mass selection from a limited number, perhaps even a single plant, of high-quality ancestral grains. Based on *STRUCTURE* clustering, location in the PCA result, pairwise F_{ST} and within-accession genetic uniformity we identified a group of 16 accessions that we call "True Chevalier" (Table 1, blue in Figure 2).

The True Chevalier group contained both accessions simply called Chevalier, but also accessions designated as Chevalier re-selections. These include the historical 'Hallet' (NM.0406184), 'Melon' (NM.0406219), and 'Lerchenborg' (NM.0406133) Chevaliers from Säbyholm as well as Californian Chevalier (GE1) and the extant accessions of

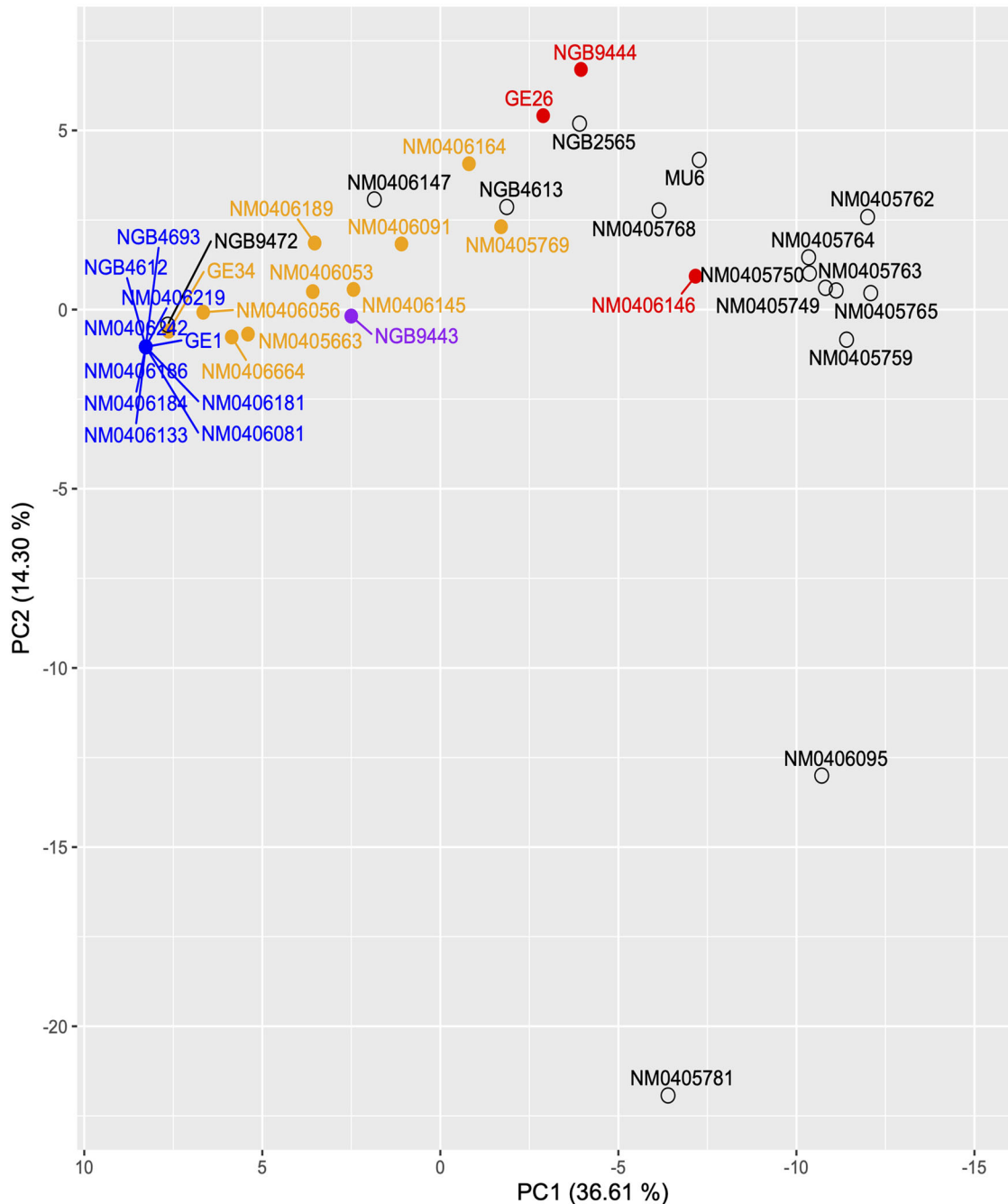


FIGURE 3 Principal component analysis (PCA) of Chevalier accessions with Nordic origin analyzed together with Nordic landraces of two-row barley. Nordic landraces are colored black and Chevalier accessions colored as in Figure 2. The *x*-axis has been reversed for clarity

Neuhaus Chevaliers (HOR3003, HOR3639), French Chevalier (NGB4612), and Mettes Chevalier (HOR20821). All of these were identical with our marker set which suggests that these types of Chevalier are indeed re-selections from original Chevalier stock.

It should be noted that the accessions studied here were only genotyped for a very limited number of markers (100), partially a consequence of the degraded nature of the DNA

of the historical samples (Leino et al., 2009). We therefore cannot tell whether the Chevalier barley and its derivatives in the True Chevalier group were truly monomorphic or truly identical to each other. Re-selection from genetically uniform populations of individuals is, from an improvement point of view, a fruitless exercise. Hence, two scenarios are possible: (a) The original Chevalier was variable in parts of the genome not captured by our limited set of markers, parts that were

subsequently fixed for desirable genetic variation in the essentially derived cultivars. (b) The original Chevalier was indeed completely homogenous and environmentally induced variation was mistaken for a source of useful variation for improvement schemes. At the time when these re-selections arose, prior to the rediscovery of the Mendelian laws of inheritance, the understanding of the interplay of heritage and environment was still incomplete (Roll-Hansen, 2000). Genotyping a larger number of markers in selected extant Chevalier accessions may shed further light on the matter.

4.2 | Seed mixtures

The probability of identifying an accession as a seed mixture depends on the relative proportion of seeds of different origins in the accession studied. In a mixture of seeds with two origins (i.e., Chevalier and landrace seed) the maximum probability is reached when the two types occur in equal proportions (Supplemental Figure S2). Genotyping six grains, as in this study, the probability of sampling both types is .969 when two types occur at equal frequencies. The probability is .738 when the less common type occurs at 20% but only .468 when the less common type occurs at 10%. A study aiming to detect seed mixtures where one of the types occurs at low frequencies hence must genotype a much larger number of grains than what we have done here. For example, if genotyping 20 grains there is a .878 probability of sampling both types when the rarer type occurs at a frequency of 10%.

Bearing in mind our limited power to detect seed mixtures when one type is rarer than 20%, several of the historical accessions nonetheless proved to consist of seed mixtures with varying frequencies of True Chevalier grains (blue in Figure 2). The True Chevalier content ranged from a single individual True Chevalier seed (NM.0406164) to single non-Chevalier seeds (NM.0406056, NM.0406664, and NM.0405663). The previously genotyped historical landrace accession NM.0406147 was not labelled as a Chevalier, but nonetheless had a Chevalier seed mixture signature.

Not only Chevalier barley cultivated on different farms turned out to be seed mixtures. The accession labelled Chevalier “Kinver” from Säbyholm estate (NM.0406189) and the two Chevaliers harvested at the experimental fields of the Royal Swedish Academy of Agriculture in 1865 (NM.0406664) and 1889 (NM.0405663), respectively, were of mixed origin. It thus seems as if leading agricultural scientists of the late 19th century in Sweden used and retained Chevalier seed of mixed origin over as much as three decades, although it should be noted that the non-Chevalier seeds in the two seed samples from the experimental fields differed in almost a third of the markers. To what extent seeds of different origin were mixed for the purpose of improving yield or

the result of more unconscious merges of available seed we do not know.

Seed mixtures were primarily found among the historical accessions. It appears as if Chevalier barley, after its introduction to the Nordic countries, was rapidly introgressed into the local seed systems and mixed with local landraces. However, the seed mixtures are not totally random. Although barley cultivation in Sweden at this time was dominated by six-row barley, none of the mixture grains genotyped for a six-row phenotype. Six- and two-row barley had different end-uses and farmers carefully kept these seed lots apart (Leino, 2017).

The mixtures of modern and traditional cultivars have present-day parallels. In an outbreeding crop like maize (*Zea mays*), gene flow through pollen spread is widespread, and Rojas-Barrera et al. (2019) found that Mexican landraces are influenced by gene-flow from modern cultivars leading to loss of genetic variation. Bellucci et al. (2013) showed that also in an inbreeding crop like Sardinian barley, landraces were subject to low, but significant gene flow from modern cultivars through seed mixture. When the uniform Chevalier was introduced to the Nordic countries in the 19th century, it probably resulted in loss of genetic variability of two-row barley through seed mixture and the subsequent replacing of genotypes in local landraces. Genetic erosion in the Nordic countries thus likely predates modern plant improvement with several decades.

4.3 | Crosses

With the exception of one individual (heterozygous for two loci), none of the genotyped individuals was heterozygous for more than a single locus such that would be expected from the early generation progeny of an outcrossing event. Outcrossing events followed by multiple generations of inbreeding will, however, result in homozygous individuals where different parts of the genome originate from the two different parents in the cross. We could detect several such accessions from our STRUCTURE analysis. We classified accessions within which all the individual seeds partially clustered in both of the two clusters as “crosses”. We chose to consider accessions with less than 10% of the genetic markers from the Chevalier cluster as non-Chevaliers, resulting in a Chevalier component of between 46 and 74% in the crosses. This is comparable to a past single cross and a cross, followed by a backcross to the Chevalier parent, respectively.

The five accessions indicated as crosses included the two Chevallier Tyst accessions (PI361614 and HOR12097) and the Chevallier Tystofte accession (NGB9443). The designation of these accessions suggests a shared origin. Although the three are not genetically identical they have low pairwise F_{ST} values and group closely in the PCA. Another cross was Chevallier Australia (B4838). About 74% of the

genetic markers of this accession belonged to the Chevalier cluster. The origin and pedigree of this accession is unknown, but its genetic setup suggests that originates from a Chevalier cross, backcrossed to Chevalier.

The last accession classified as a cross was ‘Brage’ (PI266127). In 1899 the Swedish plant breeder Hans Tedin isolated hundreds of pure lines from a seed lot of Chevalier displayed at a malting barley exhibition (Tedin, 1925). After decades of test cultivation and multiplications one of these lines was released as the cultivar Brage in 1925. Tedin (1925) describes its characteristics as similar to Chevalier but with significant differences. Under Swedish conditions Brage out-competed Chevalier in terms of yield. Our results show that Brage must have been a spontaneous cross between Chevalier and another genetic background, possibly a local landrace.

None of the historical accessions had the hybrid origin indicative of being the result of a cross. However, the historical accession NM.0406189, in addition to being a seed mixture, contained a grain of hybrid origin. This individual was completely homozygous but clustered with both clusters in the STRUCTURE analysis. We hypothesize that this individual is the descendant of a naturally occurring outbreeding event in a field with mixed genotypes grown together, similar to the outbreeding event giving rise to Brage. Rare seeds of hybrid origin, that is, homozygous individuals clustering with both clusters in the STRUCTURE analysis, were also found in three of the extant accessions (HOR20516, B7742, and B3431). The accession B3431 is listed as Chevallier Chile. Interestingly, the similarly described historical accession GE34 (Chili Chevalier Byg [= barley]) show the same distribution of True Chevalier seeds ($n = 5$) and hybrid seeds ($n = 1$). The two have a low pairwise F_{ST} value and are closely located in the PCA and likely have a shared origin.

4.4 | Non-Chevalier accessions

Both among the historical and the extant material we found accessions that were clearly not of Chevalier ancestry (red in Figures 1 and 2). Among the extant accessions it is possible that these were originally mixtures between True Chevalier and non-Chevalier seeds, but have, over time through genetic drift lost the Chevalier seeds. It is also possible that they have remained seed mixtures, but that the True Chevalier component is so small that it was not detected among the six grains sampled in this study. A third alternative is that the dominance of Chevalier in the 19th century eventually led to a classification of all two-row barleys as “Chevalier”, independent of their true origin (Beaven, 1936). ‘Scotch Common’ (PI467671) is described as a reselection of Chevalier but was shown here to have little similarity to the True Chevaliers. Based on its low genetic diversity ($h = .087$), we suggest instead that this cultivar originated from a seed mixture of

Chevalier and another two-row barley and was selected to become a pure line of the non-Chevalier type. The historical NM.0406146 (Chevalier from Ekeby farm in county Södermanland) and GE26 (English barley Norfolk Chevalier) were both clearly located among the landrace accessions along PC1 (Figure 2). These accessions had much higher genetic diversity than ‘Scotch Common’ (NM.0406146 $h = .258$; GE26 $h = .191$). Comparing them with landrace two-row barley it is likely that they, and maybe also extant accessions classified as Chevalier, are of landrace origin but were classified as “Chevalier” based on their phenotypic characteristics rather than their genetic ancestry or breeding pedigree.

4.5 | The role of mass selections in 19th-century agriculture

From our results it is clear that the designation “Chevalier” was applied rather loosely in the 19th century. The six investigated accessions from county Östergötland in Sweden illustrate this phenomenon very well. Of the three accessions from Östergötland labelled Chevalier one (NM.0406181) could be classified as True Chevalier and the remaining two as seed mixtures. NM.0406145 had a 50:50 proportion of each type while NM.0401664 was predominantly of the non-Chevalier type. The previously genotyped landrace accession NM.0406095 (labelled Borstad) proved to indeed be of landrace origin, whereas the previously genotyped landrace accession NM.0406147 (from the municipality Sturefors) turned out to be a seed mixture consisting of equal proportions landrace seed, True Chevalier seed and hybrid seeds from spontaneous outcrossing. The extant accession NGB9472 (‘Östgöta flättring’), believed to be of landrace origin, show high similarity to the True Chevaliers.

Our sample of historical grains originate from the late 19th century and predates the onset of modern scientific plant breeding based on Mendelian genetics (Roll-Hansen, 2000). The finding of Chevalier barley as pure and mixed seed in Nordic farmer’s fields demonstrate the impact farmer-driven breeding activities had over crop seed at this time. A parallel situation can be found in oats where the German and French ‘Probsteier’ and ‘Ligowo’ where mixed into Nordic landrace oats during the 19th century (Granhall, 1938; Leino & Hagenblad, in preparation). In contrast, locally cultivated six-row barley appear to have maintained genetic integrity to a much higher extent (Aslan et al., 2015; Forsberg et al., 2015; Leino & Hagenblad, 2010).

We postulate that enterprising 19th-century farmers in Sweden procured seeds from early cultivars, such as Chevalier, and mixed these with their locally sourced seeds. Barley has an outbreeding rate of about 2% (Abdel-Ghani et al., 2004) and when cultivar seeds and landrace seeds were sown together spontaneous outbreeding events gave rise to seed of

hybrid origin. This generated new diversity from which farmers could obtain their future seed, in this case locally adapted Chevalier strains. From experiments with composite crosses in barley, it is known that adaptation, also when only from natural selection can result in increased yields in such materials (Soliman & Allard, 1991). This process was not so different from the plant improvement methods developed in the early 20th century, when genotypes superior in yield or quality were combined with locally adapted material to create the first modern cultivars.

DATA AVAILABILITY STATEMENT

The dataset generated during the current study is available in Supplemental Table S2.

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AUTHOR CONTRIBUTIONS

Jenny Hagenblad: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Visualization; Writing-original draft; Writing-review & editing. Matti W. Leino: Conceptualization; Funding acquisition; Investigation; Methodology; Visualization; Writing-original draft; Writing-review & editing.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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REFERENCES

- Abdel-Ghani, A. H., Parzies, H. K., Omary, A., & Geiger, H. H. (2004). Estimating the outcrossing rate of barley landraces and wild barley populations collected from ecologically different regions of Jordan. *Theoretical and Applied Genetics*, 109(3), 588–595. <https://doi.org/10.1007/s00122-004-1657-1>
- Aslan, S., Forsberg, N. E. G., Hagenblad, J., & Leino, M. W. (2015). Molecular genotyping of historical barley landraces reveals novel candidate regions for local adaption. *Crop Science*, 55(6), 2766–2776. <https://doi.org/10.2135/cropsci2015.02.0119>
- Beaven, E. S. (1936). Barley for brewing since 1886. *Journal of the Institute of Brewing*, 42(6), 487–495. <https://doi.org/10.1002/j.2050-0416.1936.tb05686.x>
- Beaven, E. S. (1947). *Barley: Fifty years of observation and experiment*. Duckworth.
- Bellucci, E., Bitocchi, E., Rau, D., Nanni, L., Ferradini, N., Giardini, A., Rodriguez, M., Attene, G., & Papa, R. (2013). Population structure of barley landrace populations and gene-flow with modern varieties. *Plos One*, 8(12), e83891. <https://doi.org/10.1371/journal.pone.0083891>
- Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, 14(8), 2611–2620. <https://doi.org/10.1111/j.1365-294X.2005.02553.x>
- Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics*, 164(4), 1567–1587. <https://doi.org/10.1093/genetics/164.4.1567>
- Fischbeck, G. (2003). Diversification through breeding. In *Diversity in barley* (pp. 29–52). Elsevier.
- Forsberg, N. E., Russell, J., Macaulay, M., Leino, M. W., & Hagenblad, J. (2015). Farmers without borders-genetic structuring in century old barley (*Hordeum vulgare*). *Heredity*, 114(2), 195–206. <https://doi.org/10.1038/hdy.2014.83>
- Goddard, R., de Vos, S., Steed, A., Muhammed, A., Thomas, K., Griggs, D., Ridout, C., & Nicholson, P. (2019). Mapping of agronomic traits, disease resistance and malting quality in a wide cross of two-row barley cultivars. *Plos One*, 14(7), e0219042. <https://doi.org/10.1371/journal.pone.0219042>
- Granhall, I. (1938). Studier över svensk lanthavre. *Sveriges Utsädesförenings Tidskrift*, 48, 63–151.
- Harwood, J. (2015). Did Mendelism transform plant breeding? Genetic theory and breeding practice, 1900–1945. In *New perspectives on the history of life sciences and agriculture* (pp. 345–370). Springer.
- He, C., Holme, J., & Anthony, J. (2014). SNP Genotyping: The KASP Assay. In D. Fleury & R. Whitford (Eds.), *Crop breeding: Methods and protocols* (pp. 75–86). Springer.
- Jakobsson, M., & Rosenberg, N. A. (2007). CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, 23(14), 1801–1806. <https://doi.org/10.1093/bioinformatics/btm233>
- Johannsen, W. (1903). *Über Erblichkeit in Populationen und in reinen Linien*. Gustav Fischer Verl.
- Kota, R., Varshney, R., Prasad, M., Zhang, H., Stein, N., & Graner, A. (2008). EST-derived single nucleotide polymorphism markers for assembling genetic and physical maps of the barley genome. *Functional and Integrative Genomics*, 8(3), 223–233. <https://doi.org/10.1007/s10142-007-0060-9>
- Leino, M. W. (2017). *Spannmål: Svenska lantsorter*: Nordiska museets förlag.
- Leino, M. W. (2021). "Primitive" landraces: Swedish farmers' perception on seed traits before the era of crop improvement. In A. Rabo, B. G. Karlsson (Ed.), *Seedways. The circulation, control and care of plants in a warmer world* (pp. 75–88). Royal Swedish Academy of Letters, History and Antiquities.
- Leino, M. W., & Hagenblad, J. (2010). Nineteenth century seeds reveal the population genetics of landrace barley (*Hordeum vulgare*).

- Molecular Biology and Evolution*, 27(4), 964–973. <https://doi.org/10.1093/molbev/msp308>
- Leino, M. W., Hagenblad, J., Edqvist, J., & Strese, E. M. K. (2009). DNA preservation and utility of a historic seed collection. *Seed Science Research*, 19(3), 125–135. <https://doi.org/10.1017/S0960258509990055>
- Lempiäinen-Avci, M., Lundström, M., Huttunen, S., Leino, M. W., & Hagenblad, J. (2020). Archaeological and historical materials as a means to explore Finnish crop history. *Environmental Archaeology*, 25(1), 37–52. <https://doi.org/10.1080/14614103.2018.1482598>
- Moragues, M., Comadran, J., Waugh, R., Milne, I., Flavell, A. J., & Russell, J. R. (2010). Effects of ascertainment bias and marker number on estimations of barley diversity from high-throughput SNP genotype data. *Theoretical and Applied Genetics*, 120(8), 1525–1534. <https://doi.org/10.1007/s00122-010-1273-1>
- Neergaard, T. B. V. (1889). *Hvilka anspråk bör ställas på ett godt brygerikorn, och huru fyllas de?* Allmänna Svenska Utsädesföreningen.
- Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America*, 70(12), 3321–3323. <https://doi.org/10.1073/pnas.70.12.3321>
- Nordborg, M., Hu, T. T., Ishino, Y., Jhaveri, J., Toomajian, C., Zheng, H., Bakker, E., Calabrese, P., Gladstone, J., Goyal, R., Jakobsson, M., Padhukasahasram, B., Plagnol, V., Rosenberg, N. A., Shah, C., Wall, J. D., Wang, J., Zhao, K., Kalbfleisch, T., ... Bergelson, J. (2005). The pattern of polymorphism in *Arabidopsis thaliana*. *PLOS Biology*, 3(7), e196. <https://doi.org/10.1371/journal.pbio.0030196>
- Palladino, P. (1994). Wizards and devotees: On the Mendelian theory of inheritance and the professionalization of agricultural science in Great-Britain and the United-States, 1880–1930. *History of Science*, 32(98), 408–444. <https://doi.org/10.1177/007327539403200403>
- Persson, G., & Hansson, J. -A. (1986). Barley. In G. Olsson & E. Åkerberg (Eds.), *Svalöf 1886–1986: Research and results in plant breeding* (pp. 117–123). LT.
- Peterson, G., & Foster, A. (1974). Malting barley in the United States. *Advances in Agronomy*, 25, 327–378. [https://doi.org/10.1016/S0065-2113\(08\)60784-1](https://doi.org/10.1016/S0065-2113(08)60784-1)
- Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155(2), 945–959. <https://doi.org/10.1093/genetics/155.2.945>
- R Core Team. (2018). *R: A language and environment for statistical computing*. RStudio.
- Reitemeier, A. (1905). *Geschichte der Pflanzenzüchtung [PhD dissertation, Universität Breslau]*. Universität Breslau.
- Rojas-Barrera, I. C., Wegier, A., Sanchez Gonzalez, J. J., Owens, G. L., Rieseberg, L. H., & Pinero, D. (2019). Contemporary evolution of maize landraces and their wild relatives influenced by gene flow with modern maize varieties. *Proceedings of the National Academy of Sciences of the United States of America*, 116(42), 21302–21311. <https://doi.org/10.1073/pnas.1817664116>
- Roll-Hansen, N. (2000). Theory and practice: The impact of Mendelism on agriculture. *Comptes Rendus De L Academie Des Sciences. Serie III, Sciences De La Vie*, 323(12), 1107–1116. [https://doi.org/10.1016/S0764-4469\(00\)01259-2](https://doi.org/10.1016/S0764-4469(00)01259-2)
- Rosenberg, N. A. (2004). DISTRUCT: A program for the graphical display of population structure. *Molecular Ecology Notes*, 4(1), 137–138. <https://doi.org/10.1046/j.1471-8286.2003.00566.x>
- Soliman, K., & Allard, R. (1991). Grain yield of composite cross populations of barley: Effects of natural selection. *Crop Science*, 31(3), 705–708. <https://doi.org/10.2135/cropsci1991.0011183X003100030032x>
- Sparrow, D., & Doolette, J. (1975). Barley. *Australian Field Crops: Wheat and Other Temperate Cereals*, 1, 431–480.
- Tedin, H. (1925). Svalöfs Bragekorn. *Sveriges Utsädesförenings Tidsskrift*, 35, 8–17.
- Walton, J. R. (1999). Varietal innovation and the competitiveness of the British cereals sector, 1760–1930. *Agricultural History Review*, 47(1), 29–57.
- Wieland, T. (2006). Scientific theory and agricultural practice: Plant breeding in Germany from the late 19th to the early 20th century. *Journal of the History of Biology*, 39(2), 309–343. <https://doi.org/10.1007/s10739-006-0006-4>
- Wright, S. (1951). The genetical structure of populations. *Annals of Eugenics*, 15(4), 323–354. <https://doi.org/10.1111/j.1469-1809.1949.tb02451.x>

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