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Genetic diversity in remnant Swedish hop yards from the 15th to 18th century

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Abstract

Hop (*Humulus lupulus* L.) is a perennial plant cultivated for its use in beer production. The plant is dioecious and the female plants produce cones containing substances that enhance the taste and durability of beer. Beer was long an essential part of food supply in Northern Europe, and hop has thus been a very important crop during the last 1000 years. In Sweden, hop cultivation was, by law, mandatory for farmers from 1414 till 1860. Today, Swedish hop cultivation is negligible but historical remnant hop plants can still be found as feral populations. Using historical maps and document we have located ten historical hop yards from the 15th to 18th century where hop plants still persist as now feral populations. Some fifteen plants of each population were sampled and genotyped with ten SSR markers and one marker diagnostic for sex type. In addition 25 genebank preserved clones of older landraces and cultivars from Europe were genotyped. Genotyping results show abundant clonality and low rates of sexual reproduction within the feral populations. Two of the populations had markedly higher genetic diversity and a higher number of haplotypes, and in these populations a mix of female and male plants was also found. The populations were all clearly differentiated with no haplotypes shared between populations and little evidence of exchange of genetic material. These results indicate that natural spread and genetic recombination is uncommon or slow in Sweden and that the feral plants could be remnants of the original historical cultivations. In the assembly of European genebank clones several clones showed identical genotypes and overall limited genetic diversity. The Swedish populations were in most cases genetically clearly different from the genebank clones. This contrasts with historical records of massive introductions of hop clones from continental Europe during the 19th century and shows that these imports did not replace the original hop cultivated. A possible better adaption of the Swedish hops and primitive historical breeding are discussed.

Humle (*Humulus lupulus* L.) är en flerårig växt som odlas för användning i ölproduktion. Växten är tvåbyggare och honplantorna producerar kottar som innehåller ämnen som förbättrar ölets smak och hållbarhet. Öl var länge en viktig del av livsmedelsförsörjningen i norra Europa, varför humle varit en mycket betydelsefull gröda under de senaste 1000 åren. I Sverige var humleodling enligt lag obligatorisk för jordbrukare från år 1414 till 1860. I dag är den svenska odlingen av humle försumbar men förvildade humleplantor från historiska odlingar kan fortfarande hittas. Med hjälp av historiska kartor och dokument har vi återfunnit tio historiska humleodlingar från 1400- till 1700- talet där humleplantor kan återfinnas. Prov togs från ett femtontal plantor av varje population och genotypades med tio SSR markörer och en markör diagnostisk för kön. Dessutom genotypades 25 kloner av gamla lantsorter och sorter från Europa bevarade i genbanker. Resultaten visar på hög grad av klonalitet och låg frekvens av sexuell reproduktion inom populationerna. Två populationer hade markant högre genetisk diversitet och ett större antal haplotyper, i dessa populationer förekom också en blandning av han- och honplantor. Populationerna var alla tydligt differentierade från varandra och inga haplotyper förkom i mer än en population, vilket påvisar lågt utbyte av genetiskt material. Dessa resultat tyder på att naturlig spridning och genetisk rekombination är ovanligt eller långsamt i Sverige och att de nu förvildade växterna kan vara rester av de ursprungliga historiska odlingarna. Analysen av de europeiska klonerna från genbanker visade att flera kloner hade identiska genotyper och totalt sett begränsad genetisk diversitet. De svenska populationerna var i de flesta fall genetiskt klart särskiljbara från genbanksklonerna. Detta resultat motsäger historiska dokument som beskriver massiv introduktion av humlekloner från kontinentala Europa under 1800-talet och visar att denna import inte ersatte den humle som tidigare odlats. En möjlig bättre klimatanpassning av de ursprungliga svenska humlesorterna och en möjlig primitiv historisk förädling diskuteras.

Introduction

Hop (*Humulus lupulus* L.) is a climbing perennial plant. It is cultivated for the production of its cones, to be used in beer brewing. The plant is dioecious with separate male and female plants, and female plants are exclusively cultivated as they alone produce the cones. Male plants, in contrast, have throughout history been banned from the neighborhoods of hop yards (Barth et al., 1994; Bromelius, 1687). Propagation of hop under cultivation is mainly performed vegetatively, through cuttings, the plants are competitive and clones can potentially be very old. However, like many plants with a strong vegetative reproduction, in particular dioecious plants, questions regarding the longevity and population dynamics are yet unanswered (de Witte and Stocklin, 2010; Solé, 2003).

In beer producing countries in Europe hops was long an essential part of food supply. Historically, beer was not just a thirst-quenching beverage, but also a calorie rich food, a "liquid bread" (Barth et al., 1994). In the Nordic region, with its short growing season and cool and humid climate, it was often difficult to harvest cereals fully ripe and dry. Grain with pre-harvest sprouting could not be stored, but was instead used for brewing and thus not wasted. Hops did not only add flavor to the beer but also had preservative effects from substances produced by glands in the cone. Consequently, hop was during more than 1000 years an indispensable crop in Northern Europe (Unger, 2004).

It is not known how long hop has been cultivated in Sweden, but hop pollen from 7th century (Lagerås, 2003) and macro fossils from the 8th century (Heimdahl, 1999; Heimdahl, 2002) have been found. The earliest written historical primary information is from the 12th century (Tollin and Karlsson Strese, 2007). In Sweden, hop cultivation was mandatory for each

farmer and prescribed by law from 1414 till 1860. As a consequence of this law, hop cultivation was frequently reported in financial, juridical and many other historical documents. Historical sources from the 17th century and onwards, provide information about the introduction of hop cuttings into Sweden from areas mainly in current Germany, Poland and the Czech Republic (Bromelius 1687; Broocman 1736; Granhall 1951). During the 17-18th centuries hop culture was at its most extensive. During the 1800s extensive trials were conducted, often initiated by agricultural societies, who imported and planted large numbers of cuttings especially of the variety Saarz (Anonymous, 1858; Jonsson, 1883; Spaak, 1880). Cultivation of hops declined in Sweden towards the end of the 19th century. During World War I and II the access to imported hop was limited and a breeding program and cultivation was initiated in the southeast of Sweden (Granhall, 1951). After the wars hop cultivation ceased completely and the last commercial cultivation and breeding programs ended in 1959.

In the year 2000 the national program for diversity of cultivated plants (POM) was established in Sweden. The purpose was to ensure the long-term survival and sustainable use of cultivated plant resources in Sweden. The main tasks of the program are to inventory, collect and preserve clonally propagated cultivated plants. For the inventory of hop the wealth of historical source data was used to localize and verify remnants of cultivated hop (Karlsson Strese et al., 2010). At the location of many old hop yards, nowadays abandoned and covered by extensive forests, hop still grows untended by man.

A range of questions can be asked regarding the now feral hop populations and their development since active cultivation was discontinued. Although plants grow on the same sites as where historical cultivations once occurred, the plant material might have changed over time. Remnant plant material might also have been over stamped by later introductions

and re-plantations of hop yards. Another critical question is whether the populations have clonal growth, are the product of sexual propagation or a mix of the two, as is the presence of male plants and what impact they have on the genetic diversity of the populations.

Here we report of the examination of ten sites, described in historical documents from the 15th to 18th century and with remnant hop plants. Through genetic analyses we explore the origin and genetic composition of historical hop in Sweden and compare the genetic variation in Swedish remnant hop with a set of continental European landraces and varieties.

Materials and methods

Study populations

We searched for remnants of cultivated hops based on information from different historical sources such as large-scale maps from the 1630s and 1655, medieval charters and documents from the expeditions of Linnaeus in the 1730s and 1740s (Figure 1). The most important source was the map database GEORG, a digital primary source publication of original geometrical maps from 1630-1655 (Tollin, 2004). The database contains, besides the maps, transcriptions of the explanatory map text (*Notarium Explicatio*), e.g. number of hop gardens or number of hop poles. Coordinates for hamlets, villages and also thematic features like farmsteads and hop yards are specified in a Geographic Information System (GIS) and the information was transferred to a present day map and used for localization of remnant hop yards (Karlsson Strese et al., 2010). In total ten sites where hops grew abundantly were chosen for genetic analysis (Table 1, Figure 2). Although populations with male plants are rarely observed we actively choose to include two populations where no female plants were observed as contrasting material. All sites differed in shape and the occurrence of hop plants.

The estimated area that today is covered with hop varies from 25 to 200 m^2. As we aimed to explore the genetic composition of historical hop we specifically included isolated hop populations, such as population SWE9, found on an island, and SWE54 and I167 both located fairly isolated in woodlands. A total of 20 leaf samples were collected evenly distributed over the area of each site. Leaf tissue was stored in microcentrifuge tubes with silica gel until used for DNA extraction.

As a reference material, samples from 25 genebank clones representing older European landraces and cultivars were included (Table 2). These were kindly provided by the Hop Research Center Hüll, Germany and Hop Research Institute Co., Ltd., Žatec, Czech Republic. Some of the genebank clones had the same or similar names suggesting a common identity.

DNA extraction and SSR analysis

From each population, DNA was isolated from 14-16 individuals. From the genebank maintained clones DNA was isolated from a single individual of each accession. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen), E-Z 96 Plant DNA Kit (Omega Biotek Inc., Norcross, GA, US) or CTAB extraction.

SSR analysis was performed with ten markers (Table 3). PCRs were run using 1 U of DreamTaq DNA polymerase (Thermo Scientific), 1xDreamTaq buffer (Thermo Scientific), 0.2 mM of each dNTP (Thermo Scientific) and 0.1 µM each of forward and reverse primers respectively. Amplification products were analysed by capillary gel electrophoresis and confocal laser scanning on an ABI 3500xl Genetic Analyzer (Applied Biosystems, CA, US). Sizing of fragments was performed using the software GeneMapper version 4.0.

Data analysis

Genetic diversity, measured as Nei's h was estimated according to Nei (1973) using a purpose-written perl script. Principal component analysis (PCA) was carried out with the software R (R Development Core Team, 2007) using the *prcomp* command. In the PCA the number of copies of each allele at each locus for each individual were treated as independent variables. As random PCR failure will lead to genetically identical clones clustering apart in a PCA all individuals with missing data in one or more loci (N = 34) were deleted before PCA.

The software Structure v 2.2 (Falush et al., 2003; Pritchard et al., 2000) was used to analyze the genetic data for geographic clustering. We used a model with correlated allele frequencies among populations with no admixture. Non-amplifying markers were treated as missing data. The software was run with a burn-in length of 20 000 iterations followed by 50 000 iterations for estimating the parameters. This was repeated ten times for each K (the number of predetermined clusters) until the likelihood values for the runs no longer improved. The software CLUMPP v 1.1. (Jakobsson and Rosenberg, 2007) was used to compare the results of individual runs and to calculate similarity coefficients and the average matrix of ancestry. In CLUMPP the FullSearch algorithm was used for comparing runs with $K < 4$, whereas the Greedy algorithm was used for higher Ks. The number of clusters observed in the dataset was also evaluated by calculating ΔK according to Evanno et al. (2005) and by comparing the H' values calculated by CLUMPP. Graphical representation of the results was obtained using the DISTRUCT software v 1.1 (Rosenberg, 2004).

Results

Genotyping quality

A total of 150 samples from ten Swedish hops populations were genotyped for ten SSR markers. In addition we also genotyped 25 genebank preserved clones of older European landraces and cultivars. The markers used had between 83 and 98 % success rate and showed little indication of null alleles as would have been suggested by failure to amplify in some populations, but not in others. As hop is to a high degree clonally propagated detection of null alleles from departure from Hardy-Weinberg frequencies (excess homozygosity) is not possible. Although null alleles seem to be rare they cannot be ruled out as a cause of non-amplification in addition to random failure of amplification during the PCR process.

Distribution of genetic diversity suggests high within-population clonality

The majority of the studied populations consisted of a single haplotype with the exception of population SWE9 and I105 (two haplotypes each), SWE42 (five haplotypes) and SWE33 (seven haplotypes) (Table 4). Although rare, the none-amplifying loci due to random PCR failure for some individuals, could mask additional diversity. For that reason we also calculated the theoretical maximum number of haplotypes for each population. In this case the number of haplotypes in each population ranged from two in the populations SWE9 and SWE24 to nine in population SWE33 (Table 4). Genetic diversity (Table 4), calculated as Nei's h, was highly correlated with the number of haplotypes (c = 0.704 when assuming individuals with missing data had identical genotypes). The genetic diversity ranged from 0.250 in population SWE54 to 0.557 in population SWE33.

We compared all genotyped individuals pairwise to identify potential clonality, defined as two samples being genetically identical at all studied loci. In all cases the identified potential clonality was between individuals from the same population and no between-population potential clonality was observed.

Several of the studied genebank clones had identical genotypes for all the ten loci studied. Clones from the two genebanks with the same or similar names, i.e. Aromat (two samples), Fuggle (two samples), Saazer (two samples), Hallertauer Mittelfrüher and Hallertauer (one sample each), Hersbrucker Spät and Hersbrücker (one sample each) and Lubelski and Lupelski (one sample each), all had identical genotypes showing the consistency of our genotyping and scoring protocol. In contrast, the clones Spalter and Belgischer Spalter were not identical. A large group of clones, Saazer, Lubelski/Lupelski, Aromat, Spalter, Striesselspalt, Rannij and Svalöf 525-17, were all genetically identical to each other. A second group of identical genotypes consisted of Fuggle and Savinskij Golding.

Presence of male plants in feral populations

Genotyping with sex specific markers failed to give fully consistent results and thus needs to be interpreted with caution. However, two populations showed a high proportion of potential males: I105 (13 out of 15) and I167 (11 out of 15) which was consistent with visual sex determination. In an additional three populations genotyping with the sex specific marker suggested the presence of males: population SWE33 (2 out of 16), SWE40 (1 out of 15) and SWE42 (4 out of 14). In general, populations potentially including male plants tended to have higher genetic diversity (average 0.310 vs 0.253) and more haplotypes (average 3.2 vs 1.2) than did populations without males, although not significantly so (one-sided ttest, genetic diversity $p = 0.094$, haplotypes $p = 0.069$).

Strong population differentiation between hop populations

We explored population structure of the Swedish material using the software Structure. Structure uses a Bayesian clustering algorithm to assign individuals to a population, or, if of a

mixed ancestry, populations, based on their multilocus genotype. The software's assumption of within population Hardy-Weinberg equilibrium is highly violated in a clonally propagated plant such as hop. However, in the presence of both male and female hop plants, mating can be expected to be random within a population. The clonal propagation in between mating events mean we can view samples sharing the same genotypes ("clones") as a single, long-lived, repeatedly sampled individual and the clustering obtained through Structure analysis allows similarities between populations to be explored.

The H' value obtained from the software CLUMPP suggested K = 18 as the number of clusters best describing the data while ΔK supported 2 and 17 clusters. In the K = 17 model (Figure 3a) the high clonality within populations and differentiation between populations is very visible. Noteworthy are the two separate but related genotypes in population SWE9 and the mix of genotypes within SWE33 and SWE42. The model also suggests closer genetic relationship between SWE9 and I167. At lower number of clusters (data not shown) K = 2 clustered the populations in one cluster containing populations SWE9 and SWE40 and one containing populations SWE24, SWE42 and SWE54. At this level of clustering, the majority of the populations (SWE33, SWE36, I105, SWE45 and I167) showed a fairly evenly mixed clustering. When increasing the number of clusters to three, SWE9 and I167 clustered together as did SWE24 and SWE54. The later cluster remained intact until K = 6 while the SWE9 – I167 cluster remained intact also at higher levels of clustering. At K = 9 or higher population tended to cluster independently from each other although with varying degrees of mixed ancestry.

The inclusion of the genebank clones had little effect on the clustering of the Swedish populations. Genebank clones were grouped together up until K = 5, and clustered closely

with SWE36 up until K = 4 (Fig 3b). At K = 4 we noticed a similar clustering of SWE45 and the two genebank clones Hersbrücker and Hersbrucker spät.

We further explored the genetic structuring of the populations and genebank clones through Principal Component Analysis (PCA) (Figure 4). The first and second principal components explained 11.5 and 10.2 % of the variation respectively. PC1 separated most Swedish populations from the genebank clones. The exception is SWE36 which was distinctly separated from the other Swedish populations, but along PC2 also from the genebank clones. In the populations containing more than one genotype individuals clustered together, indicating close genetic similarity within these populations. In general the Swedish populations were more dispersed than the group of genebank clones although these represent a broad geographical area. Within the Swedish populations a subgrouping of SWE42, SWE54 and I105 was suggested.

High similarity between genebank clones

We used PCA to explore the relationships between the genebank clones studied. The first two PCs together explained 42.5 % of the variation (25.1 and 17.4 % respectively). The Hersbrücker clones clustered closely with Northern Brewer and Belgischer Spalter (figure 5). The Belgischer Spalter did, however, not show any close relationship with Spalter that instead belonged to the big group of Saazer-clones. A second, more dispersed cluster contained Serebrianker, Serebrianka, Urozani, Fuggle and Savinskij Golding, while a third loosely defined group contained the two Nordgaard clones (978 and 1478), Backa, Hallertauer Mittelfrüher and Hallertauer (figure 5).

Discussion

The majority of the studied remnant hop populations appear to consist of a single clone, in spite of growing over considerably large areas. These historical hop yards, where only a single clone is found can be explained by two alternative theories. Either, the hop yard was only planted once and never replanted with new material. If replantation has occurred we would instead expect to find a mix of clones. Alternatively, the sites might once have contained more genotypes but over time only one clone has survived and then spread asexually.

In the case of SWE9 two clones were found, though the two clones grow separately, about 200 m apart. The genetic similarity between the clones suggests that one of them arose from the other. For all loci the two clones share one allele but differ in the other, suggesting a parent - offspring relationship following an outcrossing event. However, today no sign of sexual reproduction is visible and the two clones reproduce solely vegetatively without physical contact with each other. In contrast, two populations (SWE33 and SWE42) consist of mixtures of genotypes, likely with both sexual and asexual reproduction. These two populations also contain both male and female plants according to both field observations and a sex identification DNA marker developed by Polley et al. (1997).

The DNA sex testing also suggested presence of both female and male plants in the populations SWE40, I105 and I167, contrary to field observations of SWE40 as a pure female population and I105 and I167 as pure male populations. The diversity in these three populations does not suggest any presence of sexual reproduction either. Likely, the few cases of plants with unexpected sex are the result of incomplete linkage between the genetic marker and actual sex determination gene of the plant. High but not complete linkage of the Polley et al. (1997) marker with sex was also reported by Patzak et al. (2002). The incompleteness of

sex identification markers (see also Danilova & Karlov, 2006) might be due to sex chromosome distortions and influence from autosomes on sex determination. In our case, sex markers might be used to identify populations as overall either male, female or mixed, but identification of individual plants should be interpreted with caution.

Generally, sexual reproduction of hop, appears to be relatively rare in Sweden. If pollen flow and seed spread were more common, the strict population differentiation observed here would not be seen. Even between populations fairly close geographically (e.g. SWE24 and SWE33; SWE36 and SWE40) we see no signs of exchange of genetic material. Other outbreeding crops in Sweden, such as rye (Hagenblad et al. 2012) and turnip (Persson et al., 2001) display exchange of genetic material over large geographic areas, high within-population diversity and low population differentiation. In *Arabidopsis lyrata*, a cross-pollinating and partially clonally propagating wild perennial plant in Scandinavia, genetic relationship between populations is highly correlated to geographical distance (Gaudeul et al., 2007). Horseradish (*Armoracia rusticana*), a mainly vegetatively propagated culture plant where little or no sexual reproduction occurs, also shows a distinct geographic distribution of genetic variation (Wedelsbäck Bladh et al., 2013). The Swedish hop populations contrast the seed-propagated crop species as well as the cultivated *Armoracia rusticana* and the wild *Arabidopsis lyrata* with a low level of sexual reproduction and probably man-facilitated migration of plants. Whether hop is to be considered as a wild plant or not in Scandinavia is a topic of debate (Karlsson Strese et al., 2012; Suominen, 1990). Our results, although from a small sample, suggest that natural spread and genetic recombination is uncommon or slow in Sweden.

Although the sites of the studied hops populations were found using historical sources, several centuries old, the age of the remnant plants is unknown. Many perennial plants can get very

old and there are examples of plant clones that are several thousands of years old (Molisch, 1938; Stebbins, 1958). Clonally propagated plants do not age in the strict gerontological sense. Aging is a function of meristeme determinacy in combination of cell death and as long as at least one apical meristem from the plant survives, the plant clone can persist from year to year (Thomas et al., 2000). Evolutionary studies on clonal species have been strongly limited by the difficulty in assessing the number, size and longevity of genetic individuals within a population (Arnaud-Haond et al., 2007). Hop is generally considered an extremely long-lived perennial (Korpelainen, 1998) but no records with dating are available. In this aspect the plants from population I167 "Hummelloken" are particularly interesting. This site was found in Northern Sweden in a today very isolated forested area at a high altitude. Only male plants were found on the site and this was mainly confirmed by the sex identification genetic marker. Interestingly, the same observation is noted on a map from 1765, where the site is indicated with the wording "Här växer humle vildt" (Here wild hop is growing). From old Swedish literature it can be inferred that "wild" hop refers to male plants wherease "tame" hops refers to the cone producing female plants (Bromelius, 1687; Broocman, 1736). It seems likely that, in the absence of female plants and sexual reproduction as well as the extremely low probability of seed spread, the site is still populated with the same clone as in 1765 when the map was drawn giving it an age of at least 250 years.

In spite of the massive introduction of hop cuttings from central Europe, primarily from the Saaz district, to Sweden during the 19th century (Cederborgh, 1861; Jonsson, 1883; Spaak, 1883) we find at the historical sites almost no trace of the introduced plant material, nor of the Nordic varieties bred in the early 20th century. The Central European clones and Nordic breeds all cluster separately from the Swedish populations (Figure 3, 4). The only exception is population SWE36 that segregates from the other Swedish population and more resemble the

cultivars. In this case the historical population might have disappeared to later be replaced by novel, foreign plant material. This situation is however rare and the 19th century plant introductions have not left any clear genetic footprint in the older historical hop yards. One reason could be that 19th century plantations were made on arable land whereas the older yards were place on remote land such as along trenches, fences and walls. The hop yards on arable land were eliminated when hops cultivation was discontinued in the 20th century, but the older yards remained (Tollin and Karlsson Strese, 2007). Another reason for the lack of central European hop genotypes could be unsuitable flowering time and maturation traits in these hop cultivars. In Sweden the growth season is short and early flowering and cone maturation is crucial for obtaining a harvest.

The majority of authors of older books on hops cultivation write solely about vegetative propagation. Only very few, like the Englishman Lance, recommend to raise plants from seeds, to then be able to select desired genotypes (Lance, 1837)). The practice to incorporate sexual progeny into stocks of vegetative propagules is common in many "traditional" farming systems (McKey et al., 2010). Possibly, also Swedish farmers tried to improve the hops clones and made an active selection for early maturity in the genetic variation that was available. We have observed in test cultivations (article in preparation) that many of the historical Swedish populations are significantly earlier maturing than Central European cultivars. Over the years this may have meant that the foreign material have not proven sufficient yield stability and have been culled from cultivations.

Our set of European landraces and cultivars show surprisingly little genetic variation in spite of the large geographical area of clone origin represented. With our set of ten SSR markers several of the cultivars were not distinguishable from each other. Genetic clustering (Figure 5)

reflect pedigree data (Table 2) and show how breeding rely heavily on a few clones (Saarz, Hersbrucker and Fuggle) that have been transferred over Europe and included in breeding efforts in several countries (Biendl, 2012). Our data also support the studies by Hartl and Seefelder (1998), Jakše et al. (2004) and Javornik et al. (2005) where limited genetic diversity within cultivated European hops was found.

A Swedish hops genebank is presently being established. This study provides important aspects on the choice of material to include for preservation. The extensive clonality within sites but high differentiation between sites suggest that, in order to save maximum genetic diversity, single plants from many locations should be preserved rather than many plants from a limited number of locations. On the other hand, collecting genetic material from a limited geographic area, such as for example the county Västergötland (the origin of the populations SWE36, SWE40, SWE45 and I105), might yield as much diversity as collecting material from the margin of the cultivation area. The genetic diversity found at the sites from Northern Sweden, for instance, is not more separated than other Swedish clones are from each other. The material described here is highly valuable to preserve both for historic and plant breeding purposes. A crucial plant trait for the successful cultivation of hops is the timing of maturation, a trait determined by complex genotype – temperature – light interactions (Biendl et al., 2012 and references therein), and cones that are not fully mature only yield a fraction of the substances desirable in brewing. The clear distinctiveness of the Swedish populations from the European material, possibly adapted to Nordic climatic light and temperature conditions, mean they provide a promising resource for local hops cultivation and breeding.

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Tables

Table 1. Populations included in the study with historical source reference and present status.

Population (genebank no)	**Location**	**Coordinates (WGS84[a])**	**Historical source**	**Year**	**Remarks**	**area (m^2) with hop plants today**
SWE9	Tåkenön, Sörmland	59.18682, 15.81976	Julita, Royal estate (Map, LSA[b] C35-62:1)	1735	Two sites	120 + 25
SWE24	Tryteke, Småland	56.55314, 14.75098	Karl Karlsson Gyllenhielms Atlas (Map, GEORG[c] Nya Bergkvara: 60)	1637		40
SWE33	Utnäs, Småland	56.55206, 14.75829	Karl Karlsson Gyllenhielms Atlas (Map, GEORG Nya Bergkvara: 59)	1637	Single male plant observed	200
SWE36	Ossala, Västergötland	58.23279, 13.49499	Gudhems parish (Map, GEORG P2: 6-7)	1642		110
SWE40	Böksnäs, Västergötland	58.62112, 14.27652	Medieval charters from the abbey of Vadstena	1447		105
SWE45	Håkanskila, Västergötland	57.54916, 12.41557	Öresten parish (Map, GEORG 56 B)	1649		25

I105	Hälsingegården, Västergötland	58.28369, 13.56688	Gudhems parish (Map, GEORG P2:63)	1642	No female plants observed	80
SWE42	Klockarberget, Västernorrland	62.27772, 17.36065	Carl von Linné, his diary during his Lapponian journey	1732		40
SWE54	Näs, Uppland	60.485664, 17.630632	"Tierp´s Jordebok" (Map, GEORG A3 227-228)	1640-41		25
I167	Humelloken, Jämtland	63.36417, 14.74161	Lits parish (Map, REG[d] 23-lit-71)	1765	No female plants observed	33

[a] WSG84 = World Geodetic System 1984

[b] LSA= Land Survey Board Archive - Lantmäteristyrelsens arkiv, http://www.lantmateriet.se/sv/Kartor-och-geografisk-information/Historiska-kartor/

[c] GEORG = Database of the oldest Geometrical Maps, http://www.riksarkivet.se/geometriska

[d] REG= Regional Archive - Lantmäterimyndigheternas arkiv, http://www.lantmateriet.se/sv/Kartor-och-geografisk-information/Historiska-kartor/

Table 2. Studied genebank clones. Materials were obtained from Hop Research Center Hüll, Germany (HRCH) and Hop Research Institute Co., Ltd, Zatec, Czech Republic (HRIZ).

Genebank	Name	Origin	Breeding history (reference)
HRCH	Aromat	former Czechoslovakia	Variety, bred in 1977. (EVIGEZ, acc. no 08X9000097)
HRCH	Belgischer Spalter		
HRCH	Fuggle	Great Britain	Variety bred in1861 of Mr Fuggle in Kent (Biendl et al., 2012)
HRCH	Hallertaue Mittelfrühe	Germany	Original landrace from Hallertau, (Barth, 2011)
HRCH	Hersbrucker spät	Germany	Landrace from Hersbruck (Barth, 2011)
HRCH	Lubelski	Poland	Landrace from Lublin, originating from Saazer (Barth, 2011)
HRCH	Northern Brewer	Great Britain	Variety bred in 1944 in Canterbury, cross East Golding x male seedling of Brewers Gold (Patzak, 2002)
HRCH	Saazer	Czech Republic	Landrace from Saaz
HRCH	Seribriancer	Russia	Selection from landrace of Siberian hop (Patzak, 2002)
HRCH	Urozâni	Russia	
HRIZ	Aromat	former Czechoslovakia	Variety, bred in 1977. (EVIGEZ, acc. no 08X9000097)
HRIZ	Backa	Serbia	Landrace from Backa Region, derived from Hersbruck (Patzak, 2002)

HRIZ	Fuggle	Great Britain	Variety bred in1861 of Mr Fuggle in Kent (Biendl et al., 2012)
HRIZ	Hallertauer	Germany	Landrace from Hallertau
HRIZ	Hersbrücker	Germany	Landrace from Hersbruck
HRIZ	Lubelski	Poland	Landrace from Lublin, originating from Saazer (Barth, 2011)
HRIZ	Nordgaard 1478	Denmark	Breeding material, cross Nordgaard978 x Bramling (EVIGEZ, acc. no 08X9000090)
HRIZ	Nordgaard 978	Denmark	Breeding material, cross Spalter x unknown (EVIGEZ, acc. no 08X9000089)
HRIZ	Rannij	Russia	
HRIZ	Saazer	Czech Republic	Landrace from Saaz
HRIZ	Savinskij Golding	Slovenia	Same genotype as Fuggle introduced in the 19th century (Biendl et al., 2012)
HRIZ	Serebrianka	Russia	Selection from landrace of Siberian hop (Patzak, 2002)
HRIZ	Spalter	Germany	Landrace from Spalt, same genotype as Saazer. (Biendl at al., 2012)
HRIZ	Striesselspalt	France	Landrace from Elsass, same genotype as Hersbrucker (Biendl et al., 2012)
HRIZ	Svalöf 525-17	Sweden	Breeding material (Saazer x Swedish male) (Granhall, 1951)

Table 3. Genetic markers used.

Marker	Reference	Annealing temperature (°C)	Type
STS	Polley et al. (1997)	54	male chromosome specific
GT1-K1-4	Jakše et al. (2008)	touchdown, 52-47 (-0.8°C/cycle)	SSR
GA4-P11-9	Jakše et al. (2008)	55	SSR
GA5-G3-10	Jakše et al. (2008)	55	SSR
GA7-I6-16	Jakše et al. (2008)	55	SSR
GA8-K15-4	Jakše et al. (2008)	55	SSR
HlGA27	Stajner et al. (2005)	touchdown, 56-52 (-0.6°C/cycle)	SSR
Hl-ACA3	Stajner et al. (2005)	touchdown, 52-47 (-0.6°C/cycle)	SSR
HlAGA1	Stajner et al. (2005)	touchdown, 52-47 (-0.6°C/cycle)	SSR
Hl-AGA6	Stajner et al. (2005)	55	SSR
Hl-AGA7	Stajner et al. (2005)	touchdown, 56-52 (-0.6°C/cycle)	SSR

Table 4. Measures of genetic diversity of the studied populations.

Population	**No of haplotypes**	**Theoretical max no of haplotypes**	**Genetic diversity (Nei's h)**	**Potential males[a]**
SWE9	2	2	0.525	
SWE24	1	2	0.350	
SWE33	7	9	0.557	2/16
SWE36	1	3	0.350	
SWE40	1	4	0.397	1/15
I105	2	4	0.350	13/15
SWE42	5	8	0.478	4/14
SWE45	1	3	0.348	
SWE54	1	4	0.250	
I167	1	8	0.450	11/15

[a] Plants suggested to be male through genotyping with sex specific markers.

Figures

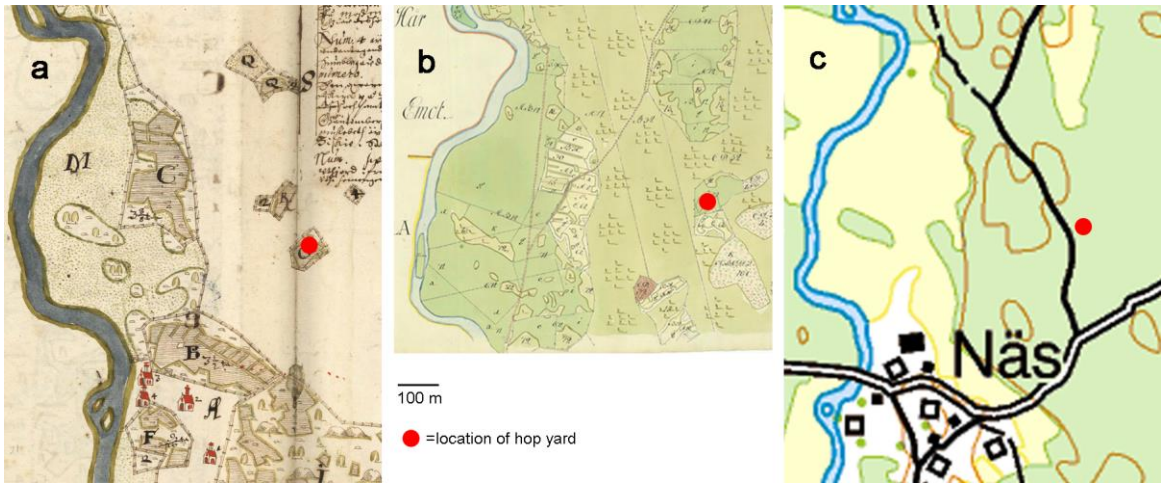

Figure 1. Strategy used for locating hops populations. Maps from different time periods showing historical hop yards are compared with modern maps to identify the location of the historical hop yard. Exampe from Näs (SWE54). a) Map from 1640-41 (GEORG: A3:227-29) where the hop yard was indicated. b) Map from 1800 (LSA: B81-14:5) used to locate persisting landscape features to enable transfer to the modern map. c) a modern topographic map from the 1980s. The hop yard is marked with a red dot on the different map generations.

Figure 2. Map of Southern and Central Sweden with studied populations indicated.

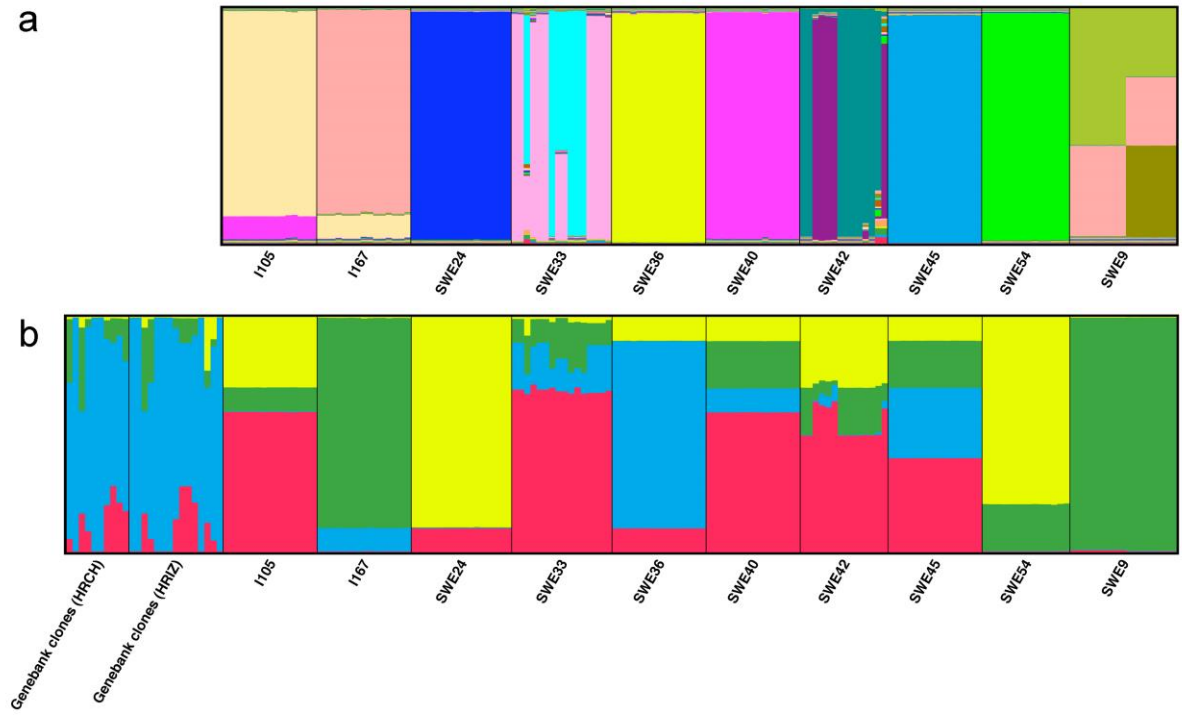

Figure 3. Clustering of hop individuals based on multilocus analysis using STRUCTURE. The individuals are organized by population. Each individual is represented by a vertical line divided into colored sections, each representing a different cluster. The length of each section is proportional to the estimated membership coefficient (Q) of the individual to each cluster. The black vertical lines are separators between the different populations. a) Swedish populations (150 individuals) at 17 clusters, b) Swedish populations and genebank clones (175 individuals) at 4 clusters.

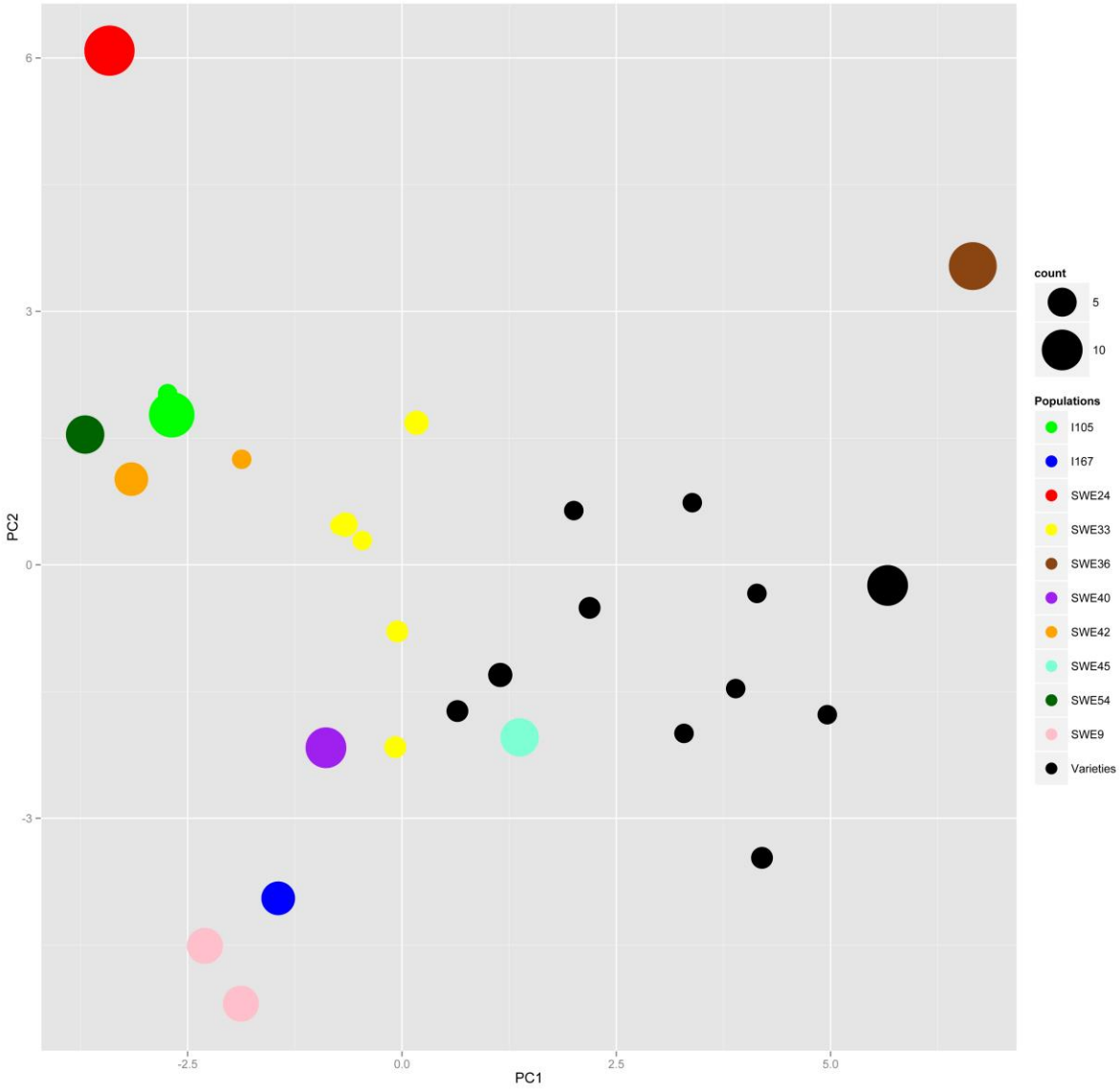

Figure 4. Plot of the 1st and 2nd components of a PCA analysis of all genotyped individuals with complete marker score. Each point is a genotype and size of the point relates to number of individuals with the genotype. The different colors refer to the populations as indicated in the legend.

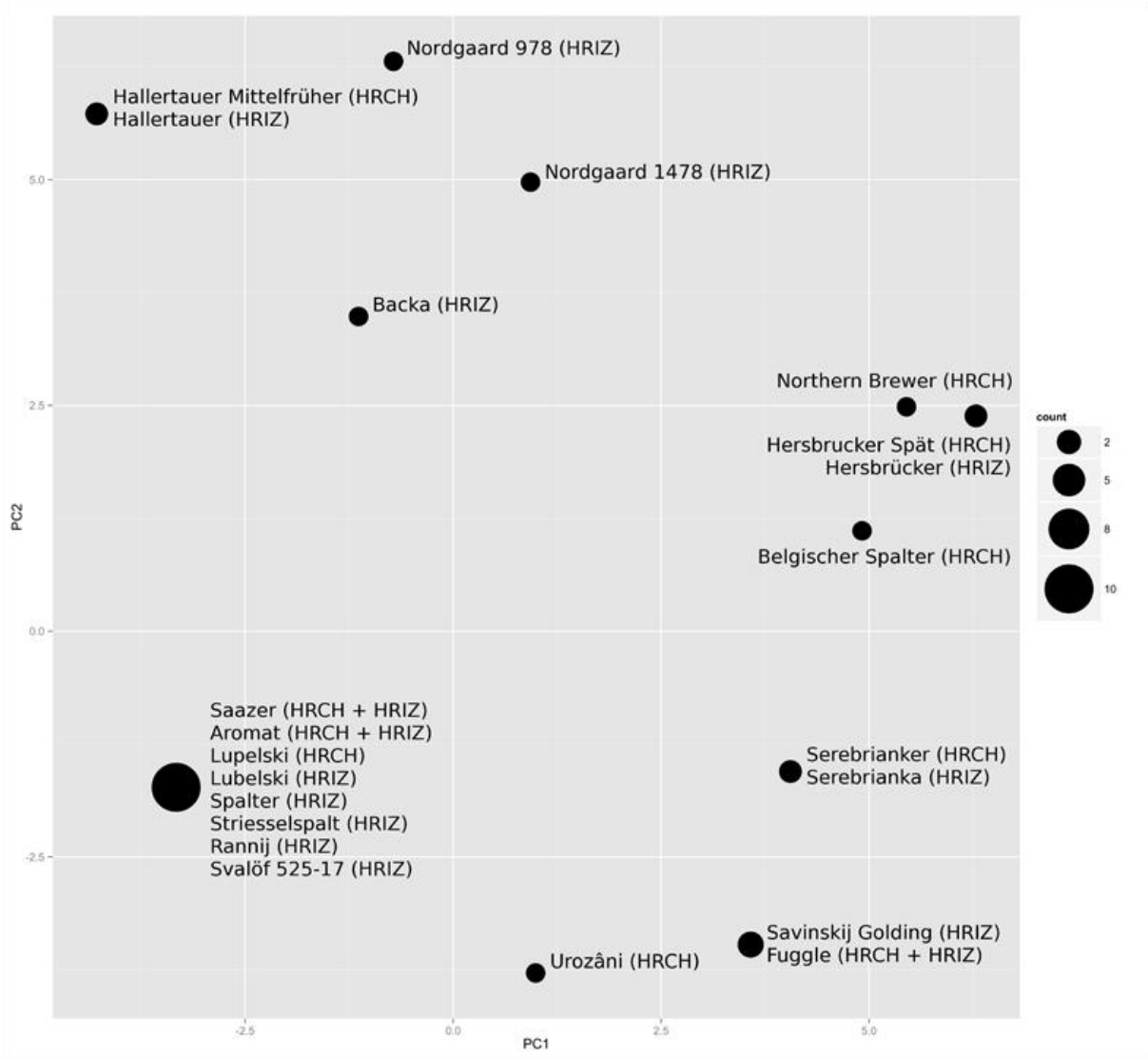

Figure 5. Plot of the 1st and 2nd components of a PCA analysis of the genebank clones. Each point is a genotype and size of the point relates to number of clones with the genotype.