Linköping Studies in Science and Technology
Dissertation No. 1959

Exploring Fennoscandian agricultural history through genetic analysis of aged crop materials

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Linköping 2018

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Linköping Studies in Science and Technology, Dissertation No. 1959

ISSN: 0345-7524

ISBN: 978-91-7685-194-4

Maria Lundström was funded by the Berit Wallenberg Foundation

Front cover: six-row barley (upper left), rye (upper right), turnip (lower left), emmer wheat (lower right).

Pictures: Painted by Henrietta Sjöberg. Digitalised by photographer Kentaroo Tryman; The Nordic Museum archives.

The digitalisation of the pictures was funded by the Berit Wallenberg Foundation and the Nanna Bergmansson, f. Wirén, Foundation, KSLA. For further information, see: Larsson, P. (2015). Sveriges kulturväxter - Ett ofullbordat praktverk. Bulletin För Trädgårdshistorisk Forskning. 22–24.

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“Success is the ability to move from one failure to another without loss of enthusiasm”

- Winston Churchill

Abstract

Crop plants have undergone a multitude of genetic changes during and following their domestication. The spread of agriculture brought the crops to new geographic regions exposing them to new environments and selection pressures along the way. This gave rise to many local variants with traits favoured both by agricultural practices and the environment.

Agriculture was introduced in Fennoscandia (Norway, Sweden, Finland and Denmark) around 4000 BC. The composition of the archaeobotanical record gives some clues as to which species were cultivated, but macroscale analyses rarely reach beyond that. Therefore, methods like genetic analysis are necessary to expand our knowledge about the history of crop cultivation. Under optimal conditions, DNA can survive in biological samples for several hundred thousand years. The preservation of plant specimens in the Fennoscandian climate has, however, rarely been explored. This thesis therefore attempts to dive deeper into the Fennoscandian cultivation history through genetic analyses of aged plant materials from both museum collections and archaeological sources. Cereal grains from a range of preservation conditions were evaluated to find which ones might be of interest for genetic investigations. Desiccated materials gave the highest success rates, in agreement with previous studies. Waterlogged materials appeared to contain small amounts of endogenous DNA, whereas genetic analysis of charred cereals failed completely in all samples.

Population structure was investigated in 17-19th century materials of both barley and rye from Sweden and Finland. Northern and southern populations of Finnish six-row barley were distinct from one another. In southern Sweden, genetic analysis suggested conserved population structure extending over 200 years. The genetic composition of rye also seemed mostly conserved, but rye did not show geographic population structure across the investigated region in Sweden and Finland.

A long-standing question in Fennoscandian crop history has been the interpretation of historical written records mentioning *Brassica* (cole crops, turnips and mustards), as well as the species identity of archaeobotanical finds of *Brassica* seeds. Thus, Next Generation Sequencing (NGS) was applied to identify which *Brassica* types that were cultivated in 17th century Kalmar, Sweden. The analysis corroborated morphological species classification in two of the investigated subfossil seeds, whereas no conclusions could be drawn from the remaining samples. The genome coverages were too low to allow subspecies identification.

Wheat has been cultivated in Fennoscandia since the introduction of agriculture but has increased dramatically in importance over the last century. The functional allele of the wheat nutrition gene *NAM-B1* was found to be particularly prominent in Fennoscandian wheats, likely associated with its effect on grain maturation time. Here the evolutionary history of *NAM-B1* was investigated to see if it could truly be considered a domestication gene as suggested in a previous study. By studying extant landrace materials of Mediterranean tetraploid wheat, it was found that the non-functional allele showed signs indicative of a selective sweep. This selection did not, however, appear to have occurred during domestication.

In conclusion, aged plant specimens from both museum and archaeological contexts could contribute greatly to our knowledge about historical cultivation, extending the investigated period into the mid 17th century. Subfossil and waterlogged archaeobotanical materials do contain endogenous DNA, suggesting that they are better suited for genetic analysis than charred ones, at least as far as cereals are concerned. There is potential for classifying archaeological *Brassica* remains using NGS, even though further optimisation of sample and library preparation may be necessary. And finally, despite *NAM-B1* showing signs of selection it should not be considered a domestication gene in tetraploid wheat.

Populärvetenskaplig sammanfattning

Genom arkeologiska fynd av bevarade växtdelar, främst frön, går det att avgöra vilka slags växter som odlades vid olika tidsperioder under historien. De arkeologiska fröfynden i sig säger dock sällan mer än vilken art som odlades. Vilka ytterligare egenskaper växterna hade, samt information om släktskap och ursprung, kan sällan utrönas. Upptäckten att också gamla "döda" material kan innehålla DNA har öppnat upp nya vägar att erhålla mer kunskap om dåtidens jordbruksväxter. Genom tekniska framsteg har möjligheterna att analysera också gammalt och skadat DNA förbättrats avsevärt under de senaste tre decennierna.

Genetiska studier av jordbruksväxternas historia har ofta fokuserat på domesticering, alltså processen där vilda växter tämjdes och medvetet började brukas av människan. Även hur växterna spridits över världen och de genetiska förändringar de genomgått som anpassning till nya miljöer och användningsområden i samband med denna spridning har undersökts. De historiska jordbruksväxterna i Skandinavien och Finland har dock mestadels hamnat utanför dessa studier. De första arterna, som korn och vete, introducerades i de sydligaste delarna redan för ca 6000 år sedan. Bristen på genetiska studier av arkeologiska växtfynd från Skandinavien och Finland kan till viss del tillskrivas vårt klimat som medför att de torkade växtmaterial som anses vara bäst lämpade för ändamålet är få på dessa breddgrader. Därför förbises skandinaviska och finska material ofta helt, trots att jordbruksväxterna just på grund av denna nordliga utbredning långt ifrån deras ursprungsområden i östra medelhavsområdet och Asien potentiellt kan bära på särskilda egenskaper som reflekterar deras särskilda historia.

Denna avhandling fokuserar därmed på genetiska studier av växtmaterial primärt från Sverige och Finland. Här undersöks bland annat vilka typer av åldrade växtmaterial som kan vara av intresse för genetiska analyser, samt vilken typ av genetisk information som kan utvinnas ur dessa. Framförallt undersöks material av korn, men också råg, vete och kålväxter behandlas.

I avhandlingen konstateras att de sällsynta fynd som görs av torrt bevarade frömaterial är värdefulla källor för genetiska studier, både sådana som härstammar från arkeologiska källor samt sådana som bevarats i herbarier och museisamlingar. Också frömaterial som bevarats subfossilt, alltså i blöta syrefria miljöer, visar sig kunna bevara DNA i hundratals år. Däremot är sådant DNA kraftigt påverkat av nedbrytning. Samtliga försök att analysera DNA från frömaterial som bevarats i förkolnad form misslyckades.

Vidare visas att korn från slutet av 1600-talet som härstammar från området kring Lund i Sverige är genetiskt mycket snarlikt 200 år yngre korn från samma sydliga region. Detta passar också in med ett tidigare observerat mönster i korn från Skandinavien och Finland där breddgrad är mer avgörande för hur närbesläktade korn från olika geografiska områden är än ursprungsland. Också inom finska kornmaterial noterar vi en skillnad mellan nordliga och sydliga populationer. Sådana geografiska skillnader kan däremot inte upptäckas i råg, trots att också rågen uppvisar få genetiska skillnader under tidsperioden från mitten av 1600-talet till slutet av 1800-talet då den moderna växtförädlingen inleddes. Därefter vidtog odling av förädlade rågsorter med ursprung från andra delar av Europa, vilket lämnade synbara genetiska spår i råg från tidigt 1900-tal.

Också kålväxter har varit viktiga grödor i Nordeuropa, och enligt arkeologiska fynd har kålväxter i någon form odlats i de sydligaste delarna av Sverige sedan åtminstone 400 e. Kr. Gruppen kålväxter är dock mycket bred och inkluderar alltifrån ätliga rötter som rova och kålrot till grönsaker som vitkål, grönkål, kålrabbi och blomkål samt växter som primärt odlas för oljeproduktion såsom raps och rybs. Skriftliga källor beskriver odling av rovor, svartsenap alternativt senap och någon slags kål med ätliga blad redan från medeltid och framåt, men tolkningen av texterna och dåtidens namn och klassificeringar är mycket osäker. Längre än så sträcker sig inte kunskapen om vilka kålväxter som faktiskt odlades. Därför undersöks här möjligheterna att med genetiska metoder identifiera vilka typer av kålväxter som odlats historiskt. I försöket används subfossilt bevarade kålfrön från en 1600-tals trädgård i Kalmar. Två prover visar sig innehålla gammalt DNA som matchar kålväxter, och särskilt stora likheter återfinns mot den grupp som innefattar rova och rybs. Detta är också vad som konstaterats, med viss osäkerhet, utifrån fröernas utseende. Däremot förhindrade provernas dåliga kvalitet ytterligare bestämning av exakt typ av kålväxt. Resultatet tyder ändå på att subfossilt bevarade kålfröer kan bevara DNA i flera hundra år. Med ytterligare justeringar av metoden och noggrant val av prover skulle det alltså kunna vara möjligt att framöver identifiera vilka typer av kålväxter som odlades.

I denna avhandling studeras också näringsgenen "*NAM-B1*" i emmer- och durumvete. Denna gen förekommer i både funktionella och icke-funktionella varianter, där den funktionella varianten har konstaterats vara sparsamt förekommande i moderna vetesorter och är särskilt sällsynt utanför Skandinavien och Finland. Två icke-funktionella varianter förekommer. Dessa icke-funktionella varianter av *NAM-B1* har föreslagits ha spelat en viktig roll under perioden då vete domesticerades då de samtidigt som de minskar näringsinnehållet också ökar kärnans storlek. Större kärnor anses vara en egenskap bönderna lätt kunde känna igen och främja genom medvetet urval. Ett sådant urval skulle inte bara påverka *NAM-B1*-genen, utan även den omgivande genetiska regionen. Här presenteras bevis som stödjer att icke-funktionell *NAM-B1* främjats i urvalsprocessen. Om detta inträffat i samband med emmervetets domesticering skulle man dock väntat sig att icke-funktionell *NAM-B1* varit dominerande i den odlade formen av emmervete, vilket inte tycks vara fallet. Sannolikt inträffade således detta urval senare under vetets odlingshistoria.

Sammantaget visas i denna avhandling att åldrade växtmaterial med ursprung i Skandinavien och Finland absolut uppvisar potential för att kunna analyseras genetiskt. Tidsperioden som omfattas av genetiska studier av åldrade växtmaterial från regionen kunde därmed expanderas från sent 1800-tal till tidigt 1600-tal. Frömaterial bevarade i torkade och subfossila tillstånd visar båda tecken på att innehålla gammalt DNA. Därför föreslås att framtida studier fokuserar på frömaterial bevarade under dessa förhållanden snarare än förkolnade, då dessa inte tycks bevara DNA tillräckligt väl, åtminstone gällande spannmål. Särskilt torkade material visade sig fungera bra för genetiska analyser, och kunde även nyttjas för jämförande studier på populationsnivå. De små genetiska förändringar som noterades inom både korn och råg mellan 1600-talet och slutet 1800-tal är särskilt intressanta, då jordbruket och de metoder som tillämpades annars genomgick stora förändringar under tidsperioden.

List of publications

Paper I

Lundström M., Forsberg N.E.G., Heimdahl J., Hagenblad J., Leino M.W. (2018) Genetic analyses of Scandinavian desiccated, charred and waterlogged remains of barley (*Hordeum vulgare* L.). *Journal of Archaeological Science: Reports* 22: 11-20. DOI 10.1016/j.jasrep.2018.09.006

Paper II

Lempiäinen-Avci M., Lundström M., Huttunen S., Leino M.W., Hagenblad J. (2018) Archaeological and historical materials as a means to explore Finnish crop history. *Environmental Archaeology*. DOI 10.1080/14614103.2018.1482598

Paper III

Larsson P., Oliveira H., Lundström M., Hagenblad J., Leino M.W. Population genetic structure in Fennoscandian landrace rye (*Secale cereale* L.) spanning 350 years. *Manuscript submitted to Genetic Resources and Crop Evolution*

Paper IV

Lundström M., Hagenblad J., Heimdahl J., Leino M.W. Next Generation Sequencing of Swedish 17th century subfossil *Brassica* seeds. *Manuscript*

Paper V

Lundström M., Leino M.W., Hagenblad J. (2017) Evolutionary history of the *NAM-B1* gene in wild and domesticated tetraploid wheat. *BMC Genetics* 18(1): 118. DOI 10.1186/s12863-017-0566-7

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Not included in thesis:

Karlsson Strese E.M., Lundström M., Hagenblad J., Leino M.W. (2014) Genetic Diversity in Remnant Swedish Hop (*Humulus lupulus* L.) Yards from the 15th to 18th Century. *Economic Botany* 68(3): 231-245. DOI 10.1007/s12231-014-9273-8

Vanhala T., Normann K.R., Lundström M., Weller J.L., Leino M.W., Hagenblad J. (2016) Flowering time adaption in Swedish landrace pea (*Pisum sativum* L.). *BMC Genetics* 17(1):1-13. DOI 10.1186/s12863-016-0424-z

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Introduction

Cereal crops like wheat, barley and rye make up a large part of the overall calorie intake in humans and are important sources for animal feed. The other group of plants discussed in this thesis, of the genus *Brassica*, provides both cruciferous vegetables as well as oilseed crops. The importance of these crops in Fennoscandia can be traced through history, both in written records and even further back in time through archaeological finds.

In archaeobotany, a lot of knowledge can be gained from observing patterns and changes on the macroscale level, ranging from geographic and temporal distribution of crops down to more specific seed traits that can, for example, allow the distinction of domesticated species from wild. Technological development brought the insight that there might be more to these materials than what first meets the eye. Certain biomolecules, like DNA, can survive for several hundred thousand years under the appropriate environmental conditions. By accessing this information, we can study the spread of different crops in much greater detail, and even catch glimpses of the changes they underwent over time.

Crop domestication

Humans' transitioning from hunter-gatherer lifestyles to living in agricultural communities was an important step to form the human societies leading up to the modern era. This change in lifestyle also affected the crops, where some traits that are beneficial for plants during life in the wild were abandoned resulting in the domesticated crops.

Traits that were commonly affected during domestication of crops, and thus making up the plant "domestication syndrome" (Hammer, 1984), are: loss of natural seed dispersal, free-threshing grain, increased yield (e.g. seed size), changes in plant architecture (e.g. affecting lateral branching and tillering), improved palatability and loss of both seed dormancy and sensitivity to photoperiod as well as similar environmental cues (Harlan, *et al.*, 1973; Doebley, 2006; Purugganan and Fuller, 2009; Olsen and Wendel, 2013a; Olsen and Wendel, 2013b; Abbo, *et al.*, 2014b). Loss of seed shattering has been most widely studied in cereals, even if it is also found in peas and lentils (reviewed in Doebley, 2006; Abbo, *et al.*, 2014b), and the genes involved have been identified in rice (Konishi, *et al.*, 2006) and barley (Pourkheirandish, *et al.*, 2015). Another important trait that can be traced in archaeobotanical specimens is that of seed size, where wild grains are smaller than domesticated (Willcox, 2004). The disturbed "man-made" soils have been suggested to select for larger grain sizes in cereals (reviewed in Purugganan and Fuller, 2009).

Domestication of crops began at different times in different locations around the world; in the Near East (e.g. wheat, barley) some 12,000 years ago, in the Andes (e.g. potato, quinoa), China (e.g. rice, foxtail and broomcorn millet), Mesoamerica (e.g. maize, common bean) and Near Oceania (e.g. banana) around 10,000 years ago, in sub-Saharan Africa (e.g. sorghum, pearl millet) around 8,000 years ago and in eastern North America (e.g. sunflower) around 6,000 years ago (reviewed in Doebley, *et al.*, 2006; Meyer and Purugganan, 2013). The archaeological

record suggests that at least cereals were cultivated for 500-2,000 years prior to their domestication (Willcox, 2004; Fuller, 2007; Fuller, *et al.*, 2011; Riehl, *et al.*, 2012; Willcox, 2013).

There has been a lengthy debate about the details of the domestication process; whether it was protracted with polyphyletic or admixed ancestry (Smith, 2007; Purugganan and Fuller, 2009; Fuller, *et al.*, 2010; Allaby, *et al.*, 2015), or relatively quick with mostly monophyletic ancestry (Abbo, *et al.*, 2011; Heun, *et al.*, 2012; Abbo, *et al.*, 2014a; Abbo and Gopher, 2017). Polyphyletic ancestry means that domestication of a certain species occurred more than once, whereas admixed ancestry means that the domesticated species was formed from a large, dynamic ancestral population. Monophyletic ancestry means that domestication of a species occurred once, and all domesticated plants of a particular species can be traced back to this one origin.

Another aspect of this debate has concerned the strength of selection pressures acting on the plants during domestication. Advocates for a fast domestication process emphasize the importance of the ancient farmers' abilities to select for the desired "domestication" traits, and under such circumstances selection would be strong (Abbo, *et al.*, 2011; Abbo, *et al.*, 2014a; Abbo and Gopher, 2017). Support for this was found when it was revealed that use of sickle tools could select for non-shattering in cereals over a time period as short as 20-200 years (Hillman and Davies, 1990).

This argument has been met with evidence showing that the evolutionary rate in cereals during domestication would have been similar to, or lower than that, of natural selection (Fuller, 2007; Purugganan and Fuller, 2009). Judging by the archaeological evidence, domestication traits gradually became more common, further supporting slow domestication of cereals (e.g. Tanno and Willcox, 2006). The mechanisms involved in natural selection and domestication appear to be similar, only that the domesticates adapted to environments created and controlled by man (reviewed in Allaby, *et al.*, 2015). Hence, it has been proposed that domestication should be considered a co-evolutionary process between plants and humans, where humans provided niches that certain plant communities adapted to (reviewed in Zeder, *et al.*, 2006; Smith, 2007; Purugganan and Fuller, 2009; Fuller, *et al.*, 2010; Allaby, *et al.*, 2015).

As for the human side of the suggested co-evolution the relationship has developed over thousands of years, and Allaby, *et al.*, (2015) argues that the relationship with plants is one thing that separate hominins from other hominid lineages. Judging by hominin consumption patterns of plants with similarity to today's crops, some level of dependence on plants can be deduced that precede domestication by over 100,000 years (Mercader, 2009; Henry, *et al.*, 2014). Consumption of starchy plants, including grass seeds thought to have been of very low importance due to high labour costs associated both with collection and processing, has been confirmed in early modern humans as well as in Neanderthals by study of 10-130 thousand-year-old specimens of dental calculus and traces found on tools (Henry, *et al.*, 2011; Henry, *et al.*, 2014; Power, *et al.*, 2018). Changes in early hominin diets have been associated with reductions in the gastrointestinal system and increased brain sizes (Aiello and Wheeler, 1995). The exact role of plants in such a diet in early hominin history is difficult to make out (Fontes-

Villalba, *et al.*, 2013), but it has been suggested that plant foods might have contributed to the observed changes in hominins (Sponheimer, *et al.*, 2013a; Sponheimer, *et al.*, 2013b).

Genomic effects of domestication

The drastic changes that came with the domestication of plant crops left their mark in the plant genomes. This is in part due to that only a subset of individuals from the wild populations contributed to the domesticated populations (reviewed in Doebley, 2006). The resulting genetic bottleneck allowed for only some of the genetic diversity found in the wild population to be retained in the domesticates (reviewed in Olsen and Wendel, 2013a; Hufford, *et al.*, 2012; Wales, *et al.*, 2018).

In addition, selection for specific traits during domestication resulted in low genetic diversity in the genetic region(s) controlling those traits. Linkage disequilibrium (LD), where loci at linked sites are non-randomly associated with each other, means that selection acting on one locus also affects the other linked loci (reviewed in Slatkin, 2008). This is reflected in the genomes as selective sweeps where selection for the targeted trait, appearing in the form of reduced genetic diversity in comparison to unlinked sites, extends into the surrounding genomic region (Smith and Haigh, 1974; Nielsen, 2005; Hufford, *et al.*, 2012; Cavanagh, *et al.*, 2013). Recombination will act to decrease the amount of LD in the genomes, so whether the species is outcrossing or self-fertilizing would influence the genomic signatures of selection (e.g. Hartfield, *et al.*, 2017). Selection strength is also a major determinant of the size of the sweep (e.g. Olsen, *et al.*, 2006).

Many of the traits involved in the plant domestication syndrome have been shown to be under the control of only a few genes, so called “domestication genes”, or genomic regions of major impact (reviewed in Meyer and Purugganan, 2013; Olsen and Wendel, 2013b). Transcription factors appear to be overrepresented as targets for selection during domestication (Doebley, 2006; Meyer and Purugganan, 2013; Olsen and Wendel, 2013b). Development of new techniques and methods have allowed for better detection of genetic regions that contribute to the phenotypes, and an increasing number of regions with minor contributions have been reported (reviewed in Ross-Ibarra, *et al.*, 2007; Olsen and Wendel, 2013a).

Landraces

Once the crops had been domesticated, they spread from their regions of origin into new environments. The expansion of agriculture to Northern Europe and Fennoscandia from the Near East has been cause for some debate. It could either have occurred via transmission of cultural techniques (e.g. Morelli, *et al.*, 2010), or migrating farmers brought them along (e.g. Pinhasi, *et al.*, 2005), or possibly even a combination of the two.

In the area around the Baltic Sea and Fennoscandia it has become evident that hunter-gatherers and farmers co-existed for almost a millennium during the Neolithic. Further, the hunter-gatherers do not appear to be the direct ancestors of modern Scandinavians suggesting

that a major turnover in the human population has occurred in this region since the Neolithic (Malmström, *et al.*, 2009). Genetic analysis has shown that the ancient farmers were more closely related to other Central European farmer populations than the Northern European hunter-gatherers (Skoglund, *et al.*, 2012; Malmström, *et al.*, 2015; Mittnik, *et al.*, 2018), supporting the idea that agriculture in this region was introduced together with immigrating people.

Dispersal brought the crops to new locations, exposing them to new selection pressures enforced both by environment and climate, but also agricultural practises (Camacho Villa, *et al.*, 2005). This process is called differentiation (reviewed in Meyer and Purugganan, 2013) and the resulting, often locally adapted, plant populations are referred to as “landraces”. Contrary to present-day varieties, landraces are domesticated crops that have not undergone formal improvement. They were formed by the farmers recurrently saving part of the harvest to use as seed in the following year. For that reason, landraces are well suited for studies of crop domestication and cultivation history as the genetic traces would not be masked by those induced during improvement.

Improvement effects resulted from selection and breeding using only a small number of plants with the desired traits, followed by seed marketing and distribution over large areas outside of the region and traditions in which the original landraces formed (Jones, *et al.*, 2008b). Studies based on landrace materials have, for example, been used to examine local adaptation to climate (Jones, *et al.*, 2008a; Jones, *et al.*, 2011; Aslan, *et al.*, 2015) and population structures in order to determine the origins and spread of different crops on local scale (Pecetti and Damania, 1996; Papa, *et al.*, 1998; Leino, *et al.*, 2013; Forsberg, *et al.*, 2015) or over larger distances (Hunt, *et al.*, 2011; Jones, *et al.*, 2011; Oliveira, *et al.*, 2012a).

Mass selection of landraces for improvement purposes began during the first half of the 19th century and more systematically towards the end of the same century (Jones, *et al.*, 2008b). Many landraces were completely replaced by elite cultivars during the 20th century following the ‘Green Revolution’ of the 1960’s (Khush, 2001; Evenson and Gollin, 2003), and even sooner, around the 1930’s, for some cereals in parts of Sweden (Börjeson, *et al.*, 2014; Leino, 2017).

The traces of these events can be seen in both oat and wheat where varieties available prior to the 1960’s have higher genetic diversity than cultivars released later (Fu, *et al.*, 2003; Roussel, *et al.*, 2005; Cavanagh, *et al.*, 2013). Similarly, landrace broccoli (*Brassica oleracea* var *italica*) has been shown to carry 8.4 times as many unique alleles as its improved counterpart (Stansell, *et al.*, 2018). This pattern is less clear in barley, where reduced diversity can only be found to affect certain traits, whereas overall diversity has not changed significantly when comparing 19th century and modern varieties of Nordic and Baltic materials (Kolodinska Brantestam, 2005). Landraces and varieties preceding the extensive breeding following the 1960’s have thus been suggested to be valuable sources of genetic material for future breeding programs (Camacho Villa, *et al.*, 2005; Jones, *et al.*, 2008b; Winfield, *et al.*, 2018).

Aged, dead materials in genetic analyses: the field of ancient DNA

There have been plenty of studies comparing extant materials of modern crop varieties to their wild counterparts in order to better understand domestication, plant adaptation and diversification (e.g. Purugganan, *et al.*, 2000; Hufford, *et al.*, 2012; Weller, *et al.*, 2012; Choi, *et al.*, 2017; Hardigan, *et al.*, 2017; von Wettberg, *et al.*, 2018), often based on materials from germplasm collections. What should be kept in mind when using extant materials to trace historic events is that these only reflect what made it through to current time (Fuller, *et al.*, 2011). In addition, plant materials preserved in genebanks have been shown to not always reflect the true diversity levels of the species (e.g. Hagenblad, *et al.*, 2012b). To further improve our knowledge of the history of crop cultivation other sources of plant materials are needed, like those found during archaeological excavations or in herbaria and museum collections. These materials have normally, due to age or storage conditions, lost the ability to germinate (Leino, *et al.*, 2009) meaning that the study of them has to rely on other methods, like analysis of information stored in their genetic code.

Archaeogenetics, the study of ancient DNA (aDNA), is a field that began with Higuchi, *et al.*, (1984) reporting successful cloning and sequencing of DNA from a 140-year-old quagga skin. The term ancient is in this context referring mostly to the poor biochemical condition of the aged DNA in the samples, rather than the absolute ages of the samples themselves (Shapiro and Hofreiter, 2014). Technological advances have been pivotal to the expansion and further development of the field in order to face the challenges of working with low concentrations of degraded and damaged DNA.

A major leap forward came with the development of Polymerase Chain Reaction (PCR) for amplification of the DNA fragments by targeting specific genomic regions (Mullis, *et al.*, 1986; Pääbo and Wilson, 1988). Using PCR for amplifying aDNA got off to a shaky start due to difficulties in distinguishing endogenous DNA from contaminating sequences (e.g. Golenberg, *et al.*, 1990; Sidow, *et al.*, 1991; Lindahl and Poinar Jr, 1993; Woodward, *et al.*, 1994; Zischler, *et al.*, 1995), but routines for handling such problems were consequently developed (Pääbo, *et al.*, 1989; Austin, *et al.*, 1997; Cooper and Poinar, 2000; Poinar, 2003; Gilbert, *et al.*, 2005). These entailed such actions as wearing appropriate protective attire during lab work, only working with these materials in dedicated aDNA facilities free from PCR products and modern DNA, cleaning of area and equipment using bleach and UV irradiation, using negative controls to detect contaminants introduced during handling of the samples, emphasizing the importance of repeatability of results, and applying a critical mindset when analysing and evaluating data generated from ancient sources.

Another major step forward came with the development of Next Generation Sequencing (NGS) and its application to aDNA (Green, *et al.*, 2006; Poinar, *et al.*, 2006; reviewed in Brown, *et al.*, 2014; Shapiro and Hofreiter, 2014; Hagelberg, *et al.*, 2015; Hofreiter, *et al.*, 2015; Gutaker and Burbano, 2017). This allowed for sequencing of all available DNA in the sample, further increasing the need for appropriate routines for sample handling (Llamas, *et al.*, 2017).

All available DNA in a sample might not originate from the sample itself, but some of it will come from the environment in which it was stored and residues introduced during the

collection and processing (Gansauge and Meyer, 2014). In ancient samples, the proportion of endogenous DNA can be very low depending on the sample material type and preservation condition. Yet, some NGS libraries created from these materials have been shown to contain 80-95% of endogenous DNA (e.g. Green, *et al.*, 2010). The DNA is normally degraded into fragment sizes <250 bp, where most fragments fall within the length span of 30-100 bp (Green, *et al.*, 2008; Weiß, *et al.*, 2016; Kistler, *et al.*, 2017).

Under the right conditions, DNA can survive for several hundred thousand years as shown in some deep ice cores and bone samples (e.g. Willerslev, *et al.*, 2007; Orlando, *et al.*, 2013). Low temperatures, as well as dry or anoxic environments, appear to slow down DNA decay (reviewed in Lindahl, 1993; Kistler, *et al.*, 2017).

In addition to the short fragment lengths, damages accumulate in DNA as it decays (reviewed in Lindahl, 1993) resulting from oxidation and hydrolytic deamination (reviewed in Pääbo, *et al.*, 2004; Dabney, *et al.*, 2013; Gutaker and Burbano, 2017). Some of these modifications will prevent amplification of the DNA, whereas others are known to cause nucleotide misincorporations, primarily in the form of C-to-T substitutions in the 5'-ends of the fragments and corresponding G-to-A substitutions at 3'-ends (Hofreiter, *et al.*, 2001; Briggs, *et al.*, 2007). These substitutions create a pattern that can be used to authenticate aged DNA using modern technology (Krause, *et al.*, 2010; Ginolhac, *et al.*, 2011).

Some studies suggest that certain tissue types preserve DNA better than others, where aDNA from bone samples seem to be more resilient to degradation than aDNA from plants (Weiß, *et al.*, 2016). Among the plant specimens, seed and grain appear to preserve DNA better than leaf and stem in herbaceous species, at least judging by historic specimens (Walters, *et al.*, 2006; Lister, *et al.*, 2008). Successful analysis has, however, been reported from wood (e.g. Gómez-Zeledón, *et al.*, 2017; Lendvay, *et al.*, 2018; Wagner, *et al.*, 2018), pollen and environmental samples (reviewed in Parducci, *et al.*, 2017).

Genetic analysis of ancient plant remains: from multi-copy regions to whole genomes
Genetic regions located within organellar genomes (chloroplast or ribosomal DNA) have often been targeted for PCR amplification and subsequent analysis in archaeobotanical specimens (e.g. Hofreiter, *et al.*, 2000; Willerslev, *et al.*, 2003). As these are found in multiple copies per cell, it is considered more likely to come across intact regions within these DNA fragments in ancient samples than in nuclear DNA. It has further been suggested that nuclear DNA might degrade faster than chloroplast DNA (Weiß, *et al.*, 2016). Multi-copy regions are often conserved within taxa, making them into useful targets for species classification of archaeobotanical remains (e.g. Pollmann, *et al.*, 2005; Elbaum, *et al.*, 2006; Gismondi, *et al.*, 2012).

Nuclear regions have also been the target for traditional PCR-based analyses. The wheat high molecular weight (HMW) glutenin alleles have been used to evaluate sample quality and discriminate between tetraploid and hexaploid wheat species (Brown, *et al.*, 1994; Oliveira, *et al.*, 2012b; Fernández, *et al.*, 2013). In maize, multiple nuclear regions have been targeted in

order to study the early history of maize domestication and its subsequent dispersal (Goloubinoff, et al., 1993; Freitas, et al., 2003; Jaenicke-Després, et al., 2003; Lia, et al., 2007). In rice, both chloroplast and nuclear regions have been utilized to find the origin of rice grown in northern Japan and genetic changes that it underwent after its arrival (Tanaka, et al., 2016).

The introduction of high-throughput methods, like pyrosequencing and Kompetitive Allele Specific PCR (KASP) (Sønstebo, et al., 2010; Pruvost, et al., 2012), and their consequent application to ancient remains permitted a more thorough survey of ancient plant genomes. KASP has been used to study allele distribution in ancient populations of barley across Europe revealing geographic structuring (Lister, et al., 2013), and on materials from the Canary Islands where the same local barley varieties were shown to have been grown for centuries (Hagenblad, et al., 2017). NGS methods like large-scale parallel pyrosequencing has, for example, been applied in studies of species composition in ancient environments (Jørgensen, et al., 2012; Pedersen, et al., 2013). Massive parallel shotgun sequencing, often the method meant when referring to NGS these days, allow analysis of all DNA fragments in both single specimens and environmental samples (see e.g. Parducci, et al., 2017), and can be combined with capture assays to enrich the samples for regions of interest (Carpenter, et al., 2013; Enk, et al., 2014).

Use of shotgun sequencing has increased our knowledge about the domestication and early cultivation history of barley (Mascher, et al., 2016) and sunflower (Wales, et al., 2018), telling a story of an early domestication bottleneck and further loss of diversity over the past century in the latter. Sequencing of ancient maize has provided insight into the timing of genetic changes, showing that a 5310-year-old sample of maize was genetically closer to modern maize than its wild progenitor teosinte (Ramos-Madrigal, et al., 2016). The technology has also aided in resolving the dispersal routes for bottle gourds to explain how African, Asian and American bottle gourds relate to each other (Kistler, et al., 2014). By comparing chloroplast genomes from Cucurbita specimens, several locations for independent domestications have been suggested (Kistler, et al., 2015).

However, despite that the technological developments have permitted analysis of an increasing number of genetic regions with gradually higher resolution, archaeobotanical remains only make up a small fraction of the total number of aDNA studies (Green and Speller, 2017; Parducci, et al., 2017). This could, in part, be due to the large genome sizes of many plants. The haploid genome size of barley has been estimated to 5.3 Gb (IBGSC, 2012), and in hexaploid wheat the corresponding number is 15-16 Gb where over 85% is repetitive DNA (Appels, et al., 2018). That makes the genome of hexaploid wheat over five times as large as that of humans.

The history of crop husbandry in Sweden and Finland

Agriculture was introduced in southern Sweden with the Neolithic around 4000 BC (Myrdal and Morell, 2011). It retreated for a period around the middle Neolithic, but remained in the southernmost parts of Sweden (Rowley-Conwy and Legge, 2015). Dependence on cultivation then gradually increased and around 1100 BC it became an important food source (Myrdal and

Morell, 2011). In Finland, pollen records suggest the first introduction around 4000 BC (Alenius, *et al.*, 2017), but cultivation appears to have taken longer to become fully established (Lahtinen and Rowley-Conwy, 2013; Lahtinen, *et al.*, 2017). Cultivation, at low levels, can be continuously traced in the pollen records as of 1500 BC (Lahtinen, *et al.*, 2017). The earliest Finnish macrofossils of charred barley grains have been dated to 1690-1260 cal BC (Vuorela and Lempiainen, 1988; Asplund, 2008).

The species and variant composition of the agricultural crops changed over time, both because of climate change but also as a consequence of changing cultivation practises (Grabowski, 2011; Myrdal and Morell, 2011; Grabowski, 2013). Technological advancements, like fertilizing the soils with manure and the use of specialized sickles (500 BC), and later on (AD 1000-1700) even ploughing, allowed the establishment of permanent fields and expansion of crop cultivation in Sweden. In Finland, the archaeological evidence suggests regular use of slash-and-burn cultivation around AD 600, with intensification of swidden farming as of AD 930 in the south-eastern parts (Alenius, *et al.*, 2013). Expansion of cultivation from southern Finland has been debated, where evidence suggesting expansion as late as the Medieval period (AD 1300-1600) (e.g. Taavitsainen, *et al.*, 1998) has been proposed to result from a lack of written documentation preceding this time rather than a lack of cultivation (Lahtinen, *et al.*, 2017). Instead, Iron Age (AD 100 and onwards) is suggested to be an important period for the expansion of cultivation in Finland.

The agricultural revolution during the period AD 1700-1870 (Olsson and Svensson, 2010) brought technological advancements allowing more efficient production as well as further expansion of the areas that could be utilized for cultivation, with subsequent increases in production. The following period from 1870 to present time included industrialisation and modern plant breeding, and cultivation where the locally adapted landrace crops mostly was replaced by commercially available elite cultivars (Camacho Villa, *et al.*, 2005; Jones, *et al.*, 2008b; Börjeson, *et al.*, 2014).

Barley

Barley (*Hordeum vulgare* L.) is a diploid species, primarily self-fertilizing (Brown, *et al.*, 1978; Wagner and Allard, 1991) and fairly hardy as it can grow in many different environments, including the colder temperatures in the north (Newton, *et al.*, 2011). Genome size has been estimated to about 5.3 Gb, with the full genome being composed of $2n = 2x = 14$ chromosomes (IBGSC, 2012). Genetic evidence suggests that barley underwent multiple domestication events (Morrell and Clegg, 2007; Dai, *et al.*, 2012; Ren, *et al.*, 2013; Wang, *et al.*, 2016), or possibly was formed from a mosaic of wild progenitors from a wide geographic region (Poets, *et al.*, 2015). One of the geographic origins for domesticated barley was located in the Near East where domestication is estimated to have occurred some 10,000 years ago (Zohary, *et al.*, 2012). Another region of importance to barley domestication was located on the Tibetan Plateau (Dai, *et al.*, 2012; Ren, *et al.*, 2013; Wang, *et al.*, 2016).

The spikes of barley come in different forms; two-rowed or six-rowed, depending on the number of fertile spikelets on each of the rachis nodes. Each node has three spikelets, and the nodes are positioned on alternate sides of the central rachis. If only the central spikelet is fertile, meaning that one spikelet per node fills up and forms a grain, it results in the ancestral two-rowed spike. The six-rowed spike results from all spikelets being fertile, a trait shown to be under control of several *Vrs*-alleles (Komatsuda, *et al.*, 2007; Koppolu, *et al.*, 2013; Casas, *et al.*, 2018). The size of the lateral spikelets is further modified by *INT-C*, which also appears to be associated with the level of tillering (the production of side-shoots) in two-row barley (Ramsay, *et al.*, 2011).

Barley is further classified as being either hulled or free-threshing. This relates to traits of the mature barley grains that are either entirely encased by an adhering hull (hulled barley) or are free-threshing from the hull (hulless or naked barley). The latter form results from a deletion in the *nud* gene (Taketa, *et al.*, 2008).

Barley was one of the earliest crops cultivated in Fennoscandia. It spread from the Near East to the Balkans around 6,000 years ago (Bogucki, 1996). From there it continued along the Mediterranean coast to north-western Europe and via another route along the Danube and Rhine river valleys through central Europe to the northern plains (Bogucki, 1996; Davison, *et al.*, 2006). This expansion pushed for adaptation to the changing climate (Jones, *et al.*, 2012; Jones, *et al.*, 2013; Pasam, *et al.*, 2014). Such adaptation came, for example, in the form of loss of daylight responsiveness in many northern barleys under control of the *Ppd-H1* gene (Turner, *et al.*, 2005; Jones, *et al.*, 2008a; Lister, *et al.*, 2009), as well as temperature tolerance and vernalization requirements under influence of *VRN* (Francia, *et al.*, 2004; von Zitzewitz, *et al.*, 2005; Cockram, *et al.*, 2007; Aslan, *et al.*, 2015).

At first mostly naked barley was grown in southern Fennoscandia, but hulled barley came to make up a notable part around 1000 BC (Myrdal and Morell, 2011; Kirleis, *et al.*, 2012; Grabowski, 2014). This was also around the time when cultivation expanded northward along the eastern coast in Sweden. Then, over just a few hundred years, hulled barley became the crop dominating cultivation in Fennoscandia and world-wide (Lister and Jones, 2013). Hulled barley remained the most important cereal crop throughout the Middle Ages and early modern period. In some parts it is the most important cereal even today (Jansson, 2011).

Six-row barley was dominating in early cultivation. Two-row varieties, despite this being the ancestral form found in wild barley, was not described in the literature until 17th century but likely came to this region earlier (Hjelmqvist, 1996). Judging by the literature, two-row barley was also grown in Finland during the 17th century (Gadd, 1770). It was, however, not widely used until the agricultural revolution in the 19th century, except in Öland and Gotland where two-row barley has dominated at least since the 18th century (Leino, 2017).

Figure 1. The different wheat species and how they relate to each other. Mutations are shown as arrows within the same ploidy level, and hybridisations as arrows between ploidy levels.

Wheat

Wheat (*Triticum* spp.) was also domesticated in the Near East, about the same time as barley. There are multiple wheat species, including diploid, tetraploid and hexaploid types, some of which have been domesticated. Tetraploid wheats have $2n = 4x = 28$ chromosomes, with the genome structure AABB to symbolize its allotetraploid origin, and a genome size estimated to >10 Gb (Avni, *et al.*, 2017). Hexaploid wheats have the genome structure AABBDD, with $2n =$

6x = 42 chromosomes, and an estimated genome size of >15 Gb (Zimin, *et al.*, 2017; Appels, *et al.*, 2018).

Wild wheat species are found both among the diploid and tetraploid wheats, where the allotetraploid wild emmer wheat is the ancestor of the tetraploid and hexaploid wheats commonly grown today, see Fig. 1. As in the case of barley, the events leading to domestication of tetraploid wheats has been debated where both monophyletic (Haudry, *et al.*, 2007; Luo, *et al.*, 2007) and polyphyletic (Feldman and Kislev, 2007; Thanh, *et al.*, 2013) ancestry has been suggested. Yet another theory proposes admixed origin of domesticated wheat (Civáň, *et al.*, 2013). The free-threshing hexaploid wheats, e.g. bread wheat, arose from a domesticated free-threshing tetraploid wheat crossing with the wild diploid *Aegilops tauschii* (genome: DD) (Huang, *et al.*, 2002; Dvorak, *et al.*, 2012). Following that event some of the hexaploid wheats, like spelt wheat, regained the hulled phenotype (Dvorak, *et al.*, 2012).

The free-threshing trait has been shown to be under the control of the genes *Q* (Simons, *et al.*, 2006) and *Tg* (Faris, *et al.*, 2014). The mutation resulting in the *Q* allele conferring the free-threshing trait only appear to have occurred once, probably in tetraploid wheat, and then spread via hybridisation into hexaploid wheat (Simons, *et al.*, 2006). In addition, *Q* has been found to also influence spike shape, rachis fragility and glume toughness but both the mutated *Q* and *Tg* are required to allow the kernels to fully separate from the otherwise adherent glumes (Kerber and Rowland, 1974; Faris, *et al.*, 2014). Other important traits in domesticated wheats are the non-brittle rachis that prevent shattering of grains at maturity, where the *Br-A2* and *Br-A3* genes are major contributors (Nalam, *et al.*, 2006), and grain size which is influenced by multiple genes (Gegas, *et al.*, 2010).

Einkorn and emmer wheat have, according to archaeological evidence, been grown in Sweden since about 4000 years BC (Ericson, *et al.*, 2009; Grabowski, 2011). Hexaploid wheat was introduced around 2200 BC in the form of spelt wheat, and during the Iron Age free-threshing hexaploid wheat gradually replaced most other wheats (Viklund, 1998; Grabowski, 2011). A small proportion of the cereal cultivation, at least in southern and central Sweden, seem to have been dedicated to wheat throughout history (Leino, 2017), but production increased dramatically as of the 20th century (Jansson, 2011; Börjeson, *et al.*, 2014). This was an effect of wheat improvement and changed cultivation practises allowing effective wheat cultivation in this region, as well as technological development allowing cultivation to become industrialised.

Rye

Same as barley and wheat, rye (*Secale cereale* L.) is also a cereal with a suggested origin in the Near East (Khush, 1963; Behre, 1992). Initially it probably grew as a weed in the wheat and barley fields and was therefore likely domesticated after these other two cereals. Rye is a diploid, outcrossing crop with an estimated genome size of 7.9 Gb with $2n = 2x = 14$ chromosomes (Bartoš, *et al.*, 2008; Bauer, *et al.*, 2017). It is a hardy crop that grows well also on poor soils. Winter rye has even been shown to be more drought tolerant than winter varieties of both wheat and barley (Schittenhelm, *et al.*, 2014).

Rye was introduced in Sweden around year 500 BC (Grabowski, 2011), and possibly around the same time in Finland as indicated by the pollen records (Alenius, *et al.*, 2009). Rye cultivation gradually increased during the late Iron Age (AD 800-1100) in Sweden, being more commonly grown in the south-eastern parts of the country than in the south-west (Viklund, 1998; Grabowski, 2011). Rye production remained at a relatively low level until around AD 1500 when it became so common that it made up more than half of the harvest in the eastern parts of Sweden.

A similar increase of rye cultivation is found in Finland leading up to the medieval period (Alenius, *et al.*, 2013). Unlike in Sweden, where rye cultivation was often associated with permanent fields and crop rotation together with barley (reviewed in Grabowski, 2014), in Finland it appears to have been cultivated through slash-and-burn cultivation to a much higher extent (Alenius, *et al.*, 2013). From the early modern period until the beginning of the 20th century rye was the economically most important cereal in Sweden and the main cereal for bread-making (Börjeson, *et al.*, 2014; Leino, 2017)). Rye was even more important in Finland, and for a long period Finland was a major exporter of rye seed to Sweden (Ahokas, 2009). In both Sweden and Finland rye was also the major crop for cultivation in slash-and-burn agriculture.

Brassica

The genus *Brassica* contains a diverse group of agronomically and horticulturally important crops that are grown worldwide for many different purposes. There are multiple *Brassica* species, some considered natural variants and others that result from intentional breeding (Al-Shehbaz, *et al.*, 2006). Some of these crops are grown as cruciferous vegetables, utilizing the leaves as vegetables as is the case for the cabbages (*B. oleracea* var. *capitata*), see Fig. 2. In others it is the inflorescences that are used, as in broccoli (*B. oleracea* var. *italica*) or cauliflower (*B. oleracea* var. *botrytis*), or the enlarged stem like kohlrabi (*B. oleracea* var. *gongylodes*). Some brassicas are grown for their roots, like swede (*Brassica napus* subsp. *napobrassica*) and turnip (*B. rapa* subsp. *rapa*), and yet others are oilseed crops like rape (*Brassica napus* subsp. *oleifera*) and turnip rape (*Brassica rapa* subsp. *oleifera*), see Fig. 3.

The relationship between the different natural *Brassica* species was described by Nagaharu U (1935) in “the triangle of U”. There it was shown that the three diploid species *B. rapa* (genome: AA), *B. nigra* (genome: BB) and *B. oleracea* (genome: CC) hybridized and gave rise to the three allotetraploid species *B. juncea* (genome: AABB), *B. napus* (genome: AACC) and *B. carinata* (genome: BBCC). However, also the now diploid species result from a whole-genome triplication that was followed by reshuffling and losses of genetic material (Parkin, *et al.*, 2005; Chalhoub, *et al.*, 2014; Lin, *et al.*, 2014). This means that there is a lot of synteny among the different *Brassica* genomes, even between the above-mentioned natural species (Liu, *et al.*, 2014; Yang, *et al.*, 2016a; Yang, *et al.*, 2016b).

Figure 2. Examples of *Brassica oleracea* subspecies. From left to right: Cabbage (*B. oleracea* var. *capitata*), kale (*B. oleracea* var. *sabellica*) and brussels sprouts (*B. oleracea* var. *gemmifera*). Both cabbage and kale are grown for their edible leaves, whereas in brussels sprouts it is the lateral shoots that are consumed. Photo: Matti Leino

Figure 3. Examples of *Brassica napus* (left) and *Brassica rapa* (right) subspecies. Upper left: rape (*B. napus* ssp. *oleifera*). Upper right: turnip rape (*B. rapa* ssp. *oleifera*). Lower left: swede (*B. napus* ssp. *napobrassica*). Lower right: turnip (*B. rapa* ssp. *rapa*). Both the rape and turnip rape are grown as oilseed crops, whereas the swede and turnip are grown for their enlarged roots. Photo: Matti Leino.

The three diploid *Brassica* species described in the triangle of U are outcrossing, whereas the allotetraploids are to a fairly large extent inbreeding (Gupta, 2013). The estimated size of the *B. rapa* genome is 442.9 Mb with $2n = 2x = 20$ chromosomes (Wang, *et al.*, 2011; Zhang, *et al.*, 2018). The *B. nigra* genome has an estimated size of 591 Mb with $2n = 2x = 16$ chromosomes (Yang, *et al.*, 2016a). The last of the three natural diploids, *B. oleracea*, has $2n = 2x = 18$ chromosomes with an estimated genome size of around 630-648 Mb (Liu, *et al.*, 2014; Parkin, *et al.*, 2014) of which 587 Mb have been sequenced and assembled into a pangenome (Golicz, *et al.*, 2016).

B. nigra can be found growing as an annual herb on the stony beaches around the Mediterranean, suggesting that it might originate from this region (Dixon, 2006). Genetic analysis of *B. rapa* accessions representing different morphotypes placed the domestication of this species in the region around Europe/ West Asia/ North Africa, whereas cultivation and breeding in other parts in Asia are suggested to have contributed to the diversification (Guo, *et al.*, 2014). Another suggestion is that *B. rapa* was domesticated twice; once in Europe leading to turnip and turnip rape, and again in China resulting in types like pak choi (Song, *et al.*, 1990). Based on the current existence of wild-growing *B. rapa* in the region of Iran/ Irak/ Turkey, this has been the proposed area of origin (Dixon, 2006).

For *B. oleracea*, two alternative locations have been suggested as origins for the domesticated species: either on the European Atlantic cliffs or in the area around the Mediterranean. Traditionally it is the European Atlantic cliffs that have been associated with *B. oleracea* domestication. However, linguistic analysis and study of ancient literature propose that domestication in the Mediterranean region is more accurate (Maggioni, 2015; Maggioni, *et al.*, 2018). If so, *B. oleracea* escaped from cultivation and became “re-naturalized” once it arrived at the European Atlantic area, which accounts for the occurrence of this species in this region.

Brassica cultivation seems to have reached Northern Europe with the expansion of the Roman Empire (Heimdahl, 2010; Rohde Sloth, *et al.*, 2012). Judging both by written sources and the archaeological record, brassicas remained important agricultural and horticultural crops from sometime around AD 1-400 (Roman Iron Age) and into the 19th century (Larsson and Ingemark, 2015; Hallgren, 2016). The exact *Brassica* types grown are only partially known from the literature and archaeobotanical record. The types that can be recognised among the archaeological samples as some of the early species introduced to Northern Europe are: cole crops (*B. oleracea*), black mustard (*B. nigra*), turnip (*B. rapa*) and rape (*B. napus*). Additionally, according to written sources swedes (*B. napus subsp. napobrassica*) have been grown in Finland since 17th century and since 18th century in Sweden (Ahokas, 2002). Even today brassicas are important in oilseed production, and several *Brassica* species are common horticultural crops.

General aims

Evaluate Fennoscandian archaeobotanical cereal remains from different preservation conditions for their potential in genetic studies

Study temporal patterns of population structures across Fennoscandia to learn more about the cereal cultivation history of this region

Find a method that allows for genetic species and subspecies identification of archaeobotanical specimens of the historically important horticultural crops belonging to the genus *Brassica*

Find out if there is a selective sweep targeting the wheat nutrition gene *NAM-B1*, and resolve its status as a suggested domestication gene

Results and Discussion

The study of genetic information from extant plant material can tell us a lot about agricultural history and the evolution of crop plants; i.e. through comparisons between wild species and their domesticated counterparts either in the form of landrace materials or modern improved varieties (e.g. Doebley, *et al.*, 1997; Burke, *et al.*, 2002; Burke, *et al.*, 2005; Paper V). If one only draws conclusions based on extant materials, that essentially represent what has made it through to present time, there is a risk that one misses relevant pieces of information. By adding information retrieved from aged plant materials it is possible to tell a more complete story about the spread and development of crop cultivation and the ability of crops to adapt to new environments (e.g. Leino, *et al.*, 2013; Roullier, *et al.*, 2013; Kistler, *et al.*, 2014; reviewed in Bieker and Martin, 2018).

Fennoscandia represents one of the northernmost expansions of crop cultivation. Previous studies have shown that both the barley and rye grown in this region are genetically distinct from the ones grown in southern and central Europe (Jones, *et al.*, 2011; Hagenblad, *et al.*, 2016). For barley, it has been shown that these structures were present already in late 19th century (Aslan, *et al.*, 2015; Forsberg, *et al.*, 2015) but the time-depth of these structures beyond that has remained unknown. One of the limiting factors has been the lack of suitable historical plant materials available for genetic analysis. The climate in Europe is hardly optimal for long-term preservation of archaeobotanical materials as it is neither dry allowing preservation through desiccation, nor constantly frozen that would also slow down degradation. Thus, Fennoscandian plant remains fall into a category of materials that has been mostly overlooked in genetic studies.

DNA preservation in Fennoscandian cereals from different conditions

Charred

Plant materials preserved through charring are by far the most common archaeobotanical materials (Dennell, 1976). Their prevalence in the archaeobotanical record in combination with them often being morphologically well-preserved has made them into attractive targets for genetic analyses. Genetic analyses of such materials have, however, produced mixed results (Boscato, *et al.*, 2008; Bunning, *et al.*, 2012; Oliveira, *et al.*, 2012b; Bilgic, *et al.*, 2016; Castillo, *et al.*, 2016). Nistelberger, *et al.*, (2016) evaluated charred materials of barley, grape, maize and rice using whole-genome sequencing with and without capture, which showed that the chances of successful analysis are exceptionally low and hardly worthwhile. They further suggested that the proposed endogenous DNA in previous studies of charred cereals could be spurious and should not be trusted.

This thesis adds to the growing list of studies reporting failure to analyse charred cereal grains. Paper I included 46 charred barley grains and Paper II included 27 charred barley grains, where all either failed to produce PCR products of the short multi-copy marker located within the P6 loop of the chloroplast *trnL* intron region or did not generate a nucleotide sequence

matching that expected from *Hordeum*. Seven of the charred Finnish grains from Paper II also underwent KASP genotyping but failed entirely.

It would appear like the level of charring needed to prevent degradation by microorganisms in soil also leaves the cereal grains devoid of endogenous DNA (Threadgold and Brown, 2003; Braadbaart, 2004). There is still the possibility that remains from other types of plant species could preserve DNA better than cereals, as indicated by a partially charred corncob (Nistelberger, *et al.*, 2016) and successful genetic analysis of charred peas (Smýkal, *et al.*, 2014).

Waterlogged

For waterlogged archaeobotanical remains there are several reports of successful analysis from seeds and pits with strong fruit endocarps like grape, olive and apple (Manen, *et al.*, 2003; Elbaum, *et al.*, 2006; Cappellini, *et al.*, 2010; Schlumbaum, *et al.*, 2012; Wales, *et al.*, 2016). Few have attempted to analyse waterlogged cereal grains, and of the studies found none had done so successfully (Brown, *et al.*, 1993; Fernández, *et al.*, 2013).

In total, 19 samples of waterlogged barley were analysed (Paper I). Of these, two samples (both assemblies of grain fragments) could be successfully identified as *Hordeum* by genetic barcoding through PCR amplification of the P6 loop of *trnL*. KASP genotyping was, however, not very successful as genotype calls could only be obtained from 11% of the investigated SNPs in the most successful waterlogged sample, and in 0-2% in the other samples. Even though some waterlogged materials of cereal grains did appear to contain endogenous DNA, the levels were too low for efficient KASP genotyping. More sensitive methods would be required to generate enough data for population level analyses. As genetic sequence matching *Hordeum* could be found in some of the specimens, which was not possible in charred, this still suggest that waterlogged archaeobotanical materials could be of interest in future studies.

Desiccated

Desiccated plant materials are without doubt the archaeobotanical material used most successfully in genetic analyses (e.g. Li, *et al.*, 2011; Oliveira, *et al.*, 2012b; Hagenblad, *et al.*, 2016; Li, *et al.*, 2016; Mascher, *et al.*, 2016). This type of material is, however, very scarce in the Fennoscandian archaeobotanical record. The nine barley grains and six rye grains recovered from the grave of bishop Peder Winstrup, who died in December 1679, therefore represent a rare opportunity to analyse such materials with a Scandinavian origin.

Out of the nine desiccated barley grains (Paper I), five were identified as *Hordeum* with PCR-based methods targeting the P6-loop of *trnL*. With KASP, success rates ranged from 8 to 94% for the individual samples and 54% overall. The Winstrup barleys have also undergone whole-genome sequencing on Illumina HiSeq2500 (paired-end, 2x125 bp), with double-stranded library construction according to Meyer and Kircher (2010) with slight modifications as described in Nistelberger, *et al.*, (2016) (unpublished results). Following bioinformatic analysis, including removal of adapters (AdapterRemoval2.0; Schubert, *et al.*, 2016), alignment

to the barley reference genome (Ensembl Plants: Hv_IBSC_PGSC_v2) with BWA aln (Li and Durbin, 2009) and seeding disabled as described in Schubert, *et al.*, (2012), and removal of duplicate reads (Picard: MarkDuplicates), it was found that the individual libraries had endogenous DNA contents ranging from 0 to 31%. These reads also presented the expected DNA degradation patterns when tested with mapDamage2.0 (Jónsson, *et al.*, 2013), further supporting their authenticity.

The three samples with the highest amounts of endogenous DNA (17-31%) on the Illumina HiSeq2500 were also the most successful in KASP genotyping (81-94% of SNPs successfully genotyped) and produced *trnL* PCR-fragments matching *Hordeum* following standard sequencing, see Table 1. This strongly suggest that DNA survival can vary greatly between samples found within the same archaeological context and emphasises the importance of including multiple specimens in genetic analysis whenever possible.

Interestingly, the desiccated rye specimens also obtained from the Winstrup grave (Paper III) were not as successful in KASP analysis as the barley, despite identical age and storage conditions. This appears true even when success rates are re-calculated to be more directly comparable, where 68 out of 90 SNPs (75%) gave genotype calls in barley when only keeping

Table 1. Comparison between methods used to analyse the 17th century desiccated barley samples originating from Bishop Peder Winstup’s grave. NGS: Percentages shown are the proportion of library reads mapping to the barley reference genome relative to the total number of reads obtained from each sample. KASP: Percentages are given as the total number of SNPs that produced a genotype call in the consensus sample (combining results from sample duplicates). PCR and standard sequencing: The data in this column is based on whether the resulting PCR fragments targeting the P6 loop of *trnL* could be identified as *Hordeum* or not.

Sample ID	NGS: Endogenous DNA content	KASP: Proportion of successfully genotyped SNPs	Identified as <i>Hordeum</i> by PCR and standard sequencing
Win.mat_1	31%	94%	Yes (x2)
Win.mat_2	2%	36%	Yes (x1)
Win.mat_3	17%	92%	Yes (x2)
Win.mat_4	0%	8%	No
Win.mat_5	23%	81%	Yes (x1, + BLAST hits against other grasses in the same tribe as <i>Hordeum</i>)
Win.mat_6	3%	59%	No
Win.mat_7	8%	67%	No
Win.pill_1	12%	71%	Yes (x1, + BLAST hits against other grasses in the same tribe as <i>Hordeum</i>)
Win.pill_2	5%	55%	No
Neg. control	0%	0%	No

SNPs where at most one out of the five samples included in population analyses failed. This would give individual barley success rates (within these 68 SNPs) of 90, 96, 100, 100 and 100% respectively. The corresponding numbers in rye was 21 out of 38 SNPs (55%) with individual success rates of 13, 34 and 53% for each of the three successfully analysed samples.

It is possible that this is some species-specific trait, or that the husk still attached to the barley grains could provide some protection against DNA degradation that the naked rye grains lacked. Species-associated differences in DNA preservation have been noted in historic museum specimens, but between legumes and cereals rather than between different cereal species (Leino, *et al.*, 2009). Hulled cereals have been shown to be better protected than naked, at least during short-term storage of extant specimen (White, *et al.*, 1999), and the role of the seed testa for protection against microbial, oxidative and UV damage is well established (Rajjou and Debeaujon, 2008).

Historic barley population structures across Sweden and Finland

KASP genotyping of 100 SNPs, of which three SNPs failed in all samples, was used to analyse barley materials from both Sweden and Finland (Paper I and II, respectively). The desiccated Winstrup barley specimens mentioned above were the only Swedish samples with high enough success rates to be included in population analyses. The Finnish barley samples were made up of herbarium and museum specimens, collected in the period from 1872-1921. The Finnish materials included 14 single grains sampled from as many spikes stored at the Turku University Herbarium and the Botanical Museum of the Finnish Museum of Natural History, Helsinki, as well as six grains from each of six different Finnish barley accessions stored at the Swedish museum of cultural history (Leino, *et al.*, 2009).

SNPs from the KASP genotyping assay associated with functional traits were analysed separately. For *Ppd-H1* (Jones, *et al.*, 2008a) the allele associated with unresponsiveness to increased day length, meaning that flowering would not have been induced by long-day light conditions, was most common in both Swedish and Finnish barleys. This was rather expected, considering that this allele has been proposed to be an important adaptation in cultivated barley to the long growth season in the north (Jones, *et al.*, 2008a; Lister, *et al.*, 2008; Jones, *et al.*, 2011). The *Vrs1* and *Int-c* genes (Komatsuda, *et al.*, 2007; Ramsay, *et al.*, 2011) are both involved in the control of row-type and size of lateral grain sizes in barley. In the Swedish Winstrup barley population, the genotypes of these genes indicated six-row phenotype of the spikes. The Finnish materials, on the other hand, contained specimens of either two-row and six-row barley types according to the genotyping results. These data did show some inconsistencies with the phenotypes noted for the corresponding herbarium and museum materials, which could be ascribed to that there are many other alleles influencing this trait that were not genotyped here (see Casas, *et al.*, 2018). The Swedish Winstrup barleys were found to cluster separate from two-row barley in PCoA plots based on data from <60 SNPs, similar to the pattern observed for Finnish barley in Paper II, further supporting their classification as six-row barleys (unpublished results).

The remaining genotyping datasets were trimmed to remove samples and SNPs with high levels of missing data before they were compared to the corresponding SNP subsets from 19th century landrace materials in Forsberg, *et al.*, (2015). Previously observed patterns of population structures across Fennoscandia could not be fully confirmed within the full set of samples originating from Finland (Paper II). But when only six-row barleys were included, northern and southern populations separated from each other. Some level of clustering was found also in Finnish two-row barleys, possibly relating to their growth habit as either spring or winter barley, but as that trait was not investigated here this has yet to be confirmed. The Swedish barley samples collected from the bishop's grave in Lund showed a great deal of genetic similarity with 19th century barley populations from southern Sweden, suggesting temporal conservation of population structures extending across 200 years (Paper I).

Temporal preservation of population composition in Fennoscandian rye
Fennoscandian rye was also genotyped using KASP to look for presence of population structures in historic materials (Paper III). If any structures could be found another goal was to see if there were any detectable signs of the slash-and-burn rye varieties mentioned in the historical records (see Leino, 2017). This outcrossing cereal crop was introduced in Fennoscandia around 500 BC, and then increased in importance as of late Iron Age (Viklund, 1998; Grabowski, 2011; Alenius, *et al.*, 2013). In the period from 16th to 20th centuries rye was one of the economically most important cereals in both Sweden and Finland (Ahokas, 2009; Leino, 2017), and the most common cereal grown in slash-and-burn cultivation.

Previous studies, that relied on extant rye materials preserved in genebanks, failed to detect population structures in Fennoscandian rye (Persson, *et al.*, 2001; Persson and von Bothmer, 2002; Hagenblad, *et al.*, 2016). Thus, rye in this region was concluded to be made up of one large meta-population. What could not be discerned in previous studies was if this was entirely an effect of poor representation of rye varieties, in combination with loss of genetic variation during *ex situ* maintenance of genebank rye (e.g. Chebotar, *et al.*, 2003). Here, by using a sample set consisting of 25 extant landrace rye cultivars, rye data from Hagenblad, *et al.*, (2016), 35 accessions from museum specimens dated from late 19th to early 20th centuries, 1 accession from the grave of Bishop Peder Winstrup from year 1679 and 1 accession originating from Leppävirta, Northern Savonia, Finland from year 1648 it was possible to study temporal effects on population structure in Fennoscandia.

Both the museum specimens of rye and extant genebank materials, most of which had been collected during the 20th century, showed similar levels of genetic diversity. Further, museum specimens and extant genebank materials also clustered together in the PCA plot, indicating that few changes had occurred during *ex situ* preservation from the time when they were collected. No clear distinction could be made between rye accessions from 17th century, 19th century or extant materials. Thus, the results presented here rendered further support to the conclusions in previous studies (Persson, *et al.*, 2001; Persson and von Bothmer, 2002; Hagenblad, *et al.*, 2016); Fennoscandian rye appear to make up one large meta-population. This is quite likely a consequence of rye being a primarily outcrossing species and pollen having the

ability to spread across large distances. There was also no genetic evidence to suggest that rye used for slash-and-burn cultivation would have been different from rye grown on permanent fields.

Early improved cultivars, however, separated from the Fennoscandian meta-population of landrace rye. These cultivars rather seemed to share genetic similarity with landraces from central Europe. This could be considered genetic evidence that rye from central Europe was indeed used for crop improvement as suggested by the literature (Gunnarsson, 1997).

Using genetic analysis methods for identification of *Brassica* remains

The genus *Brassica* contains several agricultural and horticultural crops that have been important both historically but also in present time. The earliest archaeological finds from Sweden have been dated to around 1-400 AD (Larsson and Ingemark, 2015). Which of the multitude of *Brassica* species and subspecies that were grown has, however, remained unknown as morphological examination of archaeological seeds can only, with some uncertainty, separate between some major groups of seeds within this genus such as *B. rapa*, *B. oleracea*, *B. nigra* and *B. napus* (Berggren, 1981; Tomlinson and Hall, 1996; Cappers, *et al.*, 2009).

Considering the very different end uses of the different *Brassica* variants, where some are grown for their roots or as cruciferous vegetables, and others as oilseeds or spices, identifying which species and subspecies archaeological finds belong to would contribute greatly to our understanding of historical horticulture. Historic literature gives some clues as turnips, cole crops with edible leaves and mustard are mentioned already in medieval times, but the poor descriptions and names used leaves room for much speculation (e.g. Larsson, 2010). The project described in Paper IV therefore had the aim to identify archaeological specimens of *Brassica* seeds preserved under subfossil conditions (wet and anoxic, but without water fully penetrating the tissue) using genetic analysis methods.

Genetic barcoding has been used successfully with other archaeobotanical materials allowing for identification of species even in waterlogged specimens (e.g. Pollmann, *et al.*, 2005; Gismondi, *et al.*, 2012; Gismondi, *et al.*, 2016). Two commonly used barcoding regions, the nuclear ribosomal internal transcribed spacer ITS and the chloroplast intergenic spacer *trnH-psbA* (Hollingsworth, *et al.*, 2009), were therefore sequenced in 63 accessions of extant *Brassica*, including in total 12 different *Brassica* types (four species, eleven subspecies). This revealed that the resolution provided by these two regions would not suffice for subspecies identification (Paper IV, Fig. 2). A larger number of genetic regions have been used for this purpose in extant *Brassica* (Izzah, *et al.*, 2013), but considering the low success rates with PCR-based methods on waterlogged samples (Paper I; Schlumbaum, *et al.*, 2012) and the low resolution provided by the multi-copy barcoding regions investigated here, the archaeological *Brassica* specimens were instead whole-genome sequenced using Illumina HiSeq2500.

A total of eight subfossil *Brassica* samples, one extraction blank and one library blank were sequenced. Of the resulting sequencing reads only a very small proportion, 0.004-0.039%,

could be assigned to any of the three diploid *Brassica* reference genomes (*B. rapa*, *B. oleracea* and *B. nigra*). In two samples, both morphologically identified as *B. rapa*-type, the reads mapping to *Brassica* showed the expected DNA damage patterns suggesting them to be authentic aged DNA. They also had a higher proportion of reads mapping to the *B. rapa* reference genome than to the other references, supporting the morphological species classification of the material.

The Illumina reads from these two samples were aligned to the genomes of three different *B. rapa* subspecies; Chinese cabbage, oilseed rape and turnip, to see if they had a higher resemblance to either of the three. Comparisons showed a slightly higher number of unique reads assigned to Chinese cabbage for both samples, despite a higher total number of reads assigned to oilseed rape and turnip. There was, however, no clear difference in the total number of reads assigned to either oilseed rape or turnip. Judging by the literature (Lyttkens, 1981; Hallgren, 2016), Chinese cabbage was likely not grown in Sweden during this time, which suggests that this is a consequence of the low number of reads available for analysis in these samples.

The low proportion of reads that could be assigned to either *Brassica* reference genome raised the question of where the other reads present in the samples originated from. The full set of Illumina reads from each of the samples therefore underwent metagenomic analysis to assign them into taxonomic groups. This revealed that a large part of the genetic material in the samples had bacterial origin, and only a small proportion of the reads could be assigned to Eukaryota. Of the eukaryotic reads most belonged to either Fungi or Metazoa. Only two samples and the extraction blank contained any reads that could be mapped to Viridiplantae (green plants). In the extraction blank six reads could be mapped as far as Pentapetalae. In the two samples one contained 3407 reads that could be mapped to Viridiplantae, but not further. The other was one of the samples both morphologically and genetically identified as *B. rapa*. It contained 559 reads that could be mapped as far as rosids, which is the clade that among its several subcategories also includes *Brassica*. This suggest that this particular sample is of a slightly better quality than the others, which is further supported by the fragment length distribution for the ancient reads present in this sample (Paper IV, Figure 3D).

In summary, whole-genome sequencing of subfossil *Brassica* remains did support the morphological classification of species. The sample quality was too poor to allow subspecies identification in this case, but further optimisation of methods and use of target capture (Carpenter, *et al.*, 2013; Enk, *et al.*, 2014) in combination with NGS might improve the chances of successful subspecies identification. Further, careful selection of samples and immediate processing after excavation is strongly advisable to reduce contamination and the effects of DNA degradation.

Evolutionary history of the wheat nutrition gene *NAM-B1* in tetraploid wheat

The allele distribution of the *NAM-B1* gene, controlling grain mineral and protein content as well as maturation time (Uauy, *et al.*, 2006b; Distelfeld, *et al.*, 2007; Asplund, *et al.*, 2013), has

been rather thoroughly explored in hexaploid wheats (Asplund, *et al.*, 2010; Hagenblad, *et al.*, 2012a), but not so much in the tetraploid forms (Uauy, *et al.*, 2006b). The functional wildtype allele, WT, confers high protein and mineral content in the grain as well as a faster maturation time and, in some environments, smaller grains (Uauy, *et al.*, 2006a; Uauy, *et al.*, 2006b).

There are two non-functional alleles; one deletion whose extent of >200 kb had not been mapped prior to the study described in Paper V, and a frameshift mutation in the gene itself resulting in a non-functional protein (Uauy, *et al.*, 2006b). The distribution of the different alleles in tetraploid wheat was investigated (Paper V). Wild emmers were found to mostly, with one exception, carry the WT allele. In domesticated emmer all three allele types could be found, whereas durum only had non-functional alleles.

Regions within and at different distances away from *NAM-B1* were screened to see if they contained traces indicative of a selective sweep. Low nucleotide diversity, an expected consequence of selection, was found both within but also surrounding the *NAM-B1* gene in wheats with the frameshift allele. This pattern did not extend into the reference genes that should be unlinked to *NAM-B1*, associating the observed pattern with the *NAM-B1* region. The tests for neutrality; Tajima's D, Fu and Li's D and F, mostly failed to detect significant deviance from variation expected under neutral theory, in part due to the complete lack of diversity in some of the investigated fragments. Overall, the evidence suggested that there had been selection acting on the non-functional allele, to some extent camouflaged by population events masking the signals detected by the neutrality tests. The fact that all three alleles appeared rather common in domesticated emmer wheat, however, speaks against *NAM-B1* being a domestication gene but rather suggest that selection occurred sometime during the diversification phase of wheat cultivation.

To determine the time scale for when the allele variants arose, the *NAM-B1* genotypes were investigated in 39 ancient tetraploid wheats. All specimens were obtained from Egyptian collections at Swedish museums (Medelhavsmuseet, Östergötlands museum and Vänersborgs museum). Of these samples, 31 were desiccated and eight charred, with radiocarbon dating estimating the ages to 2120-2955 cal BP whereas some had been estimated to 6500 BP based on archaeological context. Neither PCR-amplification followed by pyrosequencing, nor traditional cloning and Sanger sequencing would allow genotyping of *NAM-B1* in these ancient samples (unpublished results). The PCR primers that produced specific products in both extant tetraploid and 150-year-old museum specimens of wheat were unsuccessful in the ancients.

The genetic region surrounding *NAM-B1* is known to carry a lot of repetitive DNA (Uauy, *et al.*, 2006b) and this might be why, together with the DNA degradation found in ancient samples, this region could not be successfully amplified. The PCR-based methods attempted here were obviously not sensitive enough, especially not in a tetraploid genome. However, considering the success rates with KASP genotyping in barley it is possible that this could be a successful strategy also in this case. Another possibility, thanks to the improved quality of published reference genomes for both tetraploid and hexaploid wheats (Avni, *et al.*, 2017; Appels, *et al.*, 2018), could be NGS with target enrichment to obtain high enough cover of the *NAM-B1* region within the >10 Gb of genetic sequence in the tetraploid wheat genome.

Conclusions

Out of the investigated archaeobotanical cereal specimens the desiccated grains by far excel in genetic analyses, even allowing KASP genotyping and population level analyses.

Genetic analysis suggests centuries-long temporal preservation of population composition in both rye and barley across Sweden and Finland, with population structures following a latitudinal pattern in barley. This is particularly interesting as the 17-19th centuries were times that saw several changes to farming practises and the beginning of the agricultural revolution.

No traces of endogenous DNA could be found in charred cereals, implying that the charring protecting the grains from degradation by microflora in the soil also reduces the DNA content to undetectable levels.

For waterlogged cereal grains, PCR-based analysis methods rendered limited success even if the materials appeared to contain traces of endogenous DNA. This type of material could, however, be of interest for genetic analysis in geographic regions where more well-preserved materials are unavailable.

DNA fragments showing the expected degradation profile associated with authentic ancient DNA were identified in subfossil 17th century *Brassica* seeds using NGS.

Identification of *Brassica* subspecies was not possible with the materials at hand, but assignment of sequence reads to species did show a pattern corresponding with morphological classification leaving great promise for future studies.

In regions like Fennoscandia, where climate does not allow for good preservation of archaeobotanical materials, museum collections and herbaria are particularly important sources of materials for genetic exploration of cultivation history.

The *NAM-B1* gene, despite likely having been the target of a selective sweep, should not be considered a domestication gene in tetraploid wheats.

Challenges and future prospects

The technological improvements have greatly boosted the possibilities for analysis of aDNA. Increased use of high-throughput sequencing techniques in recent years has allowed for analysis of samples with low DNA quantities and damage that could not have been analysed before. The huge amounts of data these methods generate must be properly sorted and evaluated, creating a need for bioinformaticians with good understanding for the concerns relating to work with aDNA.

NGS shows great potential, even more so with the increasing amount of high-quality reference genomes available for many agriculturally important species. The large genomes of many of our crop plants, often rich in repetitive sequence, have so far limited analysis of many archaeobotanical finds. Possible ways around this problem would include limiting the analysis to certain regions, either by target enrichment or using a method like KASP genotyping. Judging by the results presented in this thesis, the latter would only be advisable in samples with a relatively high endogenous DNA content.

The fact that the waterlogged and subfossil samples of barley and *Brassica*, respectively, both showed signs indicative of endogenous DNA, whereas analyses of charred grains failed entirely, would suggest that future archaeogenetic studies of botanical specimens should pay more interest to materials preserved under these conditions. As shown here, there might not even be a need to restrict these analyses to samples with tough seed endocarps as has mostly been the case up until this point. This is of course of particular relevance when studying materials from regions, like Fennoscandia, where desiccated materials are exceptionally rare in the archaeobotanical record.

One major goal of this thesis was to evaluate archaeobotanical materials that have often been overlooked up until this point to see what kind of genetic information could be obtained from them. The value of this does not only lie in what we have learned about Fennoscandian agricultural history, but maybe just as much in providing insight into which materials are worth pursuing and what outcomes one can expect. The latter could give a foundation for decision-making in future analyses of archaeobotanical materials to ensure that costs and expected outcomes are better matched.

Acknowledgements

I would especially like to thank my main supervisor, Jenny Hagenblad, for her guidance over the years. Not to mention the understanding that sometimes all one really needs is a geeky comic strip, a cool science fact or a funny (cat) video.

Matti Leino, my co-supervisor, who led me onto this path many years ago – thank you! I may have regretted it many times but somehow I have always bounced back, and I'm convinced that your inspiring passion and enthusiasm for science and plants had something to do with it.

Jens Heimdahl, my go-to archaeobotanist, also deserves a special mentioning here and my most sincere thanks!

I would also like to acknowledge the Berit Wallenberg foundation that made this journey possible. And Prof. Robin Allaby and Dr. Sanne Boessenkool with co-workers that kindly allowed me into their aDNA facilities and shared a lot of useful advice on how to work with ancient samples.

To the other members of my research group over the years; Per Larsson, Nils Forsberg and Hugo Oliveira: thanks for the company, fika breaks, Styrofoam boxes (no worries Hugo, I took care of them all...), shared wisdom and laughs. It sure got empty when you all left (and not just because I threw away the Styrofoam boxes).

My teaching buddies, Johan and Martin, I certainly would not have been able to keep my cool without you. Or laughed nearly as much. And to all other fellow PhD students, past and present: who can better understand and share the joys and troubles of PhD life? On that note I would particularly like to mention my office mate Laura, but also send many great big thanks to all of you for sharing those cups of tea, coffee and beers over the years.

Thank you Tove, Ann-Charlotte and Chun-Xia for keeping everything running, both in teaching and research labs as well as the Physics clean room.

To the colleagues at Biology and Theoretical Biology, I am very happy that I got to spend the past 7.5 years getting to know you all. I have enjoyed our lovely geeky discussions – scientific and non-scientific – tremendously!

Frida and Marie, too few people realise that the only way to stay sane is to allow a certain level of crazy. Thanks for keeping me sane (or well, close enough)! That, together with the amazing movie nights packed with high-energy snacks provided everything needed to keep me going. We'll come to you next time Sofia!

My dear friends from back home, Nina and Elin, you have remained my connection to "the real world" and I'm most grateful that we've managed to stay in touch over the years.

Gyuri, my infinite distraction, what would I possibly have done these past few months without you? More walks and books and a lot less packing and chaos in the time to come, I promise!

Så slutligen, stort tack till mina alltid lika stöttande föräldrar. Min far, som sannolikt la grunden till mitt intresse för biologi och vetenskap. Och min mor, som visat att det aldrig är för sent att lära sig något nytt även om det går tungt emellanåt.

Och till mina syskon med familjer: David, Alionka, Nova och Olle – tack för sällskap, mat, bus, pyssel, bakning och någonstans gömt mitt i allt kaos; avkoppling. Therese, Roger och Leo som ivrigt hejat på, skickat underhållande kattbilder och stöttat inte bara mitt doktorerande utan också mitt växande kaffeberoende. Mattias, många tips på bra filmer och serier har det blivit genom åren och diskussioner om allt möjligt. Men en sak tror jag att vi måste reda ut en gång för alla – jag är inte liten, jag är stor.

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My contributions

Paper I

Planned the project together with the other co-authors, performed most of the laboratory work and data analysis, and wrote the manuscript together with Jenny Hagenblad and Matti W. Leino.

Paper II

Performed large parts of the laboratory work, analysed the data resulting from the ancient samples (the other samples were analysed by Jenny Hagenblad and Mia Lempiäinen-Avci) and participated in writing the manuscript together with the other authors.

Paper III

Performed parts of the laboratory work together with Per Larsson and Hugo R. Oliveira, and participated in writing the manuscript together with the other authors.

Paper IV

Planned the project together with Matti Leino and Jens Heimdahl, performed the laboratory work and data analyses and wrote the manuscript together with the other co-authors.

Paper V

Performed the laboratory work, analysed the data and wrote the manuscript together with Jenny Hagenblad and Matti Leino.

Papers

The papers associated with this thesis have been removed for copyright reasons. For more details about these see:

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