*agronomy*

Article

Toward an Investigation of Diversity and Cultivation of Rye (*Secale cereale* ssp. *cereale* L.) in Germany: Methodological Insights and First Results from Early Modern Plant Material

Sofia Filatova [1,*], Benjamin Claassen [2], Guillermo Torres [3], Ben Krause-Kyora [3], Eva Holtgrewe Stukenbrock [2] and Wiebke Kirleis [1]

1   Institute for Prehistoric and Protohistoric Archaeology, Kiel University, 24118 Kiel, Germany; wiebke.kirleis@ufg.uni-kiel.de
2   Max Planck Institute for Evolutionary Biology, 24306 Plön, Germany; claassen@evolbio.mpg.de (B.C.); estukenbrock@bot.uni-kiel.de (E.H.S.)
3   Institute of Clinical Molecular Biology, Kiel University, 24118 Kiel, Germany; g.torres@ikmb.uni-kiel.de (G.T.); b.krause-kyora@ikmb.uni-kiel.de (B.K.-K.)
*   Correspondence: s.filatova@ufg.uni-kiel.de

Abstract: Rye (*Secale cereale* ssp. *cereale* L.) is a secondary domesticate, considered to have originated as a weed in wheat fields and to have developed traits of domestication by evolving similar physiological and morphological characteristics to those of wheat. Although it migrated into Europe as a weed possessing domestication traits, it became one of the most significant crops grown in large parts of Europe from the medieval period onward. Within the modern borders of Germany, rye was grown using at least two divergent cultivation practices: eternal rye monoculture and three-field rotation. The straw of rye was used to produce Wellerhölzer, which are construction components in traditional half-timbered houses that have enabled a desiccated preservation of the plant remains. In order to assess the impact of cultivation practices, local environmental conditions and genetic variation on the genetic diversification of rye, we seek to integrate well-established archaeobotanical methods with aDNA sequencing of desiccated plant remains obtained from Wellerhölzer from Germany. In the current contribution, we present a proof of concept, based on the analysis of plant remains from a Wellerholz from the Old Town Hall of Göttingen. We use arable weed ecology to reconstruct cultivation practices and local environmental conditions and present a phylogenetic analysis based on targeted loci of the chloroplast and nuclear genome. Our results emphasise that the study of desiccated remains of plants from Wellerhölzer offer a unique opportunity for an integration of archaeobotanical reconstructions of cultivation practices and local environment and the sequencing of aDNA.

1. Introduction

Cultivated rye (*Secale cereale* ssp. *cereale*) is a well-known example of a secondary domesticate, which are crops that have undergone a divergent pathway of domestication compared to primary domesticates. Rather than developing traits of domestication directly from the wild, secondary domesticates originate as weeds in fields of crops and achieve a domesticated form by evolving similar physiological and/or morphological characteristics, enabling them to be harvested and sown alongside the intentionally cultivated crops [1,2] (p. 25; p. 167). Rye, and other secondary domesticates, dispersed outside of their areas of origin as weeds possessing domestication traits and was subsequently taken into cultivation independently in various regions [3] (pp. 94–95). While the history of rye domestication is relatively well studied, the process of the diversification of rye after having entered Europe as a weed possessing domestication traits, has not received as much attention (see below).

The process enabling secondary domesticates to develop domestication traits is known as crop mimicry [3]. Crop mimicry results in the formation of landraces of arable weeds that are specifically adapted to local agricultural practices, enabling them to escape eradication through practices such as weeding and herbicide destruction [3] (p. 258). Several crops, including rye, common oat (*Avena sativa*) and gold-of-pleasure (*Camelina sativa*), are known to have undergone "mimetic evolutionary trajectories" [4] (pp. 94–95). The speed and nature of the mimicry process is considered to be governed by the genetic variation of the respective species and is closely tied to artificial selection through practices of cultivation such as weeding, winnowing and harvesting [3] (p. 257). Mimicry can lead to the diversification of weeds and crops, resulting in the formation of landraces [3] (p. 257). The process of diversification of crops and weeds into landraces is further known to be dependent on local environmental conditions [5] (p. 9).

The process through which secondary crops become domesticated (i.e., crop mimicry) and through which crops and weeds in general diversify, are thus driven by similar factors (i.e., genes, environment and artificial selection) [3,5] (p. 258; p. 9). In order to illuminate the mechanisms of these processes, we argue that it is relevant to combine well-established archaeobotanical methods for the reconstruction of cultivation practices and local environment with ancient DNA (hereafter aDNA) sequencing. On the long term, the objective of our project is to investigate the potential impact of cultivation practices on the genetic diversification of cultivated rye after domestication. In the current contribution, we aim to present a proof of concept through the study of well-preserved plant remains from a Wellerholz dating to the (Early) Modern Period. We first investigate the suitability of these plant remains for a comprehensive reconstruction of cultivation practices using different approached from weed ecology and, second, test the quality of the remains for aDNA sequencing by sequencing the chloroplast genome and conducting a phylogenetic analysis based on targeted loci of the chloroplast and nuclear genome.

1.1. Insights into Rye Domestication

The genus *Secale* is distributed over a large area, including the Balkans, Anatolia, the Caucasus and Central Asia, and is a common component of the Mediterranean flora [6] (p. 303). It constitutes three species, of which the *Secale cereale* species contains annual species of rye in wild, weedy and cultivated forms [6] (p. 302). *Secale cereale* ssp. *vavilovii*, which most likely finds its origin in Armenia and Eastern Turkey, is regarded the ancestor of cultivated rye, based on both archaeobotanical [7] (p.65) and genetic [8,9] (p. 16; p. 15) investigations.

The earliest evidence for domestication traits in rye appears by the middle of the seventh millennium BC in the Aceramic Neolithic site of Can Hasan III in South Central Anatolia and the Aceramic Neolithic levels of Tell Abu Hureyra [10,11]. In both cases, the small quantities in which rye appears suggest that it was growing as an admixture in fields of domesticated einkorn (*Triticum monococcum* ssp. *monococcum*) and emmer (*Triticum turgidum* ssp. *dicoccon*) [12] (p. 71), alongside admixtures of free-threshing wheat (*Triticum aestivum/turgidum*) that was not yet cultivated as a crop [10] (p. 168). It is argued that rye developed its traits of domestication by mimicking wheat and, furthermore, that though-rachised free-threshing wheats and domesticated rye evolved in response to similar agricultural conditions [6,10] (p. 306; p. 169). The close relationship between free-threshing wheat and cultivated rye has recently been confirmed in studies of the rye genome and introgression with rye chromatin has played an important role in improvement of yields of bread wheat [13] (p. 565).

The first signs of cultivation of domesticated rye originate from the Bronze Age levels of Alaca Höyük in Central Anatolia, dating between the fourth and second millennium BC [7,10] (p. 65; p. 163). Archaeobotanical data suggest that the domesticated form of rye entered Europe as a (tolerated) weed [7,14] (p. 65; p. 149) as early as LBK times, ca. 4400 cal. BC [14] (p. 142). It was cultivated in certain regions during the pre-Roman Iron Age and is found throughout entire Europe around year zero, becoming prominent especially east

and north of the river Rhine during the fourth and fifth centuries AD [15] (pp. 621–626). Explanations for the sudden spike in rye cultivation include a change in harvesting heights (i.e., harvesting close to the ground, thereby preventing it to be separated from wheat) [14] (p. 149) and the inclusion of rye into Mediterranean bread-baking techniques during the Roman period [16] (p. 110). Rye eventually developed to become one of the main crops in many parts of Europe during the Middle Ages (e.g., [14,15,17–21]) and it is argued to have emerged as a crop independently at different times and places [10,14].

Genetically, rye is a diploid cereal and has the largest genome of all Triticeae, with 90% of the genome comprising repetitive sequences [9,13,22] (p. 1; p. 565; p. 853). It is of special interest to plant breeders due to its agronomic traits, which include tolerance to frost and ability to grow in poor soils under stress conditions [22] (p. 854). Genetic studies of landraces of rye conducted by Hagenblad et al. [8] show that cultivated rye has an exceptionally high genetic diversity that is most likely the result of gene flow between cultivated, weedy and wild species. In addition, studies by Persson and van Bothmer [22] and Hagenblad et al. [8] showed that some landraces can be distinguished geographically and that the clusters might reflect routes of migration and contact. Examples of clusters are the Finnmarken area (which is well known for slash-and burn cultivation of rye; see [23]) and Scotland and Morocco (argued to reflect seed exchange). Studies of historical museum and herbarium specimens of rye by Larsson et al. [23] have confirmed the clustering of the Fennoscandian meta-population, but have not been able to illuminate a finer geographical distinction between different landraces within the Fennoscandian region. Studies by Schreiber [24] have contradicted the existence of geographical distinction based on genetic studies and they suggest that the scattered genetic information in their analysis can be the result of the multiple centres of domestication of rye.

Cultivation Practices and Technical Use of Rye in GERMANY

Within the modern borders of Germany and from the medieval period onward, rye was a significant component of the three-field system due to its winter hardiness [25] (p. 75). In this system, one part is sown in autumn, one part is sown in spring, one part is fallow, and the fields are rotated every growing season. Three-field rotation was introduced to western and central Europe during the second part of the 8th century AD [26] (p. 143) and remained in use throughout Europe during the 19th century AD [25] (p. 75). The mouldboard plough was a crucial component of the three-field system [16] (206) and was in use as early as the first century BC in Feddersen Wierde (Lower Saxony, Germany) [27]. It is a heavy plough that was able to turn the wet soils of the autumn and enable successful cultivation of winter rye and winter wheat, both of which grow well in fields that are prepared intensively [15,25] (p. 206; p. 76).

In addition, areas in the north of today's Lower Saxony practiced rye monoculture [26,28] (p. 145; p. 217). Rye is an autotolerant plant, which means that the yield does not decrease when it is grown on the same field uninterrupted [29] (p. 39). Monoculture of rye in Germany included cultivation of rye for up to 20 years in a row, manured by *plaggen* soils, but without fallow or rotation, a practice that is known as eternal cultivation of rye (Ewiger Roggenbau in German) [26] (p. 145).

Within the German tradition of half-timbered house construction (Middle Ages until ca. 19th century AD) in, amongst others, Lower Saxony, Nordrhein-Westfalen, Brandenburg, Bavaria, and to some extent Baden-Württemberg, rye straw was used for the production of so-called Wellerhölzer or Lehmwickel [30–34]. These construction components consist of wooden planks that were wrapped with straw of a winter cereal (e.g., rye or spelt), covered by loam and placed between the ceiling and above-lying floor for the purpose of insulation and stabilisation [32] (pp. 120–121). The stable conditions created by the loam around the Wellerhölzer enable the preservation of plant materials in a desiccated state, preserving the plant remains in a form that does not otherwise occur in regions with a temperate climate [30] (p. 456). Archaeobotanical investigations of Wellerhölzer from diverse regions in Germany have indicated the exceptional preservation of the plant

remains used to produce the Wellerhölzer and have enabled reconstructions of, amongst other things, the local environment and cultivation practices [30–34].

1.2. Toward the Investigation of Rye Diversification—In the Past and Present

The acquisition of domestication traits by secondary domesticates, as well as the formation of landraces of weeds and crops, are suggested to be governed by genetic diversity, local environmental conditions and cultivation practices [3,5] (p. 257, p. 9). In the case of crops, the formation of landraces can be understood as a process of diversification following domestication. Based on these observations, our hypothesis therefore is that combining well-established ecological methods to reconstruct the local environment and cultivation practices with the study of aDNA of rye can help illuminate the suggested impact of environmental and artificial selective pressures on the genetic diversification of cultivated rye, following domestication. Ultimately, gaining a better understanding of the factors that drive diversification might contribute to a better understanding of the domestication process of rye and other secondary domesticates.

In archaeobotanical studies, the composition of arable weeds is widely used as indication of cultivation practices (e.g., [35–42]), which is considered to be especially defined by crop rotation and the sequence and timing of soil-tillage [43] (pp. 626–629). In Germany in particular, rye was cultivated using at least two divergent methods: eternal rye cultivation and the three-field system (see above) [26,28,30]. The use of rye straw in the production of Wellerhölzer presents an opportunity for a comprehensive reconstruction of cultivation practices and the desiccated preservation of the material makes it particularly suitable for the sequencing of aDNA [44]. Our envisaged future objective is to be able to compare the genetic diversity of rye that was grown using divergent cultivation practices in various environmental conditions, based on targeted or whole-genome sequences obtained from plant material from Wellerhölzer.

In addition to their nuclear genome, plants harbour genomes of their cellular organelles. Abundantly found in plant cells, chloroplasts bear the potential of preservation of multiple copies in a single cell [45]. Due to its abundance, compact size and the fact that most chloroplast genomes are structured in a similar way [46], chloroplasts provide excellent material for first target for aDNA studies in plants. While the chloroplast genome does not comprise the same extent of genetic variation as the nuclear genome, it contains sufficient information to properly distinguish closely related species and has therefore served as markers for species identification and for illuminating population structures of closely related crop and wild species (e.g., [7,47,48]).

We consider the high quality of aDNA data obtained from chloroplast sequencing a demonstration of the potential of whole-genome sequencing of historic specimens of rye. In the future, data from whole-genome sequencing may provide novel insights into the genomic changes associated with secondary rye domestication and subsequent diversification, including signatures of selection in domestication-related traits and, for example, pathogen resistance. Ancestral genomes of barley [49] and maize [50] have been successfully reconstructed and have provided insights into potential routes of dispersal [49] and the gradual rate of the domestication process [50]. A recently published reference genome for the winter rye variety Lo7 [13] provides new opportunities for rye genomics, allowing the alignment and analyses of ancient samples.

In this paper, we present the first results of combined archaeobotanical and genetic investigations of rye from a Wellerholz retrieved from the Old Town Hall in Göttingen (Germany). The aim of our paper is to test the suitability of desiccated remains of rye from Wellerhölzer for the reconstruction of cultivation practices, local environmental conditions and sequencing of aDNA. We reconstructed cultivation practices and local environmental conditions based on arable weed ecology and on physical characteristics of the rye in the sample. We further tested the ability of generating high quality aDNA data based on shotgun-sequencing of this particular material. Here we show the use of the sequence data to determine the phylogenetic relationship between ancient and present-day rye based

on chloroplast- and chromosomal-encoded genes. Our results suggest that the excellent preservation of desiccated plant remains from Wellerhölzer offers unique opportunities for an integration of archaeobotanical reconstructions of local environmental conditions and cultivation practice with the sequencing of plant aDNA.

2. Materials and Methods

2.1. Archaeobotanical Analysis

2.1.1. Sampling for aDNA Sequencing and Radiocarbon Dating

Wellerholz GVAC_06 from the Old Town Hall in Göttingen was chosen for the current study. The sampling of the material used to extract aDNA from rye took place prior to the archaeobotanical processing the sample. Approximately 10 tubes were filled with spikelets, ears or straw of rye (with ears or rachises attached). One grain of rye was sampled for radiocarbon dating that took place at the Leibniz Laboratory for radiometric dating and stable isotope research at Christian-Albrechts-University of Kiel.

2.1.2. Processing, Sorting and Quantification

In preparation for the archaeobotanical analysis, the Wellerholz was soaked in a large tub (up to 100 L) filled with tap water until the clay covering the straw had become saturated and the straw had started to separate from the wooden plank. Any excess clay was carefully rubbed off and the straw was subsequently unwrapped from the plank. Special care was taken not to break the straw in order to preserve its original length. The straw was taken out of the tub in small portions and rinsed with tap water over two stacked sieves with mesh sizes of 1 mm and 0.3 mm, respectively. The straw, the sediment remaining in the sieves and the wooden plank were collected and dried in a drying cabinet for approximately one week.

In order to facilitate the sorting of the sample, the finer fragments of straw and sediment were separated into four size fractions using a sieve tower with mesh sizes of 2 mm, 1 mm, 0.5 mm and 0.3 mm. The fractions were analysed using a stereo microscope with a magnification of ×0.8–×4. Selected fragments of wood and charcoal were analysed using a high-power reflected-light microscope with light and dark fields and a magnification of ×10–×100. The coarser material, i.e., the straw of the Wellerholz, was sorted and identified without separation into size fractions.

The finds are recorded according to material category, taxon/type of material, plant part/preserved part and type of preservation. The remains are recorded in a descending order based on the absolute quantity of finds. The plant remains were identified using identification manuals [51–53] and the nomenclature for taxa and plant parts follows Cappers et al. [51]. In addition, the reference collection of the Institute for Prehistoric and Protohistoric Archaeology at the Christian-Albrechts-University of Kiel was used for comparison. Remains of insects and animals have not been identified at this stage.

The majority of finds (e.g., grains, leaves, insect heads) was quantified according to the absolute number in which they occurred. Ears and ear fragments of cereals were quantified according to the number of spikelets or, on those areas of the ear where spikelets were missing, rachis internodes. The remains of cereal stems and cereal straw nodes are indicated as present (+). Additional data recorded for the plant remains are measurements of the length of rye straw.

2.1.3. Reconstructing Cultivation Practices and Soil Conditions

The reconstruction of cultivation practices and soil conditions in this study relies on a combination of approaches that can be described under the umbrella of arable weed ecology. We apply autecology, functional autecology and synecology in our reconstruction and we consider these to be complementary to one another, providing a detailed insight into local environmental conditions and applied cultivation practices. Autecology is the study of individual taxa in relation to their environment, whereby taxa are described and coded according to their responses to various climatic and edaphic factors (e.g., [43].)

Functional autecology relies on functional attributes, which are attributes of plants, such as canopy height, canopy diameter and timing of the flowering period, that are related to the use of, and response to, resources in the environment [37,54,55]. In this study, the method used is known as the Functional Interpretation of Botanical Surveys (FIBS (e.g., [37,38]). Studies of autecology and functional attributes rely on the notion that weeds are indicative of particular climatic conditions (e.g., temperature, light), soil conditions (e.g., pH, nitrogen, moisture) and activities of soil disturbance (e.g., digging, trampling). By studying these indicators, a range of plant-cultivation practices can be reconstructed, including sowing time, the use of manure and the practice of weeding. Synecology is the study of entire communities of plants [43] and includes the classification of arable weeds into communities that strongly relate to sowing time. Although caution is advised in the application of synecology in archaeobotanical studies due to changes taking place in communities over time [39,43] (p. 186; p. 625), we have chosen to include a consideration of synecology due to the young date of our material.

Only those taxa that were identified to the level of species (including uncertain species identifications, designated as cf.) were included in the analysis. Descriptive values and multi-variate analysis were applied to reconstruct cultivation practices and soil-related conditions in the field. For the descriptive values, the attributes, classifications and associated cultivation practices were used as listed in Table 1. Sowing and ploughing time were reconstructed using two attributes: flowering onset and duration of flowering [42,55–57]. The degree of disturbance was reconstructed using five attributes and classifications: Raunkiaer life form, lifespan, type of reproduction, flowering duration, and strategy type [2,41,42,55,58,59] (p. 145; pp. 344–346; p. 50; p. 139; p. 188, p. 810). The onset and length of flowering were thereby classified after the criteria presented by Charles et al. [55] (p. 132), as listed in Table 2. Additional autecological and physical characteristics of plants used in the analysis are the edaphic factors and the minimum and maximum growing heights of weeds and wild plants. The former was used to indicate the characteristics of the soil in the cultivated field. The latter was used to reconstruct harvesting height and were subdivided into three categories (short: 0–40 cm; medium: 41–80 cm; high: >81 cm) as proposed by Kreuz et al. [41]. The data on autecology, classification and functional attributes were collected from [43] and BiolFlor [60] and the minimum and maximum heights of the weeds and wild plants were extracted from Rothmaler [61].

Table 1. Autecological characteristics and functional attributes used in this paper to reconstruct sowing season and cultivation intensity (adapted from: [2,41,42,55–59]); Abbreviation: sro *: subterranean reproductive organs.

Cultivation Practice	Associated Plant Characteristic	Definition of Plant Characteristic	Indication for Practice
Sowing time	Flowering onset	Early	Autumn sowing
	Flowering onset	Intermediate	Autumn sowing
	Flowering onset	Late	Spring sowing
	Flowering duration	Short	Autumn sowing
	Flowering duration	Medium	Inconclusive
	Flowering duration	Long	Autumn sowing
Degree of disturbance	Runkiaer lifeform	Therophyte	High disturbance
	Lifespan and reproduction	Annual	High disturbance
	Lifespan and reproduction	Perennial with sro *	High disturbance
	Lifespan and reproduction	Perennial without sro *	Low disturbance
	Flowering duration	Long	High disturbance
	Flowering duration	Short	Low disturbance
	Strategy type	Ruderal	High disturbance
	Strategy type	Competitor	Low disturbance
	Strategy type	Mixture	Inconclusive
Ploughing season	Flowering onset and duration	Early onset and/or short duration	Autumn ploughing
	Flowering onset	Late	Spring ploughing

Table 2. The criteria used to define flowering onset and flowering duration (adapted from: [42,55]).

Description	Period/Length
Early flowering onset	January–April
Intermediate flowering onset	April–June
Late flowering onset	July or later
Short flowering duration	1–3 months
Medium flowering duration	4–5 months
Long flowering duration	>5 months

The multivariate analysis relied on the functional trait database and discriminant function presented in Bogaard et al. [62]. The plant-functional attributes used in the analysis are listed in Table 3 and are based on results of modern field surveys conducted by Bogaard et al. [62] in Asturias (Spain) and Haute Provence (France), aimed at distinguishing between high-intensity cultivation regimes and low-intensity cultivation regimes, respectively. Four of five attributes are associated to habitat productivity, with an emphasis on fertility, while one indicates the level of disturbance. The discriminant function of Bogaard et al. [62], which is based on data of functional attributes of arable weeds, was used to classify the intensity of cultivation of the rye from Wellerholz GVAC_06. One taxon, *Myosoton aquaticum*, was eliminated from the analysis because it likely represents a contamination, possibly from the clay used to seal the straw around the Wellerholz. IBM SPSS Statistics 28.0.0.0 was used to perform the discriminant analysis.

Table 3. Weed functional attributes used in the discriminant analysis to define intensity of cultivation practices and productivity of the habitat (adapted from: [62]).

Functional Attribute	Related Ecological Attribute	Related Habitat Conditions
Specific leaf area (leaf area/dry leaf weight)	Plant growth rate	Positively correlated with potential productivity of habitat
Leaf area per node/leaf thickness	Plant growth rate	Positively correlated with potential productivity of habitat
Logarithmic scale of canopy height	Maximum plant size, the product of growth rate and period of growth	Positively correlated with potential productivity of habitat; negatively correlated with disturbance of habitat
Logarithmic scale of canopy diameter	Maximum plant size, the product of growth rate and period of growth	Positively correlated with potential productivity of habitat; negatively correlated with disturbance of habitat
Length of flowering period	Duration of life cycle and potential to regenerate from seed	Positively associated with high disturbance

2.2. Ancient DNA (aDNA) Analysis

2.2.1. DNA Extraction, Preparation and Sequencing

All pre-PCR work, sample collection, DNA extraction and library preparation up to the index PCR, was performed in cleanrooms of the aDNA laboratory in Kiel, Germany, following published guidelines for working with aDNA.

For DNA extraction, 0.1–0.2 g of rye material was treated with bleach for 5 min to remove surface contaminants. The purified plant material was then homogenised using a mixing mill. DNA extraction was performed according to the published PTB protocol by Wales and Kistler [63]. Double-stranded half-UDG DNA sequencing libraries were prepared according to an established protocol for multiplex high-throughput sequencing [64,65]. A unique index was added to both library adapters via amplification with two index primers. Sequencing was performed on the Illumina NovaSeq6000 platform at the Institute of Clinical Molecular Biology, Kiel University, using the NovaSeq S1 Kits (200cycles) and the manufacturer's protocol for multiplex sequencing.

2.2.2. Sequence Analysis

Sequence quality and adapter content was assessed using FastQC. Standard Illumina TruSeq adapters were trimmed using cutadapt 2.1. Good quality reads were used to reconstruct *Secale cereale* chloroplast genome employing the Geneious Alignment Tool (REF) and the sequence KC912691 obtained from the NCBI database (REF) as template. To perform phylogenetical analysis the genes maturase K (matK, Ref: NC_021761), ribulose bisphosphate carboxylase large chain (rbCl, Ref: HQ600458) and the ribosomal internal transcribed spacer region (ITS, Ref: HQ600533) were included.

2.2.3. Phylogenetic Analysis

Phylogenetic trees were constructed for two different loci located on the chloroplast, rbCl and matK, and nuclear locus ITS. Multiple sequence alignments of each locus was performed with MEGAX (REF) including six reference sequences of homology plants. Alignments were carried out using MUSCLE with a gap open penalty of −400 and the unweighted pair group method with arithmetic mean (UPGMA) as clustering algorithm. Phylogenetic relationships were inferred using the Maximum Likelihood method and the Tamura-Nei evolutionary model. Bootstrapping with 1000 iterations was performed for all trees.

3. Results and Interpretations

3.1. General Characteristics of the Plant Remains in Welleholz GVAC_06

4412 remains were identified in sample GVAC_06 (Tables 4 and 5) of which the majority is desiccated. The finds are subdivided into eight material categories: cereals, oil and fibre crops, weeds and wild plants, wood and charcoal, indeterminate plants, animals and insects, fungi, and other. The cereal category represents a clear majority of the finds, with an absolute quantity of 3737 (84.78%; Table 4). The bulk of the quantified cereal remains consists of cereal straw and rye ears (Table 5). Other finds of rye include grains, rachises and anthers (Figure 1a,b). Cereals were further represented by grains, rachises and spikelets of common oat (*Avena sativa*), six-row hulled barley (*Hordeum vulgare* ssp. *vulgare*, hulled) and bread wheat (*Triticum aestivum* ssp. *aestivum*). The group of oil and fibre crops included two finds belonging to two taxa: a fruit fragment of flax/linseed (*Linum usitatissimum*, Figure 1c) and a seed of opium poppy (*Papaver* cf. *somniferum*).

Table 4. Absolute quantities and percentages of the material categories identified in Wellerholz GVAC_06.

Material Category	Absolute Quantity	Percentage
Cereals	3737	84.78
Oil and fibre crops	2	0.05
Weeds and wild plants	368	8.35
Wood and charcoal	3	0.07
Indeterminate plants	213	4.83
Animals and insects	31	0.70
Fungi	47	1.07
Other	2	0.05

Table 5. Taxa/types of finds identified in Wellerholz GVAC_06, sorted according to the associated material category.

Plant Part	Plant Part	Preservation	Absolue Quantity
Cereals			
Secale cereale ssp. *cereale*	Rachis internode/spikelet	Desiccated	3435
Hordeum vulgare ssp. *vulgare*	Empty spikelet	Desiccated	74
Hordeum vulgare ssp. *vulgare*	Rachis	Desiccated	61
Secale cereale ssp. *cereale*	Grain	Desiccated	52
Avena sativa	Floret	Desiccated	39
Avena cf. *sativa*	Husk	Desiccated	24
Triticum aestivum ssp. *aestivum*	Rachis internode/spikelet	Desiccated	23
Secale cereale ssp. *cereale*	Rachis	Desiccated	14
Hordeum vulgare ssp. *vulgare*	Grain	Desiccated	7
Secale cereale	Anther	Desiccated	3
Triticum aestivum ssp. *aestivm*	Rachis	Desiccated	3
Triticum aestivum/durum	Grain	Desiccated	2
Cerealia	Stem	Desiccated	+
Cerealia	Straw nodes	Desiccated	+
Oil and Fibre Crops			
Linum usitatissimum	Fruit fragment	Desiccated	1
Papaver cf. *somniferum*	Seed	Desiccated	1
Weeds and Wild Plants			
Papaver dubium/rhoeas	Seed	Desiccated	111
Cirsium	Leaf fragments	Desiccated	60
Odontites vernus	Seed	Desiccated	28
Chenopodium album	Seed	Desiccated	19
Avena sp.	Floret	Desiccated	12
Polygonum aviculare	Seed	Desiccated	12
Odontites vernus	Fruit and perianth	Desiccated	11
Papaver sp.	Fruit cap	Desiccated	6
Myosotis cf. *arvensis*	Seed	Desiccated	5
Papaver rhoeas	Fruit	Desiccated	4
Sonchus asper	Seed	Desiccated	4
Stellaria media	Seed	Desiccated	4
Cirsium	Flower head	Desiccated	3
Persicaria lapathifolia	Seed	Desiccated	3
Veronica/Asperula	Seed	Desiccated	3
Agrostemma githatgo	Seed	Desiccated	2
Avena fatua	Floret	Desiccated	2
Camelina microcarpa	Seed	Desiccated	2
Carex sp.	Seed	Desiccated	2
Cirsium	Stem and leaves	Desiccated	2
Fallopia convolvulus	Seed	Desiccated	2
Myosotis cf. *arvensis*	Floret	Desiccated	2
Poa annua	Seed	Desiccated	2
Sonchus oleraceus	Seed	Desiccated	2
cf. *Anthemis arvensis*	Seed	Desiccated	1
cf. *Atriplex* sp.	Seed	Desiccated	1
Brassica/Sinapis	Seed	Desiccated	1
cf. *Carex*	Urticulus	Desiccated	1
Centaurea cyanus	Seed	Desiccated	1
Galium I	Seed	Desiccated	1
Galium II	Seed	Desiccated	1
Galium II	Seed	Desiccated	1

Table 5. *Cont.*

Plant Part	Plant Part	Preservation	Absolue Quantity
Weeds and Wild Plants			
Lithospermum arvense	Seed	Desiccated	1
Medicago lupulina	Seed and seedcoat	Desiccated	1
Myosoton aquaticum	Seed	Desiccated	1
Papaver cf. *argemone*	Seed	Desiccated	1
Papaver dubium	Fruit	Desiccated	1
Papaver cf. *dubium*	Fruit (unripe)	Desiccated	1
Potamogeton sp.	Seed	Desiccated	1
cf. Ranunculus sp.	Fruitlet	Desiccated	1
Sambucus sp.	Seed	Desiccated	1
Schoenoplectus tabernaemont	Seed	Desiccated	1
Scleranthus annuus	Floret	Desiccated	1
Sonchus arvensis	Seed	Desiccated	1
Spergula arvensis	Seed	Desiccated	1
Taraxacum officinale	Seed	Desiccated	1
Caryopyllaceae II	Seed	Desiccated	19
Fabaceae	Pod fragments	Desiccated	8
Caryopyllaceae I	Seed	Desiccated	7
Fabaceae small I	Seed	Desiccated	4
cf. Cannabinaceae	Seed fragment	Desiccated	1
Fabaceae small II	Seed	Desiccated	1
Poaceae small	Fruit	Desiccated	1
Poaceae large	Fruit	Desiccated	1
Poaceae	Stem and ear	Desiccated	1
Wood and Charcoal			
Fraxinus sp.	-	Desiccated	3
Betula sp.	-	Desiccated	1
Salix/Popolus sp.	-	Desiccated	1
cf. Sorbus sp.	-	Charred	1
Indeterminate Plants			
Indeterminata	Wood	Charred	104
Indeterminata	Seed/fruit fragments	Desiccated	56
Indeterminata	Stem fragment	Desiccated	12
Indeterminata	Twig	Desicctaed	11
Indeterminata	Wood	Desiccated	8
Indeterminata	Unknown	Desiccated	7
Indeterminata	Leaf fragments	Desiccated	4
Indeterminata	Seed/fruit	Desiccated	2
Indeterminata	Seed/fruit	Charred	2
Indeterminata	Bark fragment	Desiccated	2
Indeterminata	Fruit endocarp fragment	Desiccated	1
Indeterminata	Anther (small)	Desiccated	1
Indeterminata	Flowerbud	Desiccated	1
Indeterminata	Stem and inflorescence	Desiccated	1
Indeterminata	Amorphous remains	Charred	1
Animals and Insects			
Insect	Body	Desiccated	13
Insect	Fragment	Desiccated	9
Trichoptera	larvae case	-	4
Mollusc	-	-	4
Bone	Fragment	Desiccated	1

Table 5. *Cont.*

Plant Part	Plant Part	Preservation	Absolue Quantity
Weeds and Wild Plants			
Fungi			
Fungus	On stem fragments	Desiccated	44
Fungus	On *Avena sativa* floret	Desiccated	1
Fungus	On *Trititicum aestivum/durum* grain	Desiccated	1
Fungus	On wood fragment	Desiccated	1
Other			
Ceramic fragment	-	-	2
Plaster fragment	-	-	1
Yarn/thread	-	Desiccated	1
Total			4408
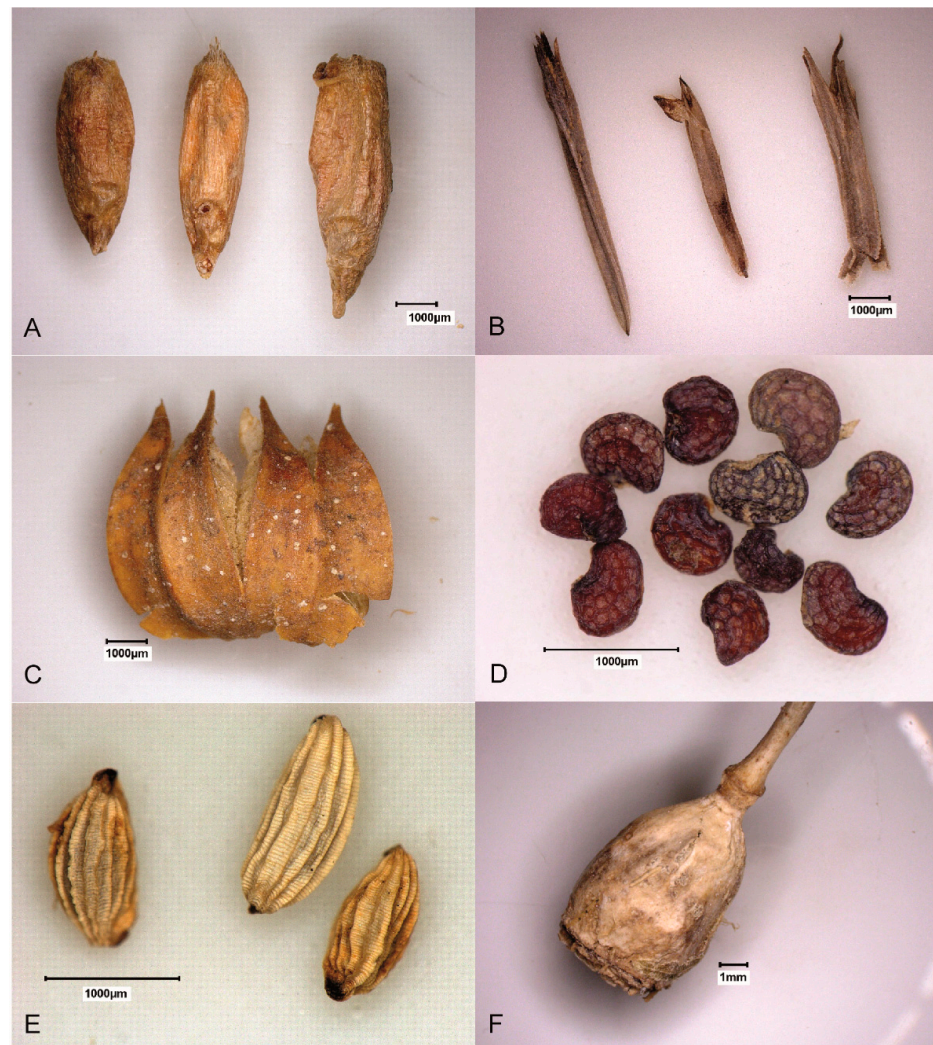
Figure 1. Selected taxa from the GVAC_06 Wellerholz from the Old Rathaus in Göttingen; (**A**) Grains of rye (*Secale cereal* ssp. *cereale*); (**B**) Anters of rye; (**C**) Capsule fragment of linseed (*Linum usitatissimum*); (**D**) Seeds of long-headed poppy/common poppy (*Papaver dubium/rhoeas*); (**E**) Seeds of red bartsia (*Odontites vernus*), (**F**) Capsule of common poppy (*Papaver rhoeas*).

The category of weeds and wild plants is the second most abundant category, with 368 finds (8.35%) and includes the highest diversity of taxa (*n* = 50) of all plant categories. Seeds of long-headed poppy/common poppy (*Papaver dubium/rhoeas*, Figure 1d) are represented by the highest absolute quantity and other frequent finds include red bartsia (*Odontites vernus*, Figure 1e) and white goosefoot (*Chenopodium album*). Seeds and fruits form the majority of plant parts, but several taxa were also represented by flower heads, fruits, pods and leafs (Figure 1f).

The category of indeterminate plants included a variety of remains, of which small fragments of charcoal were the most abundant. The group of animals and insects mainly consisted of diverse fragments of various insects, but also included bone fragments and caddisfly (*Trichoptera*) cases. The category of fungi consists of straw remains of different cereal taxa containing dark "lesions" that were likely caused by an infection with an unidentified fungus. Finally, the category of other materials included single finds of plaster, ceramics and yarn.

The rich diversity of plant taxa and plant parts in Wellerholz GVAC_06 attests to the excellent preservation conditions of the plant remains. The predominance of straw, ears and spikelets of rye indicate that it is likely that the plant material used to wrap the Wellerholz represents a harvest of rye. The high diversity of taxa of weeds and wild plants, the variety of plant parts (including flower heads, capsules and nearly complete plants) as well as the rarity of cereal grains, suggest that the plant material represents the by-products of the earliest stages of crop-processing of rye (i.e., between stages one and three as described by Stevens [66]. Considering that the aim of crop processing is to remove impurities from the harvest, the farther one progresses through the crop-processing sequence, the less reliable the data will be with respect to reconstruction of weed ecology and cultivation practice (especially the reconstruction of sowing season; e.g., [57]. In the case of GVAC_06, it can thus be assumed that the weeds and wild plants represent a significant part of the arable weed flora and that the data resulting from their analysis will not be severely biased.

3.2. Reconstructing Sowing Time and Cultivation Intensity

Figure 2 and Table S1 summarise the characteristics collected on plant-sociological groups, classification, physical properties, autecology and (a part of the) functional attributes of 26 taxa of weeds and wild plants from GVAC_06. The vast majority of taxa of weeds and wild plants are part of Ellenberg's classification [43] of modern segetal communities (in this case, classes 3.3 and 3.4). Most of the taxa (*n* = 12) are classified as Secalietea (class 3.4), a smaller portion as Chenopodietea (class 3.3; *n* = 7), while a few individual taxa belong to various other groups. The bulk (*n* = 21) is classified as indicator species of different levels of the plant-sociological groups. The dominant lifeform is therophyte-hemicryptophyte (*n* = 13), followed by therophyte (*n* = 9), hemicryptophyte (*n* = 2), hemicryptophyte-geophyte (*n* = 1) and hydrophyte-geophyte (*n* = 1). Annual taxa are dominant (*n* = 19), followed by taxa that are able to grow both as annuals and perennials (*n* = 3) and, lastly, perennials (*n* = 2). The reproduction type of the taxa is for the largest part by seed (*n* = 21) and a few (*n* = 5) are able to reproduce by seed as well as vegetatively through subterranean reproductive organs. Most taxa (*n* = 21) have a strategy type that is a mixture between ruderals, competitors and stress-tolerators, and six taxa are classified as ruderals. The most common flowering onset is intermediate (*n* = 19), with nearly equal amounts of taxa having a late (*n* = 4) and early (*n* = 3) flowering onset. Half of the taxa have a medium flowering duration (*n* = 13), while the other half have a short (*n* = 8) or a long (*n* = 5) duration.
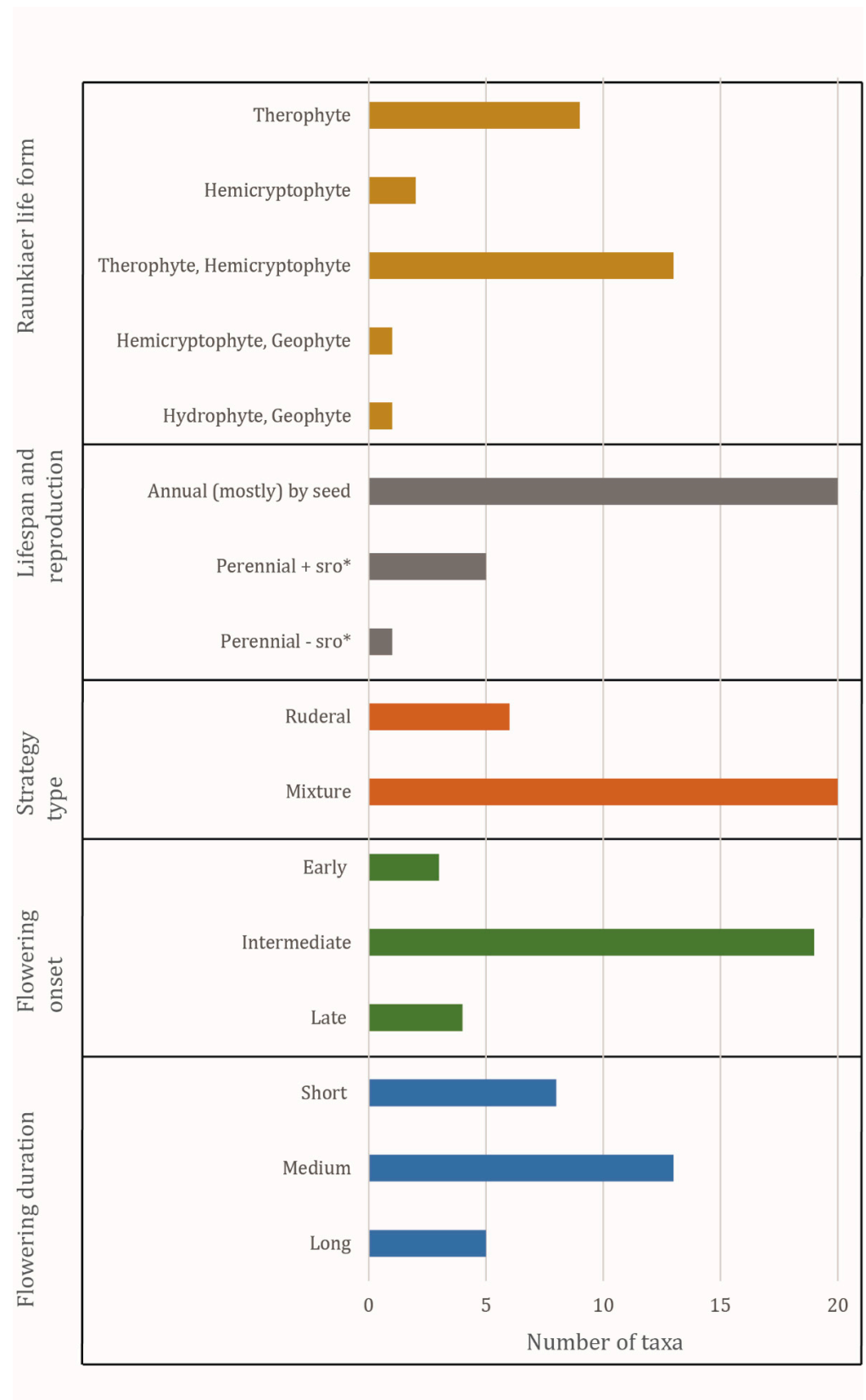
Figure 2. Summary of autecological data and functional attributes used to characterise the cultivation intensity and sowing time of the taxa of wild plants and weeds from GVAC_06. The acquired data has been collected from Ellenberg [43] and BiolFlor [60]. Abbreviation: sro *: subterranean reproductive organs.

The sowing time of the rye in the sample can be reconstructed from data based on the flowering onset and duration of flowering of the arable weed taxa (Tables 1 and 2). Autumn sowing is typically indicated by early and intermediate onset of flowering and a short flowering duration (e.g., [55,67]). In the case of the arable weeds in Wellerholz GVAC_06, the former indicators are predominant, while the latter indicator is the second most common; the flowering onset is thus clearly indicative of autumn sowing, while the flowering duration leaves some room for arguing. According to studies conducted by Bogaard et al. [67], indicators of spring sowing (i.e., late flowering onset and long flowering duration) are able to grow in autumn sown fields, while indicators for autumn sowing are usually not able to grow in spring-sown fields due to their inability to regenerate after spring ploughing. When autumn-sowing indicators occur in combination with spring-sowing indicators, it is therefore most likely that the data represent autumn sown crops. In the case of the arable weed taxa in Wellerholz GVAC_06 it can therefore be assumed that the rye in the sample was grown as a winter cereal.

The intensity of cultivation in this study is reconstructed with two sets of data and approaches: (i) descriptive values of autecological data and functional attributes, and (ii) discriminant analysis based on functional attributes. The former includes considerations of the flowering onset, the flowering duration, the Raunkiaer lifeform, the lifespan, the reproduction type and the strategy type (Table 1 and Table S1). The data on flowering onset and flowering duration proved to be inconclusive due to the predominance of a medium flowering onset and an intermediate flowering duration (Figure 2; Table S1). The data on the lifespan and reproduction type are more conclusive: the predominance of an annual lifespan and reproduction by seeds are typical indicators of cultivation practices that include a high degree of disturbance [2,41,58] (p. 145, pp. 344–346, p. 188). The most abundant Raunkiaer life form is represented by therophytes-hemicryptophytes, while therophytes form the second most abundant group. The latter is characteristic for highly disturbed habitats [59] (p. 810) and there are indications that some hemicryptophytes are as well able to grow in disturbed habitats, albeit in lower proportions [68] (p. 38). It is unknown to the authors whether the transitional lifeform of therophytes-hemicryptophytes thrives in disturbed habitats, and therefore therophytes, the second most abundant lifeform of the arable weeds in GVAC_06, are, on their own, seen as tentative indicators for a high degree of disturbance. The strategy type of the majority of the taxa was represented by a mixture between ruderals, competitors and stress-tolerators, and six taxa were classified as ruderals. Ruderals are typical indicators of high cultivation intensity, but, similar to the Raunkiaer lifeform, due to their relatively low representation in this dataset, they are seen as complementary to the data on lifespan and reproduction type. Altogether, the descriptive values can thus be interpreted as indicative of a high cultivation intensity.

The results of the discriminant analysis (classified with a high probability of 99.7%) show that the GVAC_06 sample is grouped within the low-intensity cultivation spectrum of the Haute Provence fields, toward the high-intensity cultivation spectrum of the Asturias fields (Figure 3). The results thus seemingly contradict those described above by suggesting cultivation practices with a low degree of disturbance. The low-intensity fields from Haute Provence were ploughed, harrowed and rarely to never manured, while the high-intensity fields from Asturias were hand weeded, ploughed, harrowed and manured regularly [62]. The data collected on the functional attributes of the weeds in the respective regions, however, especially relate to the productivity of the habitat, and thus to the degree to which the fields were manured [62]. It is therefore likely that the results of the discriminant analysis of Wellerholz GVAC_06 mainly reflect a lack of manuring, while not necessarily excluding a more intensive practice of soil tillage and/or weed control such as ploughing and harrowing. A likely practice in winter-rye cultivation is the use of the mouldboard plough [15,25] (p. 206, p. 76), which is known to have been used in the surroundings of Göttingen from the 12th century onward [30] (p. 458). In contrast to the ard, this is a type of heavy plough has been observed to intensively disturb the soil, effectively eliminating perennial arable weeds [2] (p. 145). Hamerow et al. [69] (p. 597) for example

describe that in Stafford (England) the arable floras of extensively cultivated fields reflect an increasing intensity from the 12th century onward as a result of the more common use of the mouldboard plough. The arable weed dataset of Wellerholz GVAC_06 indeed predominantly consists of annual taxa (Figure 2; Table S1), indicating intensive tillage of the soil. This observation, combined with the results of the discriminant analysis therefore suggest that the field in which the rye from Wellerholz GVAC_06 was grown was intensively tilled with the mouldboard plough, but was likely not hand weeded or manured.
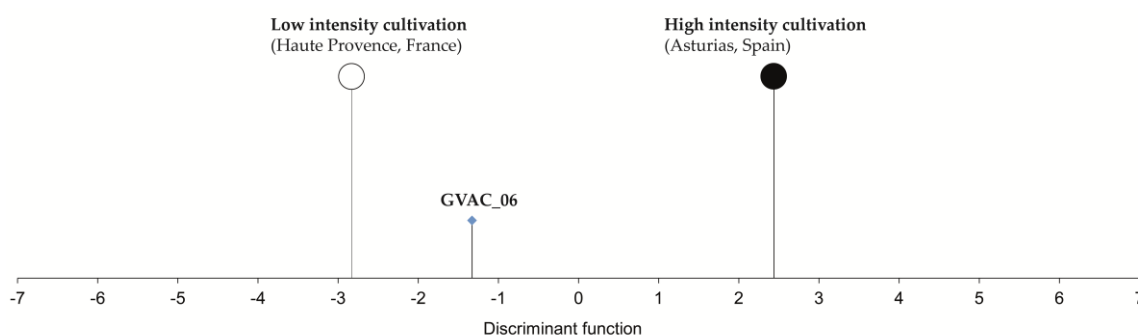
Figure 3. Results of the discriminant analysis of GVAC_06, using the discriminant function resulting adapted from studies of modern cultivation practices by Bogaard et al. [62] in Haute Provence, France (low intensity cultivation) and Asturias, Spain (high intensity cultivation).

3.3. Reconstructing Soil Conditions

The results of Ellenberg's indicator values for edaphic factors of the taxa of weeds and wild plants include a rather diverse range of values (Figure 4; Table S1). The water values show that most taxa prefer dry to moist ($n = 7$) and moist ($n = 6$) conditions. Five taxa are indifferent, while fewer prefer moist to damp ($n = 3$) and damp ($n = 2$) conditions. Extremely dry and dry conditions are represented by a single taxon each. The reaction values of the taxa indicate that 10 taxa are indifferent to soil pH. Five and three taxa, respectively, prefer to grow in weakly acidic to weakly basic and weakly basic to basic conditions. Fairly acidic to weakly acidic conditions are preferred by one taxon and fairly acidic conditions by three taxa. Acidic and extremely acidic conditions are preferred by one and two taxa, respectively. The reaction value of one taxon is unknown. The nitrogen values illustrate that eight taxa are indifferent to the nitrogen content of the soil. High to very high and high nitrogen contents are preferred by five and three taxa, respectively. Four plants prefer to grow in soil with average to high nitrogen content and three plants prefer an average nitrogen content.

On average, the values indicate moist or damp conditions (water value), more basic than acidic conditions (reaction value) and a moderate nitrogen content (nitrogen value). The water values and reaction values of the taxa correspond well to the soils and environmental conditions that prevail in the surroundings of Göttingen: the climate is temperate with a sufficient amount of rainfall to grow crops, and the typical soil in the surroundings of Göttingen is calcareous loess [30] (pp. 457–458). The nitrogen values can be interpreted in the light of the results of the discriminant analysis described above, suggesting that manuring was not practiced on a regular basis. The diverse range of indicator values might further be interpreted as indicative of a low level of competition for minerals and water. Notably, low levels of competition are characteristic for highly disturbed habitats, especially among ruderals [70] (p. 1171). The results of the indicator values can thus be seen to further support an interpretation of intensive soil tillage (see above).
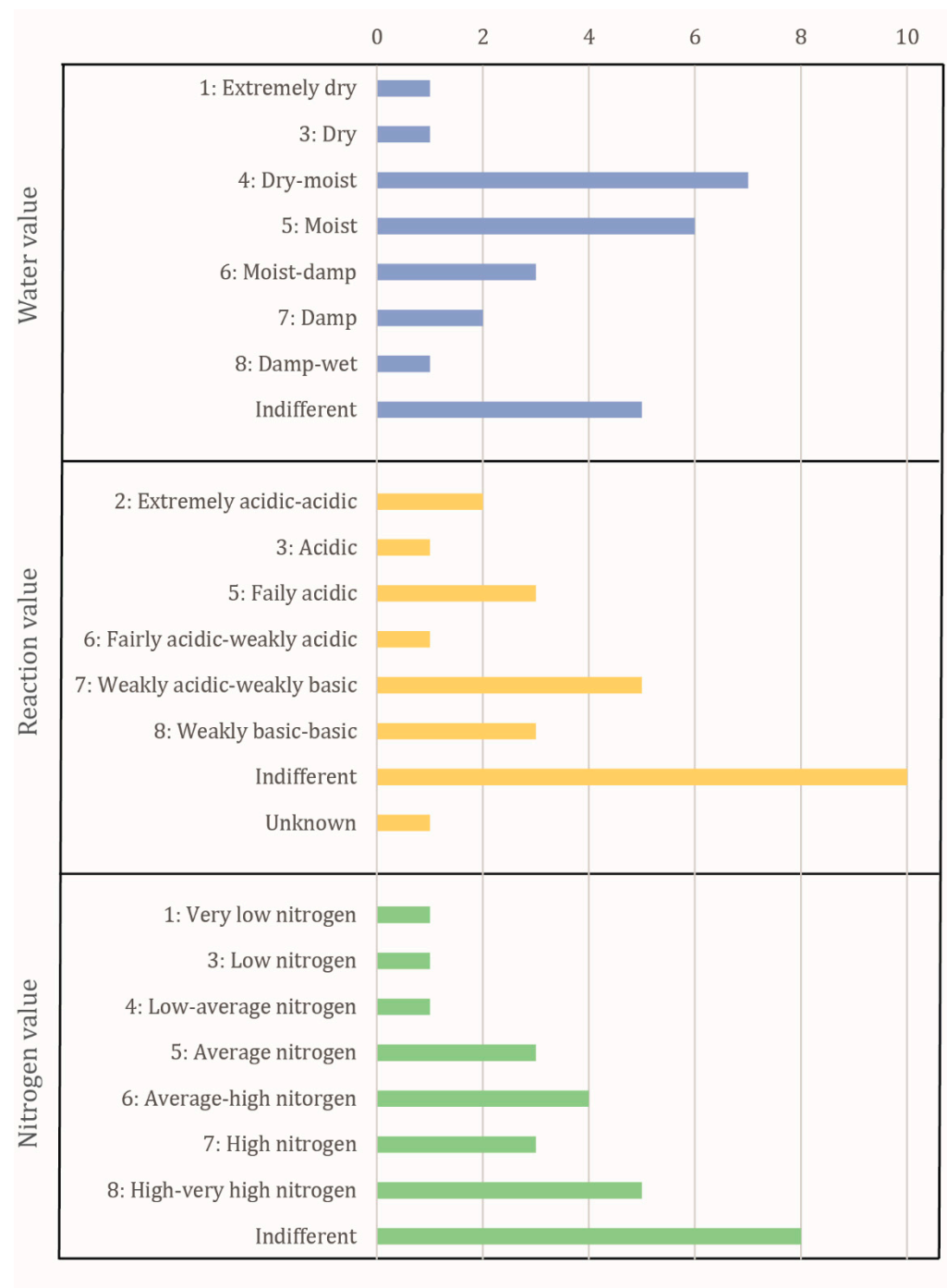
Figure 4. Summary of the indicator values for the three edaphic factors water value, reaction value (pH) and nitrogen value. The values were adapted from Ellenberg [43].

3.4. Indications for Crop Rotation

It is interesting to note that most arable weeds in GVAC_06 are classified as Secalietea (community 3.4; Table S1), which today are considered indicators of autumn sowing and/or extensive cultivation, while a smaller portion are classified as Chenopodietea, which today are typical of spring sowing and/or intensive cultivation [43] (p. 627). Segetal communities are known to have altered in composition through time, developing parallel to crops over thousands of years [39,43] (p. 186; p. 625). The communities of arable weeds that today are indicative of specific sowing times and intensity of cultivation therefore do not necessarily express similar conditions in the past [39] (p. 186). In the case of Wellerholz GVAC_06,

however, the analysis of autecological data and functional attributes mostly correspond to the Ellenberg classification, suggesting that at the time when the rye was cultivated, the arable weed flora had already achieved the typical composition that it has today.

Despite the existing division into communities of Secalietea and Chenopodietea, many of the taxa are not strictly confined to the respective communities and therefore variations do exist in their composition [43] (p. 628). The occurrence of portions of Chenopodietea in extensive and/or autumn-sown fields could, for instance, be an indication of crop rotation with summer, and/or intensively cultivated, crops [31,43] (p. 60; p. 627). In Wellerholz GVAC_06, a small portion of the arable weed assemblage is indeed classified as Chenopodietea, which can be considered an indication of crop rotation rather than rye monoculture. A further indication of crop rotation is the presence of a small portion of cultivated crops other than rye, which include bread wheat, hulled barley, common oat, flax and opium poppy (Table 4). According to Martin and Sauerborn [29] (p. 135), crops cultivated in previous rotation are able to emerge as part of the arable flora in the following rotations, although contradicting ethnographic observations by Jones and Halstead [71] (p. 112) suggest that minor admixtures of other crops are more likely the result of contamination on the threshing floor rather than contamination from previous rotations. In this case, the crop taxa observed as admixtures in Wellerholz GVAC_06 fit into the typical model of the three-field system in the surroundings of Göttingen during the Middle Ages and Early Modern period, whereby rye, wheat and possibly barley were grown as winter crops, and oat, flax and poppy were grown as summer crops [30] (pp. 457–458). It is therefore likely that, combined with the occurrence of different classes of arable weeds, the admixtures of crops in the rye harvest should be seen as evidence for the practice of the three-field system.

3.5. Implications for Harvesting Height

The minimum and maximum growing heights of the weeds and wild plants are shown in Figure 5 and Table S1. Low minimum growing heights (0–40 cm) are the most common and low and medium (41–80 cm) maximum growing heights are equally represented. Four taxa, respectively, have a medium minimum growing height and a high maximum growing height.
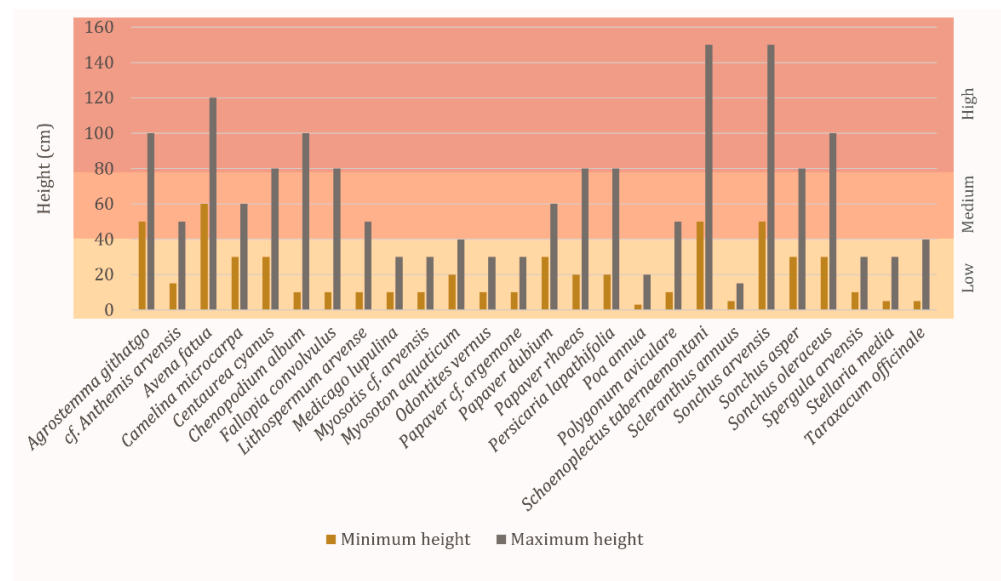
Figure 5. Summary of the minimum and maximum growing heights of the weeds and wild plants from Wellerholz GVAC_06. The heights have been adapted from [61] and subdivided into the following arbitrary categories adapted from Kreuz et al. [41]: short: 0–40 cm; medium: 41–80 cm; high: >81 cm.

A total of 260 individual straw remains were measured that could be identified as rye straw due to the presence of rye ears. The measurements were subdivided into seven height categories 0–20 cm, 21–40 cm, 41–60 cm, 61–80 cm, 81–100 cm, 101–120 cm and 121–140 cm (Figure 6). The majority of the straw was fragmented into short segments and thus fall into category A. The following categories are represented by less counts, with the tallest category (category G) containing a single measurement.
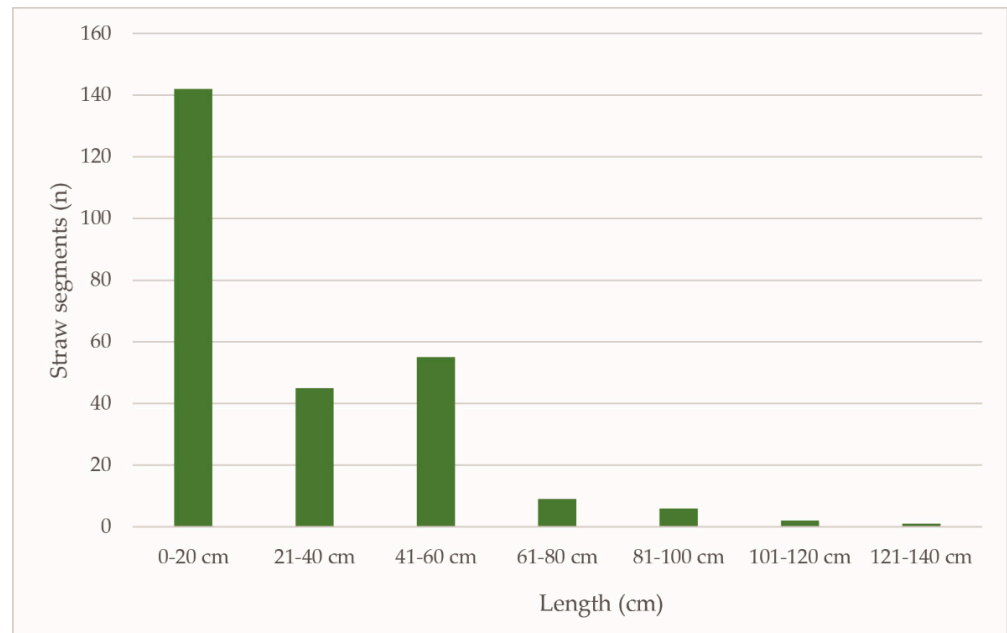

Figure 6. Length categories of rye straw measured from Wellerholz GVAC_06.

The minimum and maximum growing heights of the weeds and wild plants are various, ranging from short-growing plants (e.g., *Medicago lupulina* and *Poa annua*) to tall-growing plants (e.g., *Schoenoplectus tabernaemontani* and *Sonchus arvensis*). The maximum height of rye straw attested in Wellerholz GVAC_06 reached a height of 140 cm. The diversity in growing heights of weeds and wild plants alongside the presence of long straw segments of rye, suggests that the rye had been harvested by cutting close to the ground.

3.6. Sequence Analysis

We were able to reconstruct and annotate the complete chloroplast sequence of the rye sample obtained from Wellerholz GVAC_06 by mapping to a modern reference (Figure 7). The sequencing of the GVAC_06 sample resulted in 45,076,991 high quality reads. The chloroplast sequence consists of 114,874 base pairs and 57 open reading frames (ORFs) larger than 400 basepairs, including all known genetic domains, with a mean coverage of $29.3\times$ (Table S2). Compared to the reference sequence, we found 235 variable sites.

Phylogenetic analysis of the nuclear Internal Transcribed Spaces (ITS) locus (Figure 8) as well as the chloroplast matK region (Figure 9) show the GVAC_06 Wellerholz-sample forming a clade with the *Secale cereale* reference, indicating close relationship between the two, while the analysis of the rbCL-region (Figure 10) suggests more ambiguities between the Wellerholz sample and the *Hordeum vulgare* and *Secale cereale* references. Higher bootstrapping values for ITS and matK phylogenetic trees establish higher confidence in their accuracy in presenting the most likely phylogenetic relationship. This suggests that matK and ITS markers are more suitable for phylogenetic analysis of the Triticeae tribe than the rbCL region. The ITS region has a mean coverage of $130.8\times$, out of the total 807 covered sites, 105 were variable and 34 parsimony informative.
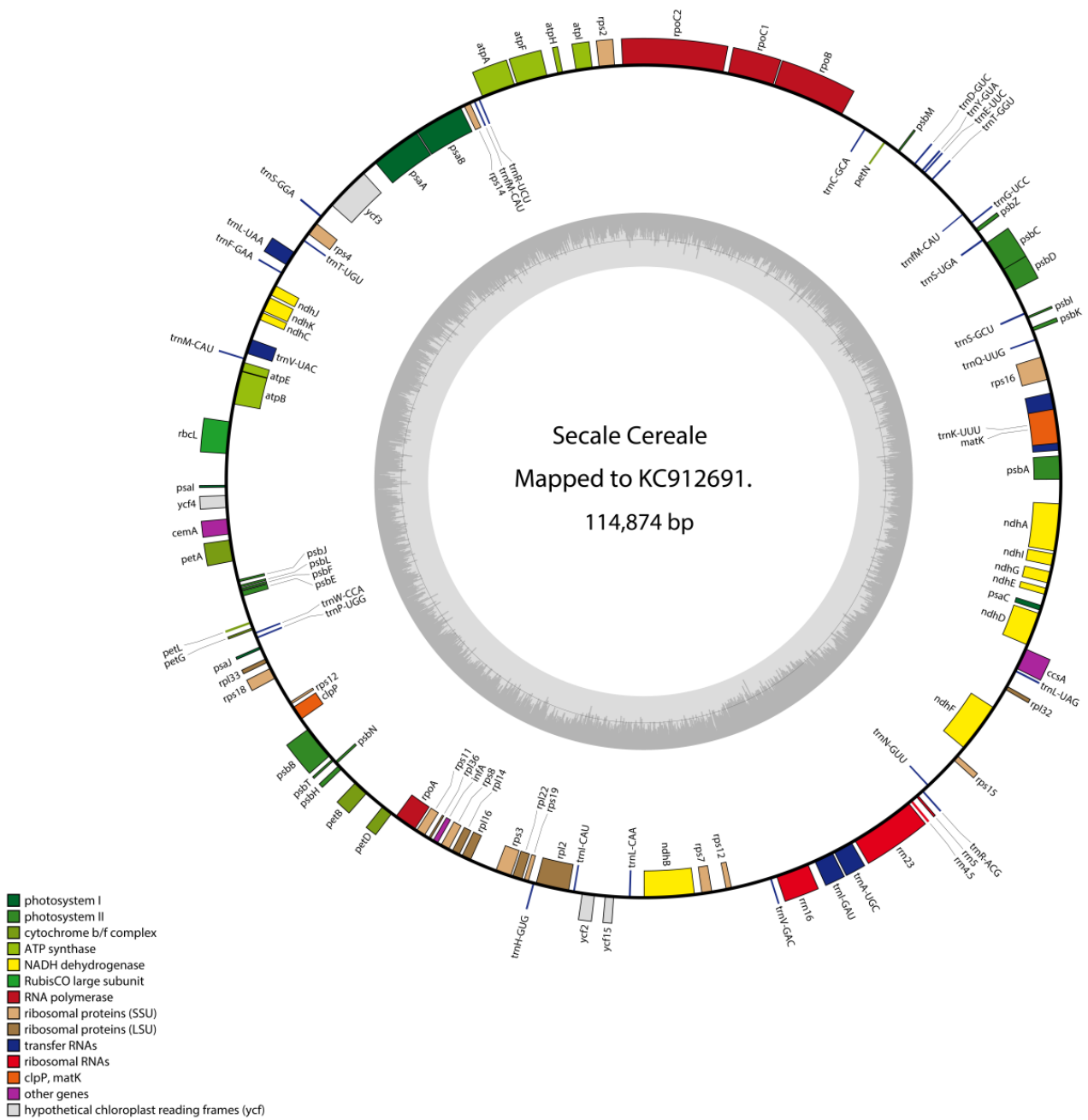
Figure 7. Chloroplast map from rye samples collected from Wellerholz GVAC_06. Domains are color-coded according to legend. The samples from Wellerholz GVAC_06 were mapped to Secale cereale chloroplast reference KC912691 using the Geneious pairwise alignment algorithm. The chloroplast map was created using the OrganellarGenomeDRAW tool (OGDRAW) version 1.3.1.
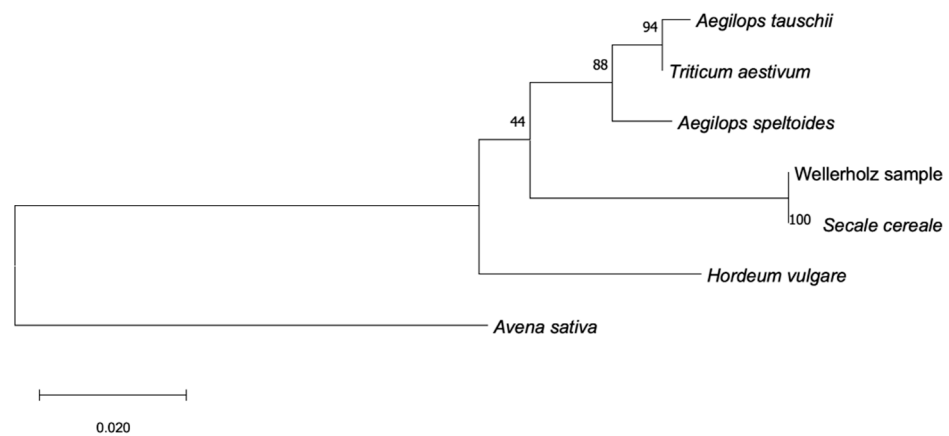
Figure 8. Genetic relationship of selected crop species based on the ITS region: The phylogenetic relationship was inferred by using Maximum Likelihood method and Tamura-Nei model. A sample collected from Wellerholz GVAC_06 is included labelled "Wellerholz sample". The tree with the highest log likelihood is shown. Branch length indicates substitutions per site. Bootstrapping values from 1000 iterations are displayed at the nodes.
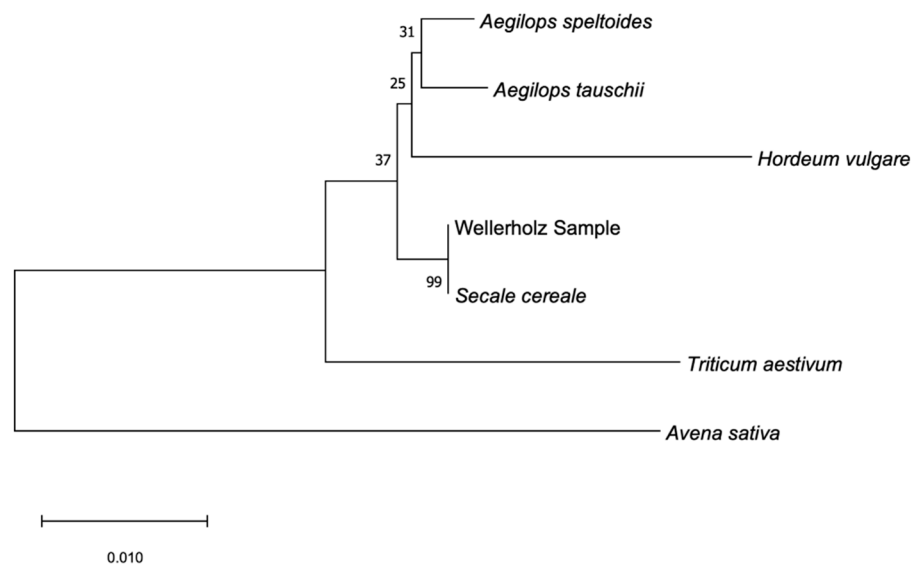
Figure 9. Genetic relationship of selected crop species based on the matK region: The phylogenetic relationship was inferred by using Maximum Likelihood method and Tamura-Nei model. A sample collected from Wellerholz GVAC_06 is included labelled "Wellerholz sample". The tree with the highest log likelihood is shown. Branch length indicates substitutions per site. Bootstrapping values from 1000 iterations are displayed at the nodes.

3.7. Radiocarbon Measurements

The radiocarbon measurements of one grain of rye resulted in four possible dates: 1637–1680 cal. AD, 1740–1753 cal. AD and 1763–1800 cal. AD and 1840–1954 cal. AD (Table 6 and Figure S1). This segmentation is caused by the Maunder minimum and the Suess effect, both of which had a considerable impact on the 14C content in the atmosphere [72–74], obscuring radiocarbon dates of material that dates between 1650 and 1950. As a result, in the case of Wellerholz GVAC_06, the remains of rye can only broadly be dated to between 1637–1954 cal. AD.
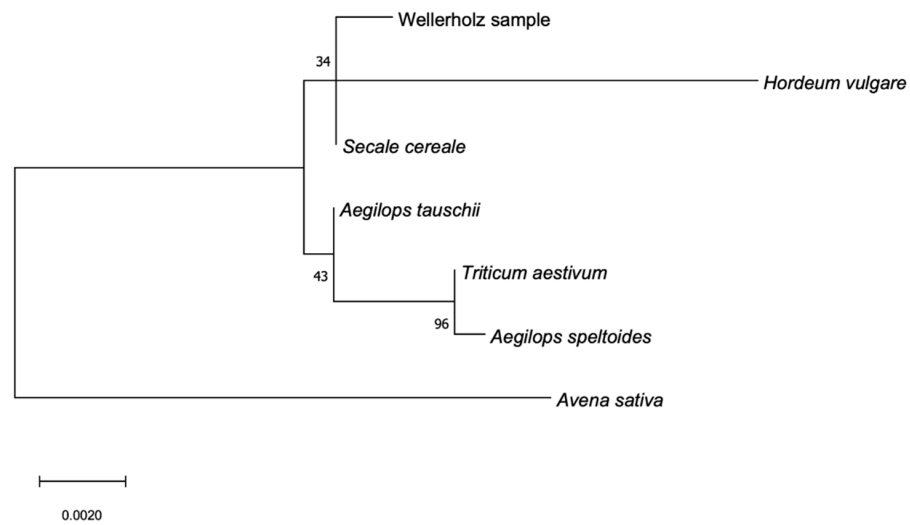
Figure 10. Genetic relationship of selected crop species based on the rbCL-region: The phylogenetic relationship was inferred by using Maximum Likelihood method and Tamura-Nei model. A sample collected from Wellerholz GVAC_06 is included labelled "Wellerholz sample". The tree with the highest log likelihood is shown. Branch length indicates substitutions per site. Bootstrapping values from 1000 iterations are displayed at the nodes.

Table 6. Radiocarbon date of one rye grain from GVAC_06 (95.4% probability), showing four possible dates: 1637–1680 cal. AD (49.6% probability), 1740–1753 cal. AD (2.7% probability), 1763–1800 cal. AD (33.3% probability) and 1940–1954 cal. AD (9.9% probability).

Number	Absolute Date (cal. AD)	Probability (%)
1	1637–1680	49.6
2	1740–1753	2.7
3	1763–1800	33.3
4	1940–1954	9.9

4. Discussion

The excellent preservation conditions of the remains from Wellerholz GVAC_06 presented in this study were attested in the diverse range of plant taxa and plant parts represented in the sample as well as in the successful sequencing of the chloroplast genome and phylogenetic analysis of rye. The preservation of a substantial portion of arable weeds, alongside indications for their origin from early crop-processing stages of rye, enabled a reconstruction of cultivation practices and provided insight into the conditions in the field. Taken together, the analysis of the autecological data, functional attributes and edaphic factors can be seen to reflect (i) autumn sowing of winter rye, (ii) extensive cultivation of rye (i.e., without the practice of manuring and hand weeding) that included intensive soil tillage (i.e., ploughing with the mouldboard plough and harrowing), (iii) the practice of crop rotation (most likely three-field system), and (iv) harvesting of rye close to the ground. The results of the analysis of the arable weed flora reinforce previous investigations of Wellerhölzer from the district of Göttingen, which likewise suggest winter cultivation of rye, harvesting close to the ground and the practice of the three-field system with the resulting presence of mixed classes of arable weeds [30,31].

The results of the aDNA analysis suggest that next generation shotgun sequencing methods can be used to acquire sufficient genetic information to study genetic variation at targeted loci as well as for entire genomic regions. The combined ITS and matK marker regions were suitable to accurately identify the plant remains from Wellerholz GVAC_06 on a species level. The positive experience from the assembly of next generation sequencing data to smaller genetic regions, such as barcode regions, as well as regions larger than 100 kilobases, such as

the chloroplast, signifies a great perspective for future sequencing projects of desiccated plant remains from Wellerhölzer and other historical specimens of rye.

In the last few decades, a wide array of (archaeo) genetic studies have been conducted on archaeological, historical and extant specimens (including landraces) of crops (for an elaborate consideration of this topic, see [44]). These studies have provided a multitude of significant insights into the history of domesticated plants, which include possible routes of dispersal [47,49,75–78], population structure of crops and their (wild) relatives e.g., ([8,23,24,47]), de-domestication of crops in novel habitats (e.g., [76]), loss of genetic diversity due to cultivation practices (e.g., [79]), continuous cultivation of locally adapted varieties of crops (e.g., [47,80]) and indications for the rate of evolution of domestication traits (e.g., [50]). Combined, the results of these studies emphasise the great potential of genetic and genomic analyses of ancient and modern crop specimens for answering archaeological questions about the origin, spread and diversification of domesticated plants. Although the genetic results presented in the current contribution are preliminary, they are promising for our envisaged future investigations on the potential role of selective pressures from local environmental conditions and cultivation practices in the genetic diversification of cultivated rye. Our results indicate that it is likely that the plant remains from Wellerholz GVAC_06 originate from the same field and the excellent preservation of the plant remains have enabled both the retrieval of the chloroplast genome, as well as a comprehensive reconstruction of the related cultivation practices and local environmental conditions. To our knowledge, no previous studies have focused on a combined consideration of aDNA and the arable weed flora directly related to the sequenced specimen and, based on the results presented in the current study, we strongly believe that desiccated plant remains from Wellerhölzer are particularly suitable for this purpose.

Although it is debated whether artificial selection induced through cultivation practices, harvesting and crop processing was conscious (e.g., [81]) or unconscious (e.g., [82,83]), it is a point where domestication studies find agreement; human agricultural practices are considered to have, at least partially, stimulated genetic changes in plants that eventually led to the development of domestication traits, regardless of whether this was a rapid, centralised event [81,84], a process that was more protracted in space and time [82,83], or an extensive process initiated by a shift from megafaunal seed dispersal of wild progenitors to human seed dispersal during the Late Pleistocene (for a recent overview, see [85,86]). Aiming at illuminating the relationship between environmental selective pressures, artificial selective pressures, genetic variation and their mutual role in the genetic diversification of rye, we believe that our results can also contribute empirical data to the discussion concerning the role of selective pressures and genetic diversity within the development of domestication traits. Following Zeder [87], we further emphasise the importance of studying direct human impact on the genetic diversification of plants and related alterations to existing habitats within current research on crop improvement and sustainability of biodiversity. Lastly, we anticipate that our future investigations will add a Central European perspective to the understanding of the genetic diversification of cultivated rye (e.g., [8,20–24]).

The ambiguous date of the plant material from Wellerholz GVAC_06 poses somewhat of a challenge with respect to the chronological contextualisation of the plant remains. At this stage, the material from Wellerholz GVAC_06 can only broadly be dated to between 1637–1954 cal. AD, falling within the Early Modern and Modern period. Due to the Maunder Minimum and the Suess effect (see Section 3.6) any material dating between 1650 and 1950 will be prone to a similar uncertainty in radiocarbon dates, corresponding to a large portion of the period in which Wellerhölzer were frequently used in traditional German half-timbered house construction [30–34]. However, we currently do not expect the lack of a precise date to obscure our attempt to illuminate the relationship between the diversification of rye and potential selective pressures from cultivation practices and the local environment. It is likely that once a sufficient amount of data has been gathered, potential genetic variation will be evident regardless of the date of the remains. Unfortunately, Early Modern and Modern material from

Wellerhölzer will not be suitable to address questions of mutation rates and it will be required to refine the current dates or use older remains for such a purpose.

In order to assess the impact of cultivation practices, local environmental conditions and genetic diversity on the genetic diversification of cultivated rye, a greater number of samples will be analysed in future investigations. Of special interest will be the comparison of arable weed and genetic data from Wellerhölzer that originate from areas where enduring rye monoculture (or eternal cultivation of rye) was practiced as opposed to areas where (three-field) crop rotation was practiced. In addition, our future research will include dendrochronological dates for those Wellerhölzer that have sufficient year rings and dull edges. This will generate a larger amount of chronological data and allow for a higher precision in the dating of the remains. Altogether, our results emphasise that the study of desiccated remains of plants from Wellerhölzer offer a unique opportunity for an integration of archaeobotanical reconstructions of cultivation practices and local environment and the sequencing of aDNA.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11122451/s1, Table S1: Data on synecology, autecology and functional attributes, Table S2: Sequencing statistics, Figure S1: Radiocarbon dates of one rye grain from the GVAC_06 Wellerholz showing four possible dates for the plant material (Image by: C. Hamann).

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