Crop Wild Relatives

– Population inventories in Denmark 2023

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Stråsø landscape with shrubs and selected CWR species: Fragaria vesca, Rubus sect. Rubus, and Vaccinium vitis-idaea. (Photos by JTD and BM)

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Summary

In this report we survey a selected list of Crop Wild Relative (CWR) plant species in Denmark. The survey was done at four sites, i.e., Husby, Stråsø, Mols, and Kattrup, which represent a west to east gradient across Denmark, and therefore include different environmental conditions, species pools, and genetic variation.

At each site, plot locations have been generated completely randomized, i.e. including no stratification of vegetation types or environmental condition. Each plot was a circular area with a radius of 15m, where we recorded the presence of CWR species, counted the number of individuals (population density), and assessed in-situ the viability of the population of each CWR species.

Across all four sites, we surveyed a total of 212 plots and found 54 CWR species. To improve the area covered for this inventory, we chose to include occurrence data from other vegetation monitoring thus adding 306 plots (of smaller plot sizes) to the total survey data pool. These additional plots added few species to the total species pool of each area but supported the less intensively surveyed area of Husby for a more comprehensive inventory. The majority of populations were viable, and the four sites represent candidates for future protection of CWR populations. The sites represent large differences in past land-use from a lesser managed coastal dune landscape and heathland to plantations and abandoned crop fields. Therefor we expect that the CWR populations of this inventory might represent a wide range of genetic resources.

This survey found many of the expected species at each site. We therefore conclude that randomized plot selection is successful in capturing the occurrence of CWR, and this simplistic methodology of occurrence and population counts represents a possibility for future monitoring efforts of e.g., population trends of CWR species. However, the monitoring protocol could be further improved by adding a stratification of vegetation and landscape types thus ensuring survey effort of vegetation types that have disproportionate low cover within the total surveyed area.

We propose that the survey methodology used and presented here could be implemented in citizen science efforts and approaches, firstly because of its simplicity in both needed equipment and the field protocol and secondly because it can be adapted to contribute monitoring of not only CWR species but also locally rare or red-listed species.

Introduction

Crop wild relatives (CWR) are plant species closely related to crop species, including livestock fodder grasses, fruit producing trees and shrubs, and medicinal plants. CWR include natives with long site persistence and local adaptation but also naturalized species that can withstand the climatic and competitive conditions when introduced to a new area. It is of special interest to preserve genetically unique populations that potentially can withstand future changes in environmental conditions from e.g. climate change and other global change drivers. Thus, CWR species with certain traits that are adapted to the climate and soil type of a particular region can be a potential resource for the improvement of crop species. By cross breeding or transferring specific genotypes from CWR species into crop species – such as pathogen resistance, tolerance to cold, drought, high salinity, or adaptation to long day lengths – the robustness of food production can be increased which is relevant in the context of a rapidly changing climate with potential pathogen outbreaks (Brozynska et al., 2016).

Identifying areas of hotspots of CWR species, both in species numbers and unique genetic variation is a central part of working with CWR, a thorough inventory of species occurrence and population status is needed to effectively point out areas in need of protection and conservation to maintain CWR populations. Protecting areas of high number of CWR species ensures that there will be sustainable populations that currently, but also into the future will provide valuable genotypes that can be available to breeders of crop species.

Another key emphasis in the designation of CWR protection sites is, that the populations protected need to be complementary in regards to the genetic variation found in a given region (García et al., 2017) e.g., if a Finnish CWR population exhibits unique traits not found elsewhere in northern Europe it would be necessary to protect a sufficient number of sites to cover the unique genetic variation found here, and not exclusively protecting sites in e.g., Norway as a representative for the whole Nordic region.

Currently there is a lack of sites dedicated to *in situ* conservation of CWR species in the Nordic region of Europe. However, most CWR species (mainly natives) are expected to be protected through conventional nature conservation areas and biodiversity management schemes. Sites of national protection such as national parks are thus expected to contain high species diversity, including CWR species, and serve as obvious candidates for CWR conservation and seed sampling activities. Some CWR species are non-native and mostly occur in ruderal habitats that conventionally are not included in area conservation and could be targeted by specific CWR conservation sites in the future.

There has not been established any standardized methodology for mapping and monitoring CWR populations even though this is a prerequisite to establishing potential CWR reserves, since the status and species richness of a site should be thoroughly examined before making protection plans. Further, to compare population trends between countries it would be highly beneficial if standardized surveying methods and protocols would be followed.

The areas visited in these four reports of CWR population surveys in Denmark include the two Nature National Parks Stråsø and Husby Klitplantage (designated but under establishment) owned by the Danish state and two areas of primarily privately owned land (Mols Laboratory and Kattrup Vildnis). Both privately owned areas contain natural areas protected by the Danish Nature protection law and parts are included in the Natura-2000 network. Recently, both landowners have committed to optimize conditions to benefit

natural processes in the two areas. The sites represent a gradient between western and eastern Denmark, in order to cover a large variety of the Danish species pool of CWR with the current survey. Two sites in the west represent sandy soils with heathlands and coastal habitats, one site in central Denmark includes extensive grazing in a rewilding context on sandy, nutrient-poor grasslands, shrublands, and deciduous forests close to a coast, and the fourth site in eastern Denmark represents abandoned arable lands on sandy-clayish soils and old deciduous forests.

Aims and objectives

The main objective was to survey the occurrences of CWR species and estimate their population sizes at four sites in Denmark in a standardized and repeatable way. In parallel, the overall goal of the project is to evaluate the status of CWR populations within each area. We further aim for ensuring optimal prerequisites for future conservation and monitoring of CWR species in the areas by developing a randomized inventory approach that can potentially be scaled and implemented across all Danish nature areas.

Methods

CWR inventory design

The species inventoried were vascular plants on the Nordic CWR priority list (Fitzgerald et al., 2018). The species were identified to highest possible taxonomic resolution that could confidently be identified by the observer.

To select the CWR sampling locations, we first generated a national grid layer covering the whole of Denmark with cell sizes of 30x30m. We then randomly selected sampling subsets of locations for each of the inventory areas. The random sampling strategy was chosen to firstly reduce observers bias and secondly support potential discoveries of previously unknown populations of CWR species. For practical reasons, the sampling effort was performed in subsets of generally 10 plots per time, representing the whole. When visiting the inventory areas for multiple days, several subsets were pooled for increasing time efficiency. The strategy forced the surveyors to visit areas otherwise potentially missed in a regular walk-through of the area. A plot was skipped if the random plot was situated on adjacent private land or on inaccessible water.

We decided on a relatively small size for the surveyed plots allowing them to be inspected by a single observer while maintaining a high level of detail and security in determination. Thus, the delimited plot areas allowed for a comprehensive and standardized inspection across surveyed areas. Each plot was sampled from the centre of a grid cells in a circle with radius 15 meters. All CWR species occurring in the circle were noted. Thus, due to the circular sampling strategy, we did not survey the whole area of a 30x30m grid cell. In this report a population is defined as the individuals occurring in each circular sampling plot. For graminoids a complete examination of all vegetative shoots would be very time consuming, so primarily flowering graminoids and easily recognisable vegetative species were noted. For species with stolon growth one individual was represented by clusters of plants in the counting thus the same plant individual possibly is counted several times, but the method still provides an estimate of population density without having to dig up root systems (practically impossible for most species e.g., *Prunus spinosa*), thus it is a compromise between what is practically feasible and what still provides information

on population density. We did not expect to find populations of more than 1000 individuals within the current plot size. For each plot, viability and threat categories were noted for each species and assigned to a status level. Due to the small area size of a plot level, we evaluated the general viability and threats for the CWR species for each area (See tables 3-6). The categories of the different observations are shown in table 1. For species specific maps illustrating population density and counts, see Appendix 1.

Table 1: Parameters measured in population surveys.

Parameter	Values & descriptions
Population counts within plot	Intervals of 1-10, 11-100, 101-1000. One count is defined as individual plants, for rhizome and creeping species each plant cluster is counted.
Viability	Is a species observed in a plot occurring at densities and life stages that seem viable (1), vulnerable (2), or highly vulnerable (3)
Main threat and severity	If evident, what is the main threat a species observed in a plot suffers from: Shading from natives, or invasives, grazing, anthropogenic disturbances, or low population numbers (gathered from IUCN Threats Classification Scheme).
Status	Is the vegetation of a plot wild, semi-natural, weedy, or different (Alercia et al., 2021)

Stationary monitoring plots

To increase the number of observations on CWR presences we included vegetation data gathered from already existing and stationary monitoring plots to assess trends in plant diversity. The locations of these plots were all distributed randomly with stratification and thus, should represent the landscape by covering gradients of vegetation structure, topography, productivity, and land-use intensity, derived from remote sensing products. This approach is expected to aid the fully random sampling strategy, to increase the probability of sampling in habitat types with smaller proportional coverage in the landscape, which may provide habitat for more rare or unique species. The data from the stationary plots were collected in years 2021-2022 from the Kattrup, Mols, and Husby areas. Since, these plots are related to ongoing projects, only the presence and absence of CWR species could be considered, i.e. recordings of population size, threats, and viability were not assessed (NA) to provide in the dataset. However, the observations from the stationary plots could support our evaluation of threats and viability in general for each of the sites.

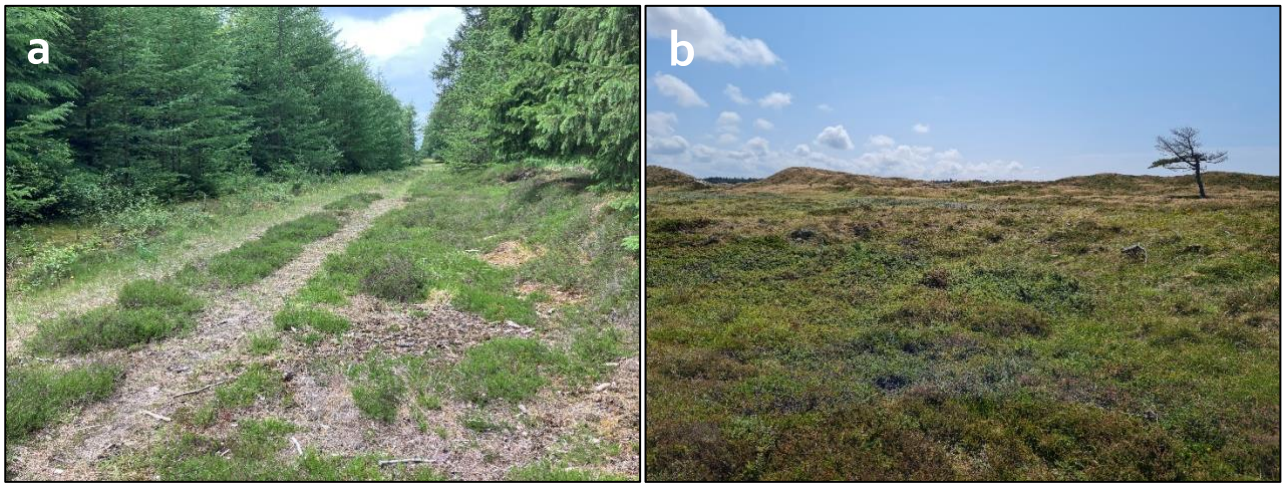

Figure 1: a) A rarely used road in Stråsø along *Picea* plantations with dwarf shrubs growing on the open road. b) Coastal heathlands and dunes with dwarf shrubs in Husby.

Figure 2: a) Abundant *Rubus* species in the forests in Mols. b) Abandoned farmlands in Kattrup with abundant thistles but also many CWR species of Poaceae.

Area descriptions

Mols

Mols Laboratory (from here on Mols), a research station owned by the Natural History Museum of Aarhus, is located within the national park Mols Bjerge in eastern Jutland as a part of a Natura-2000 site that consists primarily of semi-natural grasslands and shrubland, deciduous forests, and a marine coastline. The habitat types on the habitat directive found here are large areas of acidic grasslands, oak forests, dry heaths, and rich fens. A large part of this site has since 2016 been a designated rewilding area with all-year grazing of horses and cattle. The rewilding area is an enclosure of 120 ha but adjacent natural areas outside of the enclosure were also inventoried to include a larger variety of nature types such as coastal areas. The surrounding areas visited are also owned by Mols Laboratory (towards the coast) or by the state (Danish nature agency). The state-owned areas to the south and west of Mols are a part of a future designated nature national park meaning these areas will become a part of an about 800 ha large, connected area prioritized for natural dynamics to occur. The site contain nature types on the habitat directive such as acidic grasslands (with *Vicia spp.*, *Festuca spp.*), alkaline fens (with *Mentha aquatica*, *Schedonorus*

pratensis), deciduous forests with abundant shrub vegetation (with *Prunus spp.*, *Ribes spp.*, *Rubus spp.*, *Malus spp.*, fig 2a) thus a large variety of CWR species are expected to be found, furthermore, the coastline has distinct species such as *Leymus arenarius*, *Crambe maritima*, and *Beta vulgaris subsp. maritima*.

Management of many natural sites often has focus on specific parts of biodiversity e.g., either entirely open-adapted species thus management often centres around clearing woody plants, or at forested sites actively keeping deer out of forest plantings to maximize tree growth and prevent browsing damages. The rewilding strategy of the site has meant a transition away from the typical regime of keeping habitats in fixed states (with associated management plans and schemes e.g., continual clearing of woody plant encroachment), towards allowing the ecosystem to develop spontaneously on its own accord and habitat types vary through time. The introduction of large herbivores to roam freely year-round facilitate habitat heterogeneity by grazing certain areas intensely keeping these short and delaying the closing of forest glades by browsing shrubs especially in the winter months, thus creating variation (Pringle et al., 2023). This has created a landscape with abundant shrubs especially *Cytisus scoparius* and with many solitary trees of *Quercus*, *Betula*, and *Prunus*, further the gradients between open and shaded areas have become much wider meaning species thriving in such intermediate areas, such as *Vaccinium myrtillus* and *Rubus spp.*, are supported.

Stråsø

Stråsø is a state-owned area in western Jutland of 3500 ha containing large areas of mainly conifer plantations and heathlands (fig 1a), Natura-2000 covers mainly open areas of the site including habitat types such as heaths, inland dunes, and bogs, further small areas of oak forests are appointed. Stråsø was designated in 2021 to become Nature National Parks with biodiversity as a primary objective (Naturstyrelsen, 2022a). About half of the area is open vegetation with some of the largest sites of protected land in Denmark (of the Danish nature protection law) on the vast heathlands. The remaining area is mainly constituted of conifer plantations established in the beginning of the 20th century currently making up 70% of the forests, of this European conifer species dominate with e.g., *Picea abies*, *Pinus sylvestris* & *Abies alba*. This means most of the original oak forests have been replaced with more productive timber species, and the forest stands are mostly even-aged and dark. Historically, unvaluable trees and shrubs have been eradicated thus there is possibly a lack of CWR populations of e.g., *Prunus* and *Malus* (Naturstyrelsen, 2022a). The vast areas of heathlands support especially *Vaccinium* species which are CWR, however few other species are found in such habitats, and in the forested areas where forestry practices have created dense conifer forests few CWR are able to thrive here. There are however, some remnants of acidic grasslands, and an area of previously arable lands of almost 200 ha furthest to the SW has been developed rather quickly towards species rich grasslands since the state bought these areas in the 1990s (Naturstyrelsen, 2022a). Increasing the area of grasslands means potentially larger areas of habitat for many of the CWR Poaceae such as *Poa spp.* and *Festuca spp.*, and also Fabaceae such as *Vicia spp.* and *Trifolium spp.*

A part of the NNP designation is also that the forests are planned to be converted to "untouched forests", meaning an active effort is put into creating natural variation in the plantations by making forest clearings, veteranizing tree e.g., ring-barking trees to lighten the forest floor and create standing dead wood. Resultingly it is expected in the future for the forests to facilitate higher species richness of forests herbs and shrubs, which also is beneficial for CWR species. Further the introduction of free roaming horses and cattle will prevent succession on the open areas, thus facilitating CWR species which mainly constitute of open-adapted species. Furthermore, deciduous native trees are prioritized from now on, meaning a broader biodiversity is expected to be promoted rather than forests constituted of exclusively conifers.

Husby

Husby Klitplantage is a state-owned area of 960 ha by the coast of western Jutland containing coastal dunes, heaths, and conifer plantations. The site overlaps with a Natura-2000 area with typical coastal habitat types of white dunes, grey dunes, heaths, and wet dunes with or without *Salix repens* (fig 1b). The N-2000 area overlaps little with the conifer plantation. Husby was designated in 2022 to become Nature National Parks (NNP) with biodiversity as a primary objective (Naturstyrelsen, 2022b). Of the total national park area more than 600 ha is forests where 50% of these are invasive conifers (*Pinus contorta* & *Picea sitchensis*). About 360 ha is open areas of mainly coastal heaths. The conifer plantations closest to the sea have characteristics resembling shrub forests, the intense winds and moving sands hinder the trees from growing tall and often kill the trees at a young age. However, species such as *Pinus contorta* (native to NW North America) & *P. mugo* have proven to easily propagate on the heaths thus posing a threat to the light demanding species associated with dune ecosystems. The invasive species *Rosa rugosa* has invaded most coastal dunes of Denmark including the study area and propagates easily, there are active mitigation strategies such as digging up plant populations funded by e.g. EU Life projects (Strandby, 2019). Only about 400 ha is currently fenced and has been grazed by horses and cattle since the summer of 2022 (this grazing project was set in motion before the announcement of the NNP), potentially more area could in the future be included in the designated NNP especially the several 100 ha conifer plantation to the east of the enclosure. In the 2010s over 100 ha of conifers were cleared in the southern part of the area to increase the area of coastal dunes (Strandby, 2019). This exposed parable dunes and allowed sand dynamics once again to occur e.g., vegetation blow-outs and sand deposition, and which are distinct of coastal ecosystems. As in Stråsø the forests of Husby are converted to "untouched forests" meaning there is an ongoing active restoration of the forests, see more further above.

Of the CWR species pool the areas of heath support *Vaccinium* species, however few other species are often found here. Also, in the forested areas where forestry practices have created dense conifer forests few CWR are able to thrive. The coastline can support species such as *Leymus arenarius*, but the other CWR coastal species are more associated with the eastern coasts that are less wind-disturbed thus likely absent from Husby.

Kattrup

The study site Kattrup Vildnis is a large privately owned area in western Zealand of ca. 900 ha, it contains deciduous forests, large areas of former agricultural fields, and small areas of species rich meadows and grasslands. This area overlaps with two Natura-2000 sites that run along the rivers of Åmose å and Halleby å and the adjacent forests of habitat types of beech on dry soils and alluvial forests with alder and ash on the wetter soils, to the west is a wide river valley of open vegetation but no habitat types are found here. Currently a large nature reserve is being developed involving introduction of large herbivores (horses, boars, elks etc.) and abandonment of agricultural and forestry practices (Kattrup Vildnis, 2022). About half of the planned rewilding area is forested and the other half is open vegetation of mainly well-drained conditions. 78% of the open area were agricultural crop fields until 2021, and the remaining area is mainly meadows and bogs.

The vast areas of former crop fields have been colonized by various species especially of ruderal traits, in the summer 2023 large areas were covered by tall thistle species (*Cirsium arvense* & *vulgaris*, *Carduus crispus*, fig 2b), the abundant nutrients, large invadable areas of bare soil, and decrease in disturbances have allowed these to become dominant. The sandier fields have been colonized by both ruderal species but also grassland specialists, *Helichrysum arenarium* was found in several populations and *Pilosella*

officinarum and *Hypochaeris radicata* were frequently observed. Woody plants were not appearing to be colonizing the crop fields, perhaps due to the large populations of fallow, red, and roe deer in the area but as time progresses shrubs will likely invade the fields. The forested area is predominantly deciduous forest, mainly beech (*Fagus sylvatica*), by two thirds of the area while conifer plantations make up the rest of the area including Christmas-tree fields. The beech forests are especially of richer soils and well-developed vegetation with species such as *Lamium galeobdolon* and *Epipactis purpurata*. Kattrup Vildnis only contains 0.6 ha of protected dry grasslands (Kattrup Vildnis, 2022), thus CWR species belonging to this vegetation are not expected to be abundant in the general area, however the vast areas of former crop fields, along with the recent abandonment of farming, means many ruderal species establish and gain large populations. Many CWR species of e.g., Brassicaceae belongs to ruderal habitats (*Diplotaxis spp.*, *Brassica spp.*, *Barbarea spp.*) and Poaceae (*Lolium spp.*, *Schedonorus spp.*, *Avena fatua*), thus abandoned crop fields are potentially hot spots of CWR, especially as a source of the non-native ones. In the future many of these fields potentially develop towards diverse grasslands, but the process is expected to be slow especially because of the nutrient rich soils as a legacy of many years of farming (Fagan et al., 2008).

The cessation of agricultural practises (e.g., ploughing, herbicide use) is expected to drastically improve conditions of most plant populations. Further the plan of introducing large herbivores such as horses and wild boar will aid in creating habitat heterogeneity thus facilitating more species to coexist, grazing keeps some areas low thus promoting grazing tolerant shorter species, boar activities such as soil disturbances when foraging promotes ruderal annuals and in general allows for improved seedling germination.

Crop Wild Relatives in four Danish natural areas

In total 212 plots were visited across all 4 sites (table 2). Adding stationary vegetation monitoring data yielded 306 more plots to describe the distribution of CWR. We found a total number of 54 CWR species in the inventory campaign of four areas. A large part of the observed populations were assessed as viable in the areas, but the observations of population sizes varied in number of recordings for the number of individuals (table 3-6). We did not observe any populations with more than 1000 defined individuals within the designated plots of 30x30 m.

Table 2: Summary statistics of the study sites containing number of CWR species found of each site, the average CWR species number of the plots (Mean), standard deviation (SD), and the minimum (Min) and maximum (Max). Also showing how many CWR and stationary (St.) plots were associated with each site.

Location	CWR species	Mean	SD	Min	Max	CWR plots	St. plots
Mols	33	3.36	2.79	1	16	55	22
Stråsø	23	2.19	1.96	0	11	75	0
Husby	4	1.43	1.00	0	5	14	115
Kattrup	35	3.37	2.14	0	14	68	169
					Total:	212	306

Inventory of Crop Wild Relatives at Mols

Relative to its area Mols was the most intensively surveyed site of the four inventory areas with 55 plots surveyed on an area of 120 ha (excluding the buffer area) (fig 3) where a total of 33 CWR species were found, adding the monitoring data added 22 plots to the inventory pool. As expected, the heterogenous

and shrub rich areas of Mols offers a high frequency and abundance of *Rubus spp.* and *Prunus spinosa*, and the large areas of grasslands offer frequently occurring *Festuca rubra*, *Poa pratensis*, and *Trifolium repens*. *Rubus fructicosus* occurred in 82% of plots thus is a very widespread species, the other common *Rubus* species (*idaeus* and *caesius*) were also frequently occurring. Of Poaceae CWR there was a high diversity e.g., nutrient poor adapted species *Phleum pratense subsp. nodosum*, *Festuca ovina*, and *brevipila*, and species of more nutrient rich sites *Dactylis glomerata* and *Lolium perenne*. Mols also harboured high richness and abundance of *Trifolium* species e.g., *repens*, *pratense*, *arvense*, and few *medium*. The latter preferred roadsides outside the grazing enclosure likely less grazing tolerant than the others. Of woody species *Prunus* was well represented with *spinosa* being the most frequent, but *avium* and *cerasifera* are also widespread, the latter especially with solitary trees in the grasslands. *Malus sylvestris* is common at Mols and has many old trees, this species is elsewhere threatened by genetic flow between the naturalized *Malus domestica* often dispersing from gardens (Wagner et al., 2014). Further *Corylus* is quite frequent especially at the wet forested areas to the east. The two coastline plots covered the species *Beta vulgaris subsp. maritima* and *Leymus arenarius*. *Crambe maritima* was not found in the survey plots but is also known and recently observed from this coast according to databases of public species observations (Arter.dk). However, we have not included public databases in our inventory data to avoid the risk of publishing unvalidated observations. Of other CWR coastal species *Angelica archangelica* is known from the region and might appear at the site. The most species rich plot was close to a road thus ruderal species were present (*Medicago sativa subsp. sativa*, and *Lolium perenne*), but the plot was also partly shaded with trees and shrubs (*Malus sylvestris*, *Ribes spp.*, and *Prunus spp.*) consequently achieving high species richness.

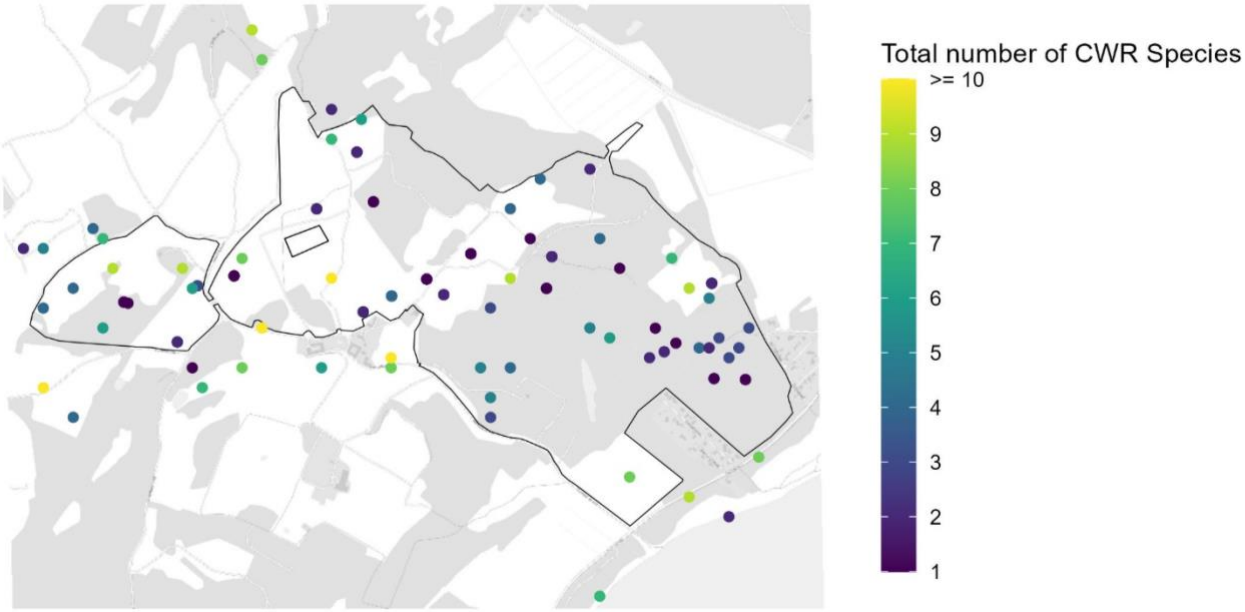

Figure 3: Number of Crop Wild Relative species of plots in Mols, data from both Crop Wild Relative inventory and vegetation monitoring. (Background map from Styrelsen for Dataforsyning og Infrastruktur, Denmark).

Population densities

The population density of species found at Mols is shown in table 3. The population density survey reveals some species that are possibly vulnerable/infrequent at Mols. Of non-woody species *Medicago spp.* was both rare and had low population numbers, these species prefer more alkaline soils thus the study site represents marginal habitat for such species. Further *Phleum pratense* (+ *subsp. nodosum*) and *Schedonorus pratensis* both were infrequent but is possibly overlooked, for the latter species more plots at the alkaline

meadows might result in additional findings, although this is speculation. Mols appears as a very suitable site for many CWR woody species, only *Ribes spp.* seem rare and in low numbers with *R. uva-crispa* the most common, there is abundant wet forests thus suitable habitat for this genus, but they appear to be rare. The genus *Rubus* is quite dominant at Mols, but the species *R. caesius* has an odd distribution especially along the roads, and is much rarer than the other *Rubus*, like *Medicago* it prefers more alkaline soils thus is quite absent from the acidic soils that dominate at Mols.

Monitoring data

No new species were found by adding monitoring data from 22 plots.

Threats and population robustness

Summarizing for Mols it seems to contain a large part of the Danish CWR species pool, the management change towards spontaneous developing vegetation where woody encroachment is not actively cleared means a significant increase in shrub vegetation. Thus, species strictly associated with open habitats could decline. However, open areas are still in large part maintained by the cattle and horses (albeit with a substantial proportion of woody species) by counteracting woody encroachment, further they browse the understory of the oak forests increasing light availability for the forest-floor vegetation to the benefit of e.g., *Vaccinium myrtillus*. Woody vegetation provides a perhaps unappreciated effect of shade by lowering soil evaporation, which during drought periods especially affects sandy grasslands, thus alleviating herbaceous vegetation of stress (Breshears, 2006). Additionally, the site has abundant thorny shrubs (*Rosa spp.*) and trees (*Prunus spinosa*) that can act as grazing repellents thus plants vulnerable to grazing can find refuge here in cages of thorns.

In the future there is not expected to be serious threats on the CWR populations at Mols, the area is not expected to experience land-use or management change thus the ecosystem can exist on its own premises without excessive human control.

Table 3. Frequency of CWR species in Mols. Ranked from the most abundant, including the frequency of absence of CWR. The percentage of populations in each density category is included. Viability summarizes number of occurrences and *in situ* assessment, * indicates species that are generally more common than the inventory shows and may be less of a concern than indicated here. Threats summarizes evident categories that might extirpate a species from the site.

Species	Frequency %	Pop. 1-10 (%)	Pop. 11-100 (%)	Pop. 101-1000 (%)	Viability	Threats status
Rubus fructicosus	81.8	33.3	51.1	15.6	Viable	No threats
Festuca rubra	47.3	34.6	50	15.4	Viable	No threats
Rubus idaeus	41.8	39.1	56.5	4.3	Viable	No threats
Poa pratensis	38.2	47.6	42.9	9.5	Viable	No threats
Prunus spinosa	38.2	47.6	52.4	0	Viable	No threats
Dactylis glomerata	30.9	47.1	47.1	5.9	Viable	No threats
Trifolium repens	32.7	66.7	22.2	11.1	Viable	No threats
Corylus avellana	27.3	80	20	0	Viable	No threats
Lolium perenne	25.5	42.9	42.9	14.3	Viable	No threats
Malus sylvestris	25.5	100	0	0	Viable	No threats
Trifolium pratense	23.6	38.5	53.8	7.7	Viable	No threats
Poa trivialis	18.2	30	60	10	Viable	No threats
Prunus cerasifera	16.4	100	0	0	Viable	No threats
Festuca ovina	16.4	55.6	44.4	0	Viable	No threats

Species	Frequency %	Pop. 1-10 (%)	Pop. 11-100 (%)	Pop. 101-1000 (%)	Viability	Threats status
Prunus avium	12.7	85.7	14.3	0	Viable	No threats
Rubus caesius	10.9	33.3	66.7	0	Viable	No threats
Vaccinium myrtillus	10.9	33.3	66.7	0	Viable	No threats
Ribes uva-crispa	9.1	100	0	0	Viable	No threats
Trifolium arvense	9.1	80	20	0	Viable	No threats
Sinapis arvensis	3.6	100	0	0	Concern	Unknown
Mentha aquatica	3.6	0	0	100	Concern*	No threats
Festuca brevipila	3.6	50	50	0	Viable	No threats
Trifolium medium	3.6	50	50	0	Viable	No threats
Vicia sativa subsp. nigra	3.6	100	0	0	Concern*	No threats
Leymus arenarius	3.6	50	50	0	Concern*	No threats
Phleum pratense	1.8	100	0	0	Concern*	More data needed
Rubus armeniacus	1.8	100	0	0	Concern*	More data needed
Vicia sativa	1.8	0	100	0	Concern*	More data needed
Medicago sativa subsp. sativa	1.8	0	100	0	Concern	More data needed
Medicago lupulina	1.8	100	0	0	Concern	More data needed
Ribes spicatum	1.8	100	0	0	Concern	More data needed
Phleum pratense subsp. nodosum	1.8	100	0	0	Concern	More data needed
Trifolium striatum	1.8	100	0	0	Viable	More data needed
Beta vulgaris subsp. maritima	1.8	100	0	0	Concern*	More data needed
Schedonorus pratensis	1.8	100	0	0	Concern*	More data needed
Poa humilis	1.8	100	0	0	Concern	More data needed
Ribes nigrum	1.8	100	0	0	Concern	More data needed

Inventory of Crop Wild Relatives at Stråsø

Relative to its area Stråsø was the least intensively surveyed of the 4 inventory areas with 75 plots surveyed out of the large area of about 3500 ha but nonetheless had the most total CWR survey plots (fig 4). At Stråsø a total of 23 CWR species were found across 75 plots on the ca. 3500 ha area. Many of the plots have low number of CWR species due to the habitats of Stråsø i.e., heathlands and conifer plantations, harbour a low part of the species pool of CWR. The most frequent species were *Vaccinium vitis-idaea* and *uliginosum* occurring at 60% and 42.7% of plots respectively (table 4), both are characteristic of heathlands the latter of wetter conditions and the former of dry, both can tolerate shade thus were also found in mature conifer forests. Other frequent species were *Festuca rubra*, *Poa spp.*, and *Trifolium spp.*, these occurred especially at plots intersecting with roadsides or at grasslands. The species *V. oxycoccos* was only found at one plot, it grows in acidic bogs or wet heaths which are abundant at Stråsø but possibly not covered sufficiently in this field survey, *V. myrtillus* was found to be rarer than expected, it thrives in open forests and possibly declines in intensive forestry areas with practices such as clear-cuts and disruption of habitat continuity. Woody CWR species were rare in the survey such as *Prunus spp.* and *Corylus avellana*. The one plot with *Malus domestica* was fenced to prevent browsing and thus planted and does not represent a naturalized population. The lack of woody CWR could be explained by the historic practice of active removal of non-valuable timber species to reduce competition to the desired conifers (Naturstyrelsen, 2022a), but also the

nutrient poor sandy areas of Stråsø are not the preferred habitat for *Prunus spp.* and *Corylus avellana*. Further Red deer are abundant at the site, and they browse heavily on young deciduous trees thus challenging the regrowth of these (Speed et al., 2013). The farthest southwestern part of the area were historically a mixture of arable lands and grasslands that in the 1990s stopped being farmed and appear to harbour a higher number of CWR. The plot of the highest number of CWR was in a hedgerow next to an open area that has been farmed for hay (still is mown), thus potentially has a history of being sown with fodder grasses and fertilized to increase yields. There was an absence of CWR species in 13.3% of plots, which is the highest compared to the other study areas (Husby is an exception with a lack of plots), many zero species plots were situated inside conifer monocultures where dense shade and clear-cut management extirpate understory vegetation of both herbaceous and woody plants.

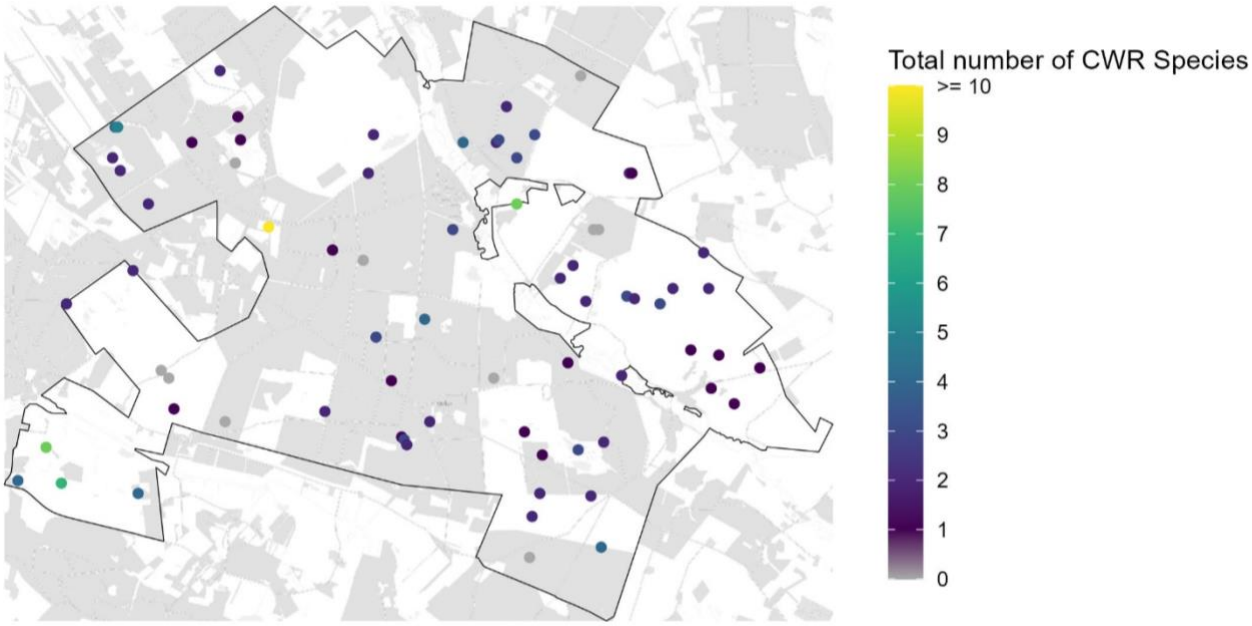

Figure 4: Number of Crop Wild Relative species of plots in Stråsø, data from both Crop Wild Relative inventory and vegetation monitoring. (Background map from Styrelsen for Dataforsyning og Infrastruktur, Denmark).

Population densities

The population density of species found at Stråsø is shown in table 4. The present woody CWR seem rare and occur in low densities of 1-10 individuals however, density alone is a poor indicator of viability of woody species, because of their size they rarely reach more than 10 individuals at each plot and since few mature individuals are more valuable than 10 seedlings that are vulnerable to browsing or shading. The infrequency of these species is on the other hand concerning. Of the herbaceous species it is difficult to infer, only the grasses *Festuca rubra* and *Poa pratensis* reaches densities of 101-1000 individuals. *Trifolium arvense* and *Festuca ovina* characteristic of acidic grasslands and heaths were uncommon and occurred often in low abundance, surprising since there is abundant cover of their habitat.

Threats and population robustness

Summarizing for Stråsø it seems to contain a moderate-small part of CWR total species pool. The management is shifting from conifer plantation to a focus on biodiversity by ceasing forestry practices and introducing large herbivores. Dominant, coarse grasses such as *Molinia caerulea* that encroach wet heaths

are often avoided by deer species, but the large grazers planned to be introduced may prevent these plant species from becoming dominant thus favouring CWR *Vaccinium oxycoccos* and *uliginosum*.

Drastic changes are on its way for Stråsø because of the National Park designation with the clearing of large stands of invasive conifers, but this is not expected to threaten any CWR on the contrary it will likely increase the area of suitable habitat for multiple CWR species e.g., heath and grassland specialists (*Vaccinium spp.* and *Festuca spp.*). Further species not found in this survey might invade the forest clearings thus there is potential for developing new species populations here. The introduced large herbivores will promote open habitats, but their effects will depend on the densities they are released in, and the State Nature Agency has plans of releasing few animals at a start to ensure enough food for all individuals and to allow the animals to familiarize with the area, thus some areas of grassland or heath might be grazed too infrequently resulting in encroachment. The newly adopted wilder regime inevitably results in areas previously open developing into areas with varying wooded cover. Large grazers such as horses and cattle can affect heaths by increasing the cover of graminoids and converting homogeneous dwarf shrub vegetation to mosaics of grassland-heath vegetation (Bokdam and Gleichman, 2001), however this change is not expected to be so drastic that the heath-associated CWR species are to be threatened. On the contrary this increase in vegetation heterogeneity might support higher number of CWR species such as *Festuca ovina* and *Trifolium spp.* increasing in frequency. The open heathlands are threatened by woody encroachment by both conifers and deciduous species such as *Populus tremula*, thus the management at Stråsø has been to cut or burn large areas of heaths to both rejuvenate the Calluna while also destroying young emerging trees (Naturstyrelsen, 2022a). This has maintained the large heath areas void of trees, the management has shifted and will from now on aim for a wilder ecosystem with less human control, however some interventions is planned to take place if conifers encroach excessively on the protected open heaths.

Table 4: Frequency of CWR species in Stråsø. Ranked from the most abundant, including the frequency of absence of CWR. The percentage of populations in each density category is included. Viability summarizes number of occurrences and *in situ* assessment, * indicates species that are generally more common than the inventory shows and may be less of a concern than indicated here. Threats summarizes evident categories that might extirpate a species from the site.

Species	Frequency %	Pop. 1-10 (%)	Pop. 11-100 (%)	Pop. 101-1000 (%)	Viability	Threat status
Vaccinium vitis-idaea	60	8.9	84.4	4.4	Viable	No threats
Vaccinium uliginosum	42.7	25	59.4	15.6	Viable	No threats
Festuca rubra	17.3	23.1	46.2	30.8	Viable	No threats
No CWR species	13.3	-	-	-	-	-
Poa pratensis	12	33.3	55.6	11.1	Viable	No threats
Trifolium repens	10.7	50	50	0	Viable	No threats
Rubus idaeus	6.7	20	80	0	Viable	No threats
Poa trivialis	5.3	0	100	0	Concern*	No threats
Festuca ovina	5.3	75	25	0	Concern*	No threats
Vicia sativa subsp. nigra	5.3	100	0	0	Concern	Unknown
Lolium perenne	5.3	25	75	0	Concern*	More data needed
Rubus fructicosus	5.3	75	25	0	Concern*	More data needed
Vaccinium myrtillus	5.3	50	50	0	Concern*	
Dactylis glomerata	5.3	25	75	0	Concern*	More data needed
Trifolium pratense	4	33.3	66.7	0	Concern*	More data needed
Trifolium arvense	4	66.7	33.3	0	Concern*	More data needed
Corylus avellana	4	100	0	0	Concern	More data needed

Species	Frequency %	Pop. 1-10 (%)	Pop. 11-100 (%)	Pop. 101-1000 (%)	Viability	Threat status
Prunus spinosa	2.7	100	0	0	Concern*	More data needed
Prunus cerasifera	2.7	100	0	0	Concern*	More data needed
Vaccinium oxycoccos	1.3	0	100	0	Concern	More data needed
Raphanus raphanistrum	1.3	100	0	0	Concern	More data needed
Malus domestica	1.3	100	0	0	Concern	More data needed
Rubus laciniatus	1.3	100	0	0	Concern	More data needed
Schedonorus pratensis	1.3	100	0	0	Concern	More data needed

Inventory of Crop Wild Relatives at Husby

Compared to the other CWR survey areas Husby was the least surveyed with 14 plots on an area of about 400 ha, adding the monitoring data adds 115 plots to the survey pool (fig 5). In the CWR field survey a total of 4 species was found at Husby (table 5), the area contains vast areas of coastal heaths and conifer plantations which harbour a low part of the species pool of CWR. The northeastern part of the area contains a system of meadows that likely have more CWR that were not found in this survey, more plots especially in this area especially would be valuable, adding monitoring plots contribute with this. Surprisingly only 1 species of *Vaccinium* was found (*uliginosum*) during the survey, when including the monitoring data *Vaccinium oxycoccos* was also found in the area but *V. vitis-idaea* and *myrtillus* were not, however they likely have populations in the area as well. Of the two *Rubus* species found, *fructicosus* and *laciniatus*, the latter is a North American species that is scattered naturalized on sandy areas in Denmark.

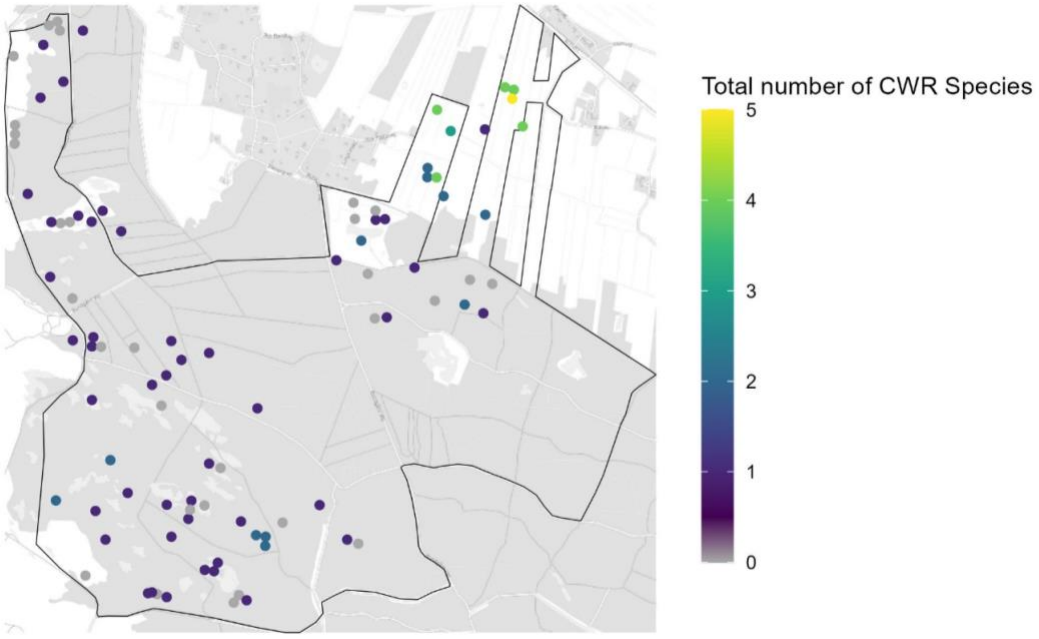

Figure 5: Number of Crop Wild Relative species of plots in Husby, data from both Crop Wild Relative inventory and stationary monitoring plots. (Background map from Styrelsen for Dataforsyning og Infrastruktur, Denmark).

Population densities

The population density of species found at Husby is shown in table 5. The species *Vaccinium uliginosum* is the most widespread and abundant in this CWR survey and is most often found with 11-100 individuals. The other species have few occurrences, i.e. *Rubus laciniatus* was found once with only 1-10 individuals,

and *Festuca rubra* also only once but with 11-100 individuals. *Rubus fructicosus* was found twice with 11-100 individuals and is not expected to be vulnerable. The population estimation survey only gathered 14 plots at Husby which is too few to generalize about the viability of CWR species at Husby, however the widespread occurrence of species such as *Vaccinium uliginosum* is an indication that it thrives at this area.

Monitoring data

Including data from the monitoring in the area resulted in 5 more species being found at Husby i.e., *Poa pratensis*, *P. trivialis*, *Trifolium pratense*, *T. repens*, and *Vaccinium oxycoccos*. All but the latter are associated with mesic-meadows which are found in the central-northern part of the area.

Threats and population robustness

Many things explained at the Stråsø section of threats is applicable to Husby as well i.e. the consequences of converting conifer plantations to semi-open habitats and the encroachment of woody vegetation to heaths. As a coastal site Husby represents habitat for CWR species such as *Leymus arenarius*, but the invasive *Rosa rugosa* thrives here and creates dense populations in the dunes threatens species in coastal habitats, however it is questionable that *R. rugosa* will extirpate CWR species at the site also because it is being actively repelled. The large herbivores will keep the meadows in the northern part of the area more open and prevent litter from shading shorter species such as *T. repens* and *pratense*.

Table 5: Frequency of CWR species in Husby. The bottom species are found in stationary monitoring only. Ranked from the most abundant, including the frequency of absence of CWR. The percentage of populations in each density category is included. Viability summarizes number of occurrences and *in situ* assessment. * indicates species that are generally more common than the inventory shows and may be less of a concern than indicated here. Threats summarizes evident categories that might extirpate a species from the site.

Species	Frequency %	Pop. 1-10 (%)	Pop. 11-100 (%)	Pop. 101-1000 (%)	Viability	Threat status
Vaccinium uliginosum	71.4	20	80	0	Viable	No threats
Rubus fructicosus	14.3	0	100	0	Concern*	More data needed
No CWR species	14.3	-	-	-	-	-
Rubus laciniatus	7.1	100	0	0	Concern	More data needed
Festuca rubra	7.1	0	100	0	Viable	No threats
Poa pratensis	-	-	-	-	Viable	No threats
Poa trivialis	-	-	-	-	Viable	No threats
Trifolium pratense	-	-	-	-	Viable	No threats
Trifolium repens	-	-	-	-	Viable	No threats

Inventory of Crop Wild Relatives at Kattrup

At Kattrup the CWR specific monitoring yielded 68 plots, and monitoring data adds 169 plots to this survey pool (fig 6). Kattrup is abundant in overall CWR with 35 species found across 68 plots randomly placed across the ca. 900 ha. The nutrient-rich soils of the previous arable fields coupled with forest edges and older deciduous forests appear to offer diverse habitat for CWR species. Most of the expected species of Poaceae were found and appeared abundant across Kattrup, species such as *D. glomerata*, *P. trivialis*, and *L. perenne* all are characteristic of nutrient rich and open areas (table 6). *Elymus caninus* characteristic of beech forests was also quite frequent. Of the genus *Festuca* only *rubra* was found to be frequent and abundant by our survey, especially on the former arable fields, the species *F. ovina* and *brevipila* possibly can be found on the small remnant areas of dry and nutrient poor grasslands but these habitats are rare at

Kattrup. Further the genus *Rubus* was well represented by all the common species (*idaeus*, *fructicosus*, & *caesius*), additionally the non-native CWR *R. armeniacus* was found in several plots. Of woody species the genus *Prunus* was frequent and represented by *avium*, *cerasifera*, and *spinosa*, and further *Corylus* and *Humulus* were found on several occasions. Fifteen plot observations of *Dactylis* were included in the analysis at a genus level, because the observer did not note whether it was *D. glomerata* or *D. polygama* (the latter is not accepted as a species by all floras and is missing from the Nordic Priority CWR List, Fitzgerald et al., 2018). However, the other observers primarily found *glomerata* thus these 15 observations are likely this species. Regardless the genus *Dactylis* is very abundant at Kattrup being present at 48 plots (71 %).

Population densities

The population density of species found at Kattrup is shown in table 6. Most of the common species have many populations with over 11 individuals, and are not vulnerable, however the infrequent species that often only have populations for 1-10 individuals are more vulnerable to extirpation e.g., *Daucus carota subsp. carota*, *Trifolium pratense*, *Mentha arvense* etc.

Monitoring data

Including data from the monitoring in the area resulted in 8 more species found at Kattrup i.e., *Malus domestica*, *Setaria viridis*, *Schedonorus pratensis*, *Ribes nigrum*, *Trifolium medium*, *T. arvense*, *Medicago sativa subsp. falcata* and *Vicia sativa subsp. nigra*. The first 3 are associated with anthropogenic landscapes and are often planted, sown, or dispersed from gardens. The *Trifolium spp.*, *Medicago*, and *Vicia* are associated with protected open habitats.

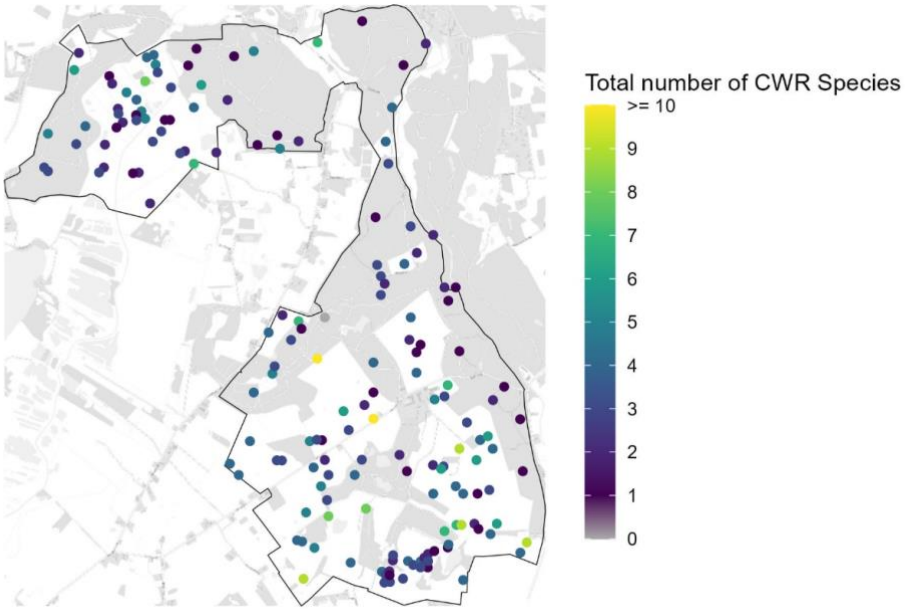

Figure 6: Number of Crop Wild Relative species of plots in Kattrup, data from both Crop Wild Relative inventory and vegetation monitoring. (Background map from Styrelsen for Dataforsyning og Infrastruktur, Denmark).

Threats and population robustness

The cessation of agricultural activities means that large areas have become available as habitat thus ruderal species are expected to increase their populations drastically. The areas of protected grasslands and bogs will benefit from less nutrient run-off which is positive for CWR species associated with these

mostly nutrient poor habitats. There is going to be introduced large grazing herbivores in the coming year which is expected to be positive for the broad CWR diversity because the eutrophic crop fields have high productivity thus few dominant species easily outcompete shorter plants. Currently much of the former fields are covered with especially *Cirsium arvense*. By introducing the function of non-selective grazing and trampling with heavy animals the dominant species are expected to be reduced in cover. However, both the cessation of cultivation and the introduction of large herbivores will likely result in drastic landscapes changes with certain species showing substantial shifts in abundance. All in all, the cessation of intensive land-use with crop fields of annual crops will in time create habitats more robust and less prone to degradation due to unusual weather patterns.

Table 6: Frequency of CWR species in Kattrup. The bottom species are found in stationary monitoring only. Ranked from the most abundant, including the frequency of absence of CWR. The percentage of populations in each density category is included. Viability summarizes number of occurrences and *in situ* assessment, * indicates species that are generally more common than the inventory shows and may be less of a concern than indicated here. Threats summarizes evident categories that might extirpate a species from the site.

Species	Frequency %	Pop. 1-10 (%)	Pop. 11-100 (%)	Pop. 101-1000 (%)	Viability	Threat status
Rubus idaeus	50	26.5	67.6	2.9	Viable	No threats
Dactylis glomerata	48.5	36.4	57.6	3	Viable	No threats
Poa trivialis	42.6	17.2	44.8	34.5	Viable	No threats
Lolium perenne	39.7	11.1	48.1	40.7	Viable	No threats
Rubus fructicosus	38.2	61.5	34.6	0	Viable	No threats
Festuca rubra	30.9	28.6	61.9	9.5	Viable	No threats
Phleum pratense	17.6	50	50	0	Viable	No threats
Elymus caninus	16.2	27.3	72.7	0	Viable	No threats
Prunus avium	13.2	88.9	11.1	0	Viable	No threats
Rubus caesius	11.8	62.5	37.5	0	Viable	No threats
Trifolium repens	11.8	12.5	50	37.5	Viable	No threats
Prunus cerasifera	10.3	100	0	0	Viable	No threats
Corylus avellana	10.3	100	0	0	Viable	No threats
Prunus spinosa	8.8	100	0	0	Viable	No threats
Lolium multiflorum	7.4	0	40	60	Viable	No threats
Mentha aquatica	5.9	0	100	0	Viable	No threats
Rubus armeniacus	5.9	50	50	0	Concern*	No threats
Ribes uva-crispa	5.9	50	50	0	Concern*	No threats
Humulus lupulus	4.4	66.7	33.3	0	Viable	No threats
Lactuca serriola	4.4	100	0	0	Concern	More data needed
Daucus carota subsp. carota	4.4	100	0	0	Concern*	More data needed
No CWR species	2.9	-	-	-	-	-
Ribes rubrum	2.9	50	50	0	Concern	More data needed
Poa palustris	2.9	50	50	0	Concern*	More data needed
Poa pratensis	2.9	0	100	0	Viable	No threats
Medicago sativa subsp. sativa	2.9	50	50	0	Viable	No threats
Medicago lupulina	2.9	0	100	0	Viable	No threats
Schedonorus arundinaceus	2.9	100	0	0	Concern	More data needed
Sinapis arvensis	1.5	100	0	0	Concern	More data needed
Barbarea stricta	1.5	0	100	0	Concern	More data needed
Vicia sativa	1.5	100	0	0	Concern*	More data needed
Trifolium pratense	1.5	100	0	0	Viable	No threats
Barbarea vulgaris var. arcuata	1.5	100	0	0	Concern	More data needed

Species	Frequency %	Pop. 1-10 (%)	Pop. 11-100 (%)	Pop. 101-1000 (%)	Viability	Threat status
Mentha arvensis	1.5	100	0	0	Concern	More data needed
Ribes spicatum	1.5	100	0	0	Concern	More data needed
Cichorium intybus	1.5	0	100	0	Concern	More data needed
Phleum pratense subsp. nodosum	1.5	0	100	0	Concern	More data needed
Malus domestica	-	-	-	-	Concern	More data needed
Medicago sativa subsp. falcata	-	-	-	-	Concern	More data needed
Ribes nigrum	-	-	-	-	Concern	More data needed
Schedonorus pratensis	-	-	-	-	Concern	More data needed
Setaria viridis	-	-	-	-	Concern	More data needed
Trifolium arvense	-	-	-	-	Concern*	More data needed
Trifolium medium	-	-	-	-	Concern	More data needed
Vicia sativa subsp. nigra	-	-	-	-	Concern	More data needed

Conclusions and recommendations

Many of the expected CWR species were found at each site, indicating that a randomized sampling strategy is suitable for performing inventories of CWR plant species on a landscape scale. The sampling strategy is standardized to be efficient across different landscapes with varying types of nature and area size, while the randomness assures objectivity in the plot selection. I.e. this forces the surveyor to visit areas otherwise skipped due to inconvenience or an assumed lack of species and can potentially increase the chance for discovery of previously unknown populations of CWR species. By having the spatially fixed grid cells as a prerequisite for doing an inventory, it is also straightforward to add previous observations from expert knowledge of known populations, which can be valuable to gain a more comprehensive representation of a certain area. Furthermore, the inventory strategy and effort can easily be extended to cover more area of the landscape and if needed can include also other plant species or types of organisms of interest, such as rare species. We believe however, that the method can be improved by including a stratified sampling strategy, especially when sampling effort is restricted in time, to not only include variation in a spatial dimension, but also along gradients of relevant ecological parameters.

Overall, we found the majority of observed CWR species populations to be viable in the four inventory areas and can recommend the sites as suitable refugium for in-situ preservation of genetic variation. Since all four sites are prioritized for maintaining or restoring natural processes to benefit biodiversity in general, we expect that this will also leverage the protection and potentially establishment of CWR species. While the Husby inventory resulted in the lowest diversity of CWR species (but also lowest amount of sampling points), the unique coastal conditions may still harbour essential genetic types adapted to more extreme climatic conditions and events. This assumption would, however, need to be examined through genetic analyses. Our inventory report can serve as fundamental background knowledge for designing further studies aimed at specific research questions.

As a future outlook, it will be interesting to follow the development of CWR species on the sites to understand whether restoration and prioritizing natural processes will be beneficial for CWR populations. Possibly, this might depend largely on the nativeness of the species, considering the cultivation history. Also, the species possibly will be influenced by the restored environmental conditions and processes, an adaptation to these conditions might occur eventually towards a more robust genetic composition, even if

population sizes decrease. Therefore, repeated and regularly inventories would further our knowledge, possibly supported by implementing a simplified protocol that facilitates the inclusions of citizen science in research and monitoring efforts.

Acknowledgements

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