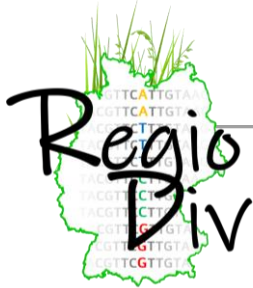


Genetic variation of herbaceous species



Sampling protocol v.2024 (RegioDiv Lux+)

15.04.2024

1.1 Project background

In Germany and other European countries, regions of provenance (regions of origin) have been defined for wild plants, in which regional seeds are produced, i.e. collected, multiplied and used for revegetation. The distribution of regions of origin is essentially based on natural geographical units. Data on the adaptation or genetic differentiation of plant species has not been taken into account. **The aim of the project is to create an empirical database on the genetic diversity of the different provenances and to create differentiation models for regional plants, or even to adapt the proposed zones of origin based on the results.**

To this end, we want to study 28 representative grassland species from all the regions of origin in several populations. The samples will be characterised using genome-representative SNP markers and the genetic structure of the populations of all target species will be studied. Sample collection in Germany has been completed. As part of the Lux+ project, in 2024 we will be extending the database to Luxembourg and the neighbouring regions of Belgium and north-eastern France.



1.2 Protocol (short version)

- **If you are interested to join, please contact one of the RegioDiv coordinators** (Walter Durka (walter.durka@ufz.de) or Stefan Michalski (stefan.michalski@ufz.de)).
- For **RegioDiv-Lux+** (Belgium, France) please contact **Maximilien Oly**, T: (+352) 26 30 36 87, Mail: max.oly@siconal.lu or **Vanessa Duprez**, T: (+352) 26 30 36 35, M: (+352) 621 647 430, Mail: vanessa.duprez@siconal.lu
- **A collection kit will be made available to facilitate sampling.**
- **You identify suitable sampling sites in your sub-region.**
- **You collect leaf material from each species (if present) at each site. The aim is to sample at least 3 sites per species in each sub-region.**
- **Once the sampling is completed, send the dried leaves** to the RegioDiv coordinators (see address below).
- **Please register the sampling sites and samples on the RegioDiv website** (<https://www.ufz.de/regiodiv/index.php?fr=47258> → Map).
- Every contribution is greatly appreciated. However, **the success of the project depends on sampling as many of the target species as possible in all the sub-regions** (insofar as they are present there). It is therefore the responsibility of those involved to collect all the species available locally. When planning your project, please let the coordinators know as soon as possible which regions/sites you can cover, and whether you can collect all the target species or just some of them!
- With the collection of samples, you are offered the right to co-author a project publication (depending on the extent of your participation in the project).

1.3 Sampling strategy

Depending on the size of the area, each species must be sampled at a minimum of 3 sites, and at least 5 sampling sites for large areas. Please check with the coordinators in your area. As it is unlikely that every species is present at every site, it must be assumed that several collection sites must be visited per region and per species to cover all species. The aim is to achieve a mostly uniform distribution across the regions.

The collection sites must be considered natural to avoid any genetic alteration of anthropic origin due to non-native seeds or crop varieties. For this reason, nature reserves would be optimal for sampling (beware of the need for permits!) as well as natural or semi-natural meadows, pastures, fallow land, orchards and borders.

1.4 Target species

Grasses

Agrostis capillaris L.

Anthoxanthum odoratum L. s. str.

Arrhenatherum elatius (L.) J. Presl & C. Presl

Bromus erectus Huds.

Corynephorus canescens (L.) P. Beauv.

Cynosurus cristatus L.

Festuca rubra L.

Herbs

Achillea millefolium L.

Agrimonia eupatoria L.

Bistorta officinalis Delarbre

Campanula rotundifolia L. s. str.

Centaurea jacea L. s. L., i.e. including *Centaurea jacea* L. s. str., *Centaurea angustifolia* Schrank, *Centaurea nigra* L. s. L. and eventually their hybrids

Euphorbia cyparissias L.

Filipendula ulmaria (L.) Maxim.

Galium album Mill.

Hypochaeris radicata L.

Knautia arvensis (L.) Coult. s. l.

Lathyrus pratensis L.

Leucanthemum vulgare agg., i.e. *L. ircutianum* & *L. vulgare*

Lotus corniculatus L.

Lychnis flos-cuculi L.

Pimpinella saxifraga L.

Prunella vulgaris L.

Ranunculus acris L.

Salvia pratensis L.

Silene vulgaris (Moench) Garcke

Thymus pulegioides L. s. l.

Tragopogon pratensis L. s. l., i.e. *T. minus*, *T. pratensis* et *T. orientalis*

1.5 Provided material

To facilitate and standardise sampling, each collector receives a kit containing the following materials:



- Labelled paper bags, for instance:

<i>Arrhenatherum elatius</i>	
Region: (Please indicate the name/number of the region)
Site:(define and write the name of the sampling site, identical to the WebGIS entry)
Number: (optionally enter the WebGIS number)

- Plastic bags
- Silica gel (to preserve leaf samples)
- Data form, see below

1.6 Sampling on site

At a collection site, only ONE plant per species is selected at random and sampled. Only in exceptional cases, when it is not possible to sample a sufficient number of sites, can several plants be sampled from one site (please using individual bags).

Healthy green leaves should be taken from each plant and placed in a (pre-)labelled paper bag. Only put leaves from a single plant or individual into each bag. Never put leaves from different plants in the same bag!

The number of leaves to be collected depends on the size of the leaf: for large leaves (e.g. *Knautia*, *Salvia*), one leaf is sufficient. Often 3-5 leaves are required (*Achillea*, *Centaurea*, *Arrhenatherum*), for small narrow leaves up to 10 (grasses, *Galium*), or whole twigs (e.g. *Euphorbia cyparissias*), or whole small plants.



Approximate minimum quantity of material per sample. Better to collect a little more!

If you are not sure about the determination of the collected species (e.g. *Festuca rubra*, *Centaurea jacea* s.l., *Thymus pulegioides*), please make a note on the picking bag. **If in doubt, it's better to collect a sample than not.** False species will be identified at the latest when the genetic data is evaluated and then eliminated. For ***Centaurea jacea***, please put a flower head in the bag.


The sample must be dried quickly, i.e. within 24 hours (in the open air, in an oven at 50°C, on silica gel, in a warm car) **if possible at temperatures below 60°C and without direct exposure to sunlight.**

For each sampling site, please record the following data on the data form: **site name, site number (according to WebGIS)**, [only in exceptional cases: GPS coordinates], **harvest date, number of plants sampled per species, assessment of site characteristics** in terms of soil moisture and nutrient content (please put a cross on the moisture and nutrient content scale).

Optionally, you can indicate 10 characteristic plant species of the sampling site.

Data Form RegioDiv Region:

(Please indicate the names of the sampling sites and the number of samples collected to the return envelope. If possible, enter data in the www.ufz.de/regiodiv database as well. V)



<https://www.ufz.de/regiodiv>

Species

	Sampling site 1	Sampling site 2
<i>Agrostis capillaris</i>		
<i>Achroanthum odoratum</i> s. str.		
<i>Arctenatherum elatius</i>		
<i>Bromus erectus</i>		
<i>Corynephorus canescens</i>		
<i>Cynosurus cristatus</i>		
<i>Festuca rubra</i>		
<i>Achillea millefolium</i>		
<i>Agrimonia eupatoria</i>		
<i>Bistorta officinalis</i>		
<i>Campanula rotundifolia</i> s. str.		
<i>Centaurea jacea</i> s.l.*		
<i>Centaurea jacea</i> s. str.*		
<i>Centaurea angustifolia</i> *		
<i>Centaurea nigra</i> *		
<i>Euphorbia cyparissias</i>		
<i>Elipendula ulmaria</i>		
<i>Galium album</i>		
<i>Hypochaeris radicata</i>		
<i>Koeleria arvensis</i> s.l.		
<i>Lathyrus pratensis</i>		
<i>Leucanthemum vulgare</i> agg.		
<i>L. scaberrimum</i>		
<i>L. vulgare</i> s. str.		
<i>Lotus corniculatus</i>		
<i>Lychnis floer-cuculi</i>		
<i>Pimpinella saxifraga</i>		
<i>Prunella vulgaris</i>		
<i>Ranunculus acris</i>		
<i>Salvia pratensis</i>		
<i>Silene vulgaris</i>		
<i>Thymus pulegioides</i>		
<i>Tragopogon pratensis</i> s.l.		
<i>T. minor</i>		
<i>T. pratensis</i> s.str.		
<i>T. orientalis</i>		

* For *Centaurea jacea* s.l., please add a flower head to the sampling bag

Data Form RegioDiv Site characterisation

(Please indicate, if possible, 10 frequent/representative companion species f properties (rather wet or dry, rather rich or poor in nutrients) with a cross. V)

Sampling site 1	Sampling site 2	Sampli
Species 1		
Species 2		
Species 3		
Species 4		
Species 5		
Species 6		
Species 7		
Species 8		
Species 9		
Species 10		

Please mark the assessment with a cross on the bar:

Comments on the sampling site

Once the collection is completed, please send all samples to:

Walter Durka
RegioDiv
Helmholtz-Zentrum für Umweltforschung
Dept. Biozönoseforschung
Theodor-Lieser-Str. 4
06120 Halle (Saale)

1.7 Entering data in the WebGIS application

The RegioDiv-WebGIS application is used to document sampling. This is where you are asked to document the sampling sites. It is also possible to record the plants collected (and optionally other accompanying species as well) at each sampling site.

1 Start RegioDiv WebGIS

<https://www.ufz.de/regiodiv/index.php?en=47258> → Map → Interactive map – Data entry login

→ Start WebGis



Username: RegioDiv2024
Password: Collection2024




2 Basic functions




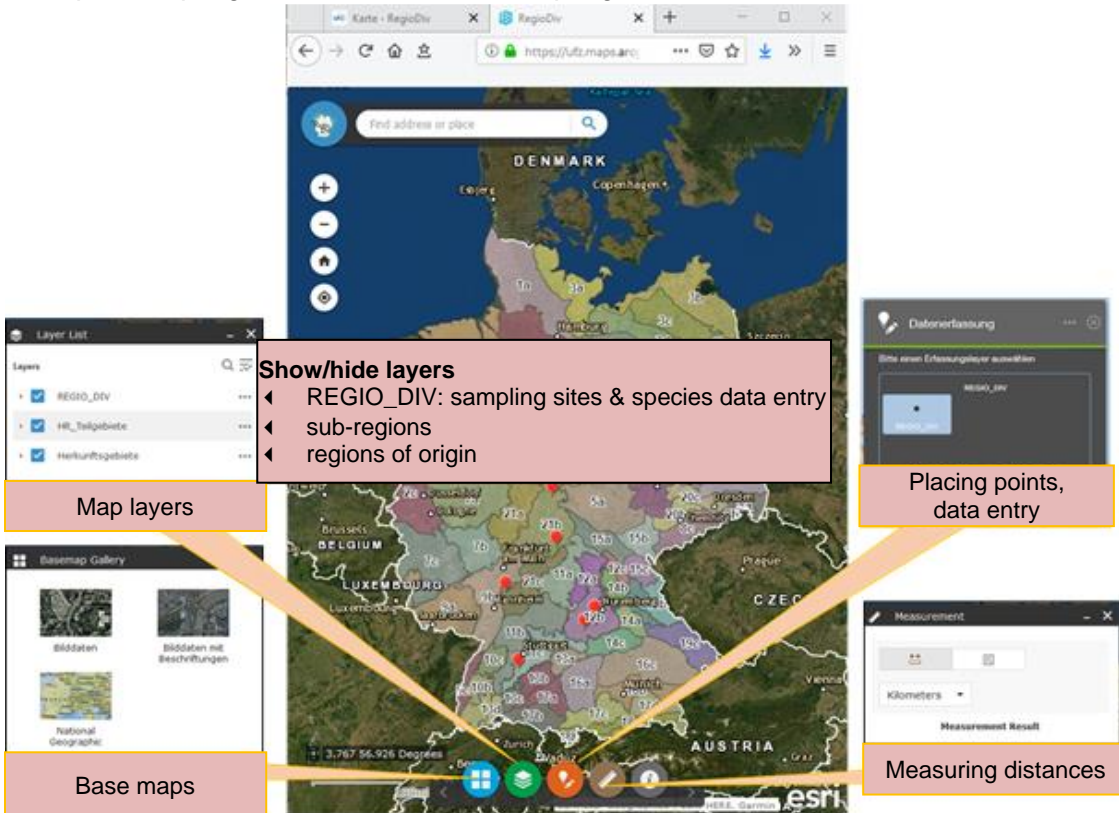
Select a base map: image data / image data with legend / National Geographic



Show / hide map layers: regions of origin / sub-regions / sampling sites

 Measure distances

 Input sampling site and document sampling



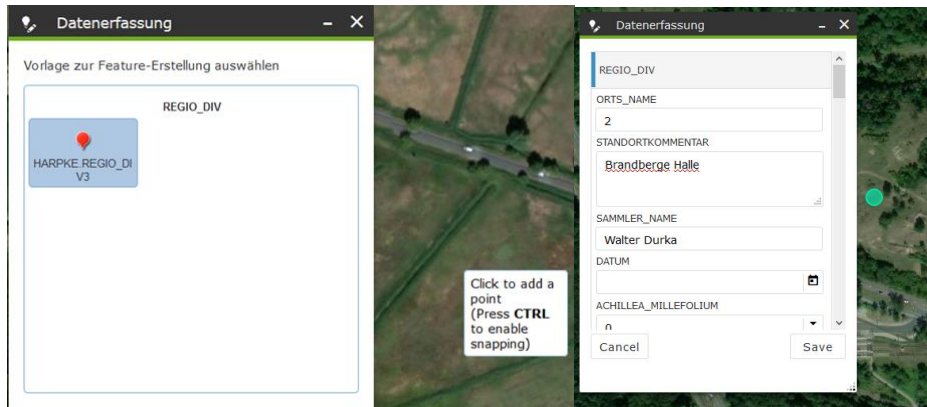
The screenshot shows the RegioDiv web application interface. The main map displays a geographical area with various regions and sampling sites marked. Several callout boxes provide details about the application's features:

- Map layers:** A callout box points to the 'Layer List' panel on the left, which shows three layers: 'REGIO_DIV', 'HL_Talgebiete', and 'Herkunftsgebiete'.
- Show/hide layers:** A callout box points to the 'Layer List' panel, listing the layers and their functions: 'REGIO_DIV: sampling sites & species data entry', 'HL_Talgebiete: sub-regions', and 'Herkunftsgebiete: regions of origin'.
- Placing points, data entry:** A callout box points to the 'Datenfassung' (Data Entry) dialog box, which prompts the user to select a data entry point.
- Measuring distances:** A callout box points to the 'Measurement' dialog box, which shows a measurement result in kilometers.
- Base maps:** A callout box points to the 'Basemap Gallery' panel, which offers different map styles like 'Bildaten', 'Bildaten mit Beschriftungen', and 'National Geographic'.

3. Input sampling sites

Ideally, as a collector, you know the sampling sites and mark them as a point on the map even before going out into the field. This gives everyone involved an overview of where sampling is planned. But you can also collect without having set a point beforehand and enter it later.

Set as many sampling points as you need. To begin with, enter only **your name** and a **name for the sampling site**. This way, it's clear who wants to collect here and the location has a name that you can use on the sample bags.



When registering a sampling site, please place the point as accurately as possible. We will use the coordinates to determine the site parameters. Complete this step either with or , but not with . After the first registration, the point will receive an identification number (ID) which will be displayed when it is reopened.

Moving points

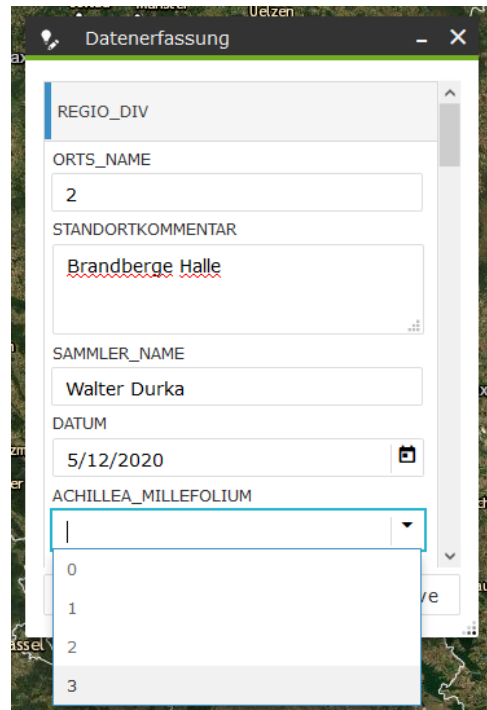
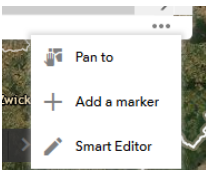
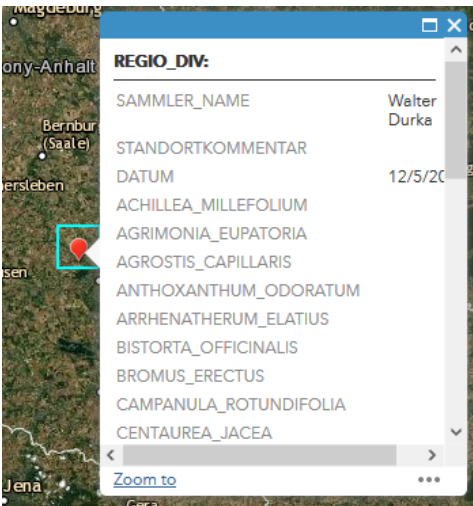
First click on the point, and a small window will appear showing the data assigned so far. Click on the three dots at the bottom right and select . The point now appears in green and can be moved with the mouse. Exit the menu with or , but not with .

Deleting points

... **does not work**. Please send a message to the coordinators indicating which point should be deleted (indicate the ID). It is best to write "Error" or "Please delete" in the site name.

4. Document the collected samples

Once you've finished sampling, you can document what you've collected for each site. You can also enter additional data, such as site name, site number, a list of accompanying species or other comments. First click on the sampling point, and a small window will appear showing the data you have entered so far. Click on the three dots at the bottom right and select .



For each species sampled, enter the number of individuals you collected in the selection window. In the "Site comments" field, you can enter the name of the site and other comments. If you have made a list of accompanying species, please also enter them in this window.

1.8 Contacts

Technical questions (WebGIS)

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