Niche Differentiation and Genetic Structure of *Bactrospora dryina* in Swiss Hardwood Floodplain and Coppice-with-Standards Forests

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Contents

1. Introduction

Niche differentiation is one of the basal ongoing evolutionary processes, causing speciation by adaptive radiation (Darwin, 1859; Benton, 1995). It explains the adaptation to new environments driven by competition between coexisting species (Armstrong & McGehee, 1980; Volterra, 1928). According to these principles, adaptation to unclaimed niches is advantageous as former limited fundamental resources do not need to be shared.
Frequent flooding events cause high structural diversity in the terrestrial vegetation which is directly linked with a high number of niches for specialised species (Fink et al., 2017).

While riverine floodplains are one of the most species-rich habitats and of high importance for ecosystem functioning, they are also of the most threatened ecosystems worldwide (Ward et al., 1999; Tockner & Stanford, 2002).
In Switzerland, 70% of the naturally occurring floodplains have disappeared since 1850 (BAFU, 2017). The main cause for this massive decline is human activities, and specifically drastic interventions to river systems. With an increasing human population size, space is one of the most valuable goods in Switzerland. In the 19th century meandering dynamic rivers were channelized to gain space for agriculture and to protect villages from flooding events. Channelization was assumed to be the most efficient way of flood protection regardless of any impact on biodiversity and ecosystem functioning (Vischer, 2003; Schudel et al., 2011). Furthermore, the level of riverbeds was lowered to increase the capacity of a river and to increase the drainage effect of a river system (Wilcock, 1991). Channelization also allowed landscapes along these rivers to be dried and used as agricultural fields. While with these interventions, fields could be used much more efficiently and harvesting yield could be increased, it had tremendous effects on habitat richness and biodiversity (Wilcock, 1991; Kennedy & Turner, 2011, Groom et al., 2006).

Figure 1 Quercus robur *in a hardwood floodplain forest at Aare river at Gippingen AG. The photo was taken in January 2018 during an intense flooding event caused by heavy rain fall and snow melting.*
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In naturally remaining flat and wide valleys with characteristically high ground water table and flooding events caused by a river, *Quercus robur, Ulmus glabra* or *Fraxinus excelsior* grow into hardwood floodplain forests (Ellenberg, 2010). This vegetation type is of higher constancy than other river-driven vegetation types such as softwood floodplain forests. The relatively moist soil in hardwood floodplain forests is typically composed of fine sediments imported from rare intense flooding events (Fink et al., 2016). Typical canopy building tree species in hardwood floodplain forests have less shading effects to the understory than other species in deciduous forests (i.e. *Fagus sylvatica*) which reduce growth of understory vegetation (Ellenberg & Leuschner, 2010). Dominant hardwood tree species are resistant against heavy flooding events and cuts caused by ice drifts. These disturbing events are the main reason for the characteristic open and mosaic patchiness structure in this forest type.

1.1 Study species

Bactrospora dryina (Ach.) Massal. (*Roccellaceae, Arthoniomycetes, Ascomycota*) is a lichen-forming fungus growing almost exclusively on the weather protected side of slightly inclined oaks older than 90 years (Scheidegger & Stofer, 2015). Old oaks offer a characteristic bark structure with deep clefts causing a specific microhabitat with a certain pH. *Bactrospora dryina* grows in symbiosis with different trentepohlioid green algae (Nadyeina et al. 2017). The crustose lichen has a white thallus and disperses via sexual spores produced in black apothecia and/or via asexual conidia (Egea & Torrente, 1993). It is one of 65 epiphytic red list lichen species in Switzerland and has a vulnerable status (VU). It also occurs in North America, Asia and northern Europe, and is described as endangered in many countries (Germany, Austria, Italy, Great Britain, Sweden) (Scheidegger & Clerc, 2002). *Bactrospora dryina* is found in old growth hardwood floodplain forests in Swiss lowlands where forest structure offers the specific requirements of this lichen.

In medieval times, coppice-with-standards type of forest management lead to a similar forest structure to present day hardwood floodplain forests. Large oaks (*Quercus robur* or *petraea*) remained in the stand and built the canopy forming layer, whereas *Carpinus betulus* (Hornbeam), *Fraxinus excelsior* (Ash) and sprouting tree species were cut occasionally. The remaining oaks were used as a source of construction wood due to their hardness and longevity, whereas wood of understory species, hornbeam and ash, was used as firewood (Nyland, 2016; Ellenberg, 2010).
The removal of understory bushes and small trees lead to a somewhat similar vegetation structure as it is seen in lowland hardwood floodplain forests today. Nowadays, floodplain areas and remaining coppice-with-standards forests are widely protected and are managed as such due to their high ecological value (Pasinelli et al., 2008; BAFU 2017). As a consequence of structural similarity of these two forest types, *B. dryina* is found in both coppice-with-standards and hardwood floodplain forests. However, it is unknown whether *B. dryina* in times of coppice-with-standards forest management spread form hardwood floodplain forest to those managed forests or if it has another colonisation origin.

1.2 Population genetics and conservation

Genetic isolation of small subpopulations can lead to genetic depletion (Elstrand & Elam, 1993; Frankham et al., 2004). Small effective population size is one of the main cause for local extinctions of a species with an impact on the genetic composition of a larger metapopulation (Harrison, 1991).

Hardwood floodplain forests along Swiss riversystems are highly fragmented. Sessile organisms such as plants or fungi which cannot migrate by themselves are even more limited in dispersal when potential habitats are fragmentated because of a decreasing chance per propagule to be transported to a suitable island (Gustafson & Gardner, 1996; Herrera & Garcia, 2010).
Therefore, according to Wrights isolation by distance (IBD) hypothesis (Wright, 1943), sessile organisms adapted to hardwood floodplain forests might tend to genetic isolation with increasing distance between sites because of habitat fragmentation. For many riparian plant species of various vegetation types, dispersal mechanisms are acting along the river corridor, either because of spore or seed transport by wind (up- and downstream) or by downstream transport of propagules in the water (Imbert & LeFevre, 2003; ; Liu et al., 2006; Werth & Scheidegger, 2014; Van der Meer & Jaquemyn, 2015). Both mechanisms could also be conceivable for *B. dryina* and other lichen or plant species occurring in hardwood floodplain forests. Hence, analysing genetic structure and differentiation of threatened species in a fragmented landscape is crucial for the development of conservation strategies for single species and threatened habitats (Manel et al., 2003).

In this study *Bactrospora dryina* acts as a model species for sessile organisms which are adapted to hardwood floodplain forests. The outcome of this study will 1) define structural criteria for forests or tree units for future *B. dryina* populations and companion species, 2) characterize genetic differentiation of *B. dryina* populations in a fragmented landscape on different special scales, 3) detect genetically impoverished populations of *B. dryina* and 4) deliver genetic data for conservation strategies in the scope of re-establishment of habitat connectedness along river systems.

1.3 Hypotheses

- *Bactrospora dryina* occurs more frequently on large oaks with deep bark clefts.
- Intermediate light conditions with a canopy coverage of about 50 % and southwards inclination of host trees are crucial for *B. dryina* to colonise a site.
- Large populations show high genetic diversity, small populations are genetically impoverished
- Subpopulations of *B. dryina* show genetic differentiation according to an isolation by distance pattern (Wright, 1943) in fragmented sites along river systems.
- Geographic separation increases colonisation limitation of *B. dryina*.

2. Materials and methods

2.1 Study area

All study sites are located in the lowland (below 600 m.a.s.l.; Figures 1-4, appendix) in north-western Switzerland in the cantons of Aargau, Solothurn, Baselland, Bern and Freiburg. The following indices were used to find structure rich forests with oaks which offer a suitable habitat for *Bactrospora dryina*:

- Coordinates of *Dendrocopos medius* (Middlespotted Woodpecker). This Woodpecker is a habitat specialist for structure rich oak forests with oaks with a stem diameter of more than 30 cm. (Pasinelli et al., 2008)
- Oak reserves (Geoportal der Schweiz, 2017)
- Satellite images; canopy structure of forests along midland rivers were screened. Forests with structure rich deciduous trees were observed (Geoportal der Schweiz, 2017).
- Forests with known *B. dryina* populations (swisslichens.ch, 2017)

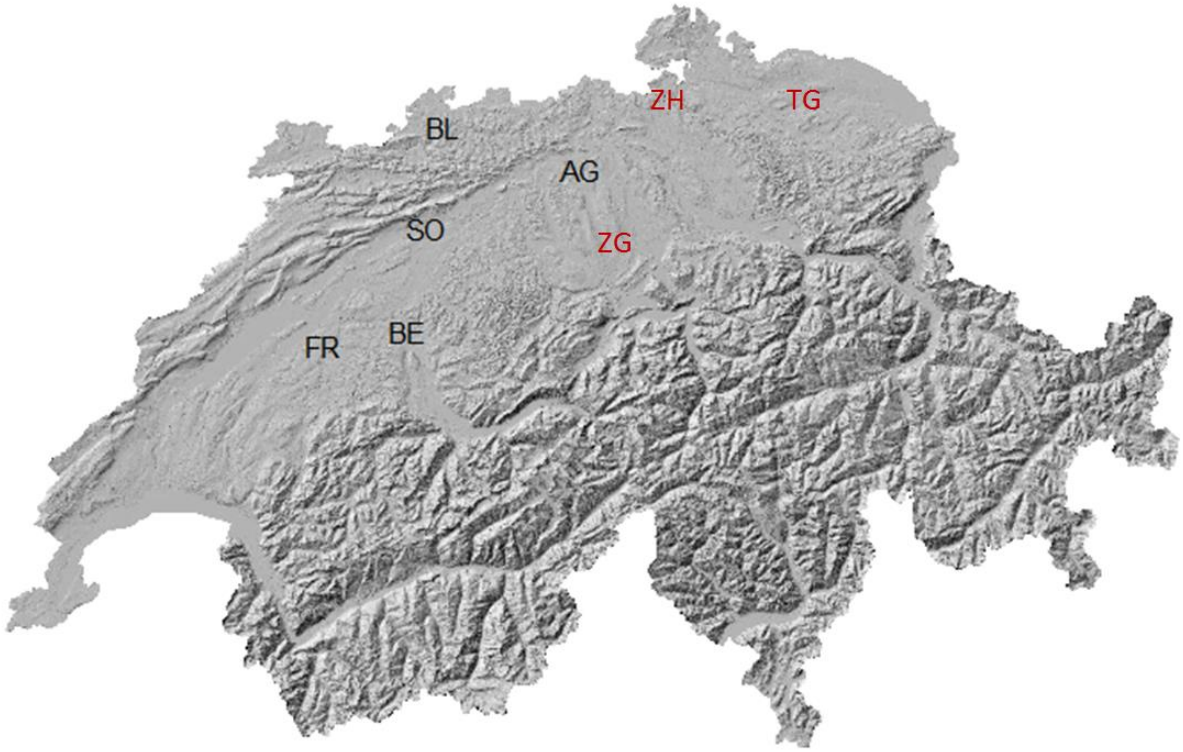

Figure 2 *Observed forests were located in the cantons Baselland (BL), Aargau (AG), Solothurn (SO), Freiburg (FR) and Bern (BE). Additional microsatellite data from Zug (ZG), Zürich (ZH) and Thurgau (TH) was provided by Nadyeina et al. (2017).*

2.2 Vegetation characteristics and niche determination

In 1 ha plots, six oaks with and six oaks without *B. dryina* were randomly chosen for closer observation and measurements. However, in many cases *B. dryina* only occurred on a few isolated trees. Therefore, this sampling design could not be applied in all location and single trees were also included, resulting in a number of completely sampled 1 ha plots and single trees in between these plots. Totally, 153 oaks with *B. dryina* and 135 without *B. dryina* were measured in the scope of this study.

The following parameters were measured for each randomly selected oak:

- Girth at breast height (GBH)
    - Old trees tend to be bigger in size. Although growth is also critically influenced by soil condition, water availability and light conditions, it can be used as a rule of thumb.
- Depth of bark crevices
    - Bark structure changes with age. Old trees tend to have deeper bark crevices than young oaks offering the needed microclimate for *Bactrospora dryina*. (Nadyeina et al., 2017)
- Percentage of broken off bark ridges
    - It delivers a further indication about the condition of the bark. Very moist bark ridges tend to break off more easily then dry ridges.
- Azimuth of the weather protected stem side
- Estimation of canopy coverage at crown border in the azimuth of the weather protected stem side explained in percentage of leaves or branches are covering the sky.

Bactrospora dryina colonies were classified into one out of five possible stati describing the development stage of the lichen:

- 1: early stages, limited to crevices, often young and largely sterile
- 2: colony with a high coverage, large parts sterile
- 3: colony with a high coverage, large parts fertile
- 4: colony fragmented, some parts fertile
- 5: colony fragmented, major parts of thallus green, overgrown with algae; fertile or not

Colonisation probability model were calculated using a multiple GLM (Generalized linear model) with the measured ecological parameter as predictors to describe the niche specificity of this species. Two dimensional plots were constructed in R studio (RStudio 1.0.44, © 2009-2016 RStudio, Inc).

Descriptive analysis of azimuth preferences was done by transforming azimuth values by trigonometric functions into levels of eastness and northness acoording to Roberts (1986).

Total number of colonies per site was estimated based on the colonies found, in relation to how comprehensive a side could be screened and how large remaining forest areas with potentially suitable structure were (based on air pictures).

2.3 Sampling design for genetic material

For each tree with a *B. dryina* colony, five samples were taken from the following positions whenever possible: top left, bottom left, center, top right and bottom right (Figure 3).
If available, small lichen fragments with apothecia were collected, because more DNA is expected in fruit bodies than in the thallus and because the major part of the apothecial tissue is composed of maternal vegetative, haploid hyphae, as suggested by Nadyeina et al. (2017). All samples were collected from March to June, when higher DNA concentration is expected due to spore production (Hilfiker, 2000). If the lichen was sterile, vegetative thallus parts of about 1 cm^2 were cut out. In thallus pieces, it is unknown how many genetically distinct individuals are collected as a colony could grow as a chimera of several individuals (Mark et al., 2016). Furthermore, it is unknown in which ploidy state the thallus cells are (Honegger & Scherrer in Nash, 2006). Only three diploid samples were detected and excluded for genetic analysis.
If *B. dryina* only covered a small area of a tree at least 3 samples were collected. All samples were put in separate tubes to avoid contamination and were stored at -18°C.

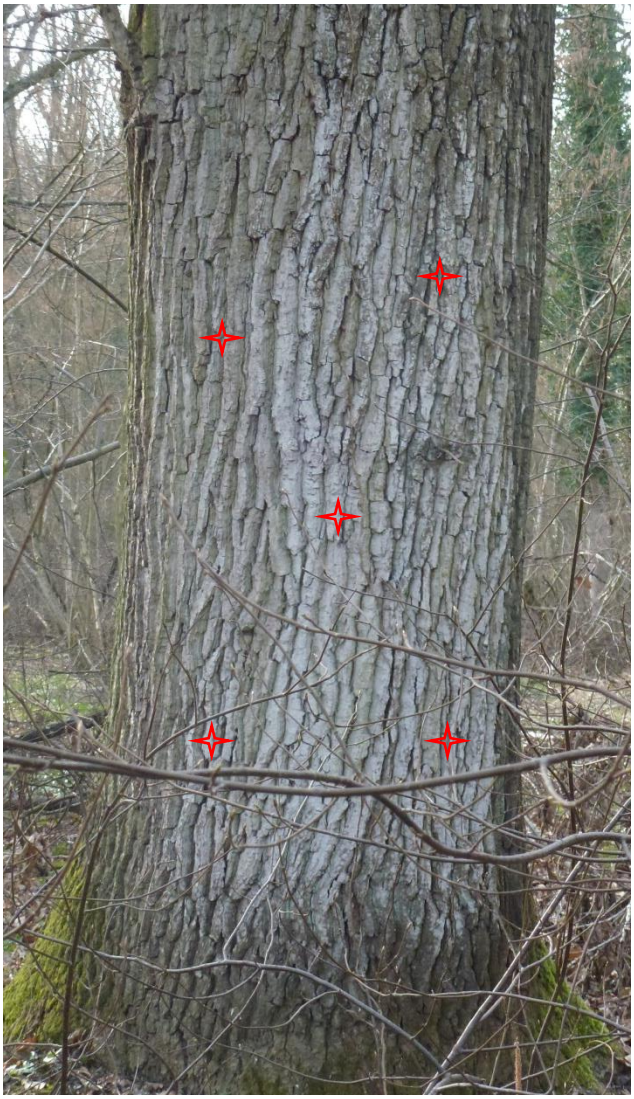

Figure 3 Bactrospora dryina *on* Quercus robur *in Allschwil BL. Red stars represent symbolically the applied sampling method at tree-level. At each of the five positions, one fruit body or thallus part was collected. © Alex Stirnemann, 2016*

2.4 DNA extraction and PCR

If present, one single apothecium per sample was isolated for DNA extraction. In samples with sterile lichen, approximately 20-50 mm^2 thallus-tissue was isolated.
The genomic DNA was extracted from specimens using PowerPlant® Pro-htp 96 Well DNA Isolation Kit (MoBio Laboratories, Carlsbad, California, USA and QIAGEN, Hilden, Germany) according to the manufacturer's instructions with minor adaptations in temperature settings and shake durations following Nadyeina et al. (2017).
For microsatellite amplification, 16 polymorphic and specific microsatellite markers were used, developed by Nadyeina et al. (2017). PCR reaction were performed in a total volume of 10 µl containing 1µl DNA template, 2 µl sterile H20, 2µl Primer Mix and 5µl Type-it Multiplex PCR Master Mix (QIAGEN, Hilden, Germany). The PCR protocol used fluorescently labelled forward primers and the reaction was performed under the following conditions:5 min at 95°C, followed by 30 cycles of 30 s at 95 °C, 90 s at 56 or 58°C (depending on the melting temperature of the primers in the different multiplexes) and 30 s at 72°C, with a final extension of 60 min at 60 °C.
PCR products were diluted with 10µl sterile H_20. 1µl of this diluted PCR product was then used for capillary electrophoresis, run on a 3130xl DNA Analyzer (Life Technologies, Carlsbad, California, USA) with 9 µl of a mix of 1 µl Hi-DiTM Formamid (Thermo Fisher Scientific) and 12 µl LIZ (Life Technologies, Carlsbad, California, USA) as the size standard for fragment analysis, according to the manufacturerer's instruction. An injection time of 6s was used to generate data. This PCR protocol is taken from Nadyeina et al. (2017). GeneMapper 5.0 (Life Technologies) was used to size alleles.
Further genetic data of Swiss *B. dryina* populations not collected by the author himself was provided by Nadyeina et al. (2017) collected in the cantons Zürich, Thurgau and in the canton of Zug (Table 2, Figure 2).

2.5 Genetic structure and variation

Gene diversity was calculated according to Nei, 1987. Fst was calculated among populations and subpopulations to detect historical gene flow. Fst significance was determined by 999 permutations. Hierarchical Analysis of Molecular Variance (AMOVA) was performed to determine genetic variation among and within areas, stands and colonies (trees). Genotype assignment test was used to identify migrated genotypes between two populations (recent gene flow). Dominant or highly frequent haplotypes were detected with Haplotype inference by expectation-maximation algorithm. All the above calculations were performed in Arlequin 3.5 (Excoffier et al., 2010).
Population genetic structuring was performed with STRUCTURE 2.3.4 (Pritchart et al., 2000). The best suitable number of genetic clusters was inferred with STURCTURE HARVESTER (Earl & von Holdt, 2012) according to "Evanno" method (Evanno et al., 2005).
All genetic analysis were performed on different spatial scales because genetic differentiation is likely to be scale dependent as shown in population genetic studies with other organisms (Angelone et al., 2011; Blair et al., 2012). Scale classes were defined to national scale, regional scale and local scale (Forests). On national scale, regional populations were summarized to four localities (Aargau/Zug, Basel, Galmiz/Messen and Marthalen/Tägerwil & Romanshorn). In geographically isolated populations regional and local scale means the same as there is no other population in the region (i.e. Galmiz).
For some subpopulations or fragmented single colonies genotype assignment test and Fst values on local scales could not be calculated due to too small sample sizes. Differentiation on regional and local scales was detected manually by comparing loci.

2.6 Isolation by distance along river network

Geographic distances between populations were calculated using the tool ODcostMatrix in ArcMap 10.1 for which the river systems built the network. Because distances between populations should represent dispersal paths or historical population connections, Euclidean distance was measured between populations growing in the same forest or in very close distance to each other (<7km) ignoring the river network. Because *B. dryina* can also occur in coppice-with-standards forests, relatedness to a river is not demanded in every case. As it is assumed that *B. dryina* originally evolved in hardwood floodplain forests, distance calculations for colonies in coppice-with-standards forests were made along the closest larger river system. IBD was tested using Mantel test of "vegan" package (Oksanen et al., 2017, RStudio 1.0.44, © 2009-2016 RStudio, Inc.).

3. Results

3.1 *Bactrospora dryina* occurrence in the study area

Table 1 *In 14 areas in Switzerland, populations of* Bactrospora dryina *were found. Total number of* Bactrospora dryina *populations were estimated in the field. Forest type only describes the actual forest stands where* Bactrospora dryina *occurs. Location names are based on villages nearby and field or forest names.*

Location	Coordinates	Forest type	No. colonies sampled	Estimated Number colonies
Brügglihau, Hermetschwil AG (later "Bremgarten Süd")	47°20'04.017"N 8°20'58.462"E	Hardwood floodplain forest	25	60
Chessel, Bremgarten AG – Talhau, Stetten AG (later "Bremgarten Nord")	47°21'48.053"N 8°20'05.432"E	Hardwood floodplain forest / deciduous mix forest	31	80
Gruemet, Mellingen AG	47°25'36.274"N 8°15'53.516"E	Deciduous mix forest / Oak forest	6	10
Niggisbüel, Mägenwil AG	47°25'00.547"N 8°14'37.109"E	Deciduous mix forest / Oak forest	1	1
Berg, Othmarsingen AG	47°24'10.413"N 8°13'41.980"E	Deciduous mix forest / Oak forest	2	2
Lind, Niederlenz AG	47°24'08.825"N 8°11'11.246"E	Deciduous mix forest	4	4
Chaibegarte, Lenzburg AG	47°22'42.539"N 8°11'34.825"E	Deciduous mix forest / Oak forest	9	20
Schlatt, Seengen AG	47°20'11.868"N 8°11'00.304"E	Hardwood floodplain forest / Coppice-with-standards	19	40
Steppberg, Magden AG	47°32'34.037"N 7°48'28.359"E	Coppice-with-standards forest / oak forest	7	50
Hard, Birsfelden BL	47°32'40.838"N 7°38'38.557"E	Hardwood floodplain forest / Coppice-with-standards forest	8	20
Asp, Muttenz BL	47°30'58.931"N 7°37'49.893"E	Old growth deciduous mix forest	3	3
Allschwiler Wald, Allschwil BL	47°32'13.064"N 7°31'01.607"E	Coppice-with-standards forest	7	50
Barhollen / Junkholz, Messen SO	47°04'48.580"N 7°27'13.584"E	Hardwood floodplain forest / deciduous mix forest	11	20
Frischenei, Galmiz FR	46°56'48.602"N 7°09'43.699"E	Hardwood floodplain forest / deciduous mix forest	20	30

The largest spreading of *B. dryina* was found along the Reuss river in the canton of Aargau. Another Argovian group of subpopulation is located along Aabach which flows in a parallel valley to the Reuss. Small scattered populations between these rivers were found, however no *B. dryina* colonies could be found along the Aare river in the canton of Aargau. Further important subpopulations are found in

the area of Basel with its possibly largest occurrence at Berg and Steppberg near Magden. The populations in Galmiz (FR) and Messen (SO) are of smaller number and are geographically located within small distances. The areas in north-eastern Switzerland were observed in a previous study by Nadyeina et al. (2017) of which genetic data was provided for this master thesis (Table 2).

Table 2 *Genetic data of eight populations in north-eastern Switzerland was provided by Nadyeina et al. (2017). Location description are based on villages nearby and field or forest names.*

Location	Coordinates
Mosholz, Marthalen ZH	47°35'50.391"N 8°44'51.207"E
Niederholz, Marthalen ZH	47°37'11.232"N 8°36'58.108"E
Hard, Marthalen ZH	47°36'44.611"N 8°39'41.286"E
Tägerwiler Wald, Tägerwilen TG	47°38'22.884"N 9°06'43.480"E
Neuwald, Romanshorn TG	47°33'56.150"N 9°20'15.698"E
Frauental, Maschwanden ZG (later "Lorze")	47°13'17.458"N 8°25'14.393"E
Zollischlag, Hünenberg ZG	47°11'08.170"N 8°24'45.419"E
Rüsshalden, Hühnenberg ZG (later "Zugsüd")	47°09'57.626"N 8°24'58.380"E

3.2 Vegetation characteristics and niche determination

Many of the forests described as hardwood floodplain forests are now managed forests. Their structure is not only formed by the flooding dynamics of rivers, but also by selective harvesting. Nevertheless, high soil moisture due to the vicinity to a river is still essential in shaping the typical species composition of floodplain forests. In Messen and Galmiz, the forests are located along a small river network with ponds, whereas in the area of Bremgarten, the large Reuss river (134 m^3/s, BAFU 2018) dominates the landscape as well as the Rhein in Birsfelden (909 m^3/s, BAFU 2018).
Small scattered *B. dryina* populations found in non-floodplain forests are mostly located in deciduous mixed forests with old oaks. Large populations in non-floodplain forests are found in oak forests with coppice-with-standard type of forest management (Table 1).

In a binomial GLM the probability of being a *B. dryina* host-tree increased significantly with increasing bark crevice depth (Figure 7), bark condition (Figure 6) and canopy coverage (marginal significant) (Table 4). Girth at breast height was only a significant parameter when tested as the only explanatory variable (Figure 4, Table 3). It becomes insignificant when bark crevice depth was included, meaning that these two variables are autocorrelated (Pearson correlation coefficient = 0.623, $p < 2.2e^{-16}$). Furthermore, bark condition was influenced by canopy coverage ($p = 0.0004$).
Most *B. dryina* colonies were growing in south or south-eastern direction. Although there were slightly more trees inclined towards south, GLM suggests northness to be negatively correlated with the probability of an oak to carry the lichen, meaning – southness is positively correlated as it is the opposite (Figure 5).

With increasing light availability, development status of *B. dryina* colonies tend to shift (Figure 8). Newly colonised trees are situated in areas with an intermediate level of light. The lichen can develop apothecia with sexually reproduced spores on a more shaded level (canopy coverage 60 %).

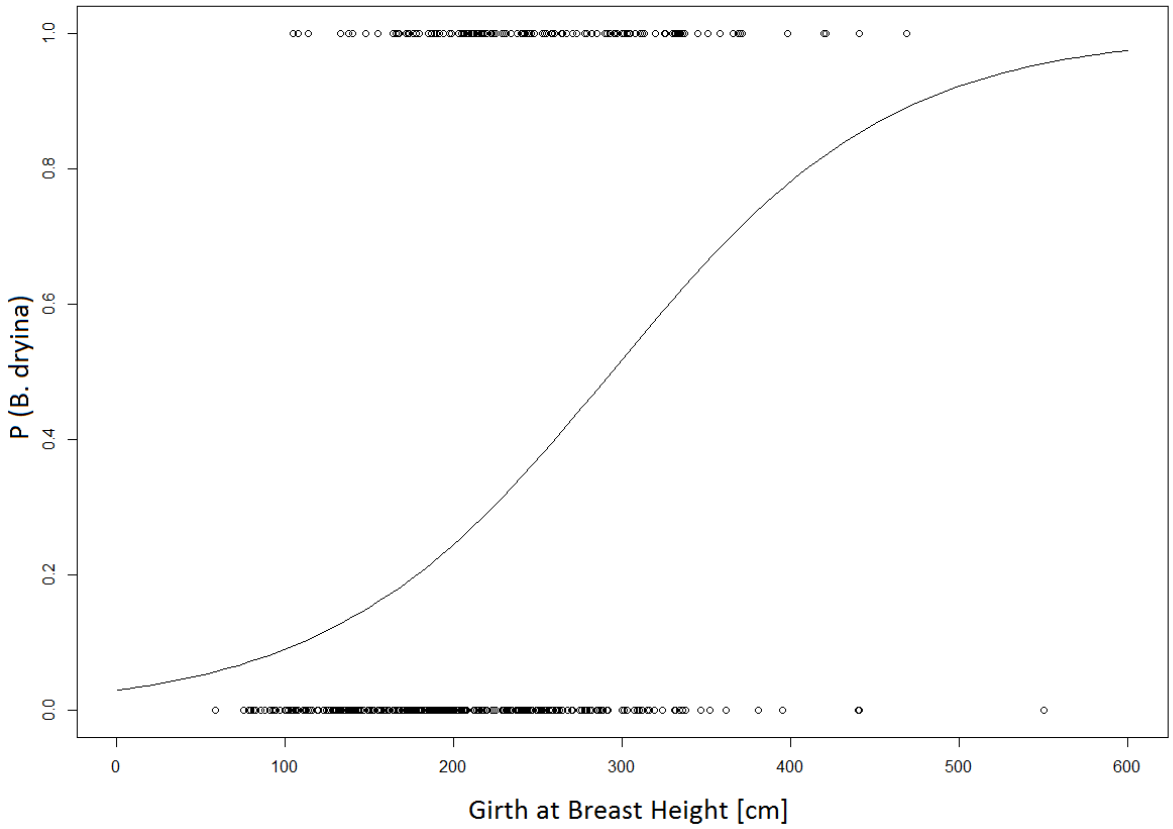

Figure 4 *Oaks are more likely to be colonised by* Bactrospora dryina *with increasing girth at breast high. Probability curve represents the significant binomial generalised linear model.*

Table 3 *Binomial generalized linear model explaining the likelihood of* Bactrospora dryina *to be present on an oak in dependence of girth at breast height (GBH). Increasing GBH significantly enhances probability of* Bactrospora dryina *to be growing on an oak.*

	Estimate	Std. Error	Z Value	Pr (>|z|)
Intercept	-3.52222	0.363382	-9.693	$< 2e^{-16}$***
GBH	0.011987	0.001531	7.828	$4.95e^{-15}$***

Table 4 *Binomial generalized linear model explaining the likelihood of* Bactrospora dryina *to be present on an oak by the azimuth of the suitable stem side (northness), bark condition, bark crevice depth, canopy coverage and girth at breast height (GBH).*

	Estimate	Std. Error	Z Value	Pr (>|z|)
Intercept	-1.104803	0.653789	-1.169	0.091057
Northness	-0.739933	0.210792	-3.510	0.000448***
Bark Condition	-0.012615	0.005458	-2.311	0.020819*
Crevice Depth	0.044583	0.021179	2.105	0.035290*
Canopy Coverage	-0.012399	0.006540	-1.896	0.057964
GBH	0.004024	0.002609	1.542	0.123004

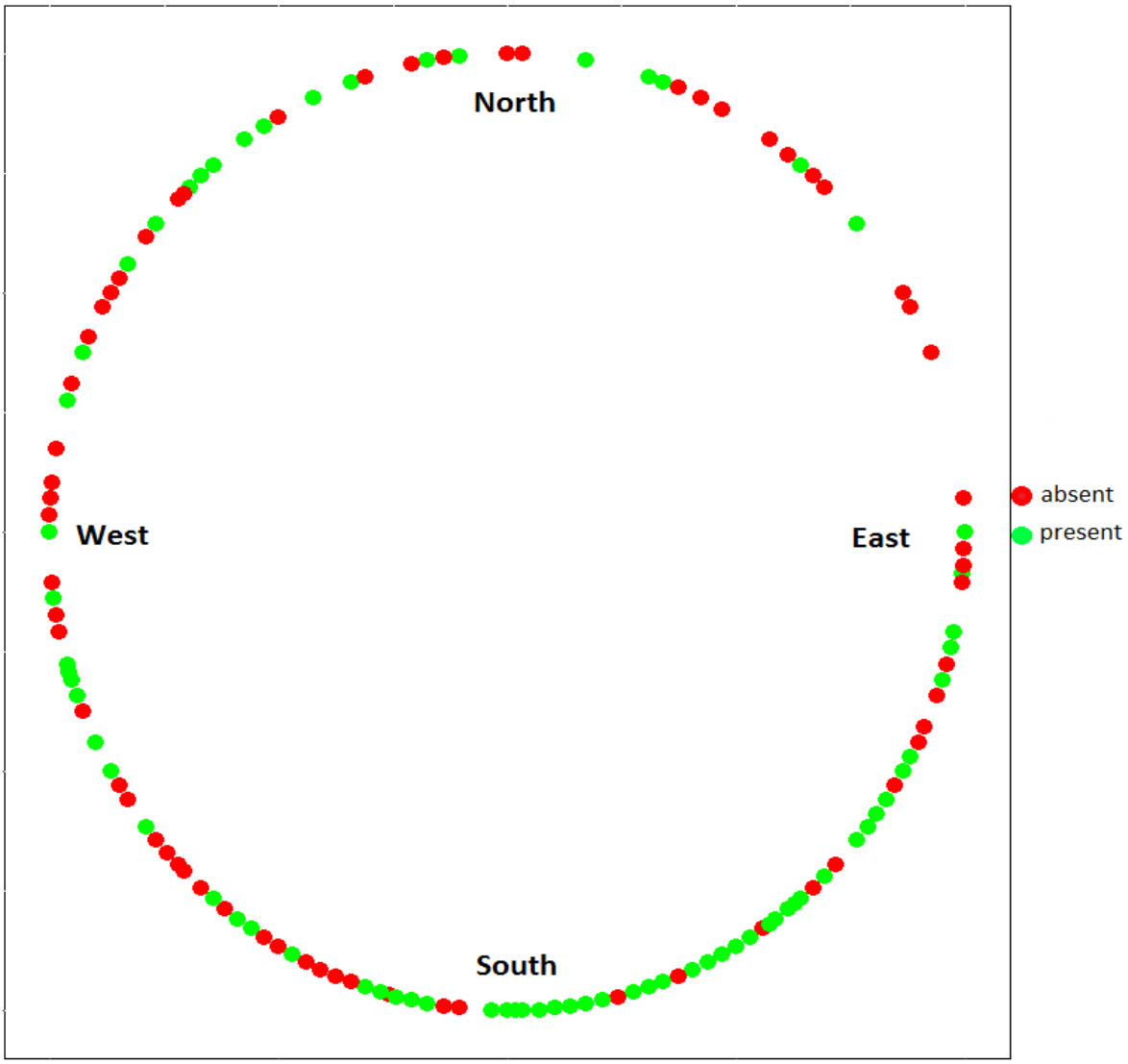

Figure 5 *Green dots represent populations of* Bactrospora dryina *on a stem whereas red dots stand for suitable dry stem sides of oaks where the lichen could not be found. Significantly more oaks were colonised by* Bactrospora dryina *when inclined southwards compared to oaks inclined towards other directions.*

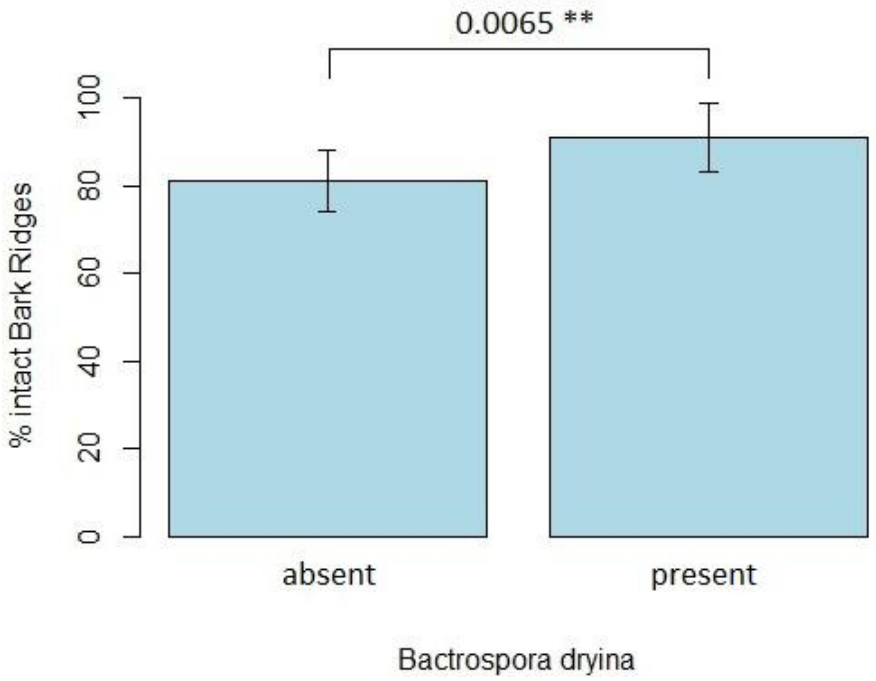

Figure 6 *Oaks colonized with* Bactrospora dryina *have largely unscathed bark ridges. Oaks without a* Bactrospora dryina *colony on its stem show significantly larger area on the suitable stem side with broken off bark ridges.Error bars represent standard error.*

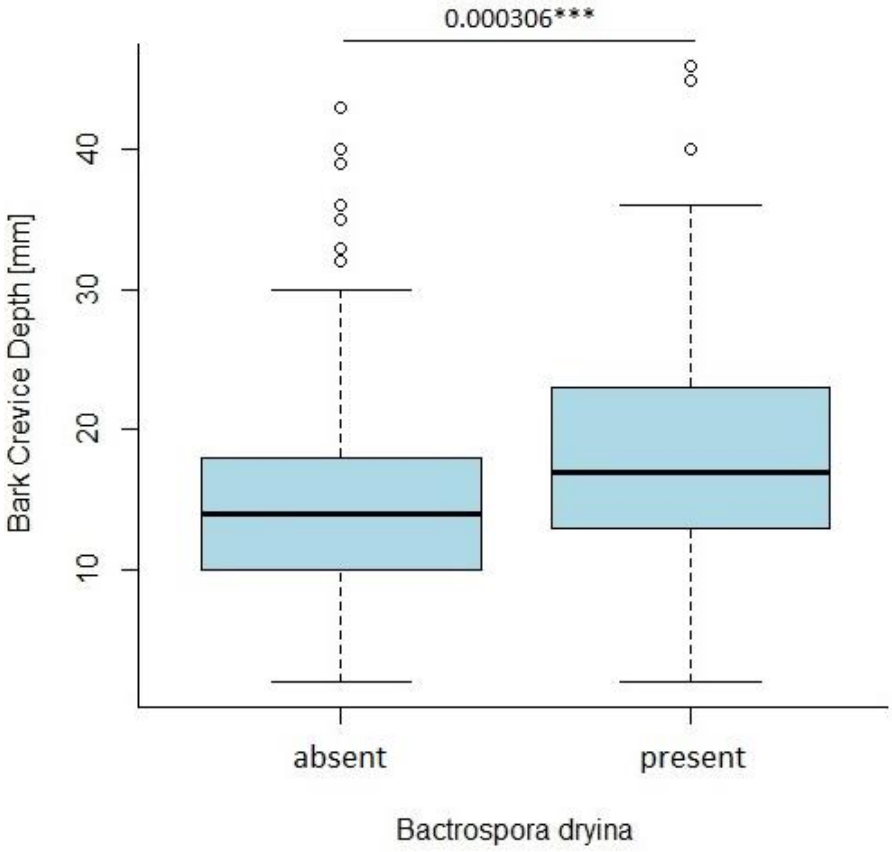

Figure 7 *Significant difference in the bark crevice depth of* Bactrospora dryina *carrying oaks to non-host-oaks growing at the same site.*

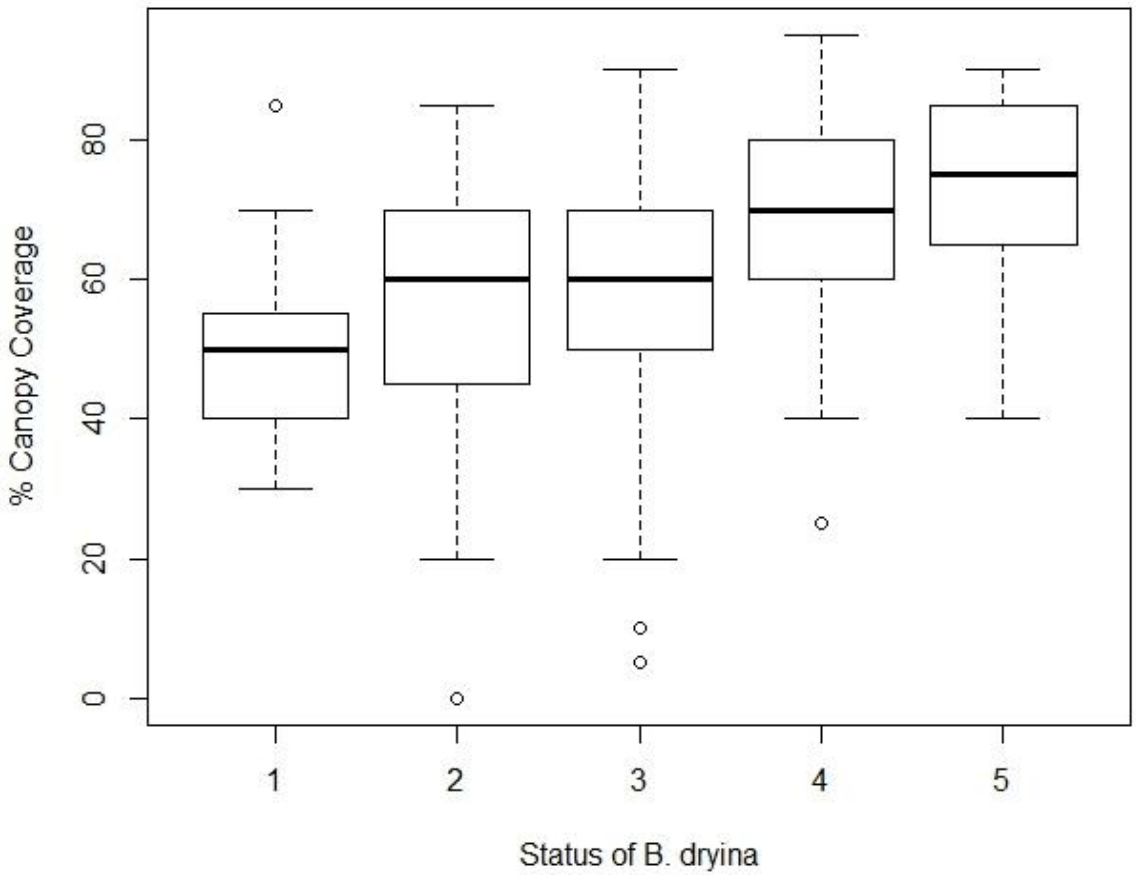

Figure 8 *Development status of* Bactrospora dryina *compared to canopy coverage. Canopy coverage is significantly different between early statuses (1, 2, 3) and late statuses (4, 5) (Table 1, appendix). Status 1) early sterile stages of colonisation, 2) large coverage of sterile* Bactrospora dryina*, 3) large coverage of fertile* Bactrospora dryina*, 4) fragmented colony with fertile parts, 5) fragmented colony overgrown with algae*.

3.3 Genetic structure and differentiation of *Bactrospora dryina* in Switzerland

3.3.1 National Scale

Hierarchical analysis of molecular variance (AMOVA) explains highest variation within the four areas and only 7% is explained by variation between localities (Table 5). As a consequence of lower variation among populations, STRUCTURE suggests to divide the haplotypes in three genetic clusters (Figure 9). Furthermore, genetic distance is not correlated with geographic distance neither along the river network ($p = 0.208$; Figure 13, appendix) nor with Euclidean distances ($p = 0.542$; Figure 12, appendix).

Pairwise Fst (Figure 15,appendix) is lowest for Galmiz/Messen and Aargau/Zug and STRUCTURE analysis on national scale (Figure 9) suggests similar genetic structure. Therefore, these two localities are analysed separately (Figure 10). However, population structure showed no explainable differentiation pattern along the river system and isolation by distance can also be rejected for these two localities ($p = 0.436$).

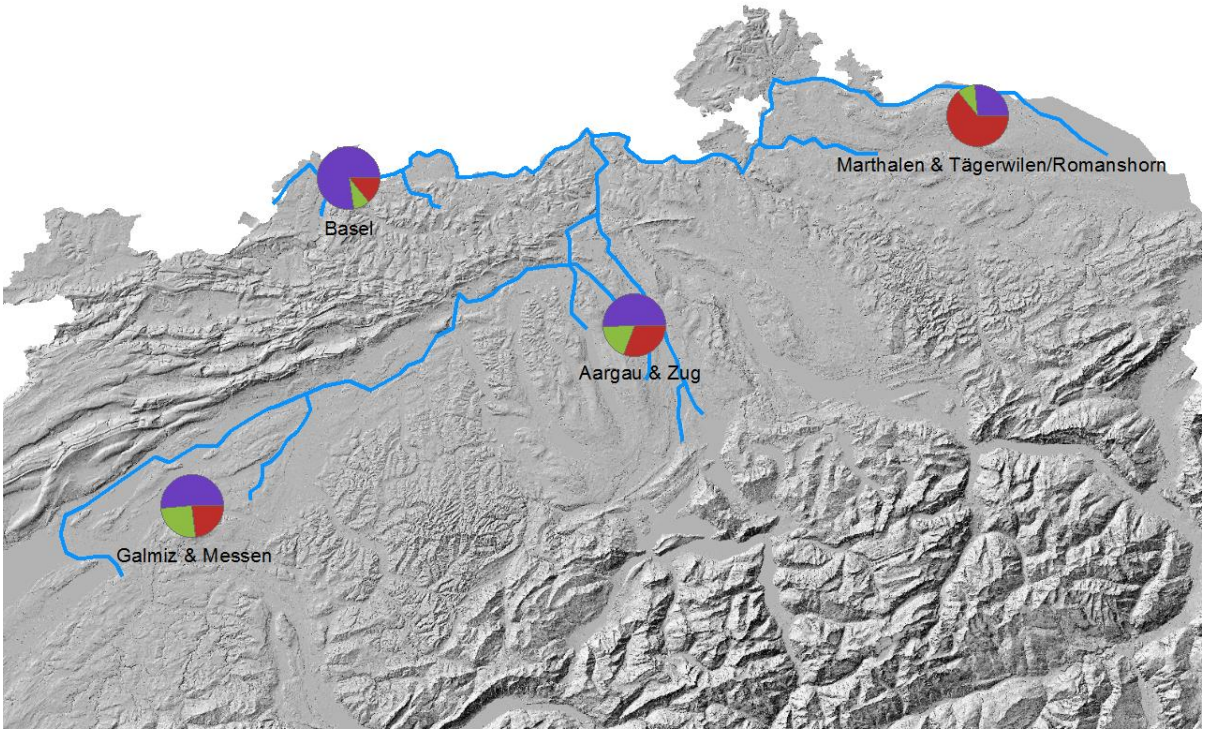

Figure 9 *Pie charts representing summarised local populations of* Bactrospora dryina *in four localities of northern Switzerland. Coloured divisions of pie charts represent the relative amount of colonies belonging to one of the three genetic clusters per site based on STRUCUTRE analysis. Blue network shows the river network of Aare, Reuss, Rhein and Aabach with minor affluents.*

Table 5 *Hierarchical analysis of molecular variance (AMOVA) of* Bactrosproa dryina *of four areas in northern Switzerland indicating low genetic variation among localities and highest variation within localities.*

Source of Variation	SS	Variance	Variation (%)	P
Among localities	623.095	0.23750	7.02	***
Within localities	13344.029	3.14569	92.98	***

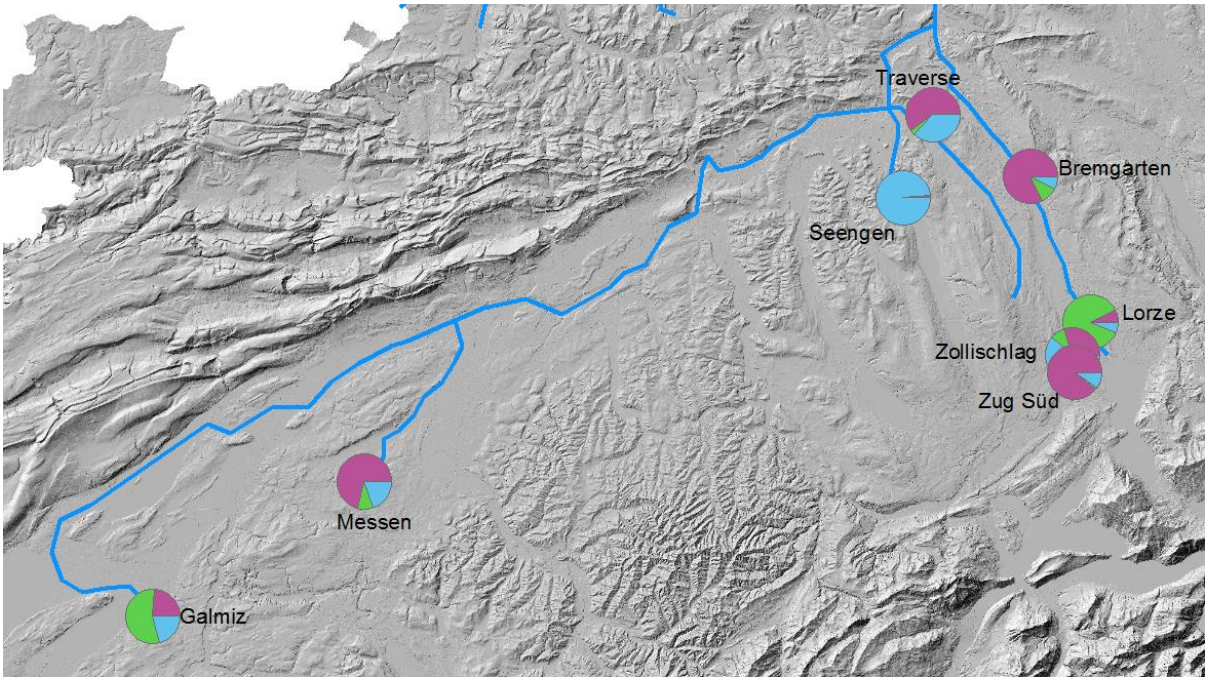

Figure 10 *Pie charts represent local populations of* Bactrospora dryina *in north-western Switzerland. Coloured divisions of pie charts represent the relative amount of colonies belonging to one of the three genetic clusters per site based on STRUCUTRE analysis. Blue network shows the river network of Aare, Reuss and Aabach with minor affluents.*

3.3.2 Regional scale

While on national scale, no differentiation could be found among regions, on regional scale differentiation among sampling sites was recovered.

Because the population are not the same size, the number of samples at each site are highly different. Therefore, the number of different haplotypes per site depends on the sample size (Ad. R^2 = 0.8229, $p < 0.001$; Figure 10, appendix). However, gene diversity is not significantly correlated with sample size (Figure 11, appendix). The lowest gene diversity is found in populations Lorze and Seengen, and the highest gene diversities are found in populations Marthalen Niederholz and Tägerwilen (Table 6).

Table 6 Eighteen Swiss populations of Bactrospora dryina that were included in the population genetic analysis of this study. Number of samples and number of haplotypes are autocorrelated.

Population	Number of Samples	No. Haplotypes	Gene diversity
Bremgarten Süd	108	23	0.36
Bremgarten Nord	132	33	0.43
Traverse	101	27	0.45
Seengen	87	13	0.14
Zollihschlag	296	24	0.38
Lorze	149	27	0.22
Zug Süd	11	3	0.33
Galmiz	90	31	0.33
Messen	45	24	0.37
Allschwil	29	13	0.42
Magden	33	19	0.45
Muttenz	6	4	0.44
Birsfelden	36	12	0.34
Romanshorn	35	6	0.39
Tägerwilen	498	109	0.47
Marthalen Mosholz	20	4	0.26
Marthalen Hard	50	32	0.41
Marthalen Niederholz	500	157	0.51

Basel

Although low Fst values are calculated between each of the four subpopulation at the area of Basel (Figure 16, appendix), there is high genetic diversity within subpopulations with a mean gene diversity of 0.48. A closer look to each sampled tree further shows that many colonies on a tree are of only one haplotype (Figure 12).

Dividing this population into genetic clusters could not definitively be done (Figure 19, appendix). According to Evanno's method (Evanno et al., 2005), population at Basel might be composed of two (Figure 11) or nine (Figure 12 a-d) genetic clusters. Increasing the number of genetic clusters would finally lead to a number of clusters equal to the number of unique haplotypes. However, in the case of 9 clusters, there are still two clusters which occur in at least three out of the four subpopulations. In the case of Basel it is the dark blue and the purple cluster in figures 12 a-d. I.e. Populations of the blue cluster (Evannos Delta K=9) are homozygous at 9 Loci and show for 6 of the remaining loci two different haplotypes (Figure 13). Hierarchical AMOVA supports this genetic structure of a highly diverse regional population with low local differentiation (table 7).

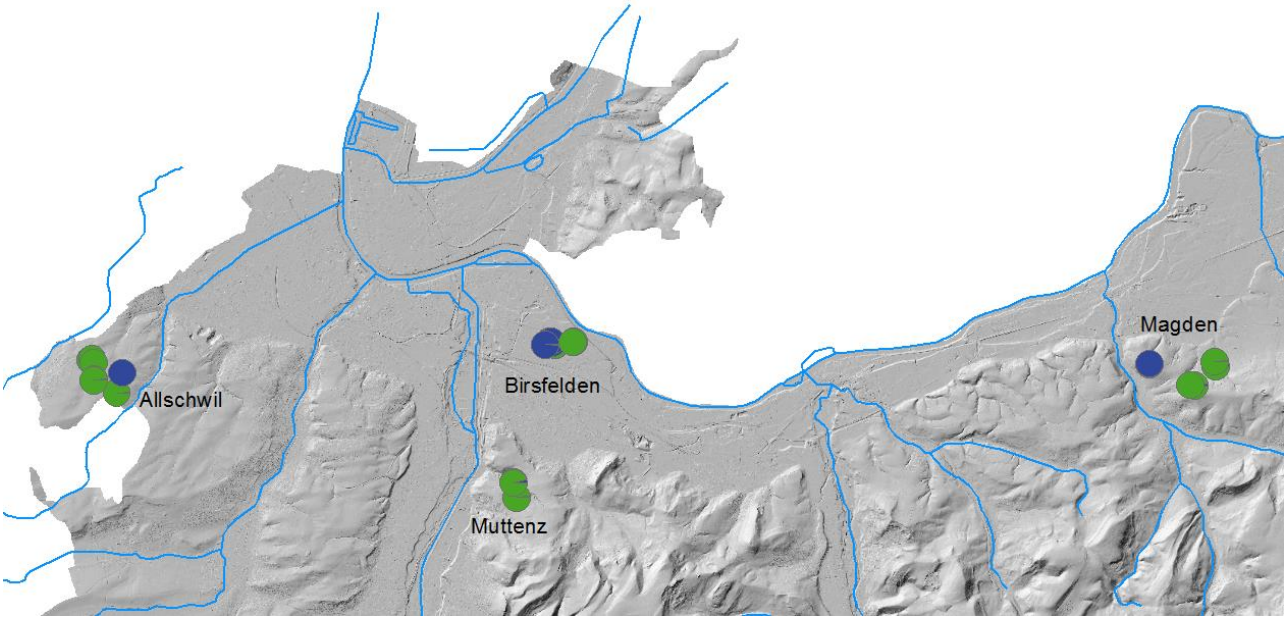

Figure 11 *Four populations in the area of Basel in north-western Switzerland were found. Pie charts represent individual colonies (trees). The local population-structure of Basel is divided into two genetic clusters, which are found in all of the four subpopulations.*

Table 7 *Hierarchical analysis of molecular variance (AMOVA) of* Bactgrospora dryina *of four subpopulations in the area of Basel indicating lowest genetic variation explained among subpopulation and among trees within subpopulation and largest variation within subpopulations.*

Source of Variation	SS	Variance	Variation (%)	P
Among subpopulations	107.191	0.39028	13.21	**
Within subpopulations	368.954	1.96531	66.53	***
Among Trees within subpopulations	94.567	0.59852	20.26	***

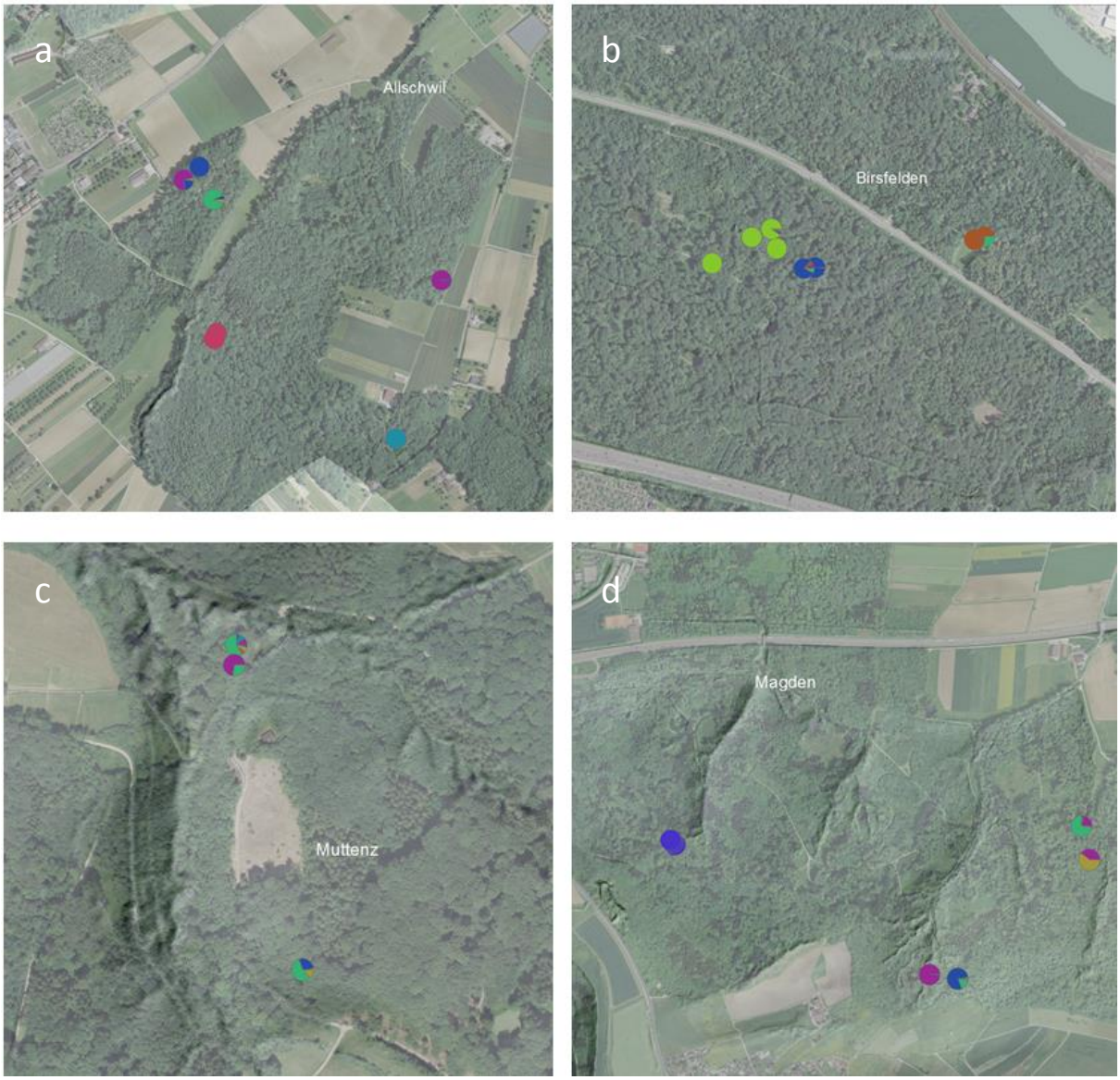

Figure 12 a-d *Pie charts represent individual colonies (trees) in the area of Basel. The local population-structure of Basel is divided into nine genetic clusters, which are highly specific to single trees or aggregation of trees. a) Allschwil, b) Birsfelden, c) Muttenz, d) Magden*

Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Allschwil	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Birsfelden	B	A	A	A	A	A	A	B	B	B	A	B	A	B	A	A
Magden	B	C	A	A	A	A	A	C	A	A	A	B	A	A	A	A

Figure 13 *Comparison of multilocus genotypes of three colonies from (Allschwil, Birsfelden and Magden) which were assigned to the same genetic cluster (royal blue in figure 12 a, c, d) when population structure at Basel is divided into nine genetic clusters. Top row indicates Loci 1-16, coloured fields /letters represent same allele length. Colours/letters used in this figure are not corresponding to letters used in other figures showing multilocus genotypes.*

Population assignment test identifies recent gene flow within this region (Figure 14). One migrant found in the population of Magden belonging genetically to Muttenz and another one in Allschwil belonging to the population in Magden.

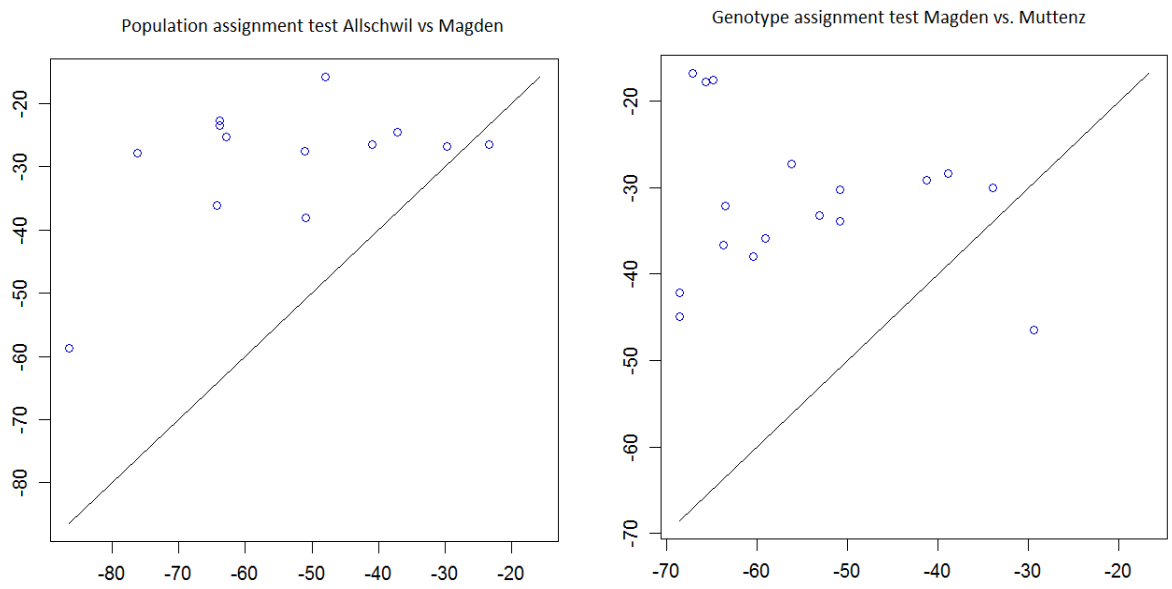

Figure 14 *Left: dots beyond the diagonal line belong to population Allschwil, dots below the diagonal line indicates recent gene flow from Magden. Right: Dots beyond the diagonal line belong to population Magden, dots below the diagonal line indicates recent gene flow from Muttenz.*

Galmiz

The population at Galmiz is restricted to a very small area. Three subpopulations were found with 5-6 colonies (trees) each within a total area of approximately 2.25 ha. Further, in this area three colonies outside the subpopulations were found and individual samples were taken. The whole population at Galmiz shows a gene diversity of 0.33. The three colonies outside these subpopulations (23, 24, 25 in figure 15) are genetically very closely related to each other and show only a second genotype on two loci. Their dominant haplotype is also found in subpopulation 32-37 on one tree (Figure 15, red circle).

STRUCTURE divides the population in two genetic clusters. Each subpopulation contains both types. Only three of the 25 colonies carry only one haplotype. Hence, genetic variation is high, even in single colonies as shown with AMOVA (Table 8). Therefore, colonies belong to one panmictic population with an unlimited gene flow between colonies and subpopulations.

Figure 15 Bactrospora dryina *population at Galmiz with three subpopulation and three scattered individual colonies. Each pie chart represents the genetic structure of a colony (tree). All subpopulations show both genetic clusters. Isolated colonies 23-25 belong to only one cluster and are composed of one haplotypes with minor mutations. This haplotype is also found on tree nr. 32 in subpopulation 32-37 (red circle).*

Table 8 *Hierarchical analysis of molecular variance (AMOVA) of* Bactgrospora dryina *population at Galmiz indicating high connectivity between subpopulations and highest genetic variation at tree level.*

Source of Variation	Sum of Squares	Variance	Variation (%)	P
Among Subpopulations	21.346	0.06457	2.84	0.149
Among Colonies (Trees) within Subpopulations	101.401	0.49798	24.75	***
Within colonies (Trees)	167.467	1.44368	72.40	***

Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
32	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
32	A	A	A	A	A	B	A	B	B	A	A	A	A	A	D	A
32	A	B	A	B	B	B	B	B	B	A	B	A	A	A	A	A
23	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
24	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
24	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
25	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
25	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

Figure 16 *Diversity pattern of multilocus genotypes in* Bactrospora dryina *of eight samples taken from four different colonies at Galmiz. Top row indicates Loci 1-16, letters/coloured fields represent same allele length. First column indicates tree number according to figure 15. Colonies 23-25 share one identical haplotype with colony 32.*

Messen

Population at Messen is composed of three small subpopulations with two to five colonised trees. These Subpopulations are found within an area of approximately 2.5 ha. Over all loci in Messen a gene diversity of 0.37 was calculated which is rather high as only eleven colonies were found. Each of the three subpopulations show heterogeneity in at least in one colony, which is also shown by STRUCTURE analysis, suggesting the population to be composed of two genetic clusters (Figure 17).

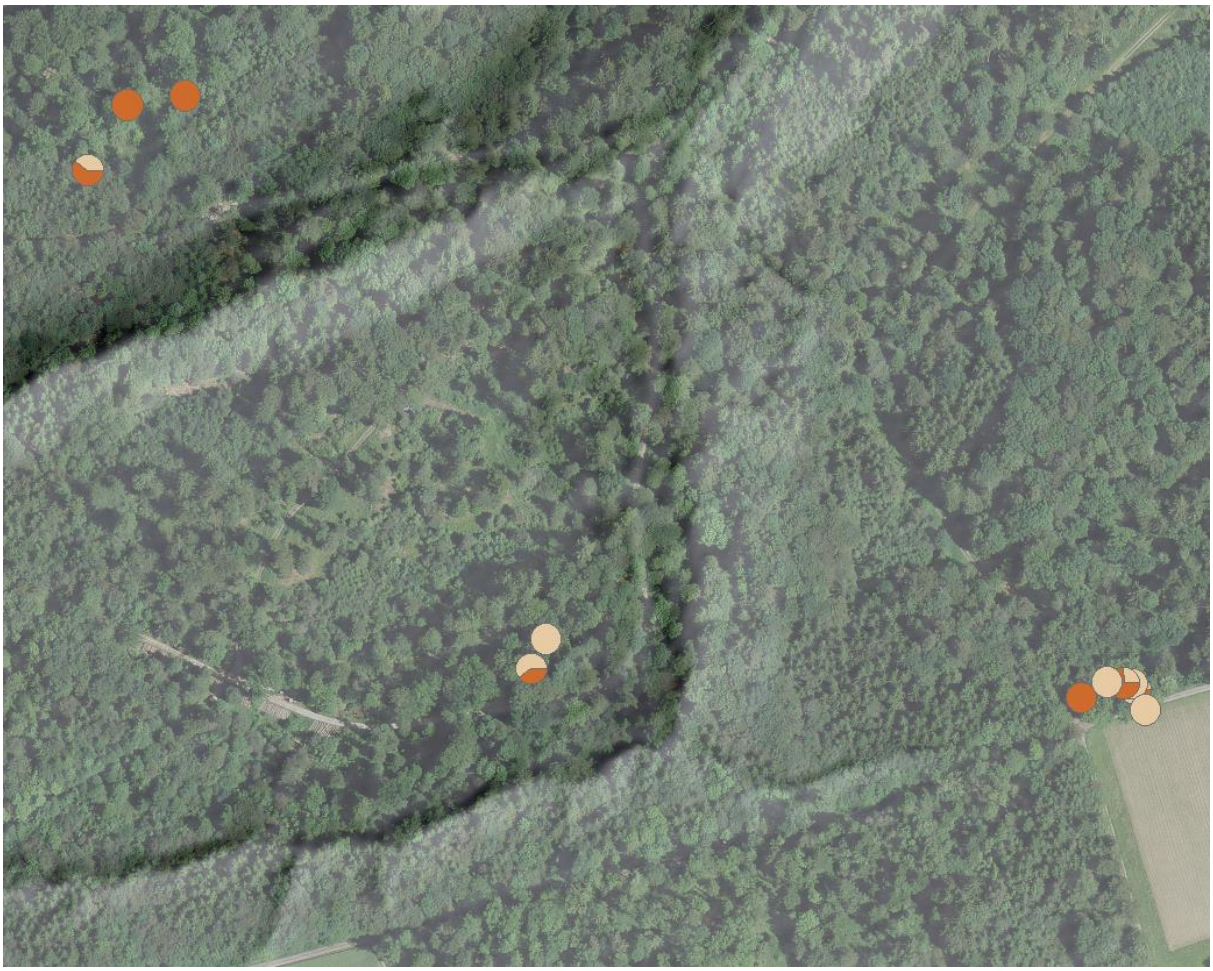

Figure 17 Bactrospora dryina *population at Messen with three subpopulation. Each pie chart represents the genetic structure of a colony (Tree). All subpopulations are composed of both clusters.*

Table 9 *Hierarchical analysis of molecular variance (AMOVA) of* Bactgrospora dryina *population at Messen indicating high connectivity between subpopulations and no significant variation among subpopulations.*

Source of Variation	Sum of Squares	Varience	Variation (%)	P
Among Subpopulations	47.373	0.48865	18.88	0.073
Among Colonies (Trees) within Subpopulations	94.101	1.20360	46.51	***
Within colonies (Trees)	62.700	0.89571	34.61	***

South-eastern Aargau

Populations in the canton of Aargau are scattered mainly along the river system of three rivers (Figure 18). There are several more or less geographically seperated populations along Reuss, one large population at Lorze, a river which is connected to Reuss, and a population at Seengen along the Aabach, a river which is through Aare connected with Reuss. A number of scattered colonies between Reuss and Aabach which are not situated along a river is in the scope of this study called "Traverse" population as it is a linkage between the two valleys (Figure 18/22). Because there was no population found in the connection area of Reuss and Aare river a potential genetic connectivity between the Reuss populations and the population at Seengen was assumed to be replaced by colonies at Traverse.

Genetic analysis show no increasing genetic distance with increasing distance (IBD) over all seven populations (Mantel statistic r = 0.2624, p = 0.18; Figure 22, appendix). Populations Zollischlag and Zugsüd are closely related to populations Bremgarten and Traverse (Fst in figure 21, appendix), although large geographic distances are in between. Significant mantel tests indicate isolation by distance if populations Zollihschlag and Zugsüd are removed (Mantel statistic r = 0.8042, p = 0.025) (Figure 19a). Significant isolation by distance is also found for populations Bremgarten to Seengen (Mantel statistic r = 0.7197, p = 0.041667) (Figure 19b).

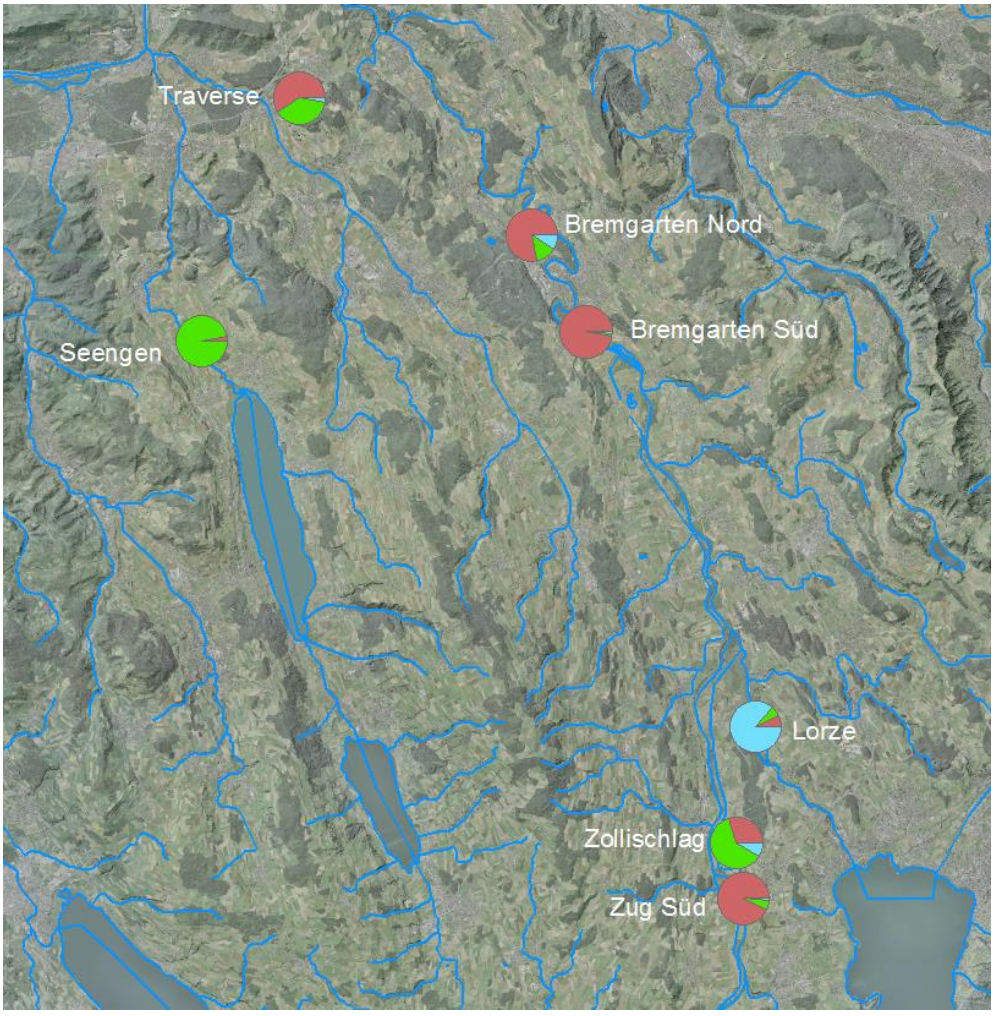

Figure 18 Bactrospora dryina *populations along Aabach and Reuss. Each pie chart represents the genetic structure of a local population. Blue network shows the river system of Aare, Reuss, Aabach and Bünz. Genetic structure of Lorze population is unique for this area.*

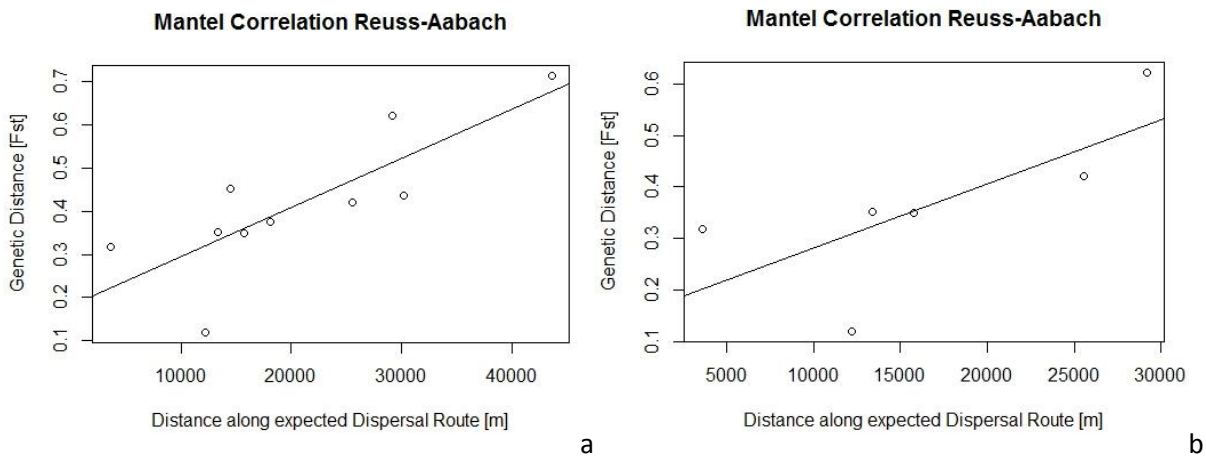

Figure 19 *a) Significant genetic isolation with increasing geographic distance is found in populations Seengen, Traverse, Bremgarten nord, Bremgarten süd and Lorze. The pattern remains significant if population Lorze is removed (b).*

Table 10 *Hierarchical analysis of molecular variance (AMOVA) of* Bactrospora dryina *populations along Reuss and Aabach. High variation among populations and rather homogeneous structures within single colonies is found.*

Source of Variation	Sum of Squares	Varience	Variation (%)	P
Among Populations	1911.58	1.22399	33.91	***
Among Colonies (Trees) within Populations	3316.321	1.84738	51.18	***
Within colonies (Trees)	753.467	0.53819	14.91	***

Seengen

Population at Seengen is divided into two genetically closely related clusters (Figure 20). Three subpopulations with 6-7 colonies were found and analysed, of which one subpopulation did not show any migrant from another subpopulation, whereas these show a heterogeneous compositions. Five out of the twenty-five colonies are heterogeneous, whereas all other colonies are composed of only one haplotype which are slightly different from each other. In total, 14 different haplotypes are detected in this forest, which is of a size of 750 ha. Two of these haplotypes are highly dominant and occur in 45% and 30% of all colonies. Other haplotypes show only few mutations at single loci. Due to the high number of homogenous colonies hierarchical AMOVA explains only 10% of genetic variation within colonies (tree-level)(Table 11). With a gene diversity of 0.14 it has a low diversity gene pool. Furthermore, genotype assignment test shows no indication of migration between the population at Seengen to other populations nearby.

The suggestion by STRUCTRUE to divide this population into two genetically distinct groups is supported by genotype assignment test for the subpopulations, where for subpopulations 140-146 and 147-152 migrating genotypes origin in subpopulation 134-139 was detected (Figure 26, appendix).

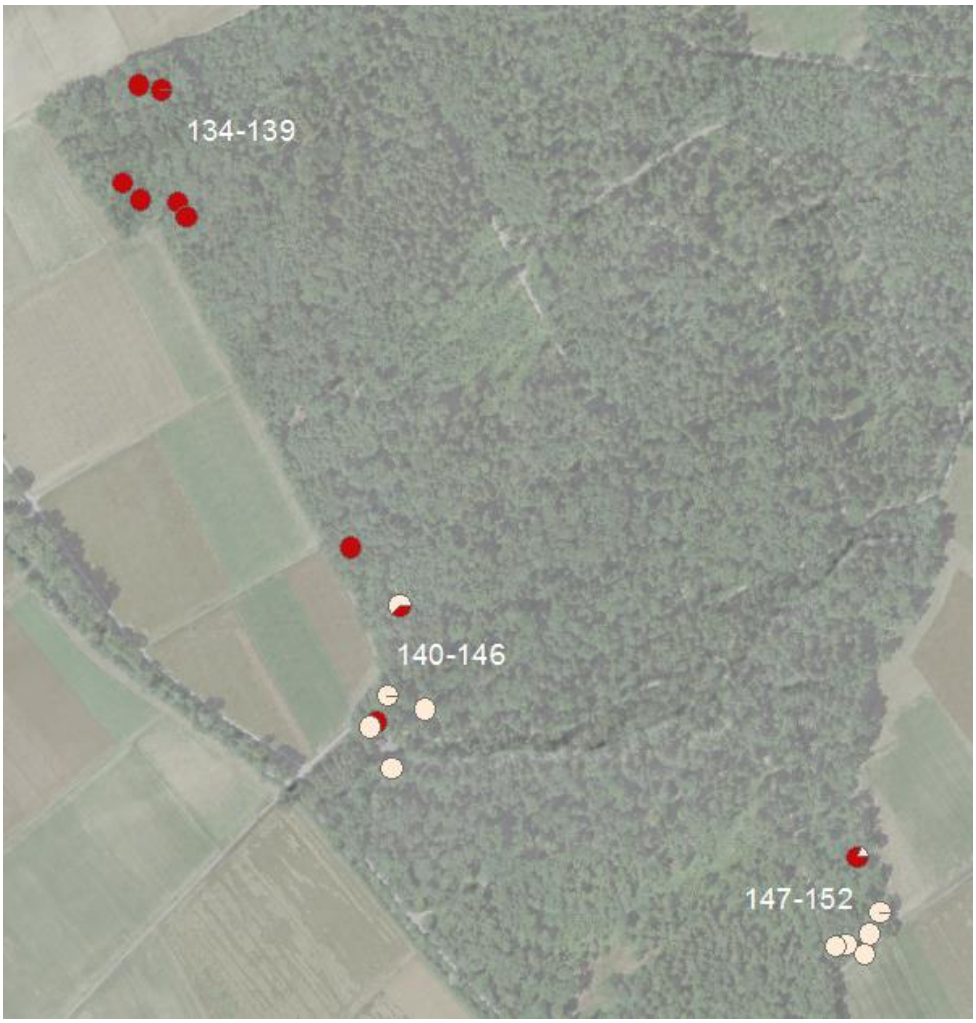

Figure 20 Bactrospora dryina *population at Seengen. Each pie chart represents the genetic structure of a colony (Tree) based on STRUCTURE analysis. Subpopulation 134-139 is only composed of genotypes belonging to one cluster.*

Table 11 *Hierarchical analysis of molecular variance (AMOVA) of* Bactgrospora dryina *population at Seengen shows low variation within a colony. Hence, colonies are widely homogeneous.*

Source of Variation	Sum of Squares	Varience	Variation (%)	P
Among Subpopulations	92.244	0.72138	55.95	**
Among Colonies (Trees) within Subpopulations	67.868	0.43892	34.04	***
Within colonies (Trees)	17.175	0.12341	9.57	***

Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
134	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
144	B	A	B	A	A	A	B	A	A	A	A	A	A	A	B	A
150	B	A	B	A	A	A	B	A	A	A	A	A	A	A	B	A

Figure 21 *Diversity pattern of mulitlocus genotypes in* Bactrospora dryina *of three samples taken from three different colonies (first column) at Seengen. Each sample is of another subpopulation according to figure 20. Top row indicates Loci 1-16. High similarity among subpopulations and low genotype diversity is indicated.*

Traverse

Subpopulations of Traverse population builds, as it is called, a traverse in terms of genetic linkage between population at Seengen and populations along Reuss. It consists of small scattered individual colonies. At Mägenwil only one single tree is carrying a *B. dryina* colony. At Mellingen six sterile colonies were found. Two colonies at Othmarsingen and the two subpopulations at Niederlenz are only composed of 2 colonies. Most colonies of Traverse population are genetically homogenous, although this homogenous haplotypes belong to different clusters (Figure 22).

Colonies at Lenzburg share genotypes with colonies at Seengen and with colonies at Bremgarten as shown in figure 23.

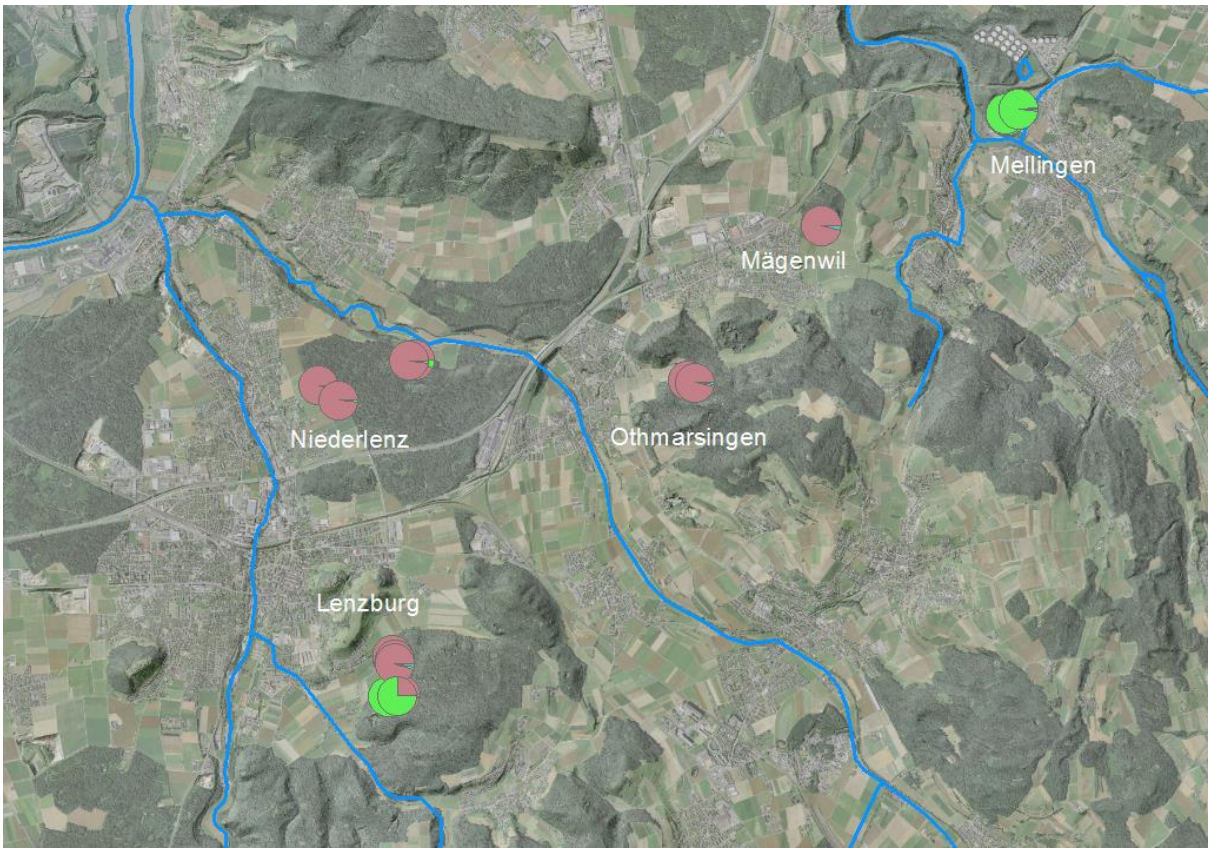

Figure 22 *Scattered colonies of* Bactrospora dryina *in Traverse population. Each pie chart represents the genetic structure of a colony (Tree-level) based on STRUCTURE analysis for canton of Aargau.*

Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Seengen	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Seengen	B	A	B	A	A	A	B	A	A	A	A	A	A	A	B	A
Lenzburg	C	A	B	A	C	C	A	A	A	A	A	A	C	A	A	A
Lenzburg	C	A	B	D	D	C	A	A	D	A	D	D	D	D	B	D
Bremgarten	C	E	E	A	A	C	A	A	E	A	A	A	A	D	B	D

Figure 23 *Diversity pattern of mulitlocus genotypes in* Bactrospora dryina *of five samples taken from three different colonies along the Traverse, Seengen and Bremgarten. Top row indicates Loci 1-16, coloured fields represent same allele length if colour/letter is the same.*

Bremgarten Nord and Bremgarten Süd

Populations arround Bremgarten are composed of aggregated colonies forming subpopulations. These are connected with scattered colonies. Populations Bremgarten Nord and Bremgarten Süd have togheter a gene diversity of 0.46. Three subpopulations however are of a remarkable genetic composition as they are genetically clearly distinct from the main population (Figure 24). Subpopulation 126-132 is built by a number of trees carrying only one haplotype. It shows high genetic distance to all closely situated subpopulations and shows no pattern of migration to or from another subpopulation. Because of the smaller sample size at this scale, Fst value of 0.6 equates to 7 different loci out of 16 (Figure 25, appendix). Hence, population 126-132 belongs genetically to Bremgarten. However, no migration is detected and a genetic depletion with a gene diversity of 0.07671 is recognised. The second distinct subpopulation (53-78) has higher gene diversity (0.36113) and shows patterns of migrated genotypes from other subpopulations of this site. However, no genotypes of this unique type is spread to another subpopulation. It shares further genotypes with population Lorze. A third remarkable subpopulation is 95-99 which shares as well several genotypes with colonies of Lorze population (Figure 25).

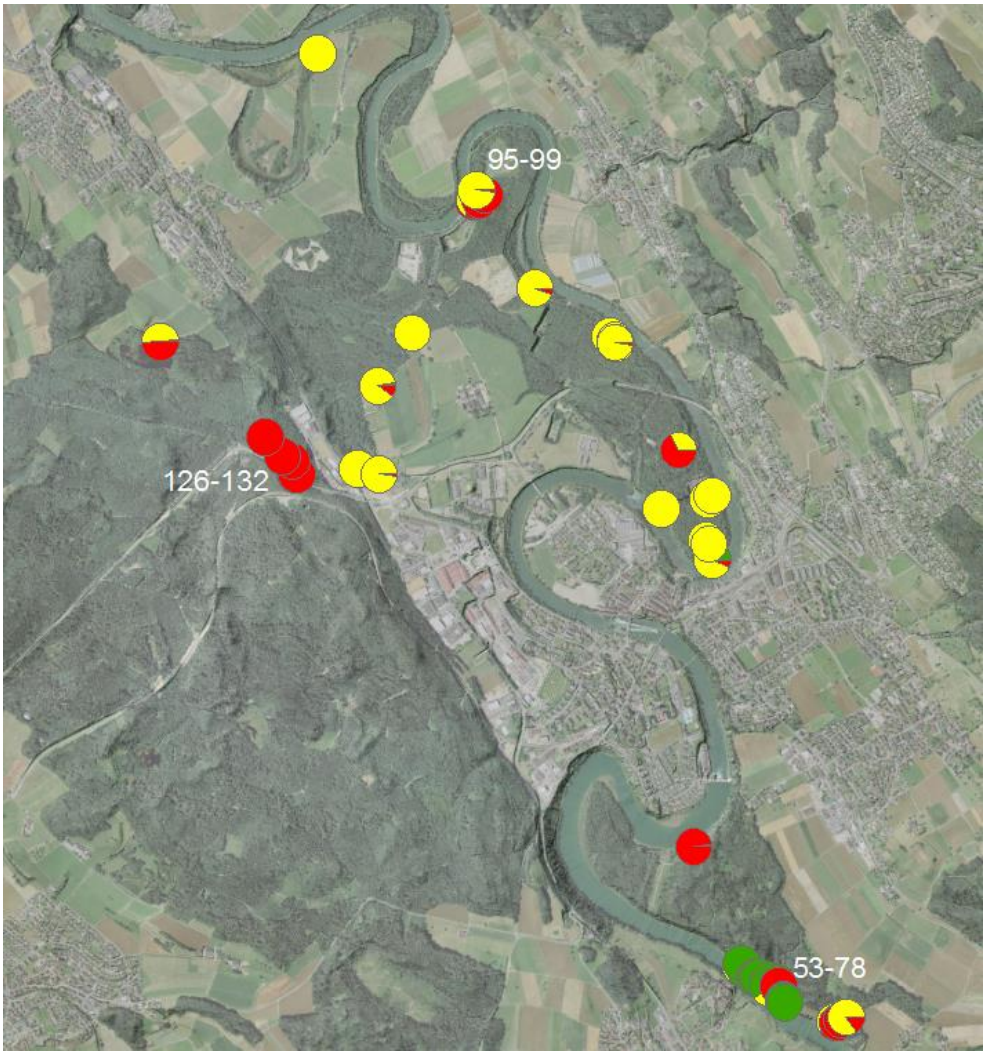

Figure 24 *Scattered colonies of* Bactrospora dryina *at Bremgarten. Each pie chart represents the genetic structure of a colony (tree). Colonies 126-132 are genetically identical and slighltly distinguished from the yellow population. So are 95-99 and 53-78 which show some genotypes found in the 12km distant Lorze population.*

Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Bremgarten Nord	B	C	A	C	A	C	I	C	F	F	C	B	C	A	A	A
Bremgarten Nord	B	A	A	G	B	C	A	B	F	F	G	B	A	B	A	A
Bremgarten Süd	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Bremgarten Süd	B	C	F	A	A	A	A	B	F	F	A	B	A	A	A	B
Zollischlag	B	C	A	C	A	C	A	C	A	H	C	B	C	A	A	A
Lorze	B	A	A	A	B	J	A	B	A	B	A	B	A	B	A	B
Lorze	G	G	G	G	G	G	G	B	A	G	G	B	A	B	A	B

Figure 25 *Diversity pattern of mulitlocus genotypes in* Bactrospora dryina *of five samples taken from three different colonies along Reuss river. Top row indicates Loci 1-16, coloured fields/letters represent same allele length. Samples from Zollischlag, Lorze and Bremgarten show several identical alleles on multiple loci.*

4. Discussion

In this study, habitat requirements of *B. dryina* could be described as highly dependent on light level, which has effects on several important parameters. This habitat is found on old oaks in structure rich forests. Weak connectedness of old growth oak forests in the Swiss lowland might be one of the main reasons for genetic differentiation and isolation of *B. dryina* populations. STRUCTURE analysis on different spatial scales revealed national uniformity and differentiation within regions.

4.1 Vegetation characterisation and niche differentiation

Bactrospora dryina was found in lowland floodplain forests, coppice-with-standards forests and in deciduous mixed forest. Here I showed that different morphological characteristics of oaks are crucial for the development and the survival of *B. dryina*. While the variables tested in a multiple GLM are not independent to each other, there are some conclusions we can draw from this analysis. This model reveals *B. dryina* to be most likely in areas with intermediate light levels. However, the effect of light itself may not be the most important characteristic of this niche, as light is linked with air humidity in the understory (Ellenberg & Leuschner, 2010). A dense canopy, and therefore less intense sun radiation leads to higher moisture in the understory. Oaks in dense forests with high canopy coverage and high moisture therefore do not offer suitable bark characteristics to *B. dryina*.
The development stage of the lichen differed in dependence of canopy coverage. In areas with dense canopy structure (canopy coverage > 80 %) and intense shading other organisms, including algae, moss, and other lichens are outcompeting *B. dryina*. Furthermore, although *B. dryina* is able to survive on stems in shaded area (canopy coverage 60-70%), colonisation to new stems is shown to be most abundant in areas of intermediate light levels (canopy coverage 50%).
Hence, our hypothesis of intermediate light level as a characteristic of B. dryina habitats was confirmed, as the first development stage is shown at on average 50 % canopy coverage. Hence, open forest structure due to disturbances by rivers or selective logging by humans are the key for a suitable light level for *B. dryina*.
The shift of development stage according to canopy coverage can be explained by higher bark damages in moist forests. Spongy ridges of oaks in dense forests tend to be more fragile than ridges in more shaded areas. Bark with broken of ridges do not show deep bark crevices and often remains spongy if humidity levels remain the same. As *B. dryina* prefers to grow on the weather protected side of oaks, spongy bark might be too moist substrate for this species to grow on.
Old oaks are mostly inclined towards the direction of highest light availability as a consequence of light competition (Loehle, 1986). Therefore, forest trees in the northern hemisphere tend to be inclined southwards or towards forest gaps. *Bactrospora dryina* was found more often growing on trees with an inclination towards the south to southeast rather than towards other directions relative to the abundance of inclination directions of sampled oaks. This is not surprising because adaptation to this niche may be simply due to higher abundance of south-leaning oaks when compared to other directions. This finding supports my hypothesis which suggested south leaning oaks to be the suitable stems for *B. dryina* colonies.
Lichen communities adapted to oak forests in late successional stages are now rare as old-growth forests are changed and management for resource extraction and therefore infrequently reach late successional stages (Ranius et al., 2008, Pallto et al., 2011). Corresponding to my hypothesis, the same is found in this study for *B. dryina*. It is showed that *B. dryina* prefers oaks with high stem diameter and deep bark crevices. These parameters are directly linked with tree age (Ranius et al., 2008). Although girth did not remain a significantly correlated variable in the multiple model, taking care of old oaks with a certain stem size can be applied most easily in forest conservation.
In many cases *B. dryina* populations in non-coppice-with-standard forests were found near or at the

edges of forests and was often missing in the centre, indicating a forest management in the centre of the forest which is not suitable for *B. dryina* although old oaks might be present.
To detect potential forests for *B. dryina* with a suitable structure offering the asked light characteristics, *Dendrocopos medius* may be a forerunner as it is found in structure rich oak forests with oaks of smaller sizes than *B. dryina* requires (>30 cm stem diameter; Pasinelli, 2008). In this study coordinates of *D. medius* breeding burrows were used to find *B. dryina* colonies, which was successful in some cases and indicate a potential habitat overlap.

4.2 Genetic structure and differentiation of *Bactrospora dryina* in Switzerland

Genetic differentiation is directly linked with connectedness of subpopulations to a main population and depends on the size of an isolated population determining the time needed for a strict differentiation. Referring to my hypotheses, I could show genetic depletion for small isolated populations and high genetic diversity in large populations. Furthermore, with the dominance of few genotypes in fragmented small sized populations my hypothesis of limited colonisation likelihood for small stand sizes is supported. Genetic isolation with increasing geographic distance along a river system which was hypothesised could be shown for only one area. However, if differentiation mechanism are simply driven by geographic distance remains questionable. Further suggestions how such a genetic structure could also occur are discussed.

4.2.1 National scale

Genetic differentiation of all investigated populations in Switzerland do not correspond to any particular national geographic structure (Figure 9). There was no clear pattern of differentiation along airways or rivers as it has been shown for plant species (Imbert & Lefèvre, 2003; Liu et al., 2006; Werth & Scheidegger, 2014; Van der Meer & Jaquemyn, 2015). Also, no distance dependent genetic differentiation could be found when focusing on the river systems of Reuss and Aare across Switzerland (figure 10). A clear differentiation of *B. dryina* along a river system might not be seen because time since geographical isolation is too short to see structural patterns on this scale. The genetic structure of *B. dryina* on a national scale shows an ancient panmictic population of which differentiation mechanisms or dispersal ways remain unknown. It might therefore be interesting to compare the Swiss population to other large scaled populations in Europe to find differentiation processes on even larger scales.

4.2.2 Regional Scale

At local scale there are clear patterns of differentiation and isolation by distance, as well as local gene flow. Furthermore, critically endangered and genetically impoverished populations can be identified. Hence, the microsatellite markers developed by Nadyeina et al. (2017) show mutation frequencies which are useful to detect genetic differentiation on local scale, indicating recent mutations.

Basel

Populations in Basel are highly diverse, despite subpopulation size in some cases being very small (i.e. Muttenz). Gene flow was detected, which indicates that these populations used to be part of a large panmictic population, or still are. Genetic variability within these subpopulations (Allschwil, Birsfelden, Muttenz and Magden) is high (Table 7). In some of these diverse populations only two or three trees were coloniesed by *B. dryina*, therefore these subpopulations are at risk of being lost if they find no suitable trees for future colonies.

The population at Muttenz is critically endangered. *Bactrospora dryina* colonies were only found on two very old and already partly dead standing oaks. In the surrounding stand, suitable trees are rare or growing in rather dark and shady places. As newly settled colonies of other Swiss populations were mainly found on trees with an intermediate light level with an open canopy structure, trees for future colonisations are lacking in immediate distance at Muttenz. However, within a distance of approximately 1 km the forest is mainly composed by oaks in intermediate density. At this site, no *B. dryina* could be found. In this case, consideration about transplantations from one site to another might be done to help this population to spread and to sustain. In between the potential and the

actual site, old-growth beech forest is separating oak dominated sites which may hinder the dispersal partially.
Population at Magden and Allschwil are estimated to consist of over 50 colonies. These forests are managed as coppice-with-standards forests. Hence, suitable light conditions are sustainably secured. As there are several uncolonised trees with smaller sizes, habitat for future *B. dryina* colonies is available. These sites are of high importance for genetic source of Bactrospora, as they are genetically very diverse and abundant.
"Hard" forest at Birsfelden has high structural diversity that includes many old oaks. Although this ~240 ha was screened very carefully, *B. dryina* colonies were only found on eight trees standing close together. Fine differentiation, as seen in figure 12b, indicate four different colonisation events of closely related source colonies or clonal dispersal over short distances and dispersal over larger distances by sexually produced diaspores. Within each cluster at Birsfelden high similarity and identical haplotypes are found, indicating successful dispersal of clonal propagules. In the area of Basel, clonal dispersal along trees within a distance of 10-150 meters are also found in Magden and Allschwil.
Structural diversity is high at Hard forest and oaks are of high abundance. Therefore, colonisation of new stems might be detected in future. If not, transplantation for ensuring an enlarging population at this site should be considered.

Galmiz
The population at Galmiz is one panmictic population with about 30 colonies within an area of ~9 ha. It is geographically isolated from other Swiss populations and shares its lowest Fst with the population Marthalen Niederholz, which is placed in an Euclidean distance of ~135 km. Therefore, it might be a relictual ancient genetic population, as no migration from closer located population is detected. Between each subpopulation there is evidence for frequent migration. Identical haplotypes on four different trees indicate clonal dispersal, which is in the case of Galmiz found for colonies located in a distances of 230-350 meters.
As there are young oaks within the stands, population at Galmiz does not seem to be critically endangered. Nevertheless, connectivity to other populations is completely lost which increases the inbreeding rate and decreases adaptability to changing environmental conditions (Frankham, 2004).

Messen
A similar situation is found at Messen, where a gene diversity of 0.37 is found, indicating a solid basement for a stable population. Nevertheless, population at Messen is critically endangered as there were only 11 colonies found and not many more expected, as vegetation structure and species composition of the forests nearby does not offer any further suitable habitat.

Intensive observation in other floodplain areas and oak rich forests in the Bernese "Seeland" and along Aare river did not lead to further *B. dryina* populations. Although, there are large-scale floodplain reserves which might provide suitable habitat. As population Galmiz and Messen are the only population in the western area of Switzerland, they might have stored the genepool of an ancient Aare population which would provide a source for establishment of a new population i.e. in the hardwood floodplain forest along the "alte Aare" at Lyss (47°04'11.640"N, 7°17'23.507"E), which is a floodplain area of national importance.

Population Aargau
Genetic structure of *B. dryina* along the river systems of Reuss, Lorze and Aabach in the canton of Aargau shows partially isolation by distance. Subpopulations in the area of Bremgarten and the southern Zollischlag may build the dominating genepool with frequent gene flow between subpopu-

lations. There is also migration indicated from the Zollischlag population to the Lorze population, which are in a distance of 4 km. At Lorze however, other genotypes are dominant which can also be found in Bremgarten. Subpopulation at Bremgarten are closely related to Traverse individuals. Some genotypes of Traverse populations are similar to the genetically impoverished population at Seengen. As a consequence, there is significant isolation by distance for populations Lorze to Seengen via Bremgarten and Traverse (figure 19a). The population at Zollischlag makes the correlation between genetic and geographic distance insignificant because they are in close distance to Lorze population, however, they are closely related to other populations along Reuss. While subpopulations along the Reuss river share very low Fst values to each other, subpopulation at Seengen (Aabach) and Lorze are genetically distinct. There are several interpretations of the structure along these three rivers.

A possible interpretation is based on the branching structure of river systems, of which each has an isolated subpopulations. There are three rivers for which each *B. dryina* population is significantly different from each other. The isolation by distance pattern which could be shown for these three rivers might be caused by an island like isolation by distance pattern (Wright, 1943). It is important to note is the linkage between populations along Reuss and the population at Seengen, which is not connected to a river system. Traverse subpopulations of small scattered stands or even single trees build the linkage between these two populations. As there was no *B. dryina* colony found in the naturally hardwood floodplain forest connection at the river crossing with aare further in the north, three explanations for this Traverse population structure are likely.

1) Traverse population is the dispersal path between the Aabach population and the populations at Reuss. Because Traverse populations are very small isolated populations, mantel test becomes significant due to a bottleneck effect in the migration towards Seengen. This bottleneck effect bases in the dispersal strategy of diaspores of *B. dryina*. While spores can be dispersed over several thousand meters (Ronnås et al., 2017), the number of spores and therefore the probability of a new colonisation decreases with increasing distance (Stoneburner et al., 1992). Hence, populations at Traverse, which occur in a distance of about 2000 meter to each other, are within a possible dispersal range, with the limitation that only few spores and therefore low number of different genes are carried from one stand to a new colony.
   This bottleneck effect of Traverse therefore explains the low gene diversity (0.14) at the end of the chain located in Seengen. (Figure 27, yellow)
2) Genetic isolation of population at Seengen is caused by a large distance to Reuss along the river system Aabach and Aare of about 40 km. Because no *B. dryina* colonies were found at the crossings of these rivers, Seengen population was cut off from an ancient population at Aare (Figure 27, orange), which might have been removed in times when landscapes were drained for agricultural use and rivers were channelized for flood protection. The Traverse population therefore might be colonised from both sides and build a new connection pathway between Aabach and Reuss instead of the ancient dispersal path via Aare.
3) Because population at Seengen shares lower Fst to Zollihschlag than to the closer population at Bremgarten (by river and by Euclidian way) a third hypothesis would suggest the origin of population at Seengen as beeing directly colonised from south by a population of Reuss valley. (Figure 27, red)

Mainly the low gene diversity of Seengen population makes it most reasonable to believe in explanation 1 where dispersal of B. dryina along scattered Traverse-subpopulation acted as an exemplary genetic bottleneck. However, explanations 2 and 3 cannot be excluded. If in the suggested alterna-

tive connection areas between populations Seengen and populations at Reuss *B. dryina* is found, genetic analysis needs to be done and could reveal the colonisation question of this isolated populations at Seengen.

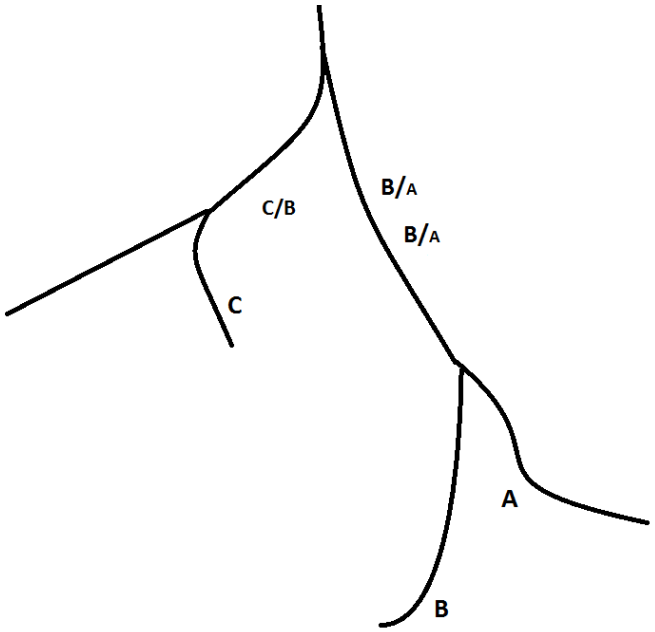

Figure 26 *Schematic figure of genetic structure of* Bactrospora dryina *populations along Aabach, Reuss and Lorze. Letters represent genetic structure of each population. Small letters indicate minor spreadings.*

Interpretation of genetic differentiation is much more difficult for populations Zollischlag and Lorze. Lorze population shares lower Fst with population near Bremgarten than with Zollischlag. There are some individual colonies in the Bremgarten population carrying multiple genotypes from Lorze, indicating a former gene flow. Zollischlag however, has even lower genetic distance to Bremgarten.

If a dispersal from north to south is expected, genetic differentiation could be explained by bottleneck effects acting in the dispersal to side valleys. Population Lorze and Zollischlag however, are situated within the same forest with a distance of 4 km between each other. Because gene flow is observed from Zollischlag to Lorze (not vice versa), an additional colonisation event from elsewhere might be the reason for the strong genetic difference of Lorze. Hence, separation from Lorze population out of the uniform Reuss population seems to be unlikely.
Hence, population along Reuss, which only shows small differences within subpopulations, is one large population limited to this valley.
Low gene diversity at Lorze population indicates that colonisation event might have been act as a founder effect at this site. Populations at Bremgarten and Zollischlag show more diversity. A dispersal pathway from south to north would answer the question of a missing Lorze genotype at Zollihschlag and a similarity to some Bremgarten subpopulations and could be explained with a dispersal by water, either sporal in times of a flooding event or by the downstream transport of whole stems after a storm. The origin of Lorze population remains questionable and is assumed to be external.

Although significant mantel tests supports the idea of Wright's isolation by distance differentiation pattern population differentiation along these three rivers might be driven by other evolutionary processes. Wright's IBD suggests differentiation because of higher meeting probability of closely situated subpopulations causing genetic drift with distance, whereas in this case genetic differences of Seengen population is rather explained by a bottleneck effect. The gene-flow of the genetically rather homogenous Lorze population to subpopulations at Bremgarten would match the idea of IBD. However, all populations along Reuss are of similar genetic composition if the influence of Lorze genotypes at Bremgarten is ignored. Hence, genetic differentiation including Lorze and the whole Reuss

populations do not belong to an isolation by distance structure, because Lorze populatin does not show any gene flow to the population at Zollischlag.

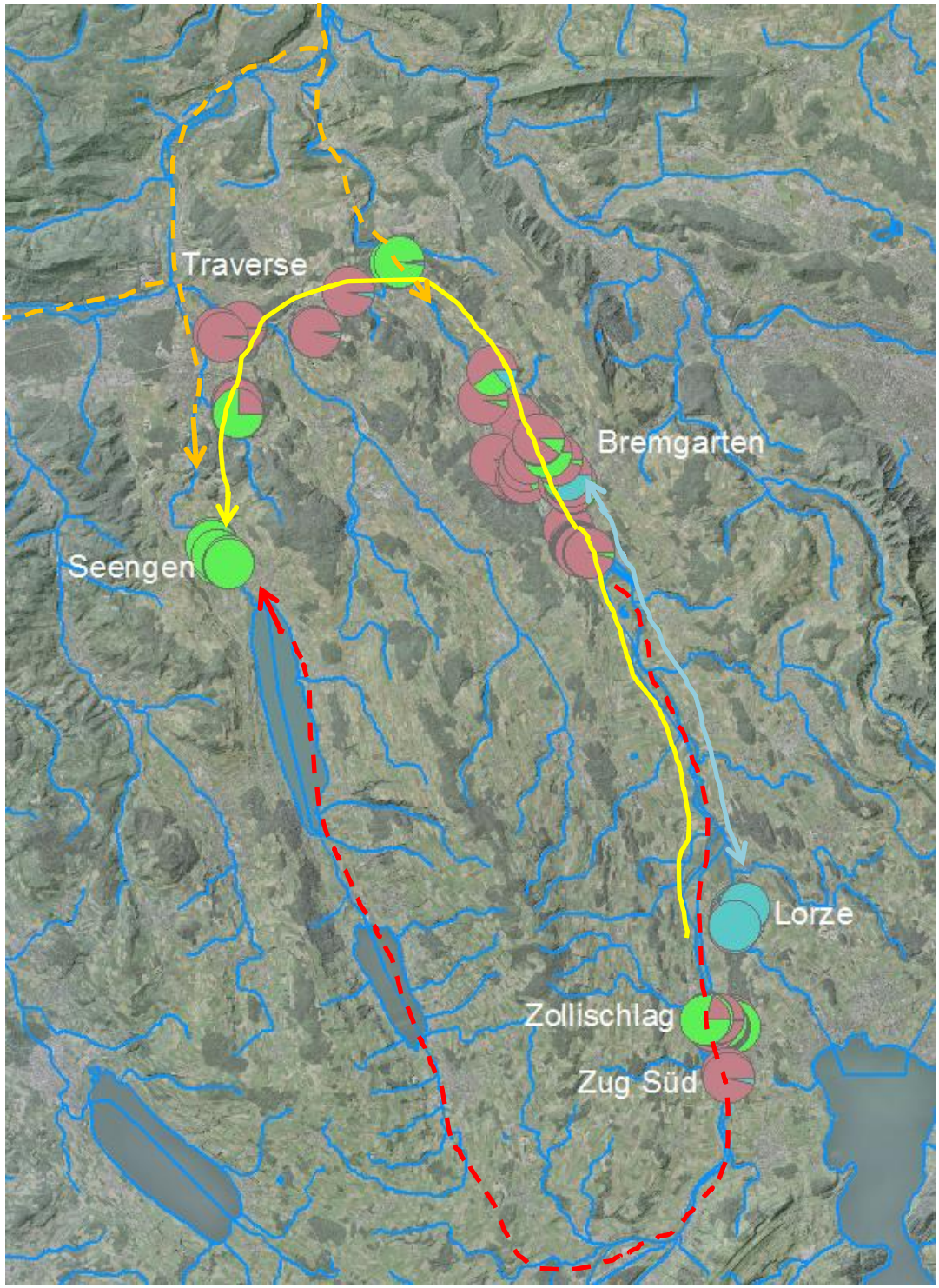

Figure 27 Bactrospora dryina *colonies along Aabach, Reuss and Lorze. Each pie chart represents the genetic structure of a colony (tree). Arrows indicate potential paths of ancient gene flow suggested by genetic structure.*

4.3 Dispersal limitation vs. colonisation limitation

Diaspores of lichenised fungi can be spread over several kilometers (Ronnås et al., 2017). With increasing distance however, the probability of founding a new colony declines. This is due to a dicrease in spore density with increasing distance (Stoneburner et al., 1992). An additional reason is the change in environment across the landscapes, particularly with fragmented habitats. In case of *B. dryina*, spores only found new colonies if they are transported to their specialised niche on old oaks in a structure-rich forest. Hence, habitat loss and fragmentation might be the fundamental reason for unsuccessful dispersal of spores as the chance of being carried to a suitable stem decreases drastically. On the other hand, recruitment with diaspores is a strategy allowing a species to colonize new habitats in very far distances. In the case of Reuss, where forests offer numerous potential oaks to be colonised, dispersal and colonization was successful several times from several parental populations. This leads to high gene diversity within each site. The opposite is found in the dispersal towards population at Seengen. Small numbers of potential trees are scattered to several small scaled forest stands. Hence, the chance of being colonised several times with spores of genetically different parents is very small. Colonies along Traverse show low gene diversity as haplotypes for several colonies are identical (i.e. Mellingen).

Based on the assumption of a bottleneck along the Traverse population and the linkage between Reuss and Aabach populations, *B. dryina* might not be limited in dispersal distance via spore dispersal. The limiting factor for *B. dryina* might be the lack of large suitably structured forests with numerous oaks of old ages. This would increase the chance of various colonisation events from origins of different genetic compositions. As a consequence, gene diversity is larger and as recombination is possible in non-homogenous populations, new haplotypes are generated. This is seen in population at Bremgarten where intermediate genotypes of Bremgarten and Lorze are found.

4.4 Microsatellite limitations

Although microsatellite markers allow research on a very fine scale, every method has its limitations (Selkoe & Toonen, 2006).

As well as in other genetic analyses, characters that are the same are assumed to be homologous, even though they may not actually be. For microsatellites in particular, a duplication and a later deletions of one repeated DNA motif can cause the same allele length as a non-mutated allele shows.

A further limitation is shown in detecting differentiation within subpopulations on smallest scales (comparison colonies of trees within one stand). Analysis of genetic structure of *B. dryina* colonies on groups of closely standing trees might be improved by: 1) Increasing sample size per colony 2) developing microsatellite markers at loci with an even higher mutation rate or conduct genome wide analysis, which has much higher resolution than microsatellites.

Microsatellite analysis might further reach limitations as clonal dispersal might be an important reproduction strategy within closely situated colonies, causing very high similarity within a group of trees. Depending on the choice of loci and its mutation rate, colonies might be identified as clones or as sexually reproduced individuals with mutations on a very fine scale (not detectable with the chosen loci).

5. Conclusion and conservation outlook

With ongoing processes of revitalisation and renaturalization of Swiss alpine and midland rivers, terrestrial and aquatic structural diversity of river systems is increasing again. Mainly gravel bank populations and softwood floodplain forest rise shortly after structural upgradings and openings of riverbeds. Hardwood floodplain forests and its threatened species richness however, cannot benefit from small scale revitalisations (Hughes et al., 2003). Sometimes, openings of channelized rivers are done at the expense of remaining hardwood floodplain forests nearby a channelized river.
Scheidegger et al. (2012) suggests a minimal area of 10 ha, which is needed for an entirely functioning hardwood floodplain forest.
Large forests with high genetic diversity are very important genetic reserves for *B. dryina*. The development of such a forest needs at least 40 years and requires a long-term strategy for the development of new sites (Scheidegger et al., 2012). Sporal dispersal over kilometers is shown for other lichen species (Ronnås et al., 2017). Recent gene flow between distant sites could also be shown for *B. dryina* in this study. However, to support and enlarge a threatened metapopulation, large scaled hardwood floodplain or managed forests are needed to found new colonies with larger and less vulnerable effective population sizes. In addition, connectivity along riverine floodplain forests needs to be increased to enable gene flow between sites.
Open structure, offering suitable light conditions, such as those found on forest edges, as occurring in naturally dynamic hardwood-floodplain-forests and as reached in coppice-with-standards forests by management, needs to be promoted in former hardwood floodplain forests which show tendency to be overgrown when detached from the dynamic of a river system. Conserving remnant intact sites is the foundation for the promotion of hardwood-floodplain forests in future as they contain populations of specialist species of a local gene pool.

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Appendix

Study Area

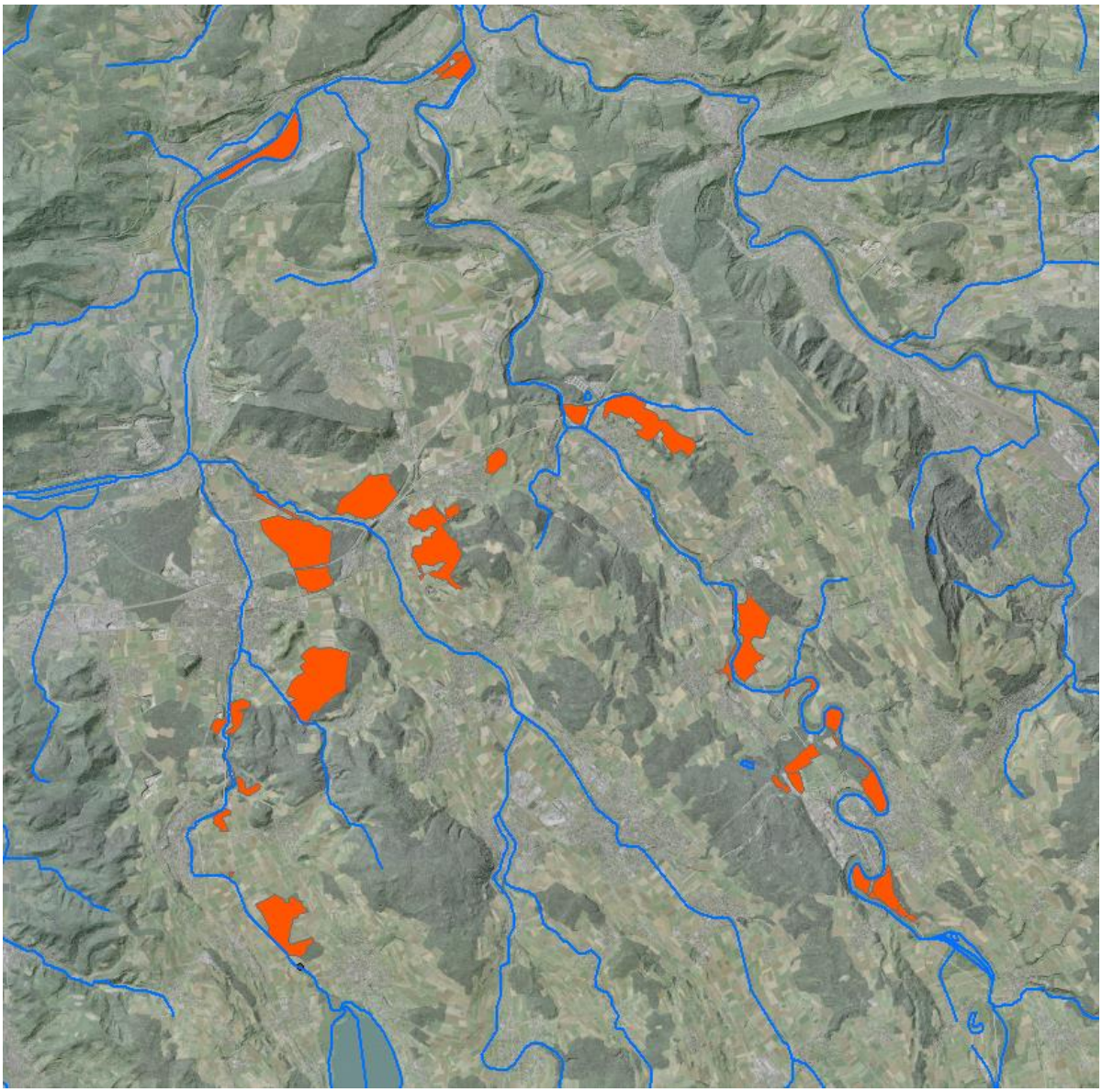


Figure 1 *Study area along Aabach, Bünz, Reuss and Aare in the canton of Aargau. Red areas were screened for* Bactrospora dryina*.*

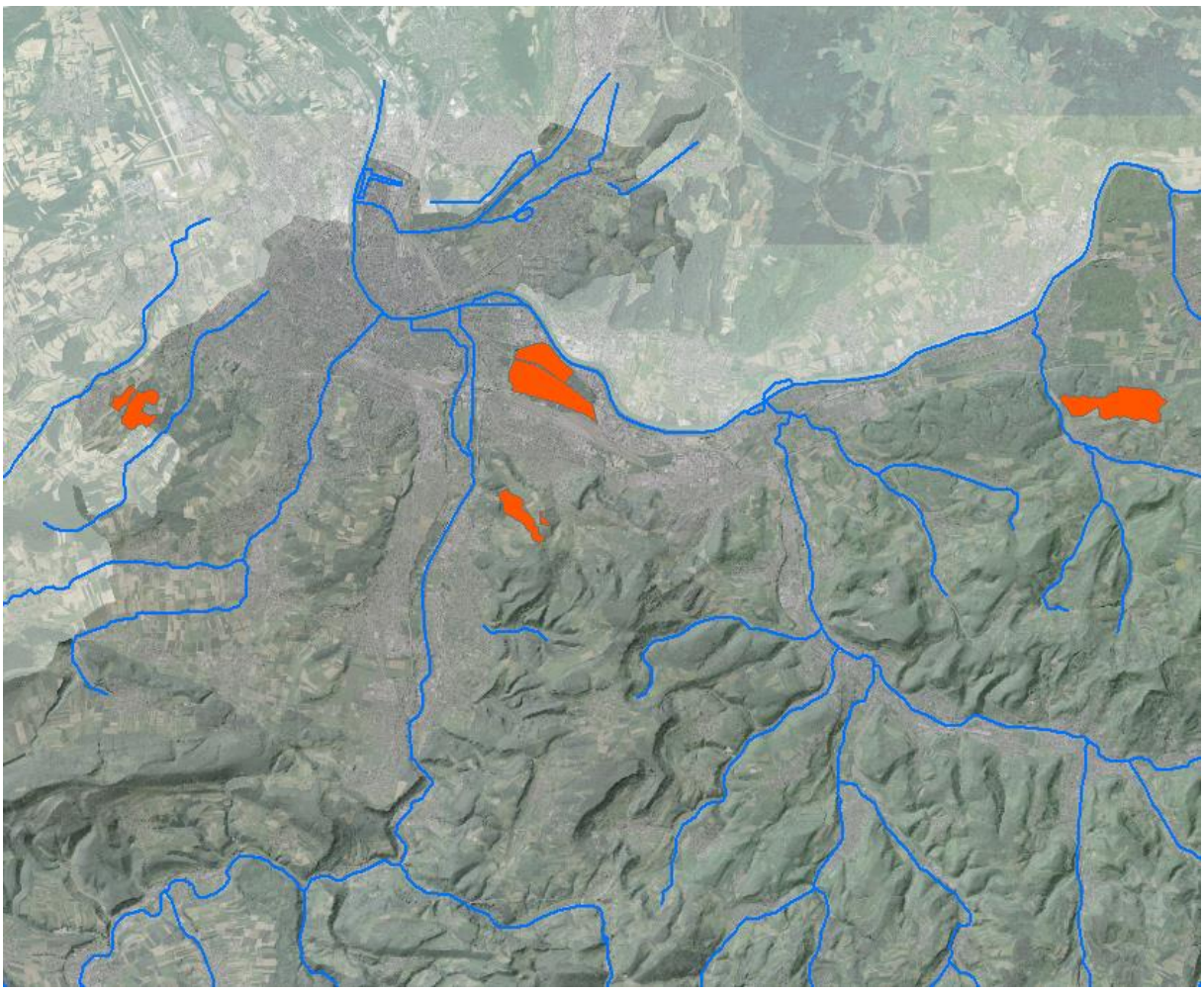


Figure 2 *Study are canton at Basel. Red areas were screened for* Bactrospora dryina.

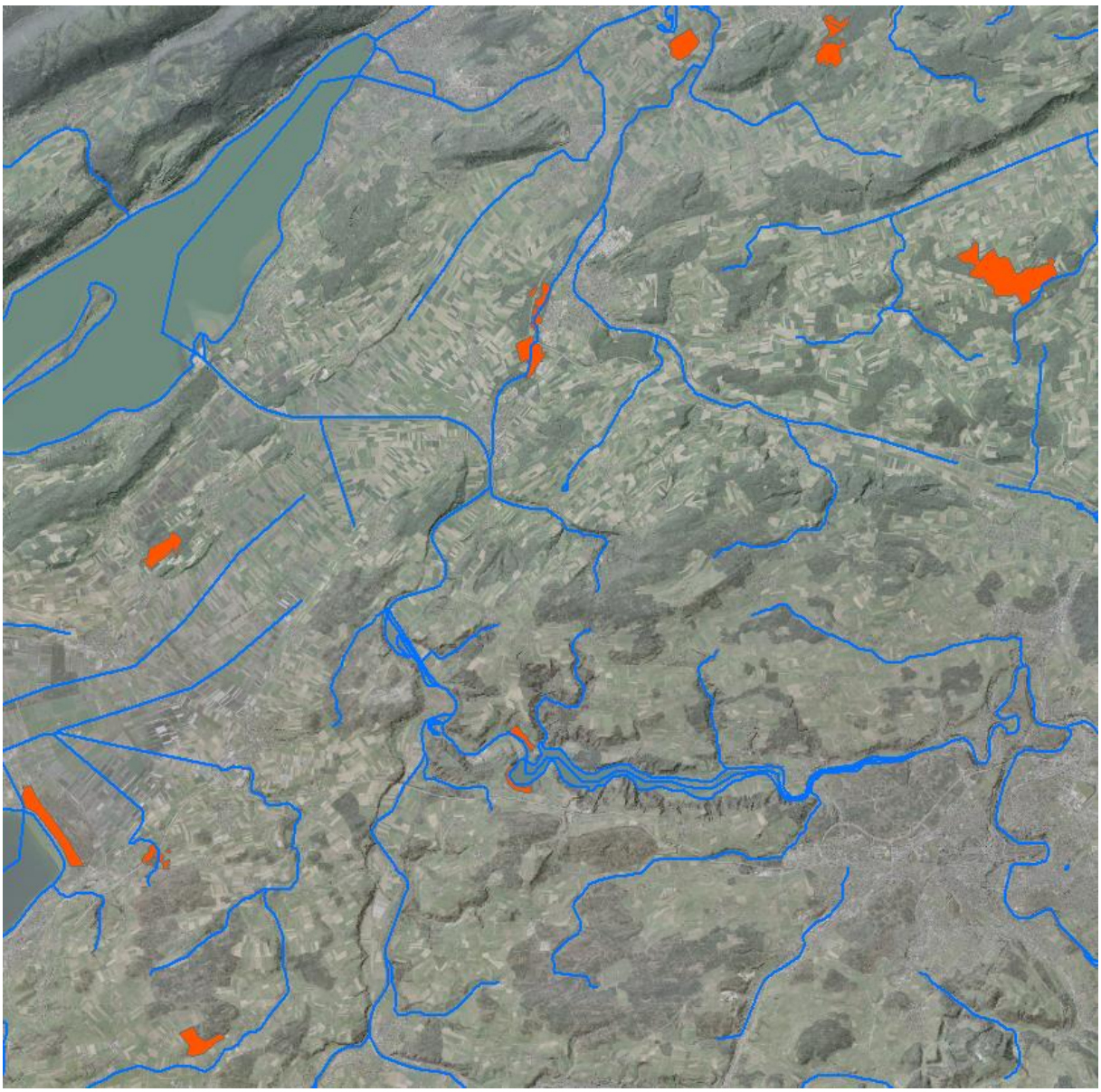


Figure 3 *Study area of canton Bern, Freiburg and Solothurn. Red areas were screened for* Bactrospora dryina.

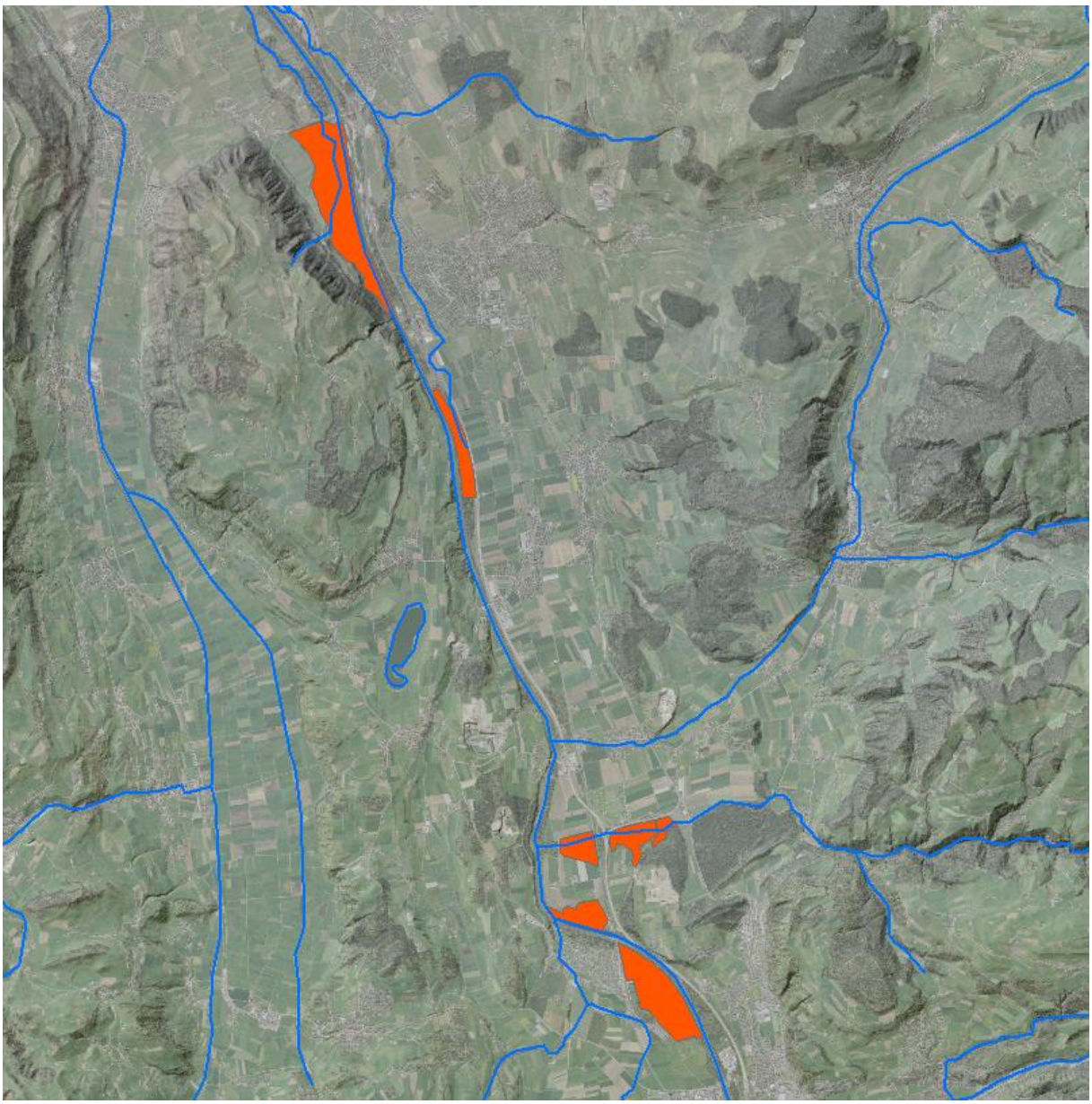
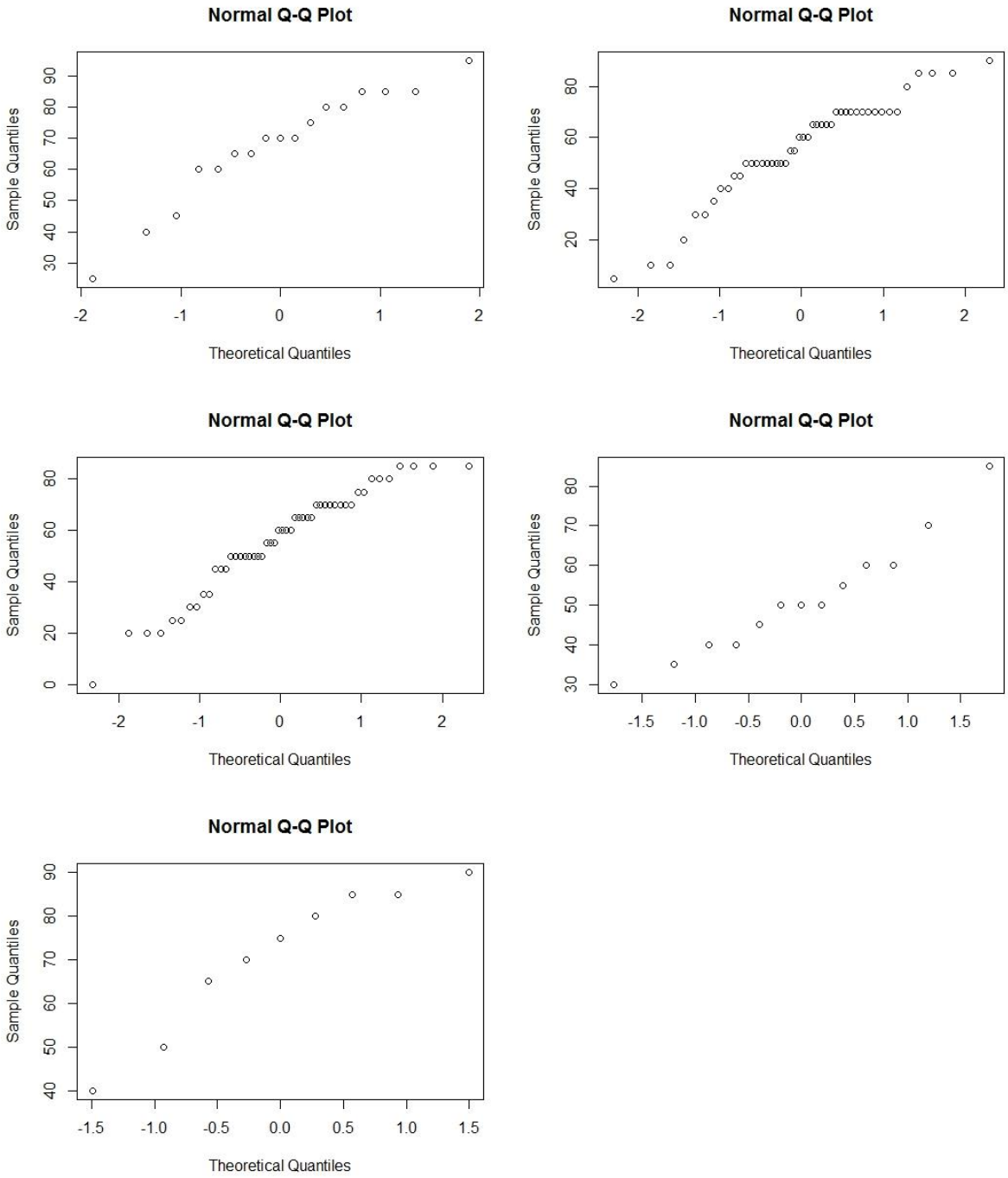


Figure 4 *Observed area at Aare valley between Bern and Thun. Surveyed by Giotto Roberti in 2017. Red areas were screened for* Bactrospora dryina*. However, the lichen could not be found in this area.*



Figures 5-9 *QQ plots of canopy coverages of five development statuses of* Bactrospora dryina *colonies showing normal distribution.*

Table 1 *Significant differences in mean canopy coverages between early and late development statuses of* Bactrospora dryina*. Data was normaly distributed as seen in figures 5-9 in appendix.*

Welch two sample t-test		
Canopy Coverage for colonies with:		**P - value**
Status 1	**Status 2**	**0.3928**
Status 1	**Status 3**	**0.4013**
Status 1	**Status 4**	**0.01118 ***
Status 1	**Status 5**	**0.01295 ***
Status 2	**Status 3**	**0.9941**
Status 2	**Status 4**	**0.02833 ***
Status 2	**Status 5**	**0.03226 ***
Status 3	**Status 4**	**0.02934 ***
Status 3	**Status 5**	**0.03256 ***
Status 4	**Status 5**	**0.6629**

Genetic structure and differentiation

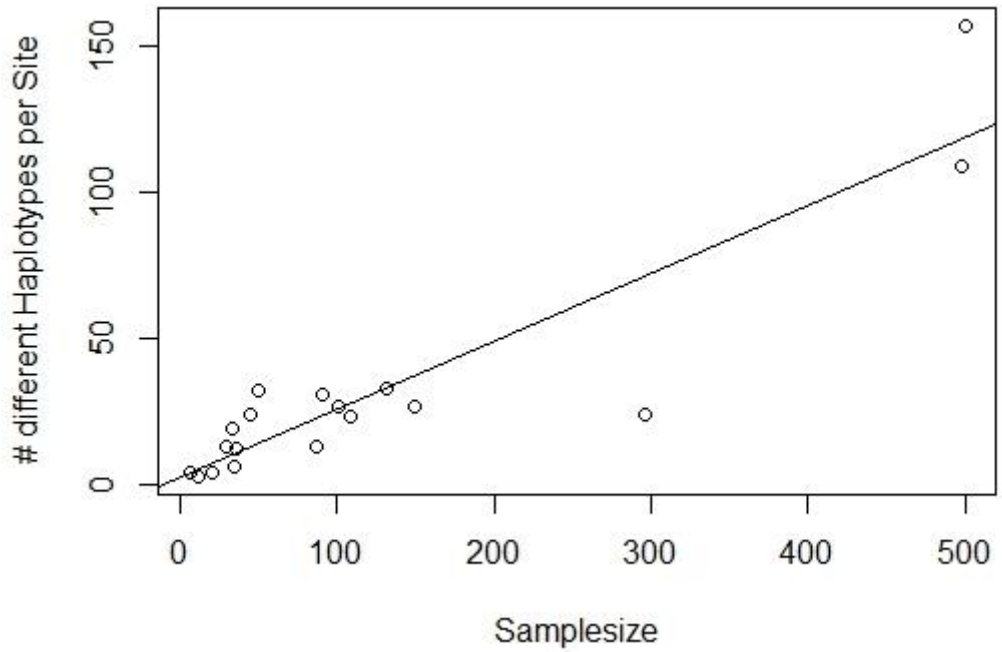


Figure 10 *Sample size is positively correlated with the amount of different haplotypes (Adj. R-squared 0.8229, p = 2.07e^{-7}).*

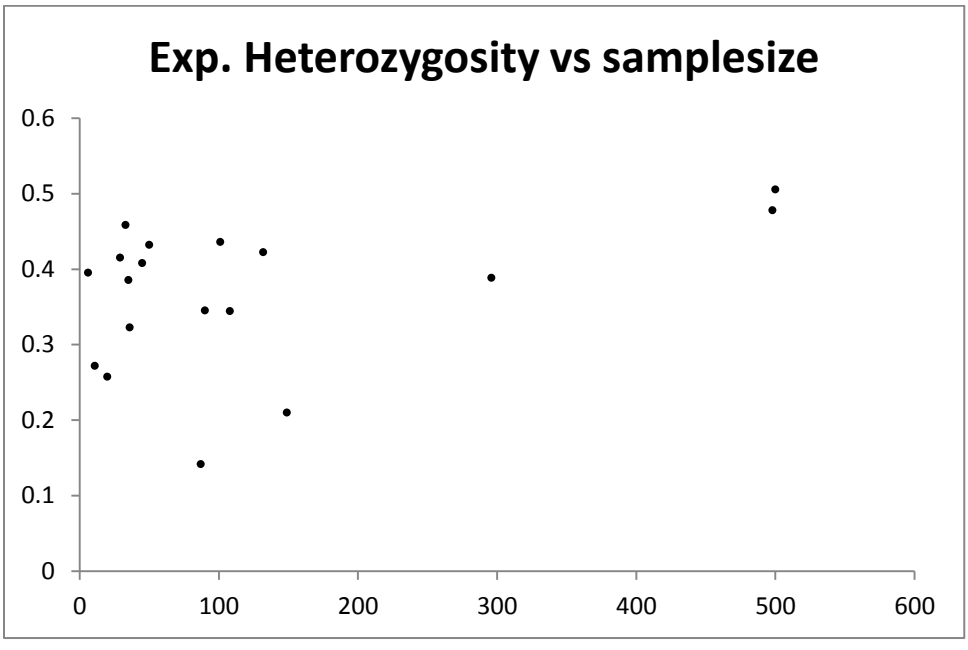


Figure 11 *Expected heterozygosity (y) does not correlate with samplesize (x).*

National Scale

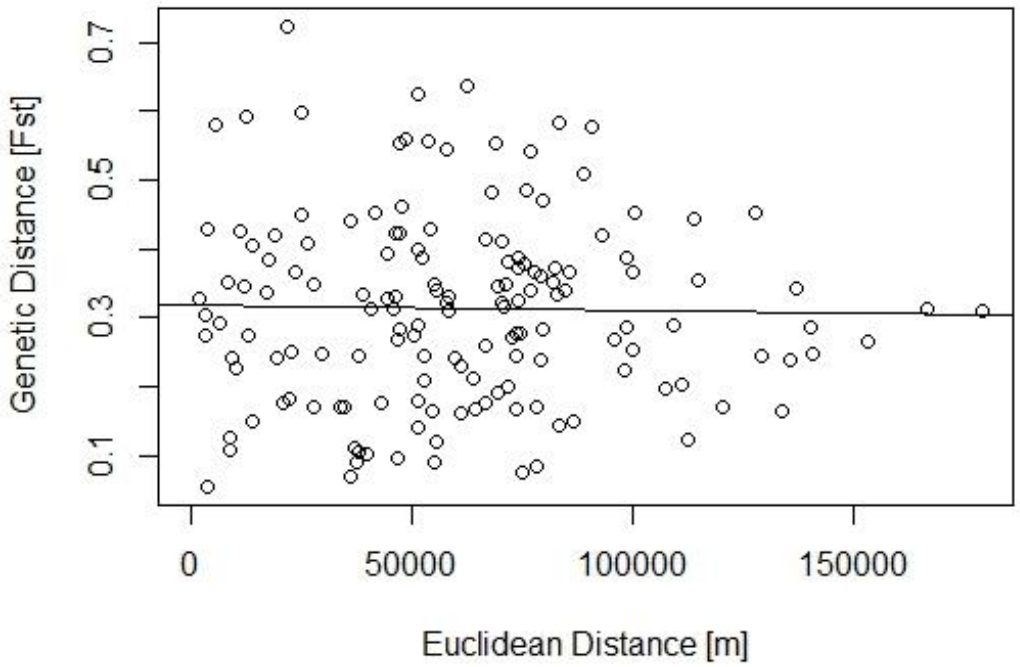


Figure 12 *Populations on a national scale do not correspond to an isolation by distance pattern if Euclidean Distance is used (r = -0.01927, p = 0.542).*

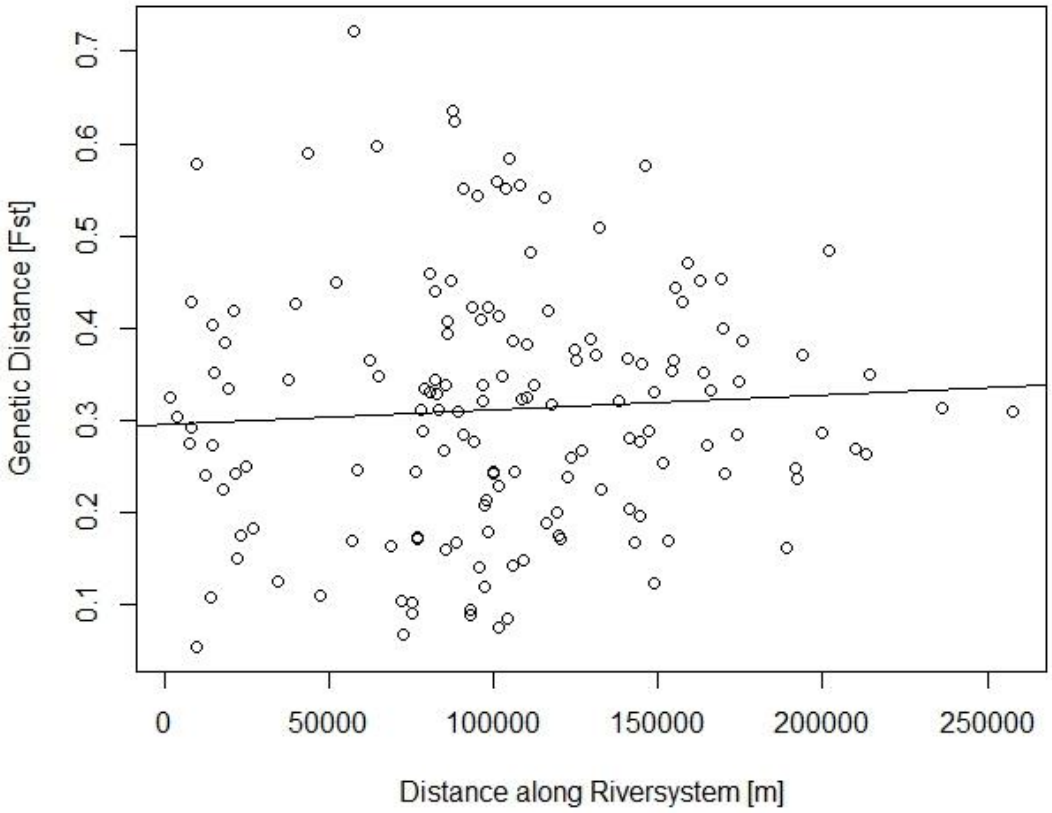


Figure 13 *Populations on a national scale do not correspond to an isolation by distance pattern if distance is measured along river systems (r = 0.4795, p = 0.208).*

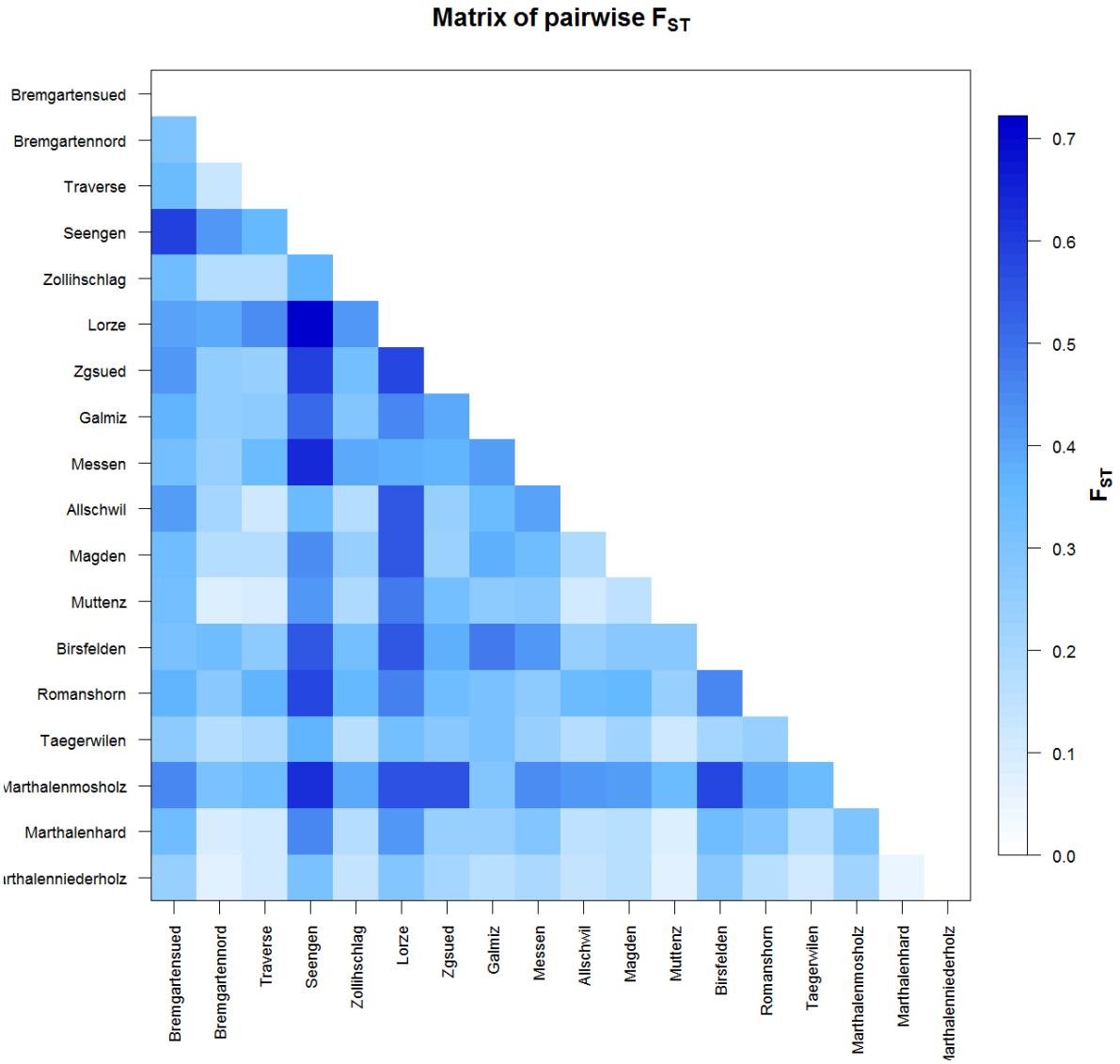


Figure 14 *Pairwise Fst matrix of all observed 18* Bactrospora dryina *population in Switzerland, which were analysed in this study. Bright blue colour represent high genetic similarity or recent gene flow, dark blue colour stands for low genetic similarity and few or no gene flow.*

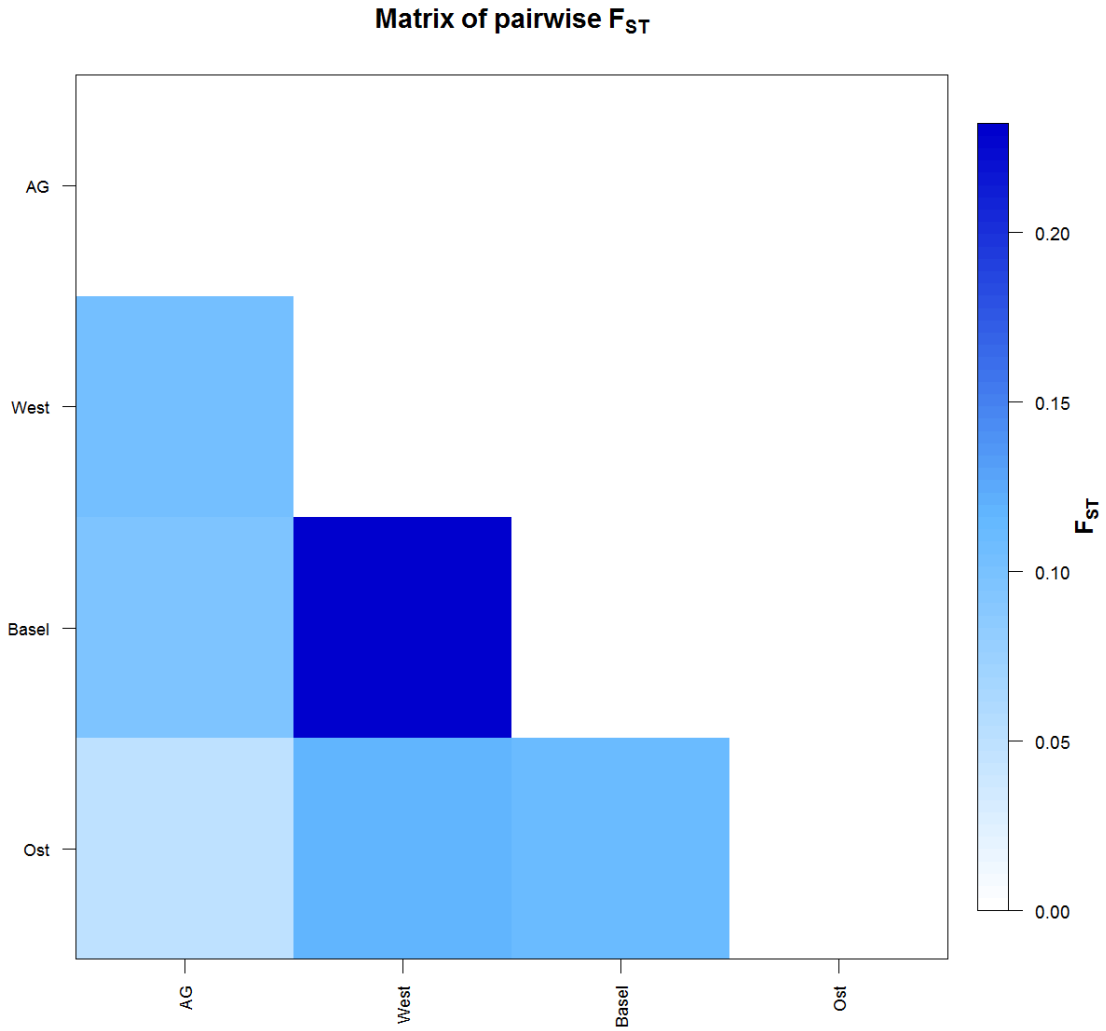


Figure 15 *Pairwise Fst matrix of all observed 18* Bactrospora dryina *population in Switzerland which are summarized into four groups. Ost populations represents Marthalen, Tägerwilen and Romanshorn, West represents Galmiz and Messen. Bright blue colour represent high genetic similarity or recent gene flow, dark blue colour stands for low genetic similarity and few or no gene flow.*

Regional scale

Basel

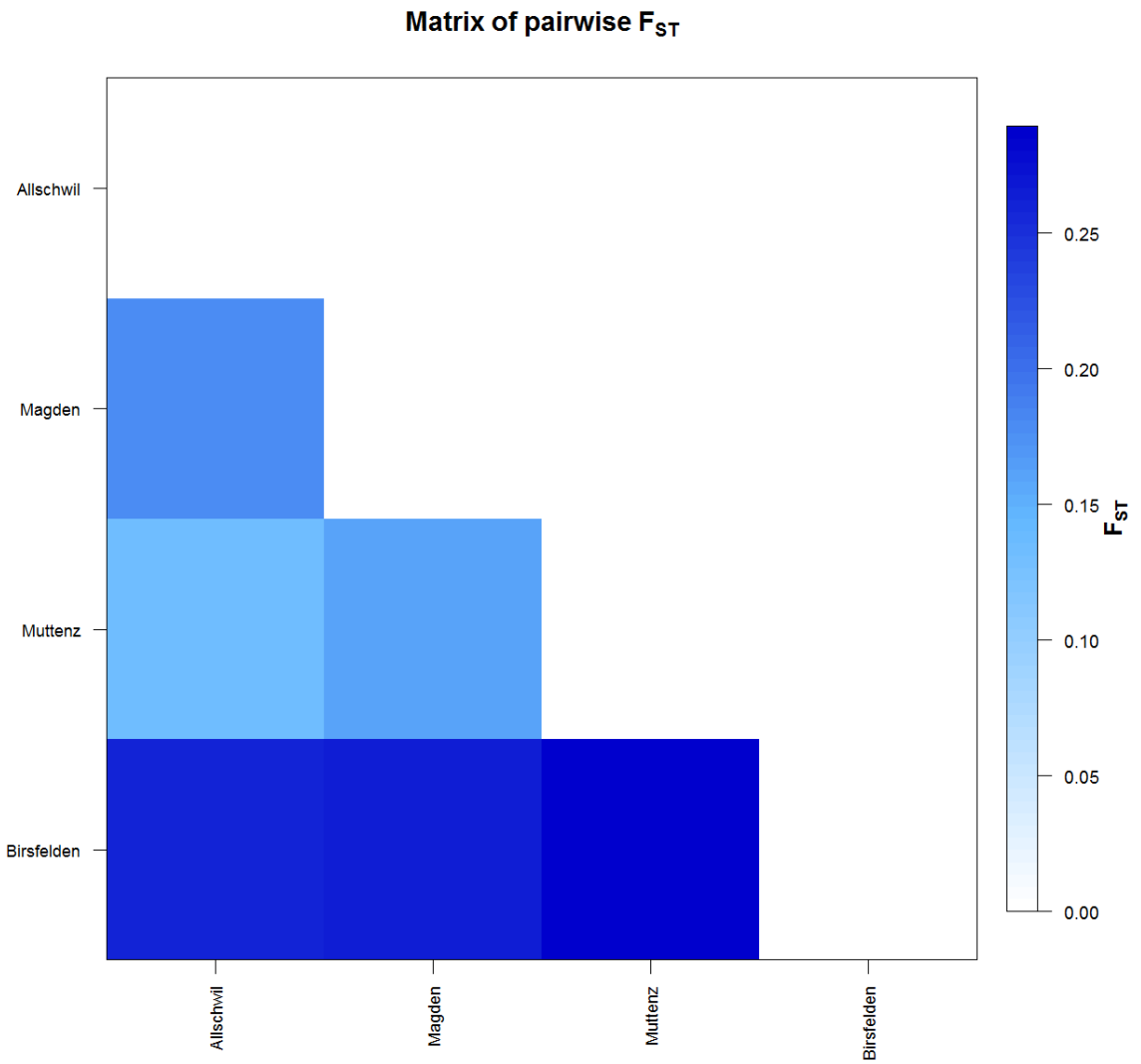


Figure 16 *pairwise Fst matrix of four subpopulation at the area of Basel. Bright blue colour represent high genetic similarity or recent gene flow, dark blue colour stands for low genetic similarity and few gene flow.*

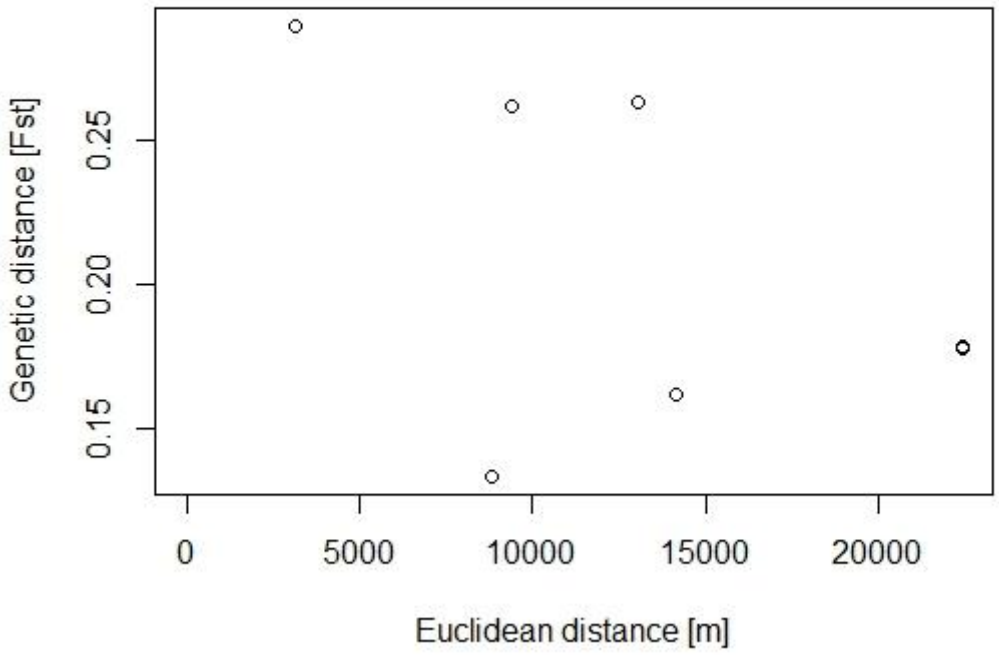


Figure 17 *Subpopulation at Basel do not correspond to an isolation by distance pattern if Euclidean distance is used (r = -0.4653, p = 0.70833).*

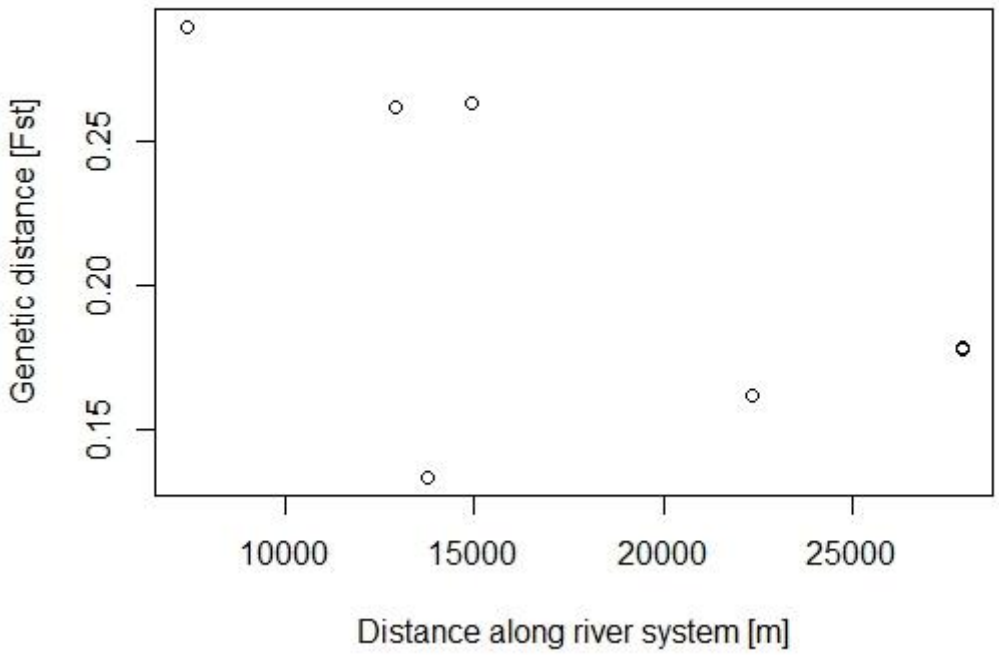


Figure 18 *Subpopulation at Basel do not correspond to an isolation by distance pattern if geographic distance is measured along river system (r = -0.6032, p = 0.83333).*

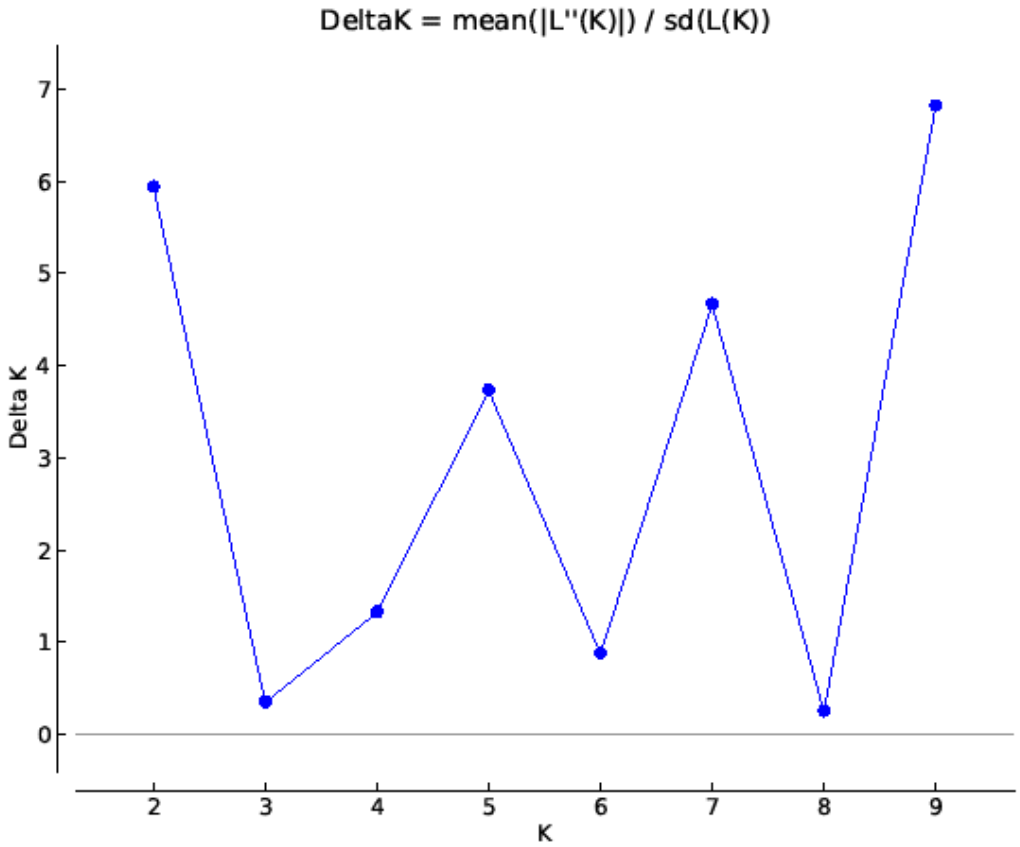


Figure 19 *Delta K diagram for population at Basel according to Evanno's Method to determine cluster number. Highest delta K value would indicate the best expected cluster number. In the case of Basel, delta K was very high for several cluster numbers (K).*

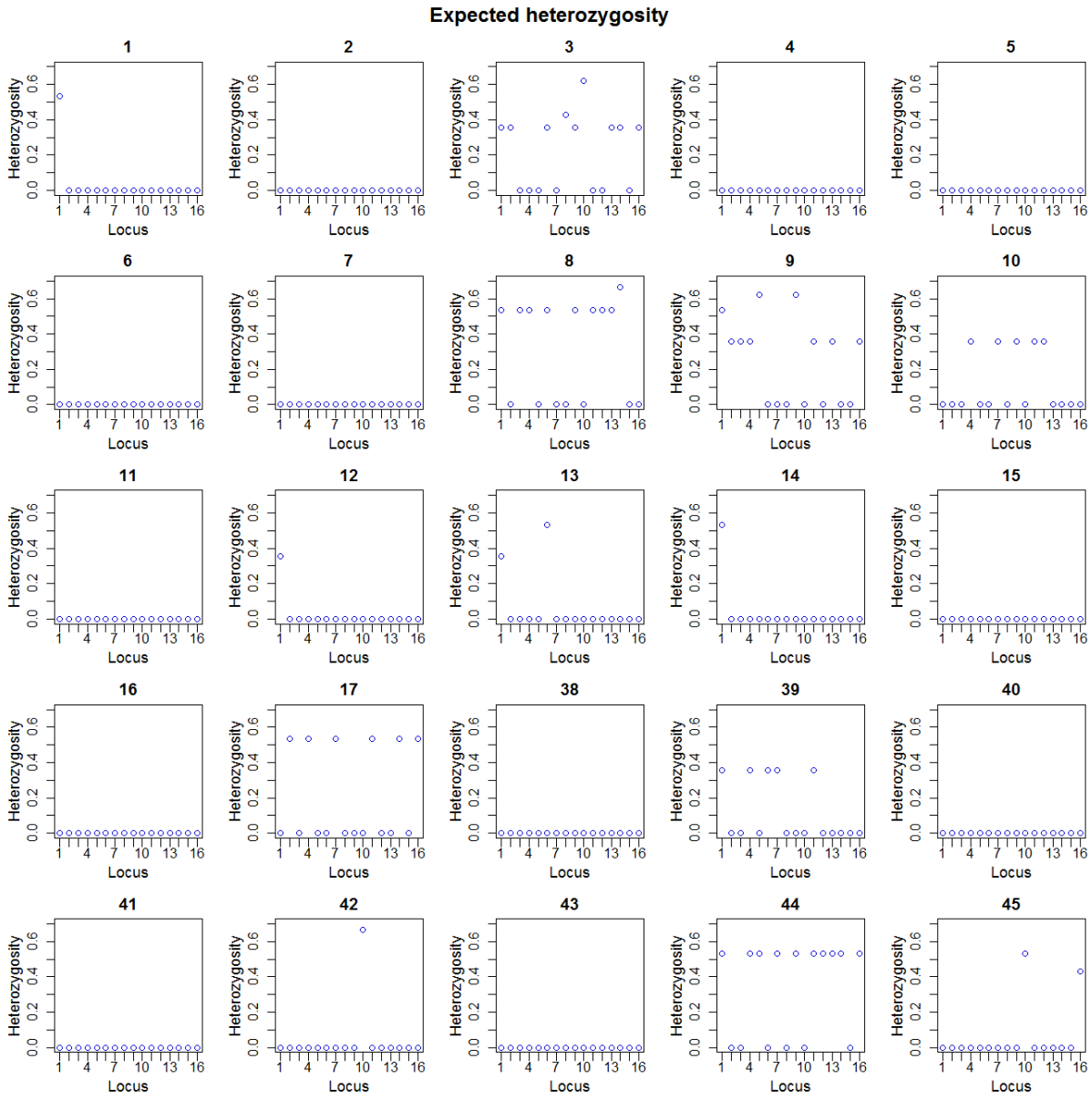


Figure 20 *Expected heterozygosity of each colony (tree) and each locus at the area of Basel. 15 of 25 colonies are homozygous or have only a polymorphism at one locus. 1-7 Allschwil, 8-14 Magden, 15-17 Muttenz, 38-45 Birsfelden.*

Aargau

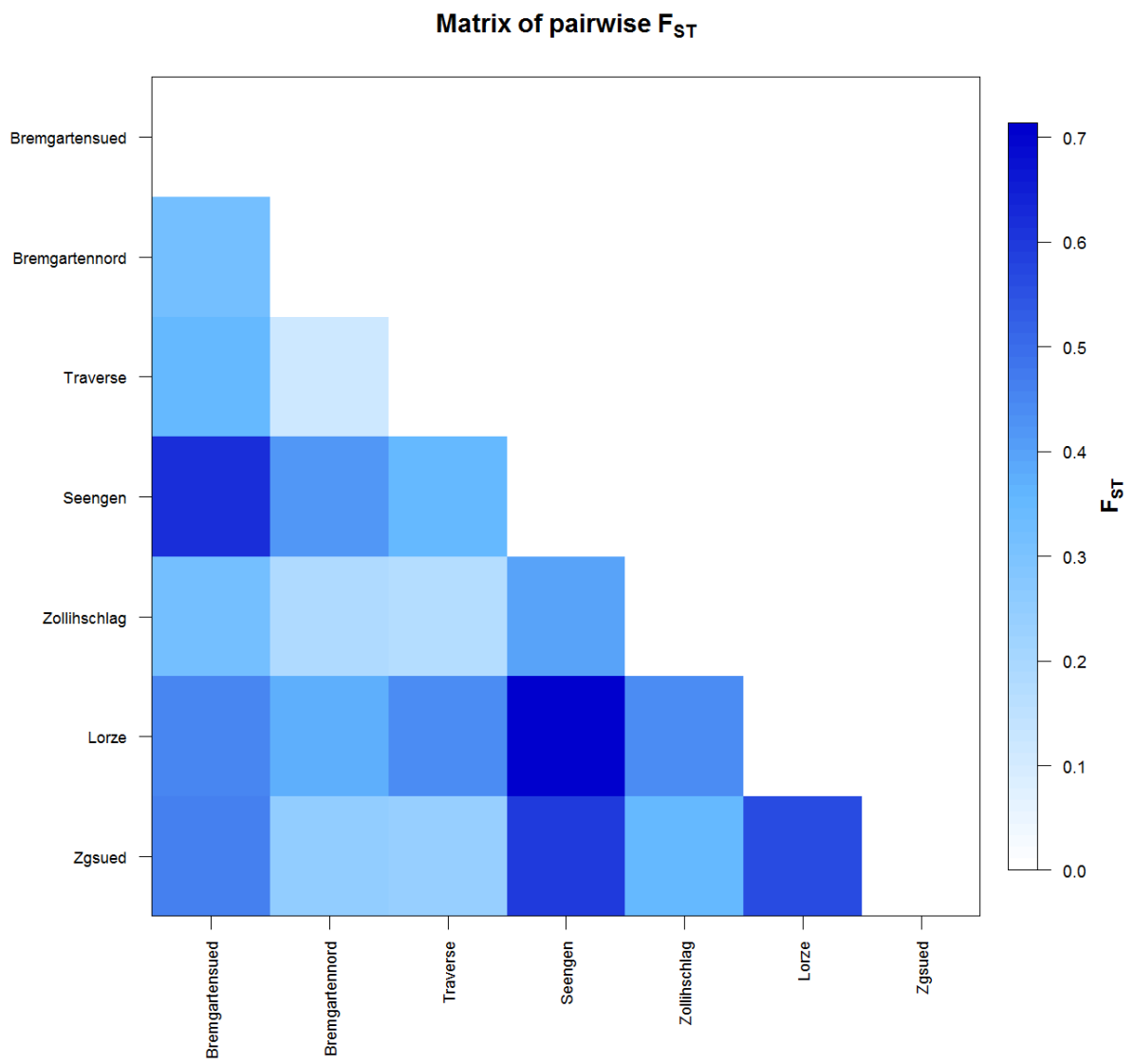


Figure 21 *Pairwise Fst matrix of 7* Bactrospora dryina *population along Reuss and Aabach, which were analysed in this study. Bright blue colour represent high genetic similarity or recent gene flow, dark blue colour stands for low genetic similarity and few or no gene flow.*

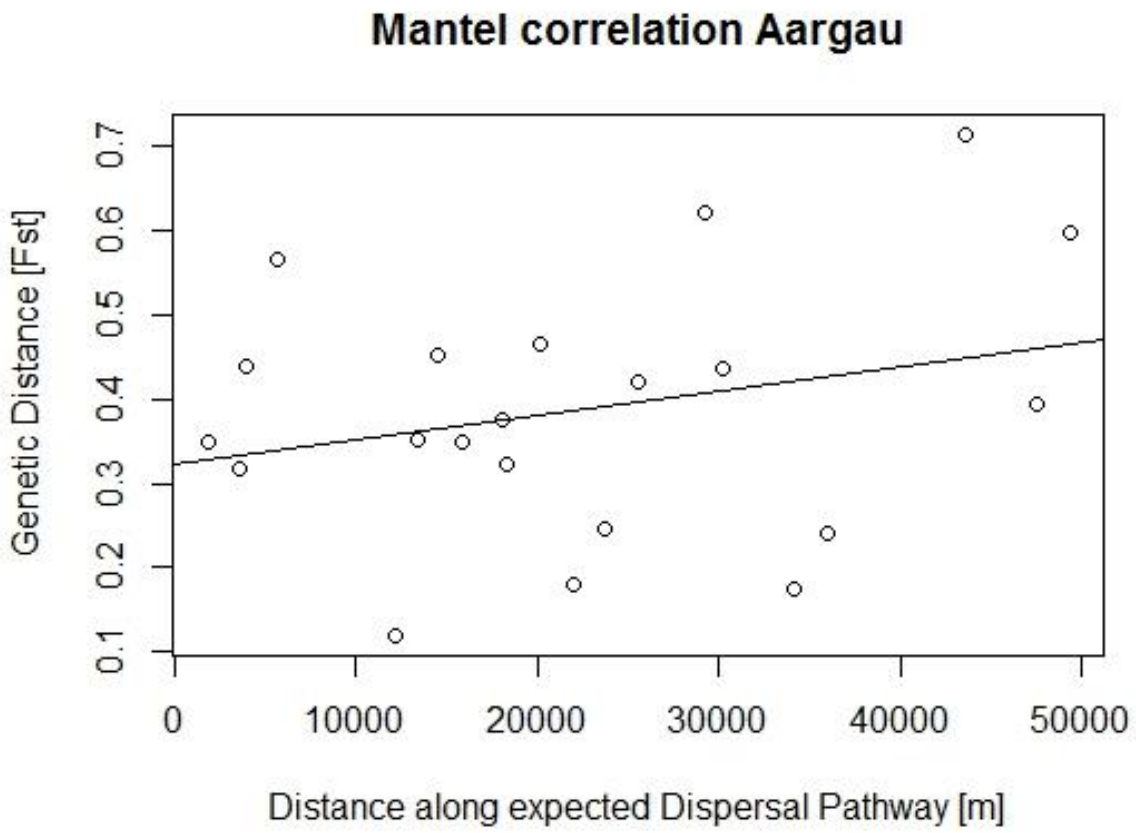


Figure 22 *Isolation by distance was not confirmed by mantel test for all populations along Reuss and Aabach (r = 0.2624, p = 0.185).*

Bremgarten

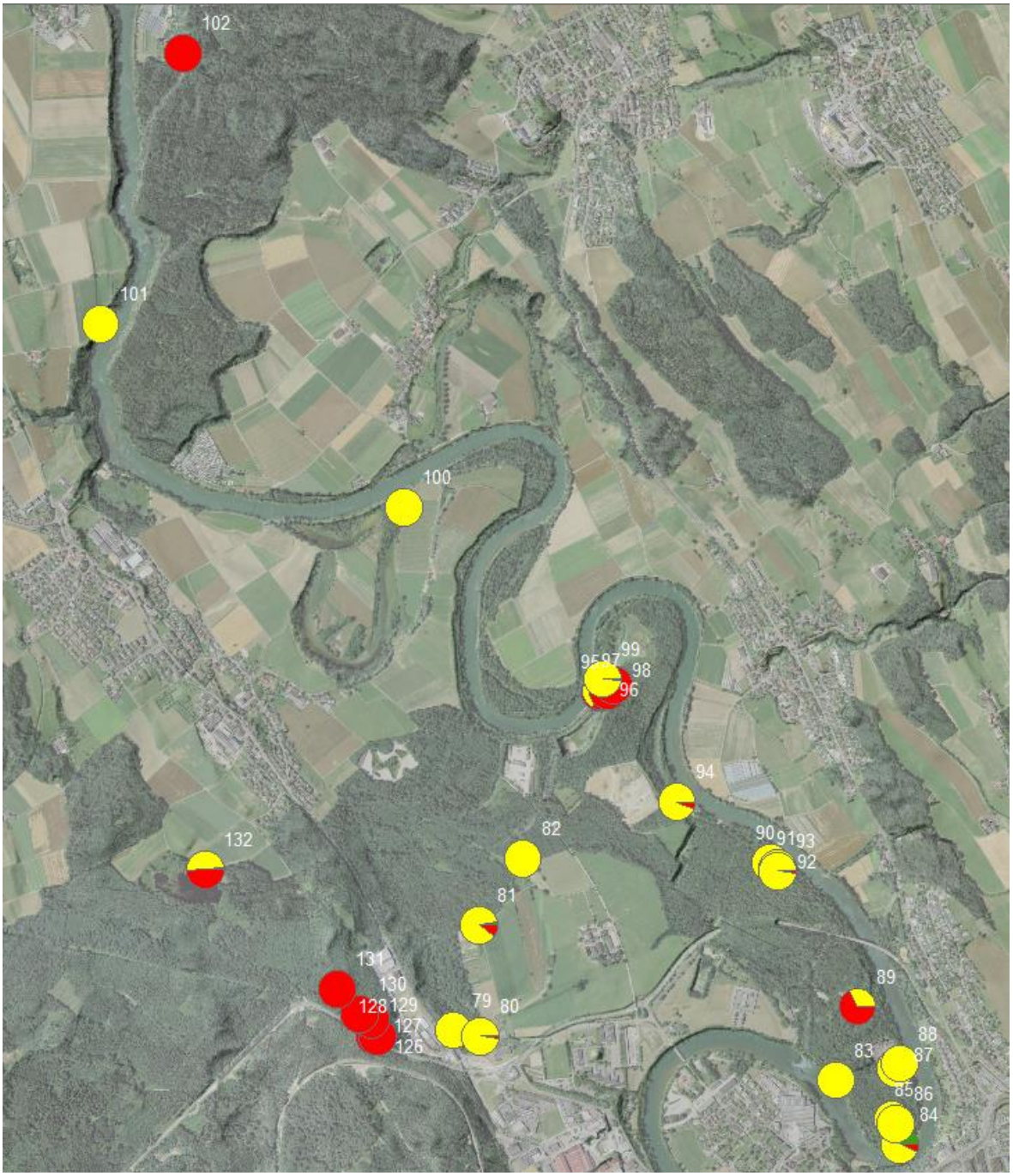


Figure 23 *Population of* Bactrospora dryina *at Bremgarten Nord. Each pie chart represents one colony. Colours represent clusters to which a colony belongs to. Numbers of colonies are according to figure 25 in appendix.*

Figure 24 *Population of* Bactrospora dryina *at Bremgarten Süd. Each pie chart represents one colony. Colours represent clusters to which a colony belongs to. Numbers of colonies are according to figure 25 in appendix.*

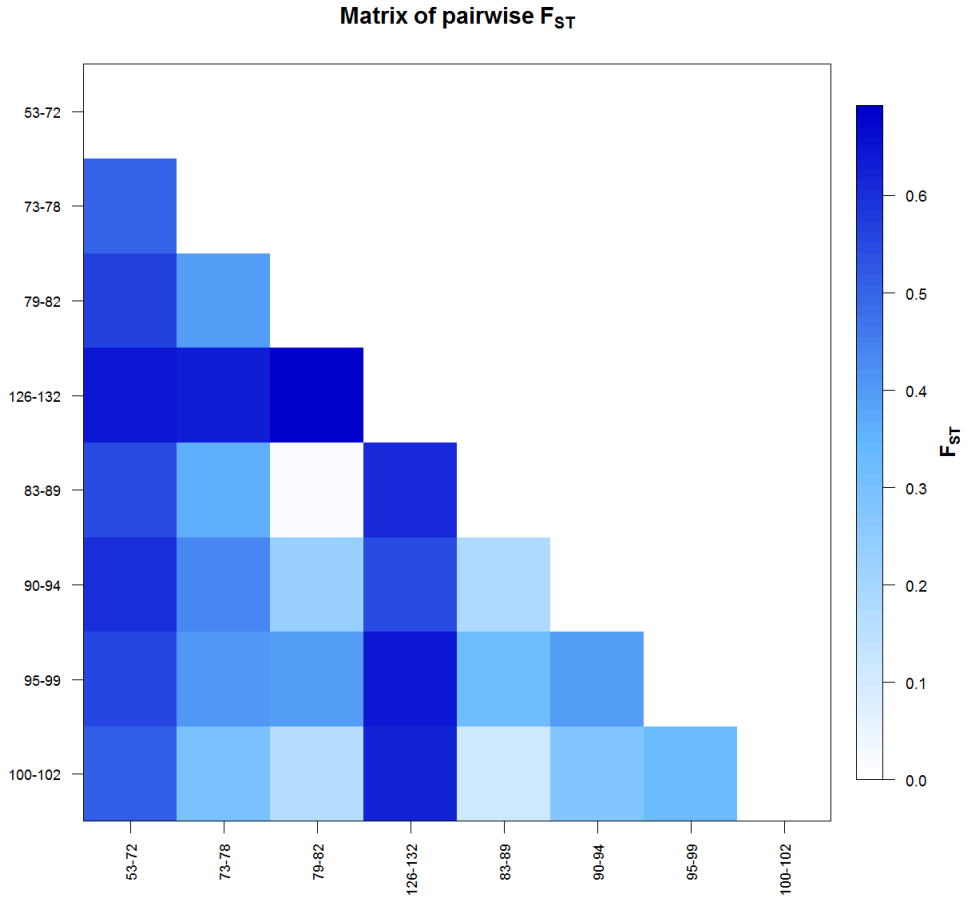


Figure 25 Pairwise Fst matrix of subpopulations in the area of Bremgarten. Bright blue colour represent high genetic similarity or recent gene flow, dark blue colour stands for low genetic similarity and few or no gene flow.

Seengen

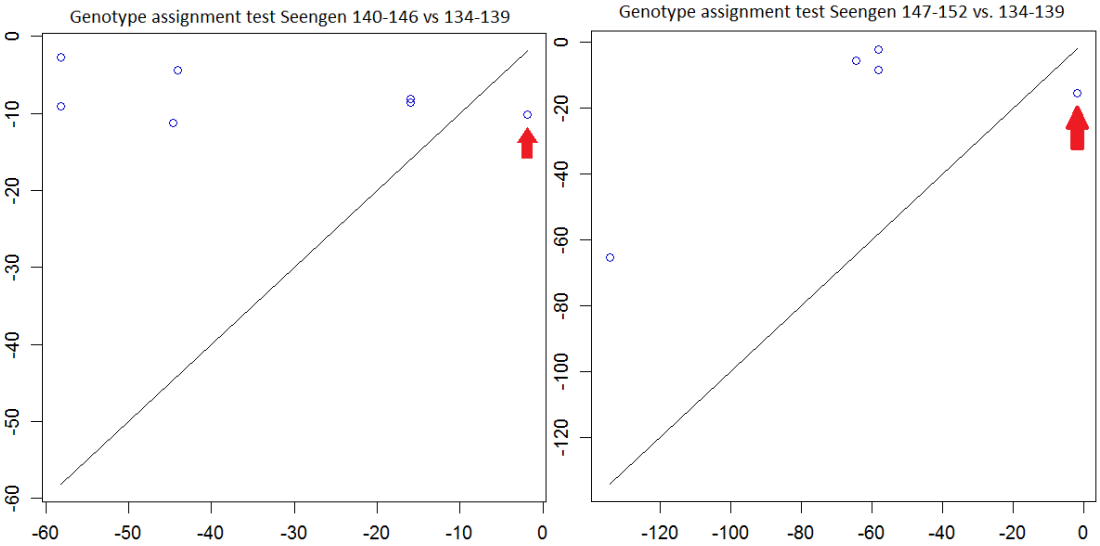


Figure 26 Genotype assignment test of Bactrospora dryina population at Seengen is corresponding to the genetic structure as shown in Figure 20 (main thesis). There is geneflow observed from subpopulation 134-139 to the other two subpopulations 140-146 and 147-152.

Erklärung

gemäss Art. 28 Abs. 2 RSL 05

Name/Vorname: Stirnemann Alex

Matrikelnummer: 13-116-348

Studiengang: Ecology and Evolution, with special qualification in Plant Ecology

Bachelor ☐ Master ☑ Dissertation ☐

Titel der Arbeit: Niche Differentiation and Genetic Structure of Bactrospora dryina in Swiss Hardwood Floodplain and Coppice-with-Standards Forests

LeiterIn der Arbeit: Prof. Dr. Christoph Scheidegger

Ich erkläre hiermit, dass ich diese Arbeit selbständig verfasst und keine anderen als die angegebenen Quellen benutzt habe. Alle Stellen, die wörtlich oder sinngemäss aus Quellen entnommen wurden, habe ich als solche gekennzeichnet. Mir ist bekannt, dass andernfalls der Senat gemäss Artikel 36 Absatz 1 Buchstabe r des Gesetzes vom 5. September 1996 über die Universität zum Entzug des auf Grund dieser Arbeit verliehenen Titels berechtigt ist. Ich gewähre hiermit Einsicht in diese Arbeit.

Wohlen, 23.02.2018

Ort/Datum

Unterschrift

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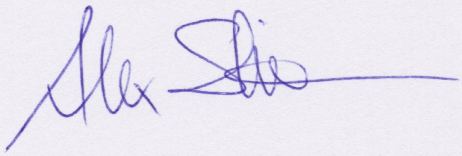

Unterschrift