

JULIUS-MAXIMILIANS UNIVERSITÄT
WÜRZBURG



Quantitative Genetics:
From Genome Assemblies to Neural Network
aided Omics Based Prediction of Complex
Traits

Doctoral thesis at the Graduate School of Life Sciences

Section Integrative Biology

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Affidavit

I hereby confirm that my thesis entitled "*Quantitative genetics - from genome assemblies to neural network aided omics based prediction of complex traits*" is the result of my own work. I did not receive any help or support from commercial consultants. All sources and or materials applied are listed and specified in the thesis. Furthermore, I confirm that this thesis has not yet been submitted as part of another examination process neither in identical nor in similar form.

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“Wit beyond measure is man’s greatest treasure”

Rowena Rawenclaw

Acknowledgements

Thanks to everyone who has supported me over the course of the last three years.

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List of Abbreviations

| | |
|------------------|---|
| Adadelata | Adaptive delta |
| Adagrad | Adaptive Gradient Algorithm |
| Adam | Adaptive Moment estimation |
| ANN | Artificial Neural Network |
| API | Application Programming Interface |
| AUC | Area Under the Curve |
| BLUE | Best Linear Unbiased Estimator |
| BLUP | Best Linear Unbiased Predictor |
| BP | Base Pair |
| BRR | Bayesian Ridge Regression |
| CPU | Core Processing Unit |
| DH | Doubled Haploid |
| DNA | DeoxyriboNucleic Acid |
| RNA | RiboNucleic Acid |
| EMMA | Efficient Mixed Model Associations |
| FCL | Fully Connected Layer |
| GBLUP | Genomic Best Linear Unbiased Predictor |
| GD | Gradient Descent |
| GEBC | Genomic Estimated Breeding Values |
| GPL | General Public License |
| GP | Genomic Prediction |
| GPU | Graphical Processing Unit |
| GRM | Genomic Relationship Matrix |

| | |
|----------------|---|
| GS | Genomic Selection |
| GUI | Graphical User Interface |
| GWAIS | Genome Wide Interaction Association Studies |
| GWAS | Genome Wide Association Studies |
| HDF | Hierarchical Data Format |
| HL | Hidden Layer |
| HPC | High Performance Computing |
| IR | Inverted Repeat |
| LCL | Locally Connected Layer |
| LD | Linkage Disequilibrium |
| LMM | Linear Mixed Model |
| LSC | Large Single Copy |
| MAF | Minor Allele Frequency |
| MBP | Mega Base Pair |
| MCMC | Markov Chain Monte Carlo |
| MLP | Multi Layer Perceptron |
| ML | Machine Learning |
| MSE | Mean Square Error |
| Nadam | Nesterov-accelerated Adaptive Moment Estimation |
| NAG | Nesterov Accelerated Momentum |
| NCBI | National Center for Biotechnological Information |
| QTL | Quantitative Trait Locus |
| ReLU | Rectified Linear Units |
| RKHS | Reproducing Kernel Hilbert Spaces |
| RMSE | Root Mean Square Error |
| RMSProp | Root Mean Square Propagation |
| ROC | Receiver Operating Characteristics |
| RSS | Residual Sum of Squares |

| | |
|------------|--|
| SGD | S tochastic G radient D escent |
| SNP | S ingle N ucleotide P olymorphism |
| SRA | S equence R ead A rchive |
| SSC | S mall S ingle C opy |
| TRN | T Rai N ing subset |
| TST | T e S Ting subset |
| WGS | W hole G enome S equencing |
| XOR | e X clusive O R |

For/Dedicated to/To my...

1 General introduction

Plant breeding is a process that started as early as agriculture itself around 10,000 BC. Even after twelve millennia many of its aspects are still obscured behind complex genetics and our lack to thoroughly comprehend them. But never were the challenges the vast as today. In the year 2050 this world's agriculture will be responsible for feeding 9 to 10 billion people GERLAND et al., 2014, next to other inflicted duties of replacing fossil fuels with regenerate energy from plants, providing fibers for industrial textile production and pharmaceutical applications.

According to WALLACE, RODGERS-MELNICK, and BUCKLER, 2018 the history of breeding can be divided into four main epochs that always utilized to technologies available to them in a specific era. The majority of this time breeding, as a succinct field of science, did not exist and breeding was executed by simple phenotypic selection by local farmers leading to dramatic changes in ca. 7000 cultivated crop species KHOURY et al., 2016. The next era in plant breeding was sparked by the upcoming of new statistical methods and the rediscovery of Mendelian genetics in the late 19th and early 20th century, which combined let the development of quantitative genetics TSCHERMAK, 1900; FISHER, 1919; FISHER and MACKENZIE, 1923; FALCONER and MACKAY, 1996. With it came the discovery of inbreeding and inbreeding depression, schematic design of field trials, the concept of variance component analysis, hybrid breeding and others.

The third stage, the genomic era of plant breeding, began with the discovery of molecular markers, leading up to marker-assisted breeding, linkage and QTL mapping. As marker arrays grew larger and sequencing costs dramatically reduced, they were succeeded by the more sophisticated and precise methods of

whole genome regression and genome-wide association studies (GWAS) with high-density marker maps HAYES and GODDARD, 2001; KORTE and FARLOW, 2013. Those technological advances allowed plant breeders to provide farmers with cultivars, which were able to feed the exponentially growing world's populations since the 1950s. However, like any century before the 21st imposes great challenges on humankind. Climate change leads to different stresses in the environments and plant breeders need to adapt to the specific requirements on high-yielding cultivars, maybe quicker than ever before, as drought and flooding occur more often around the world each year.

Artificial selection has similar effects as bottlenecks in natural selection do on populations. Of the 7000 cultivated plants in agricultural history, today only three provide the major source of food around the world. All three of them – maize (*Zea mays*), wheat *Triticum aestivum* and rice *Oryza sativa* – are closely related from the family of *poaceae*. Furthermore during the course of breeding the elite cultivars have lost the majority of the genetic diversity of its ancestral wild populations WALSH and LYNCH, 2018b. To continuously adapt and improve crop plants all the methods of quantitative genetics, genomics and genome editing need to be combined in the modern age of Breeding 4.0 WALLACE, RODGERS-MELNICK, and BUCKLER, 2018.

2 Benchmarking of Chloroplast Genome Assembly tools

This chapter is oriented on FREUDENTHAL et al., 2019b. Only the chapters from the publication which the author majorly contributed to are included. If not cited otherwise the plots were designed and generated by the author of this thesis.

2.1 Introduction

2.1.1 Motivation

Some organelles like mitochondria and chloroplasts contain their own genetic information from which they are able to synthesize certain proteins independent of the nucleus genome. Evolutionary this developed during endosymbiosis, a process which underlying theory seeks to explain how eukaryotic cells formed from prokaryotes MERESCHKOWSKY, 1905; KUTSCHERA and NIKLAS, 2005. This widely acknowledged hypothesis explains how in the early evolution of eukaryotes other cells were incorporated, which ultimately became organelles. The most likely precursors of today's chloroplasts were photosynthetic bacteria or similar organisms ARCHIBALD, 2015. This process left its traces in the structure of chloroplast genomes until today, which resemble more closely prokaryotic genomes than that of its eukaryotic host cells. A typical chloroplast genome consists of circular DNA with a size between 120 kbp to 160 kbp PALMER, 1985, while plant core genomes are linear, organized in chromosomes and larger by multiples of hundreds to tens of thousands.

The first chloroplasts have been sequenced as early as 1986 and were isolated from *Marchantia polymorpha* and *Nicotiana tabacum* OHYAMA et al., 1986; SHINOZAKI et al., 1986. Complete reviews on the structure of chloroplast genomes were authored by GREEN, 2011 and WICKE et al., 2011. Chloroplast genomics is widely applied in evolutionary studies aiding to elucidate the processes involved in endosymbiosis and the development of photosynthetic plants MARTIN et al., 2002; XIAO-MING et al., 2017. Over the course of natural adaptation, the genome has been reduced in size through endosymbiotic gene transfer, a form of horizontal gene transfer where fractions of plastid genomes are incorporated in the core DNA MARTIN et al., 2002; DEINER et al., 2017. This mechanism of evolution is still ongoing and can be observed *in vitro* BOCK, 2017; FUENTES et al., 2014; STEGEMANN and BOCK, 2009.

In the case of *Arabidopsis thaliana*, this process resulted in 14 % of the core genome's genes previously being located on the chloroplast, while 100-120 genes remain on the chloroplast itself WICKE et al., 2011, which by far would not suffice to allow the chloroplast to function independently of its host cell. The fact that organelle genomes are much smaller and highly conserved with a large gene content leads to polymorphisms being more likely to cause functional changes in physiological processes. Another difference between organelle and core genomes is that single chloroplasts contain up to hundreds of copies of its own genome KUMAR, OLDENBURG, and BENDICH, 2014; BENDICH, 1987. Considering that photosynthetic active cells again contain multiple chloroplasts means that the number chloroplast genomes, therefore is considerably higher than the number of core genomes per cell.

Structurally, chloroplast genomes are made up of four distinct regions. Two inverted repeats (IR), IR_A and IR_B , ranging from 10 kbp to 76 kbp in size, that divide the circular genome into two regions: the large single copy (LSC) and the small single copy (SSC) as shown in figure 2.1 PALMER, 1985.

Taking into account that the majority of assembly tools has been designed to assemble linear core genomes, the structure of chloroplast genomes is an obstacle for the assembly pipelines to overcome. This holds true especially for solving and aligning the IRs correctly WANG et al., 2018.

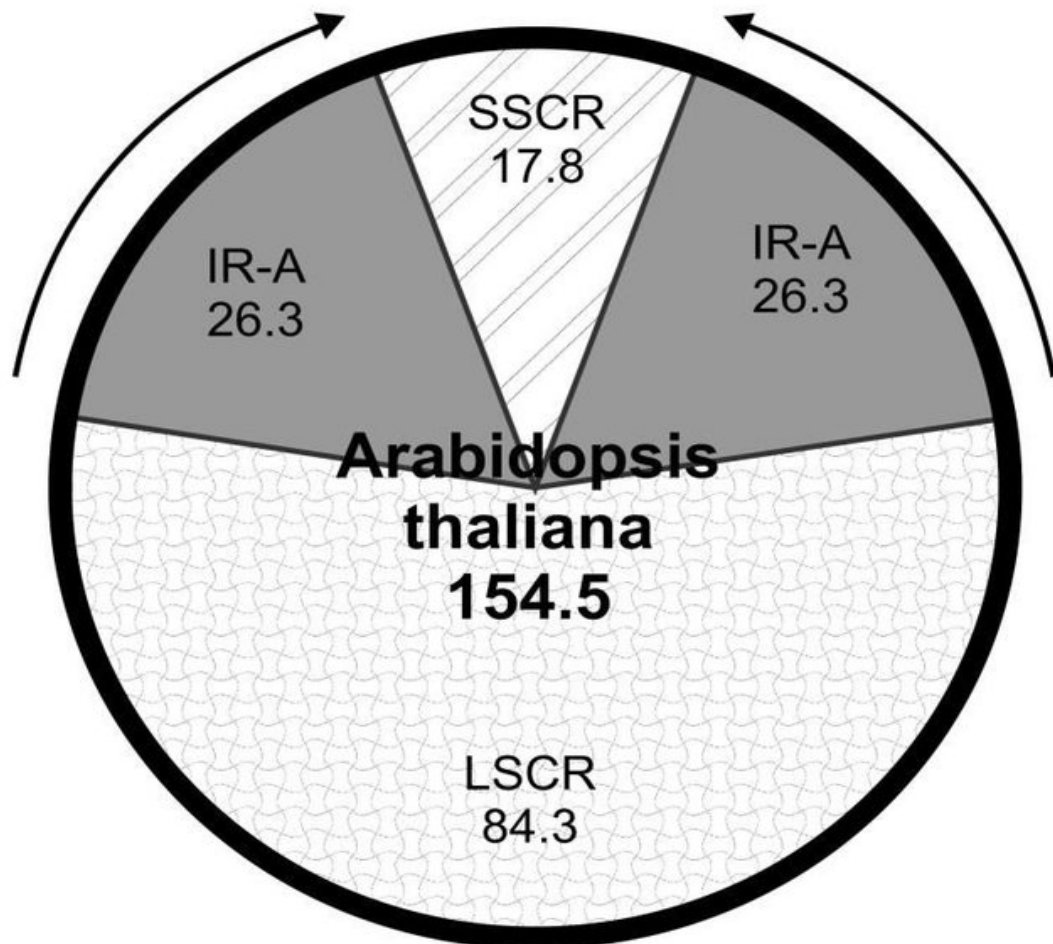


FIGURE 2.1: Structure of the chloroplast genome of *A. thaliana* with small single copy region (SSCR) and large single copy region (LSCR). The number denotes the length of the genome and its parts in kilo base pairs (kbp). Graphic from OLEJNICZAK et al., 2016

Another difficulty for the assembly is heteroplasmy, which describes the phenomenon of co-existence of multiple versions of the chloroplast's genome in a single organism and even single cells of that respective organism. Heteroplasmy

complicates genome assemblies and ongoing from there the downstream analyses CORRIVEAU and COLEMAN, 1988; CHAT et al., 2002. The underlying evolutionary mechanisms behind heteroplasmy are not fully elucidated and existing fitness advantages fueling heteroplasmy cannot be explained satisfactory by standard evolutionary methods SCARCELLI et al., 2016.

Derived from a multitude of plant genome projects, there is a large variety of databases, containing short read data for species without assembled organelle genomes available, e.g. NCBI's sequence read archive (SRA) LEINONEN et al., 2010. Because most plant DNA extraction protocols applied to procure raw input for sequencing use green leaf tissue as their basis, they also contain a large amount of plastid DNA, providing a valuable basis for organelle genome assemblies pipelines. In the course of this chapter, the performance of these pipelines will be assessed.

Having larger numbers of assembled and annotated chloroplast genomes publicly available will be beneficial for evolutionary studies and are a useful addition to bar-coding and super-barcoding COISSAC et al., 2016, aside from other biotechnological applications DANIELL et al., 2016. To obtain those there is a variety of tools available. In the course of this chapter the availability, usability and overall performance of seven of those assembly pipelines will be assessed. Ultimately, the newly gained insights will be utilized to attempt to *de novo* assemble more than 100 chloroplasts.

2.1.2 Extraction of chloroplast reads from whole genome data and general assembly workflow

There is large array of strategies to assemble chloroplast genomes from raw sequencing data TWYFORD and NESS, 2017. In general the process of chloroplast genome assembly involves three steps:

- (i) extraction of plastid reads from the whole-genome sequencing (WGS) data
- (ii) assembly of the plastid genome
- (iii) solving the circular structure of the genome with the IRs.

There are three approaches to address step (i). The first one is to map all reads to a reference chloroplast VINGA et al., 2012, which works reasonably well if there is one available for the same or at least a closely related species. The second approach is to make use of the much larger coverage of chloroplast DNA compared to core DNA, with a k-mer analysis CHAN and RAGAN, 2013, this is for example done by `chloroExtractor` ANKENBRAND et al., 2018. The third way to accomplish plastid DNA extraction is to combine both approaches (i) and (ii) as done by `NOVOPlasty` DIERCKXSENS, MARDULYN, and SMITS, 2017. Figure 2.2 shows the general workflow of chloroplast assembly tools with the bifurcation at step (ii).

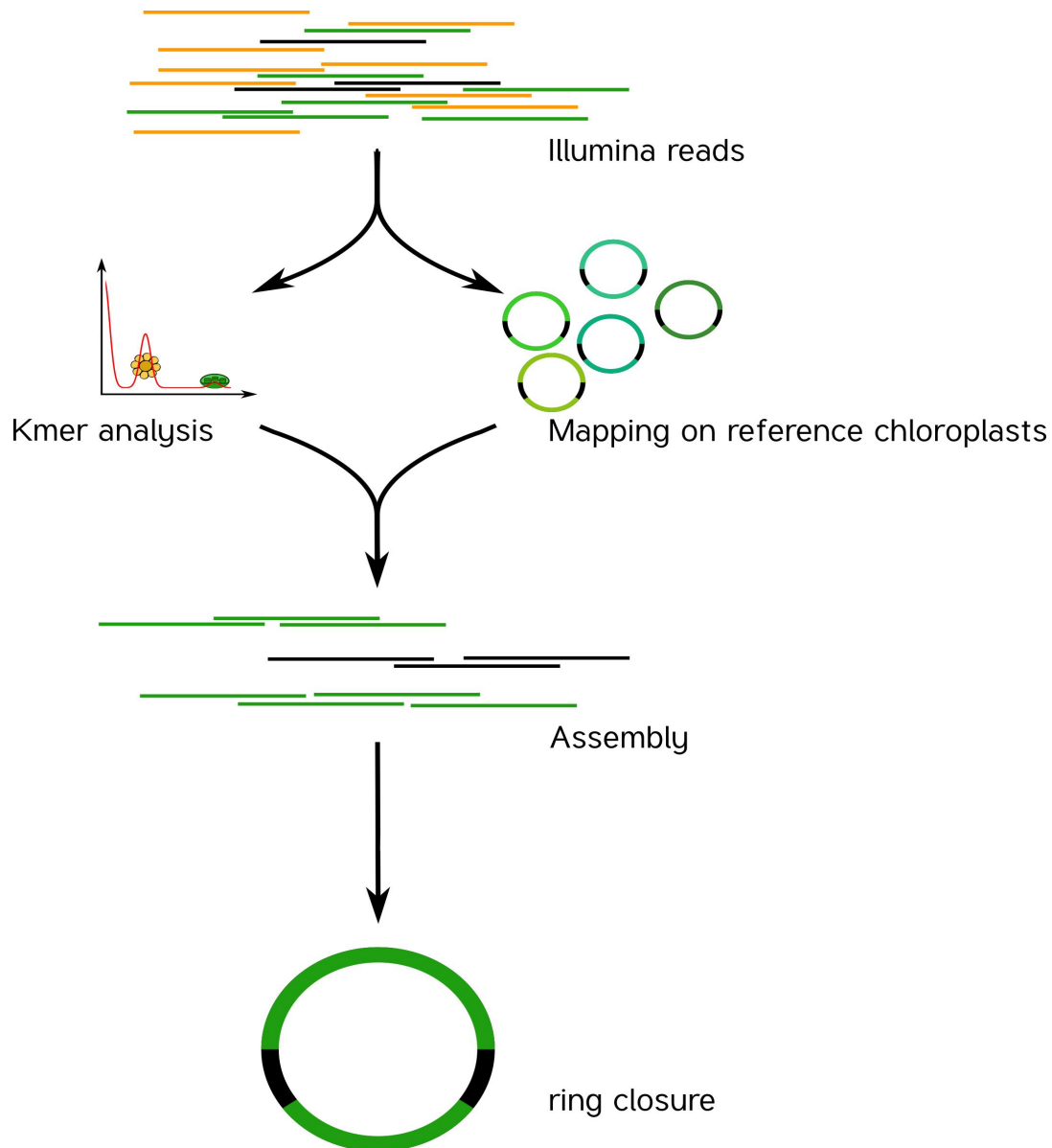


FIGURE 2.2: Standard workflow of chloroplast genome assembly.

Graphic from ANKENBRAND et al., 2018

Purpose and scope of benchmarking the landscape of chloroplast assembly tools

The purpose of this study is to provide insights into the landscape of chloroplast assembly tools, to recommend best practices for organelle genome assemblies and to contribute *de novo* assemblies for many species and families without a reference chloroplast available so far to the scientific community.

2.2 Material and Methods

2.2.1 Methods

Data and code availability

All the source code and data used is publicly available under the terms of the MIT-License. The source code has been published on github [GitHub Repository for Benchmark Project](#) and archived on zenodo FÖRSTER and ANKENBRAND, 2019 . The docker images are available on dockerhub [Docker Hub Group for Benchmark Project](#).

Tools

To be included into this study, the software, including the source code, must be publicly available and published under the terms of a liberal, free open source software license e.g. GPL or MIT-license. The study was further restricted to paired-end Illumina data sets as their sole input source, because they were abundantly available for this benchmark. The only technical requirement was being able to assemble chloroplast genomes from paired-end Illumina reads. The other requirements were dictated by reproducibility: (i) The software must be open-source and available under the terms of a liberal software license and the software must be able to be operated from a command line, since GUI-only tools are not suited for highly repetitive, automated analyses. In total there were seven tools that met those conditions:

- (i) chloroExtractor ANKENBRAND et al., 2018
- (ii) Chloroplast assembly protocol SANCHO et al., 2018
- (iii) GetOrganelle JIN et al., 2018

- (iv) ORG .Asm COISSAC et al., 2016
- (v) IOGA BAKKER et al., 2016
- (vi) Fast-Plast MCKAIN and AFINIT, 2017
- (vii) NOVOPlasty DIERCKXSENS, MARDULYN, and SMITS, 2017

Standardization and reproducibility

Along with the study, easy and ready-to-use versions of all the involved programs, working with standardized input, were published. For this purpose docker containers MERKEL, 2014 were implemented. To work with the containers in a closed HPC environment they were transformed into related singularity containers KURTZER, SOCHAT, and BAUER, 2017. To use the programs users simply need to provide two files: one for the forward reads (`forward.fq`) and one for the reverse reads (`reverse.fq`) and run the containers without any need for further configuration or installation besides docker or singularity itself, which can be easily done on all popular operating systems. Both files are required to be in FASTQ format. Besides the individual output files recording the process of the respective program, all programs write the assembly products into files called `output.fa` in FASTA format. For the quantitative and consistency measurements the singularity containers were run on the Julia HPC-cluster of the University of Würzburg using the SLURM workload manager JETTE, YOO, and GRONDONA, 2002. All runs for all assemblies were set with a time limit of 48 hours. This was necessary because some assemblers e.g. IOGA, if they did not finish after at least 12 hours, showed the tendency not to finish, even after weeks of running.

2.2.2 Data

Three different data sets were used:

- (i) simulated data from *A. thaliana* chloroplasts

- (ii) real data with known reference chloroplast to rate the success of the assemblies
- (iii) novel data sets from NCBI's SRA without a known reference chloroplast to apply the gained knowledge for the *de novo* assembly of more than 100 chloroplasts.

Simulated

To allow full control over all the parameters involved, we started with the simulated data. In the present case the data's input parameters, thought to be influential on the outcome, were: The read length, the ratio between chloroplast and core genome reads as well as the total size of the data set. The data simulations were based on real data from the TAIR10 genome of *A. thaliana* BERARDINI et al., 2015 and spawned using `seqkit` SHEN et al., 2016. Core to chloroplast ratios simulated were: 0:1, 1:10, 1:1000 and 1:1000, with read length of 150 and 250 bp. The artificial data consisted either of 2 million read pairs or the full data available. The simulation process was documented and the code and the data is available on github and zenodo ANKENBRAND and FÖRSTER, 2019.

Real data set

Real data was selected from the SRA database. Table 2.1 lists the search parameters that had to be met for a plant to be included in the study from SRA.

TABLE 2.1: Data selection criteria for real data sets from SRA

| Choice | Option | Explanation |
|------------|--------------|--|
| Organism | green plants | include only photosynthetic plants e.g. no algae |
| Strategy | wgs | only data from wgs projects included |
| Platform | Illumina | include only paired-end Illumina reads |
| Properties | biomol DNA | include only biomol. DNA samples (e.g. no RNA) |
| Layout | paired | exclude single-end reads |
| Selection | random | |
| Access | public | Only publicly available data included |

In total this resulted in 369 data sets representing a broad variety of the plant kingdom with many different families and genera included.

Novel data sets

To assess the performance of assemblies without a published chloroplast on *CpBase* *CpBase* 105 data sets were selected from SRA. It was emphasized that the chosen read libraries were as distant as possible related to the next relatives with a reference chloroplast, related as possible in taxonomic terms according to NCBI *NCBI Taxonomy*. This was achieved by a phylogenetic analysis of the accessible data sets on SRA by Frank Förster described in FREUDENTHAL et al., 2019b.

2.2.3 Evaluation

Quantitative

Where explicable, each assembly from each assembler was compared to their respective reference genome by alignment using *minimap2* LI, 2018. Based on those alignments scores were calculated following equation 2.1 from 0 to 100, with 100 being a perfect score.

$$score = \frac{1}{4} \cdot \left(cov_{ref} + cov_{qry} + \min \left\{ \frac{cov_{qry}}{cov_{ref}}, \frac{cov_{ref}}{cov_{qry}} \right\} + \frac{1}{n_{contigs}} \right) \cdot 100 \quad (2.1)$$

Four different metrics contributed equally to the final score:

- (i) the coverage of the assembled genome compared to the reference genome cov_{ref} as an estimate for the completeness
- (ii) the vice versa case cov_{qry} as a measure for the correctness of the assembly
- (iii) the success of resolving the IR correct, estimated by difference from the reference and the newly assembled genome $\min \left\{ \frac{cov_{qry}}{cov_{ref}}, \frac{cov_{ref}}{cov_{qry}} \right\}$
- (iv) the number of total contigs were weighted as $\frac{1}{n_{contigs}}$ giving a chloroplast with one contig the optimal score.

While it is difficult to evaluate the success or failure of assemblies on a continuous scale, equation 2.1 allows for objective and unbiased measurements. SNPs or other small variants do not influence the outcome of the score, because they are more likely due to in-species variation between plastid genomes and not caused by the assembly itself. Even if the latter is true it would be difficult to determine.

Consistency

For any given bioinformatical application consistency is a desired trait. Software ideally should repeatedly yield the same output when provided with the same input and assembly tools are exception. To evaluate the reproducibility of the 7 tools, for all the 369 real data sets described in section 2.2.2, they were assembled and scored twice with each assembler. The correlations between the first and the second run's scores were used as the measure for the robustness of a program.

2.3 Results

2.3.1 Quantitative

Simulated data

The simulated data sets were assembled and scored with all the tools as described above. Figure 2.3 shows a tile plot with the results displaying a color scale from orange over light green to dark green representing the scores from 0 to 100. Blank spaces indicate the failure to produce any output in the given time frame of 48 hours.

While at first sight there is no clear correlation between the input data sets and the score, it is clearly visible that there are grave differences between the assemblers. Two programs, namely Chloroplast assembly protocol and IOGA, failed to correctly assemble a single chloroplast's genome. IOGA even fails to provide an output at all for the majority of the data sets. While those two stand out as negative examples, Fast-Plast and GetOrganelle stand out as positive examples, perfectly or nearly perfectly assembling all the data sets, with GetOrganelle surpassing the performance of Fast-Plast. In the middle of the field are chloroExtractor, ORG.Asm and NOVOPlasty performing reasonably well, but sometimes lacking to solve the IRs and the circular structure.

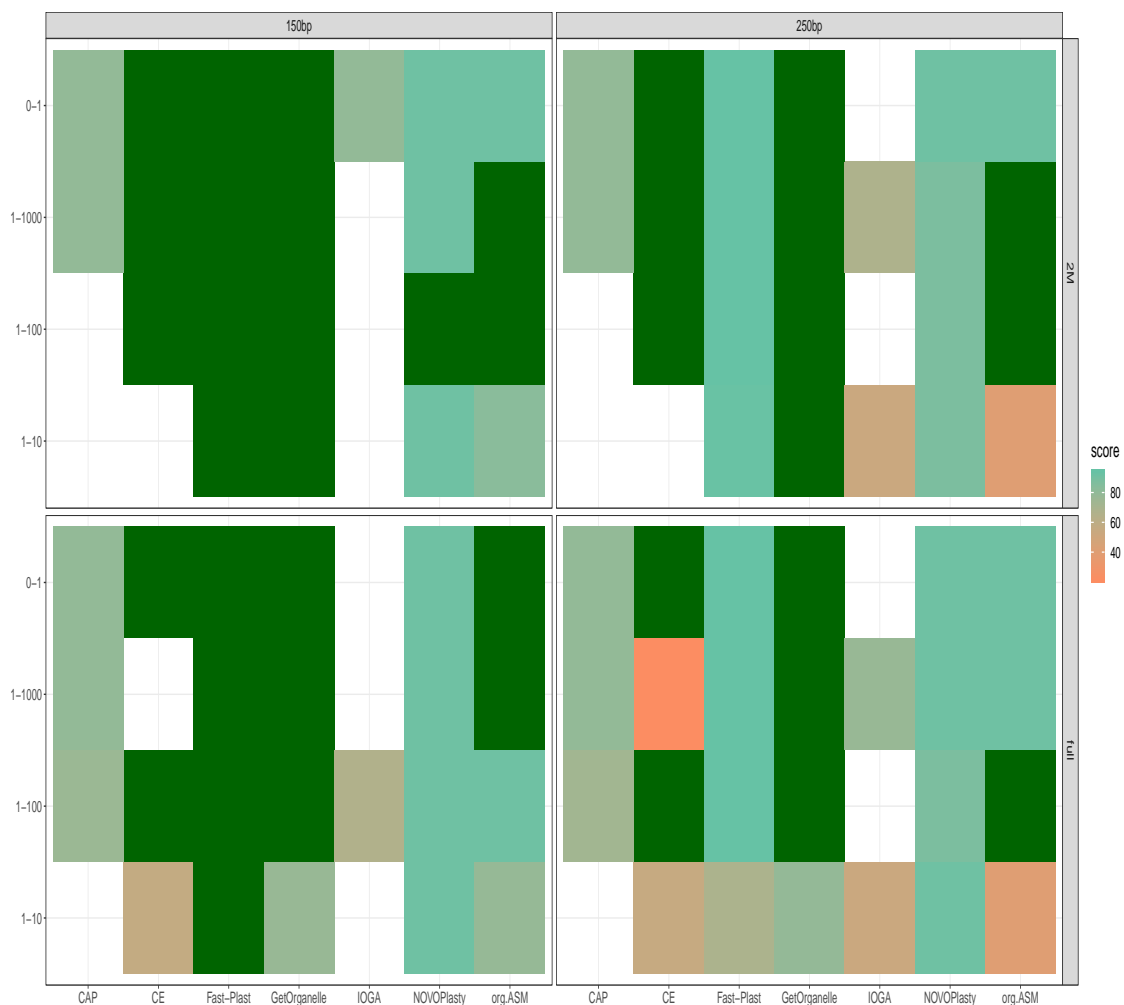


FIGURE 2.3: Results of assemblies executed with simulated data sets. The tile color from orange to green indicate the score from 0 to 99. Dark green tiles are score >99. Blank tiles point to assemblies, which failed to provide an output file. The axis on the left shows the ratio between nucleic and plastid DNA, the one on the right the size of the data sets. On the top the read length in base pairs (bp) is given and in the bottom the seven different assemblers

There is a significant difference between the performance of the assemblers in general. The varying input parameters, however, do not have as grave an influence as the choice of the assembler. While *Fast-Plast* deals with the shorter reads of 150 bp much better than with the longer reads of 250 bp, the scores of the other assemblers do not seem to be influenced by the read length. There is no difference between the full and the subsampled data sets. And while all assemblers appear to be more challenged by low chloroplast to core genome ratios of

1:10, beyond a ratio of 1:100 it does not affect the quality of the assemblies. Table 2.2 shows all the individual results for all data sets and assemblers. For the fields with no entry the respective assembler failed to provide an output.

TABLE 2.2: Scores of assemblies of simulated data. with CAP = Chloroplast assembly protocol; CE = chloroExtractor; FP = Fast-Plast; GO = GetOrganelle; NP = NOVOPlasty; oA = ORG.Asm; length in base pairs (bp).

| Set | Length | Ratio | CAP | CE | FP | GO | I0GA | NP | oA |
|------|--------|--------|-------|--------|--------|--------|-------|--------|--------|
| full | 150 | 0-1 | 79.10 | 100.00 | 99.48 | 100.00 | | 91.52 | 100.00 |
| 2M | 150 | 0-1 | 79.10 | 100.00 | 99.72 | 100.00 | 79.10 | 91.52 | 91.50 |
| full | 150 | 1-10 | | 56.44 | 100.00 | 76.98 | | 91.52 | 78.00 |
| 2M | 150 | 1-10 | | | 99.97 | 100.00 | | 91.52 | 82.72 |
| full | 150 | 1-100 | 75.72 | 100.00 | 99.48 | 100.00 | 66.09 | 91.52 | 91.50 |
| 2M | 150 | 1-100 | | 100.00 | 99.47 | 100.00 | | 100.00 | 100.00 |
| full | 150 | 1-1000 | 79.10 | | 99.72 | 100.00 | | 91.52 | 100.00 |
| 2M | 150 | 1-1000 | 79.10 | 100.00 | 99.72 | 100.00 | | 91.52 | 100.00 |
| full | 250 | 0-1 | 79.10 | 100.00 | 93.82 | 100.00 | | 91.52 | 91.50 |
| 2M | 250 | 0-1 | 79.10 | 100.00 | 93.83 | 100.00 | | 91.52 | 91.50 |
| full | 250 | 1-10 | | 54.98 | 68.45 | 78.89 | 52.71 | 91.52 | 40.20 |
| 2M | 250 | 1-10 | | | 93.00 | 100.00 | 52.67 | 87.40 | 40.20 |
| full | 250 | 1-100 | 72.81 | 100.00 | 93.82 | 100.00 | | 87.40 | 100.00 |
| 2M | 250 | 1-100 | | 100.00 | 93.83 | 100.00 | | 87.40 | 100.00 |
| full | 250 | 1-1000 | 79.10 | 21.30 | 93.83 | 100.00 | 76.96 | 91.52 | 91.50 |
| 2M | 250 | 1-1000 | 79.10 | 100.00 | 93.83 | 100.00 | 67.55 | 87.40 | 100.00 |

Real data sets

Table 2.3 summarizes the results from the assemblies of 369 data sets with seven the assemblers. Similar to the scores of the previous section there is a significant difference between the tools. Likewise GetOrganelle is the most successful assembler by a large margin with 210 of 369 chloroplast genomes perfectly assembled. It completely fails to provide output for only 9 data sets, resulting in a median score >99. Contrary Chloroplast assembly protocol and I0GA both failed to completely assemble a single genome. The performance of Fast-Plast is reasonably well in comparison, completing approximately half as many genomes as GetOrganelle and being the only other tool whose average score surpasses 90.

Similar to the trials with the simulated data in chapter 2.3.1 chloroExtractor, NOVOPlasty and ORG.Asm are in the middle of the field.

TABLE 2.3: Median scores of chloroplast genome assemblers with inter-quartile range (IQR) and the number of perfect scores (n_perfect) compared to the total number of assemblies (n_tot) providing an output

| Assembler | Median | IQR | n_perfect | n_tot |
|-------------------------------|--------|-------|-----------|-------|
| Chloroplast assembly protocol | 45.25 | 50.19 | 0 | 369 |
| chloroExtractor | 56.55 | 71.50 | 14 | 369 |
| Fast-Plast | 92.80 | 23.59 | 113 | 369 |
| GetOrganelle | 99.83 | 20.94 | 210 | 360 |
| IOGA | 71.10 | 11.21 | 0 | 338 |
| NOVOPlasty | 75.95 | 48.69 | 58 | 369 |
| ORG.Asm | 67.35 | 91.69 | 46 | 348 |

Figure 2.4 emphasizes the large differences between the assemblers shown in table 2.3. The swarm plots show distinct bands for some assemblers e.g. NOVOPlasty and ORG.Asm, suggesting that multiple assemblies fail to be solved into a single contig genome at a certain point. As thoroughly discussed in section 2.4, solely from the swarm plot, it is debatable if all the tools should be recommended to be used for the purpose they were designed for.

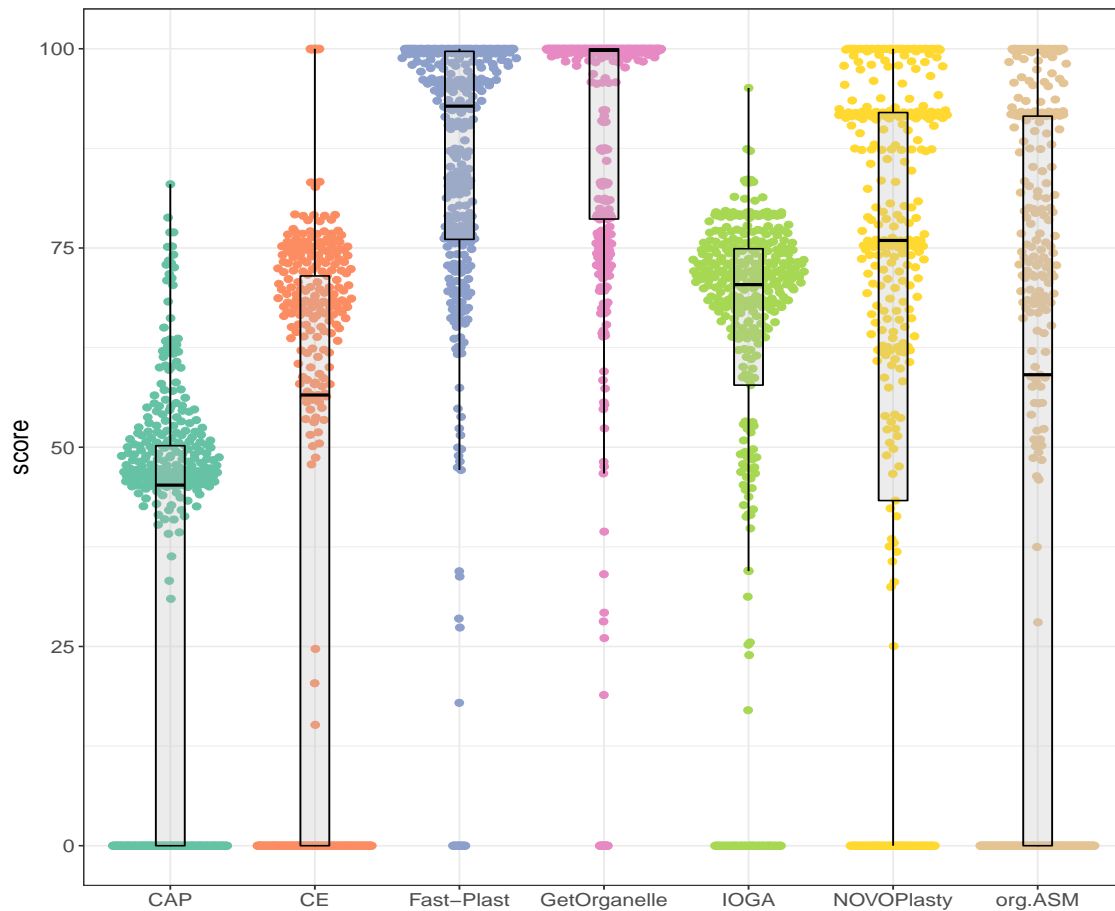


FIGURE 2.4: Box and swarm plots depicting the results from scoring of the assemblies for the real data sets as calculated by equation 2.1

Consistency

Consistency testing was done by re-running every assembly for the real data sets and comparison of the two scores. `chloroExtractor` was the only tool that was 100 % consistent over both runs. Followed by `Fast-Plast` and `NOVOPlasty`. The consistency plot (figure 2.5) for both of them results in arrowhead shaped plots. With differences between the first and second run appearing in assemblies with the best scores. All other assemblers appear to produce the same output in the two runs, except if either run failed to complete the assembly at all. This is less pronounced for `Chloroplast assembly protocol` and `GetOrganelle` and is a grave issue for `ORG.Asm` and `IOGA`.

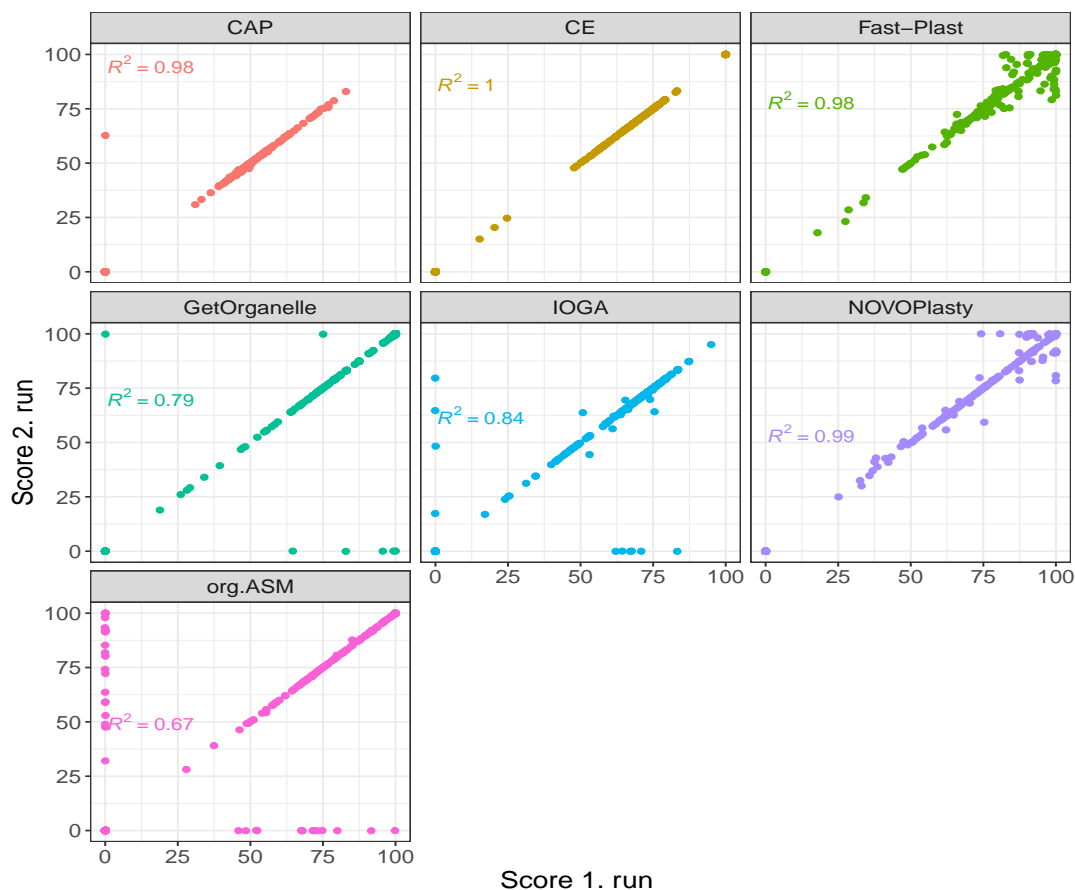


FIGURE 2.5: Swarm plots depict the results from the scoring shown in 2.1 for two independent runs for each assembler on each of the data sets

Novel

The final assessment in the evaluation of the assemblers was to test them on novel data sets without a published chloroplast. This step is important for two reasons. (i) It is possible that certain tools perform well on known chloroplasts because they have knowledge of their structure, which would lead to a lack of generalization on unknown genomes. (ii) To apply and test the gained insights with the goal of providing the scientific community with a larger variety of published chloroplast genomes.

As in previous evaluations the most successful assembler was GetOrganelle, with 49 out of 105 novel data sets completely assembled.

Lacking a reference genome for alignment, the success had to be defined differently and equation 2.1 was not suitable to evaluate the novel assemblies. Metrics influencing the score of the novel assemblies were the number of contigs, solving the IRs and the size of the SSC and LSC. This, known to the author, might be biased and not true for all chloroplasts and assumes that all chloroplast genomes evolved according to the general structure described in chapter 2.1. Figure 2.6 compares the results of the assemblies with at least one successful assembly.

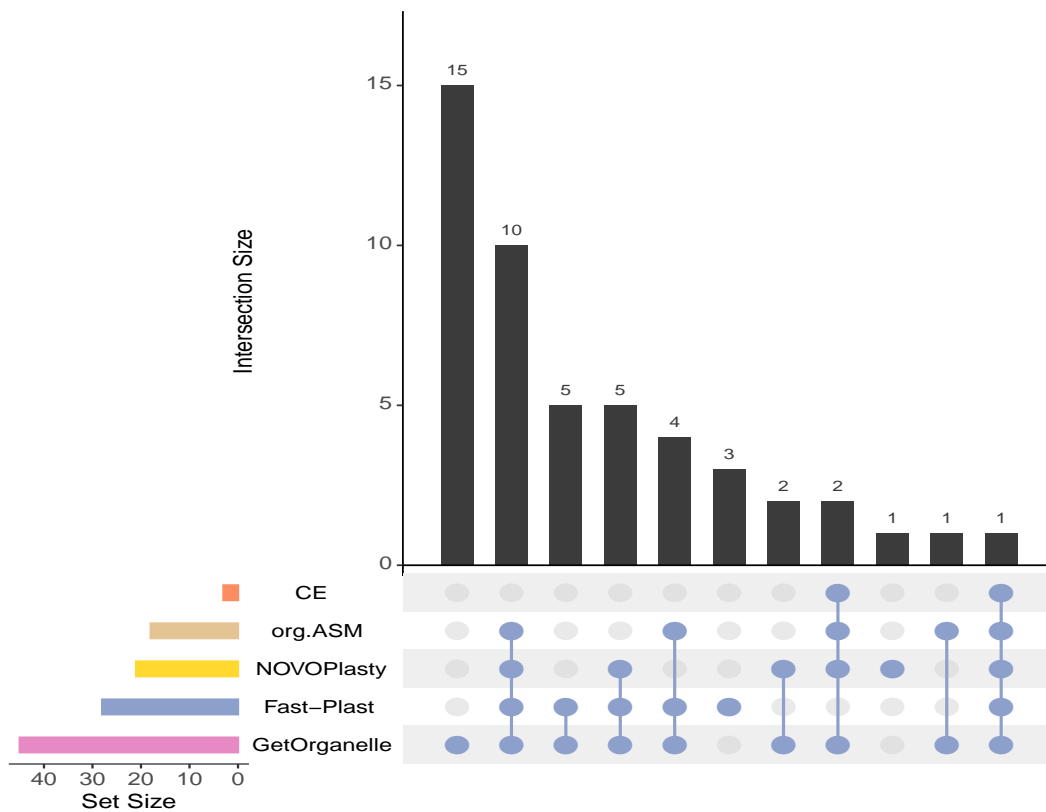


FIGURE 2.6: Upset plot comparing the success (single contig, length *geq* 130 kbp, ir *geq* 17 kbp) rates of the different assemblers for the novel data sets. The colored, horizontal bar plot show the total amount of successful assemblies for each assemblers. The black, vertical barplot the size of the intersection indicated by the dots in the middle

2.4 Discussion

The study presented in this chapter so far consists of two goals:

- (i) to assess the overall performance of a variety of tools designed specifically for the assembly of circular chloroplast genomes from paired-end Illumina reads and
- (ii) to *de novo* assemble a variety of yet unpublished chloroplast genomes from existing data.

To accomplish the first goal 16 simulated and 369 real data sets were used adding up to a total of 5166 assemblies for the real data sets and 112 for the simulated data, along 735 assemblies for the novel data sets, thus underlying the statistical powers of this benchmarking study.

The most successful tools were `GetOrganelle` and `Fast-Plast`, which are recommended to be used complementary because, as shown in figure 2.7, they succeed for most data sets compared to other assemblers and accomplish to satisfactory assemble chloroplast genomes where the other fails. If both of them fail it might be worthwhile to repeat the runs because other results could be expected as shown in the scatter plots of figure 2.5, especially `Fast-Plast` might be able to improve the previously reached score. Only if both of them fail it might, even though improbable, that `NOVOPlast` might lead to a successful assembly. The other assemblers should be used with caution. While `chloroExtractor` might be good for a quick overview due to its relatively low demand in computational time FREUDENTHAL et al., 2019b; Chloroplast assembly protocol, ORG.Asm and IOGA are not recommended to be used as the primary tools in organelle genome assembly projects.

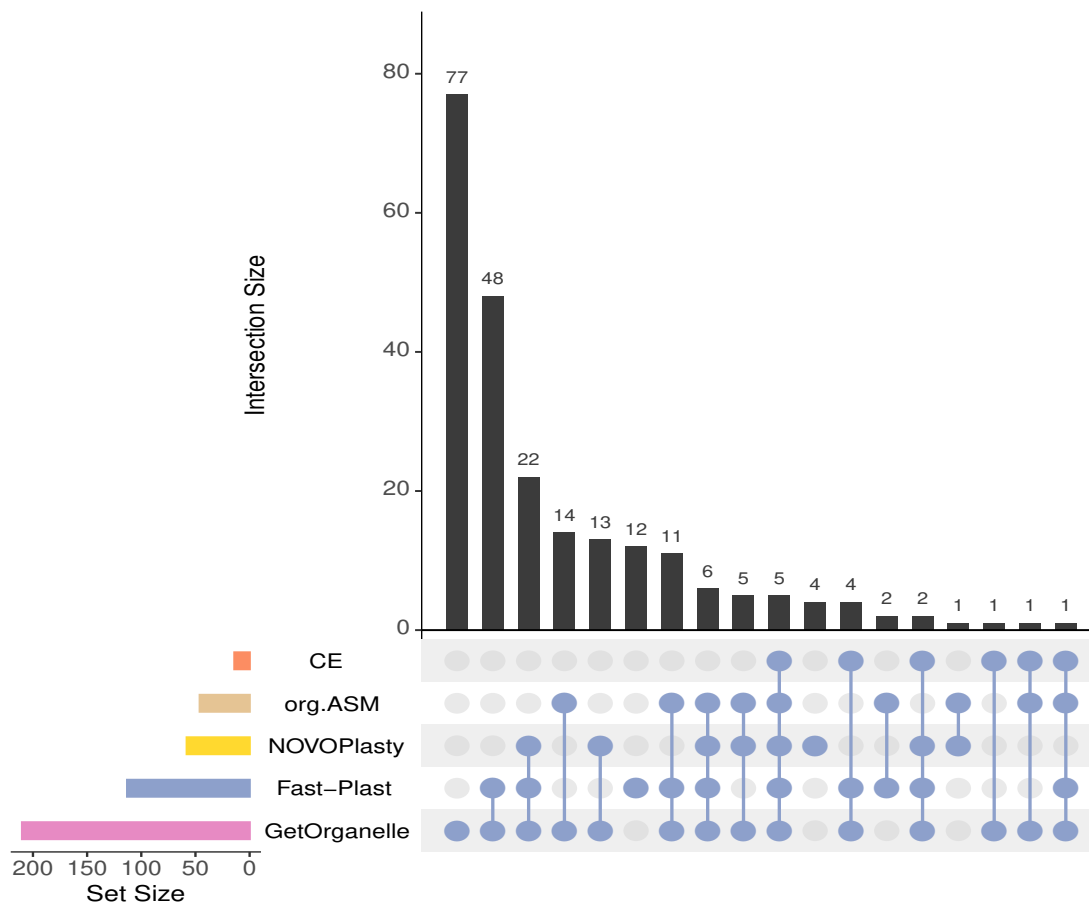


FIGURE 2.7: Upset plot showing the intersections of success rates between assemblers. A successful assembly was defined by a score >99 according to equation 2.1. The colored, horizontal barplot indicate the total number of successful assemblies for an individual tool. The black, vertical barplot gives the magnitude of the intersection between the assemblers indicated by the dots in the middle. Therefore the first and second vertical bars are to be interpreted as follows: 77 data sets were only successfully assembled by chloroExtractor, likewise 48 genomes were assembled completely by GetOrganelle and Fast-Plast and so on.

It might be possible that overall performance of a specific tool might change significantly by fine tuning the input parameters of the tool, which was purposely

not done in the scope of the present study because this study was designed to mimic the behavior of end-users and not developers of such tools. It is assumed that users with little experience in bioinformatics are inclined to use the basic configurations of such a tool.

While there are huge differences between all assemblers, they are presented with the same challenges and the bottlenecks are similar for all of them. However, the success rate of passing those differs. Figure 2.8 shows the alignment of the genomes, assembled with the seven tools, of *Oryza brachyantha*, a grass distantly related to cultivated rice *Oryza sativa*, and the respective reference genome. For the need of a linear representation of the circular genome the convention is to present chloroplast genomes in the order LSC - IRa - SSC -IRb. *O. brachyantha* was chosen because multiple tools successfully or at least almost assembled the full genome. Chloroplast assembly protocol is singled out, which only managed to assemble a few fragments on the SCC and the IRs on many contigs. A common mistake is to return 3 contigs as IOGA did. They represent the LSC the SSC and one IR but failed to resolve those regions into a one circular contig. GetOrganelle and Fast-Plast were able to reproduce the structure of the reference, while chloroExtractor flipped the LSC and NOVOPlasty and ORG.Asm were not able to construct the single contig into the conventional structure. All of these are common mistakes appearing more or less rare in all the assemblers. This could be a good starting point for the developers to further improve their tools. In this example all but Chloroplast assembly protocol were able to construct all the parts of the chloroplast's genome, and the most common error was to resolve the structure of the genome into a circular, one contig version.

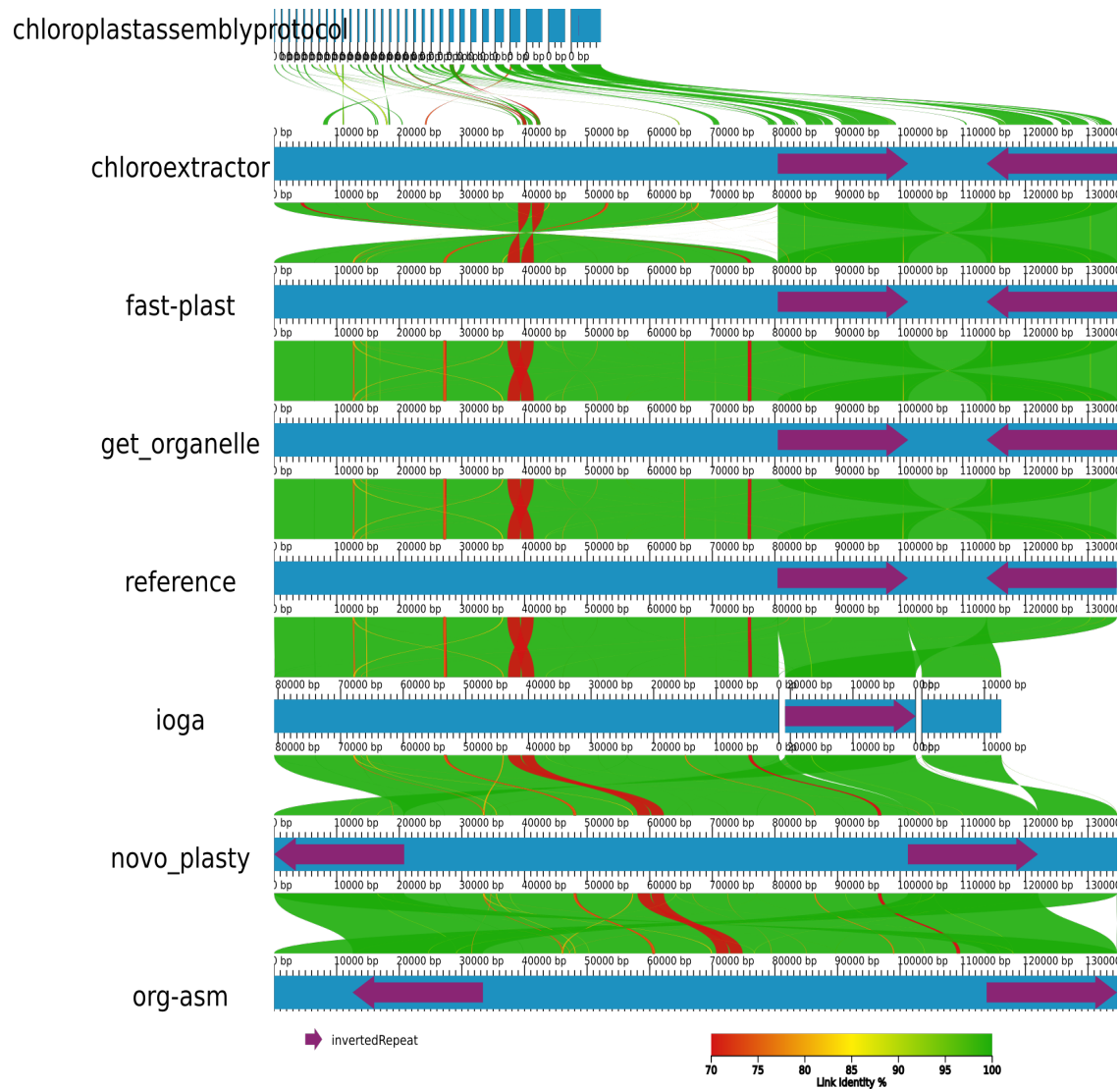


FIGURE 2.8: AliTV plot ANKENBRAND et al., 2017 from FREUDENTHAL et al., 2019b showing the alignments of *Oryza brachyantha* chloroplast genomes for all seven assemblers. Regions in adjacent assemblies are connected with colored ribbons. The color codes of for the similarity between regions. The purple arrows indicate the IR regions

2.5 Conclusion & outlook

Organelle genomics is a promising field in plant genetics. As described in section 2.1 chloroplast genomes are well-suited for applications in evolutionary sciences, taxonomy and barcoding applications. Alike for its mother branch core

genomics, for comparative chloroplast genomics it is just as crucial to obtain high quality genomes. The quality is mainly influenced by two factors: the quality of the genome sequencing protocol and the quality of the assembly. As shown the latter varies massively between tools and not all tools are recommend equally from the conclusions drawn from the experiments described above. All tools have room for improvement. This is not meant to criticize the respectable work of the developers, but to encourage them to further develop tools and publish them under terms of liberal software licenses for the greater benefit of the entire scientific community.

3 GWAS-Flow a gpu-accelerated software for large-scale genome-wide association studies

The following chapter has been published in a similar version on the bioRxiv preprint server FREUDENTHAL et al., 2019a and has been submitted for publication. The experiments and the software were designed and conducted by the author. The manuscript has been prepared by the author, with minor corrections from Prof. Arthur Korte & Prof. Dominik Grimm. All authors approved of the final manuscript.

3.1 Introduction

Genome-wide association studies, pioneered in human genetics HIRSCHHORN and DALY, 2005 have become the predominant method to detect associations between phenotypes and the genetic variations present in a population, in the last decade. Understanding the genetic architecture of traits and mapping the underlying genomic polymorphisms is of paramount importance for successful breeding, both in plants and animals, as well as for studying the genetic risk factors of diseases. Over the last decades the costs for genotyping have been reduced dramatically. Early GWAS consisted of a few hundred individuals, which have been phenotyped and genotyped on a couple of hundreds to thousands of genomic markers. Nowadays marker densities for many species easily exceed millions of genomic

polymorphisms. Albeit commonly SNPs are used for association studies, standard GWAS models are flexible to handle different genomic features as input.

The *Arabidopsis* 1001 genomes project features for example 1135 sequenced *Arabidopsis thaliana* accessions with over 10 million genomic markers that segregate in the population ALONSO-BLANCO et al., 2016. Other genome projects also yielded large amounts of genomic data for a substantial amount of individuals, as exemplified in the 1000 genomes project for humans SIVA, 2008, the 2000 yeast genomes project or the 3000 rice genomes project LI, WANG, and ZEIGLER, 2014. Thus, there is an increasing demand for GWAS models that can analyze these data in a reasonable time frame.

One critical step of GWAS is to determine the threshold at which an association is termed significant. Classically the conservative Bonferroni threshold is used, which accounts for the number of statistical tests that are performed, while many recent studies try to set significance thresholds that are based on the false-discovery rate (FDR) STOREY and TIBSHIRANI, 2003. An alternative approach to determine permutation-based thresholds CHE et al., 2014. Permutation-based thresholds estimate the significance by shuffling phenotypes and genotypes before each GWAS run, thus any signal left in the data should not have a genetic cause, but might represent model mis-specifications or uneven phenotypic distributions. Typically this process is repeated hundreds to thousands of times and will lead to a distinct threshold for each phenotype analyzed TOGNINALLI et al., 2017. The computational demand of permutation-based thresholds is immense, as per analysis not one, but at least hundreds of GWAS need to be performed. Here the main limitation is the pure computational demand. Thus, faster GWAS models could easily make the estimation of permutation-based thresholds the default choice.

3.2 Methods

GWAS Model

The GWAS model used for GWAS-Flow is based on a fast approximation of the linear-mixed-model described in KANG et al., 2010; ZHANG et al., 2010, which estimates the variance components σ_g and σ_e only once in a null model that includes the genetic relationship matrix but no distinct genetic markers. These components are thereafter used for the tests of each specific marker. Here, the underlying assumption is that the ratio of these components stays constant, even if distinct genetic markers are included into the GWAS model. This holds true for nearly all markers and only markers, which possess a big effect will alter this ratio slightly, where now σ_g would become smaller compared to the null model. Thus, the p-values calculated by the approximation might be a little higher (less significant) for strongly associated markers.

The GWAS-Flow Software

The GWAS-Flow software was designed to provide a fast and robust GWAS implementation that can easily handle large data and allows to perform permutations in a reasonable time frame. Traditional GWAS implementations that are implemented using Python VAN ROSSUM and DRAKE JR, 1995 or R R CORE TEAM, 2019 cannot always meet these demands. We tried to overcome those limitations by using TensorFlow, a multi-language machine learning framework published and developed by Google ABADI et al., 2015. GWAS calculations are composed of a series of matrix computations that can be highly parallelized and easily integrated into the architecture provided by TensorFlow. Our implementation allows both the classical parallelization of code on multiple processors (CPUs) and the use of graphical processing units (GPUs).

GWAS-Flow is written using the Python TensorFlow API. Data import is done with *pandas* MCKINNEY, 2010 and/or *HDF5* for Python COLLETTE, 2013. Preprocessing

of the data (e.g filtering by minor Allele count (MAC)) is performed with *numpy* OLIPHANT, 2006. Variance components for residual and genomic effects are estimated with a function slightly altered from the Python package *limix* LIPPERT et al., 2014. The GWAS model is based on the following linear mixed model that takes into account the effect of every marker with respect to the kinship:

$$Y = \beta_0 + X_i\beta_i + u + \epsilon, u \sim N(0, \sigma_g K), \epsilon \sim N(0, \sigma_e I) \quad (3.1)$$

From this LMM the residual sum of squares for marker i are calculated as described in 3.2

$$RSS_i = \sum Y - (X_i\beta_0 + I_i\beta_1) \quad (3.2)$$

The residuals are used to calculate a p-value for each marker according to an overall F-test, which compares the model including a distinct genetic effect to a model without this genetic effect:

$$F = \frac{RSS_{env} - R1_{full}}{\frac{R1_{full}}{n-3}} \quad (3.3)$$

Apart from the p-values that derive from the F-distribution, GWAS-Flow also reports summary statistics, such as the estimated effect size (β_i) and its standard error for each marker.

Calculation of permutation-based thresholds for GWAS

To calculate a permutation-based threshold essentially n repetitions ($n \geq 100$) are computed of the GWAS on the same data with the sole difference that before each GWAS phenotypic values are randomized. Thus any correlation between the phenotype and the genotype will be broken and indeed for over 90% of these analyses the estimated pseudo-heritability is close to zero. On the other hand, the

phenotypic distribution will stay unaltered by this randomization. Hence any remaining signal in the GWAS has to be of a non-genetic origin and could be caused by e.g. model mis-specifications. Now the lowest p-value (after filtering for the desired minor allele count) is taken for each permutation and the 5% lowest value is set as the permutation-based threshold for the GWAS.

Benchmarking

For benchmarking of GWAS-Flow data from the *Arabidopsis* 1001 Genomes Project ALONSO-BLANCO et al., 2016 was used. The genomic data used were subsets of the full data set containing between 10,000 and 100,000 markers. Subsets that exceed 100,000 markers were not included because there is a linear relationship between the number of markers and the computational time demanded, as all markers are tested independently. Phenotypic data for flowering time at ten degrees (FT10) for *A. thaliana* was used, downloaded from the AraPheno database SEREN et al., 2016. Down- and up-sampled sets were generated to obtain phenotypes for sets between 100 and 5000 accessions. For each set of phenotypes and markers 10 permutations were run to assess the computational time necessary.

All analyses have been performed with a custom R script that has been used previously TOGNINALLI et al., 2017, GWAS-Flow using either a CPU or a GPU architecture and GEMMA ZHOU and STEPHENS, 2012. GEMMA is a fast and efficient implementation of the mixed model that is broadly used to perform GWAS. All calculations were run on the same machine using 16 i9 virtual CPUs. The GPU version ran on an NVIDIA Tesla P100 graphic card. Additionally to the analyses of the simulated data, the times required by GEMMA and both GWAS-Flow implementations for > 200 different real data sets from *A. thaliana* were compared, which also have been downloaded from the AraPheno database SEREN et al., 2016 and have been analyzed with the available fully imputed genomic data set of ca. 10 million markers, filtered for a minor allele count greater five.

3.3 Results

The two main factors influencing the computational time for GWAS are the number of markers incorporated in such an analysis and the number of different accessions, while the latter has an approximate quadratic effect in classical GWAS implementations ZHOU and STEPHENS, 2012. Figure 3.1 shows the time demand as a function of the number of accessions used in the analysis with 10,000 markers. Exponential increases in the time demand are clearly visible for the custom R implementation, as well as for the CPU-based GWAS-Flow implementation and *GEMMA*. The GWAS-Flow implementations and *GEMMA* clearly outperform the R implementation in general. For a smaller number of accessions GWAS-Flow is slightly faster than *GEMMA*.

For the GPU-based implementation the increase in run-time with larger sample sizes is much less pronounced. While for small ($< 1,000$ individuals) data there is no benefit compared to running GWAS-Flow on CPUs or running *GEMMA*, the GPU-version clearly outperforms the other implementations if the number of accessions increases.

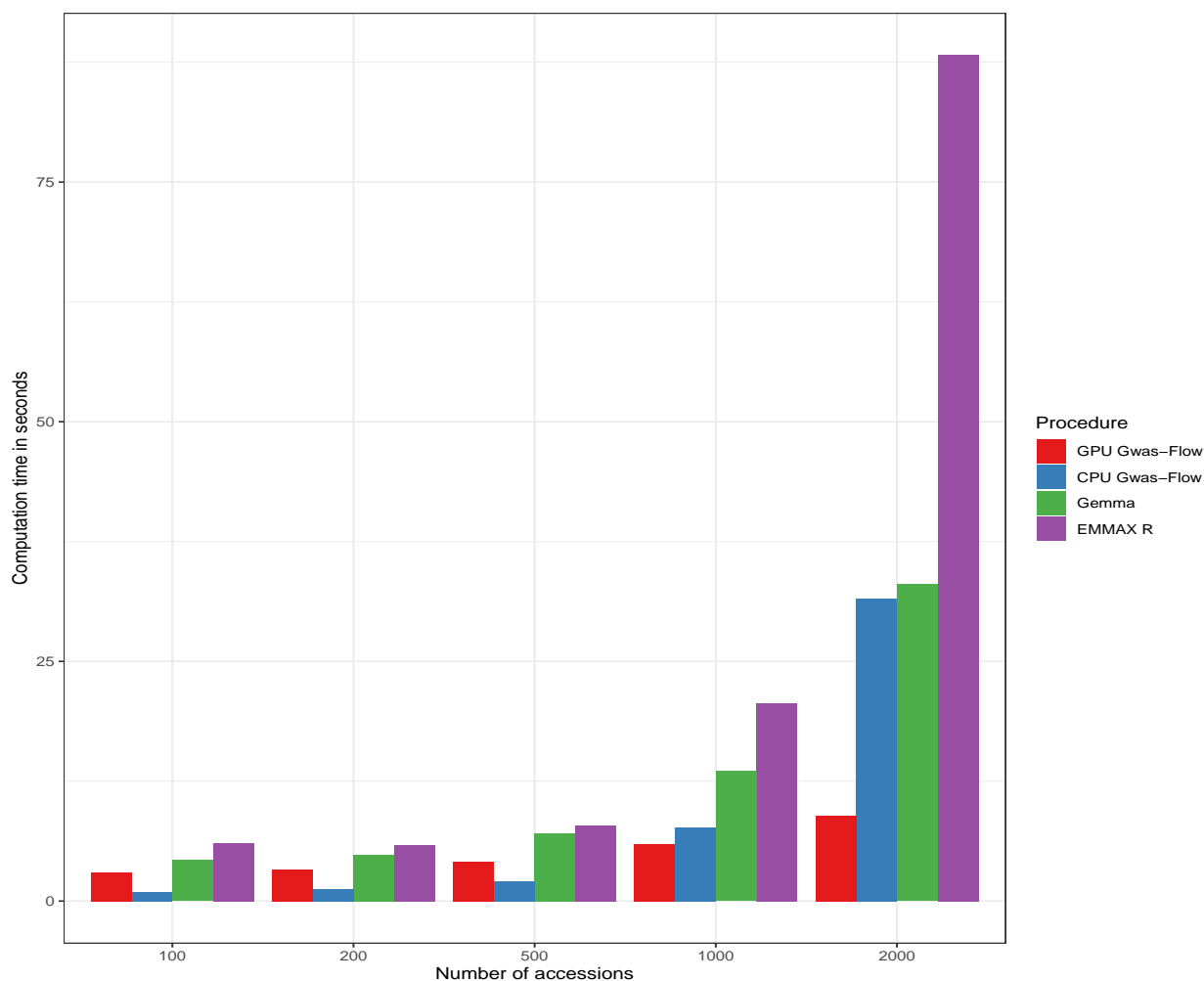


FIGURE 3.1: Computational time as a function of the number of accessions with 10000 markers each.

Figure 3.2 shows the computational time in relation to the number of markers with a fixed population of 2000 accessions for the two different GWAS-Flow implementations. Here, a linear relationship is visible in both cases.

To show the performance of GWAS-Flow not only for simulated data, both implementations were also run on more than 200 different real data sets of *A. thaliana*.

Figure 3.3 shows the computational time demands for all analyses comparing both GWAS-Flow implementations to GEMMA. Here, the CPU-based GWAS-Flow performs comparable to GEMMA, while the GPU-based implementation outperforms both if the number of accessions is above 500. Importantly all obtained

GWAS results (p-values, beta estimates and standard errors of the beta estimates) are nearly (apart from some mathematical inaccuracies) identical between the three different implementations.

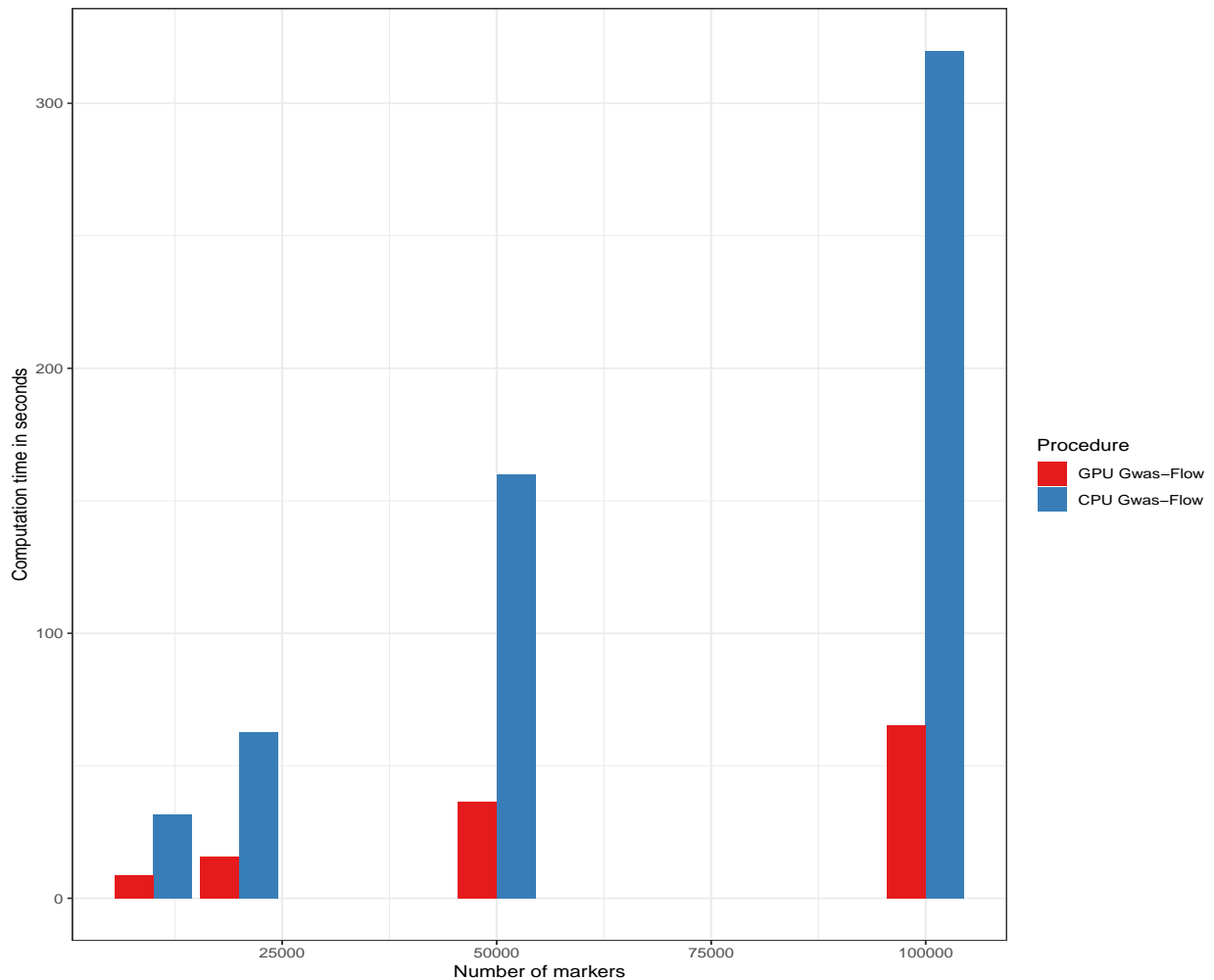


FIGURE 3.2: Computational time as a function of the number of genetic markers with constantly 2000 accessions for both GWAS-Flow versions

3.4 Discussion

To cope with the increasing computational demand in analyzing large GWAS data sets, recent developments of computational architecture and software were utilized to develop GWAS-Flow. With GWAS-Flow both a CPU- and a GPU-based

version of the classical linear mixed model, commonly used for GWAS, is provided. Both implementations outperform custom R scripts on simulated and real data. While the CPU-based version performs nearly identical compared to *GEMMA*, a commonly used GWAS implementation, the GPU-based implementation outperforms both, if the number of individuals, which have been phenotyped, increases. For analyzing big data, here the main limitation would be the RAM of the GPU, but as the individual test for each marker is independent, this can be easily overcome programmatically.

The presented GWAS-Flow implementations are markedly faster compared to custom GWAS scripts and even outperform efficient fast implementations like *GEMMA* in terms of speed. This readily enables the use of permutation-based thresholds, as with GWAS-Flow hundred permutations can be performed in a reasonable time frame even for big data. Thus, it is possible for each analyzed phenotype to create a specific, permutation-based threshold that might present a more realistic scenario. Importantly the permutation-based threshold can be easily adjusted to different minor allele counts, generating different significance thresholds depending on the allele count. This could help to distinguish false and true associations even for rare alleles.

GWAS-Flow is a versatile and fast software package and currently is and will remain under active development to make the software more versatile. This will e.g. to include the compatibility with TensorFlow v2.0.0 and to enable data input formats, such as PLINK PURCELL et al., 2007. The whole framework is flexible, so it is easy to include predefined co-factors e.g. to enable multi-locus models SEGURA et al., 2012 or account for multi-variate models like the multi-trait mixed model KORTE et al., 2012.

Standard GWAS are good in detecting additive effects with comparably large effect sizes, but lack the ability to detect epistatic interactions and their influence on complex traits MCKINNEY and PAJEWSKI, 2012; KORTE and FARLOW, 2013. To catch the effects of these gene-by-gene or SNP-by-SNP interactions, a variety

of genome-wide association interaction studies (GWAIS) have been developed, thoroughly reviewed in RITCHIE and VAN STEEN, 2018. Here, GWAS-Flow might provide a tool that enables to test the full pairwise interaction matrix of all SNPs. Although this would be a statistic nightmare, it now would be computationally feasible.

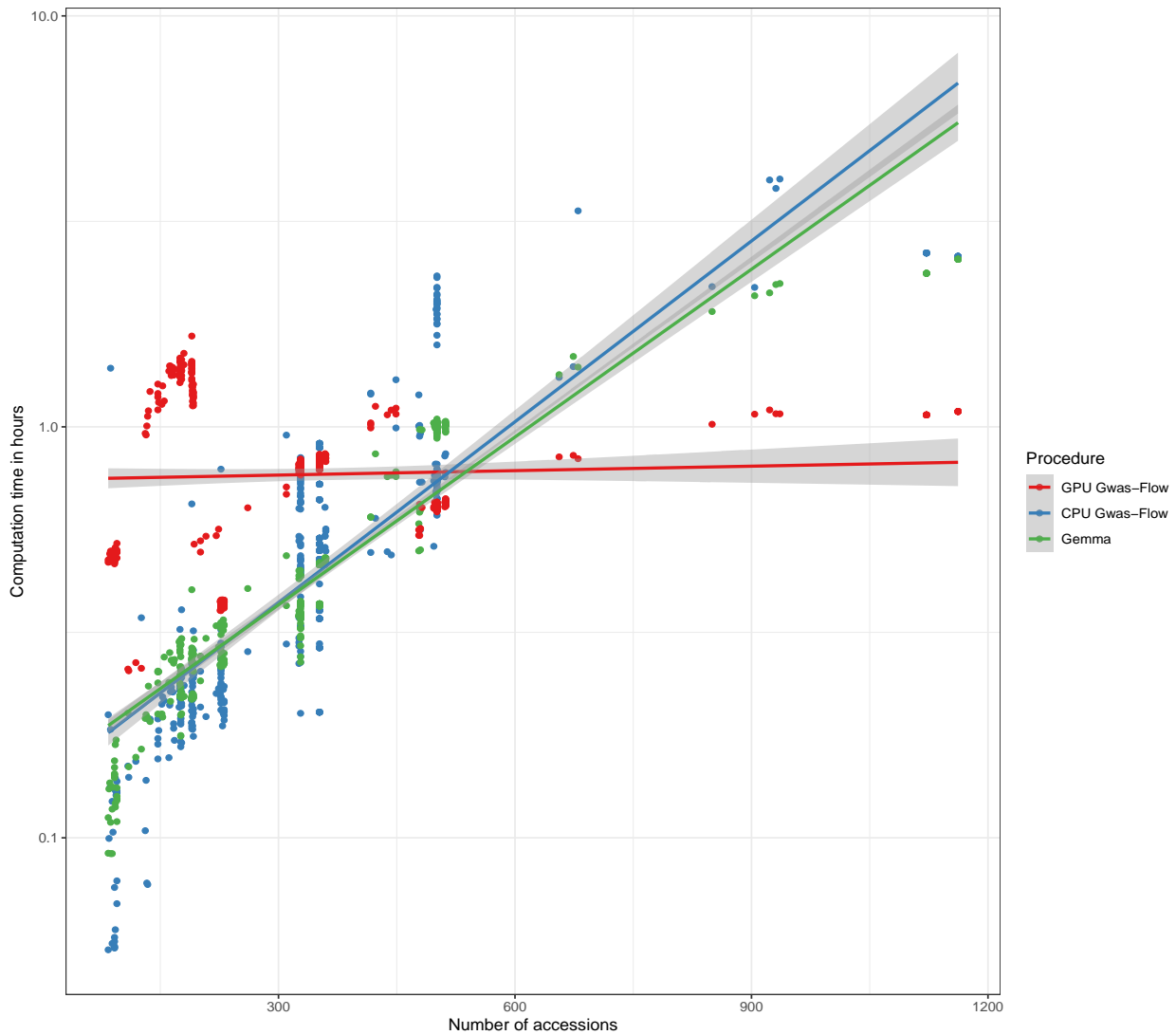


FIGURE 3.3: Comparison of the computational time for the analyses of > 200 phenotypes from *Arabidopsis thaliana* as a function of the number of accessions for GEMMA and the CPU- and GPU-based version of GWAS-Flow. GWAS was performed with a fully imputed genotype matrix containing 10.7 M markers and a minor allele filter of $MAC > 5$

4 Genomic prediction of phenotypic values of quantitative traits using artificial neural networks

4.1 Introduction to machine learning

4.1.1 A brief history of machine learning

The basic perceptron model

While machine learning, neural networks and deep learning became essential tools for many applications in more recent years, their mathematical principals date back to the early 1950s and 1960s. Figure 4.1 schematically shows the basic perceptron model as proposed by Rosenblatt, one of the founders of machine learning, as the set of related statistical algorithms would be defined today. Rosenblatt designed his perceptron to mimic the information flow in biological nervous systems ROSENBLATT, 1961.

This basic perceptron, which contrary to perceptrons used nowadays, does not have an embedded activation function, takes n binary inputs x_1, x_2, \dots, x_n and produces a single, likewise binary, output y after being processed. To achieve this Rosenblatt introduced the concept of weights, which determine a certain input's relative importance to the outcome of the output w_1, w_2, \dots, w_n . The output y is determined by the weighted sum of the weights $\sum_i w_i x_i$. If a certain threshold

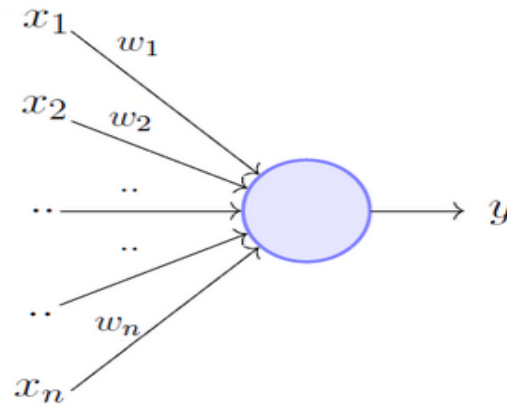


FIGURE 4.1: Basic perceptron model as proposed by Rosenblatt
ROSENBLATT, 1961

value is met the neuron is either activated and outputs 1 or not activated resulting in and output of 0. This is algebraically represented in equation 4.1:

$$0 = \text{if } \sum_i^n w_i x_i - \theta \leq 0 \quad (4.1a)$$

$$1 = \text{if } \sum_i^n w_i x_i - \theta > 0 \quad (4.1b)$$

Next to the weights w_n and the inputs x_n , a third term θ is introduced in equation 4.1, which represents the activation threshold. A single perceptron is a linear classifier and can only be trained on linearly separable functions and can be applied, as shown by ROSENBLATT, 1961, to solve simple logical operations as AND, OR and NOT. The basic perceptron fails, however, due to non-linearity to perform XOR operations, which was proven by MARVIN and SEYMOUR, 1969. This discovery led to a near stillstance in the research of artificial neural networks in the 1970s. That time period is now often referred to as the first AI-winter. Another reason that massively hindered the applications and research of machine learning during that span was the, compared to modern times, incredibly small

amount of computational power available NGUYEN and WIDROW, 1990.

More complex decision making, like solving XOR problems, requires more complex structures than a single perceptron can provide. Continuing the trend of mimicking human neural networks, multiple artificial neurons were stacked into layers and these layers were connected to each other allowing communication between the many perceptrons in such a network. Figure 4.2 schematically shows the basic structure of an artificial neural network, now harboring three types of layers.

- (i) the input layer
- (ii) one or more hidden layers
- (iii) the output layer, which in this case only consists of only one neuron

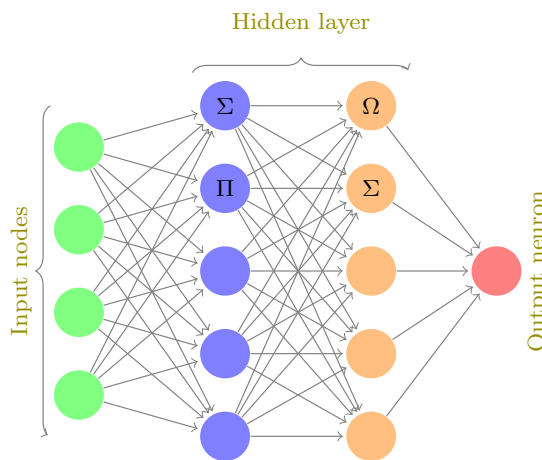


FIGURE 4.2: Schematic layout of a simple multi-layer perceptron

In the sample layout of figure 4.2 the neurons in the first column weigh the inputs and pass the gathered information to the neurons into the second layer. In the case above neurons in the first layer are connected to all neurons on the second layer. Such layers are referred to as fully-connected layers (FLC) and their resulting networks are often called multi-layer perceptrons (MLP) or fully-connected

networks. This architecture enables the network to perform more complex calculations resulting in more abstract decision making than single neurons or single layer architectures.

There are other architectures, where neurons in the previous layer are only connected with neighboring neurons in the succeeding layers. Those are known as locally-connected layers (LCL). Related to them are convolutional layers which share weights between selected neurons, building convolutional neural networks (CNN) LECUN et al., 1999.

Activation functions

The neurons discussed so far are only capable of outputting binary results, depending on whether threshold values are being reached or not. For more complex estimations it is desirable that small changes in the input also result in small changes of the output. This requirement cannot be easily met with binary outputs. Activation functions for a given node provide more sophisticated rules for the output in accordance to their inputs ŽILINSKAS, 2006.

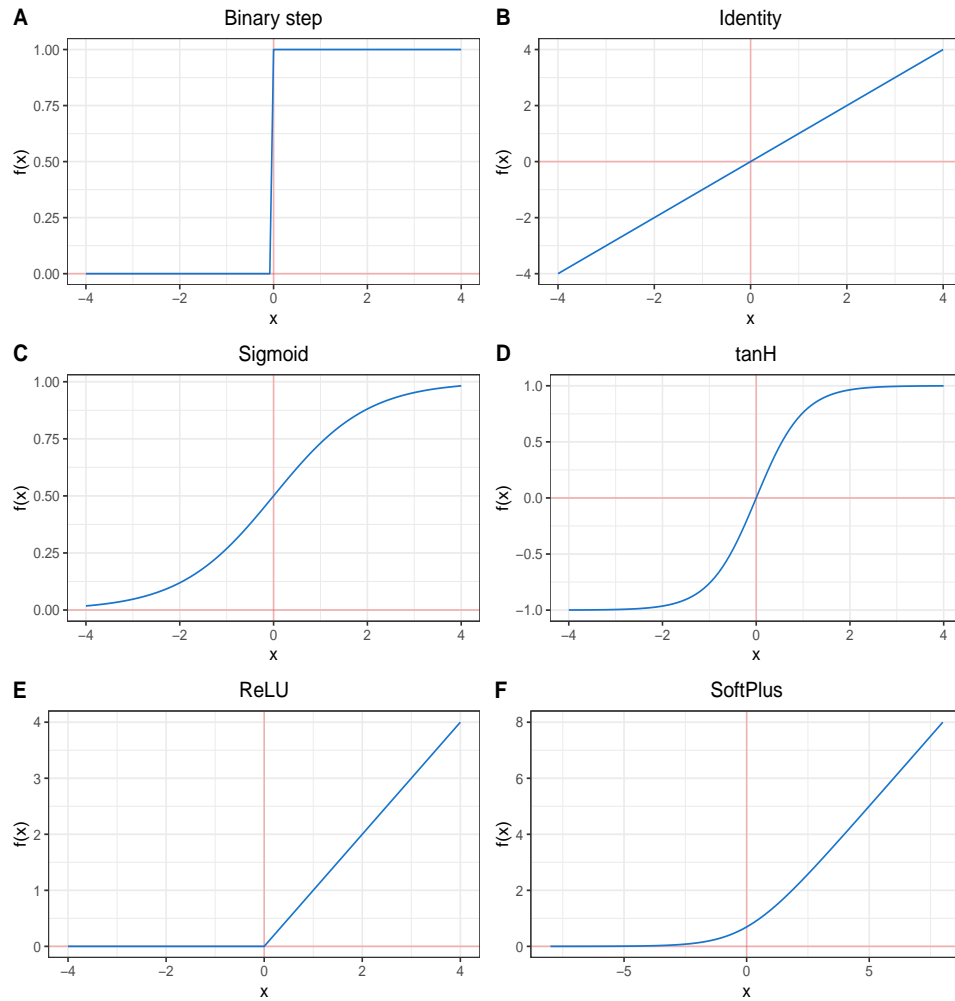


FIGURE 4.3: Popular activation functions used in neural networks.

A Binary step activation function**B** Identity activation function**C** Sigmoid or logistic activation function**D** tangens hyperbolicus activation function**E** rectified linear units activation function**F** SoftPlus activation function

Figure 4.3 shows six of the most commonly used activation functions WARNER and MISRA, 1996. The simplest one, the binary step activation was already introduced (function A in equation 4.2), which properties have been discussed along the perceptron model. All other activation functions produce continuous outputs from given inputs.

Next to a binary step function any mathematical function is able to serve as an

activation function in neural nets, starting with a simple identity function (equation 4.3, figure 4.3 B). The sigmoid function (figure 4.3 C, equation 4.4) and tanh (figure 4.3 D, equation 4.5), when $x \rightarrow \infty$ or $x \rightarrow -\infty$ have similar properties as the binary function, but produce continuous output around threshold values of 0.

$$f(x) = \sigma(x) = \begin{cases} 0 & \text{for } x < 0 \\ 1 & \text{for } x \geq 0 \end{cases} \quad (4.2)$$

$$f(x) = \sigma(x) = x \quad (4.3)$$

$$f(x) = \sigma(x) = \frac{1}{1 + e^{-x}} \quad (4.4)$$

$$f(x) = \sigma(x) = \frac{e^x - e^{-x}}{e^x + e^{-x}} \quad (4.5)$$

$$f(x) = \sigma(x) = \begin{cases} 0 & \text{for } x < 0 \\ x & \text{for } x \geq 0 \end{cases} \quad (4.6)$$

$$f(x) = \ln(1 + e^x) \quad (4.7)$$

ReLU (equation 4.6) and the SoftPlus (equation 4.7) share similar properties as well, the latter one being a smoothed version of ReLU. Rectifiers as activation functions have been introduced in the 2000s HAHNLOSER et al., 2000 and have since then overtaken all others as the most popular activation functions in neural networks and deep learning LECUN, BENGIO, and HINTON, 2015. They have proven to be superior in many deep-learning applications over sigmoid or logistic functions. One of the advantages leading to the superiority of ReLUs is that with randomly initialized weights only half of the ReLU neurons are activated at start compared to tanh and sigmoid activation GLOROT, BORDES, and BENGIO,

2011. All activation functions shown in figure 4.3, but the binary step function, share one common property: a small change of the input weight will result in small changes of the output, while a small change of the input for the binary step function leads to either no or a complete change of the output, except for ReLU when $x < 0$. This property is, as described below, is an important prerequisite for networks being able to learn.

Gradient descent algorithm

Let a network alike the one shown in figure 4.2 be designed for the classification of an arbitrary phenotype like blue petals with $x_1 \dots x_4$ on the input layers being genetic markers as features. The output layer displays value from 0 to 1 giving the probability of the petals being blue or not. To quantify how well the network performs on predicting the color of the petals a loss function is applied SCHMID-HUBER, 2015.

There is a large variety of different loss functions available for neural networks like mean squared error (MSE), root mean squared error (RMSE) and cross-entropy among others. In general MSE and RMSE are used for regression problems, with the latter being less popular, and cross-entropy also called log-loss is used for binary or multi-class classification settings JANOCHA and CZARNECKI, 2017. Since all problems presented in due course are regression problems that use MSE as their loss function this will be the only loss function further elaborated upon in. MSE or the quadratic loss function can be written as:

$$MSE = \frac{1}{n} \sum_{i=1}^n (y_i - \hat{y}_i)^2 \quad (4.8)$$

Equation 4.8 shows the MSE function which is the sum of the squares of the differences of all the predicted \hat{y} and the real values y_i . The same function can be

rewritten with the previously used terminology of weights and biases in equation 4.8 with $L(w, b)$ as the loss.

$$L(w, b) = \frac{1}{2n} \sum_x \|y(x) - y\|^2. \quad (4.9)$$

With w and b as the collection of all the weights and the biases in the network used to optimize the function $y(x)$. Giving the quadratic nature of the function $L(w, b)$ will always be non-negative. If $L(w, b) \rightarrow 0$ the loss is minimal, meaning that the real and predicted values are close together and the network found weights and biases that approximate the output well.

A widely used function to find the optimum for such a loss function by finding its minimum is gradient descent (GD) BOTTOU, 1991. The idea behind GD or other optimization algorithms is to start with randomly initialized weights and biases and repeatedly move them in direction Δw and Δb . This results in a change of the loss function which can be represented using partial derivatives as shown in equation 4.10.

$$\Delta L = \frac{\partial L}{\partial w} \Delta w + \frac{\partial L}{\partial b} \Delta b \quad (4.10)$$

Ideally ΔL is negative and the optimization algorithm found Δw and Δb that lead to a reduction of the loss. To simplify this problem let Δd be the vector of changes: $\Delta d = (\Delta w, \Delta b)^T$ and ∇L the vector of the partial derivatives as in equation 4.11.

$$\nabla L = \left(\frac{\partial L}{\partial w}, \frac{\partial L}{\partial b} \right)^T \quad (4.11)$$

Having defined ∇L and Δd the term 4.10 can be simplified to:

$$\Delta C = \nabla L * \Delta d \quad (4.12)$$

Now the task of gradient descent is to find Δd that results in ΔC being negative as shown in equation 4.13

$$\Delta d = -\eta \nabla L \quad (4.13)$$

Here η is a small positive decimal number, commonly referred to as the learning rate, which usually, but not exclusively, ranges from 0.1 to 0.001. Having found a way to ensure that ΔL always is negative according to equation 4.13 it can be utilized to repeatedly update the gradient ∇L over time steps T . To make the gradient descent algorithm efficient the learning rate η has to be chosen correctly. If η is too large the gradient ΔL possibly ends up being larger than zero leading to an increase of the loss. If the learning rate is too small convergence will either take too long or not take place at all BERGSTRA et al., 2011. In practical machine learning approaches different learning rates are tested. There are also algorithmic approaches to select the learning rate available. Equation 4.11 only accounts for two inputs features but it can be generalized to compute n inputs as shown in equation 4.14.

$$\nabla L = \left(\frac{\partial L}{\partial w_1}, \dots, \frac{\partial L}{\partial w_n} \right)^T \quad (4.14)$$

Equation 4.15 shows the gradient descent how it is used to repetitively update the weights and biases to optimize the loss function $L(c, w)$ with w and b as the weight and bias matrices and the learning rate η . In machine learning each iterational update of the network is often called epoch or training epoch.

$$w = w_i - \eta \frac{\partial}{\partial w} L(w) \quad (4.15a)$$

$$b = b_i - \eta \frac{\partial}{\partial b} L(b) \quad (4.15b)$$

Substituting the partial differentials with ∇L equation 4.15 a and b likewise simplifies to:

$$w = w_i - \eta \nabla L \quad (4.16)$$

Optimizers

The previous section introduced the concept of gradient descent, an algorithm to minimize the loss function of the weights and biases of a neural network. All other optimizers introduced in the following chapter are either variations or extensions of the basic gradient descent algorithm shown in equation 4.15.

One disadvantage of gradient descent is that if the data set grows larger the demand in memory for computation increases exponentially. Taking into consideration that machine learning is a popular method in big data applications this is a serious drawback.

Methods to solve that issue are stochastic gradient descent and mini batch gradient descent. The idea behind the latter is to randomly divide the entity of the training data into subsamples called mini batches BOTTOU and BOUSQUET, 2008. The network is then trained iteratively with all mini batches. The batch size has a significant influence on the accuracy and the training speed of the network and is a hyperparameter, which has to be tuned by iteratively testing different settings. If the batch size is one mini batch GD is also referred to as stochastic gradient descent (SGD).

During the optimization process optimizers can descent into local minima of the cost function without being able to overcome them enabling to reach the global minimum. An algorithm extending GD to accelerate the search of the global minimum is momentum, which allows the GD to speed up when the loss is decreasing and to carry on even when the loss function $L(w, b)$ is temporally increasing. This is achieved by accounting for the gradient of the previous step in the

calculation of the current step. This concept was introduced by POLYAK, 1964 and re-popularized alongside the introduction of backpropagation learning by RUMELHART, HINTON, and WILLIAMS, 1988 an algorithm to efficiently update the weights and biases.

$$w = w_i - \eta \nabla L + \alpha \Delta w \quad (4.17)$$

Equation 4.17 shows how the momentum is mathematically represented in GD to update the weights w or likewise the biases. The delta of the weights multiplied by an coefficient α is the momentum. α is usually ranges from 0.1 to 0.9 and is another parameter to be tuned for successful training. If the momentum is too small the GD will not be able to overcome local minima and if α is too large the loss functions tends to oscillate without ever finding an optimum LE-CUN, BENGIO, and HINTON, 2015.

For both the momentum and the learning rate it is impractical to maintain the same level during all training epochs. After each epoch the loss function is either closer or further away from its global minima and depending on the distance to that minimum it is desirable to have larger or smaller learning rates and momenta. This can be achieved with naive approaches, for example using a step function to gradually decrease those values after each iteration, or to utilize algorithmic approaches MICHIE, SPIEGELHALTER, and TAYLOR, 1994. There is a large variety of optimizers trying to find optimal values for α and η and till today this field is under active research GOODFELLOW, BENGIO, and COURVILLE, 2016. Popular among those are: RMSprop HINTON, SRIVASTAVA, and SWERSKY, 2012; Nesterov momentum DOZAT, 2016; Adadelata ZEILER, 2012; Adagrad RUDER, 2016 and Adam KINGMA and BA, 2014. With Adam being the most popular optimizer today.

Nesterov momentum is a slight change to the normal momentum algorithm, capable of having huge impacts in practical applications, because it helps avoiding

oscillations around the minimum by using intermediate information to adapt the momentum.

RMSProp - root mean square propagation - is a method aiming to adapt the learning rate algorithmically, by choosing η for each iteration. Lastly, the wide-spread Adam optimizer combines both of the features of momentum and RMSProp and adapts the learning rate as well as the momentum iteratively KINGMA and BA, 2014.

Regularization parameters and overfitting

A common problem in machine learning is over parameterize the model on the training data and losing the ability to generalize on validation data. This issue occurs because neural networks have hundreds of thousand of free parameters to be trained. Deeper neural networks even have billions or trillions of parameters. If training of the neural net continues for enough epochs eventually the loss function will approach a minimum. As $L(w, b) \rightarrow 0$ the initially drawn conclusion could mislead to assuming that training was successful. However, when trying to apply the trained network on the training data set (TRN) to a testing data set (TST) the loss of TST is very large and the accuracy of prediction of TST is accordingly small. This phenomenon is known as overfitting and a lot of fine tuning of hyperparameters is devoted to minimizing this effect TETKO, LIVINGSTONE, and LUIK, 1995. Figure 4.4 visualizes this effects during training GOODFELLOW, BENGIO, and COURVILLE, 2016 of a neural networks.

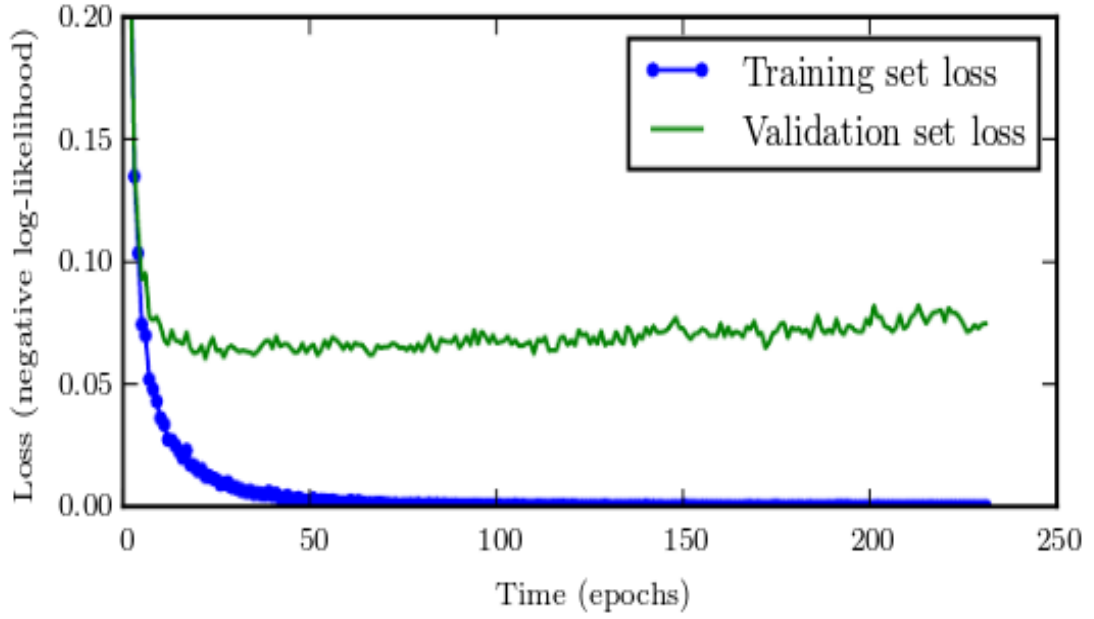


FIGURE 4.4: Learning curves showing how a loss function changes during training in the training and validation data set. While the training loss approaches 0 the validation loss starts increasing after hitting a minimum. This effect is due to overfitting on the training data set. Figure from GOODFELLOW, BENGIO, and COURVILLE, 2016.

Cross-validation

A method that is used in basically every training of neural networks is splitting up the data in multiple subsets. More specifically in a training set (TRN) and a testing set (TST). The training set is used to minimize the loss functions and its success is evaluated by comparison of the predicted values \hat{y} and the real values in TST y . For all neural nets in this study Pearson's correlation coefficients were chosen as performance metric, calculated according to equation 4.18 SOPER et al., 1917.

$$\rho(y, \hat{y}) = \frac{\text{cov}(y, \hat{y})}{\sigma_y \sigma_{\hat{y}}} \quad (4.18)$$

There are other popular performance metrics, especially for classification problems, like AUC (area under the curve) and ROC (receiver operating characteristics), which evaluate the success of learning by weighing sensitivity and specificity.

In cross-validation compared to single validation the initial data set is split into TRN and TST multiple times, e.g. if the ratio is 80:20 five times, and each TRN-TST pair is evaluated individually. Sometimes it is necessary to use a third subset - the validation data set. Because hyperparameter tuning is performed with the TRN and TST sets, a third portion of the data is needed to assess whether the neural network is able to generalize on global data or not.

4.2 Introduction to genomic selection

4.2.1 On the nature of quantitative traits

According to the omnigenic model, which is an extension of the polygenic model, proposed by BOYLE, LI, and PRITCHARD, 2017 and thoroughly reviewed in TIMPSON et al., 2018, all traits or phenotypic values are influenced by a great number or even all genes in the genome. This therefore results in traits following gradual statistical distributions instead of being binned in classes or binary.

Intuitively, this might be contradicting to the theoretical foundation of modern genetics - Mendel's three laws. They were derived from observations, which were mainly influenced by one locus. Using one of Mendel's phenotypes as an example - the round or wrinkled surfaces of peas (*Pisum sativum*)- an assessment of a couple of thousands peas would inevitably lead to the conclusion that from the "roundest" to the "wrinkliest" pea any gradual step between those two classes is possible and observable.

Mendel's third law of independent segregation also only holds true under certain assumptions. The simplest one being that the traits under investigation have to be located on different linkage groups. Otherwise the seven traits used in Mendel's initial studies would not have segregated independently. The odds of seven randomly selected traits being on seven different linkage groups are rather small, especially taking into account that the genome of the *P. sativum* consists of only seven chromosomes itself KALO et al., 2004. Mendel most likely knew about traits not following his own laws, as well as being aware of the quantitative nature of traits such as the constitution of pea's surface or the color of its petals. However, being the pioneer of a then rather unexplored field of science, some of which big questions we fail to satisfactory answer today, he did not have the resources or the knowledge to explain traits that were not "mendeling".

Initially thought to be contradicting to Mendel's ideas, Darwin proposed the concept of evolution due to natural selection, which introduced the idea of traits following a gradual distributions DARWIN, 1859. This contrast led to a long lasting debate in the scientific community in the early 1900s between the Mendelians and the biometricians, who believed in the quantitative nature of continuous traits. This conflict has eventually been solved by Fisher's fundamental work published in 1919 FISHER, 1919. His theories combined the then in all fields of science popular research on statistical distributions and genomics. He mathematically proved that traits influenced by many genes, with randomly-sampled alleles follow a continuous normal distribution in a population.

While this combined the ideas of Mendel and of the biometricians it opened another long debated question of effect sizes and the overall genetic architecture of complex traits. While in the theory of monogenic traits the effect size of the single gene on the trait is 100%, with an increasing number of genes influencing a complex trait the *per se* contribution of single genes has to decrease with an increasing number of loci determining the value of a given trait. Until the 1990s it has been believed that complex traits are predominantly controlled by few genes with a large to medium effect size, while others supposedly have a minimal influences ZHANG et al., 2018.

With the upcoming popularity of GWAS as the favored method to decipher genetic architectures of traits, having pioneered in human genetics, it became clear that the majority of effect sizes are tiny ($< 1\%$), while there are very few loci which have a moderate effect on the phenotypic variance of a population with around 10% or less KORTE and FARLOW, 2013; STRINGER et al., 2011. This nature of quantitative traits presents great challenges to animal GODDARD and HAYES, 2009 and plant breeding WÜRSCHUM, 2012 in further improving crop or livestock performances, as well as complicating the decomposition of genomic causes for diseases like schizophrenia or autism in human medicine DE RUBEIS et al., 2014; PURCELL et al., 2014.

While the complex nature of the architecture of quantitative traits provides enough challenges as is, all traits are also influenced by the environment surrounding the individual.

Therefore the distribution of trait values in a given population can be expressed as the addition of the variances of its genetic and the environmental effects 4.19.

$$\sigma_P = \sigma_G + \sigma_E \quad (4.19)$$

The genomic and the environmental effects do not only influence the phenotypic variance directly, but the environment also has an influence on gene expression, methylation of DNA bases etc. and therefore the equation 4.19 extends by the variance of the gene-environment interactions $\sigma_{G \times E}$ to equation 4.20 LYNCH and WALSH, 1998; WALSH and LYNCH, 2018b.

$$\sigma_P = \sigma_G + \sigma_E + \sigma_{G \times E} \quad (4.20)$$

Equation 4.20 shows the decomposition of the phenotypic variance. To thoroughly understand the complex genetic architectures of traits the genetic variance needs to be decomposed further in its additive, dominance and epistatic components as in equation 4.21.

$$\sigma_G = \sigma_A + \sigma_D + \sigma_I \quad (4.21)$$

The additive effects are caused by single, for this model mostly homozygous, loci while the variance caused by dominance effects is caused by heterozygous loci with their resulting interactions being full-, over-, co- or underdominant. Lastly, the interaction effects are a result of two or more genes only having an impact if they co-occur in a certain state. The resulting variance is commonly known as the gene-gene interaction or epistasis FALCONER and MACKAY, 1996.

Since possible interactions in a genome can appear between additive or dominant or a combination of those loci, the variance due to interaction effects σ_I can be further dissembled into the variances resulting from additive-additive σ_{AA} dominant-dominant σ_{DD} and additive-dominant σ_{AD} interactions as shown in equation 4.22.

$$\sigma_I = \sigma_{AxA} + \sigma_{DxD} + \sigma_{AxD} \quad (4.22)$$

Knowledge of the variance components involved in the expression of a trait in a given population leads up to the estimation of the total influence of all genetic variances and the environmental variance the phenotypic distribution. This concept is called heritability.

The heritability of a trait H^2 accounts for the proportion of the phenotypic variance controlled by the total genetic variance as shown in equation 4.23. This is also referred to as broad sense heritability, because all genetic effects, including additive, dominance and epistatic effects, are included BROOKER, 1999.

$$H^2 = \frac{\sigma_A + \sigma_D + \sigma_I}{\sigma_P} \quad (4.23)$$

The concept of narrow-sense heritability 4.24 is similar to the broad-sense heritability, but only the additive genetic effects are included in the equation. This differentiation is important for natural and artificial selection and thus is commonly used in evolutionary genomics and breeding. Because in diploid species each parent only passes down a single allele of a given locus, dominance effects or interaction effects are not commonly inherited from one parent. Therefore mainly the additive genetic effects of a parent influence its offspring. While the dominance and epistatic variances are controlled by the combination of the parents FALCONER and MACKAY, 1996, WALSH and LYNCH, 2018b.

$$h^2 = \frac{\sigma_A}{\sigma_P} \quad (4.24)$$

4.2.2 Artificial selection in plant and animal breeding in the genomics era

Introduction to genomic selection

Genomic prediction has been applied to almost all relevant crop and model species. This includes among others:

A.thaliana; SHEN et al., 2013; HU et al., 2015.

Alfalfa (*Medicago sativa*) LI and BRUMMER, 2012; ANNICCHIARICO et al., 2015; LI et al., 2015; BIAZZI et al., 2017; HAWKINS and YU, 2018.

Barley (*Hordeum vulgare*) ZHONG et al., 2009; OAKEY et al., 2016; NEYHART, LORENZ, and SMITH, 2019.

Cassava (*Manihot esculenta*) ELIAS et al., 2018a; ELIAS et al., 2018b. Cauliflower (*Brassica oleracea* spp) THORWARTH, YOUSEF, and SCHMID, 2018.

Cotton (*Gossypium* spp.) GAPARE et al., 2018.

Maize (*Zea mays*) RINCENT et al., 2012; WINDHAUSEN et al., 2012; TECHNOW, BÜRGER, and MELCHINGER, 2013; RIEDELSHEIMER et al., 2013; GUO et al., 2013; PEIFFER et al., 2014; TECHNOW et al., 2014; LEHERMEIER et al., 2014; OWENS et al., 2014; MONTESINOS-LÓPEZ et al., 2015; BUSTOS-KORTS et al., 2016a; KADAM et al., 2016; SCHOPP et al., 2017a; SCHOPP et al., 2017b; SOUSA et al., 2017; BRAUNER et al., 2018; SCHRAG et al., 2018; MOEINIZADE et al., 2019; ALLIER et al., 2019.

Potato (*Solanum tuberosum*) ENCISO-RODRIGUEZ et al., 2018; ENDELMAN et al., 2018.

Rape seed (*Brassica napus*) SNOWDON and INIGUEZ LUY, 2012; WÜRSCHUM, ABEL, and ZHAO, 2014; QIAN, QIAN, and SNOWDON, 2014; JAN et al., 2016; LUO et al., 2017; WERNER et al., 2018.

Rice (*Oryza sativa*) XU, 2013; GRENIER et al., 2015; HASSEN et al., 2018; MOMEN et al., 2019.

Rye (*Secale cereale*) BERNAL-VASQUEZ et al., 2014; WANG et al., 2014; AUINGER et al., 2016; MARULANDA et al., 2016; BERNAL-VASQUEZ et al., 2017. Sugar beet (*Beta vulgaris*), WÜRSCHUM et al., 2013; BISCARINI et al., 2014.

Sugar cane (*Saccharum officinarum*) GOUY et al., 2013.

Soybean (*Glycine max*) JARQUIN, SPECHT, and LORENZ, 2016; XAVIER, MUIR, and RAINEY, 2016; STEWART-BROWN et al., 2019.

Switchgrass (*Panicum virgatum*) RAMSTEIN et al., 2016; POUDEL et al., 2019; RAMSTEIN and CASLER, 2019.

Wheat (*Triticum aestivum*) THAVAMANIKUMAR, DOLFERUS, and THUMMA, 2015; LOPEZ-CRUZ et al., 2015; SUKUMARAN et al., 2016; BUSTOS-KORTS et al., 2016b; GIANOLA et al., 2016; CROSSA et al., 2016; RINCENT et al., 2018; NORMAN et al., 2018; BELAMKAR et al., 2018; OVENDEN et al., 2018; CUEVAS et al., 2019a; HOWARD et al., 2019; KRAUSE et al., 2019.

As well as various tree species HOLLIDAY, WANG, and AITKEN, 2012; RESENDE et al., 2012; ZAPATA-VALENZUELA et al., 2013; JARAMILLO-CORREA et al., 2014; KUMAR et al., 2015; EL-DIEN et al., 2016; RINCENT et al., 2018; RATCLIFFE et al., 2017; KAINER et al., 2018; ALMEIDA FILHO et al., 2019.

Even though GS finds broad application in plant breeding it has been originally developed for the use in animal breeding HAYES and GODDARD, 2010; GODDARD, HAYES, and MEUWISSEN, 2011. The gold standard is a method known as genomic BLUP VANRADEN, 2008, which utilizes a relationship matrix based on the co-occurrence of genetic markers. This method is derived from the pre-genomic era in animal breeding, where the relationship matrix was constructed after pedigrees according to the best linear unbiased predictors (BLUP) based on the linear mixed model equations developed by HENDERSON, 1975.

GBLUP accounts only for additive-genetic effects VANRADEN, 2008. There are other methods that are able to account for more complex genomic effects that are non-additive. Popular among those are for example Reproducing Kernel Hilbert Spaces (RKHS) GIANOLA and KAAM, 2008. Alternatively to Henderson's linear mixed models, a large variety of different Bayesian methods became popular HAYES and GODDARD, 2001; GIANOLA et al., 2009; HABIER et al., 2011; GIANOLA, 2013; CROSSA et al., 2017.

Genomic prediction in recurrent selection and the breeders equation

While the quantitative genetic methods breeders utilize are complex their goals can be defined in one sentence: genetically improve plant germplasms for agriculture. The breeding process started at the same time as farming around 10,000 BC in the region between the Euphrat and Tigris rivers known as the fertile crescent KINGSBURY, 2009. This changed the phenotypic appearance of the early crops dramatically to the point where they share little external traits with their wild ancestors. Those changes have been deeply carved into the genomes, which underwent serious alterations, including hybridization, duplications etc. This led to most crop plants not having any wild ancestors with whom they could naturally mate. For example wheat (*T. aestivum*), one of the three most important sources of food on a global scale, underwent multiple hybridization steps OZKAN, LEVY, and FELDMAN, 2001. Wheat is a hybrid from either the diploid emmer (*T. diccoides*) or durum wheat (*T. durum*) and *Aegilops tauschii*, while emmer and durum are hybrids derived from wild emmer, which is a hybrid of wild grass of the genus of *Aegilops* and *T. urata* FRIEBE et al., 2000; FELDMAN and LEVY, 2012.

While being ignorant of modern genetics early “plant breeders” must have had an intuitive, yet naive, understanding of the general concept of heritability in a way that they must have comprehended that offsprings share properties with their parents, which motivated them to regrow individuals with desired traits generations after generation. This induced many changes including that artificial selected plants are commonly largely inbred. That process could be considered an early form of recurrent truncation selection. Truncation selection on a normal distributed phenotype is shown in figure 4.5.

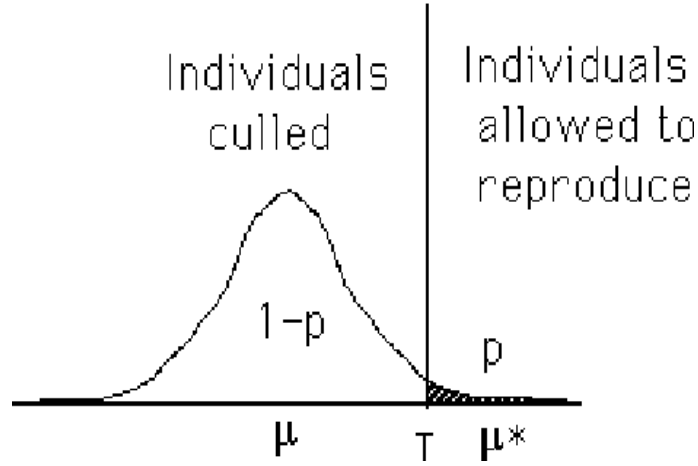


FIGURE 4.5: Truncation selection from a normal distributed phenotype with selection a threshold value of T , μ as the mean of the total population and μ^* as the mean of the selected phenotypes. Graphic from WALSH and LYNCH, 2018a

Like the early breeders modern breeders have to determine a selection threshold T to divide the total population with the mean μ into two groups: the individuals culled and the ones allowed to reproduce with the mean μ^* . The difference between those two is the selection differential S :

$$S = \mu^* - \mu \quad (4.25)$$

In the case of normal distributed data as depicted in figure 4.5 S can be expressed as:

$$S = \varphi\left(\frac{T - \mu}{\sigma}\right) \frac{\sigma}{p} \quad (4.26)$$

From which we can obtain the selection intensity i , which makes i solely a function of p .

$$i = \frac{S}{\sigma} = \frac{\varphi(z_{1-p})}{p} \quad (4.27)$$

With recurrent truncation selection over many generations the population

mean of the trait μ will change (hopefully in the desired direction) if the heritability (in this case the narrow sense heritability) $h^2 > 0$. It is impossible to breed for traits that do not contain any genetic components in their architecture WALSH and LYNCH, 2018b.

Next to i the selection intensity and h^2 the accuracy of the selection process r_{uA} is important for the success of a breeding program. Those three terms can be applied to estimate the gain of selection R over one generation (equation 4.28). Due to its importance in the evaluation of breeding schemes it is known as the breeder's equation MOUSSEAU and ROFF, 1987; FALCONER and MACKAY, 1996; KINGSOLVER et al., 2001.

$$R = ir_{uA}\sigma_A \quad (4.28)$$

The accuracy r_{uA} of equation 4.28 in cases when only phenotypic selection is conducted is the narrow-sense heritability and in cases where the selection process is aided by genomic prediction it is the prediction accuracy ¹. According to the breeder's equation there are three parameters, which can be influenced through genomic prediction.

- (i) The prediction accuracy, which is usually smaller than the heritability, varies for different prediction equations and an increase in the accuracy will lead to an proportional increase in R . For this reason since 2001, in quantitative genetics one very active field of research was and still is to find new, better algorithms for GS as presented in the next chapter (4.2.2. As later evaluated on more than 150 phenotypes in chapter 4.7 h^2 is always larger than r_{uA} . Which if it was the only variable factor in equation 4.28, would make genomic selection inferior to phenotypic selection, which from a certain point

¹The prediction accuracy in the literature is sometimes used synonymously with predictive ability, sometimes the predictive ability is defined as the prediction accuracy divided by the narrow or broad sense heritability. In the present study they will be used synonymously or termed $\rho(y, \hat{y})$ as the correlation after Pearson between the real y and the predicted values \hat{y}

of view it is. Phenotypic trials are better approximations for phenotypic appearance as GEBVs. However, as the cost of genotyping has decreased dramatically in the last 20 years, phenotyping with field trials remains tedious, laborious and vastly expensive. Taking into account that field trials have to be repeated in several years and locations to produce reliable accounts it becomes clear that genotyping 10s of thousands of accessions is much cheaper than conducting field trials with 1000 of them.

- (ii) The selection intensity can be much stricter if the total population that is selected from is larger. In genomic prediction settings they are, because breeders can select from two pools. First the pool of plants with known phenotypes and known genotype information and from those were just genomic data is available. When selecting from a pool of 1000 with $p = 0.05$ with the goal to keep 50 plants in the next breeding cycle, the same goal can be reached when genomically selecting from a pool of 10000 with and intensity of $p = 0.005$.
- (iii) The decrease in time per generation is probably the largest advantage of genomic selection, when applied to breeding. While in field trials it is only possible to have one generation per year, genomic selection does not require the plants to be grown in the field. For GS it is only necessary to grow the plants large enough, that DNA can be extracted from the tissues and evaluated. After selection only the ones above the threshold are grown until they bear seeds (or other reproductive organs) and be used for the next selection cycle, allowing up to ten generations per year. This development has lead to the rise to a new branch of breeding: speed breeding GHOSH et al., 2018; WATSON et al., 2018. In practical, company-level breeding, genomic prediction has largely contributed to an increase by a factor of 2 to the gain in selection in recent years (personal communication with breeding company employees).

The last term in equation 4.28, the additive genetic variance σ_A , is not directly, yet heavily influenced by the described breeding scheme. Artificial selection has similar effects on the genetic variance as bottlenecks do in natural selection: it decreases, thus making it harder to increase R in later selection cycles WALSH and LYNCH, 2018b.

Genomic BLUP and Bayesian methods

All methods share a common statistical obstacle, which is commonly referred to as the $n \gg p$ problem, which arises because the number n of markers is usually a multitude larger than the number of observations p . In practical applications it is not uncommon to have more than 100k markers while the number of phenotypes is no larger than 100. This does not allow to obtain genomic estimated breeding values (GEBV) by single marker regression as done by GWAS, which estimates highly inflated SNP-effects KORTE and FARLOW, 2013. One possibility is to include effect sizes as random effects and make prior assumptions about their distribution. The difference in prior distribution is the main distinction between the many methods of the Bayesian alphabet introduced in the following chapter GIANOLA, 2013.

Genomic BLUP

In the early years of research on genomic prediction algorithms were not solely benchmarked against each other, but had to compete with the previously popular pedigree methods. Quickly in the course of the first decade of this millennium the superiority of the genomic methods were elucidated in livestock and plant breeding HABIER, FERNANDO, and DEKKERS, 2007; VANRADEN, 2008; VANRADEN et al., 2008; HARRIS, JOHNSON, and SPELMAN, 2009. While the genomic methods are superior to non-genomic methods, there is no clear evidence that either of the genomic methods are superior to each other and there is lack of empirical evidence that the Bayesian methods generally outperform GBLUP MOSER et al.,

2009; BERNARDO, 2010; AZODI et al., 2019.

Like pedigree BLUP for genomic BLUP the co-variance between related individuals is used for the predictions. In the latter case it is calculated from marker information.²

The general genomic prediction model (equation 4.29) is derived from mixed models HENDERSON, 1975; VANRADEN, 2008 and implemented as:

$$Y = X\beta + Zu + \varepsilon \quad (4.29)$$

where Y is a $n \times 1$ vector of phenotypic observations, X the matrix of the fixed effects and β the vector of the fixed effects. Z is the incidence matrix for the combined marker effects and u is a $n \times 1$ vector of the additive genetic effect the vector of the residuals ε .

To construct a GBLUP model lets assume a matrix of size $(n \times m)$ with n individuals and m loci M containing marker information for three individuals on four loci, thus being of size 3×4 . The four markers of matrix 4.30 can take values of $-1, 0$ and 1 , translating into minor allele, heterozygous locus and major allele.³

$$M = \begin{pmatrix} -1 & 0 & 1 & -1 \\ -1 & 0 & 0 & 0 \\ 0 & 1 & 1 & -1 \end{pmatrix} \quad (4.30)$$

The M matrix contains all the information that are necessary for the computation of the K matrix and other viable genetic parameters. The MM' matrix of size $n \times n$ (4.31) bears additional parameters.

²In the GWAS terminology the relationship matrix is referred to as K for kinship, while in GS circumstances it is called GRM (genomic relationship matrix) or abbreviated as G . This study will remain consistent with the circumstantial literature and therefore purposely inconsistent within itself. In the chapters addressing GWAS it will be called K for kinship matrix and in the following chapter elucidating GS it will be referred to as G .

³This example calculation has been adapted from ISIK, 2013.

$$MM' = \begin{pmatrix} 3 & 1 & 2 \\ -1 & 1 & 0 \\ 2 & 0 & 3 \end{pmatrix} \quad (4.31)$$

The diagonal shows the number of homozygous loci per individual, while the other elements of the matrix indicate the number of markers shared by related individuals. This is an indicator for the distance of the relationship between individuals, as defined by identity-by-descent VANRADEN, 2008; MISZTAL et al., 2013. While matrix 4.31 calculates the metrics per individual, the $M'M$ matrix (4.32) accounts for metrics per marker. Likewise the diagonal contains the number of homozygous individuals per marker.

$$M'M = \begin{pmatrix} 3 & -1 & 0 & 0 \\ -1 & 1 & 1 & 1 \\ 0 & 1 & 2 & 1 \\ 0 & 1 & 1 & 2 \end{pmatrix} \quad (4.32)$$

The next step is to obtain a matrix of the allele frequencies at each locus also of size $n \times m$ like matrix M . For the design of matrix P (4.33) let the minor allele frequencies $p_1 \dots p_4$ be $\{0.3, 0.2, 0.1, 0.15\}$. The allele frequency of the i^{th} column of P is expressed according to the n^{th} marker of matrix M as $P_i = 2(p_i - 0.5)$ resulting in:

$$P = \begin{pmatrix} -0.4 & -0.6 & -0.8 & -0.7 \\ -0.4 & -0.6 & -0.8 & -0.7 \\ -0.4 & -0.6 & -0.8 & -0.7 \end{pmatrix} \quad (4.33)$$

The allele frequencies, as in this simulated example, should be drawn from the entire population and not only the subsample used for the calculation VANRADEN, 2008. The final step to obtain the Z matrix for the us in equation 4.29 is to subtract the P matrix from the M matrix $Z = M - P$ resulting in:

$$Z = \begin{pmatrix} 1.4 & 0.6 & 1.8 & -0.3 \\ -0.6 & 0.6 & 0.8 & 0.7 \\ 0.4 & 1.6 & 1.8 & -0.3 \end{pmatrix} \quad (4.34)$$

In Z the mean values of the allele effects are set to 0 and the subtraction of P emphasizes the effect of rare variants VANRADEN, 2008. There is a large variety of methods to generate the genomic relationship matrices and here lies the major difference between different genomic BLUP methods, but K is always of size $n \times n$.

- (i) The naive approach is to iterate over each individual and count the common markers with every other individual. This approach is suited for inbred or doubled-haploid populations, less so for outcrossed populations with high degrees of heterozygosity, because as in the sample implementation it does only account for homozygous loci. This method becomes computationally intense when the data sets grow larger as common today (personal observation).
- (ii) Probably the most popular method in GS is to obtain K as proposed by VANRADEN, 2008 designed after Wright's WRIGHT, 1922 equations for the covariance in structured populations, as described by equation 4.35 with Z as in 4.36.

$$G = \frac{ZZ'}{2\sum p_i(1 - p_i)} \quad (4.35)$$

In the above example this would result in the following kinship matrix:

$$G = \begin{pmatrix} 4.8 & 0.6 & 4.1 \\ 0.6 & 1.6 & 1.7 \\ 4.1 & 1.7 & 5.2 \end{pmatrix} \quad (4.36)$$

- (iii) The unified additive relationship G_{UAR} according to YANG et al., 2010 and equation 4.37

$$G_{UAR} = A_{jk} = \frac{1}{N} \sum_i A_{ijk} = \begin{cases} \frac{1}{N} \sum_i \frac{(x_{ij} - 2p_i)(x_{ik} - 2p_i)}{2p_i(1 - p_i)}, j \neq k \\ 1 + \frac{1}{N} \sum_i \frac{x_{ij}^2(1 + 2p_i)x_{ij} + 2p_i^2}{2p_i(1 - p_i)}, j = k \end{cases} \quad (4.37)$$

where p_i is the allele frequency at locus i and x_{ij} the genotype for the j^{th} individual at the i^{th} locus. Another method also proposed by YANG et al., 2010 is to adjust G_{UAR} with β as in equation 4.38

$$G_{UARadj} = \begin{cases} \beta A_{jk}, & j \neq k \\ 1 + \beta(A_{jk} - 1), & j = k \end{cases} \quad (4.38)$$

- (iv) Another approach is to weigh marker by the reciprocals of their expected variance according to the model 4.39. This was originally designed to investigate population structures in human genomics LEUTENEGGER et al., 2003; AMIN, VAN DUIJN, and AULCHENKO, 2007.

$$G = ZDZ', \text{ with} \quad (4.39)$$

$$D_{ii} = \frac{1}{m|2p_i(1 - p_i)|}$$

- (v) Other methods like the gaussian kernel compute kinship between individuals by the euclidean distance between the respective genotypes MOROTA and GIANOLA, 2014.

$$K(x_i, x_j) = \exp(-\theta d_{ij}^2) \quad (4.40)$$

$$= \prod_{k=1}^m \exp(-\theta (x_{ik} - x_{jk})^2)$$

with $d_{ij} = \sqrt{(x_{i1} - x_{j1})^2 + \dots + (x_{ik} - x_{jk})^2 + \dots + (x_{im} - x_{j,a})^2}$ and $x_{ik}(i, j = 1, \dots, n, k = 1, \dots, m)$ and x_{ik} as the i^{th} individual at SNP k .

The linear model of equation 4.29 $Y = X\beta + Zu + \varepsilon$, with β as the vector fixed effects and u as the vector of additive genetic effects, can be solved to obtain genomic estimated breeding values as:

$$\begin{pmatrix} X'X & X'Z & 0 \\ Z'X & Z'Z + G^{11} & G^{12} \\ 0 & G^{21} & G^{22} \end{pmatrix} \begin{pmatrix} \hat{b} \\ \hat{y}_1 \\ \hat{y}_2 \end{pmatrix} = \begin{pmatrix} X'y \\ Z'y \\ 0 \end{pmatrix} \quad (4.41)$$

with G^{12} as the part of G^{-1} containing individuals with phenotypic data and with G^{22} as the part of G^{-1} containing individuals without phenotypic data and just marker information available.

This can be algebraically solved to compute the GEBV of the unknown phenotypes \hat{y}_2 as:

$$\hat{y}_2 = - \left(G^{22} \right)^{-1} G^{21} \hat{y}_1 \quad (4.42)$$

GBLUP is fairly easy compared to more complex Bayesian methods and can be quickly implemented in any programming language capable of solving liner equations like R or Python R CORE TEAM, 2018; VAN ROSSUM and DRAKE JR, 1995. Computational as the number of phenotypes in the study increases in numbers the timed demand grows exponentially, because the kinship matrix quadruples in size and it becomes more complicated to compute the inverse of G (personal observations).

Bayesian methods

Next to the universal GBLUP a set of related algorithms became popular for solving the mixed models involved in genomic selection, known as the Bayesian alphabet GIANOLA et al., 2009; GIANOLA, 2013. They are all based on Bayes' fundamental theorem (equation 4.43).

$$P(\theta|y) = \frac{P(\theta)P(y|\theta)}{P(y)} \quad (4.43)$$

with $P(\theta)$ as the prior distribution, $P(y|\theta)$ as the likelihood and $P(y)$ as the marginal density of y . The prior distribution in GS assume that y was drawn from a certain distribution. Infinitesimal models assume that the genetic effects follow a normal distribution LEGARRA, LOURENCO, and VITEZICA, 2018, while the Bayesian frameworks, however, will assume non-normal distributed marker effects. This can be explained by a two-step hierarchical distribution. Stage one assumes that every marker has *a priori* a different variance LEGARRA, LOURENCO, and VITEZICA, 2018.

$$p(a_i|\sigma_{ai}^2) = N(0, \sigma_{ai}^1) \quad (4.44)$$

The second stage assumes prior distributions for the variances.

$$p(a_i|variable) = P(\dots) \quad (4.45)$$

with *variable* standing for the large variety of prior distributions. In total there are more than >20 different Bayesian models known to the author. Their main difference "simply" lies in the *a priori* assumptions of prior distributions. This change can make some methods mathematically much more complicated than others. As shown in later chapters none of the methods is completely superior over others in terms of prediction accuracy.

Approximation to the solution of the linear equations is usually performed by Gibb's sampling using Markov Chain Monte Carlo (MCMC) simulations DE LOS

CAMPOS et al., 2009; CAMPOS and RODRIGUEZ, 2016. Table 4.1 summarizes commonly applied Bayesian methods for genomic prediction indicating their key differences.

TABLE 4.1: Overview of properties of a variety of commonly applied Bayesian methods for genomic prediction. Table altered after KÄRKKÄINEN and SILLANPÄÄ, 2012

| Name | Reference | Prior | Indicator | Hierarchy | Hyperprior | Estimation |
|--------------|-------------------------|---------|-----------|-----------|------------|------------|
| BayesA | HAYES and GODDARD, 2001 | Student | No | Yes | No | MCMC |
| BayesB | HAYES and GODDARD, 2001 | Student | Yes | Yes | No | MCMC |
| BayesC | VERBYLA et al., 2009 | Student | Yes | Yes | No | MCMC |
| BL | XU, 2010 | Laplace | No | Yes | No | EM |
| BayesD π | HABIER et al., 2011 | Student | Yes | Yes | Yes | MCMC |

The name is given by the author. The prior column tells which shrinkage prior is used.

4.2.3 Genomic selection using artificial neural networks

As mentioned in 4.2.2 genomic selection (GS) has been successfully applied in animal HAYES and GODDARD, 2010; GIANOLA and ROSA, 2015 and plant breeding CROSSA et al., 2010; HEFFNER et al., 2010; DESTA and ORTIZ, 2014; CROSSA et al., 2017 as well as in medical applications since it was first reported HAYES and GODDARD, 2001. Since then the repertoire of methods for predicting phenotypic values has increased rapidly e.g. DE LOS CAMPOS et al., 2009; HABIER et al., 2011; GIANOLA, 2013; CROSSA et al., 2017. Genomic prediction has repeatedly been proven to outperform pedigree-based methods CROSSA et al., 2010; ALBRECHT et al., 2011 and is nowadays used in many plant and animal breeding schemes. It has also been shown that using whole-genome information is superior to using only feature-selected markers with known QTLs for a given trait BERNARDO and YU, 2007; HEFFNER, JANNINK, and SORRELLS, 2011 in most cases. A more recent study AZODI et al., 2019 compared 11 different genomic prediction algorithms with a variety of data sets and found contradicting results, indicating that feature selection can

be useful for some cases when whole genome regression is performed by neural nets.

While every new method is a valuable addition to the toolbox of genomic selection, some fundamental problems remain unsolved and are the same for every algorithm, of which the $n \gg p$ problematic stands out. Usually in genomic selection settings the size of the training population (TRN) with n phenotypes is substantially smaller than the number of markers p FAN, HAN, and LIU, 2014, making the number of trainable features immensely large. Furthermore every marker is treated as an independent observation neglecting collinearity and linkage disequilibrium (LD) between them. More difficulties arise through non-additive, epistatic and dominance marker effects. The main issue with epistasis in quantitative genetics is the almost infinite amount of different marker combinations, which cannot be represented within the size of TRN in the thousands. The same problems arises in GWA studies KORTE and FARLOW, 2013. With already large p the number of possible additive SNP-SNP interactions potentiates to $p^{(p-1)}$. Methods that attempt to overcome those issues are EG-BLUP, which us an enhanced epistatic kinship matrix and reproducing kernel Hilbert space regression (RKHS) JIANG and REIF, 2015; MARTINI et al., 2017.

In the past 10 years, due to increasing availability of high performance computational hardware with decreasing costs and parallel development of free easy-to-use software, most prominent being googles library TensorFlow ABADI et al., 2016 and Keras CHOLLET, 2015, machine learning (ML) has experienced a renaissance. ML is a set of methods and algorithms used widely for regression and classification problems. Popular among those are e.g. support vector machines, multi-layer perceptrons (MLP) and convolutional neural networks. ML has been widely applied in many biological fields MIN, LEE, and YOON, 2017; LAN et al., 2018; MAMOSHINA et al., 2016; ANGERMUELLER et al., 2016; WEBB, 2018; RAMPASEK and GOLDENBERG, 2016.

A variety of studies assessed the usability of ML in genomic prediction GONZÁLEZ-CAMACHO et al., 2018; GONZÁLEZ-CAMACHO et al., 2016; OGUTU, PIEPHO, and SCHULZ-STREECK, 2011; MONTESINOS-LÓPEZ et al., 2019a; GRINBERG, ORHOBOR, and KING, 2018; CUEVAS et al., 2019b; MONTESINOS-LÓPEZ et al., 2019b; MA et al., 2017; QIU et al., 2016; GONZÁLEZ-CAMACHO et al., 2012; LI et al., 2018. Through all those studies the common denominator is that there is no such thing as a gold standard for genomic prediction. No single algorithm was able to outperform all the others tested in a single of those studies, let alone in all. While the general aptitude of ML for genomic selection has been repeatedly proven, there is no evidence exists that neural networks can generally outperform mixed-model approaches as GBLUP HAYES and GODDARD, 2001.

In other fields like image classification neural networks have up to 100s of hidden layers HE et al., 2016. The commonly used fully-connected networks in genomic prediction tend to have one to three hidden layers. With one layer networks often being the most successful among those. Contradicting to the idea behind machine learning in genomic selection one hidden layer networks will be inapt to capture interactions between loci and thus only account for additive effects. As shown in AZODI et al., 2019 convolutional networks perform worse than fully-connected networks in genomic selection, which again is contradicting to other fields where convolutional layers are applied successfully, e.g. natural language processing DOS SANTOS and GATTI, 2014 or medical image analysis LITJENS et al., 2017. Instead of using convolutional layers and fully-connected layers only, as shown in Pook et al 2019, we also propose to use locally-connected layer in combination with fully-connected layers. While CL and LCL are closely related they have a significant difference. In CL weights are shared between neurons and in LCLs each neuron as its own weight. This leads to a reduced number of parameters to be trained in the following FCLs and should therefore theoretically lead to a decrease in overfitting. To evaluate the usefulness of machine learning in GS the data sets generated in the scope of the 1001 genome project of *A. thaliana*

ALONSO-BLANCO et al., 2016 and the MAZE project were used.

4.3 Proof of concept for ANN-based genomic selection

Having established the quantitative architecture of traits in section 4.2.1 and the basics of machine learning and neural nets in section 4.1.1, that knowledge can be used to provide a proof of concept that neural networks are a candidate for GP. Table 4.2 provides all the possible genotypes $G_1 \dots G_4$ that can be derived by two bi-allelic markers M_1, M_2 on a fictional haploid organism. In this simulation the effect sizes for each marker β_1 and β_2 are constant with a value of 1.

TABLE 4.2: Simple simulated phenotypes and genotypes for genomic prediction with genotypes $G_1 \dots G_4$, Markers M_1 and M_2 and phenotypes based on additive effects or *and*, *or*, *xor* logic gates.

| | M_1 | M_2 | Y_{ADD} | Y_{AND} | Y_{OR} | Y_{XOR} |
|-------|-------|-------|-----------|-----------|----------|-----------|
| G_1 | 0 | 0 | 0 | 0 | 0 | 0 |
| G_2 | 0 | 1 | 1 | 0 | 1 | 1 |
| G_3 | 1 | 0 | 1 | 0 | 1 | 1 |
| G_4 | 1 | 1 | 2 | 1 | 1 | 0 |

The four phenotypes Y_{ADD} , Y_{AND} , Y_{OR} and Y_{XOR} , which were derived from their respective marker effects, were used for GP. Y_{ADD} is a phenotype with purely additive effects. So in the nomenclature introduced in chapter 4.2.1 $\sigma_A = \sigma_G$ and $\sigma_I = 0$. Since the hypothetical organism is haploid there are no dominance effects to be accounted for $\sigma_D = 0$ and since all the genetic effects are caused by additive effects and there are also no environmental effects σ_E . The narrow sense heritability h^2 - equation 4.24 - and the broad sense heritability H^2 - equation 4.23 - are equally 1. The other three phenotypes are base on epistatic effects σ_I , generated by passing the markers M_1 and M_2 through their respective logic gates. This

theoretically results in $h^2 = 0$ and $H^2 = 1$ because there should be no additive effects. For y_{AND} , however, $h \approx 0.5$ because there is a correlation between Y_{ADD} and Y_{AND} . In practical applications this allows methods like GBLUP, designed to account for additive genetic effects, to capture some of the epistatic effects of σ_I VIEIRA et al., 2017.

According to chapter 4.1.1 a single perceptron fails to solve *xor* gates. While a network with multiple nodes and layers should be able to overcome that deficit. A relatively simple neural network with two fully-connected hidden layers with 10 and 5 nodes was trained for the prediction of the phenotypes. To keep the simulation as simple possible, no regularization parameters like dropout etc. were included. The activation function was ReLU (4.6) with an Adam optimizer. The results of the prediction are shown in table 4.3.

TABLE 4.3: Results of genomic prediction from phenotypes and genotypes in table 4.2

| | M_1 | M_2 | \hat{Y}_{ADD} | \hat{Y}_{AND} | \hat{Y}_{OR} | \hat{Y}_{XOR} |
|-------|-------|-------|-----------------|-----------------|----------------|-----------------|
| G_1 | 0 | 0 | 0.01 | 0.00 | 0.00 | 0.01 |
| G_2 | 0 | 1 | 0.99 | 0.01 | 0.99 | 0.98 |
| G_3 | 1 | 0 | 0.99 | 0.00 | 0.99 | 1.01 |
| G_4 | 1 | 1 | 1.99 | 0.98 | 1.01 | 0.02 |

Not surprisingly, the simple network is able to solve all four problems and predict the phenotypes accurately. The task was rather easy because the training data set and the testing data set were the same, but it served the purpose of showing that neural networks are generally apt to solve different marker interactions. *In natura* those interactions and the overall genetic architecture are much more complex, of course. Effect sizes are not constant and epistasis may be caused by interactions with more than just two markers. With an increasing number of markers n the number of possible two way interactions increases even more to 2^{n-1} . Smaller interaction effects could be obscured under larger additive effects.

Gene-environment interactions might have a significant influence resulting in a model that does not converge.

4.4 Material

Two different data sets were used for the genomic prediction trials. A set of doubled-haploid (DH) populations derived from maize landraces and *A. thaliana* data sets with genomic data procured along the 1001 genomic project ALONSO-BLANCO et al., 2016 and various phenotypic trials SEREN et al., 2016.

4.4.1 DH populations derived from maize landraces

The DH populations were produced, propagated and phenotyped in the scope of the MAZE project phase I, funded by the Federal Ministry of Education and Research (BMBF) (Funding ID: 031B0195, project “MAZE”) as well as the KWS SAAT SE, by various project partners at the Technical University of Munich, University of Hohenheim and the KWS. A thorough description of the germplasm selection and phenotyping was recently published by HÖLKER et al., 2019.

Modern maize cultivars are almost exclusively high-performing hybrids from two inbreed lines originating from different heterotic pools. Commonly hybrids are derived from a cross of European Flint and American Dent maize SANTOS DIAS et al., 2004; BRAUNER et al., 2019. Before hybrid breeding became the predominant method in maize breeding in the 1960s, landraces were grown by farmers. Landraces are dynamic, open-pollinated, locally highly-adapted populations. They did not derive from modern breeding, but from locally confined selection and adaption by farmers to often very specific needs ARTEAGA et al., 2016. The hybrids grown today are derived from just a few landraces as founder lines, while the majority of landraces has been nearly forgotten. This and high intensity selection over many generation has led to a loss of genetic diversity σ_G in modern maize cultivars.

The landrace germplasm present an important and essential stock of genetic variability for continuous success in maize breeding. The utilization of those germplasms would be impossible without the invaluable work of institutions like the IPK

Gartersleben, whose goal as genebanks is to maintain and store genetic material for long time periods. From European three landraces representing large phenotypic and genetic heterogeneity were chosen to be assessed in the scope of the MAZE project:

- (i) Kemater Landmais Gelb (KE, Austria)
- (ii) Petkuser Ferdinand Rot (PE, Germany)
- (iii) Lalin (LL, Spain).

They represent 95% of the molecular variance in a set of 35 landraces analyzed in a preceding project by MAYER et al., 2017.

In total 1015 DH lines (516 KE, 432 PE, 67 LL) were produced with *in vivo* haploid induction with an inducer line as described in ROEBER, GORDILLO, and GEIGER, 2005.

Genomic maize data

The genomic maize data was provided by the TUM as described by HÖLKER et al., 2019.

Genotyping was performed with the 600k Affymetrix® Axiom® Maize array UNTERSEER et al., 2014. The markers were quality filtered and missing values were imputed individually for each landrace population using Beagle 5.0 BROWNING and BROWNING, 2007; BROWNING, ZHOU, and BROWNING, 2018. After LD pruning and further quality control 29833 markers remained for 471 Kemater and 403 PE DHs. LL was excluded from further analyses due to insufficient amounts of genotypes.

Phenotypic maze data

The phenotype data was provided by the TUM as described by HÖLKER et al., 2019.

The traits were evaluated with lattice design in 6 different locations across Europe. Those traits were:

- (i) early Vigor (EV) at three different stages (V3, V4, V6)
- (ii) plant height (PH) at two developing stages (V4,V6)
- (iii) the final plant height (PH_final)
- (iv) male flowering time: days till tasseling (DtTAS)
- (v) female flowering time: days till silking (DtSILK))

To account for GxE best linear unbiased estimators were calculated according to Henderson's model HENDERSON, 1975 and used for further prediction. Once the BLUEs were calculated across all environments and once they were calculated for the DHs in the six environments individually.

4.4.2 *A. thaliana*

Genomic data

The genomic data was generated during the course of the 1001 genome project of *A. thaliana* ALONSO-BLANCO et al., 2016 producing completed sequenced and assembled genomes for 1035 ecotypes along 600k marker data for 1307 accessions with an overlap between those groups resulting in a total of 2029 genotyped accessions. Totaling in more than 10 mio. SNPs and Indels on the 5 chromosomes of *A. thaliana*. Imputation of missing data and upsampling of the 600k subsets was performed with Beagle3 BROWNING and BROWNING, 2007.

For every one of the 164 phenotypes used for prediction subsets were sampled, LD pruned and MAF filtered. LD pruning was executed with the R-package SNPRelate ZHENG, 2013 with a relatively strict LD threshold of 0.65 and a $MAF > 10$. This resulted in data sets with approximately 150.000 markers for each phenotype.

Phenotypic data

A complete list of the phenotypes used can be found in Appendix B with the according study references. The phenotypic trials ranged from 100 to more than 1000 accessions per data set ATWELL et al., 2010; LI et al., 2010; MEIJÓ et al., 2014; STRAUCH et al., 2015.

4.5 Methods

The theoretical backgrounds of the methods used for genomic prediction were described in section 4.1.1 for the ANNs and section 4.2.2 for the Bayesian methods and GBLUP. The next sections are devoted to explaining how those methods were adapted and implemented for the prediction of maize and *Arabidopsis* traits.

4.5.1 Validation scheme

The validation approach in this study was a little different than common five fold cross validation. All predictions were run 50 times with different splits of TST and TRN. For the full data sets randomly 20% were assigned to TST and 80% to TST. This process was repeated 50 times reducing the chance of biases due to any TST-TRN combination being randomly more predictable for one or the other method. The validation scheme was generated *a priori* and stored in cross-validation files to allow reusing the validation sets.

4.5.2 ANN

The scripts for ANN based GS were written in python using the lower level API TensorFlow ABADI et al., 2016 and the higher level API Keras CHOLLET, 2015. Both are very versatile, well-documented and are capable of performing a large variety of machine learning applications. For those reasons they are among the most used ML libraries. Another advantage is that they work well on GPUs, which

allows ML algorithms to run in a reasonable amount of time compared to CPU-based calculations.

Prior to training the data was split into TRN and TST. The markers of TRN served as the input layer for the network while the phenotypes were values trained upon in the output node. Preliminary trials showed that Adam is the superior optimizer for GS and hence was the only one further used. Likewise relu was the activation of choice being superior to sigmoid or other non-rectifiers. All the weights and the biases of the kernel were initialized with truncated normal distributed values. The loss function used was always MSE.

Having a few hyperparameters fixed the remaining ones were optimized via a grid search. For each training set multiple networks were trained to fine tune the input parameters. Those were the number of layers, the nodes per layer, the magnitude of the dropout, the type of dropout used, whether the first layer was locally-connected for fully-connected and the duration of training via the training epochs. This amounted to a total of almost 260000 trained networks for the 145 *A. thaliana* data sets alone.

After another set preliminary runs LCL as the first layer appeared to result in higher prediction accuracies than FLC and where henceforth exclusively used and applied with a stride length of 7. The stride length determines how many nodes of the input layer, in this case markers, are combined in the first hidden layer. The type of drop out used (alpha dropout, Gaussian noise or normal dropout) did not show any effect therefore the normal dropout function was used further. The network's training was iterated over the different number of epochs, architectures, drop out values and the cross validation cycles, thus explaining the tremendous amount of total networks trained. Epochs from 5 to 60 in steps 5 and several 1, 2 or 3 Layer architectures, following the locally-connected layer, were tested.

Single environment prediction

Next to the across environment BLUEs used for prediction the single environment BLUEs were used for prediction to able to gain insights of the structure of $\sigma_{G \times E}$ of the maize traits. This resulted in 2246 genotype x environment combinations for Kemater and 1975 for Petkuser with at least one data point. This number is lower than the maximum number of n DHs per populations times the 6 environments, because naturally not all genotypes yielded reliable data in the environments. Each DH x environment was treated as an individual in for the across environment prediction. The marker matrix was enhanced with the environmental origin as cofactors as show in table 4.4 with one-hot encoded markers.

TABLE 4.4: Schematic representation of the enhanced genotype matrix for across environment prediction of maize phenotypes with DHs 1-2 with markers M 1-2 in environments E1-2

| | M-1 | M-2 | E-1 | E-2 |
|--------|-----|-----|-----|-----|
| DH1-E1 | 0 | 1 | 1 | 0 |
| DH2-E1 | 1 | 0 | 1 | 0 |
| DH1-E2 | 0 | 1 | 0 | 1 |
| DH2-E2 | 1 | 0 | 0 | 1 |

4.5.3 GBLUP and Bayesian methods

The evaluation of the genomic BLUP and the Bayesian methods was performed with the R-package BGLR CAMPOS and RODRIGUEZ, 2016. To allow pairwise comparison of the individual validation runs the same validation scheme as for the ANNs was used with the same TST and TRN sets. BGLR implements GBLUP as Bayesian ridge regression (BRR), which mathematically has the same results as GBLUP, but uses and Bayesian approach CAMPOS and RODRIGUEZ, 2016. For further comparison of the prediction methods, not only ANN and GBLUP were compared, but also five different Bayesian methods were applied to the maize data sets:

- (i) BayesA
- (ii) BayesB
- (iii) BayesC
- (iv) Bayesian Lasso
- (v) BRR / GBLUP

Besides the actual prediction algorithms the number of markers and the number of accessions will influence the final accuracy. To assess the prediction accuracy as related to the number of markers, the full Petkuser genotype matrix was subsampled five times each into 1k, 2k, 5k, 10k and 20k subsets. To analyze the prediction accuracy as a function of the number of accessions the Kemater data set was sampled into 50,100,200,300 and 400 accession subsets randomly 10 times. Both trials were run with 50 fold validation with 80% TRN and 20% TRN.

4.6 Results

Results of *A. thaliana* prediction

Table 4.5 shows the results for genomic prediction for 145 *A. thaliana* phenotypes with ANNs and GBLUP and the architecture, determined via grid search, yielding the highest prediction accuracies.

TABLE 4.5: Prediction accuracies of *A. thaliana* phenotypes for GBLUP and ANN

| Phenotype | GBLUP | ANN | Architecture | Epochs |
|----------------------------------|--------|--------|--------------|--------|
| FT16 | 0.8237 | 0.8215 | 100 | 10 |
| 2W | 0.8156 | 0.8205 | 50, 30 | 35 |
| FT10 | 0.8249 | 0.8191 | 48 | 50 |
| LD | 0.8128 | 0.8159 | 150 | 30 |
| DTF sweden 2009 (1st experiment) | 0.8063 | 0.8141 | 48 | 30 |
| DTF sweden 2009 (2nd experiment) | 0.8035 | 0.8091 | 50, 30 | 20 |
| DTF sweden 2008 (2nd experiment) | 0.7986 | 0.8057 | 150 | 25 |
| 4W | 0.795 | 0.8052 | 50, 35, 15 | 30 |

| | | | | |
|-----------------------------------|--------|--------|------------|----|
| FT22 | 0.8009 | 0.8043 | 150 | 15 |
| DTF spain 2008 (2nd experiment) | 0.7975 | 0.8032 | 150 | 40 |
| LN16 | 0.7996 | 0.7999 | 50, 30 | 20 |
| DTF spain 2009 (2nd experiment) | 0.7917 | 0.7988 | 150 | 55 |
| LDV | 0.8158 | 0.7975 | 150 | 15 |
| 0W GH FT | 0.7873 | 0.7942 | 50, 30 | 15 |
| DTFmainEffect2009 | 0.7794 | 0.7855 | 50, 35, 15 | 35 |
| SD | 0.7905 | 0.7848 | 48 | 30 |
| DTFplantingSummer2008 | 0.75 | 0.7746 | 50, 30 | 20 |
| FT GH | 0.7693 | 0.7702 | 50, 30 | 15 |
| DTFlocSweden2009 | 0.7595 | 0.7626 | 50, 30 | 60 |
| DTFplantingSummer2009 | 0.7521 | 0.7584 | 50, 30 | 50 |
| 0W | 0.7488 | 0.7473 | 48 | 40 |
| DTF spain 2009 (1st experiment) | 0.7691 | 0.7425 | 48 | 40 |
| DTF sweden 2008 (1st experiment) | 0.727 | 0.728 | 50, 30 | 20 |
| DTFlocSweden2008 | 0.7161 | 0.7271 | 50, 30 | 55 |
| Seed Dormancy | 0.7014 | 0.7241 | 50, 30 | 35 |
| DTFmainEffect2008 | 0.7102 | 0.7142 | 50, 30 | 20 |
| 8W | 0.7259 | 0.7083 | 150 | 50 |
| LN22 | 0.7004 | 0.7069 | 50, 30 | 20 |
| Size sweden 2009 (1st experiment) | 0.6905 | 0.6994 | 48 | 50 |
| LN10 | 0.6934 | 0.698 | 50, 30 | 20 |
| DTF spain 2008 (1st experiment) | 0.6944 | 0.677 | 150 | 25 |
| SDV | 0.6775 | 0.6728 | 150 | 15 |
| 8W GH FT | 0.7001 | 0.6546 | 48 | 40 |
| 0W GH LN | 0.6568 | 0.654 | 50, 30 | 20 |
| Storage 7 days | 0.6496 | 0.65 | 50, 30 | 25 |
| Storage 28 days | 0.6627 | 0.6483 | 50, 30 | 55 |
| 8W GH LN | 0.671 | 0.6434 | 48 | 70 |
| Size sweden 2009 (2nd experiment) | 0.6114 | 0.6268 | 48 | 50 |
| SizeLocSweden2009 | 0.6144 | 0.619 | 150 | 35 |
| FLC | 0.6118 | 0.6161 | 50, 30 | 30 |
| LFS GH | 0.6178 | 0.6136 | 150 | 35 |
| FT Field | 0.7324 | 0.6112 | 150 | 60 |
| LY | 0.6072 | 0.6088 | 150 | 60 |
| Storage 56 days | 0.6085 | 0.5788 | 150 | 15 |
| LES | 0.56 | 0.5764 | 150 | 50 |
| M216T665 | 0.5155 | 0.5674 | 50, 30 | 50 |
| LC Duration GH | 0.5799 | 0.5664 | 150 | 55 |
| M172T666 | 0.5165 | 0.5487 | 150 | 60 |
| Trichome avg JA | 0.588 | 0.5343 | 150 | 55 |
| Secondary Dormancy | 0.5184 | 0.5264 | 150 | 30 |
| SizeMainEffect2009 | 0.52 | 0.5171 | 48 | 50 |
| DSDS50 | 0.4754 | 0.5006 | 50, 30 | 60 |
| avrPphB | 0.5054 | 0.4942 | 150 | 60 |
| Hypocotyl length | 0.4934 | 0.4807 | 150 | 50 |
| Size spain 2009 (1st experiment) | 0.5121 | 0.4751 | 150 | 50 |

| | | | | |
|-----------------------------------|--------|--------|------------|----|
| Yield spain 2009 (1st experiment) | 0.5205 | 0.4719 | 50, 30 | 50 |
| Leaf serr 10 | 0.4636 | 0.4683 | 150 | 55 |
| Size spain 2009 (2nd experiment) | 0.471 | 0.4623 | 48 | 50 |
| Trichome avg C | 0.4617 | 0.4385 | 48 | 40 |
| Germ in dark | 0.4447 | 0.4382 | 150 | 15 |
| YieldMainEffect2009 | 0.505 | 0.4345 | 150 | 30 |
| FT Diameter Field | 0.5004 | 0.4274 | 150 | 15 |
| Bacterial titer | 0.5406 | 0.417 | 150 | 55 |
| FRI | 0.4011 | 0.4119 | 48 | 30 |
| Rosette Erect 22 | 0.3973 | 0.3934 | 48 | 30 |
| Area sweden 2009 (1st experiment) | 0.4203 | 0.3895 | 50, 35, 15 | 30 |
| Width 10 | 0.3932 | 0.3784 | 50, 30 | 60 |
| Silique 22 | 0.4339 | 0.377 | 50, 30 | 50 |
| avrRpt2 | 0.3757 | 0.3737 | 50, 30 | 30 |
| M130T666 | 0.4381 | 0.3733 | 150 | 60 |
| SizePlantingSummer2009 | 0.3769 | 0.3615 | 150 | 5 |
| Area sweden 2009 (2nd experiment) | 0.359 | 0.3542 | 48 | 45 |
| FW | 0.3397 | 0.3522 | 50, 30 | 25 |
| P31 | 0.3632 | 0.3419 | 50, 30 | 45 |
| MT GH | 0.4016 | 0.3397 | 150 | 50 |
| avrB | 0.3304 | 0.3384 | 50, 30 | 30 |
| avrRpm1 | 0.361 | 0.3368 | 50, 30 | 20 |
| Seed bank 133-91 | 0.3446 | 0.3334 | 150 | 5 |
| Mg25 | 0.5321 | 0.3288 | 50, 30 | 60 |
| Leaf roll 10 | 0.3558 | 0.3272 | 48 | 40 |
| Yield spain 2009 (2nd experiment) | 0.4184 | 0.3197 | 20, 10 | 40 |
| Noco2 | 0.3051 | 0.3174 | 48 | 30 |
| Emwa1 | 0.3226 | 0.3124 | 50, 30 | 30 |
| FT Duration GH | 0.2659 | 0.3123 | 48 | 5 |
| Leaf serr 22 | 0.3021 | 0.3108 | 150 | 60 |
| Anthocyanin 10 | 0.3198 | 0.3107 | 50, 35, 15 | 60 |
| Cd114 | 0.3345 | 0.3069 | 50, 30 | 50 |
| Leaf serr 16 | 0.2895 | 0.3011 | 48 | 40 |
| Fe56 | 0.2802 | 0.3006 | 150 | 35 |
| YieldLocSweden2009 | 0.3431 | 0.2993 | 150 | 60 |
| Width 16 | 0.3463 | 0.2983 | 150 | 50 |
| Co59 | 0.2738 | 0.2953 | 50, 35, 15 | 25 |
| K39 | 0.3036 | 0.2952 | 50, 30 | 60 |
| Leaf roll 16 | 0.3072 | 0.2886 | 150 | 15 |
| DTFplantingLoc2008 | 0.2971 | 0.275 | 50, 30 | 5 |
| SizePlantingSummerLocSweden2009 | 0.2803 | 0.2704 | 50, 30 | 60 |
| Mn55 | 0.2775 | 0.2662 | 50, 30 | 20 |
| Anthocyanin 22 | 0.2731 | 0.2635 | 150 | 15 |
| As75 | 0.254 | 0.2619 | 50, 30 | 35 |
| Na23 | 0.2564 | 0.2598 | 50, 30 | 15 |
| Ni60 | 0.2894 | 0.2539 | 150 | 25 |
| Mo98 | 0.2765 | 0.2537 | 50, 30 | 35 |

| | | | | |
|------------------------------------|---------|---------|------------|----|
| Chlorosis 22 | 0.2622 | 0.2453 | 50, 35, 15 | 10 |
| Hiks1 | 0.2441 | 0.2452 | 20, 10 | 20 |
| Zn66 | 0.2553 | 0.2444 | 150 | 35 |
| B11 | 0.2891 | 0.2392 | 48 | 40 |
| Germ 16 | 0.2987 | 0.2356 | 50, 30 | 41 |
| At2 | 0.2147 | 0.216 | 150 | 15 |
| Emco5 | 0.166 | 0.2101 | 150, 30 | 20 |
| Se82 | 0.2192 | 0.2075 | 150 | 25 |
| Mature cell length | 0.1987 | 0.2052 | 150 | 45 |
| DW | 0.2878 | 0.2048 | 50, 30 | 60 |
| Yield sweden 2009 (1st experiment) | 0.2274 | 0.2033 | 150 | 55 |
| As2 | 0.1774 | 0.1962 | 150 | 15 |
| Meristem zone length | 0.1976 | 0.195 | 150 | 50 |
| Germ 10 | 0.2073 | 0.1873 | 20, 10 | 40 |
| Anthocyanin 16 | 0.2433 | 0.1867 | 20, 10 | 10 |
| Width 22 | 0.2224 | 0.1856 | 50, 30 | 50 |
| YieldPlantingSummerLocSweden2009 | 0.2146 | 0.18 | 150 | 55 |
| DTFplantingSummerLocSweden2009 | 0.2032 | 0.1775 | 150 | 55 |
| Bs | 0.2161 | 0.1656 | 50, 30 | 60 |
| Bs CFU2 | 0.1672 | 0.1584 | 50, 35, 15 | 15 |
| Germ 22 | 0.1267 | 0.1533 | 50, 30 | 35 |
| Leaf roll 22 | 0.1135 | 0.1511 | 48 | 45 |
| RP GH | 0.1755 | 0.1458 | 150 | 15 |
| Cu65 | 0.1543 | 0.1315 | 150 | 5 |
| Li7 | 0.1611 | 0.1297 | 150 | 60 |
| As | 0.1089 | 0.1227 | 100 | 20 |
| At1 | 0.1473 | 0.1197 | 48 | 40 |
| S34 | 0.1045 | 0.11 | 50, 30 | 60 |
| YieldPlantingSummer2009 | 0.1265 | 0.0984 | 150 | 50 |
| Silique 16 | 0.2366 | 0.0884 | 50, 30 | 60 |
| Chlorosis 10 | 0.0243 | 0.088 | 50, 35, 15 | 55 |
| Ca43 | 0.3333 | 0.0732 | 50, 35, 15 | 55 |
| Seedling Growth | 0.0813 | 0.0636 | 48 | 30 |
| Vern Growth | -0.0096 | 0.0422 | 150 | 15 |
| At2 CFU2 | 0.0694 | 0.0378 | 150 | 25 |
| Yield sweden 2009 (2nd experiment) | 0.0536 | 0.0355 | 150 | 25 |
| As CFU2 | 0.0312 | 0.035 | 150 | 5 |
| At1 CFU2 | 0.0818 | 0.0319 | 50, 30 | 50 |
| Aphid number | -0.0246 | 0.029 | 50, 35, 15 | 10 |
| After Vern Growth | -0.1433 | 0.0057 | 50, 35, 15 | 5 |
| Chlorosis 16 | -0.0313 | -0.0121 | 150 | 5 |
| As2 CFU2 | 0.0504 | -0.0325 | 50, 30 | 60 |

Table 4.5 contains in total 145 phenotypes where both ANN and GBLUP yielded

successful predictions. For 60 of 145 phenotypes ANNs were able to outperform GBLUP. However, when the overall prediction accuracies are generally high $\rho(y, \hat{y}) \geq 0.75$, 16 out 20 of the ANN yield higher predictive abilities than GBLUP. At an intermediate level they perform both at the same level and at low levels $\rho(y, \hat{y}) < 0.30$ GBLUP appears to be better than the tested ANNs.

Figure 4.6 compares the average prediction accuracies of 50 validation folds for ANN and GBLUP for all the 145 phenotypes.

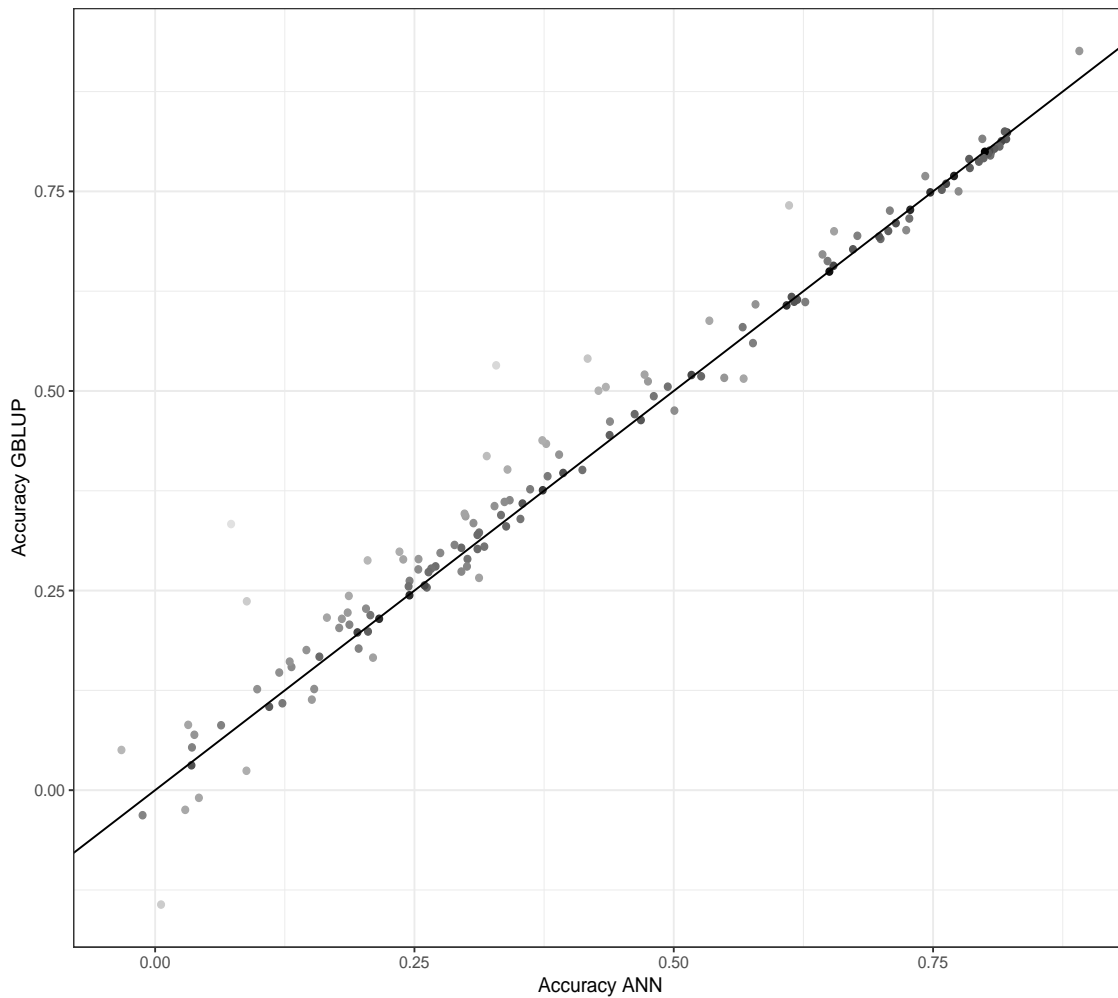


FIGURE 4.6: Scatterplot comparing prediction accuracies of ANN and GBLUP in *A. thaliana*. Greyscale indicates the magnitude of the difference between the methods

Usually the prediction accuracies across methods are closely correlated with each other. Only for a few phenotypes there is a large difference in the prediction

accuracies observable. For more than 100 of the prediction sets the difference in accuracies is smaller than 0.03. The ones with a difference in accuracies larger than 0.05 are among those with low general prediction accuracies, with extreme values of 0.2 and 0.15 going in either direction, however with the majority of those with GBLUP being dominant over ANNs. Furthermore, also visible in figure 4.6, when the predictive abilities are high and ANNs perform better, the differences between the methods becomes insignificantly small, so that for those data sets the difference is smaller than 0.01.

4.6.1 Results of maize prediction

Across environments

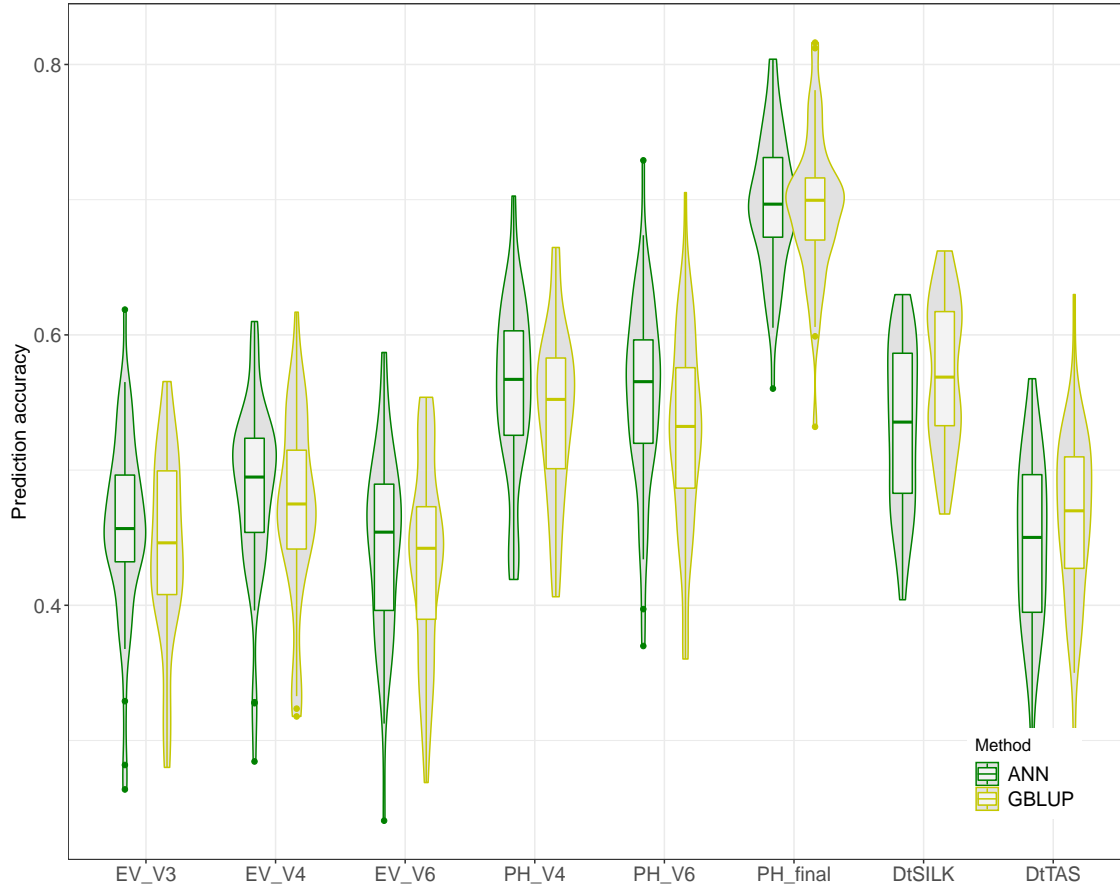


FIGURE 4.7: Violinplot comparing the results of genomic prediction in the doubled-haploid population Kemater for ANN and GBLUP for the early vigor (EV_V3,4,6) and plant height (PH_V4,V6,final) traits and days till silking (DtSILK) and days till tasseling (DtTAS).

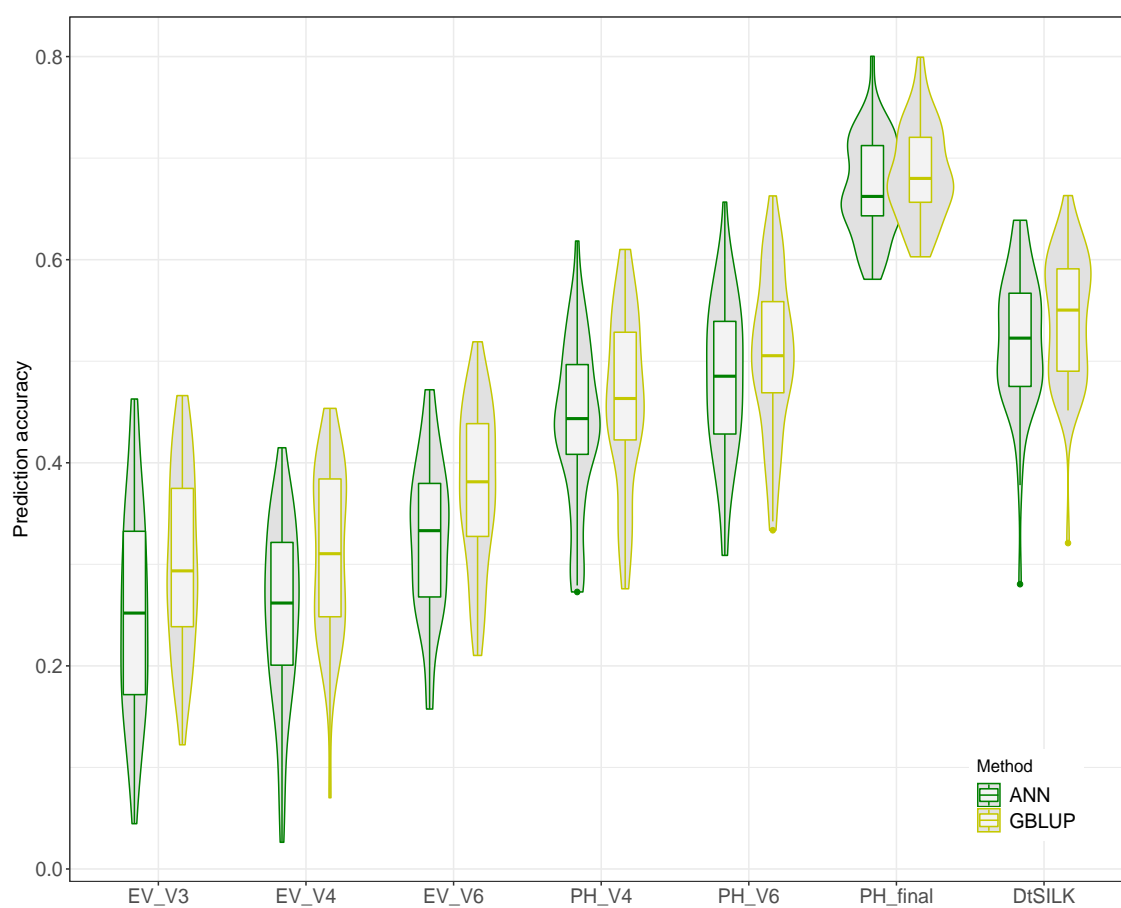


FIGURE 4.8: Violinplot comparing the results for genomic prediction in the doubled-haploid population Petkuser for ANN and GBLUP for the early vigor (EV_V3,4,6) and plant height (PH_V4,V6,final) traits and days till silking (DtSILK).

TABLE 4.6: Prediction accuracies of maize phenotypes for the doubled-haploid populations Kemater and Petkuser and the early vigor (EV_V3,4,6) and plant height (PH_V4,6,final) traits and days till silking (DtSILK).

| Phenotype | Kemater | | Petkuser | |
|------------|---------|------|----------|------|
| | GBLUP | ANN | GBLUP | ANN |
| EV_V3 | 0.44 | 0.46 | 0.31 | 0.25 |
| EV_V4 | 0.47 | 0.49 | 0.31 | 0.25 |
| EV_V6 | 0.43 | 0.44 | 0.38 | 0.33 |
| DtTAS | 0.47 | 0.44 | | |
| PH_V4_mean | 0.54 | 0.56 | 0.46 | 0.44 |
| PH_V6_mean | 0.53 | 0.56 | 0.51 | 0.48 |
| PH_final | 0.69 | 0.70 | 0.68 | 0.67 |
| DtSILK | 0.57 | 0.53 | 0.54 | 0.52 |

Single environments

The prediction of the single environment BLUEs with the environmentally enhanced marker matrix yielded substantially higher prediction accuracies than the prediction with the across environment BLUEs (previous section). The gain is higher if the prediction accuracies previously have been lower. Figure 4.9 **A** compares the results for within and across location prediction for the Kemater DHs and **B** for the Petkuser population. The overall gain of adding the environmental information to the marker matrix is higher for Petkuser, where the prediction accuracies with the across environment BLUEs have been smaller.

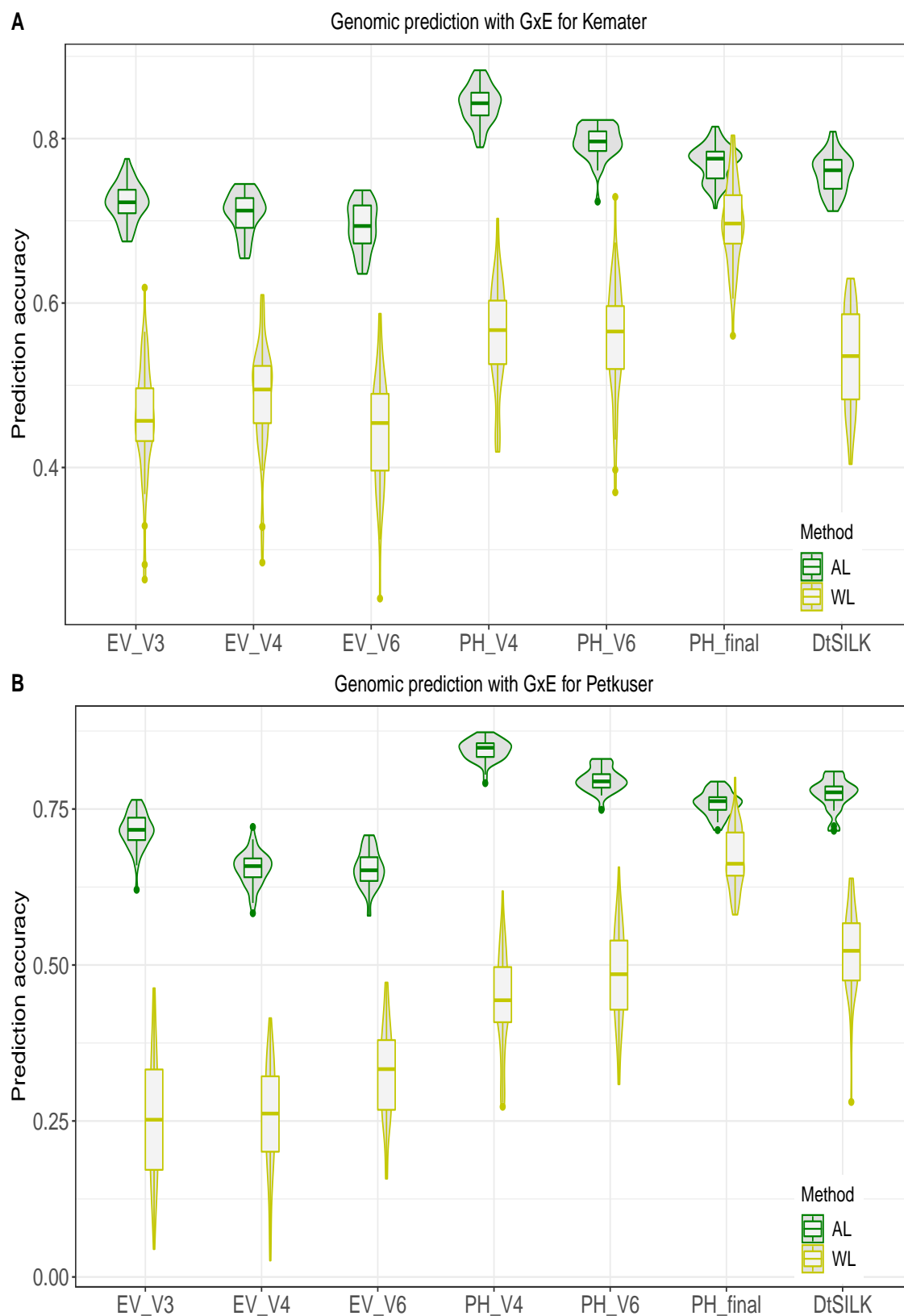


FIGURE 4.9: Results of genomic prediction across single environments for **A** Kemater and **B** Petkuser DH populations

Comparison of Bayesian methods in maize phenotype prediction

Figure 4.10 compares the results of phenotype prediction for five different Bayesian methods in terms of the respective prediction accuracy. The trials have been run for both DH populations independently. The results back those from the literature, mentioned in chapter 4.2.2.

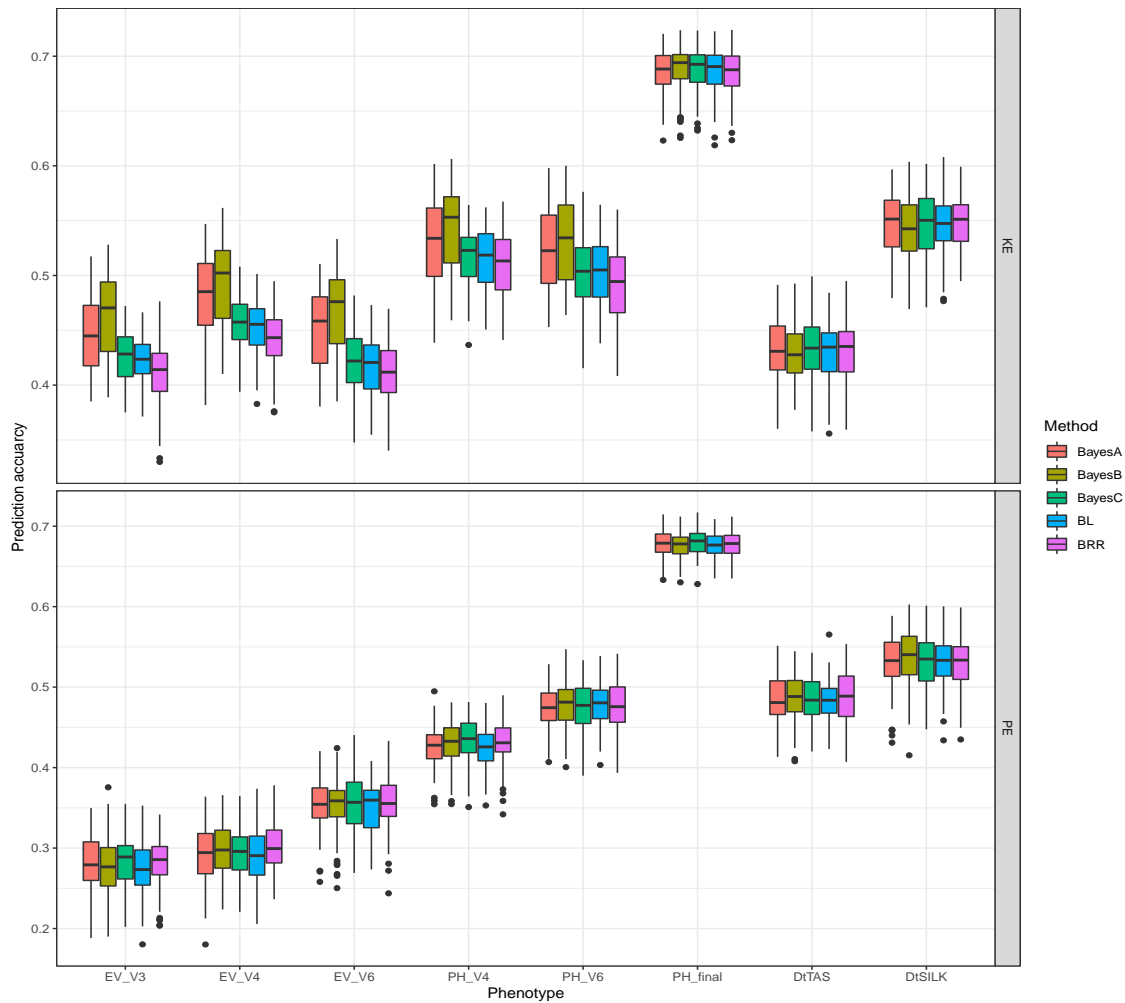


FIGURE 4.10: Results of genomic prediction of maize traits with five different Bayesian methods for eight difference traits for the DH populations Kemater (KE) and Petkuser (PE)

No single method is superior over all the others. This is more pronounced in the Petkuser subpopulation, where there is almost no difference between those methods at all, and less articulated for Kemater. At view the plot for Kemater

might suggest that BayesA and especially BayesB perform on higher levels for most trades. However, all the early vigor and plant height traits are closely correlated since they are basically the same trait measured at different time points, it is not surprising that the same algorithm that works well on one of those works well on the others as well.

Number of marker and prediction accuracy

Marker chips like the ones to analyze the genomic maize data for this study contain hundreds of thousands of SNPs and other polymorphisms UNTERSEER et al., 2014. Through LD, many of those markers do not segregate independently and are highly co-linear. In elite breeding materials LD is typical very large and cultivated maize is no exception. The markers used were already LD pruned to the remaining 28933 markers. Figure 4.11 shows the mean of prediction accuracies for the Bayesian methods as a function of the markers used. For that the complete marker set has been subsampled multiple times in 1k, 2k, 5k, 10k or 20k subsets and used in the prediction pipeline.

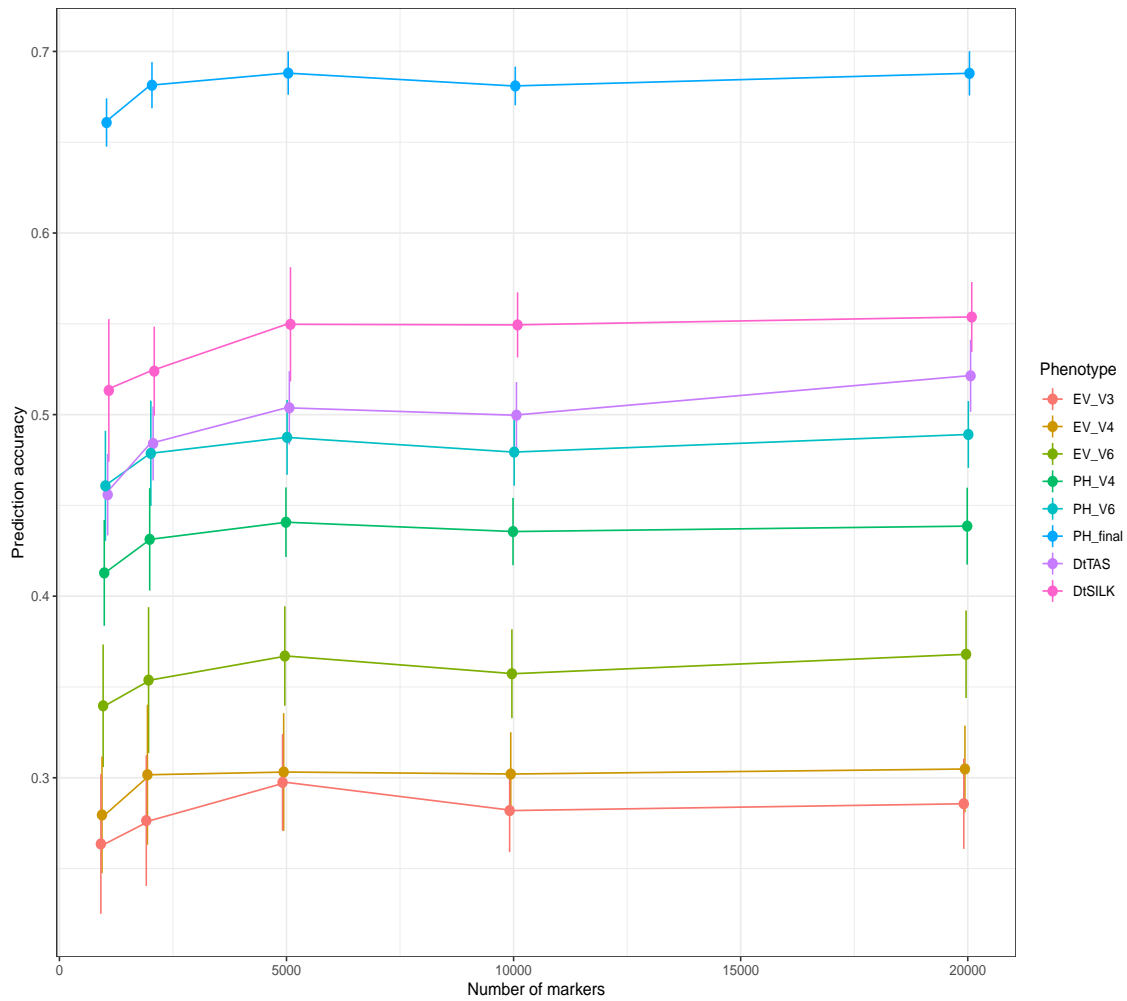


FIGURE 4.11: Predictive ability as a function of the number of markers for the Petkuser population of maize and varying amounts of markers and the Bayesian methods

As visible in figure 4.11 there is no increase in prediction accuracies after the marker sets exceed 5000 markers for all the maize traits in the Petkuser population. Furthermore, the majority of the predictive ability is already met with just 1000 genomic markers in the study, so that the increase between 1k and 5k is usually smaller than 0.05. This holds true for all the traits, and does not increase or decrease as the overall predictive ability grows larger.

Number of DHs and prediction accuracy

Figure 4.12 shows the prediction accuracy as a function as the number of Kemater DHs in the prediction set. As explained in section 4.5.3, the full 471 Kemater DH library was subsampled in 50,100, 200, 300 and 400 DH subsets 10 times each and again each subset has been split into 80% TRN and 20% TRN 50 times.

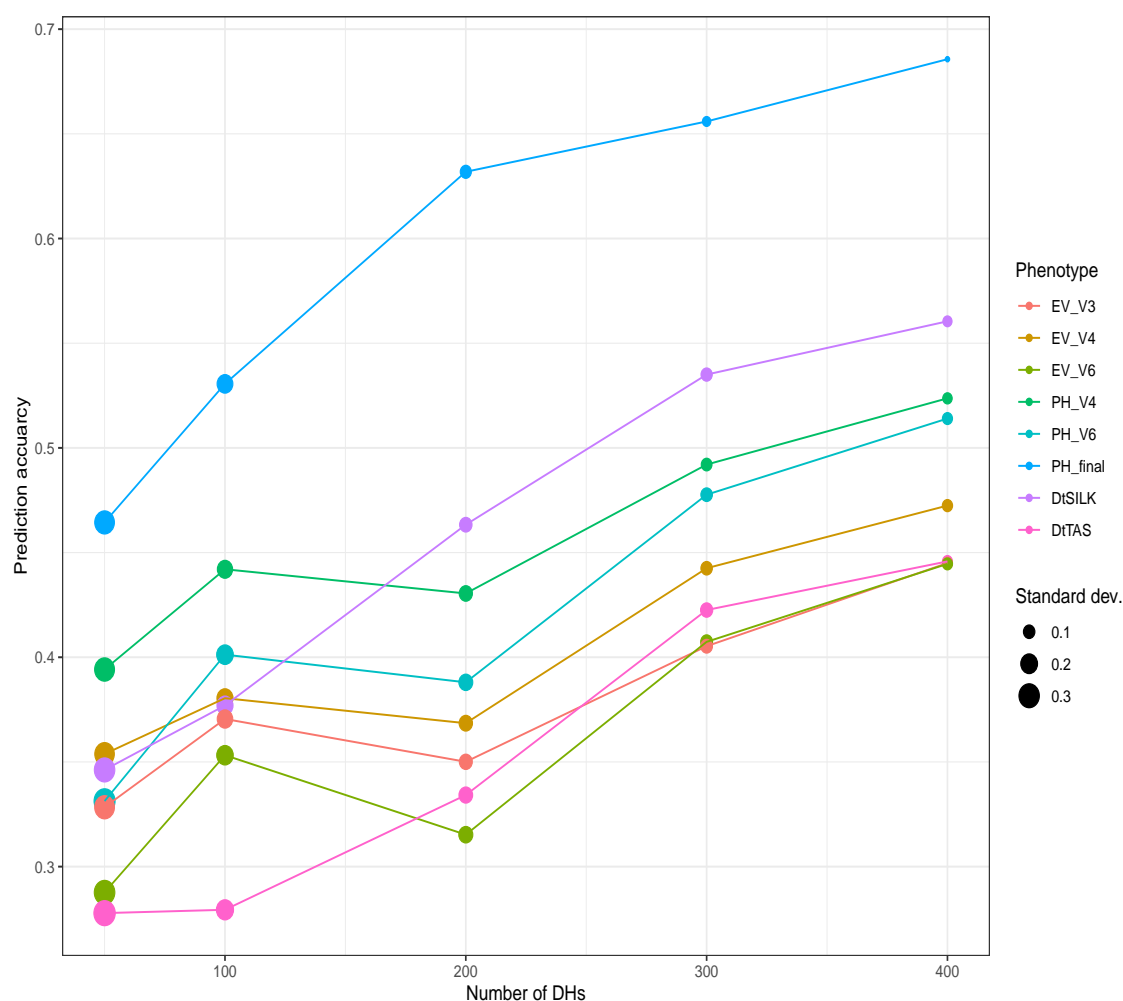


FIGURE 4.12: Predictive ability as a function of the number of phenotypes included in the prediction from the Kemater population. The dots represent the mean prediction accuracies for 10 randomly subsets with 50 fold validation each. The pointsize indicates the standard deviation

Figure 4.12 shows similar behaviors for all the eight different phenotypes assessed. With increasing number of DH the prediction accuracy will gradually

increase, while the standard deviation of a total of 500 predictions for each subset and phenotype decreases. Figure 4.11, addressing $\rho_{(y,\hat{y})}$ as a function as the number of markers shows a plateau just after a couple of thousand markers. For the number of DHs a similar effect for $\rho_{(y,\hat{y})}$ is not observable. Even though the largest increases are realized between 50 and 200 DHs, between 200 and 400 DHs there appears to be a linear increase of the predictive ability, which does not hit a plateau yet. This will be thoroughly discussed in section 4.7.

4.7 Discussion

4.7.1 Correlation between heritability and prediction accuracy

The results in section 4.6 and table 4.5 show that for a large variety of different *A. thaliana* traits prediction accuracies vary from 0 to almost 0.9, depending on the trait being assessed. Next to the number for markers and phenotypes. The architectures of a trait as explained in section 4.2.1 will have an definite influence on the ability of prediction algorithms being able to produce meaningful results. Plot 4.13 compares the heritability with the results of GBLUP prediction for the 146 *A. thaliana* traits used for prediction. The heritability here is the pseudo-heritability as estimated during GWAS using REML estimations of the variance components of a trait with given genotypes.

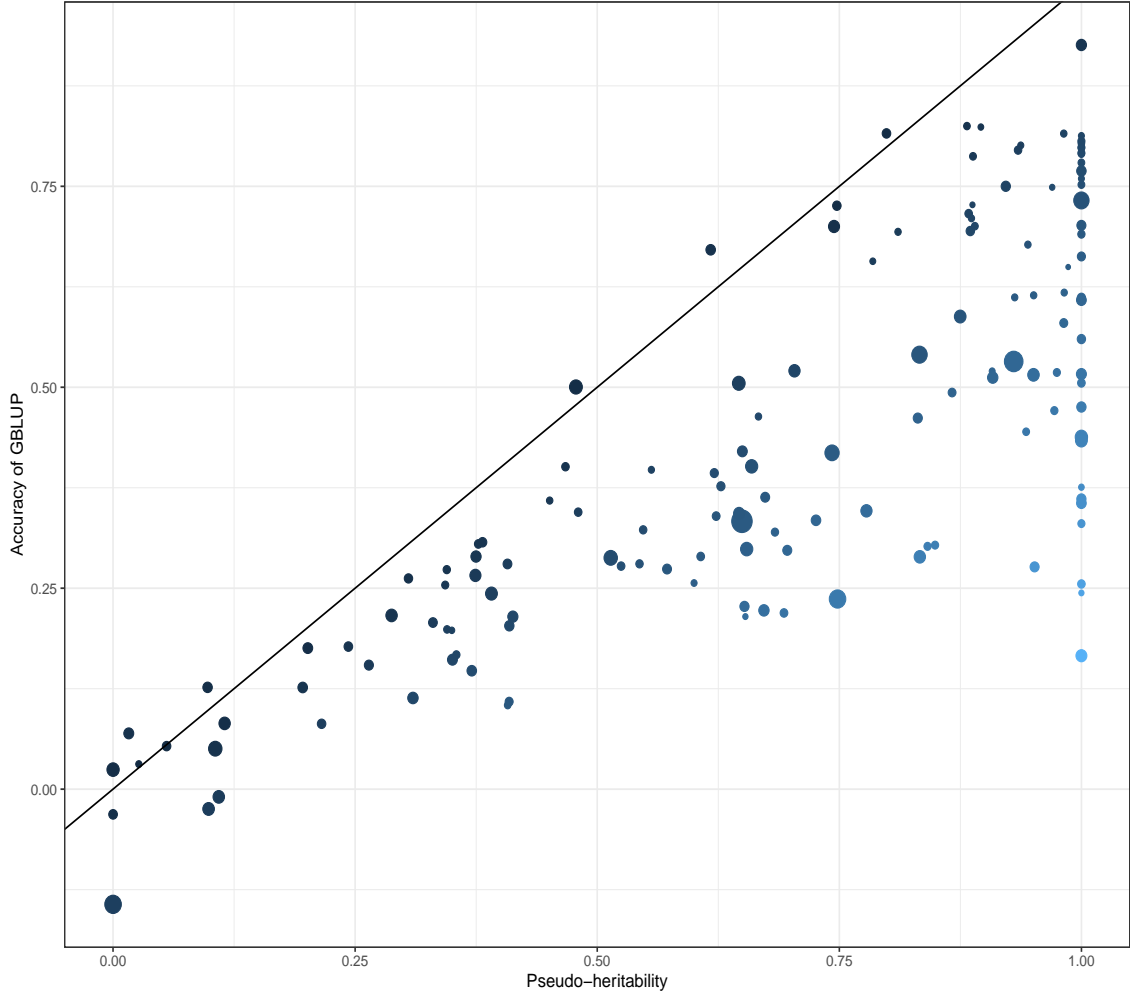


FIGURE 4.13: Prediction accuracies of GBLUP compared to the pseudo-heritability of *A. thaliana* traits. The color scale indicates the difference between the accuracy and the pseudo-heritability. The size of the dots indicates the absolute difference between ANN and GBLUP prediction. Dots on the diagonal line have the same accuracy and heritability

The predictive ability and the pseudo-heritability are highly correlated with each other and the latter can be used as a valid approximation for the accuracy of prediction. In chapter 4.2.1 it was stated that it is theoretically impossible for $\rho_{(y,\hat{y})}$ to exceed H^2 . As figure 4.13 shows this assumptions hold almost completely true. Only a few points are slightly above the diagonal and are either close to 0 for both $\rho_{(y,\hat{y})}$ and the pseudo-heritability or might be due to over- or under inflation of the REML model. Many published studies found similar results concerning the

comparison of the two metrics e.g KWONG et al., 2017; MORGANTE et al., 2018; YAP et al., 2018; PIASKOWSKI et al., 2018; ZHANG et al., 2019

4.7.2 Two or three layer networks outperform deeper ANNs

While different network architectures were tested on the ones with LCL were better performing than with just FCL architectures.

TABLE 4.7: ANN architectures resulting in highest prediction accuracies, with number of hidden layer (HL) and the total count (n)

| LCL | Architecture | HL | n |
|------|--------------|----|----|
| True | 150 | 2 | 56 |
| True | 50, 30 | 3 | 47 |
| True | 48 | 2 | 23 |
| True | 50, 35, 15 | 4 | 11 |
| True | 20, 10 | 3 | 5 |
| True | 100 | 2 | 2 |
| True | 150, 30 | 3 | 1 |

Number of phenotypes is the bottleneck

Design of training populations AKDEMIR and ISIDRO-SÁNCHEZ, 2019

4.7.3 GxE interactions have great influence on plant development traits in maize

TABLE 4.8: Comparison of prediction results of ANN within locations (WL) and across locations (AL) for Kemater and Petkuser

| Phenotype | Kemater | | | Petkuser | | |
|-----------|---------|------|----------|----------|------|----------|
| | AL | WL | Δ | AL | WL | Δ |
| EV_V3 | 0.73 | 0.46 | 0.27 | 0.72 | 0.25 | 0.47 |
| EV_V4 | 0.72 | 0.49 | 0.23 | 0.66 | 0.25 | 0.40 |
| EV_V6 | 0.70 | 0.44 | 0.26 | 0.65 | 0.33 | 0.33 |
| PH_V4 | 0.84 | 0.56 | 0.28 | 0.84 | 0.44 | 0.41 |
| PH_V6 | 0.80 | 0.56 | 0.25 | 0.80 | 0.48 | 0.31 |
| PH_final | 0.78 | 0.70 | 0.08 | 0.76 | 0.67 | 0.09 |
| DtSILK | 0.76 | 0.53 | 0.23 | 0.77 | 0.52 | 0.25 |

5 General Discussion and further Observations

5.1 Genomic data preparation is error-prone

Researching and applying quantitative genetics from genome assemblies to genomic selection is tedious with many error-prone steps involved. To obtain optimal results every step in the entire process has to be optimized individually, without losing the larger frame out of sight.

To perform analyses for quantitative genetics in general there are two types of data required: (i) genotypic and (ii) phenotypic data. Both are equally important and take many steps to procure. Figure 5.1 schematically displays the key steps involved in obtaining genomic marker matrices for downstream analyses as GWAS and GS from selection of candidate genotypes to the final numeric marker matrix. Genotyping can either be achieved by whole genome sequencing or by SNP analysis with a SNP array. The first step after sequencing, which provides raw reads, is to assemble the genome. As discussed in chapter 2 genome assembly is a complicated process. This holds true for both the assembly of core and plastid genomes. There is a large variety of tools available for core genome assembly and like the ones for plastid genomes they vary in their algorithmic approaches and likewise their accuracy ZHANG et al., 2011, which makes it hard to determine whether polymorphisms between individual genomes are due to artifacts in the genome assembly pipeline or actually are mirrored in the biological

genome. Furthermore, genome assemblies result in one dimensional representations of formerly three dimensional genomes, losing most of the spatial and epigenetic information.

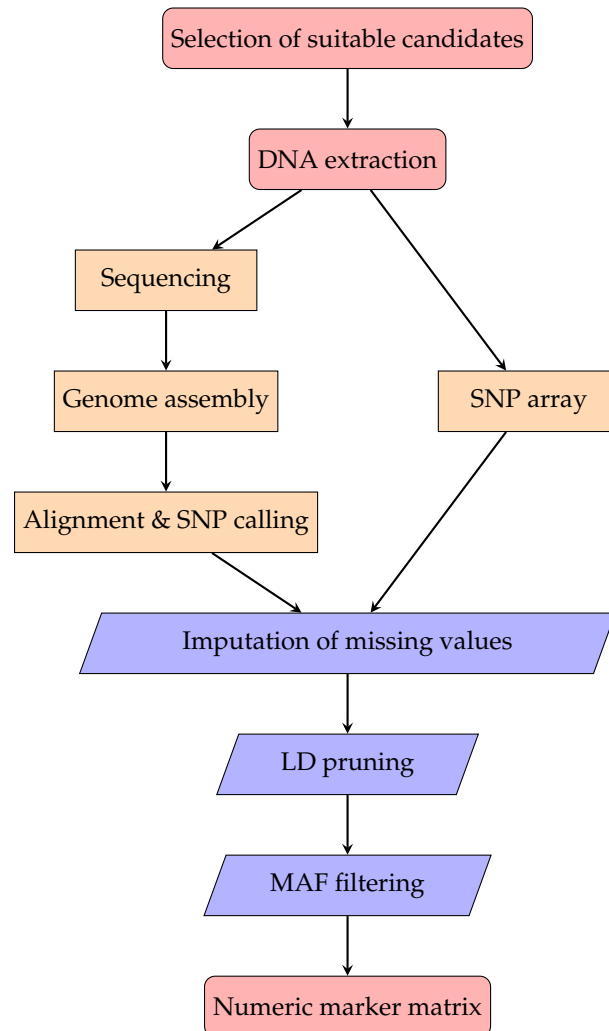


FIGURE 5.1: Schematic process of genotyping for quantitative genetics analyses with its crucial steps

After sequencing and assembling multiple genomes of a species the next step is to align them to detect genetic polymorphisms such as SNPs, InDels, etc. followed by the imputation of missing values. This takes into consideration that all the missing data are actually missing due to the assembly and not actually missing in the genome as deletions. However, this step is necessary because GWAS and genomic selection requires complete data without missing values. Again,

there is a variety of tools for the imputation of missing markers. In plant genomics the most commonly used software is Beagle BROWNING and BROWNING, 2007; BROWNING, ZHOU, and BROWNING, 2018, which is based on hidden Markov models. As thoroughly reviewed by POOK et al., 2019 the accuracy of the algorithm varies vastly depending on the population, LD structure, chromosome region, effective population size and the allele frequency, all possibly leading to errors adding up the ones already introduced to the upper branches of the entire pipeline.

5.1.1 Imputation leads to false positive GWAS results

Faulty imputation and SNP calling can possibly result in false positive GWAS results as shown in the following example. Data from phenotypic trials with 330 fully sequenced *A. thaliana* for carbon isotope discrimination were used to perform GWAS with a marker matrix with 10 million SNPs imputed with Beagle 3.0 DITTBERNER et al., 2018. This resulted in one marker with a significant p value on the fourth chromosome. Upon further investigation of the chromosomal region in question using the unimputed data a complex haplotype structure was revealed as shown in figure 5.2.

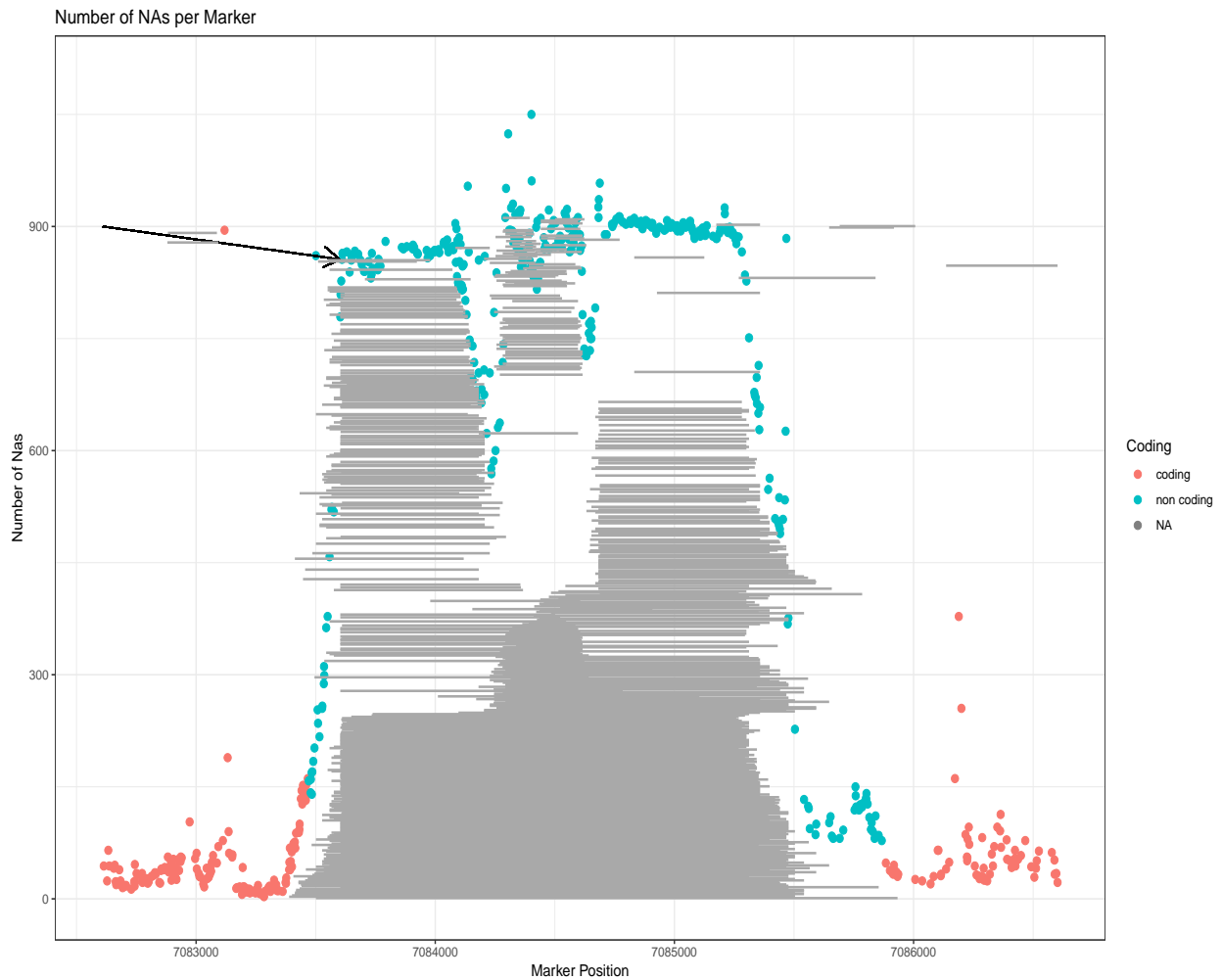


FIGURE 5.2: Haplotype structure on a 1 kb window of chromosome 5 of *A. thaliana*. On the vertical axis the number of NAs in the population of 1135 accessions for a given marker is displayed. The horizontal axis gives the physical position on the chromosome. Red markers are located in coding and blue markers in non-coding regions according to the TAIR10 annotation RHEE et al., 2003. The gray bars indicate more than five coherent missing values for one accession. The arrow points to the location of the significant GWAS hit.

The significant SNP is located in a region where up to 80% of the data were originally missing values and were filled with Beagle 3.0. Additionally a complex structure of longer or shorter deletions is present, completely cutting out the non-coding region between the two coding ones. Taking a look at figure 5.2 it

immediately becomes obvious that imputation in this region has to be wrong because the complex haplotype structure is a clear indication for the missing values not being due to sequencing errors, but that they are actually mirrored in the biological genomes. The possibility of imputation leading to false positives has been discussed by LIN et al., 2010. The present case provides an practical example of the phenomenon.

Further in the scope of the study it was assessed whether the phasing algorithm used in Beagle 3.0 detected some signal from the haplotype structure that led to the faulty imputation. The different haplotypes and deletions were coded as pseudo-markers for further association studies, all resulting in non-significant p-values. The plots in figure 5.2 provide a good example to show how the information loss about complex genomic structures can lead to false statistical assumptions.

5.1.2 Numeric marker matrices cannot represent the complexity of genomes

Figure 5.3 shows the complex haplotype structure of chromosome one of *A. thaliana*. The plots for chromosome two to five are included in appendix C.2. They basically all follow a similar pattern. The region directly flanking the centromere is more polymorphic than the telomeres at the p and q arms of the chromosomes, independent if the chromosome is metacentric like chromosome one and five, telocentric as chromosome two and four acrocentric as chromosome three. The centromere itself is highly conserved and generally coding regions have less haplotypes than non-coding regions. E.g. on chromosome one over a 1 kbp window in 1135 accessions there are ca. 78 different haplotypes in general and 98 in coding and 62 in non-coding regions on average. The most polymorphic regions, however, are often coding regions, like a region on the q arm of chromosome one located at around 22 Mbp, which has more than 700 segregating haplotypes

in the 1 kbp window. The region harbors a locus containing disease resistance genes CHENG et al., 2017, over the evolutionary advantages or disadvantages for those regions being highly polymorphic can only be speculated.

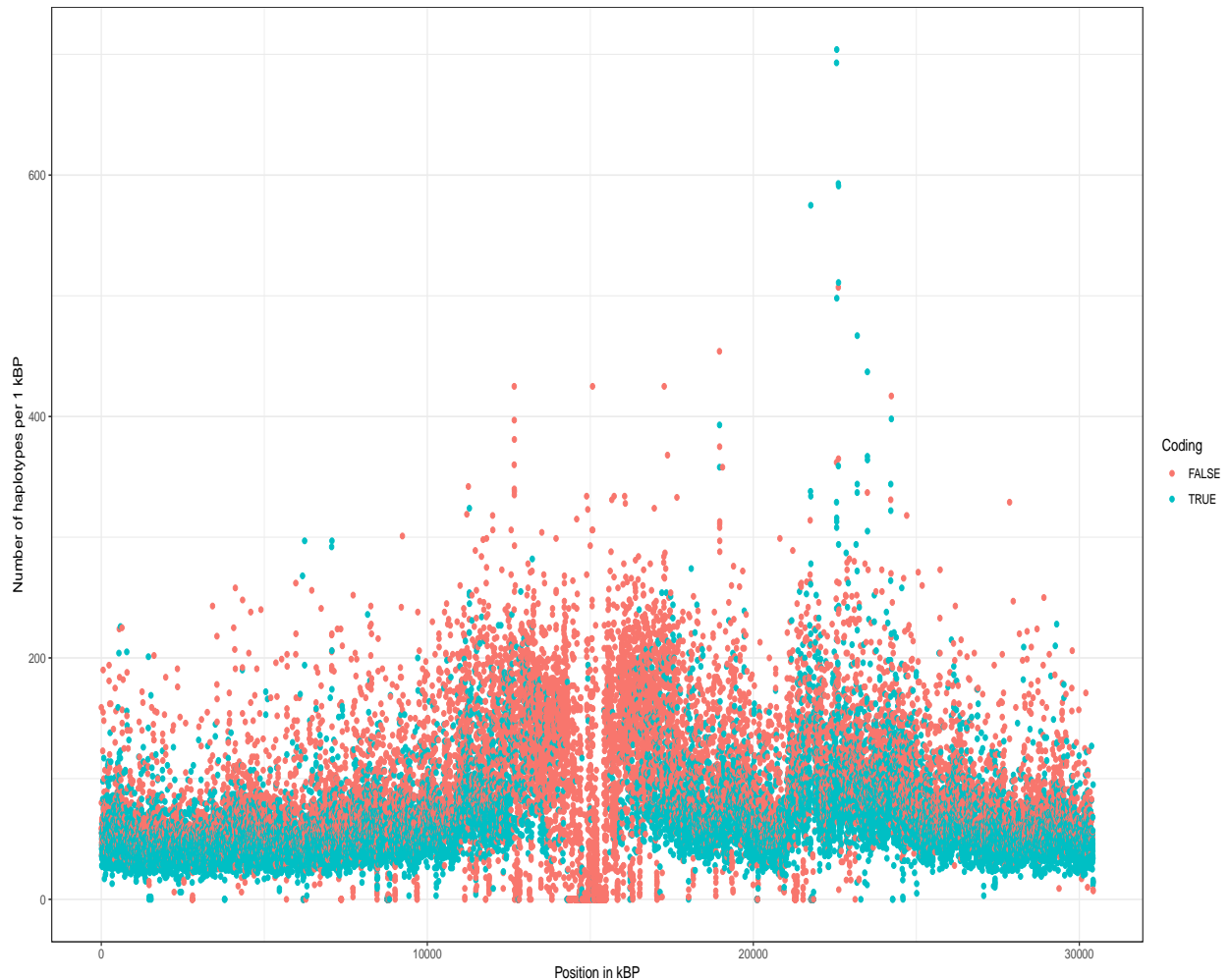


FIGURE 5.3: The number of segregating haplotypes with a polymorphism in at least one position over a stretch of 1 kbp on chromosome one of *A. thaliana*.

Next to highly polymorphic regions there are regions, which are completely conserved and do not have a single polymorphism in a 1 kbp window. Around the centromere there are regions longer than 10 kbp with no SNPs. Intuitively one would assume that this would indicate important household genes that do not allow for any alterations in the amino acid sequence, however, the majority, around 75% of those regions are considered to be non-coding. Conserved

non-coding sequences (CNS) have been widely studied and shown great evolutionary importance and were witnessed across species with millions of years of evolutionary distance BURGESS and FREELING, 2014.

The haplotype analysis allows to visualize another interesting evolutionary artifact. Chromosome one of *A. thaliana* was derived from a fusion of two chromosomes of its next relative *A. lyrata*. Next to the active centromere located in the middle of the chromosome, at around 20 mbp there is a region that shares some properties of a centromere, where one of the *A. lyrata* centromeres was located KOCH and MATSCHINGER, 2007.

The haplotype as well as the LD structure overall or for special regions e.g flowering time associated loci LI et al., 2014, is too complex to be represented sufficiently in a binary marker matrix. If this holds true, as shown, for *A. thaliana* it matters even more so in plants whose genomes are much larger, underwent multiple whole genome duplications and consist of many more chromosomes. Like the diploid *Z. mays* with 10 chromosomes, the allotetraploid *B. napus* a product of hybridization with 19 chromosomes from the two ancestral species *B. rapa* and *B. oleracea* LIU, SNOWDON, and CHALHOUB, 2018, or the even more complex allohexaploid genome of *T. aestivum* CONSORTIUM, 2018.

5.1.3 Input data for GWAS and GS

Phenotypic trials can only ever represent a sub sample of whole populations. Even larger trials in the 1001 genome project only feature a bit more than 1000 accessions ATWELL et al., 2010; ALONSO-BLANCO et al., 2016. In practical trials it is common to randomly pick accessions, cultivars or genotypes in the hope that they will segregate for a certain trait. Sometimes this can be backed by a PCA or an analysis of molecular variance to choose suitable candidates HÖLKER et al., 2019 but this is not common practice. This results in allele frequencies in the subpopulations not following those of the global populations and phenotypic values

not following normal distributions. For the 402 tested *A. thaliana* traits analyzed only 72 follow a normal distribution (own observation), according the Shapiro-Wilk test SHAPIRO and WILK, 1965. Taking into account that many statistical tests assume normal distributed data, this is an another source of errors in the genomic analysis pipelines, leading to over or under inflation of p-values. This effect can become very large for imbalanced and/or binary phenotypes like YEL (appendix B). One method to overcome this problematic is to use permutation-based thresholds for significance, which can account better for phenotypic distributions than Bonferroni thresholds (chapter 4). In the given example the permutation threshold is around 10^{-16} and the Bonferroni threshold is approximately 10^{-8} , potentially leading to a larger number of number of false positive markers.

Due the many sources of statistical inaccuracies that can be possibly introduced in the whole genome analysis pipeline all results have to be carefully evaluated, which often times is not done sufficiently. For each significant marker that has been detected the raw genomic information needs to assessed to validate the results.

5.2 Prospects in Genomic Selection and Plant Breeding and Conclusion

Plant breeding, like in the last decades and centuries, will utilize the technology of its time. Many new tools including genome editing were recently added to the tool box of breeding and despite regulatory issues of quickly found its way to revolutionize modern plant biotechnology ARAKI and ISHII, 2015. While in the future GWAS and its relatives or progeny will be used to further elucidate the nature of quantitative traits, genomic selection and genome editing will allow further improvement of the worlds major crop plants RODRÍGUEZ-LEAL et al., 2017, aiming to provide germplasms with the yield potential required by the demand

of the growing world's population.

Following the current trends in bioinformatics for plant breeding this full lead to a further increase in the dimensionality of data, as genotyping costs will further decline and modern automated phenotyping techniques allow for larger trials, swallowing less resources.

A Source code

A.1 GWAS-Flow

A.1.1 gwas.py

```
1 import os
2 import sys
3 import time
4 import numpy as np
5 import pandas as pd
6 import main
7 import h5py
8
9 # set defaults
10 mac_min = 1
11 batch_size = 500000
12 out_file = "results.csv"
13 m = 'phenotype_value'
14 perm = 1
15 mac_min= 6
16
17 X_file = 'gwas_sample_data/AT_geno.hdf5'
18 Y_file = 'gwas_sample_data/phenotype.csv'
19 K_file = 'gwas_sample_data/kinship_ibs_binary_mac5.h5py'
20
21
22
23 for i in range (1,len(sys.argv),2):
24     if sys.argv[i] == "-x" or sys.argv[i] == "--genotype":
25         X_file = sys.argv[i+1]
26     elif sys.argv[i] == "-y" or sys.argv[i] == "--phenotype":
```

```

27     Y_file = sys.argv[i+1]
28     elif sys.argv[i] == "-k" or sys.argv[i] == "--kinship":
29         K_file = sys.argv[i+1]
30     elif sys.argv[i] == "-m":
31         m = sys.argv[i+1]
32     elif sys.argv[i] == "-a" or sys.argv[i] == "--mac_min":
33         mac_min = int(sys.argv[i+1])
34     elif sys.argv[i] == "-bs" or sys.argv[i] == "--batch-size":
35         batch_size = int(sys.argv[i+1])
36     elif sys.argv[i] == "-p" or sys.argv[i] == "--perm":
37         perm = int(sys.argv[i+1])
38     elif sys.argv[i] == "-o" or sys.argv[i] == "--out":
39         out_file = sys.argv[i+1]
40     elif sys.argv[i] == "-h" or sys.argv[i] == "--help":
41         print("-x , --genotype :file containing marker information
in csv or hdf5 format of size")
42         print("-y , --phenotype: file container phenotype
information in csv format" )
43         print("-k , --kinship : file containing kinship matrix of
size k X k in csv or hdf5 format")
44         print("-m : name of columnn containing the phenotype :
default m = phenotype_value")
45         print("-a , --mac_min : integer specifying the minimum
minor allele count necessary for a marker to be included.
Default a = 1" )
46         print("-bs, --batch-size : integer specifying the number of
markers processed at once. Default -bs 500000" )
47         print("-p , --perm : single integer specifying the number
of permutations. Default 1 == no perm ")
48         print("-o , --out : name of output file. Default -o results
.csv ")
49         print("-h , --help : prints help and command line options")
50         quit()
51     else:
52         print('unknown option ' + str(sys.argv[i]))
53         quit()
54
55

```

```

56
57 print("parsed commandline args")
58
59 start = time.time()
60
61 X,K,Y_,markers = main.load_and_prepare_data(X_file,Y_file,K_file,m)
62
63
64 ## MAF filterin
65 markers_used , X , macs = main.mac_filter(mac_min,X,markers)
66
67 ## prepare
68 print("Begin performing GWAS on ", Y_file)
69
70 if perm == 1:
71     output = main.gwas(X,K,Y_,batch_size)
72     if( X_file.split(".")[ -1] == 'csv'):
73         chr_pos = np.array(list(map(lambda x : x.split("- "),
74 markers_used)))
75     else:
76         chr_reg = h5py.File(X_file,'r')['positions'].attrs['
chr_regions']
77         mk_index= np.array(range(len(markers)),dtype=int)[macs >=
mac_min]
78         chr_pos = np.array([list(map(lambda x: sum(x > chr_reg
[: ,1]) + 1, mk_index)), markers_used]).T
79         my_time = np.repeat((time.time()-start),len(chr_pos))
80         pd.DataFrame({
81             'chr' : chr_pos[:,0] ,
82             'pos' : chr_pos[:,1] ,
83             'pval': output[:,0] ,
84             'mac' : np.array(macs[macs >= mac_min],dtype=np.int) ,
85             'eff_size': output[:,1] ,
86             'SE' : output[:,2]}) .to_csv(out_file,index=False)
87 elif perm > 1:
88     min_pval = []
89     perm_seeds = []
90     my_time = []

```

```
90     for i in range(perm):
91         start_perm = time.time()
92         print("Running permutation ", i+1, " of ", perm)
93         my_seed = np.asscalar(np.random.randint(9999, size=1))
94         perm_seeds.append(my_seed)
95         np.random.seed(my_seed)
96         Y_perm = np.random.permutation(Y_)
97         output = main.gwas(X, K, Y_perm, batch_size)
98         min_pval.append(np.min(output[:, 0]))
99         print("Elapsed time for permuatation", i+1, " with p_min",
100               min_pval[i], " is", ":", round(time.time() - start_perm, 2))
101         my_time.append(time.time() - start_perm)
102         pd.DataFrame({
103             'time': my_time,
104             'seed': perm_seeds,
105             'min_p': min_pval }).to_csv(out_file, index=False)
106
107
108 end = time.time()
109 eltime = np.round(end - start, 2)
110
111 if eltime <= 59:
112     print("Total time elapsed", eltime, "seconds")
113 elif eltime > 59 and eltime <= 3600:
114     print("Total time elapsed", np.round(eltime / 60, 2), "minutes")
115 elif eltime > 3600:
116     print("Total time elapsed", np.round(eltime / 60 / 60, 2), "hours")
117
118
```

A.1.2 main.py

```
1     import pandas as pd
2     import numpy as np
3     from scipy.stats import f
4     import tensorflow as tf
```

```

5     import limix
6     import herit
7     import h5py
8     import limix
9     import multiprocessing as mlt
10
11     def load_and_prepare_data(X_file,Y_file,K_file,m):
12         type_K = K_file.split(".")[1]
13         type_X = X_file.split(".")[1]
14
15         ## load and preprocess genotype matrix
16         Y = pd.read_csv(Y_file,engine='python').sort_values(['
17         accession_id']).groupby('accession_id').mean()
18         Y = pd.DataFrame({'accession_id' : Y.index, 'phenotype_value'
19         : Y[m]})
20
21         if type_X == 'hdf5' or type_X == 'h5py' :
22             SNP = h5py.File(X_file,'r')
23             markers= np.asarray(SNP['positions'])
24             acc_X = np.asarray(SNP['accessions'][:,],dtype=np.int)
25         elif type_X == 'csv' :
26             X = pd.read_csv(X_file,index_col=0)
27             markers = X.columns.values
28             acc_X = X.index
29             X = np.asarray(X,dtype=np.float32)/2
30         else :
31             sys.exit("Only hdf5, h5py and csv files are supported")
32
33         if type_K == 'hdf5' or type_K == 'h5py':
34             k = h5py.File(K_file,'r')
35             acc_K = np.asarray(k['accessions'][:,],dtype=np.int)
36         elif type_K == 'csv':
37             k = pd.read_csv(K_file,index_col=0)
38             acc_K = k.index
39             k = np.array(k, dtype=np.float32)
40
41         acc_Y = np.asarray(Y[['accession_id']]).flatten()
42         acc_isec = [isec for isec in acc_X if isec in acc_Y]

```

```

41     idx_acc = list(map(lambda x: x in acc_isec, acc_X))
42     idy_acc = list(map(lambda x: x in acc_isec, acc_Y))
43     idk_acc = list(map(lambda x: x in acc_isec, acc_K))
44
45     Y_ = np.asarray(Y.drop('accession_id',1),dtype=np.float32)[
idy_acc,:]
46
47     if type_X == 'hdf5' or type_X == 'h5py' :
48         X = np.asarray(SNP['snps'][0:(len(SNP['snps'])+1)],dtype=
np.float32)[:,idx_acc].T
49         X = X[np.argsort(acc_X[idx_acc]),:]
50         k1 = np.asarray(k['kinship'][:])[idk_acc,:]
51         K = k1[:,idk_acc]
52         K = K[np.argsort(acc_X[idx_acc]),:]
53         K = K[:,np.argsort(acc_X[idx_acc])]
54     else:
55         X = X[idx_acc,:]
56         k1 = k[idk_acc,:]
57         K = k1[:,idk_acc]
58
59
60     print("data has been imported")
61     return X,K,Y_,markers
62
63
64 def mac_filter(mac_min, X, markers):
65     ac1 = np.sum(X,axis=0)
66     ac0 = X.shape[0] - ac1
67     macs = np.minimum(ac1,ac0)
68     markers_used = markers[macs >= mac_min]
69     X = X[:,macs >= mac_min]
70     return markers_used, X, macs
71
72 def gwas(X,K,Y,batch_size):
73     n_marker = X.shape[1]
74     n = len(Y)
75     ## REML

```



```

76     K_stand = (n-1)/np.sum((np.identity(n) - np.ones((n,n))/n) * K)
       * K
77     vg, delta, ve = herit.estimate(Y,"normal",K_stand,verbose =
False)
78     print(" Pseudo-heritability is " , vg / (ve + vg + delta))
79     print(" Performing GWAS on ", n , " phenotypes and ", n_marker
,"markers")
80     ## Transform kinship-matrix, phenotypes and estimate intercpt
81     Xo = np.ones(K.shape[0]).flatten()
82     M = np.transpose(np.linalg.inv(np.linalg.cholesky(vg * K_stand
+ ve * np.identity(n))))).astype(np.float32)
83     Y_t = np.sum(np.multiply(np.transpose(M),Y),axis=1).astype(np.
float32)
84     int_t = np.sum(np.multiply(np.transpose(M),np.ones(n)),axis=1).
astype(np.float32)
85     ## EMMAX Scan
86     RSS_env = (np.linalg.lstsq(np.reshape(int_t,(n,-1)) , np.
reshape(Y_t,(n,-1)))[1]).astype(np.float32)
87     ## calculate betas and se of betas
88     def stderr(a,M,Y_t2d,int_t):
89         x = tf.stack((int_t,tf.squeeze(tf.matmul(M.T,tf.reshape(a
,(n,-1))))),axis=1)
90         coeff = tf.matmul(tf.matmul(tf.linalg.inv(tf.matmul(tf.
transpose(x),x)),tf.transpose(x)),Y_t2d)
91         SSE = tf.reduce_sum(tf.math.square(tf.math.subtract(Y_t,tf
.math.add(tf.math.multiply(x[:,1],coeff[0,0]),tf.math.multiply(x
[:,1],coeff[1,0])))))
92         SE = tf.math.sqrt(SSE/(471-(1+2)))
93         StdERR = tf.sqrt(tf.linalg.diag_part(tf.math.multiply(SE ,
tf.linalg.inv(tf.matmul(tf.transpose(x),x))))) [1]
94         return tf.stack((coeff[1,0],StdERR))
95     ## calculate residual sum squares
96     def rss(a,M,y,int_t):
97         x_t = tf.reduce_sum(tf.math.multiply(M.T,a),axis=1)
98         lm_res = tf.linalg.lstsq(tf.transpose(tf.stack((int_t,x_t)
,axis=0)),Y_t2d)
99         lm_x = tf.concat((tf.squeeze(lm_res),x_t),axis=0)

```

```

100         return tf.reduce_sum(tf.math.square(tf.math.subtract(tf.
squeeze(Y_t2d),tf.math.add(tf.math.multiply(lm_x[1],lm_x[2:]),
tf.multiply(lm_x[0],int_t))))))
101     ## loop over the batches
102     for i in range(int(np.ceil(n_marker/batch_size))):
103         tf.reset_default_graph()
104         if n_marker < batch_size:
105             X_sub = X
106         else:
107             lower_limit = batch_size * i
108             upper_limit = batch_size * i + batch_size
109             if upper_limit <= n_marker :
110                 X_sub = X[:,lower_limit:upper_limit]
111                 print("Working on markers ", lower_limit , " to ",
upper_limit, " of ", n_marker )
112             else:
113                 X_sub = X[:,lower_limit:]
114                 print("Working on markers ", lower_limit , " to ",
n_marker, " of ", n_marker )
115             config = tf.ConfigProto()
116             n_cores = mlt.cpu_count()
117             config.intra_op_parallelism_threads = n_cores
118             config.inter_op_parallelism_threads = n_cores
119             sess = tf.Session(config=config)
120             Y_t2d = tf.cast(tf.reshape(Y_t,(n,-1)),dtype=tf.float32)
121             y_tensor = tf.convert_to_tensor(Y_t,dtype = tf.float32)
122             StdERR = tf.map_fn(lambda a : stderr(a,M,Y_t2d,int_t),
X_sub.T)
123             R1_full = tf.map_fn(lambda a: rss(a,M,Y_t2d,int_t), X_sub.T
)
124             F_1 = tf.divide(tf.subtract(RSS_env, R1_full),tf.divide(
R1_full,(n-3)))
125             if i == 0 :
126                 output = sess.run(tf.concat([tf.reshape(F_1,(X_sub.
shape[1],-1)),StdERR],axis=1))
127             else :
128                 tmp = sess.run(tf.concat([tf.reshape(F_1,(X_sub.shape
[1],-1)),StdERR],axis=1))

```

```

129         output = np.append(output, tmp, axis=0)
130     sess.close()
131     F_dist = output[:,0]
132     pval = 1 - f.cdf(F_dist, 1, n-3)
133     output[:,0] = pval
134     return output
135
136
137

```

A.1.3 herit.py

```

1
2 def estimate(y, lik, K, M=None, verbose=True):
3     from numpy_sugar.linalg import economic_qs
4     from numpy import pi, var, diag
5     from glimix_core.glmm import GLMMExpFam
6     from glimix_core.lmm import LMM
7     from limix._data._assert import assert_likelihoood
8     from limix._data import normalize_likelihoood, conform_dataset
9     from limix.qtl._assert import assert_finite
10    from limix._display import session_block, session_line
11    lik = normalize_likelihoood(lik)
12    lik_name = lik[0]
13    with session_block("Heritability analysis", disable=not verbose
14):
15        with session_line("Normalising input...", disable=not
16verbose):
17            data = conform_dataset(y, M=M, K=K)
18            y = data["y"]
19            M = data["M"]
20            K = data["K"]
21            assert_finite(y, M, K)
22            if K is not None:
23                # K = K / diag(K).mean()
24                QS = economic_qs(K)
25            else:
26                QS = None
27            if lik_name == "normal":

```

```
26         method = LMM(y.values, M.values, QS, restricted=True)
27         method.fit(verbose=verbose)
28     else:
29         method = GLMMExpFam(y, lik, M.values, QS, n_int=500)
30         method.fit(verbose=verbose, factr=1e6, pgtol=1e-3)
31     g = method.scale * (1 - method.delta)
32     e = method.scale * method.delta
33     if lik_name == "bernoulli":
34         e += pi * pi / 3
35     v = var(method.mean())
36     return g, v, e
37
38
39
```

A.2 Genomic prediction

A.2.1 GP ANN

```
1
2 import os, sys, gc
3 import pandas as pd
4 import numpy as np
5 import timeit
6 from datetime import datetime
7 import keras
8 import tensorflow as tf
9 from keras import backend as K
10 from keras import layers
11 from keras.models import Sequential
12 from keras.layers import Dense, Dropout, GaussianNoise,
13     AlphaDropout, Reshape
14 from keras.layers import Flatten, LocallyConnected1D,
15     LocallyConnected2D
16 from keras.optimizers import Adam, Adagrad, Adadelta
17 from keras.backend.tensorflow_backend import set_session
18
19 ##set default values
```

```

18
19 learning_rate = 0.01
20 JobID = 1
21 ps = 25
22 optim = "adam"
23 X_file = "KE.geno.csv"
24 Y_file = "KE.pheno.csv"
25 CV_file = "KE_cv_pw.csv"
26 label = "DtSILK"
27 start_time = timeit.default_timer()
28 act="relu"
29 drop_rate = str('0.5,0.5,0.5')
30 arc = str('63,63')
31 DG = 'D,D,D,D,D,G'
32 LC = True
33 training_epochs = 25
34 hyp = False
35
36 ### parse command line arguments
37
38 for i in range (1,len(sys.argv),2):
39     if sys.argv[i] == "-x":
40         X_file = sys.argv[i+1]
41     elif sys.argv[i] == "-y":
42         Y_file = sys.argv[i+1]
43     elif sys.argv[i] == "-cv":
44         CV_file = sys.argv[i+1]
45     elif sys.argv[i] == "-JobID":
46         JobID = int(sys.argv[i+1])
47     elif sys.argv[i] == "-label":
48         label = sys.argv[i+1]
49     elif sys.argv[i] == "-act":
50         act = str(sys.argv[i+1])
51     elif sys.argv[i] == "-epochs":
52         training_epochs = int(sys.argv[i+1])
53     elif sys.argv[i] == "-lr":
54         learning_rate = float(sys.argv[i+1])
55     elif sys.argv[i] == "-arc":

```

```

56     arc = sys.argv[i+1]
57     elif sys.argv[i] == "-ps":
58         ps = int(sys.argv[i+1])
59     elif sys.argv[i] == "-dr":
60         drop_rate=str(sys.argv[i+1])
61     elif sys.argv[i] == "-LC":
62         LC = bool(sys.argv[i+1])
63     elif sys.argv[i] == "hyp":
64         hyp = bool(sys.argv[i+1])
65     else:
66         print('unknown option ' + str(sys.argv[i]))
67         quit()
68
69
70 x = pd.read_csv(X_file, index_col = 0)
71 y = pd.read_csv(Y_file, index_col = 0)
72 cv_folds = pd.read_csv(CV_file, index_col=0)
73
74 ## select column of phenotype file via columnname
75
76 y = y[[label]]
77 ## activity_regularizer=regularizers.l1(0.01))
78
79 def build_network(arc, drop_rate, LC, DG):
80     def add_drops(model, drop_out, k):
81         if DG[k].upper() == 'D':
82             model.add(Dropout(drop_out[0]))
83         elif DG[k].upper() == 'G':
84             model.add(GaussianNoise(drop_out[k]))
85         elif DG[k].upper() == "A":
86             model.add(AlphaDropout(drop_out[k]))
87         else:
88             pass
89         return model
90     DG = DG.strip().split(",")
91     arc = arc.strip().split(",")
92     archit = []
93     for layer in arc:

```

```

94     archit.append(int(layer))
95     layer_number = len(archit)
96     drop_rate = drop_rate.strip().split(",")
97     drop_out = []
98     for drops in drop_rate:
99         drop_out.append(float(drops))
100     model = Sequential()
101     if LC == True:
102         model.add(Reshape(input_shape=(x_train.shape[1],),
103 target_shape=(x_train.shape[1],1)))
104         model.add(LocallyConnected1D(1,10, strides=7, input_shape=(
105 x_train.shape[1],1)))
106         model.add(Flatten())
107         start = 0
108         model = add_drops(model, drop_out, start)
109     elif LC == False:
110         model.add(Dense(archit[0], kernel_initializer='
111 truncated_normal', activation=act, input_shape=(x_train.shape
112 [1],)))
113         model = add_drops(model, drop_out, start)
114         start = 1
115         for k in range(start, len(archit)):
116             model.add(Dense(archit[k], kernel_initializer='
117 truncated_normal', activation=act))
118             model = add_drops(model, drop_out, k)
119         model.add(Dense(1, kernel_initializer='truncated_normal'))
120         return(model)
121
122 config = tf.ConfigProto()
123 #config.gpu_options.per_process_gpu_memory_fraction = 0.1
124 config.gpu_options.allow_growth = True
125 set_session(tf.Session(config=config))
126
127 if not os.path.isfile("RESULTScv50.txt"):
128     out2 = open("RESULTScv50.txt", 'w')
129     out2.write('DateTime\tCompTime\ttDF\ttGenos\ttPhenos\ttCV_fold\t
130 tArchit\ttConv\ttActFun\ttEpochs\ttdrop_rate\ttAccuracy\n' )
131

```

```

126 for k in range(1,51):
127     print("Training on cv fold "+ str(k))
128     cv = cv_folds['cv_' + str(k)]
129     num_cvs = np.ptp(cv) + 1
130
131     i = 1
132     x_train = x[cv != i]
133     x_test = x[cv == i]
134     y_train = y[cv != i]
135     y_test = y[cv == i]
136
137     yhat = np.zeros(shape = y_test.shape)
138
139     model = build_network(arc,drop_rate,LC,DG)
140     model.compile(loss='mse', optimizer=Adam(lr=0.01,decay = 0.001)
141 ,metrics=['accuracy'])
142     model.fit(x_train,y_train, epochs=training_epochs , verbose=0)
143 #     score = model.evaluate(x_test, y_test, verbose=0)
144     bla = model.predict(x_test)
145     y_sub= y[np.asarray(cv == i)]
146
147     print(model.summary())
148     print('\n')
149     print(label)
150
151     comp_time = int(round(timeit.default_timer() - start_time,0))
152
153     DateTime = datetime.now().strftime('%Y-%m-%d %H:%M:%S')
154     acc = np.corrcorcoef(bla[:,0],np.asarray(y_sub)[:,0])[0,1]
155
156     out2 = open("RESULTScv50.txt", 'a')
157     out2.write('%s\t%i\t%s\t%s\t%i\t%s\t%s\t%s\t%i\t%s\t%0.5f\n'
158 , % (
159         DateTime, comp_time, label, X_file, Y_file, int(k), arc, LC
160 , act,int(training_epochs), drop_rate, round(acc,4)))
161
162     del model,bla, x_train, x_test, y_train, y_test
163     K.clear_session()

```



```

161     gc.collect()
162
163     config = tf.ConfigProto()
164     #config.gpu_options.per_process_gpu_memory_fraction = 0.1
165     config.gpu_options.allow_growth = True
166     set_session(tf.Session(config=config))

```

A.2.2 GBLUP

```

1     geno_pred <- function(phenocsv, genocsv, cvfcsv, cvf=1, mod = "BRR"
2     , label, phe)
3     {
4         my_phe <- phe
5         depends <- c("BGLR", "doBy", "doParallel", "R.utils", "BBmisc", "
6         dplyr")
7         foo <- sapply(depends,
8             function(X){if(!suppressPackageStartupMessages(
9             require(X, character.only = T))){install.packages(X)}})
10        foo <- sapply(depends, function(X){
11            suppressPackageStartupMessages(library(X, character.only=TRUE))})
12        rm(foo)
13
14        maze <- read.csv(genocsv, row.names = 1)
15        phe <- read.csv(phenocsv, row.names = 1)
16        cvffolds <- read.csv(cvfcsv, row.names=1)
17
18        X <- scale(maze)
19        y <- phe[[label]]
20        if(any(is.na(y))){
21            rms <- which(is.na(y))
22            y <- y[-rms]
23            X <- X[-rms,]
24        }
25        for(i in 1:50){
26            cvf = i
27            n=length(y)
28            seed <- sample(1:100, 1)
29
30            #set.seed(seed)

```

```

26                                     #folds=sample(1:cvf,size=n,
replace=T)
27     folds = cvffolds[,cvf]
28     yHatCV=rep(NA,n)
29
30     for(i in 1:max(folds)){
31         cat("Predicting cv-fold ",i," of ", max(folds))
32         tst=which(folds==i)
33         yNA=y
34         yNA[tst]=NA
35         fm=BGLR(y=yNA,ETA=list(list(X=X,model=mod)),verbose =F
,nIter=7000,burnIn=1000)
36         yHatCV[tst]=fm$yHat[tst]
37         cat("    done\n")
38     }
39
40     my_cor <- cor(yHatCV,y,use = "complete.obs")
41     print(c("Corrleation of GP", mod, my_cor))
42     filename = paste0(my_phe,"_gp_results.csv")
43     print(filename)
44     if(!any(dir() == filename)){
45         res <- matrix(ncol=8, nrow = 1) %>%
46             setColNames(c("geno","pheno","cv_folds","seed","
label", "cor","method","nmark"))
47         res[1,] <- c(as.character(genocsv),as.character(
phenocsv),as.character(cvf),as.character(seed),
48                     as.character(label),as.character(my_cor),
as.character(mod),dim(X)[2])
49         print("#####")
50         print(res)
51         print("#####")
52         write.csv(res,filename)
53     }else{
54         res <- read.csv(filename,row.names = 1)
55         for(i in 1:7){
56             res[,i] <- as.character(res[,i])
57         }

```

```

58         res[dim(res)[1]+1,] <- c(as.character(genocsv),phenocsv
, cvf, seed, as.character(label), my_cor, mod, dim(X)[2])
59         write.csv(res, filename)
60     }
61 }
62
63 }
64 ## execute this script with: Rscript ex.gblup.r -x genofile -y
phenofile -c cv file
65 source("~/PHD/Projects/gblup/bglr.r")
66
67
68 my.args <- commandArgs(trailingOnly = TRUE)
69 #my.args <- c("-x", "gent_genocsv", "-y" , "gent_phenocsv")
70 ### set defaults
71 #cvf.name = NA
72
73 ## parsing the command line options
74 all.opts <- c("-x", "-y", "-label", "-h", "-cv", "-phe")
75 for(i in 1:length(my.args)){
76     if( i %% 2 == 1){
77         if(!my.args[i] %in% all.opts){
78             cat("unknown option", my.args[i], "Use only", all.opts
, "\n")
79             cat("use -h for help \n")
80             quit()
81         }
82     }
83     if(my.args[i] == "-x"){
84         geno.name <- as.character(my.args[i+1])
85     } else if(my.args[i] == "-y") {
86         pheno.name <- as.character(my.args[i+1])
87     } else if(my.args[i] == "-label") {
88         my_ph <- as.character(my.args[i+1])
89     } else if(my.args[i] == "-cv"){
90         cv.name = as.character(my.args[i+1])
91     } else if(my.args[i] == "-phe"){
92         my_phe <- as.character(my.args[i+1])

```

```
93     } else if(my.args[i] == "-h") {
94         print(" This script takes as a minimum two intputs\n")
95         print(" -x genotypefile")
96         print(" -y phenotypefile ")
97         print(" -cv cross-valiadtion file : is optional if none is
specified random 5 fold cv will be used")
98         print(" -JobID : specify column number to use in your cross
validation file")
99         print(" -label : use header of phenotype file column you
want to use")
100         quit()
101     }
102 }
103
104
105 #pheno.name <- my.args[1]
106 #geno.name <- my.args[2]
107 #cvf.name <- my.args[3]
108
109 geno_pred(phenocsv = pheno.name,genocsv=geno.name, cvfcsv = cv.name
, label=my_ph,mod = "BRR", phe =my_phe)
```

B *A. thaliana* phenotypic data

| ID | Phenotype name | doi | Reference |
|----|-------------------|-----------------------|---------------------|
| 1 | FT Diameter Field | 10.21958/phenotype:1 | ATWELL et al., 2010 |
| 2 | At2 CFU2 | 10.21958/phenotype:2 | ATWELL et al., 2010 |
| 3 | Leaf serr 16 | 10.21958/phenotype:3 | ATWELL et al., 2010 |
| 4 | Seed bank 133-91 | 10.21958/phenotype:4 | ATWELL et al., 2010 |
| 5 | Na23 | 10.21958/phenotype:5 | ATWELL et al., 2010 |
| 6 | Leaf serr 10 | 10.21958/phenotype:6 | ATWELL et al., 2010 |
| 7 | Emco5 | 10.21958/phenotype:7 | ATWELL et al., 2010 |
| 8 | Leaf roll 16 | 10.21958/phenotype:8 | ATWELL et al., 2010 |
| 9 | Leaf roll 10 | 10.21958/phenotype:9 | ATWELL et al., 2010 |
| 10 | Bs | 10.21958/phenotype:10 | ATWELL et al., 2010 |
| 11 | 2W | 10.21958/phenotype:11 | ATWELL et al., 2010 |
| 12 | Rosette Erect 22 | 10.21958/phenotype:12 | ATWELL et al., 2010 |
| 13 | Cd114 | 10.21958/phenotype:13 | ATWELL et al., 2010 |
| 14 | Width 16 | 10.21958/phenotype:14 | ATWELL et al., 2010 |
| 15 | Storage 28 days | 10.21958/phenotype:15 | ATWELL et al., 2010 |
| 16 | LY | 10.21958/phenotype:16 | ATWELL et al., 2010 |
| 17 | avrRpm1 | 10.21958/phenotype:17 | ATWELL et al., 2010 |
| 18 | Width 10 | 10.21958/phenotype:18 | ATWELL et al., 2010 |
| 19 | Chlorosis 22 | 10.21958/phenotype:19 | ATWELL et al., 2010 |
| 20 | Storage 7 days | 10.21958/phenotype:20 | ATWELL et al., 2010 |
| 21 | As2 CFU2 | 10.21958/phenotype:21 | ATWELL et al., 2010 |
| 22 | Co59 | 10.21958/phenotype:22 | ATWELL et al., 2010 |

| | | | |
|----|-------------------|-----------------------|---------------------|
| 23 | FW | 10.21958/phenotype:23 | ATWELL et al., 2010 |
| 24 | Cu65 | 10.21958/phenotype:24 | ATWELL et al., 2010 |
| 25 | Bacterial titer | 10.21958/phenotype:25 | ATWELL et al., 2010 |
| 26 | Width 22 | 10.21958/phenotype:26 | ATWELL et al., 2010 |
| 27 | Storage 56 days | 10.21958/phenotype:27 | ATWELL et al., 2010 |
| 28 | YEL | 10.21958/phenotype:28 | ATWELL et al., 2010 |
| 29 | FLC | 10.21958/phenotype:29 | ATWELL et al., 2010 |
| 30 | FT16 | 10.21958/phenotype:30 | ATWELL et al., 2010 |
| 31 | FT10 | 10.21958/phenotype:31 | ATWELL et al., 2010 |
| 32 | FT Duration GH | 10.21958/phenotype:32 | ATWELL et al., 2010 |
| 33 | Se82 | 10.21958/phenotype:33 | ATWELL et al., 2010 |
| 34 | LDV | 10.21958/phenotype:34 | ATWELL et al., 2010 |
| 35 | Noco2 | 10.21958/phenotype:35 | ATWELL et al., 2010 |
| 36 | 8W GH LN | 10.21958/phenotype:36 | ATWELL et al., 2010 |
| 37 | 0W | 10.21958/phenotype:37 | ATWELL et al., 2010 |
| 38 | MT GH | 10.21958/phenotype:38 | ATWELL et al., 2010 |
| 39 | After Vern Growth | 10.21958/phenotype:39 | ATWELL et al., 2010 |
| 40 | Aphid number | 10.21958/phenotype:40 | ATWELL et al., 2010 |
| 41 | LN22 | 10.21958/phenotype:41 | ATWELL et al., 2010 |
| 42 | Bs CFU2 | 10.21958/phenotype:42 | ATWELL et al., 2010 |
| 43 | avrRpt2 | 10.21958/phenotype:43 | ATWELL et al., 2010 |
| 44 | Hypocotyl length | 10.21958/phenotype:44 | ATWELL et al., 2010 |
| 45 | Germ 22 | 10.21958/phenotype:45 | ATWELL et al., 2010 |
| 46 | Leaf roll 22 | 10.21958/phenotype:46 | ATWELL et al., 2010 |
| 47 | SD | 10.21958/phenotype:47 | ATWELL et al., 2010 |
| 48 | 8W | 10.21958/phenotype:48 | ATWELL et al., 2010 |
| 49 | FT GH | 10.21958/phenotype:49 | ATWELL et al., 2010 |
| 50 | DSDS50 | 10.21958/phenotype:50 | ATWELL et al., 2010 |

| | | | |
|----|-----------------|-----------------------|---------------------|
| 51 | Ca43 | 10.21958/phenotype:51 | ATWELL et al., 2010 |
| 52 | LC Duration GH | 10.21958/phenotype:52 | ATWELL et al., 2010 |
| 53 | 0W GH FT | 10.21958/phenotype:53 | ATWELL et al., 2010 |
| 54 | B11 | 10.21958/phenotype:54 | ATWELL et al., 2010 |
| 55 | Chlorosis 10 | 10.21958/phenotype:55 | ATWELL et al., 2010 |
| 56 | RP GH | 10.21958/phenotype:56 | ATWELL et al., 2010 |
| 57 | Chlorosis 16 | 10.21958/phenotype:57 | ATWELL et al., 2010 |
| 58 | LFS GH | 10.21958/phenotype:58 | ATWELL et al., 2010 |
| 59 | Germ 10 | 10.21958/phenotype:59 | ATWELL et al., 2010 |
| 60 | Germ 16 | 10.21958/phenotype:60 | ATWELL et al., 2010 |
| 61 | Anthocyanin 16 | 10.21958/phenotype:61 | ATWELL et al., 2010 |
| 62 | Anthocyanin 10 | 10.21958/phenotype:62 | ATWELL et al., 2010 |
| 63 | At1 CFU2 | 10.21958/phenotype:63 | ATWELL et al., 2010 |
| 64 | Ni60 | 10.21958/phenotype:64 | ATWELL et al., 2010 |
| 65 | P31 | 10.21958/phenotype:65 | ATWELL et al., 2010 |
| 66 | Emwa1 | 10.21958/phenotype:66 | ATWELL et al., 2010 |
| 67 | As75 | 10.21958/phenotype:67 | ATWELL et al., 2010 |
| 68 | Germ in dark | 10.21958/phenotype:68 | ATWELL et al., 2010 |
| 69 | FRI | 10.21958/phenotype:69 | ATWELL et al., 2010 |
| 70 | As CFU2 | 10.21958/phenotype:70 | ATWELL et al., 2010 |
| 71 | Trichome avg C | 10.21958/phenotype:71 | ATWELL et al., 2010 |
| 72 | Vern Growth | 10.21958/phenotype:72 | ATWELL et al., 2010 |
| 73 | Mo98 | 10.21958/phenotype:73 | ATWELL et al., 2010 |
| 74 | Hiks1 | 10.21958/phenotype:74 | ATWELL et al., 2010 |
| 75 | Anthocyanin 22 | 10.21958/phenotype:75 | ATWELL et al., 2010 |
| 76 | Zn66 | 10.21958/phenotype:76 | ATWELL et al., 2010 |
| 77 | Trichome avg JA | 10.21958/phenotype:77 | ATWELL et al., 2010 |
| 78 | LES | 10.21958/phenotype:78 | ATWELL et al., 2010 |

| | | | |
|-----|--------------------|------------------------|---------------------|
| 79 | Silique 16 | 10.21958/phenotype:79 | ATWELL et al., 2010 |
| 80 | Emoy* | 10.21958/phenotype:80 | ATWELL et al., 2010 |
| 81 | K39 | 10.21958/phenotype:81 | ATWELL et al., 2010 |
| 82 | 0W GH LN | 10.21958/phenotype:82 | ATWELL et al., 2010 |
| 83 | At2 | 10.21958/phenotype:83 | ATWELL et al., 2010 |
| 84 | At1 | 10.21958/phenotype:84 | ATWELL et al., 2010 |
| 85 | LN10 | 10.21958/phenotype:85 | ATWELL et al., 2010 |
| 86 | FT Field | 10.21958/phenotype:86 | ATWELL et al., 2010 |
| 87 | LN16 | 10.21958/phenotype:87 | ATWELL et al., 2010 |
| 88 | avrB | 10.21958/phenotype:88 | ATWELL et al., 2010 |
| 89 | LD | 10.21958/phenotype:89 | ATWELL et al., 2010 |
| 90 | Seedling Growth | 10.21958/phenotype:90 | ATWELL et al., 2010 |
| 91 | S34 | 10.21958/phenotype:91 | ATWELL et al., 2010 |
| 92 | Leaf serr 22 | 10.21958/phenotype:92 | ATWELL et al., 2010 |
| 93 | DW | 10.21958/phenotype:93 | ATWELL et al., 2010 |
| 94 | Seed Dormancy | 10.21958/phenotype:94 | ATWELL et al., 2010 |
| 95 | Mn55 | 10.21958/phenotype:95 | ATWELL et al., 2010 |
| 96 | Silique 22 | 10.21958/phenotype:96 | ATWELL et al., 2010 |
| 97 | avrPphB | 10.21958/phenotype:97 | ATWELL et al., 2010 |
| 98 | Fe56 | 10.21958/phenotype:98 | ATWELL et al., 2010 |
| 99 | 8W GH FT | 10.21958/phenotype:99 | ATWELL et al., 2010 |
| 100 | 4W | 10.21958/phenotype:100 | ATWELL et al., 2010 |
| 101 | Li7 | 10.21958/phenotype:101 | ATWELL et al., 2010 |
| 102 | FT22 | 10.21958/phenotype:102 | ATWELL et al., 2010 |
| 103 | As2 | 10.21958/phenotype:103 | ATWELL et al., 2010 |
| 104 | SDV | 10.21958/phenotype:104 | ATWELL et al., 2010 |
| 105 | Mg25 | 10.21958/phenotype:105 | ATWELL et al., 2010 |
| 106 | Secondary Dormancy | 10.21958/phenotype:106 | ATWELL et al., 2010 |

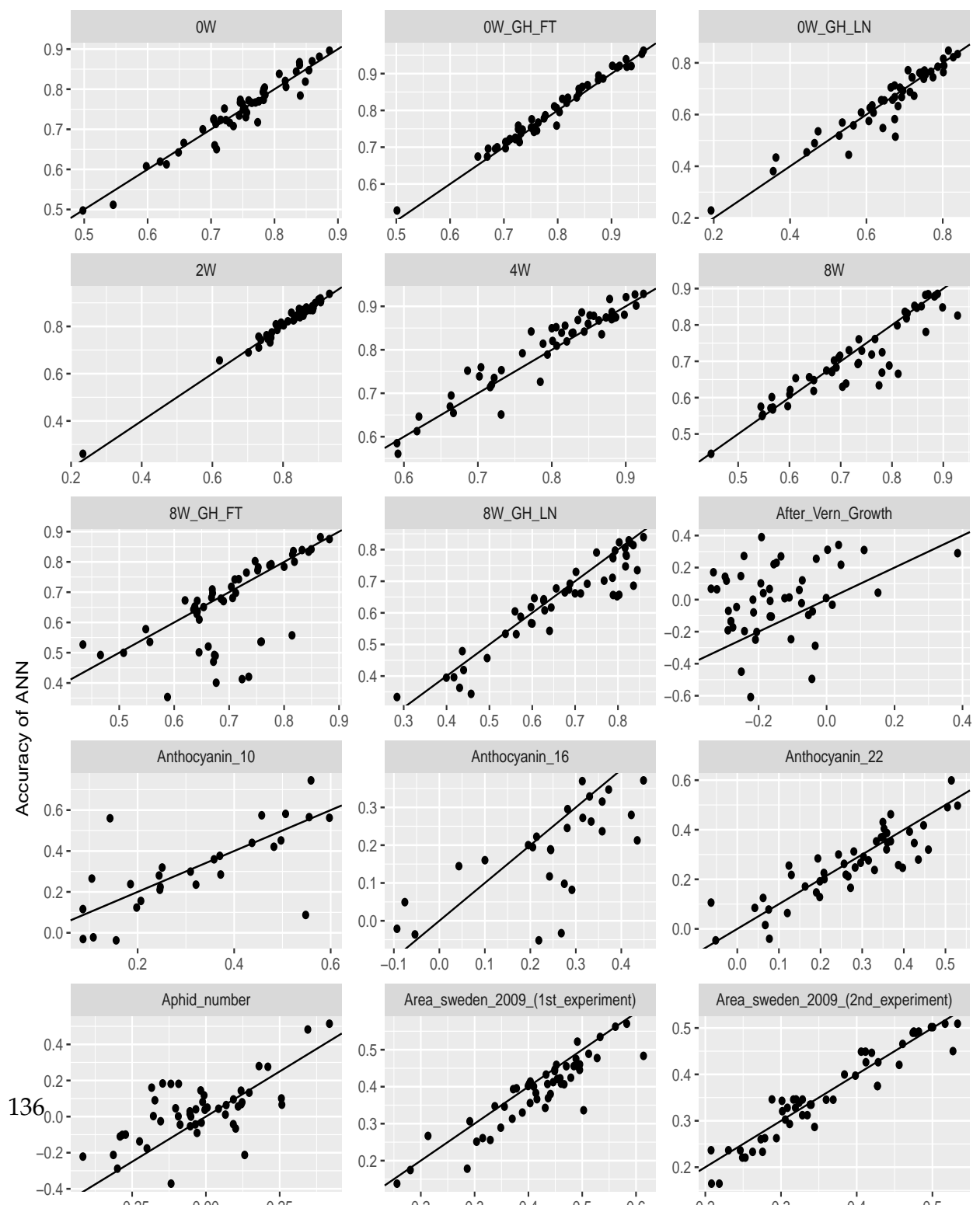
| | | | |
|-----|--------------------------------------|------------------------|---------------------|
| 107 | As | 10.21958/phenotype:107 | ATWELL et al., 2010 |
| 108 | Area Sweden 2009 (1st experiment) | 10.21958/phenotype:108 | LI et al., 2010 |
| 109 | Size Planting Summer 2009 | 10.21958/phenotype:109 | LI et al., 2010 |
| 110 | Size Sweden 2009 (2nd experiment) | 10.21958/phenotype:110 | LI et al., 2010 |
| 111 | Size Planting Summer Loc Sweden 2009 | 10.21958/phenotype:111 | LI et al., 2010 |
| 112 | Area Sweden 2009 (2nd experiment) | 10.21958/phenotype:112 | LI et al., 2010 |
| 113 | DTF Sweden 2008 (1st experiment) | 10.21958/phenotype:113 | LI et al., 2010 |
| 114 | Yield Sweden 2009 (2nd experiment) | 10.21958/phenotype:114 | LI et al., 2010 |
| 115 | Size Loc Sweden 2009 | 10.21958/phenotype:115 | LI et al., 2010 |
| 116 | DTF planting Summer Loc Sweden 2009 | 10.21958/phenotype:116 | LI et al., 2010 |
| 117 | DTF loc Sweden 2008 | 10.21958/phenotype:117 | LI et al., 2010 |
| 118 | DTF loc Sweden 2009 | 10.21958/phenotype:118 | LI et al., 2010 |
| 119 | DTF Spain 2009 (1st experiment) | 10.21958/phenotype:119 | LI et al., 2010 |
| 120 | DTF planting Loc 2008 | 10.21958/phenotype:120 | LI et al., 2010 |
| 121 | DTF Spain 2009 (2nd experiment) | 10.21958/phenotype:121 | LI et al., 2010 |
| 122 | Yield Spain 2009 (2nd experiment) | 10.21958/phenotype:122 | LI et al., 2010 |
| 123 | Size Sweden 2009 (1st experiment) | 10.21958/phenotype:123 | LI et al., 2010 |

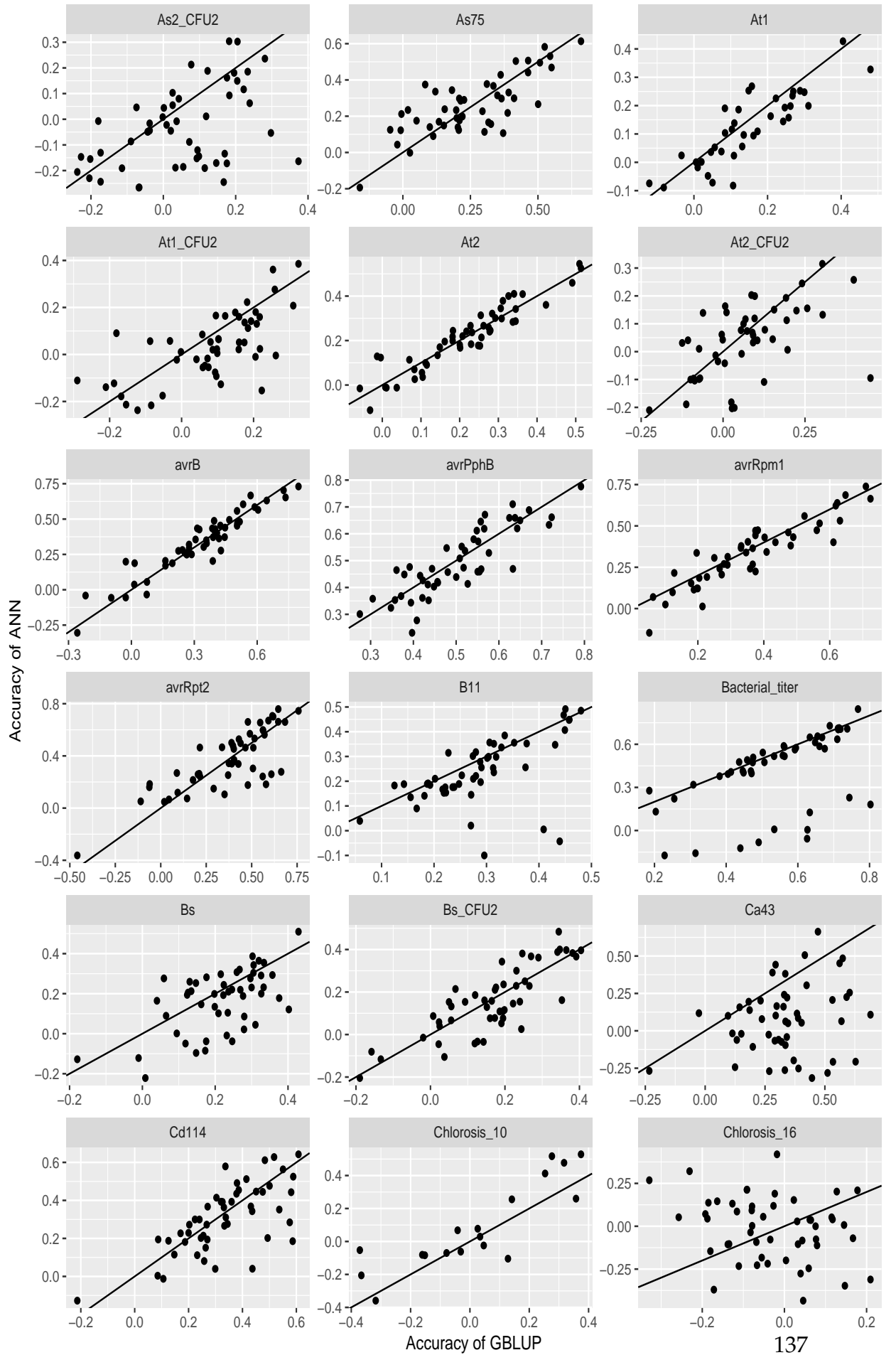
| | | | |
|-----|--------------------------------------|------------------------|-----------------|
| 124 | Yield Spain 2009 (1st experiment) | 10.21958/phenotype:124 | LI et al., 2010 |
| 125 | DTF main Effect 2009 | 10.21958/phenotype:125 | LI et al., 2010 |
| 126 | DTF main Effect 2008 | 10.21958/phenotype:126 | LI et al., 2010 |
| 127 | Size Spain 2009 (2nd experiment) | 10.21958/phenotype:127 | LI et al., 2010 |
| 128 | Size Spain 2009 (1st experiment) | 10.21958/phenotype:128 | LI et al., 2010 |
| 129 | DTF planting Summer 2009 | 10.21958/phenotype:129 | LI et al., 2010 |
| 130 | DTF planting Summer 2008 | 10.21958/phenotype:130 | LI et al., 2010 |
| 131 | Size Main Effect 2009 | 10.21958/phenotype:131 | LI et al., 2010 |
| 132 | DTF Spain 2008 (1st experiment) | 10.21958/phenotype:132 | LI et al., 2010 |
| 133 | Yield Planting Summer 2009 | 10.21958/phenotype:133 | LI et al., 2010 |
| 134 | DTF Sweden 2009 (1st experiment) | 10.21958/phenotype:134 | LI et al., 2010 |
| 135 | Yield Loc Sweden 2009 | 10.21958/phenotype:135 | LI et al., 2010 |
| 136 | DTF Spain 2008 (2nd experiment) | 10.21958/phenotype:136 | LI et al., 2010 |
| 137 | Yield Main Effect 2009 | 10.21958/phenotype:137 | LI et al., 2010 |
| 138 | Yield Planting Summer Loc Sweden 009 | 10.21958/phenotype:138 | LI et al., 2010 |
| 139 | Yield Sweden 2009 (1st experiment) | 10.21958/phenotype:139 | LI et al., 2010 |
| 140 | DTF Sweden 2009 (2nd experiment) | 10.21958/phenotype:140 | LI et al., 2010 |

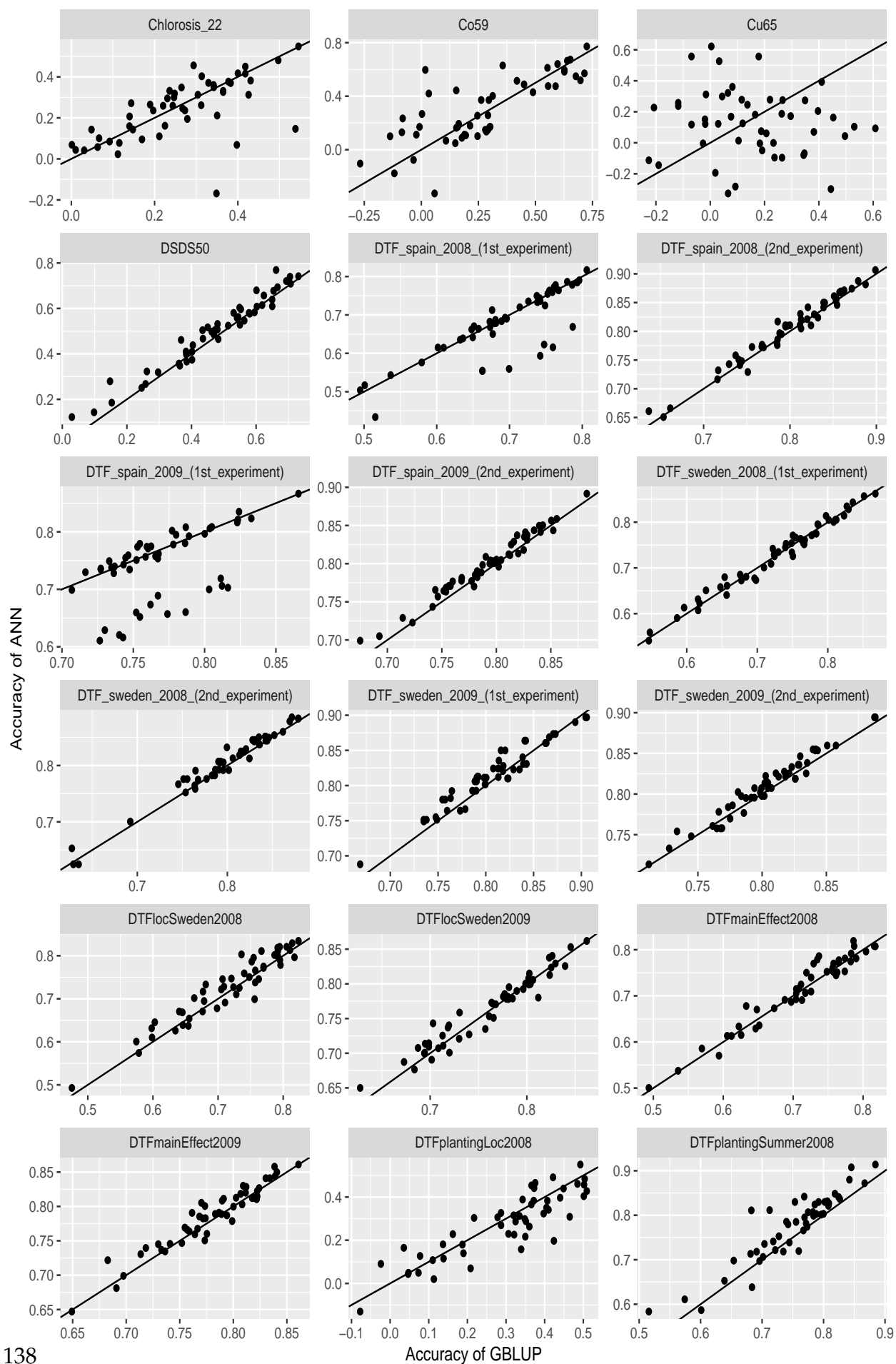
| | | | |
|-----|----------------------------------|------------------------|----------------------------|
| 141 | DTF Sweden 2008 (2nd experiment) | 10.21958/phenotype:141 | LI et al., 2010 |
| 142 | Mature cell length | 10.21958/phenotype:142 | MEIJÓ et al., 2014 |
| 143 | Meristem zone length | 10.21958/phenotype:143 | MEIJÓ et al., 2014 |
| 144 | M216T665 | 10.21958/phenotype:144 | STRAUCH et al., 2015 |
| 145 | M130T666 | 10.21958/phenotype:145 | STRAUCH et al., 2015 |
| 146 | M172T666 | 10.21958/phenotype:146 | STRAUCH et al., 2015 |
| 261 | FT10 | 10.21958/phenotype:261 | ALONSO-BLANCO et al., 2016 |
| 262 | FT16 | 10.21958/phenotype:262 | ALONSO-BLANCO et al., 2016 |
| 269 | Li7 | 10.21958/phenotype:269 | FORSBERG et al., 2015 |
| 270 | B11 | 10.21958/phenotype:270 | FORSBERG et al., 2015 |
| 271 | Na23 | 10.21958/phenotype:271 | FORSBERG et al., 2015 |
| 272 | Mg25 | 10.21958/phenotype:272 | FORSBERG et al., 2015 |
| 273 | P31 | 10.21958/phenotype:273 | FORSBERG et al., 2015 |
| 274 | S34 | 10.21958/phenotype:274 | FORSBERG et al., 2015 |
| 275 | K39 | 10.21958/phenotype:275 | FORSBERG et al., 2015 |
| 276 | Ca43 | 10.21958/phenotype:276 | FORSBERG et al., 2015 |
| 277 | Mn55 | 10.21958/phenotype:277 | FORSBERG et al., 2015 |
| 279 | Co59 | 10.21958/phenotype:279 | FORSBERG et al., 2015 |
| 280 | Ni60 | 10.21958/phenotype:280 | FORSBERG et al., 2015 |
| 281 | Cu65 | 10.21958/phenotype:281 | FORSBERG et al., 2015 |
| 282 | Zn66 | 10.21958/phenotype:282 | FORSBERG et al., 2015 |
| 283 | As75 | 10.21958/phenotype:283 | FORSBERG et al., 2015 |
| 284 | Se82 | 10.21958/phenotype:284 | FORSBERG et al., 2015 |

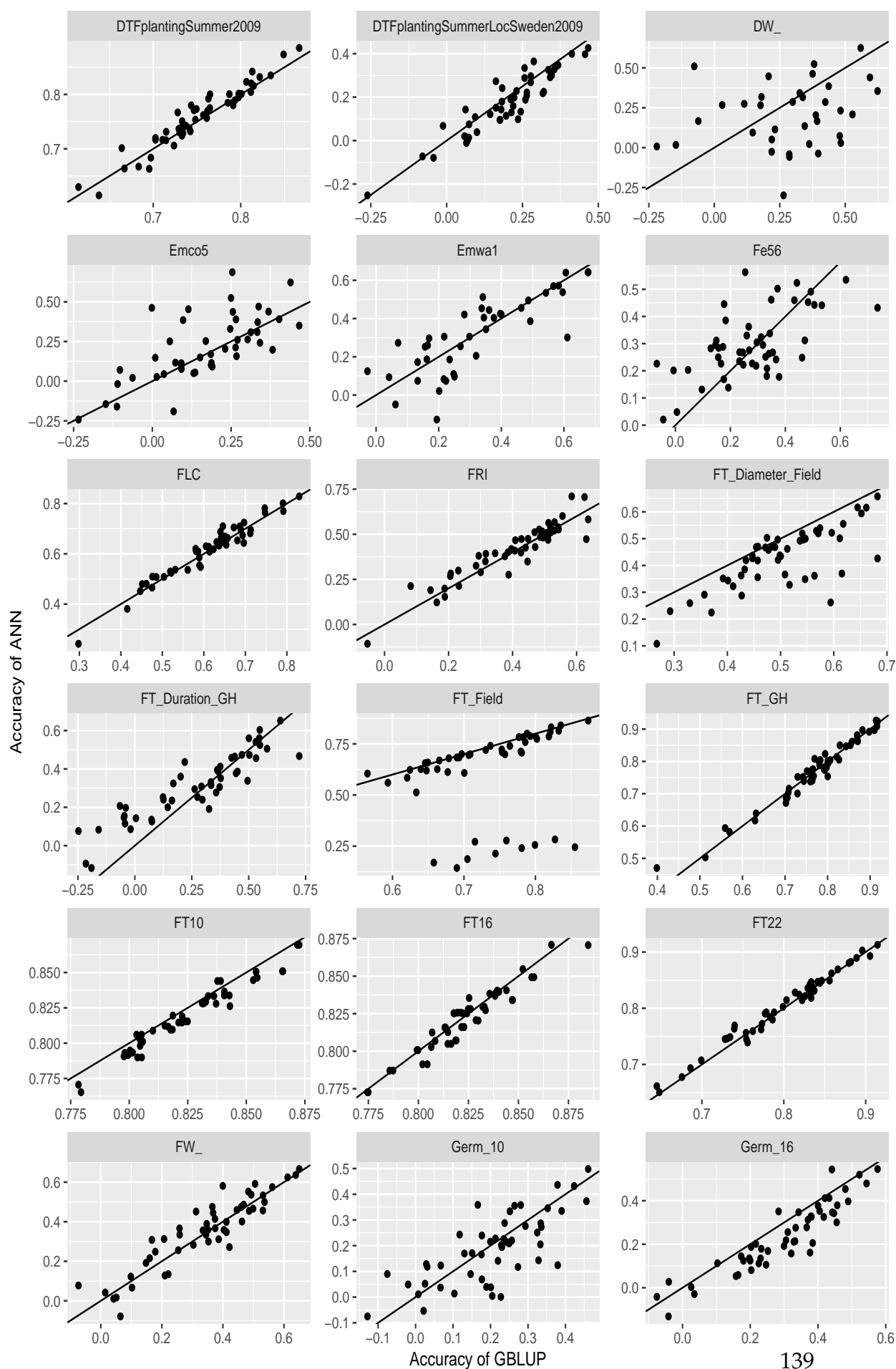
C Supplementary results

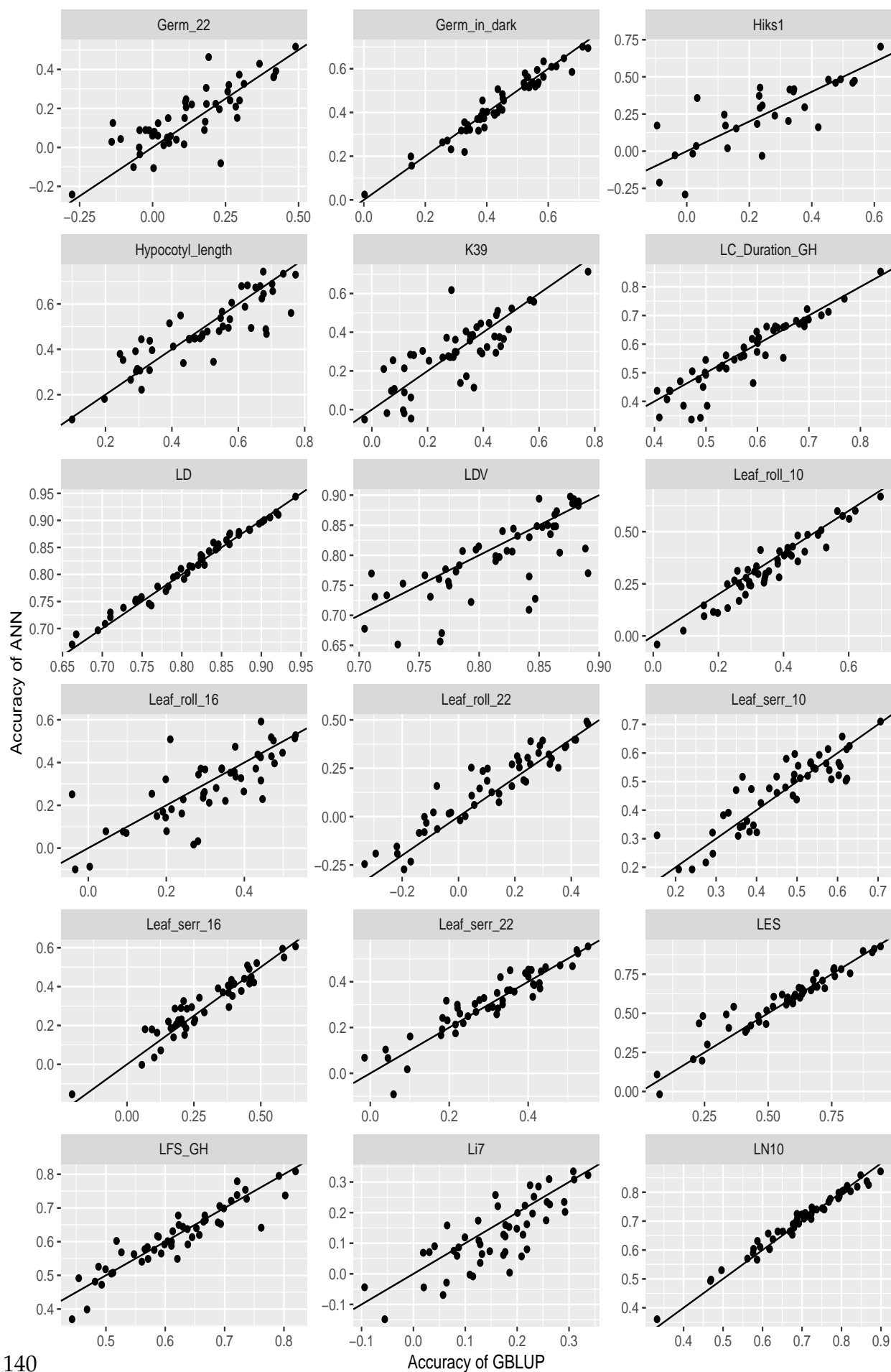
C.1 Correlation plots of *A. thaliana* GP

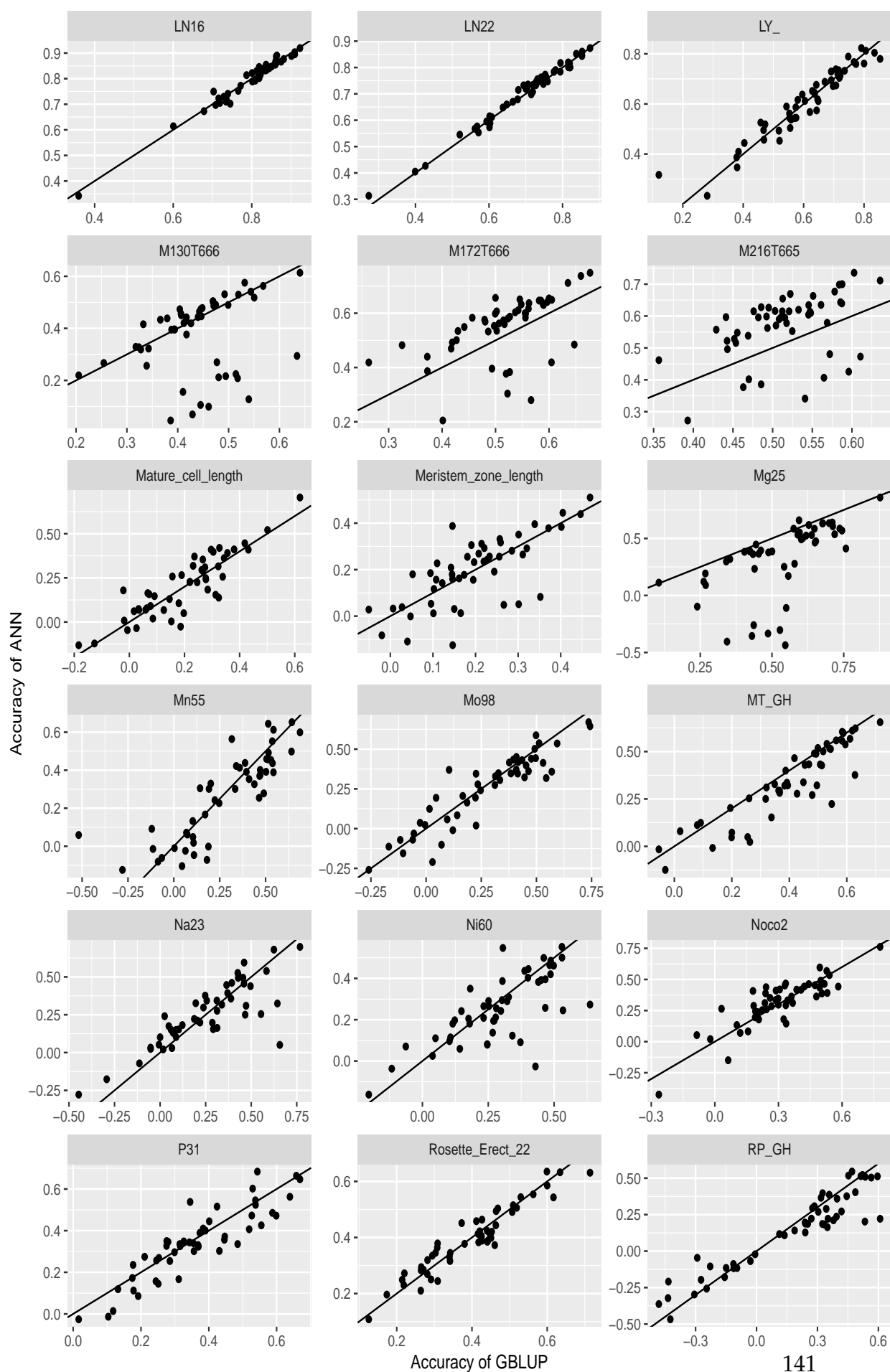


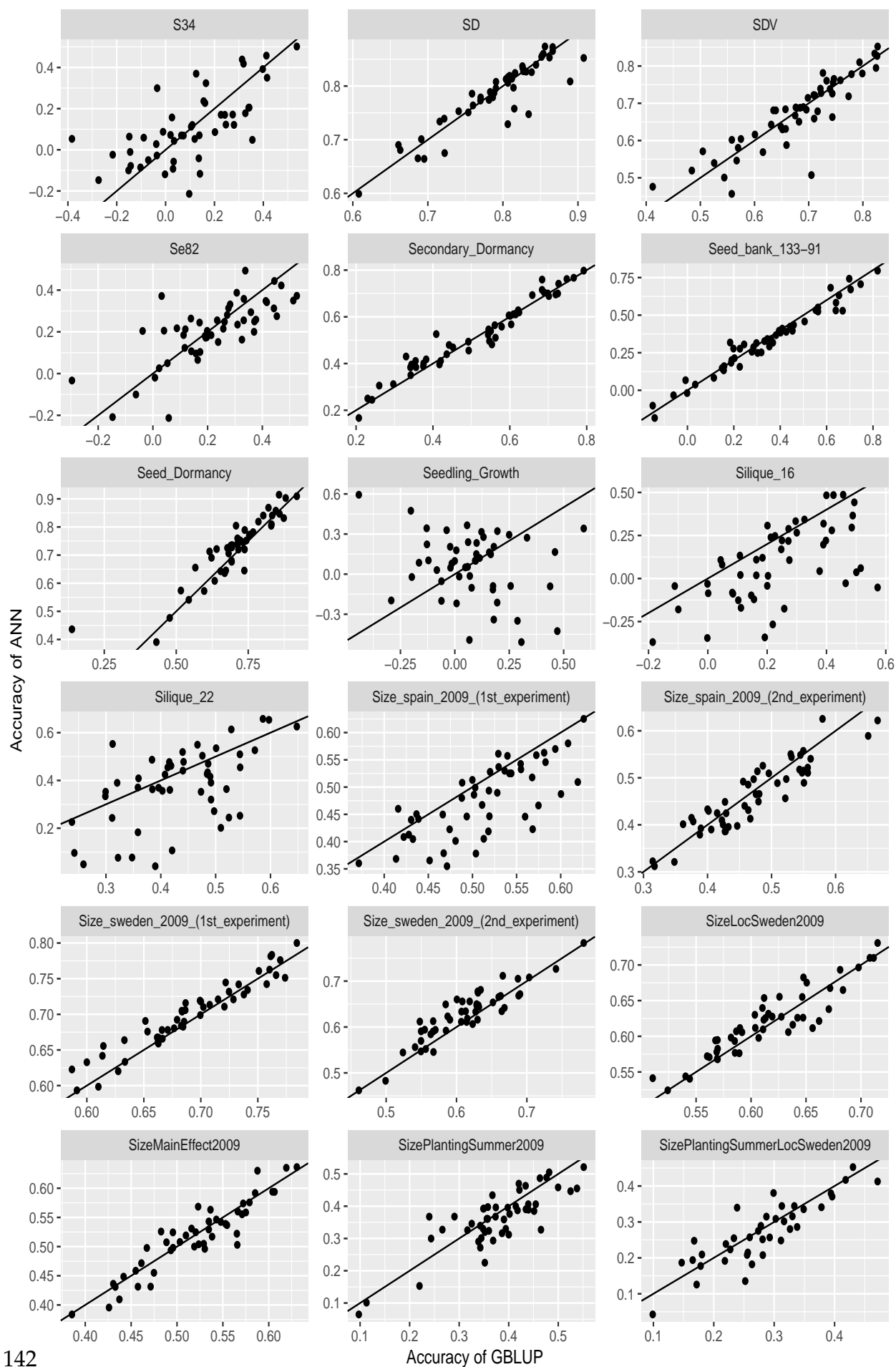


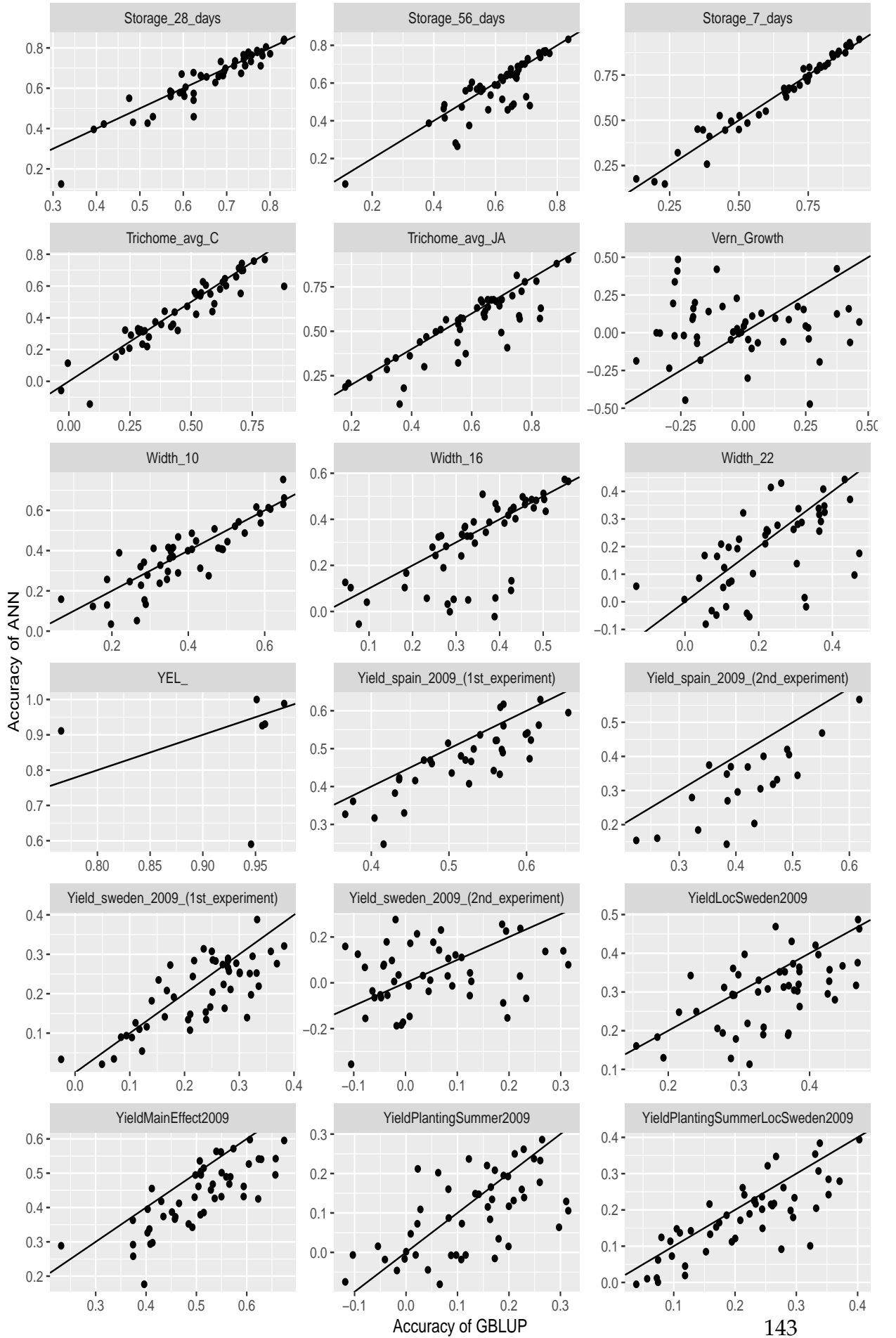


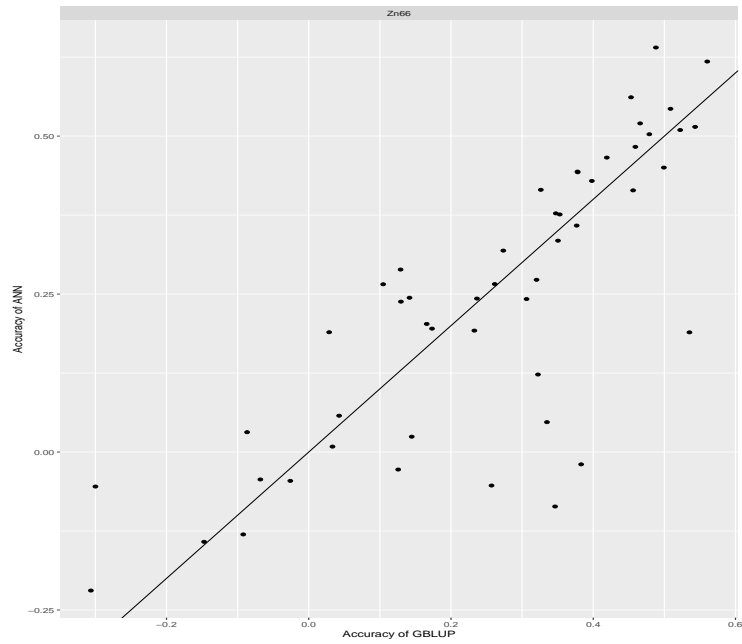












C.2 Haplotype structure of *A. thaliana*

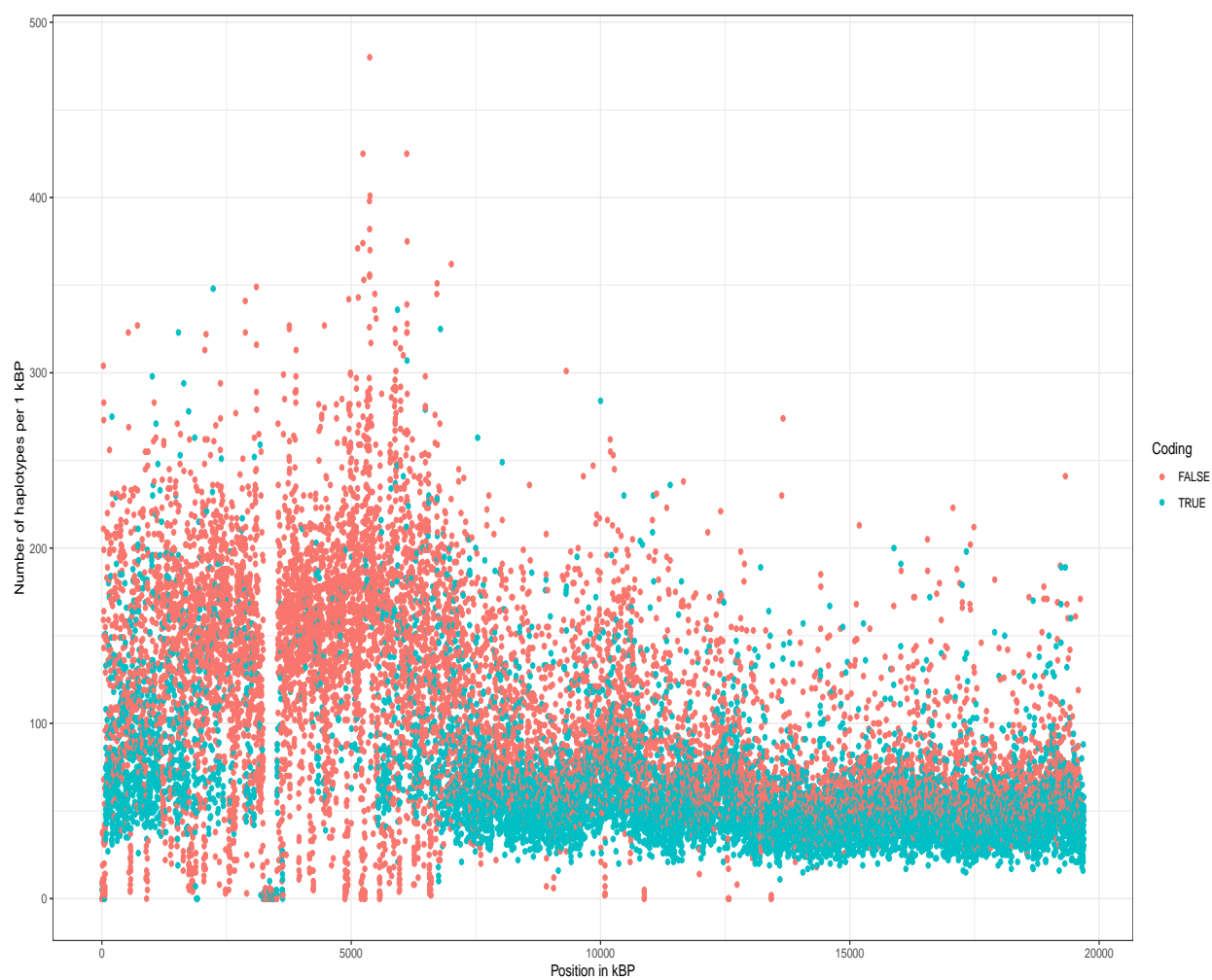


FIGURE C.1: Number of segregating haplotypes with a polymorphism in at least one position over a stretch of 1 kBP.

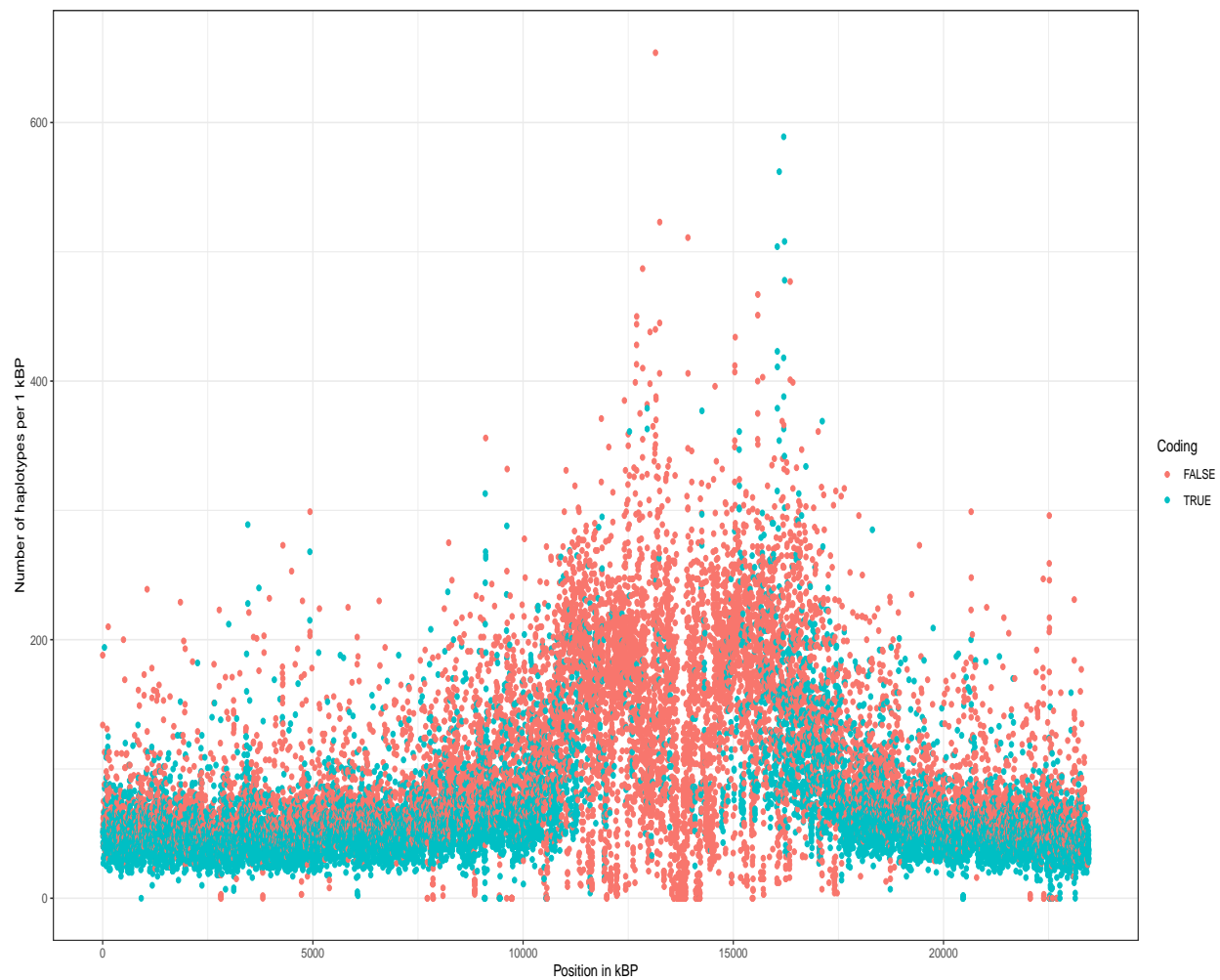


FIGURE C.2: Number of segregating haplotypes with a polymorphism in at least one position over a stretch of 1 kBP.

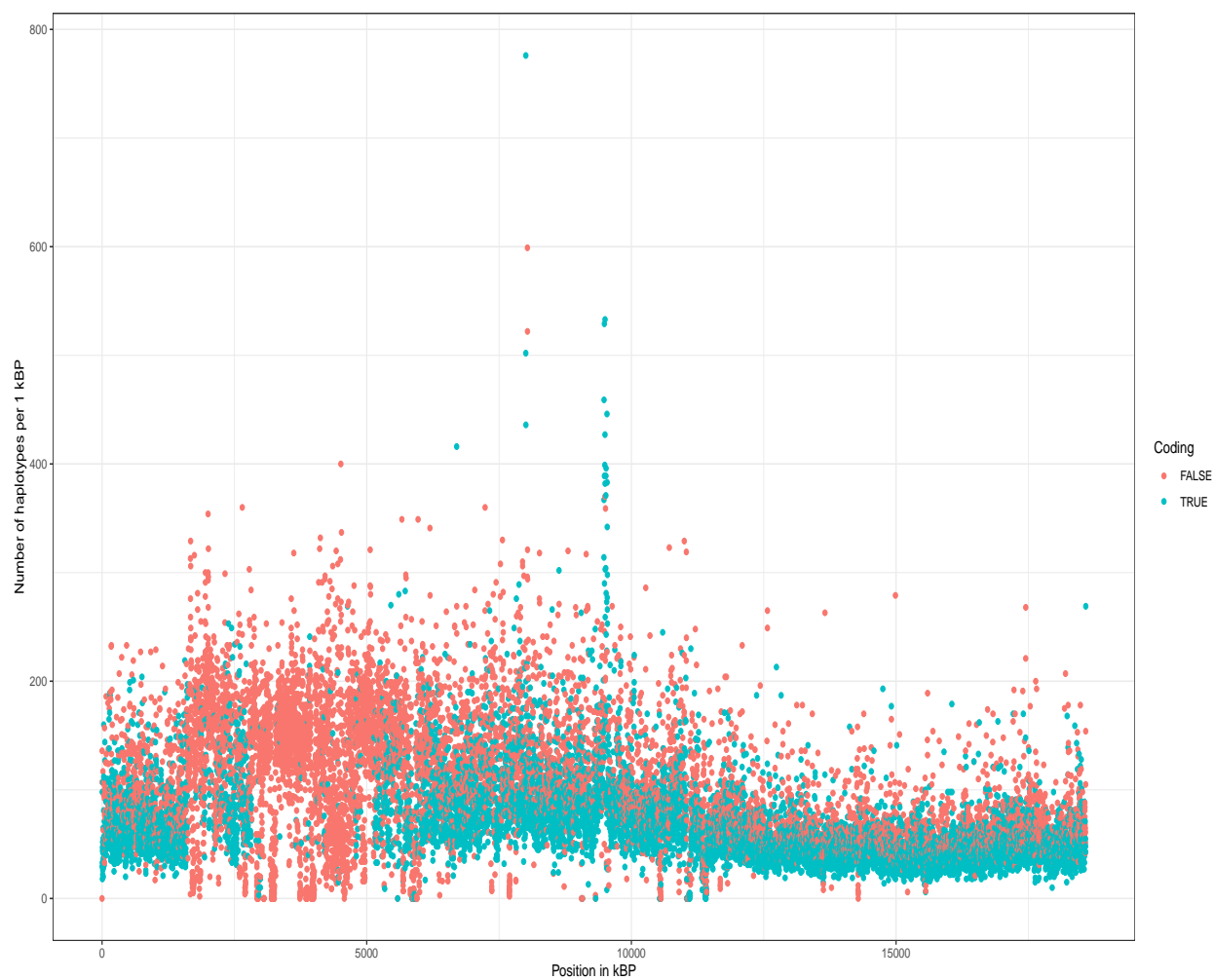


FIGURE C.3: Number of segregating haplotypes with a polymorphism in at least one position over a stretch of 1 kBP.

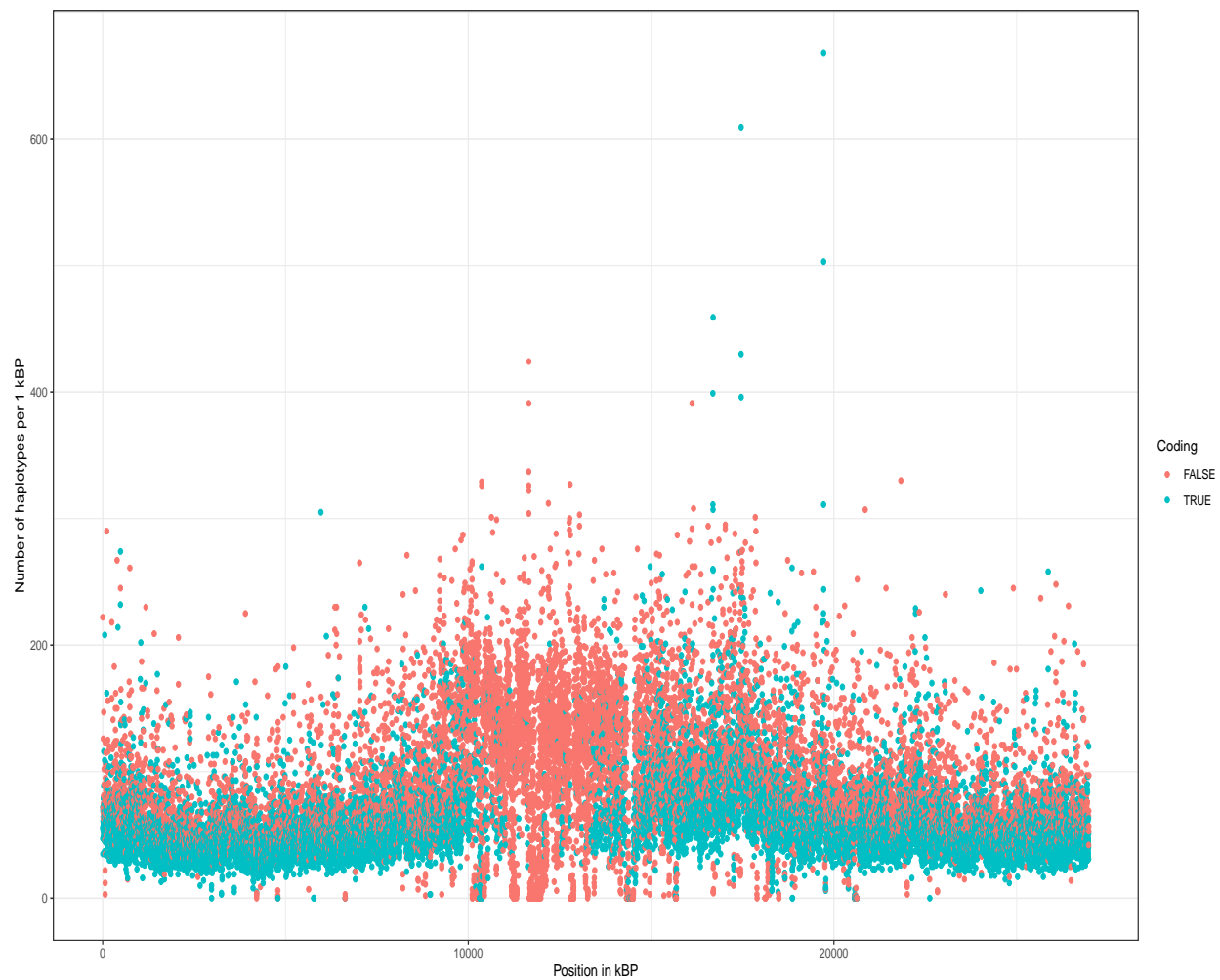


FIGURE C.4: Number of segregating haplotypes with a polymorphism in at least one position over a stretch of 1 kBP.

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Curriculum Vitae

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