

# Dissertation

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# Table of contents

<b>Preface</b>	<b>1</b>
<b>1 Introduction</b>	<b>3</b>
<b>I MAGIC</b>	<b>5</b>
Running Code . . . . .	7
<b>2 Estimate mosaic metrics MAGIC</b>	<b>9</b>
<b>3 What is the size of the recombination chunks in the MEMA population?</b>	<b>21</b>
<b>4 What is the average size of the “same genotype” chunks in the parental magic haplotype?</b>	<b>27</b>
<b>II CML</b>	<b>45</b>
Running Code . . . . .	47
<b>5 2023_Highland_experiment_design</b>	<b>49</b>
<b>6 PT</b>	<b>63</b>
6.1 Running Code . . . . .	63
<b>7 AMF</b>	<b>65</b>
7.1 Running Code . . . . .	65
<b>8 Summary</b>	<b>67</b>
<b>References</b>	<b>69</b>



# Preface

This is a Quarto book.

To learn more about Quarto books visit <https://quarto.org/docs/books>.



# Chapter 1

## Introduction

This is a book created from markdown and executable code.

See Knuth (1984) for additional discussion of literate programming.





**Part I**

**MAGIC**



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Quarto enables you to weave together content and executable code into a finished document. To learn more about Quarto see <https://quarto.org>.

## Running Code

When you click the **Render** button a document will be generated that includes both content and the output of embedded code. You can embed code like this:

```
1 + 1
```

```
[1] 2
```

You can add options to executable code like this

```
[1] 4
```

The `echo: false` option disables the printing of code (only output is displayed).



## Chapter 2

# Estimate mosaic metrics MAGIC

There are a few questions that we can make out of the MAGIC population:

- Is there any residual populations structure in the MAGIC population?
- Can we improve the parenta haplotype call in the MAGIC population if we increae the number of markers?
  - How does the choromosomal mosaic looks in the MAGIC population?
  - What's the median and maximum chromosomal chunk size in the MAGIC population?
  - What's the median and maximum “same genotype” chunk in a pair-wise comparison of the MAGIC parents?
  - What is the minimal number of markers that we need to obtain information similar to the total of 8.2 M markers?

Logic: We hypothesize that most of the NAs or mistakes in the call of the parent in the MAGIC populations are because {qtl2} can't tell apart one parent from the other with enough confidence (with  $\alpha = 0.5$ ) because the marker density is not enough to tell a parent from the other. In this case, increasing the number of markers between each chunk will help us only if the maximum size of the chunks with “same genotype” in the parents information is smaller than the recombination chunk size in the MAGIC population. IN other words, we're adding more relevant information in each of these chunks that would allow us to differentiate one parent from the other.

Loading libraries

```
library(tidyverse)
```

```
-- Attaching packages ----- tidyverse 1.3.2 --
```

```

v ggplot2 3.4.1      v purrr  1.0.1
v tibble  3.1.8      v dplyr  1.0.10
v tidyr   1.3.0      v stringr 1.5.0
v readr   2.1.3      v forcats 0.5.2

```

Warning: package 'ggplot2' was built under R version 4.2.2

Warning: package 'tidyr' was built under R version 4.2.2

Warning: package 'readr' was built under R version 4.2.2

Warning: package 'purrr' was built under R version 4.2.2

Warning: package 'dplyr' was built under R version 4.2.2

Warning: package 'stringr' was built under R version 4.2.2

Warning: package 'forcats' was built under R version 4.2.2

```

-- Conflicts ----- tidyverse_conflicts() --
x dplyr::filter() masks stats::filter()
x dplyr::lag()    masks stats::lag()

```

```
library(furrr)
```

Warning: package 'furrr' was built under R version 4.2.2

Loading required package: future

Warning: package 'future' was built under R version 4.2.2

```

plan(multisession)
library(arrow)

```

Warning: package 'arrow' was built under R version 4.2.2

Attaching package: 'arrow'

The following object is masked from 'package:utils':

```
timestamp
```

```
library(tidymodels)
```

```

-- Attaching packages ----- tidymodels 1.0.0 --
v broom      1.0.3      v rsample    1.1.1
v dials      1.1.0      v tune       1.0.1
v infer      1.0.4      v workflows  1.1.2
v modeldata  1.1.0      v workflowsets 1.0.0
v parsnip    1.0.3.9000    v yardstick  1.1.0

```

```

v recipes      1.0.4
Warning: package 'broom' was built under R version 4.2.2
Warning: package 'dials' was built under R version 4.2.2
Warning: package 'infer' was built under R version 4.2.2
Warning: package 'modeldata' was built under R version 4.2.2
Warning: package 'recipes' was built under R version 4.2.2
Warning: package 'rsample' was built under R version 4.2.2
Warning: package 'tune' was built under R version 4.2.2
Warning: package 'workflows' was built under R version 4.2.2
Warning: package 'yardstick' was built under R version 4.2.2

-- Conflicts ----- tidymodels_conflicts() --
x scales::discard() masks purrr::discard()
x dplyr::filter()   masks stats::filter()
x recipes::fixed()  masks stringr::fixed()
x dplyr::lag()      masks stats::lag()
x yardstick::spec() masks readr::spec()
x recipes::step()   masks stats::step()
* Use suppressPackageStartupMessages() to eliminate package startup messages

library(qtl2)

Warning: package 'qtl2' was built under R version 4.2.2
Attaching package: 'qtl2'

The following object is masked from 'package:readr':

  read_csv

library(tidymodels)
library(finetune)

Warning: package 'finetune' was built under R version 4.2.2

library(reshape2)

Attaching package: 'reshape2'

The following object is masked from 'package:tidyr':

```

```
smiths
```

```
library(tidymodels)
```

Clean CrossObject: Remove weird markers and individuals based on their total number of recombinations and LODerror

```
MEMA_DATA <-
  read_cross2(
    "C:/Users/sergi/Documents/SAWERS LAB/MAGIC/genetic_mapping/MEMA_CTRL_wo_weird.ya
  )
```

Warning in drop\_incomplete\_markers(output): Omitting 4738 markers that are not in both genotypes and maps

Warning in check\_cross2(output): Physical map out of order on chr 1, 2, 3, 4, 5, 6, 7, 8, 9, 10

```
snp.info.founders <- readr::read_csv(
  "C:/Users/sergi/Documents/SAWERS LAB/MAGIC/genetic_mapping/snp.info.founders.csv"
) %>%
  filter(!is.na(snp))
```

Rows: 19654 Columns: 4

-- Column specification -----

Delimiter: ","

chr (1): snp

dbl (3): chr, pos, sdp

i Use `spec()` to retrieve the full column specification for this data.

i Specify the column types or set `show\_col\_types = FALSE` to quiet this message.

```
# recombinations
#####

genmap_MEMA <- MEMA_DATA$gmap

# Estimate genotype probabilities
set.seed(100)
genoprob_MEMA <-
  calc_genoprob(
    cross = MEMA_DATA,
    map = genmap_MEMA,
    error_prob=0.002) %>%
  clean_genoprob ()
```



```

# Estimate the genotypes with maximum marginal probabilities
set.seed(100)
geno_maxprob_MEMA <-
  maxmarg(
    probs = genoprob_MEMA,
    minprob = 0.95
  ) #assigns founder code to each marker/pos

# Estimate the genotypes with maximum marginal probabilities with letters
set.seed(100)
geno_maxprob_letters_MEMA <- maxmarg(
  probs= genoprob_MEMA,
  minprob = 0.95,
  return_char = TRUE
)

```

Estimate the error lod probability for any marker for any family. Positive LOD scores suggest that there might be a mistake in the genotype probability. I'm estimating the proportion of genotype calls in each marker whose errorLOD > 0 and estimate the top 5 percentile values of the proportion distribution and remove the markers whose proportion belong to this group.

```

# Calculate genotyping error LOD scores
set.seed(100)
errorlod_MEMA <-
  calc_errorlod(
    cross = MEMA_DATA,
    probs = genoprob_MEMA
  )

errorlod_MEMA <- do.call("cbind", errorlod_MEMA)

# matrix to df
errorlod_MEMA_df <-
  errorlod_MEMA %>%
  as_tibble(rownames = "family")

# Estimate the proportion of LODerror > 0 in every marker
errorlod_MEMA_prop_marker <-
  errorlod_MEMA_df %>%
  pivot_longer(-family) %>%
  filter(!is.infinite(value)) %>%
  group_by(name) %>%
  summarise(prop = sum(value >= 0)/dim(errorlod_MEMA_df)[1])

```

```

errorlod_MEMA_prop_marker

# A tibble: 14,176 x 2
  name      prop
  <chr>    <dbl>
1 1_100088401 0.125
2 1_100224800 0.115
3 1_100225275 0.135
4 1_100229005 0.11
5 1_100344399 0.13
6 1_100344420 0.13
7 1_100344944 0.145
8 1_100384962 0.13
9 1_100385294 0.16
10 1_100388059 0.135
# ... with 14,166 more rows

# Estimate the 95 percentile of the LODerror proportion distribution
errorlod_95_perc <- quantile(errorlod_MEMA_prop_marker$prop, 0.95)

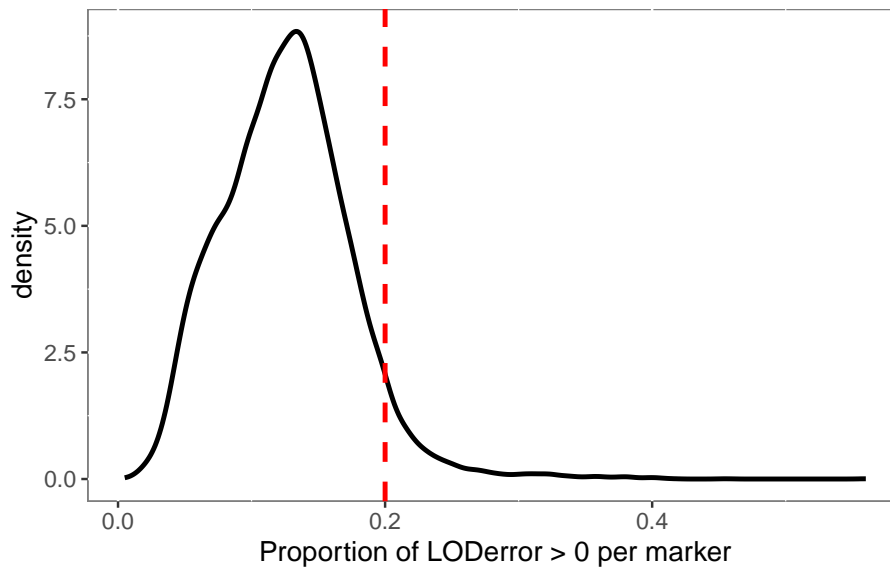
errorlod_95_perc

95%
0.2

# Remove all the markers with prop >=0.2

errorlod_MEMA_prop_marker %>%
  ggplot(data = ., aes(x = prop)) +
  geom_density(linewidth = 1) +
  xlab("Proportion of LODerror > 0 per marker") +
  geom_vline(
    aes(xintercept = errorlod_95_perc),
    color = "red",
    linetype = "dashed",
    linewidth = 1
  ) +
  theme(
    panel.background = element_rect(fill = "white", colour = "grey50"),
    text = element_text(size = 12),
    legend.position = "top"
  )

```



```
# Select markers in the 95 percentile to remove
errorlod_marker_remove <- errorlod_MEMA_prop_marker %>%
  filter(prop >= errorlod_95_perc) %>%
  .$name

#####
# Count number of recombinations per family #
#####

# Estimate the total number of recombinations per family
set.seed(100)
n_recomb_family_MEMA <-
  count_xo(geno = geno_maxprob_MEMA) %>%
  as_tibble(rownames = "family") %>%
  pivot_longer(-family) %>%
  group_by(family) %>%
  summarise(n_recomb = sum(value)) %>%
  arrange(desc(n_recomb))

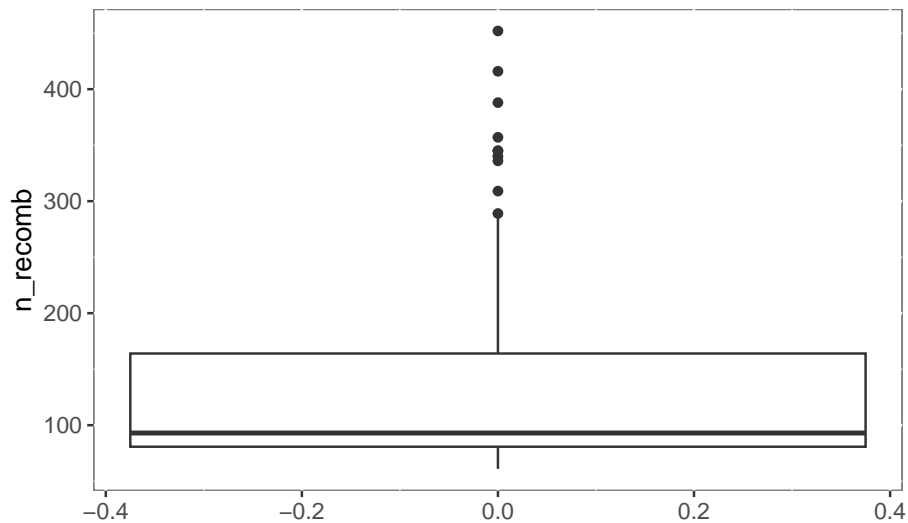
# Identify outliers in the distribution of genomewide recombination events
n_recomb_family_MEMA %>%
  ggplot(data = ., aes(y = n_recomb)) +
  geom_boxplot() +
  theme(
    panel.background = element_rect(fill = "white", colour = "grey50"),
```

```

text = element_text(size = 12),
plot.title = element_text(hjust = 0.5)
) +
ggtitle("Distribution of number of genome-wide number of recombinations per family")

```

tribution of number of genome-wide number of recombinations per family



```

# Outliers total number of recomb
outliers_lots_recomb_MEMA <-
  boxplot(n_recomb_family_MEMA$n_recomb, plot = F)$out

# non outlier families
family_normal_recomb <-
  n_recomb_family_MEMA %>%
  filter(! n_recomb %in% outliers_lots_recomb_MEMA) %>%
  .$family

MEMA_DATA_wo_weird <-
  MEMA_DATA %>%
  drop_markers(errorlod_marker_remove) %>%
  subset(x = ., ind = family_normal_recomb)

```

Is there any population structure in the MEMA population?

Approach: I'm going to estimate the kinship matrix using the overall option and a decomposition of eigenvalues of the same matrix and detect population structure.

```

# Estimate genotype probabilities
set.seed(100)
genoprob_wo_weird_MEMA <-
  calc_genoprob(
    cross = MEMA_DATA_wo_weird,
    map = genmap_MEMA,
    error_prob=0.002) %>%
  clean_genoprob ()

# Estimate Allele probabilities
set.seed(100)
allele_prob_MEMA <-
  genoprob_to_alleleprob(genoprob_wo_weird_MEMA)

# Estimate kinship matrix
set.seed(100)
kinship_wo_weird_MEMA <- calc_kinship(
  probs = allele_prob_MEMA,
  type = "overall"
)

# Estimate the eigenvalue decomposition of the kinship matrix
set.seed(100)
eigen_kinship_MEMA <-
  decomp_kinship(kinship_wo_weird_MEMA)

# correlation matrix of the kinship matrix
set.seed(100)
corr_kinship_MEMA <-
  scale_kinship (kinship_wo_weird_MEMA)

# Pairwise correlation of families
pairwise_genocorr_MEMA <- corr_kinship_MEMA %>%
  as_tibble(rownames = "family") %>%
  pivot_longer(-family) %>%
  filter(family != name) %>%
  mutate(code = map2_chr(
    .x = family,
    .y = name,
    .f = ~ paste0(.x, .y) %>% str_split("") %>% unlist() %>% sort() %>% paste(collapse = "")
  )) %>%
  group_by(code) %>%
  filter(row_number() == 1) %>%
  ungroup() %>%

```

```

select(-code)

# Estimate 99 percentile of pairwise correlation
geno_corr_99_perc <- quantile(pairwise_geno_corr_MEMA$value, 0.99)

geno_corr_99_perc

```

```

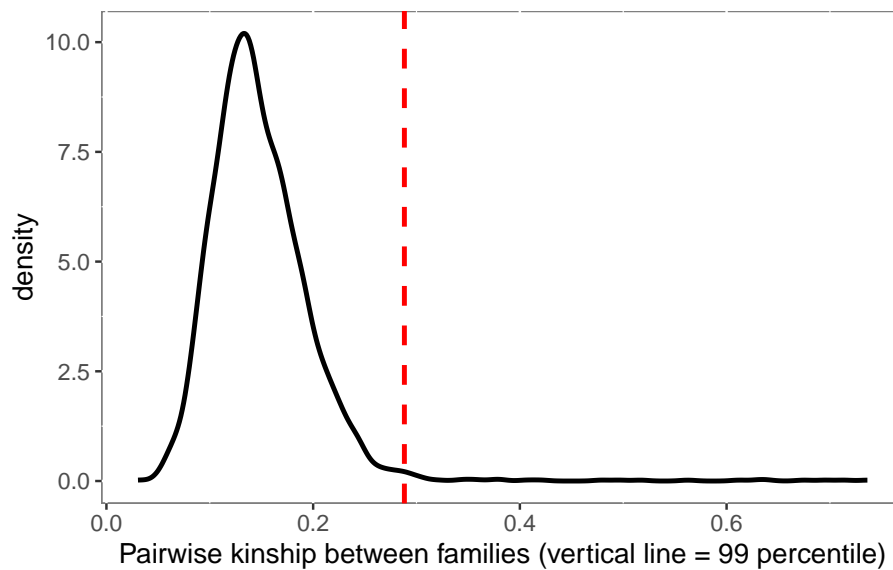
99%
0.2882856

```

```

# plot the distribution of the pairwise correlation between families
pairwise_geno_corr_MEMA %>%
  ggplot(data = ., aes(x = value)) +
  geom_density(linewidth = 1) +
  xlab("Pairwise kinship between families (vertical line = 99 percentile)") +
  geom_vline(
    aes(xintercept = geno_corr_99_perc),
    color = "red",
    linetype = "dashed",
    linewidth = 1
  ) +
  theme(
    panel.background = element_rect(fill = "white", colour = "grey50"),
    text = element_text(size = 12),
    legend.position = "top"
  )

```



```
# Identify family pairwise correlation values in the 99 percentile
families_high_corr <-
  pairwise_genocorr_MEMA %>%
  filter(value >= geno_corr_99_perc) %>%
  arrange(desc(value))
```

```
families_high_corr
```

```
# A tibble: 27 x 3
  family name value
  <chr>  <chr>  <dbl>
1 MEMA210 MEMA211 0.736
2 MEMA245 MEMA244 0.714
3 MEMA162 MEMA163 0.695
4 MEMA128 MEMA127 0.675
5 MEMA307 MEMA308 0.638
6 MEMA033 MEMA034 0.631
7 MEMA133 MEMA132 0.612
8 MEMA055 MEMA054 0.563
9 MEMA252 MEMA253 0.517
10 MEMA168 MEMA078 0.496
# ... with 17 more rows
```

```

# Select families to drop that are in the 99th percentile but doesn't have a phenoty

genotyped_fam_MEMA <-
  MEMA_DATA_wo_weird$geno$`1` %>% rownames()

families_no_pheno <-
  MEMA_DATA_wo_weird$pheno %>%
  rownames() %>%
  setdiff(genotyped_fam_MEMA, .)

families_no_pheno_high_corr <-
  famlies_high_corr %>%
  rowid_to_column() %>%
  rename(cor = value) %>%
  pivot_longer(c(family, name)) %>%
  count(value) %>%
  arrange(desc(n)) %>%
  filter(value %in% families_no_pheno) %>%
  .$value

# Drop families with high correlation and not phenotyped: c("MEMA163", "MEMA210", "M

MEMA_DATA_wo_weird2 <- MEMA_DATA_wo_weird %>%
  subset(ind = setdiff(genotyped_fam_MEMA, families_no_pheno_high_corr))

```

In the absence of population structure we can expect a correlation between families of  $1/8$ . Any significant deviation from this number would imply that there is residual population structure in the population. The distribution of the pairwise correlation values for the MEMA family is right skewed with a median value of  $\sim 0.14$ , close to  $0.125$  ( $1/8$ ) expected. This value is inflated a little bit by weird families that have a lot of correlation. We hypothesize that the high correlation values for these families (for instance families MEMA210-MEMA211;  $\text{cor} = 0.736$ ) was the result of either mistakes in the development of families: Someone mistakenly grabbed seed from an incorrect envelope that was planted and generated a very closely related family, or it could also be the result of mistakes in collecting tissue from plants for genotyping. Nevertheless, the results show that the median pairwise correlation doesn't deviate that much from the expected under the hypothesis of no population structure, so we can conclude that there is a reduced population structure in the MEMA pop. We're going to remove highly correlated individuals for whom we don't have phenotypic data.



## Chapter 3

# What is the size of the recombination chunks in the MEMA population?

```
# Estimate genotype probabilities
set.seed(100)
genoprob_wo_weird2_MEMA <-
  calc_genoprob(
    cross = MEMA_DATA_wo_weird2,
    map = genmap_MEMA,
    error_prob=0.002) %>%
  clean_genoprob ()

# Estimate Allele probabilities
set.seed(100)
geno__wo_weird2_MEMA <-
  maxmarg(
    probs = genoprob_wo_weird2_MEMA,
    minprob = 0.95
  )

# Guess the phase of each family in each chromosome
phase_MEMA <- guess_phase(MEMA_DATA_wo_weird2, geno__wo_weird2_MEMA)

# genetic map
genmap_MEMA <-
  MEMA_DATA_wo_weird2$gmap %>%
```

```

    map_df(.x = ., .f = ~ .x %>% enframe(name = "marker", value = "g_pos"))

# markers at the end of each chromosome
genmap_chr_end <-
  genmap_MEMA %>%
  separate(marker, into = c("chr", "p_pos"), sep = "_", remove = F) %>%
  mutate(
    chr = as.integer(chr),
    p_pos = as.integer(p_pos)
  ) %>%
  arrange(chr, p_pos) %>%
  group_by(chr) %>%
  filter(row_number() == max(row_number())) %>%
  mutate(p_pos = p_pos/1e6) %>%
  ungroup() %>%
  rename(chr_end = p_pos)

# Identify the recombination breakpoints in the MEMA population
set.seed(100)
recomb_location_MEMA <-
  locate_xo(phase_MEMA, map = MEMA_DATA_wo_weird2$gmap) %>%
  map_df(
    .x = .,
    .f = ~ map_df(
      .x = .,
      .f = ~ enframe(.x, name = NULL, value = "location"),
      .id = "family"
    ),
    .id = "chr"
  ) %>%
  mutate(marker = find_marker(MEMA_DATA$gmap, chr = chr, pos = location)) %>%
  mutate(chr = as.integer(chr))

# Estimate the size of the recombination chunks in the MEMA population
recomb_chunk_size_MEMA <-
  recomb_location_MEMA %>%
  group_by(family, chr) %>%
  nest() %>%
  mutate(data = map(
    .x = data,
    .f = ~ .x %>% add_row(location = NA, marker = NA)
  )) %>%
  ungroup() %>%

```

```

unnest(c(data), keep_empty = T) %>%
group_by(family, chr) %>%
mutate(
  pos = gsub("^\\d{1,2}_", "", marker) %>% as.integer() %>% "/"(1e6),
  chr = as.integer(chr)
) %>%
mutate(
  start = lag(pos, default = 0),
  end = pos
) %>%
left_join(genmap_chr_end %>% select(-marker), by = "chr") %>%
mutate(end = ifelse(is.na(pos), chr_end, end)) %>%
select(family, chr, location, marker, start, end) %>%
ungroup() %>%
mutate(chunk_size = end - start) %>%
mutate(
  p_pos_start = find_marker(MEMA_DATA$gmap, chr = chr, pos = start),
  p_pos_end = find_marker(MEMA_DATA$gmap, chr = chr, pos = end)) %>%
mutate(
  across(contains("p_pos"), ~ gsub("\\d{1,2}_", "", .) %>% as.integer() %>% "/"(1e6))
) %>%
mutate(p_chunk_size = p_pos_end - p_pos_start) %>%
filter(chunk_size > 0 & p_chunk_size > 0)

recomb_chunk_size_MEMA

```

```

# A tibble: 22,217 x 10
  family    chr location marker    start    end chunk~1 p_pos~2 p_pos~3 p_chu~4
  <chr>   <int>   <dbl> <chr>   <dbl> <dbl>   <dbl>   <dbl>   <dbl>   <dbl>
1 MEMA156     1    14.0 1_7987929    0    7.99    7.99    1.74    4.91    3.17
2 MEMA156     1    34.1 1_18969916  7.99  19.0   11.0     4.91   11.3    6.40
3 MEMA156     1    65.8 1_42129633  19.0  42.1   23.2    11.3   23.6   12.3
4 MEMA156     1    97.8 1_71985242  42.1  72.0   29.9    23.6   50.5   26.9
5 MEMA156     1    98.6 1_72903601  72.0  72.9    0.918   50.5   53.8    3.24
6 MEMA156     1    99.4 1_74159005  72.9  74.2    1.26    53.8   54.8    1.01
7 MEMA156     1   101. 1_78116605  74.2  78.1    3.96    54.8   57.4    2.69
8 MEMA156     1   104. 1_80559358  78.1  80.6    2.44    57.4   58.3    0.826
9 MEMA156     1   105. 1_81673169  80.6  81.7    1.11    58.3   59.0    0.751
10 MEMA156     1   105. 1_82472265  81.7  82.5    0.799   59.0   59.4    0.420
# ... with 22,207 more rows, and abbreviated variable names 1: chunk_size,
# 2: p_pos_start, 3: p_pos_end, 4: p_chunk_size

```

```

recomb_med_max_size <-
  recomb_chunk_size_MEMA %>%

```

```

summarise(
  min_chunk_size = min(chunk_size),
  median_chunk_size = median(chunk_size),
  max_chunk_size = max(chunk_size)
)

```

```
recomb_med_max_size
```

```

# A tibble: 1 x 3
  min_chunk_size median_chunk_size max_chunk_size
      <dbl>          <dbl>          <dbl>
1      0.0180          6.43          249.

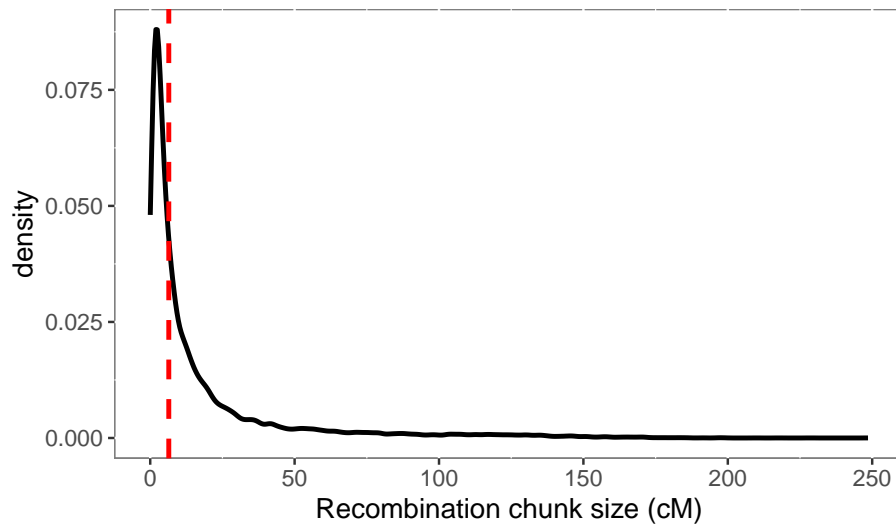
```

```

recomb_chunk_size_MEMA %>%
  ggplot(data = ., aes(x = chunk_size)) +
  geom_density(linewidth = 1) +
  xlab("Recombination chunk size (cM)") +
  geom_vline(
    data = recomb_med_max_size,
    aes(xintercept = median_chunk_size),
    color = "red",
    linetype = "dashed",
    linewidth = 1
  ) +
  theme(
    panel.background = element_rect(fill = "white", colour = "grey50"),
    text = element_text(size = 12),
    legend.position = "top"
  ) +
  ggtitle("Distribution of the recombination chunk sizes in the MEMA population")

```

Distribution of the recombination chunk sizes in the MEM



```

recomb_med_max_p_size <-
  recomb_chunk_size_MEMA %>%
  summarise(
    min_chunk_size_p = min(p_chunk_size),
    median_chunk_size_p = median(p_chunk_size),
    max_chunk_size_p = max(p_chunk_size)
  )

recomb_med_max_p_size

# A tibble: 1 x 3
  min_chunk_size_p median_chunk_size_p max_chunk_size_p
      <dbl>           <dbl>           <dbl>
1      0.00000800          4.20           235.

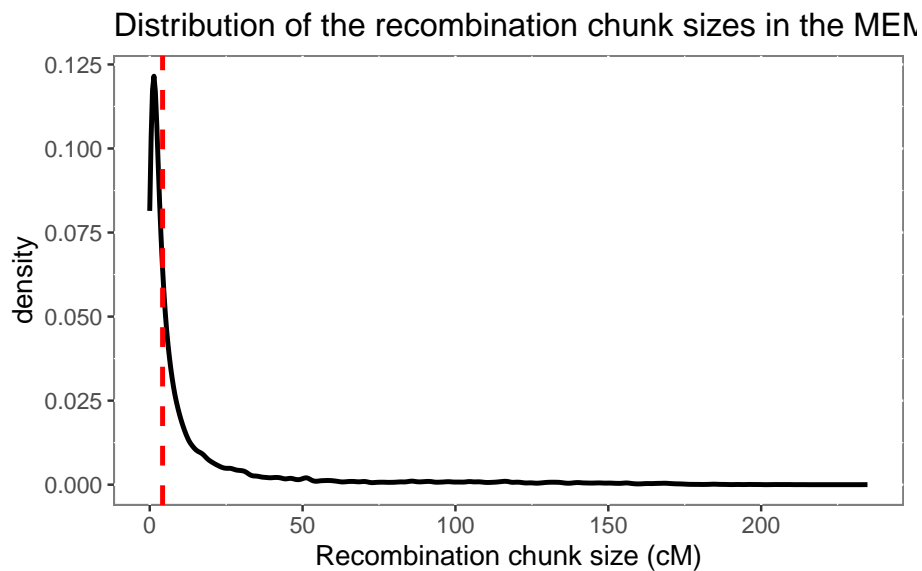
recomb_chunk_size_MEMA %>%
  ggplot(data = ., aes(x = p_chunk_size)) +
  geom_density(linewidth = 1) +
  xlab("Recombination chunk size (cM)") +
  geom_vline(
    data = recomb_med_max_p_size,
    aes(xintercept = median_chunk_size_p),
    color = "red",
    linetype = "dashed",
    linewidth = 1
  )

```

```

) +
theme(
  panel.background = element_rect(fill = "white", colour = "grey50"),
  text = element_text(size = 12),
  legend.position = "top"
) +
ggtitle("Distribution of the recombination chunk sizes in the MEMA population")

```



The median recombination chunk size is  $\sim 4.20$  MB, but they can go from almost 0 to  $>200$  MB. It is kinda weird. The most probable thing is that those very small chunks are just mistakes in the haplotype calling and therefore the chunks are very small. It is also worth to mention that the missing information in the parental haplotype calls seems to be located when one haplotype ends to when the other starts, so it seems that with this marker density ( $\sim 14K$  markers), `rqtl2` can't confidently assign a parental haplotype in these transition regions.

## Chapter 4

# What is the average size of the “same genotype” chunks in the parental magic haplotype?

We hypothesize that rqt12 is having trouble in calling parental haplotypes with confidence because there are chromosomal chunks where two or more parents have the same genotype, and with the actual marker density rqt12 cannot tell apart one from the other. So we want to assess if including a greater number of markers (that come from WGS) can reduce the size of this chunks, therefore becoming easier to identify one parent from the other. In the other hand, it might be the case that even with a greater marker density the chunks stay about the same size, so including a genotype strategy to increase the marker density in the MEMA families will not be helpful at all.

```
# Import CHIP data

mema_par_hap_raw <-
  read_delim(
    "C:/Users/sergi/Documents/SAWERS LAB/MAGIC/mosaic_estimates/gen_mosaic_estimates/magic_par",
    delim = "\t",
    escape_double = FALSE,
    trim_ws = TRUE
  )
```

Rows: 23645 Columns: 19

-- Column specification -----

Delimiter: "\t"

chr (11): rs#, alleles, strand, m\_zc, m\_nt, m\_rv, m\_gd, m\_tb, m\_mu, m\_ja, m\_pt

dbl (2): chrom, pos

lgl (6): assembly#, center, protLSID, assayLSID, panellSID, QCcode

i Use ``spec()`` to retrieve the full column specification for this data.

i Specify the column types or set ``show_col_types = FALSE`` to quiet this message.

```
colnames_WGS_data <- arrow::open_dataset(
  sources = "C:/Users/sergi/Documents/SAWERS LAB/MAGIC/mosaic_estimates/gen_mosaic_e
  )$schema$names

parent_chip_MEMA <- c("GD", "JL", "MS", "NT", "PT", "RV", "TB", "ZC")

mema_par_hap <-
  mema_par_hap_raw %>%
  select(marker = `rs#`, chrom, pos, contains("m_")) %>%
  select(marker:pos, sort(names(.))) %>%
  rename_with(.cols = contains("m_"), ~ c("GD", "JL", "MS", "NT", "PT", "RV", "TB",
  pivot_longer(-c(marker:pos), names_to = "parental", values_to = "geno")

chip_parent_comp_mema <-
  crossing(p1 = parent_chip_MEMA, p2 = parent_chip_MEMA) %>%
  filter(p1 != p2) %>%
  mutate(code = map2_chr(
    .x = p1,
    .y = p2,
    .f = ~ paste0(.x, .y) %>% str_split("") %>% unlist() %>% sort() %>% paste(collap
  )) %>%
  group_by(code) %>%
  filter(row_number() == 1) %>%
  ungroup() %>%
  select(-code) %>%
  left_join(mema_par_hap %>% rename(geno1 = geno), by = c("p1" = "parental")) %>%
  left_join(mema_par_hap %>% rename(geno2 = geno), by = c("p2" = "parental", "marker

chip_parent_chunk_info <-
  chip_parent_comp_mema %>%
  mutate(comp_group = paste(p1, p2, sep = "-")) %>%
  select(-c(p1, p2)) %>%
  group_by(comp_group) %>%
  nest() %>%
  mutate(chunk_info = future_map(
    .x = data,
```



[illegible]

[illegible]

```
chip_parent_chunk_info_pre <-
  chip_parent_chunk_info %>%
  select(comp_group, chunk_info) %>%
  ungroup() %>%
  unnest(chunk_info) %>%
  mutate(chunk_size = round(chunk_size/1e6, 2)) %>%
  filter(chunk_size != 0)

chip_parent_chunk_info_pre %>%
```

```

group_by(comp_group) %>%
summarise(
  med_size = median(chunk_size),
  max_size = max(chunk_size)
) %>%
summarise(
  med_size = median(med_size),
  max_size = median(max_size)
)

# A tibble: 1 x 2
  med_size max_size
  <dbl>     <dbl>
1    0.19    12.1

chip_parent_chunk_mema_label <- chip_parent_chunk_info_pre %>%
group_by(comp_group) %>%
summarise(
  label = sapply(c("min", "max", "median"), do.call, list(x = chunk_size)) %>%
    round(., 2) %>%
    paste(names(.), ., sep = " = ") %>%
    paste(., collapse = "\n"),
  y = 1000,
  chunk_size = 10
)

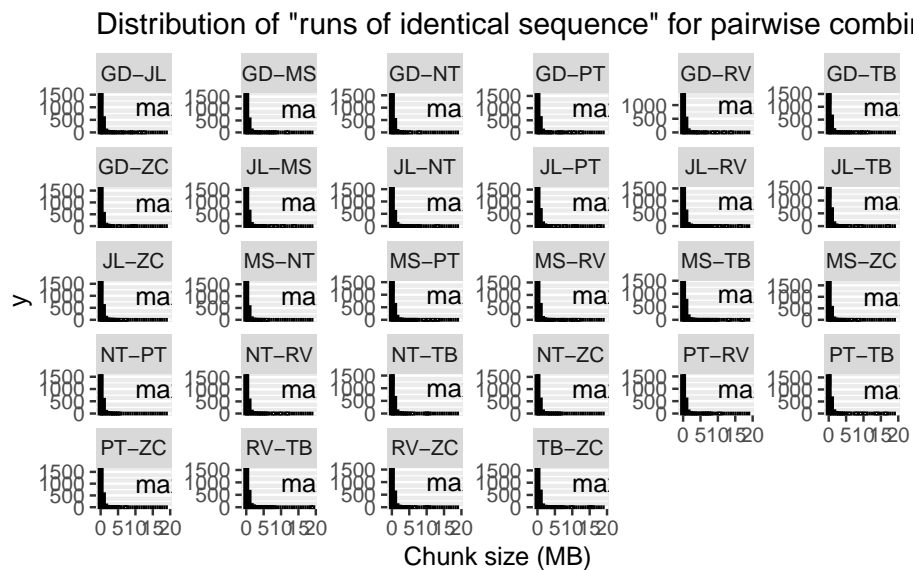
chip_parent_chunk_info_pre %>%
ggplot(data = ., aes(x = chunk_size, fill = comp_group )) +
geom_histogram(color = "black", linewidth = 0.75, bins = 30) +
geom_vline(
  data = . %>% group_by(comp_group ) %>% summarise(chunk_size = median(chunk_size)),
  aes(xintercept = chunk_size),
  linetype = "dashed",
  linewidth = 0.75
) +
geom_text(
  data = chip_parent_chunk_mema_label,
  aes(label = label, x = chunk_size, y = y, hjust = "bottom"),
) +
facet_wrap(. ~ comp_group, scales = "free_y") +
xlab("Chunk size (MB)") +
theme(
  legend.position = "none",
  panel.grid.major.x = element_blank(),

```

```

panel.grid.minor.x = element_blank(),
) +
ggtitle('Distribution of "runs of identical sequence" for pairwise combination of

```



In general the maximum size of the “same genotype” chunks seems to be 8-16 MB depending on the pairwise cross that is being analyzed. We expect to see a reduction in the size by using WGS (higher marker density)

```

wgs_mema_parquet_source <-
  "C:/Users/sergi/Documents/SAWERS LAB/MAGIC/mosaic_estimates/gen_mosaic_estimates/p

colnames_WGS_data <-
  arrow::open_dataset(sources = wgs_mema_parquet_source)$schema$names

WGS_parents_raw <-
  colnames_WGS_data[-c(1:11)] %>%
  crossing(p1 = ., p2 = .) %>%
  filter(p1 != p2) %>%
  mutate(code = map2_chr(
    .x = p1,
    .y = p2,
    .f = ~ paste0(.x, .y) %>% str_split("") %>% unlist() %>% sort() %>% paste(collapse = ",")
  )) %>%
  group_by(code) %>%

```

```

filter(row_number() == 1) %>%
ungroup() %>%
select(-code) %>%
mutate(
  cols = map2(
    .x = p1,
    .y = p2,
    .f = ~ c(colnames_WGS_data[c(1, 3, 4)], .x, .y)
  ) %>%
mutate(comp_group_label = paste0(p1, "_", p2))

downsize_scheme <- tibble(
  n_marker = c(5000, 10000, 20000, 50000, 100000, 500000, 1e6, 3e6, 5e6, 8125930),
  code = c("5k", "10k", "20k", "50K", "100K", "500K", "1M", "3M", "5M", "8.2M")
) %>%
mutate(prop = n_marker/8125930)

# for (j in 10) {
#
#   for (i in 1:dim(WGS_parents_raw)[1]) {
#
#     wgs_data_pre <- read_parquet(file = "parents_WGS_hap.parquet", col_select = all_of(WGS_p
#       rename_with(~c("marker", "chrom", "pos", "geno1", "geno2"))
#
#     if (j == dim(downsize_scheme)[1]) {
#
#       wgs_magic_chunks_data_pre <- wgs_data_pre
#
#     } else {
#
#       set.seed(100)
#       wgs_data_split <- initial_split(wgs_data_pre, prop = downsize_scheme$prop[j], strata =
#       wgs_magic_chunks_data_pre <- training(wgs_data_split)
#
#     }
#
#     wgs_magic_chunks <-
#       wgs_magic_chunks_data_pre %>%
#       group_by(chrom) %>%
#       rowid_to_column() %>%
#       filter(geno1 == geno2) %>%
#       mutate(
#         dif_inicio = lead(rowid) - rowid,

```

```

#       dif_final = lag(dif_inicio)
#       ) %>%
#       filter(dif_inicio == 1 | dif_final == 1) %>%
#       mutate(block = ifelse(dif_inicio == 1, NA, rowid)) %>%
#       fill(block, .direction = "up") %>%
#       group_by(block) %>%
#       mutate(chunk_n_marker = n()) %>%
#       filter(rowid == min(rowid) | rowid == max(rowid)) %>%
#       group_by(chrom, block) %>%
#       summarise(
#         chunk_n_marker = first(chunk_n_marker),
#         chunk_size = diff(pos),
#         chunk_name = paste(first(rowid), last(rowid), sep = "-"),
#         spans = paste(round(first(pos)/1e6, 2), round(last(pos)/1e6, 2), sep = "-")
#       ) %>%
#       ungroup() %>%
#       mutate(chunk_size = round(chunk_size/1e6, 2)) %>%
#       filter(chunk_size > 0)
#
#   SINK <- paste0(WGS_parents_raw$comp_group_label[i], "_", downsize_scheme$code[j])
#
#   write_parquet(wgs_magic_chunks, sink = SINK)
#
#   rm(wgs_data_pre, wgs_data_split, wgs_magic_chunks_data_pre, wgs_magic_chunks, SINK)
#   gc()
# }
# }

parents_mema_gws_all_data <-
  WGS_parents_raw %>%
  select(comp_group_label) %>%
  mutate(ds = list(downsize_scheme)) %>%
  unnest(ds) %>%
  mutate(file = paste0(
    "C:/Users/sergi/Documents/SAWERS LAB/MAGIC/osaic_estimates/gen_mosaic_estimates/
    comp_group_label,
    "_", code,
    "_chunk_info.parquet")) %>%
  mutate(data = map(
    .x = file,
    .f = ~ read_parquet(.x)
  ))

```

```
WGS_parents_chunk_sizes <-
  parents_mema_gws_all_data %>%
  unnest(data) %>%
  group_by(comp_group_label, n_marker) %>%
  summarise(
    min_chunk_size = min(chunk_size),
    med_chunk_size = median(chunk_size),
    max_chunk_size = max(chunk_size),
    code = first(code)
  )
```

`summarise()` has grouped output by 'comp\_group\_label'. You can override using the `.groups` argument.

```
WGS_parents_chunk_sizes %>%
  group_by(n_marker, code) %>%
  pivot_longer(contains("chunk_size")) %>%
  ungroup() %>%
  filter(name != "min_chunk_size") %>%
  group_by(code, name) %>%
  filter(value == min(value) | value == max(value)) %>%
  ungroup() %>%
  select(-comp_group_label) %>%
  distinct() %>%
  arrange(n_marker, name) %>%
  group_by(n_marker, code, name) %>%
  summarise(interval = paste(value, collapse = " - ")) %>%
  pivot_wider(names_from = name, values_from = interval)
```

`summarise()` has grouped output by 'n\_marker', 'code'. You can override using the `.groups` argument.

```
# A tibble: 10 x 4
# Groups:   n_marker, code [10]
  n_marker code max_chunk_size med_chunk_size
    <dbl> <chr> <chr> <chr>
1   5000 5k 28.76 - 66.82 0.93 - 2.02
2  10000 10k 21.82 - 56.12 0.51 - 1.01
3  20000 20k 10.53 - 41.29 0.26 - 0.55
4  50000 50K 7.57 - 22.21 0.13 - 0.23
5 100000 100K 5.35 - 19.09 0.08 - 0.13
6 500000 500K 2.46 - 7.87 0.03 - 0.05
7 1000000 1M 2.38 - 5.83 0.03
8 3000000 3M 5.49 - 1.19 0.02
9 5000000 5M 5.33 - 1 0.02
```

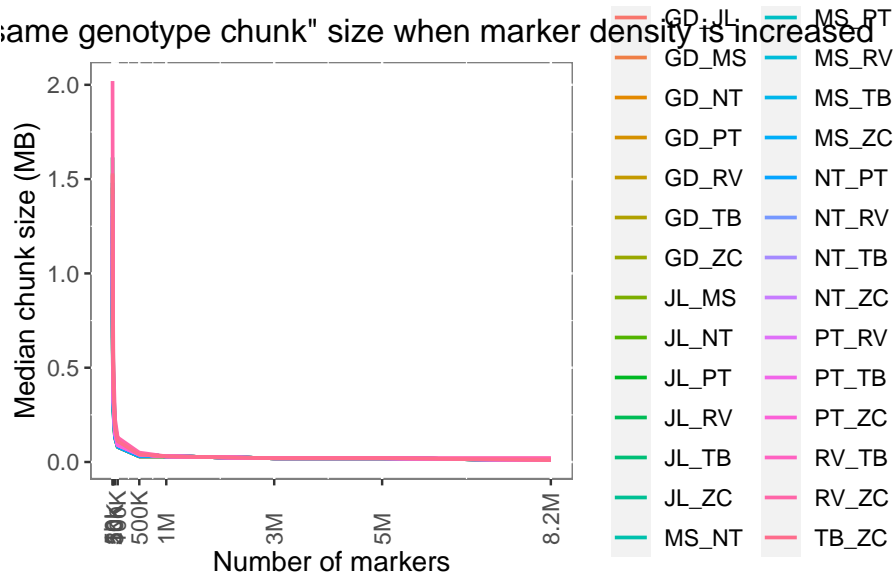
10 8125930 8.2M 0.56 - 4.13 0.01 - 0.02

```
WGS_parents_med_chunk_sizes_plot <-
  WGS_parents_chunk_sizes %>%
  mutate(
    code = as_factor(code)) %>%
  ggplot(
    data = .,
    aes(x = n_marker, y = med_chunk_size, color = comp_group_label, group = comp_group_label)
  ) +
  geom_line(linewidth = 0.75) +
  scale_x_continuous(
    breaks = unique(WGS_parents_chunk_sizes$n_marker),
    labels = unique(WGS_parents_chunk_sizes$code)
  ) +
  xlab("Number of markers") +
  ylab("Median chunk size (MB)") +
  ggtitle('Reduction in "same genotype chunk" size when marker density is increased') +
  theme(
    panel.background = element_rect(fill = "white", colour = "grey50"),
    text = element_text(size = 12),
    axis.text.x = element_text(angle = 90, vjust = 0.5, hjust = 0),
    legend.title = element_blank(),
    plot.title = element_text(hjust = 0.5)
  )

WGS_parents_med_chunk_sizes_plot
```



same genotype chunk" size when marker density is increased

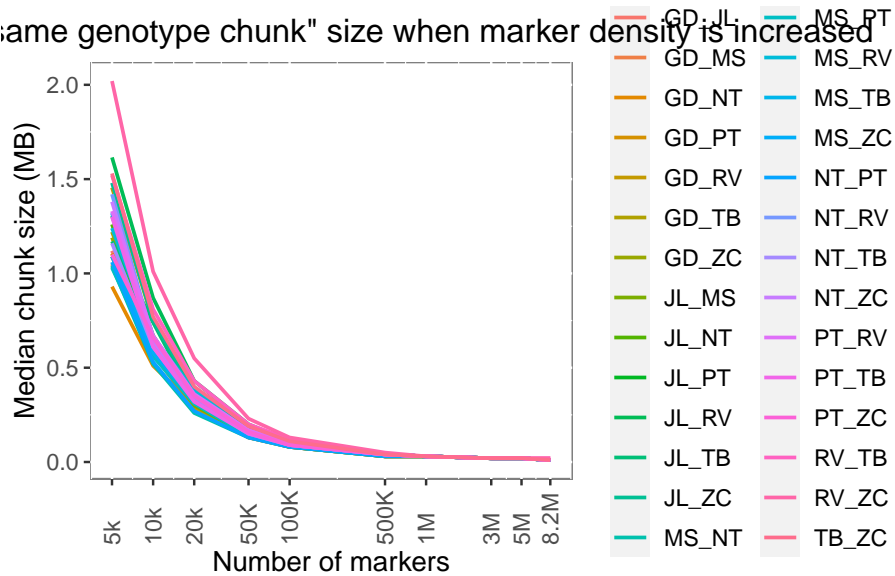


```
WGS_parents_med_chunk_sizes_plot +
  scale_x_log10(
    breaks = unique(WGS_parents_chunk_sizes$n_marker),
    labels = unique(WGS_parents_chunk_sizes$code)
  )
```

Scale for x is already present.

Adding another scale for x, which will replace the existing scale.

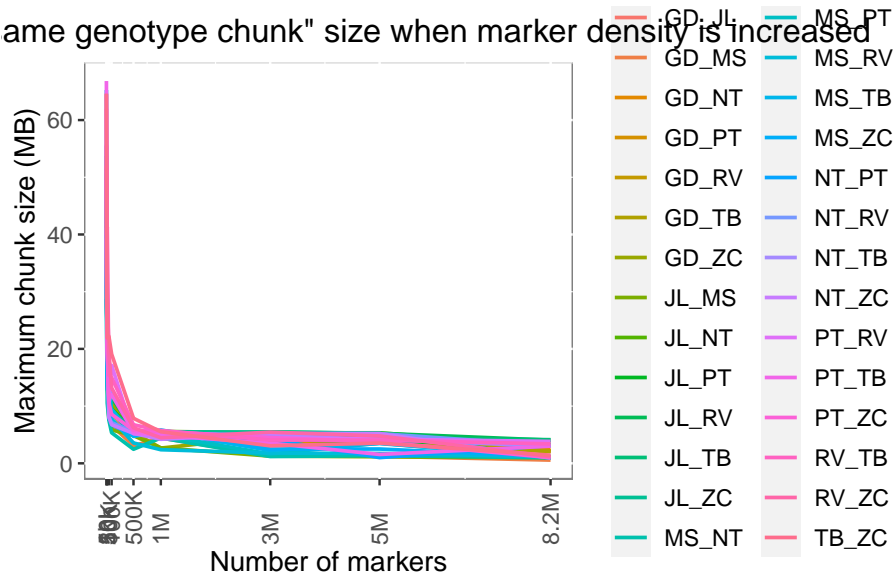
same genotype chunk" size when marker density is increased



```
WGS_parents_max_chunk_sizes_plot <-
  WGS_parents_chunk_sizes %>%
  mutate(
    code = as_factor(code)) %>%
  ggplot(
    data = .,
    aes(x = n_marker, y = max_chunk_size, color = comp_group_label, group = comp_group_label)
  ) +
  geom_line(linewidth = 0.75) +
  scale_x_continuous(
    breaks = unique(WGS_parents_chunk_sizes$n_marker),
    labels = unique(WGS_parents_chunk_sizes$code)
  ) +
  xlab("Number of markers") +
  ylab("Maximum chunk size (MB)") +
  ggtitle('Reduction in "same genotype chunk" size when marker density is increased') +
  theme(
    panel.background = element_rect(fill = "white", colour = "grey50"),
    text = element_text(size = 12),
    axis.text.x = element_text(angle = 90, vjust = 0.5, hjust = 0),
    legend.title = element_blank(),
    plot.title = element_text(hjust = 0.5)
  )

WGS_parents_max_chunk_sizes_plot
```

ame genotype chunk" size when marker density is increased

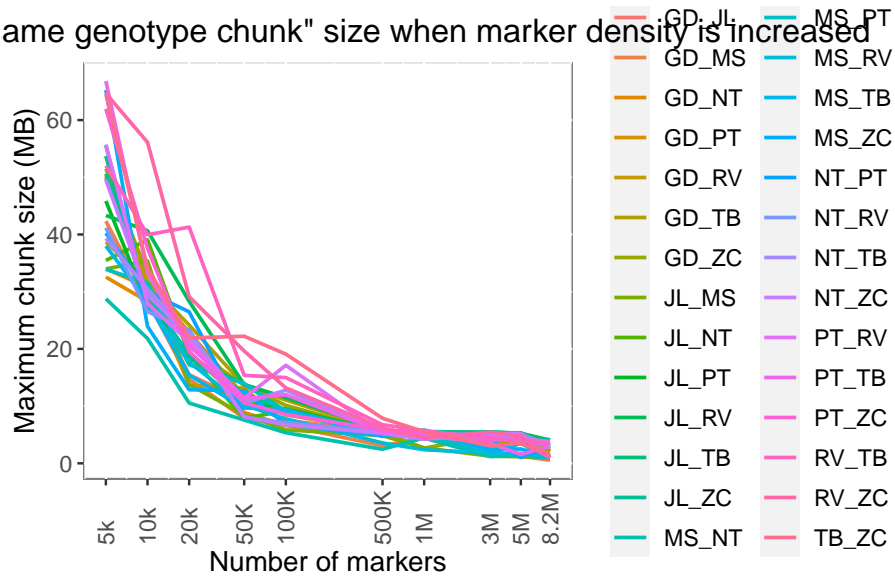


```
WGS_parents_max_chunk_sizes_plot +
  scale_x_log10(
    breaks = unique(WGS_parents_chunk_sizes$n_marker),
    labels = unique(WGS_parents_chunk_sizes$code)
  )
```

Scale for x is already present.

Adding another scale for x, which will replace the existing scale.

ame genotype chunk" size when marker density is increased

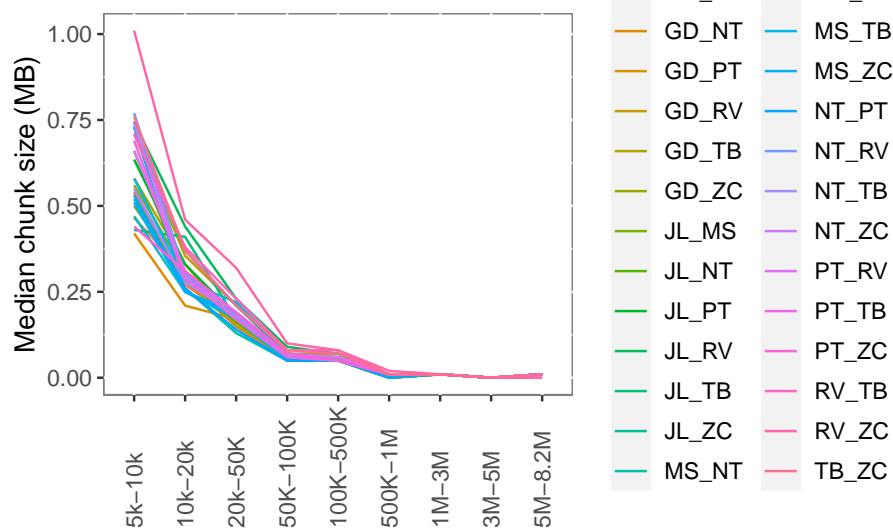


```
dif_chunk_size_par <-
  WGS_parents_chunk_sizes %>%
  group_by(comp_group_label) %>%
  mutate(
    dif_med = med_chunk_size - lead(med_chunk_size),
    dif_max = max_chunk_size - lead(max_chunk_size),
    code2 = lead(code),
    code2 = paste0(code, "-", code2)
  ) %>%
  filter(!is.na(dif_med)) %>%
  mutate(code2 = as_factor(code2))

dif_chunk_size_par %>%
  ggplot(
    data = .,
    aes(x = code2, y = dif_med, color = comp_group_label, group = comp_group_label)) +
  geom_line() +
  xlab(NULL) +
  ylab("Median chunk size (MB)") +
  ggtitle('Difference in the median chunk size as the number of markers is increased') +
  theme(
    panel.background = element_rect(fill = "white", colour = "grey50"),
    text = element_text(size = 12),
    axis.text.x = element_text(angle = 90, vjust = 0.5, hjust = 0),
    legend.title = element_blank(),
```

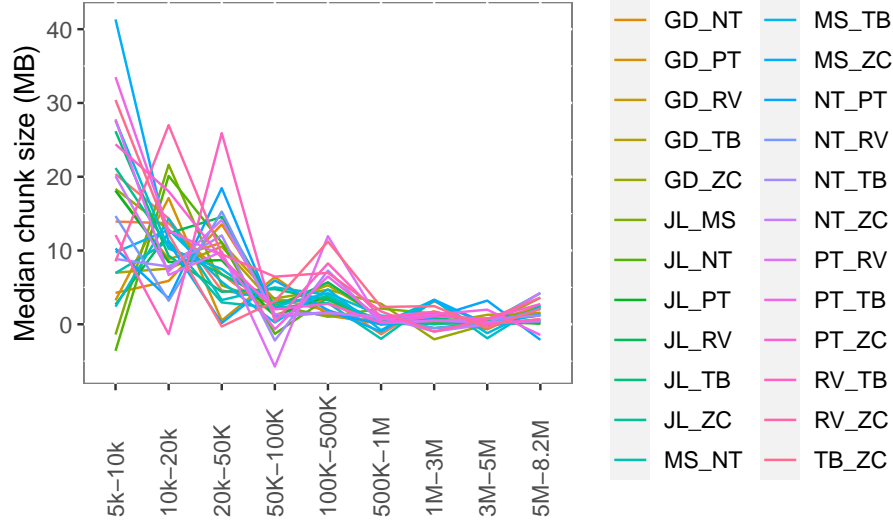
```
plot.title = element_text(hjust = 0.5)
)
```

the median chunk size as the number of markers is increased.



```
dif_chunk_size_par %>%
  ggplot(
    data = .,
    aes(x = code2, y = dif_max, color = comp_group_label, group = comp_group_label)) +
  geom_line() +
  xlab(NULL) +
  ylab("Median chunk size (MB)") +
  ggtitle('Difference in the maximum chunk size as the number of markers is increased') +
  theme(
    panel.background = element_rect(fill = "white", colour = "grey50"),
    text = element_text(size = 12),
    axis.text.x = element_text(angle = 90, vjust = 0.5, hjust = 0),
    legend.title = element_blank(),
    plot.title = element_text(hjust = 0.5)
  )
```

maximum chunk size as the number of markers is increased.



We hypothesize that the big amount of missing information in the assignment of a parental haplotypes for markers in the MEMA population is because at the actual marker density ( $\sim 15K$  markers) `rqtl2` cannot differentiate between any parent with a 95% of confidence, thus generating missing information. This leads us to think that is because there are genomic regions in the parents that have “identical genotype (IBD?)”, making difficult or impossible for `qtl2` to differentiate one parent from the other. So we think that if we can reduce the size of this “same genotype” regions by identifying polymorphic markers across each pair of parents, we can give more information to `rqtl2` to assign a parental haplotype and reduce the number of misscalling.

The question here is: how big are this regions? and If we increase the genome-wide marker density, can we reduce the size of this regions? Can we add more information with more markers? How many markers do we need to add more information and reduce the size of the “same genotype chunks”?

To answer these questions we assess the size of the “same genotype” regions in each pairwise combination of the parents of the MEMA population by using the 50K CHIP marker data ( $\sim 15K$  SNPs) and WGS data ( $\sim 8.2M$  SNPs). We use a “down-scale” simulation with the WGS data, where we randomly sampled markers across the genome with the same proportion of markers per chromosome as the original dataset, to assess if we can observe a gradient of how much information we acquire (in terms of reduction of the size of the same genotype chunks) as we increase the marker density. We selected 5000, 10,000, 20,000, 100,000, 1,000,000, 3,000,000, 5,000,000 and 8,200,000 markers to answer this question.

For the CHIP dataset, the same chunk genotype median and max size can go from 0.17-0.24 MB and 5.09-19 MB respectively. For WGS data, we can observe that an increase of the number of markers leads to the reduction of the median and max same genotype chunk size, for instance, with 50,000 markers we get a median chunk size from 0.13 - 0.23 MB and max size of 7.57 - 22.21 MB, and as we increase the marker density we observe a reduction in these values. So we can conclude that more dense marker data increases the information on the parents of the MEMA population. So it is worth to find a genotyping way that can increase the density of markers, like skim sequencing + imputation.





## Part II

## CML



Sergio Pérez-Limón

Quarto enables you to weave together content and executable code into a finished document. To learn more about Quarto see <https://quarto.org>.

## Running Code

When you click the **Render** button a document will be generated that includes both content and the output of embedded code. You can embed code like this:

```
1 + 1
```

```
[1] 2
```

You can add options to executable code like this

```
[1] 4
```

The `echo: false` option disables the printing of code (only output is displayed).



## Chapter 5

# 2023\_\_Highland\_\_experiment\_\_design

Sergio Perez-Limon

Here I use the FieldHub app to design the field evaluation of the CML F2:3 population, for the Highlands we're using a p-rep design (more info: 10.1198/108571106X154443). In total there are going to be 210 genotypes of the HI73 and HI93 populations, 320 from the HI79 and Hermes as a CHECK evaluated in two locations. For this experimental design, 70 genotypes of each biparental population are going to be replicated, and the rest (140, 140 and 250 respectively) are going as single reps. The families are selected based on the phc BLUP value. It is important to note that we're only removing a few families based on this, so there is not an important bias/selection towards the best genotypes, but it might help us to select families that are going to survive. We select the families that are going to be repeated randomly. Check is repeated 50 times. The experiment is designed for a 25 columns x 40 rows experiment.

There is an augmented block design for Ameca, a possible “no stress” environment where we can have a baseline to compare the performance of the highland site. Here, the experiment is composed of a 53 block per repetition, and 2 replications, with the same genotypes as the p-rep design. Each genotype is present only once per replication and is not repeated across block either.

Loading libraries

```
library(FieldHub)
library(google sheets4)
```

Warning: package 'google sheets4' was built under R version 4.2.2

```
library(googledrive)
```

Attaching package: 'googledrive'

The following objects are masked from 'package:googlesheets4':

```
request_generate, request_make
```

```
library(tidyverse)
```

```
v ggplot2 3.4.1      v purrr  1.0.1
v tibble  3.1.8      v dplyr  1.0.10
v tidyr   1.3.0      v stringr 1.5.0
v readr   2.1.3      v forcats 0.5.2
```

Warning: package 'ggplot2' was built under R version 4.2.2

Warning: package 'tidyr' was built under R version 4.2.2

Warning: package 'readr' was built under R version 4.2.2

Warning: package 'purrr' was built under R version 4.2.2

Warning: package 'dplyr' was built under R version 4.2.2

Warning: package 'stringr' was built under R version 4.2.2

Warning: package 'forcats' was built under R version 4.2.2

```
-- Conflicts ----- tidyverse_conflicts() --
x dplyr::filter()      masks stats::filter()
x dplyr::lag()         masks stats::lag()
x googledrive::request_generate() masks googlesheets4::request_generate()
x googledrive::request_make()   masks googlesheets4::request_make()
```

```
library(lme4)
```

Warning: package 'lme4' was built under R version 4.2.2

Loading required package: Matrix

Warning: package 'Matrix' was built under R version 4.2.2

Attaching package: 'Matrix'

The following objects are masked from 'package:tidyr':

```
expand, pack, unpack
```

```
gs4_auth("checo.spl@gmail.com")
```

Import available genotypes and genotype information

```
# raw data for genotypes
data_url <- "https://docs.google.com/spreadsheets/d/14yHP2kqQZQ-wp0IdiUgeUeQIeqorQed3-m-xcwFu_

genotypes_data_raw <-
  data_url %>%
  as_id() %>%
  range_read(sheet = "Genetic Stocks")
```

v Reading from "CML457\_459\_Populations".

v Range "'Genetic Stocks'".

```
families_url <- "https://docs.google.com/spreadsheets/d/1Mix1vFbJkeLhC3psHa0xxJscgPXsiizt-unjr

seed_availability_url <- "https://docs.google.com/spreadsheets/d/14yHP2kqQZQ-wp0IdiUgeUeQIeqor

# Import raw data for families
families_data_raw <- families_url %>%
  as_id %>%
  range_read(ss = ., sheet = "UNISEM21A", skip = 1)
```

v Reading from "21\_NCS\_PSU\_LANGEBIO\_FIELDS".

v Range "'UNISEM21A'!2:10000000".

New names:

```
* `` -> `...16`
* `` -> `...18`
```

```
seed_available <- seed_availability_url %>%
  as_id() %>%
  range_read(ss = ., sheet = "Genetic Stocks")
```

v Reading from "CML457\_459\_Populations".

v Range "'Genetic Stocks'".

```
packing_seed_info_url <- "https://docs.google.com/spreadsheets/d/14yHP2kqQZQ-wp0IdiUgeUeQIeqor

# packing seed info

packing_seed_info <-
```

```

packing_seed_info_url %>%
  as_id() %>%
  range_read(ss = ., sheet = "seed_packing_2022")

```

v Reading from "CML457\_459\_Populations".

v Range "'seed\_packing\_2022'".

```

packing_seed_info_unique <-
  packing_seed_info %>%
  select(familia, F2_Parent, sobre_UNISEM) %>%
  unique() %>%
  rename(`Female genotype` = familia, `Female parent` = sobre_UNISEM)

```

Highland site p-rep design

```

# Genotypes available for planting

available_genos <-
  genotypes_data_raw %>%
  select(family = Family_ID, pop = Population_ID, use = `Use_23 (798)`) %>%
  filter(use == 1)

# select families based on the best BLUP values
cml_pops_raw_data <-
  data_url %>%
  as_id() %>%
  range_read("22_Highland_Raw_data_tidy") %>%
  mutate(phc = as.double(phc))

```

v Reading from "CML457\_459\_Populations".

v Range "'22\_Highland\_Raw\_data\_tidy'".

```

cml_phc_example <-
  cml_pops_raw_data %>%
  select(location, family, phc, nblock) %>%
  mutate(across(c(location, family), ~ as_factor(.))) %>%
  mutate(phc = ifelse(phc > 2 | phc == 0, NA_real_, phc)) %>%
  filter(family != "HERMES")

set.seed(100)
phc_BLUP_example <-
  lmer(
    phc ~ location + (1|family) + (1|location:nblock), data = cml_phc_example

```



```

    ) %>%
  ranef() %>%
  .$family %>%
  as_tibble(rownames = "family") %>%
  rename(BLUP = `(Intercept)`)

# Best families available based on the BLUP value
prospect_families <-
  phc_BLUP_example %>%
  semi_join(available_genos) %>%
  mutate(pop = gsub("-\\d+$", "", family)) %>%
  arrange(pop, desc(BLUP)) %>%
  group_by(pop) %>%
  mutate(id = row_number()) %>%
  filter(
    (pop == "HI73" & id %in% c(1:210)) |
    (pop == "HI93" & id %in% c(1:210)) |
    (pop == "HI79" & id %in% c(1:320))
  ) %>%
  ungroup() %>%
  rename(phc_BLUP = BLUP)

```

Joining, by = "family"

```

# p-prep experimental design using FieldHub

highland_2023_prep_design <-
  partially_replicated(
    nrows = 40,
    ncols = 25,
    repGens = c(530, 210, 1),
    repUnits = c(1, 2, 50),
    planter = "serpentine",
    l = 2,
    seed = 100,
    locationNames = c("site1", "site2")
  )

```

Warning message:

Since plotNumber was missing, it was set up to default value of: 1001 2001

```

# Extracting the fieldbook from the experimental design
high23_fieldbook <-
  highland_2023_prep_design$fieldBook %>%
  as_tibble() %>%
  rename_with(~tolower(.))

# Selecting rep families by random and randomizing families within each pop
set.seed(100)
high_exp_data <-
  prospect_families %>%
  group_by(pop) %>%
  slice_sample(prop = 1) %>%
  mutate(id2 = row_number()) %>%
  ungroup() %>%
  select(family, pop, id2) %>%
  add_row(family = "CHECK", id2 = NA, .before = 1) %>%
  mutate(
    rep = case_when(
      is.na(id2) ~ 50,
      id2 %in% c(1:70) ~ 2,
      T ~ 1
    ) %>%
    arrange(desc(rep), pop, id2) %>%
    mutate(id = row_number())

treatment_family <-
  high23_fieldbook %>%
  count(location, treatment) %>%
  mutate(id = gsub("G", "", treatment) %>% as.integer()) %>%
  rename(rep = n) %>%
  arrange(id) %>%
  left_join(high_exp_data) %>%
  select(treatment, family, pop) %>%
  distinct() %>%
  mutate(pop = ifelse(is.na(pop), "CHECK", pop))

```

Joining, by = c("rep", "id")

```

high23_fieldbook_family <-
  high23_fieldbook %>%
  left_join(treatment_family)

```

Joining, by = "treatment"

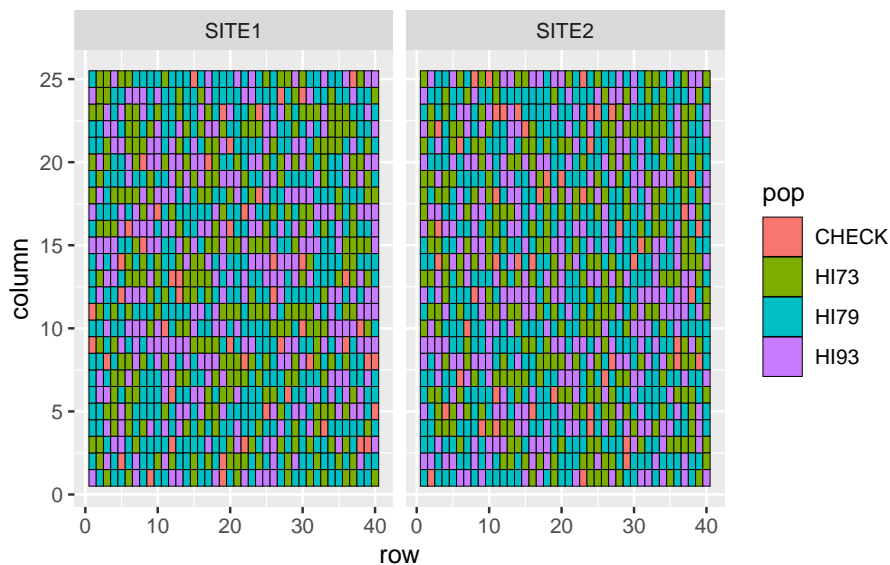
```
high23_fieldbook_family
```

```
# A tibble: 2,000 x 12
```

	id	expt	location	year	plot	row	column	checks	entry	treatment	family
	<int>	<chr>	<chr>	<chr>	<dbl>	<int>	<int>	<chr>	<dbl>	<chr>	<chr>
1	1	Expt1	SITE1	2023	1001	1	1	186	186	G186	HI93-271
2	2	Expt1	SITE1	2023	1002	1	2	0	574	G574	HI79-370
3	3	Expt1	SITE1	2023	1003	1	3	0	218	G218	HI73-028
4	4	Expt1	SITE1	2023	1004	1	4	0	508	G508	HI79-280
5	5	Expt1	SITE1	2023	1005	1	5	0	567	G567	HI79-306
6	6	Expt1	SITE1	2023	1006	1	6	115	115	G115	HI79-286
7	7	Expt1	SITE1	2023	1007	1	7	0	444	G444	HI79-321
8	8	Expt1	SITE1	2023	1008	1	8	0	384	G384	HI79-191
9	9	Expt1	SITE1	2023	1009	1	9	1	1	G1	CHECK
10	10	Expt1	SITE1	2023	1010	1	10	0	482	G482	HI79-104

```
# ... with 1,990 more rows, and 1 more variable: pop <chr>
```

```
high23_fieldbook_family %>%
  ggplot(data = ., aes(x = row, y = column)) +
  geom_tile(
    aes(fill = pop),
    color = "black") +
  facet_grid(. ~ location)
```



```

data_spreadsheet <-
  high23_fieldbook_family %>%
  select(
    `CML23-` = plot, Description = pop, `Female genotype` = family,
    row_design = row, column_design = column, checks) %>%
  mutate(
    `Origin (Package)` = "LANGEBIO",
    `Packed?` = " ",
    `Who/What` = "AL/JL",
    `Male parent` = "x sib",
    `Male genotype` = "x sib",
    `Number/Selection` = "14K",
    rep = case_when(
      checks == 0 ~ 1,
      Description == "CHECK" ~ 50,
      T ~ 2
    )
  ) %>%
  left_join(packing_seed_info_unique) %>%
  select(
    `CML23-`, `Origin (Package)`:`Who/What`, Description, `Female parent`,
    `Male parent`, `Female genotype`, `Male genotype`, `Number/Selection`,
    row_design, column_design, rep
  ) %>%
  mutate(across(contains("Female"), ~ ifelse(is.na(.), "HERMES", .)))

```

Joining, by = "Female genotype"

```
write_csv(data_spreadsheet, "23_highland_experiment_spreadsheet.csv")
```

Ameca augmented complete block design

```

prospect_families %>%
  count(pop) %>%
  mutate(prop = n/sum(n)) %>%
  mutate(a = prop*24)

```

```

# A tibble: 3 x 4
  pop      n prop    a
  <chr> <int> <dbl> <dbl>
1 HI73   210 0.284  6.81
2 HI79   320 0.432 10.4
3 HI93   210 0.284  6.81

```

```

prospect_families %>% dim %>% .[[1]]

[1] 740

Ameca_RCBD <- RCBD_augmented(
  lines = prospect_families %>% dim %>% .[[1]],
  planter = "serpentine",
  checks = 1,
  b = 53,
  repsExpt = 2,
  l = 1,
  random = TRUE,
  locationNames = c("Ameca"),
  seed = 100,
)

# 57, 53, 50, 47, 44

Ameca_fieldbook <- Ameca_RCBD$fieldBook %>%
  as_tibble() %>%
  rename_with( ~ tolower(.))

Ameca_fieldbook %>%
  filter(treatment != "CH1") %>%
  count(block) %>%
  mutate(nn = n/2) %>%
  count(nn)

# A tibble: 1 x 2
      nn      n
<dbl> <int>
1    14    53

prospect_families %>%
  count(pop) %>%
  mutate(prop = n/sum(n)) %>%
  mutate(a = prop*14)

# A tibble: 3 x 4
  pop      n prop      a
<chr> <int> <dbl> <dbl>
1 HI73    210 0.284  3.97
2 HI79    320 0.432  6.05
3 HI93    210 0.284  3.97

```

```

# Per block: 4 HI73/HI93; 6 HI79

set.seed(100)
Ameca_fieldbook_pops <-
  Ameca_fieldbook %>%
  mutate(rep = ifelse(id < 796, 1, 2)) %>%
  group_by(rep, block) %>%
  mutate(rowid = row_number()) %>%
  slice_sample(prop = 1) %>%
  mutate(
    type = ifelse(treatment == "CH1", "check", "family"),
    type = factor(type, levels = c("check", "family"))
  ) %>%
  ungroup() %>%
  arrange(rep, block, type) %>%
  mutate(what = rep(c("HERMES", rep("HI73", 4), rep("HI79", 6), rep("HI93", 4)), 100))
  filter(treatment != "Filler")

Ameca_fieldbook_pops %>%
  count(rep, what)

# A tibble: 8 x 3
  rep what      n
  <dbl> <chr> <int>
1     1 HERMES    53
2     1 HI73    211
3     1 HI79    317
4     1 HI93    212
5     2 HERMES    53
6     2 HI73    211
7     2 HI79    318
8     2 HI93    211

# Need 420 HI73/HI93; 640 HI79

set.seed(100)
extra_family_replacement <-
  Ameca_fieldbook_pops %>%
  filter(what %in% c("HI93", "HI73")) %>%
  group_by(rep, what) %>%
  slice_sample(n = 3) %>%
  filter(
    (rep == 1 & what == "HI73" & row_number() == 1) |
    (rep == 1 & what == "HI93" & row_number() %in% c(1:2)) |

```

```

      (rep == 2 & what == "HI73" & row_number() %in% c(1)) |
      (rep == 2 & what == "HI93" & row_number() %in% c(1))
    ) %>%
    mutate(what = "HI79")

set.seed(100)
Ameca_fieldbook_pops_family <- Ameca_fieldbook_pops %>%
  anti_join(extra_family_replacement, by = c("rep", "plot")) %>%
  bind_rows(extra_family_replacement) %>%
  arrange(rep, what) %>%
  group_by(rep, what) %>%
  slice_sample(prop = 1) %>%
  mutate(id2 = row_number()) %>%
  left_join(prospect_families %>% select(-phc_BLUP), by = c("what" = "pop", "id2" = "id")) %>%
  mutate(family = ifelse(what == "HERMES", "HERMES", family)) %>%
  ungroup()

Ameca_fieldbook_pops_family %>%
  arrange(id) %>%
  select(id, plot:block, rep, treatment, pop = what, family)

```

# A tibble: 1,586 x 10

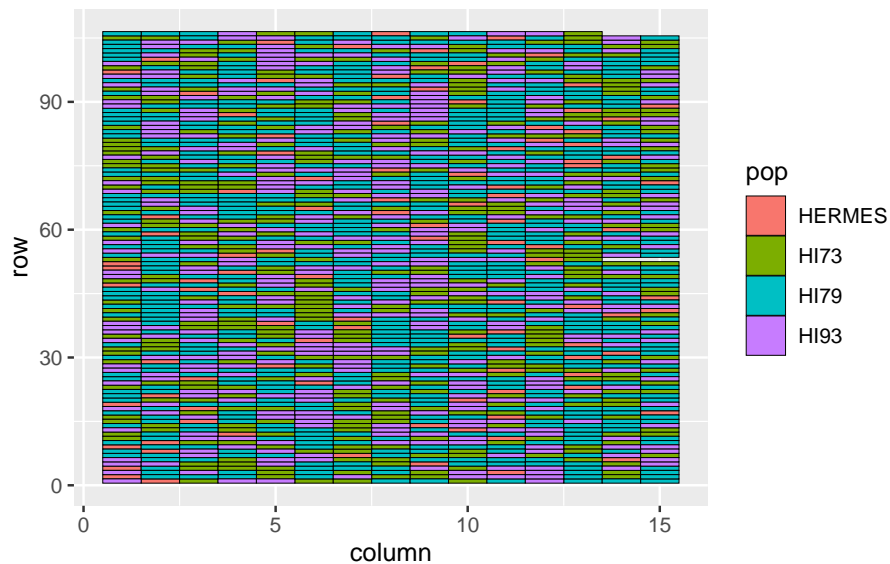
	id	plot	row	column	checks	block	rep	treatment	pop	family
	<int>	<dbl>	<int>	<int>	<chr>	<dbl>	<dbl>	<chr>	<chr>	<chr>
1	1	101	1	1	0	1	1	G658	HI93	HI93-179
2	2	102	1	2	1	1	1	CH1	HERMES	HERMES
3	3	103	1	3	0	1	1	G205	HI73	HI73-092
4	4	104	1	4	0	1	1	G133	HI93	HI93-233
5	5	105	1	5	0	1	1	G641	HI93	HI93-108
6	6	106	1	6	0	1	1	G321	HI73	HI73-137
7	7	107	1	7	0	1	1	G418	HI73	HI73-220
8	8	108	1	8	0	1	1	G44	HI79	HI79-162
9	9	109	1	9	0	1	1	G636	HI79	HI79-268
10	10	110	1	10	0	1	1	G391	HI73	HI73-248

# ... with 1,576 more rows

```

Ameca_fieldbook_pops_family %>%
  select(id, plot:block, rep, treatment, pop = what, family) %>%
  ggplot(data = ., aes(x = column, y = row, fill = pop)) +
  geom_tile(color = "black")

```



```
Ameca_data_spreadsheet <-
  Ameca_fieldbook_pops_family %>%
  arrange(id) %>%
  mutate(`CML23-` = id + 4000) %>%
  select(
    `CML23-`, Description = what, `Female genotype` = family,
    row_design = row, column_design = column, checks, rep, block) %>%
  mutate(
    `Origin (Package)` = "LANGEBIO",
    `Packed?` = " ",
    `Who/What` = "AL/JL",
    `Male parent` = "x sib",
    `Male genotype` = "x sib",
    `Number/Selection` = "14K"
  ) %>%
  left_join(packing_seed_info_unique) %>%
  select(
    `CML23-`, `Origin (Package)`:`Who/What`, Description, `Female parent`,
    `Male parent`, `Female genotype`, `Male genotype`, `Number/Selection`,
    row_design, column_design, rep, block
  ) %>%
  mutate(across(contains("Female"), ~ ifelse(is.na(.), "HERMES", .)))
```

Joining, by = "Female genotype"



```
write_csv(Amecca_data_spreadsheet, "Amecca_data_spreadsheet.csv")
```



# Chapter 6

## PT

Sergio Pérez-Limón

Quarto enables you to weave together content and executable code into a finished document. To learn more about Quarto see <https://quarto.org>.

### 6.1 Running Code

When you click the **Render** button a document will be generated that includes both content and the output of embedded code. You can embed code like this:

```
1 + 1
```

```
[1] 2
```

You can add options to executable code like this

```
[1] 4
```

The `echo: false` option disables the printing of code (only output is displayed).



# Chapter 7

## AMF

Sergio Pérez-Limón

Quarto enables you to weave together content and executable code into a finished document. To learn more about Quarto see <https://quarto.org>.

### 7.1 Running Code

When you click the **Render** button a document will be generated that includes both content and the output of embedded code. You can embed code like this:

```
1 + 1
```

```
[1] 2
```

You can add options to executable code like this

```
[1] 4
```

The `echo: false` option disables the printing of code (only output is displayed).



## Chapter 8

# Summary

In summary, this book has no content whatsoever.





# References

Knuth, Donald E. 1984. “Literate Programming.” *Comput. J.* 27 (2): 97–111.  
<https://doi.org/10.1093/comjnl/27.2.97>.

