Linkage disequilibrium analysis of hexaploid wheat (Triticum aestivum L.)

A thesis presented for the degree of Doctor of Philosophy

by

Sherri Anne Kruger

The School of Agriculture, Food and Wine The University of Adelaide

June 2007

Table of Contents

TABLE OF CONTENTS	II
LIST OF TABLES	V
LIST OF FIGURES	VIII
LIST OF ABBREVIATIONS AND ACRONYMS	XIII
ABSTRACT	XVI
DECLARATION	XVII
ACKNOWLEDGEMENTS	XVIII
CHAPTER 1: GENERAL INTRODUCTION	1
1.1 AN INTRODUCTION TO WHEAT	1
1.2 A COMPLEX GENOME	1
1.3 GENETIC TOOLS	4
1.4 MOLECULAR MARKERS	5
1.4.1 RFLPs	6
1.4.2 RAPDs	7
1.4.3 AFLPs	9
1.4.4 SSRs	10
1.4.5 SNPs	13
1.5 IDENTIFYING AND LOCALIZING TRAITS OF INTEREST	15
1.6 ASSOCIATION MAPPING BASED ON LINKAGE DISEQUILIBRIUM	17
1.7 EXTENT AND PATTERNS OF LINKAGE DISEQUILIBRIUM IN HUMAN GENETICS	20
1.8 EXTENT OF LINKAGE DISEQUILIBRIUM IN PLANTS	22
1.9 Measuring linkage disequilibrium	26
1.10 FACTORS AFFECTING LINKAGE DISEQUILIBRIUM	29
1.11 PHYSICAL FACTORS AFFECTING LD	29
1.11.1 Mutations and recombinations	29
1.12 POPULATION GENETIC FACTORS AFFECTING LD	31
1.12.1 Selection	31
1.12.2 Population dynamics	33
1.12.3 Mating systems	36
1.13 SUMMARY	38
CHAPTER 2: SEQUENCE BASED ANALYSIS OF LINKAGE DISEQUILIBRIUM	M IN
WHEAT	39

2.1 Introduction	39
2.2 Materials and methods	42
2.2.1 Plant material	42
2.2.2 Nucleic acid isolation	44
2.2.3 PCR amplification of Pina and Pinb genes	45
2.2.4 Fragment purification and sequencing of Pina and Pinb genes	46
2.2.5 Data analysis	47
2.3 RESULTS	48
2.3.1 Sequence evaluation of Pina and Pinb genes from hexaploid wheat	48
2.3.2 LD across the hardness locus of Ae. tauschii	54
2.4 Discussion	62
CHAPTER 3: POPULATION STRUCTURE WITHIN EXPERIMENTAL HEXA	APLOID
WHEAT POPULATIONS	
3.1 Introduction	67
3.2 MATERIALS AND METHODS	69
3.2.1 Plant material	69
3.2.2 Microsatellite markers	
3.2.3 PCR amplification microsatellite markers in hexaploid wheat	
3.2.4 ABI Prism 3700 DNA analyzer	
3.2.5 Microsatellite analysis	
3.2.6 Cluster analysis	
3.3 RESULTS	76
3.3.1 Establishing program parameters	
3.3.2 Deciding on the most appropriate value of K	85
3.3.3 Identifying sub-populations in Australian and UK data sets	85
3.4 DISCUSSION	89
CHAPTER 4: GENOME WIDE LINKAGE DISEQUILIBRIUM IN HEXAPLOI	D WHEAT 97
4.1 Introduction	97
4.2 Materials and Methods	99
4.2.1 Plant material and DNA isolation	99
4.2.2 Microsatellite Analysis	99
4.2.3 Linkage Disequilibrium Data Analysis	99
4.3 RESULTS	101
4.3.1 Extensive LD within South Australian hexaploid wheat germplasm	
4.3.2 LD in the absence of population stratification	104
4 3 3 LD in the absence of rare alleles	109

4.3.4 Examination of LD within linkage groups and genomes	111
4.3.4.1 LD within linkage groups	111
4.3.4.2 LD within genomes	117
4.3.4.3 LD across the Group 7 chromosomes	119
4.3.5 LD in a large European Population	124
4.4 Discussion	130
CHAPTER 5: GENERAL DISCUSSION	138
LITERATURE CITED	144
APPENDIX A	XXIII
APPENDIX B	XXIX

List of Tables

Table 1.1
A selection of genetic maps developed in T. aestivum sp. using a variety of molecular marker
systems.
Table 1.26
Overview of characteristics of the most commonly used molecular marker systems for wheat.
Table 1.323
Extent of linkage disequilibrium in plant studies.
Table 2.1
List of the Australian hexaploid wheat accessions used in evaluating sequence level LD in the
Pina and Pinb genes.
Table 2.2
Gene specific primers used to amplify the full length Pina and Pinb genes in the hexaploid
wheat varieties outlined in Table 2.1
Table 2.3
Summary of Pinb nucleotide variation and their impact on the resultant protein as observed in
the 50 Ae. tauschii lines.
T.11.21
Table 3.170
Genomic distribution of 150 microsatellite markers used in genotyping 96 Australian wheat
varieties.

Table 3.2		
Twenty-five, unlinked, SSR markers used in estimating population structure of the Australian		
and UK hexaploid wheat data sets.		
Table 3.375		
Five experiments set up in Structure in order to determine the most appropriate parameter		
settings for the program.		
Table 3.4		
Pedigree information for 22 Australian wheat lines, making up the largest sub-population		
based on the genetic clustering algorithm Structure.		
Table 3.592		
Pedigree information for 93 UK wheat lines, making up the largest sub-population based on		
the genetic clustering algorithm Structure.		
Table 4.1		
Number of pair-wise comparisons performed in the LD analysis of the complete Australian		
wheat dataset (96 lines) as well as the largest Australian sub-population as determined from		
STRUCTURE in Section 3.3.3 (22 lines).		
Table 4.2		
Number of pairwise comparisons performed in the LD analysis of the complete UK wheat		
dataset (225 lines) as well as the largest UK sub-population as determined from		
STRUCTURE in Section 3.3.3 (93 lines).		

List of Figures

Figure 1.1
Flow chart illustrating the origin of hexaploid wheat, Triticum aestivum.
Figure 1.2
Schematic representations of the effects of LD on association mapping strategies from
(Rafalski 2002).
Figure 2.149
Gel images of three group 5 nullisomic tetrasomic (Nulli-Tetra) Chinese Spring wheat lines
and a water control, amplified with the gene specific primers for Pina-D1 (A) and Pinb-D1
(B).
Figure 2.250
Gel images of 87 Australian hexaploid wheat lines amplified with the Pina gene specific
primers in addition to a glutenin gene used as an internal control in order to detect null Pina-
D1b alleles (*, Glutenin amplicon; **, Pina amplicon).
Figure 2.3
Consensus sequence of the Pina gene as determined from sequence analysis of 44 Australian
hexaploid wheat lines.
Figure 2.4
Pinb gene consensus sequences as determined from sequence analysis of 62 Australian
hexaploid wheat lines.

Figure 2.555
Consensus sequence of 6 haplotypes of the Pina gene as determined from sequence analysis
of 50 Ae. tauschii wheat lines.
Figure 2.658
Consensus sequence of 4 haplotypes of the Pinb gene as determined from sequence analysis
of 50 Ae. tauschii wheat lines.
Figure 2.760
Scatter plot of LD (r2) as a function of distance in base pairs across the 447 bases in the Pinb
gene of 50 Ae. tauschii lines.
Figure 2.861
Scatter plot of LD (r2) as a function of distance in base pairs across the hardness locus of 50
Ae. tauschii lines.
Figure 3.178
Experiment 1. Scatter plot of the log probability of the data versus the estimated K values (1
to 23) using 90 Australian wheat lines.
to 25) using 90 Austranan wheat fines.
Figure 3.279
Experiment 2. Scatter plot of the log probability of the data versus the estimated K values (1
to 23) using 90 Australian wheat lines.
Figure 3.380
Experiment 3. Scatter plot of the log probability of the data versus the estimated K values (1
to 23) using 90 Australian wheat lines.

Figure 3.482
Experiment 4. Scatter plot of the log probability of the data versus the estimated K values (1
to 23) using 90 Australian wheat lines.
Figure 3.583
Experiment 5. Scatter plot of the log probability of the data versus the estimated K values (1
to 23) using 90 Australian wheat lines.
Figure 3.684
UK data set. Scatter plot of the log probability of the data versus the estimated K values (1 to
23) using 184 UK wheat lines.
TI
Figure 3.787
Estimation of population structure for the Australian (A) and UK (B) data sets for population
estimations $K = 3$.
Figure 4.1
Scatter plot of LD (D') vs marker distance (cM) for all Australia wheat lines.
Figure 4.2105
Bar graph illustrating the distribution of average D' values for each of the four experimental
scenarios (outlined in the legend) within each of the five marker 'classes' (on the x- axis).
Figure 4.3
Bar graph illustrating the distribution of percent significant pairwise comparisons for each of
the four experimental scenarios (outlined in the legend) within each of the five marker
'classes' (on the x-axis)

Figure 4.4
Scatter plot of LD (D') vs marker distance (cM) for the largest Australian sub-population (22
lines) as determined through Structure analysis.
Figure 4.5
Scatter plot of LD (D') vs marker distance (cM) for Australian wheat lines in the absence of
population structure and rare alleles pooled.
Figure 4.6
Scatter plot of LD (D') vs marker distance (cM) for groups 1 (A), 2 (B), 3 (C), 4 (D), 5 (E), 6
(F), and 7 (G) of the whole Australian wheat data set.
Figure 4.7
Scatter plot of LD (D') vs marker distance (cM) for genomes A, B, and D using the whole
Australian wheat data set.
T' 40
Figure 4.8
Bar graph illustrating the distribution of average D' values of each genome (A, B, and D).
Figure 4.9
Scatter plot of LD (D') vs marker distance (cM) for the group 7 chromosomes (A: 7A; B: 7B;
and C: 7D) in the whole Australian wheat data set.
Figure 4.10
Bar graph illustrating the distribution of average D' values of the group 7 chromosomes (7A,
7B, and 7D).

Figure 4.11
Bar graph illustrating the distribution of percent significant pairwise comparisons of the
group 7 chromosomes (7A, 7B, and 7D).
Figure 4.12
Scatter plot of LD (D') vs marker distance (cM) for whole UK data set.
E' 412
Figure 4.13
Bar graph illustrating the distribution of average D' values for each of the four experimental
scenarios (outlined in the legend) within each of the five marker 'classes' (on the x- axis) for
the UK wheat data set.
Figure 4.14
Bar graph illustrating the distribution of percent significant pair-wise comparisons for each of
the four experimental scenarios (outlined in the legend) within each of the five marker
'classes' (on the x-axis) for the UK wheat data set

List of Abbreviations and Acronyms

 $\begin{array}{cc} \mu g & \quad \ \ Micrograms \\ \mu L & \quad \ \ Microliter \end{array}$

ABI Applied biosystems incorporated

AFLP Amplified fragment length polymorphism

Amp Ampicillin

APS Ammonium persulfate ATP Adenosine triphosphate

BAC Bacterial artificial chromosome

bp Base pairs

°C Degree celsius

CIMMYT International centre for maize and wheat improvement

CS Chinese spring cM Centimorgan

DaRT Diversity array technology

DH Doubled haploid
DNA Deoxyribonucleic acid

DNTPs Deoxynucleotide triphosphate

EDTA Ethylene diamine tetra-acetic acid

EST Expressed sequence tag
EtBr Ethidium bromide
EtOH Ethyl alcohol

g Gram

GDM Gatersleben D-genome microsatellite

GSP Grain softness protein

GWM Gatersleben wheat microsatellite

HCl Hydrochloric acid HMW High molecular weight

IAA Isoamyl alcohol

IPTG Isopropyl β-D-galactopyranoside

ITMI International triticeae mapping initiative

Kb Kilobase

L Litre

LB Luria bertani broth LBA LB with bacto-agar LD Linkage disequilibrium

LOD Log likelihood

MAS Marker assisted selection MgCl₂ Magnesium chloride

mL Millilitre mM Millimolar

MWM Molecular weight marker

N₂ Nitrogen

NaCl Sodium chloride NaOH Sodium hydroxide

NCBI National centre of biotechnology information

ng Nanogram

NIL Near isogenic line

nmol Nanomole

NT Nullisomic tetrasomic

PCR Polymerase chain reaction

pH Potential hydrogen
Pina Puroindoline a
Pinb Puroindoline b

PVPP Polyvinyl polypyrrolidone

QTL Quantitative trait locus

R40 RNaseA

RAPD Randomly amplified fragment length polymorphism

RFLP Restriction fragment length polymorphism

RNA Ribonucleic acid RNase Ribonuclease RO Reverse osmosis

SDS Sodium dodecyl sulfate

SNP Single nucleotide polymorphism
SOC Salt optimized broth + carbon
SSR Simple sequence repeats
STS Sequence tagged site

TAE Tris-HCl, acetic acid and EDTA TBE Tric-HCl, boric acid and EDTA

TE Tris-HCl and EDTA
Tm Annealing temperature

U Units UV Ultra violet V Volts

VNTI Vector NTI®

WMC Wheat microsatellite consortium

WMS Wheat microsatellite

X-GAL 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside

Abstract

There has recently been a renewed interest in using a whole-genome approach for identifying regions with relatively small effect on a particular trait of interest. One method that has proven effective in human populations is association mapping or linkage disequilibrium (LD) mapping. With focus on identifying the statistical correlations between marker allele frequency and phenotypes, association mapping, as a result, typically requires a high density marker map and a firm understanding of the extent and patterns of LD in the population.

This study assesses the feasibility of applying LD mapping in hexaploid wheat research for the fine mapping of traits. Adequate marker coverage of the large wheat genome was attained providing a framework enabling the examination of the extent of LD in this species. Results presented in this thesis illustrate how extensive LD is in locally adapted populations of hexaploid wheat, extending up to 100cM in some cases. It is also apparent that statistical associations are not limited only to markers on the same chromosome but include those on different genomes and chromosome groups. One of the main focuses of this study was to evaluate the effect of genetic and evolutionary factors on the levels of statistically significant LD. Type-1 error rate was successfully reduced by accounting for population structure and the presence of rare alleles in the data sets. This research has provided a base from which patterns of LD can begin to be understood in other populations and subsequently assess the applications of association mapping in inbreeding crop species, specifically *Triticum aestivum* L.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

	my thesis, when deposited in the University
Library, being made available in all form	ns of media, now or hereafter known.
Sherri A. Kruger	Date

Acknowledgements

I would like to sincerely thank my supervisors, Professor Peter Langridge, and Dr Jason Able for their guidance, advice and patience during my studies. They both have a passion for science and learning that is truly inspiring. Peter's insight into my project and assistance in allowing me to see the big picture is greatly appreciated. I am indebted to Jason for the seemingly endless editing of chapters and for assisting me with the submission of this manuscript. Both Peter and Jason were always enthusiastic about my project, many many thanks to you both.

Special thanks to Drs Ute Bauman and Andreas Schrieber for their statistical support and genuine interest in my project over the past 4 years. Dr Penny Henschke, and Alison Hay for their assistance in the genotyping and endless trouble shooting of the loveable 3700. I would also like to acknowledge Drs Klaus Oldach, Tim Sutton and Amanda Able for their friendship and encouragement throughout my studies. And of course my "Aussie sisters", Pat Warner and Elise Tucker, I am forever thankful for them lending me their superior lab skills over the years but most of all for their friendships. It was an absolute pleasure working with the rest of the Langridge lab group. Their support, patience and making the lab more than "just a place to work" made the transition from Canada and every year after much more manageable.

I would also like to thank Drs Greg Penner and Peter Jack for their support and for encouraging me to do a PhD in the first place. Without them I may never have gotten myself into this! I am grateful to the CRC for Molecular Plant Breeding who financially supported this project throughout my studies and who also provided me with a postgraduate research scholarship.

I wish to thank my Mom and Dad, Ma, Walt, Grannie, Darren and my two beautiful sisters Jenni and Chrissy, for absolutely everything. The messaging sessions and never ending phone calls made us seem not so far apart. The visits to Australia gave us a lot to look forward to and the care packages from home got us through many of the difficult times. (And Chrissy you still are the WR champ!).

Most importantly, I am forever grateful for the love and support of my husband, Gwynn, who stood by me every step of the way. He gave up life in Canada to allow me to realise my dreams in Australia. We had many adventures along the way and an experience of a lifetime. Go the transpositron! ©