GENETIC ANALYSIS OF PERCENT HULL IN OAT, *AVENA SATIVA* L.

BY

PHILIP RONALD

A Thesis

Submitted to the Faculty of Graduate Studies

In Partial Fulfilment of the Requirements

for the Degree of

Master of Science

Department of Plant Science

University of Manitoba

Winnipeg, Manitoba

National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file *Votre référence*

Our file *Notre référence*

ISBN 0-612-16258-3

Canada

Name Philip Ronald

Dissertation Abstracts International and *Masters Abstracts International* are arranged by broad, general subject categories. Please select the one subject which most nearly describes the content of your dissertation or thesis. Enter the corresponding four-digit code in the spaces provided.

SUBJECT TERM

Genetics

0369

SUBJECT CODE

UMI

Subject Categories

THE HUMANITIES AND SOCIAL SCIENCES

COMMUNICATIONS AND THE ARTS
- Architecture 0729
- Art History 0377
- Cinema 0900
- Dance 0378
- Design and Decorative Arts 0389
- Fine Arts 0357
- Information Science 0723
- Journalism 0391
- Landscape Architecture 0390
- Library Science 0399
- Mass Communications 0708
- Music 0413
- Speech Communication 0459
- Theater 0465

EDUCATION
- General 0515
- Administration 0514
- Adult and Continuing 0516
- Agricultural 0517
- Art 0273
- Bilingual and Multicultural 0282
- Business 0688
- Community College 0275
- Curriculum and Instruction 0727
- Early Childhood 0518
- Elementary 0524
- Educational Psychology 0525
- Finance 0277
- Guidance and Counseling 0519
- Health 0680
- Higher 0745
- History of 0520
- Home Economics 0278
- Industrial 0521
- Language and Literature 0279
- Mathematics 0280
- Music 0522
- Philosophy of 0998
- Physical 0523
- Reading 0535
- Religious 0527
- Sciences 0714
- Secondary 0533
- Social Sciences 0534
- Sociology of 0340
- Special 0529
- Teacher Training 0530
- Technology 0710
- Tests and Measurements 0288
- Vocational 0747

LANGUAGE, LITERATURE AND LINGUISTICS
- Language
  - General 0679
  - Ancient 0289
  - Linguistics 0290
  - Modern 0291
  - Rhetoric and Composition 0681
- Literature
  - General 0401
  - Classical 0294
  - Comparative 0295
  - Medieval 0297
  - Modern 0298
  - African 0316
  - American 0591
  - Asian 0305
  - Canadian (English) 0352
  - Canadian (French) 0355
  - Caribbean 0360
  - English 0593
  - Germanic 0311
  - Latin American 0312
  - Middle Eastern 0315
  - Romance 0313
  - Slavic and East European 0314

PHILOSOPHY, RELIGION AND THEOLOGY
- Philosophy 0422
- Religion
  - General 0318
  - Biblical Studies 0321
  - Clergy 0319
  - History of 0320
  - Philosophy of 0322
- Theology 0469

SOCIAL SCIENCES
- American Studies 0323
- Anthropology
  - Archaeology 0324
  - Cultural 0326
  - Physical 0327
- Business Administration
  - General 0310
  - Accounting 0272
  - Banking 0770
  - Management 0454
  - Marketing 0338
- Canadian Studies 0385
- Economics
  - General 0501
  - Agricultural 0503
  - Commerce-Business 0505
  - Finance 0508
  - History 0509
  - Labor 0510
  - Theory 0511
- Folklore 0358
- Geography 0366
- Gerontology 0351
- History
  - General 0578
  - Ancient 0579
  - Medieval 0581
  - Modern 0582
  - Church 0330
  - Black 0328
  - African 0331
  - Asia, Australia and Oceania 0332
  - Canadian 0334
  - European 0335
  - Latin American 0336
  - Middle Eastern 0333
  - United States 0337
- History of Science 0585
- Law 0398
- Political Science
  - General 0615
  - International Law and Relations 0616
  - Public Administration 0617
- Recreation 0814
- Social Work 0452
- Sociology
  - General 0626
  - Criminology and Penology 0627
  - Demography 0938
  - Ethnic and Racial Studies 0631
  - Individual and Family Studies 0628
  - Industrial and Labor Relations 0629
  - Public and Social Welfare 0630
  - Social Structure and Development 0700
  - Theory and Methods 0344
- Transportation 0709
- Urban and Regional Planning 0999
- Women's Studies 0453

THE SCIENCES AND ENGINEERING

BIOLOGICAL SCIENCES
- Agriculture
  - General 0473
  - Agronomy 0285
  - Animal Culture and Nutrition 0475
  - Animal Pathology 0476
  - Fisheries and Aquaculture 0792
  - Food Science and Technology 0359
  - Forestry and Wildlife 0478
  - Plant Culture 0479
  - Plant Pathology 0480
  - Range Management 0777
  - Soil Science 0481
  - Wood Technology 0746
- Biology
  - General 0306
  - Anatomy 0287
  - Animal Physiology 0433
  - Biostatistics 0308
  - Botany 0309
  - Cell 0379
  - Ecology 0329
  - Entomology 0353
  - Genetics 0369
  - Limnology 0793
  - Microbiology 0410
  - Molecular 0307
  - Neuroscience 0317
  - Oceanography 0416
  - Plant Physiology 0817
  - Veterinary Science 0778
  - Zoology 0472
- Biophysics
  - General 0786
  - Medical 0760

EARTH SCIENCES
- Biogeochemistry 0425
- Geochemistry 0996
- Geodesy 0370
- Geology 0372
- Geophysics 0373
- Hydrology 0388
- Mineralogy 0411
- Paleobotany 0345
- Paleoecology 0426
- Paleontology 0418
- Paleozoology 0985
- Palynology 0427
- Physical Geography 0368
- Physical Oceanography 0415

HEALTH AND ENVIRONMENTAL SCIENCES
- Environmental Sciences 0768
- Health Sciences
  - General 0566
  - Audiology 0300
  - Dentistry 0567
  - Education 0350
  - Administration, Health Care 0769
  - Human Development 0758
  - Immunology 0982
  - Medicine and Surgery 0564
  - Mental Health 0347
  - Nursing 0569
  - Nutrition 0570
  - Obstetrics and Gynecology 0380
  - Occupational Health and Safety 0354
  - Oncology 0992
  - Ophthalmology 0381
  - Pathology 0571
  - Pharmacology 0419
  - Pharmacy 0572
  - Public Health 0573
  - Radiology 0574
  - Recreation 0575
  - Rehabilitaion and Therapy 0382
  - Speech Pathology 0460
  - Toxicology 0383
- Home Economics 0386

PHYSICAL SCIENCES
Pure Sciences
- Chemistry
  - General 0485
  - Agricultural 0749
  - Analytical 0486
  - Biochemistry 0487
  - Inorganic 0488
  - Nuclear 0738
  - Organic 0490
  - Pharmaceutical 0491
  - Physical 0494
  - Polymer 0495
  - Radiation 0754
- Mathematics 0405
- Physics
  - General 0605
  - Acoustics 0986
  - Astronomy and Astrophysics 0606
  - Atmospheric Science 0608
  - Atomic 0748
  - Condensed Matter 0611
  - Electricity and Magnetism 0607
  - Elementary Particles and High Energy 0798
  - Fluid and Plasma 0759
  - Molecular 0609
  - Nuclear 0610
  - Optics 0752
  - Radiation 0756
- Statistics 0463

Applied Sciences
- Applied Mechanics 0346
- Computer Science 0984
- Engineering
  - General 0537
  - Aerospace 0538
  - Agricultural 0539
  - Automotive 0540
  - Biomedical 0541
  - Chemical 0542
  - Civil 0543
  - Electronics and Electrical 0544
  - Environmental 0775
  - Industrial 0546
  - Marine and Ocean 0547
  - Materials Science 0794
  - Mechanical 0548
  - Metallurgy 0743
  - Mining 0551
  - Nuclear 0552
  - Packaging 0549
  - Petroleum 0765
  - Sanitary and Municipal 0554
  - System Science 0790
- Geotechnology 0428
- Operations Research 0796
- Plastics Technology 0795
- Textile Technology 0994

PSYCHOLOGY
- General 0621
- Behavioral 0384
- Clinical 0622
- Cognitive 0633
- Developmental 0620
- Experimental 0623
- Industrial 0624
- Personality 0625
- Physiological 0989
- Psychobiology 0349
- Psychometrics 0632
- Social 0451

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

GENETIC ANALYSIS OF PERCENT HULL IN OAT,

Avena sativa L.

BY

PHILIP RONALD

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

Acknowledgements

The author is grateful to Drs. Doug Brown, Greg Penner and Anita Brûlé-Babel for supervision, and advice throughout the project. The author is also grateful to Solomon Kibite at Lacombe, Alberta for conducting extensive field trials in 1993.

Appreciation is extended to Wes Braun, Tanis Mayert, Tim Preston and Gordon Impy for valuable assistance with field studies and phenotypic determinations; to Graham Casey for primer synthesis and technical support; to Nick Tinker for assistance with MQTL analysis; to the members of the Penner laboratory for encouragement and assistance.

Appreciation is due to the faculty and students of the Department of Plant Science at the University of Manitoba. Especially to Professor Louis Lenz for directing me to the plant kingdom, and to Anh Phan for his helpful advice on numerous occasions.

Lastly I would like to acknowledge the support of my family, especially my parents, Wilbert and Sharon Ronald. Their encouragement and teaching have been instrumental in bringing me thus far.

This project was funded by the University of Manitoba, and an Agriculture and Agri-Food Canada/NSERC Research Partnership Support Program Grant in conjunction with SeCan Association.

TABLE OF CONTENTS

LIST OF TABLES

Measurement of Percent Hull in Oat, *Avena sativa* L.

Heritability of Percent Hull in Oat, *Avena sativa* L.

Heritability of Percent Hull in Oat, *Avena sativa* L.

Identification of RAPD Markers for Percent Hull in Oat, *Avena sativa* L.

LIST OF FIGURES

Heritability of Percent Hull in Oat, *Avena sativa* L.

Identification of RAPD Markers for Percent Hull in Oat, *Avena sativa* L.

APPENDIX 1

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Percent hull, the ratio of hull weight to total kernel weight, is considered a reliable physical indicator of oat grain quality. The objective of this study was to gain a better understanding of the genetic control of percent hull which would facilitate the development of oat cultivars with reduced hull content. Progeny populations, derived from crosses between three oat cultivars, were grown at several locations from 1992-1995. Percent hull was measured for progeny populations of three crosses, involving the cultivars Cascade, Robert and AC Marie (which are 30%, 25% and 23% hull respectively). Percent hull data were obtained for seed samples of F_5-F_7 progeny lines and parental checks by mechanically dehulling 50 primary kernels. Variance components revealed highly significant genotype and location effects, and limited genotype by location interaction for percent hull of Cascade/AC Marie F_7 lines grown in 1995. Broad-sense heritability estimates for percent hull were high, ranging from 0.44 to 0.95 for three crosses. Cascade/AC Marie progeny populations were used in conjunction with arbitrarily primed polymerase chain reaction (PCR) and bulked segregant analysis to identify markers linked to genes controlling percent hull. Three markers, $OPC13_{800}$, $OPD20_{600}$ and $OPK7_{1300}$, were identified as independently linked to quantitative trait loci for percent hull, together explaining 41% of the genetic variance across two locations in 1995. The development of markers linked to quantitative trait loci for percent hull, will facilitate genotypic selection for low hull content among early generation seedlings.

1.0 INTRODUCTION

Oat (*Avena sativa* L.) is an important cereal crop in world agriculture. Average annual world oat production from 1983-1992 was estimated at 40 million tonnes. Canada ranks third in world oat production behind the former U.S.S.R. and the United States (CGC, 1993). In Canada, oat has remained a consistent third in harvested area among cereals, behind wheat and barley. In 1995, oat was grown on 1.2 million hectares for a total production of 2.8 million tonnes (Statistics Canada, 1995).

Burrows (1986) summarized the major objectives of oat breeders as: (1) Improving grain yield potential, by increasing the number and size of seeds per panicle; (2) Protecting yield potential by reducing disease, lodging and shattering; (3) Improving grain quality, including nutritional factors, such as oil and protein, as well as physical parameters such as kernel size and shape, and percent hull.

Breeders face numerous challenges in improving the physical parameters of oat grain quality. The presence of a hull, high in crude fibre, is a constraint to the development of superior oat cultivars. The hull does not contribute to total grain energy content and decreases bulk density, making efficient storage and transport difficult (Burrows, 1986). Hulless oats may be the ideal solution, but problems with preharvest sprouting and threshability have hindered their development (Forsberg and Reeves, 1992). Moreover, the hull plays an important role in protecting the groat from damage caused by handling (Burrows,

1986) and pathogenic attack (Stanton et al., 1930; Christenson and Meronuck, 1986).

Percent hull refers to the ratio of hull weight to total kernel weight expressed as a percentage (Plourde, 1984). As the groat contributes most of the nutritional value in the kernel, reduced hull percentage is an objective of most oat breeding programs. Oat cultivars with low percent hull are desirable for both livestock feed and human food (Plourde, 1984). Ideally, percent hull should be made as low as possible, while avoiding thin and membranous hulls which separate from kernels during threshing (Burrows, 1986).

Percent hull is controlled by multiple genes with both additive (Wesenberg and Shands, 1971) and dominant (Stuthman and Granger, 1977) effects. The trait is influenced by genetic and environmental factors, and characterized by a continuous distribution of phenotypes. Percent hull can exhibit highly significant genotype by location and genotype by year interactions (Bartley and Weiss, 1951; Gullord and Aastveit, 1987). Variation in hull percentage exists among oat cultivars under ideal conditions, and is amplified when adverse environments result in decreased groat weight (Hunt, 1904).

The objectives of this study were: (1) to investigate the heritability of percent hull in progeny populations of three oat crosses, and (2) to identify randomly amplified polymorphic DNA (RAPD) markers linked to quantitative trait loci controlling the trait. An improved understanding of the genetics of hull percentage is important for the continued improvement of oat.

2.0 LITERATURE REVIEW

2.1 An Overview of the Oat Kernel

The inflorescence in oat, termed the panicle, consists of a branched rachis that is 225 to 300 cm in length. Up to 75 spikelets are borne on an individual panicle, each attached to a rachis branch by a single pedicel (Hunt, 1904). A typical *Avena sativa* L. spikelet consists of two or three flowers, two of which normally mature into primary and secondary kernels (Hunt, 1904). Primary kernels are larger and often have hull percentages 4-6% higher than those of secondary kernels. This difference in hull percentage is attributable to the primary kernel's broader base, attached rachilla and, in some cultivars, the presence of awns (Atkins, 1943).

Each oat kernel is composed of the groat or caryopsis and a membranous envelope, termed the hull or husk (Stanton, 1953). The hull consists of the large lemma and the smaller, partially enclosed palea. The groat includes the fertilized embryo, the starchy endosperm, and bran (Fulcher, 1986). In *Avena sativa* L. kernels, the groat is tightly encapsulated by the fibrous hull and is not liberated during threshing. The groat typically constitutes 65 to 75 percent of the total kernel weight (Stanton, 1953). Since hull formation is complete long before kernel filling (Hunt, 1904), conditions that are unfavourable for groat development, can significantly increase percent hull through decreased groat size and weight (Simons et al., 1979).

2.2 Milling Efficiency

The fibrous hull surrounding the oat kernel has little nutritional value to humans and must be removed to allow processing and consumption of the edible groat. The detachment of the lemma and palea from the oat groat followed by groat processing is referred to as milling (Poehlman, 1987). Physical parameters of oat kernels affect the efficiency of the milling process, such that any morphological trait that contributes to milling yield should be monitored in an oat breeding program (Atkins, 1943). Milling efficiency provides an indication of the amount of raw oats required to produce a specified quantity of milled groats (Peek and Poehlman, 1949). Several oat kernel characteristics have been shown to influence milling efficiency.

Greig and Findlay (1907) and Love (1914) stated that milling efficiency was associated with hull thickness. Oat cultivars producing well-filled kernels with thin, overlapping hulls were considered of superior milling quality. Kernel size may give a poor reflection of milling efficiency because many large seeded oat cultivars can produce kernels with thick hulls.

Atkins (1943) and Peek and Poehlman (1949) proposed percent hull and groat yield, respectively, as accurate measures of milling efficiency. Both groups also investigated the importance of the relative proportions of primary and secondary kernels in determining milling efficiency. They concluded that the size and shape of both kernel types, and their relative abundance must be considered when assessing milling efficiency.

Yarrow et al., (1992) proposed low mill yield (amount of raw oats required to produce a standard amount of product) as a suitable measure of milling efficiency, and observed percent hull to be significantly correlated with mill yield (0.53). In a separate study, Root (1979) also observed a significant positive correlation between mill yield and percent hull (0.73), suggesting that percent hull is a consistent indicator of milling efficiency.

2.3 Associations between Traits Affecting Oat Grain Quality

Many attempts have been made to determine the association between percent hull and other oat grain quality traits. The inconsistant conclusions reached by these studies suggest differential modes of inheritance among crosses and complex environmental influences for percent hull in oat.

Love (1914) and Bunch and Forsberg (1989) observed a positive correlation between hull percentage and kernel weight evidenced by the absence of desirable progeny lines with both high kernel weight and low hull percentage. However, Bartley and Weiss (1951) observed significant negative correlation between hull percentage and kernel weight and Stuthman and Granger (1977) observed the relationship between hull percentage and kernel weight to vary across populations.

Atkins (1943) observed low hull percentage to be associated with lower grain yield. This positive correlation was attributed to the negative influence of crown rust infection on groat weight. Siripoonwiwat et al., (1996) also observed significant positive correlations between hull percentage and grain yield.

However, Bartley and Weiss (1951) observed significant negative correlations between hull percentage and grain yield among early maturing cultivars. Wesenberg (1968) also observed a negative correlation between percent hull and grain yield. However, this relationship was significant in one year and non-significant in another, with no association observed within segregating populations.

Peek and Poehlman (1949) studied the relationship between hull percentage and three kernel classes for 15 oat cultivars. They observed percent hull to be lowest for secondary kernels, intermediate for primary kernels and highest for tertiary kernels. Souza and Sorrells (1988) reported a negative correlation between hull percentage and fertility of the tertiary floret. They suggested that competition between primary and supernumerary florets can cause poor grain filling in primary kernels, which typically compose the greatest proportion of seed yield.

Fore and Woodworth (1932) attempted to divide grain yield into its constituent parts. Significant correlations were observed between percent hull and both yield per panicle and branches per panicle. They suggested that highly correlated characters were inherited together, whereas those showing little association were under independent genetic control.

Murphy and Frey (1962) suggested potential benefits in the subdivision of percent hull, into its physical components. Selection for component traits can result in increased heritability of the major character when the component traits

are genetically determined at different stages of plant development. Unfavourable conditions during grain development increased percent hull largely through decreased groat weight, rather than increased hull weight. They proposed assessment of oat grain quality by groat weight, or groat width, the character with the greatest impact on groat weight.

2.4 Inheritance of Percent Hull in Oat

Few studies on the mode of inheritance for hull/groat percentage have been conducted (Plourde, 1984). Percent hull has been suggested to be a quantitative trait, characterized by a continuous range of variation (Hunter, 1935). Hull percentage is influenced by genetic and environmental effects (Kibite and Edney, 1992) and genotype by environment interactions (Atkins, 1943; Bartley and Weiss, 1951; Gullord and Aastveit, 1987). In certain crosses, genotypes with low percent hull are readily identified in early generations, suggesting that, in these cases, only a few genes control the trait (Forsberg and Reeves, 1992).

Hunter (1935), studied the inheritance of percent hull in the progeny of the winter oat cross, Argentine/Grey Winter. The mean hull percentage of the F_1 progeny approximated the midparent value. An absence of transgressive segregates was observed in the F_3 progeny. A study by de Villiers (1935), followed the inheritance of groat percentage in the F_3 progeny of the cross, Bancroft/Sunrise. However, in this study, transgressive segregation was reported for both extremes of groat percentage.

Wesenberg and Shands (1971, 1973) carried out detailed studies of groat percentage with F_1, F_2, F_3, and F_4 generations of the same three crosses, and the BC_1F_1 and BC_1F_2 of one cross. No transgressive segregation for high groat percentage was observed, and population means approximated the midparent value for each cross. The backcross progenies displayed mean groat percentages equivalent to the high value of the recurrent parent. Based on F_1 data and F_2 frequency distributions, they suggested that several genetic factors with additive effects influence groat percentage in oat.

Stuthman and Granger (1977) also attempted to characterize the inheritance of groat percentage in oat using bidirectional selection for groat percentage in three oat crosses. Bidirectional selection for groat percentage was effective in all three populations with increased or decreased groat percentage attributable to changes in groat weight, hull weight, or both. Comparison of mean groat percentage of unselected groups with parental means for each population suggested either partial dominance or additive mechanisms of inheritance.

Gullord and Aastveit (1987) evaluated the stability of percent hull among 25 oat genotypes grown at 10 locations over four years. Highly significant genotype by location and genotype by year interactions were observed for percent hull. Kibite and Edney (1992) collected data for 14 oat genotypes grown at three locations for two years. They noted highly significant effects of location, year and genotype on percent hull, with location having the greatest effect. However, first and second order interaction effects for percent hull were not

significant, suggesting that the ranking of genotypes remained essentially the same under different environments. This discrepancy between studies may be due to differences in the amount of inherent variability among the genotypes evaluated, or to the limited number of genotypes in the latter study.

2.5 Heritability of Percent Hull in Oat

Heritability is classified as broad-sense or narrow-sense depending on which components of the total genetic variance are included in the numerator. Broad-sense heritability describes the fraction of phenotypic variance that is attributable to the total genetic variance. Genetic variance includes additive variance, variance due to dominance deviation, and variance due to epistatic and genotype by environment interactions (Falconer and Mackay, 1996). Narrow-sense heritability measures the proportion of total phenotypic variation accounted for by additive genetic variance.

Pawlisch (1959) considered the inheritance of groat percentage between F_3 and F_4 progeny of the oat cross, Minhafer/Vicland. Broad-sense heritability of groat percentage ranged from 0.63 - 0.92 by variance components and 0.08 - 1.00 by regression.

In Wesenberg and Shands' (1971) study of groat percentage of primary kernels in F_1, BC_1F_1, and F_2 generations, the F_2 variances produced broad-sense heritability estimates of 0.52 - 0.76 for primary groat percentage among three crosses. They observed that heritability may be directly proportional to the level of genetic diversity within a particular population.

Wesenberg and Shands (1973) also estimated broad-sense heritability for groat percentage of F_3 and F_4 populations from the same three crosses. Heritability estimates for primary groat percentage were generally high, ranging from 0.38-0.92 by correlation, 0.74-0.93 by variance components and 0.20 - 0.85 by regression. Heritability by regression was highest when both generations were grown in the same year.

Stuthman and Granger (1977) further investigated the heritability of groat percentage in oat. F_2-derived F_4 families for three oat crosses, were used to calculate realized heritability estimates based on the mean groat percentages of selected and unselected groups grown in successive years. Narrow-sense heritability for groat percentage ranged from 0.34 - 0.75.

Heritability estimates depend on many factors including parental genotypes and the mode of calculation (Wesenberg and Shands, 1973). Nevertheless, high heritability for percent hull in oat has been reported in several independent studies, suggesting strong genetic control.

2.6 Genetic Markers

Genetic markers are used to identify differences (polymorphisms) in the genetic information of two or more individuals. A useful genetic marker must: (1) distinguish between parents, and (2) be accurately reproduced among progeny (Paterson et al., 1991a). There are three predominate classes of genetic markers: visual or morphological markers, enzyme markers and DNA-based markers. Visible markers are derived through mutations in genes with obvious

effects such as dwarfism and eye colour (Morgan, 1911). Enzymes (isozymes) are useful as genetic markers since genetically determined changes in their structure can be detected by differences in electrophoretic mobility (Markert and Moller, 1959). However, large numbers of genetic markers are best developed by direct analysis of DNA sequence variation.

Until recently, effective selection as a form of plant improvement relied completely on the expression of a plant's genetic constitution as an observed phenotype (Rafalski et al., 1991). Phenotype-based selection strategies depend on: (1) the proportion of additive genetic variation selected, and (2) the cost in time, materials and labour (Anderson et al., 1993). The use of marker-assisted selection is more effective than phenotypic selection when more additive genetic variation can be accounted for by markers associated with the trait, than by phenotype (Anderson et al., 1993). The remainder of this review will deal primarily with the use of DNA markers to identify genetic factors that contribute to the total variability associated with quantitative traits.

Restriction fragment length polymorphisms (RFLPs) (Botstein et al., 1980) have been used extensively to develop genetic maps in many plant species. Despite some technical difficulty associated with RFLPs, there are definite advantages to their use including their high level of reliability and reproducibility, and their ability to detect multiple alleles at a single locus (Waugh and Powell, 1992). The codominant property of RFLPs means that both parental alleles are represented by discrete fragments such that homozygotes are distinguishable

from heterozygotes.

Another class of DNA marker, based on the polymerase chain reaction (PCR), is the randomly amplified polymorphic DNA (RAPD) marker (Williams et al., 1990). The identification of RAPD markers is simple and rapid, requiring only small amounts of DNA and eliminating the need for radioactivity (Waugh and Powell, 1992). Unlike PCR, which depends on specific primers of considerable length, RAPD amplification is initiated wherever short primers of arbitrary sequence anneal to the DNA template (Waugh and Powell, 1992). RAPD polymorphisms may result from: (1) a single base change in DNA sequence within the primer binding site, or (2) large scale modifications (e.g., insertions, deletions, inversions) that alter amplicon size or prevent amplification (Waugh and Powell, 1992). RAPD markers tend to only positively identify one allele at a locus. The absence of an amplified band represents all other alleles at that locus, which fail to amplify (Rafalski et al., 1991).

2.7 Identification of DNA Markers

Complete genetic maps represent the simplest tool for marker identification. When a linkage map saturated with marker loci is available, the genome of a species can be systematically scanned for markers linked to genes for a specific trait (Gebhardt and Salamini, 1992). These flanking markers can be used to diagnose the presence or absence of a particular gene before the phenotype is analysed (Paterson et al., 1991a). However, because mapping requires many individuals and markers, identifying markers for a particular trait

is often easier with pooled DNA (Wang and Paterson, 1994).

Bulked segregant analysis (BSA), first suggested by Michelmore et al., (1991), involves the identification of polymorphisms between DNA, pooled from individuals of common phenotype. The bulked DNA samples contain different alleles in the chromosomal region surrounding the gene of interest, in a background of mixed, unlinked loci (Michelmore et al., 1991). Polymorphisms identified between the DNA pools often prove to be markers for the target trait, when scored across a segregating population.

Selective genotyping, first suggested by Lebowitz et al., (1987), is another method that may be used to identify DNA-based markers for a specific trait. This technique requires that only individuals with extreme phenotype be scored for marker genotype (Tinker and Mather, 1993). Markers associated with the trait of interest can then be identified by their frequency in subpopulations sampled from the phenotypic extremes of the population (Hu et al., 1995).

2.8 DNA Markers for Quantitative Traits

Quantitative traits, such as yield, quality factors and stress resistance, are often the most difficult to improve in a breeding program (Waugh and Powell, 1992). The efficiency of phenotypic selection for quantitative traits may be reduced by the effect of environment on phenotype and by complex inheritance (Rafalski et al., 1991). Moreover, the existence of specific genes underlying quantitative traits cannot be deduced using classical genetics, since discrete classes of individuals are not observed among segregating populations (Doerge

and Churchill, 1994). Nevertheless, the genes underlying quantitative traits, are identical to genes for qualitative traits, exhibiting segregation and recombination during sexual reproduction (Paterson et al., 1991a). Thus, it should be possible to find and characterize quantitative trait loci by their linkage to known markers segregating in Mendelian ratios (Gebhardt and Salamini, 1992).

Markers have been identified for many quantitative traits including pre-harvest sprouting resistance in wheat (Anderson et al., 1993), specific gravity in potato (Freyre and Douches, 1994), and vernalization requirement in *Brassica rapa* (Teutonico and Osborn, 1995). Most of these markers were identified using a map based approach, which allows determination of the chromosome location, dosage effect, phenotypic effect and environmental sensitivity of any QTL.

The use of BSA to develop markers for quantitative traits is also feasible. Waugh and Powell (1992) suggested that individuals composing the phenotypic extremes of a continuously distributed trait, differ at most of the genes controlling the character, and may be bulked to identify markers. Wang and Paterson (1994) outlined four requirements that maximize the likelihood of QTL identification using phenotype-based DNA pools: (1) crosses with extreme variation, (2) large population sizes, (3) homozygous populations such as doubled haploids, and (4) replicated phenotypic evaluations to determine true genotypic values. They observed that even when these conditions are met, the success of phenotypic pooling strategies depends on: (1) the phenotypic effect of individual QTLs, (2) the population size sampled, and (3) the influence of non-

genetic factors on phenotype.

Chalmers et al., (1993) described the first instance where BSA was successfully used to identify markers for QTLs. Bulked segregant analysis and a doubled haploid population were used to identify RAPD markers for milling energy, a quantitative trait in barley. DNA bulks were developed by choosing individuals from the extremes of the distribution for milling energy, and with alternate alleles at the *Rrn2* marker locus, previously shown to have a strong association with milling energy. Four of the six marker loci, identified between DNA bulks, defined a region surrounding the *Rrn2* locus. This region explained 45%, 20% and 15% of the genetic variance in milling energy in three successive years. Two marker loci identified between DNA bulks were unlinked to the *Rrn2* region, suggesting that genotype-based BSA does not prevent the detection of other markers for a quantitative trait.

2.9 Assessing Marker-QTL Associations

Unlike simply-inherited traits, quantitative traits are affected by many genes and, therefore, cannot be described as a single locus (Doerge and Churchill, 1994). Instead, statistical analysis is used to quantify the proportion of genetic variation in the parents contributed to their offspring by each region of the genome (Doerge and Churchill, 1994). QTL analysis refers to the detection of associations between chromosomal regions and quantitative traits. Testing for QTL effects involves comparison of genotype means for a single marker or, more commonly, interval analysis of linked markers.

Comparison of genotype means is the simplest approach to infer associations between markers and quantitative traits. However, such analysis is restricted to the detection of QTLs and has limited ability to estimate the location and effect of the locus (Doerge and Churchill, 1994). Comparison of genotype means at a single marker locus involves a comparison of phenotypic values between marker genotypes. A significant difference between class values supports linkage of that marker to a locus contributing to the quantitative trait phenotype (Freyre and Douches, 1994). Associations between single markers and quantitative traits are usually shown by *t* tests or analysis of variance.

Most current methods of QTL analysis depend on interval analysis, where hypotheses about the effects of a locus are tested across marker intervals along a genomic map (Tinker and Mather, 1995a). At least three types of interval analysis exist: (1) simple interval mapping (SIM) (Haley and Knott, 1992), (2) composite interval mapping (CIM) (Zeng, 1993; 1994), and (3) simplified composite interval mapping (sCIM) (Tinker and Mather, 1995a). All variants of simple interval mapping function in essentially the same manner, searching for a single target QTL at positions throughout a mapped genome. Composite interval mapping refines SIM estimates of QTL position and effect by searching the genome for one QTL at a time, while simultaneously accounting for the effects of other segregating QTLs for the trait.

The presence of a QTL is inferred when a significant proportion of phenotypic variation is attributed to a chromosomal region linked to a marker

locus. Whatever the method used, such inferences are quantified from the data by calculating test statistics (Tinker and Mather, 1995a). A QTL is inferred at a particular chromosomal location when a significant test statistic is calculated, based on the association of phenotype with the genotype of a local marker. Test statistics can be influenced by many factors that vary across experiments including: (1) population size, (2) genome size, (3) density of markers in the map, (4) the proportion of missing data, and (5) potential segregation distortion (Doerge and Churchill, 1994).

The test statistic must be compared with a threshold value to estimate its significance. One common problem in the analysis of quantitative traits is the difficulty associated with developing appropriate thresholds against which to compare test statistics (Doerge and Churchill, 1994). MQTL software (Tinker and Mather, 1995b), can permute the phenotypic entries to produce sets of data corresponding to the null hypothesis, that no QTL is present. Such randomized data sets reveal any chance associations that might exist under the null hypothesis (Doerge and Churchill, 1994). Based on analysis of permuted data sets, a threshold value is chosen to give a Type I error rate of 0.05. To accept the alternate hypothesis (a QTL is present), the test statistic must exceed the threshold value (Tinker and Mather, 1995b).

Determining how much phenotypic variance is explained by an inferred QTL is important. Estimates of R^2 {(variance explained) / (total variance)} for a single marker locus can be made from SIM test statistics, using the formula:

$$R^2 = 1 - 1/\exp(TS/n)$$

where; TS = the SIM test statistic for a single marker, and n = the product of the number of environments and the number of progeny (Tinker and Mather, 1995b). Based on peak test statistics in SIM and sCIM output, it is possible to identify positions where QTL estimates are to be made. A multi-locus model is then fitted to the data and test statistics for QTL main effect and QTL by environment effect determined at all positions. An estimate of the genetic variance explained by an inferred QTL is calculated by dividing the proportion of total phenotypic variance explained by the marker (R^2), with the broad-sense heritability.

2.10 Genetic Markers for Percent Hull in Oat

Selection for reduced hull content in oat is expensive and time consuming due to the necessity of obtaining laboratory measurements for each progeny line in a breeding program (Stuthman and Granger, 1977; Plourde, 1984). Furthermore, phenotypic selection for decreased hull content in oat must be delayed until later generations when the majority of the phenotypic variation is attributable to fixable, genetic differences. The efficiency of selection would be markedly increased by implementing a system where lines with low hull content could be recognized by an identifiable genetic marker.

Plourde (1984) investigated lemma colour as a visible genetic marker for percent hull among near-isogenic F_6 lines of *Avena sativa* L. For the crosses studied, red lemma colour was associated with low percent hull in the white versus red and yellow versus red comparisons. White lemma colour was also

associated with lower hull percentage in comparison with yellow coloured lemmas. Progeny lines with black lemma colour had higher hull percentages.

Kianian et al., (1996) used a linkage map of 360 RFLP markers and 71 F_7 lines, derived from the cross of *Avena byzantina* cv. Kanota and *Avena sativa* cv. Ogle, to identify four chromosomal regions with significant associations with groat percentage. One region identified, showed a significant association with groat percentage in four of five environments, whereas the remaining regions were significant in three of five environments. The logarithm of the odds' scores ranged from 2.03 to 7.87 among the four regions, which individually explained 16.2 to 58.2% of the phenotypic variance in groat percentage.

Siripoonwiwat et al., (1996) conducted another QTL study with a population of 84 recombinant inbred lines from the cross between Kanota and Ogle. A subset of the cultivated hexaploid oat RFLP linkage map (O'Donoughue et al., 1995), consisting of 252 DNA markers in 38 linkage groups, was used with groat percentage data collected at one location for three years. Markers on seven linkage groups, significant at $\propto = 0.01$, were used to construct multiple regression models. Four markers, with higher-value alleles from Ogle, explained 18% of the phenotypic variance in groat percentage. Four markers, with higher-value alleles from Kanota, accounted for 23% of the phenotypic variance in groat percentage. The authors suggested that improved estimates of QTL position and effect would be possible with: (1) a more accurate method of measuring groat percentage, (2) data from multiple locations, and (3) a larger population size.

3.0 MEASUREMENT OF PERCENT HULL IN OAT, *AVENA SATIVA* L.

3.1 ABSTRACT

Percent hull, an important indicator of oat grain quality, can be time consuming and costly to measure. Moreover, percent hull is affected by the environment and multiple evaluations of a particular genotype are often necessary for reliable estimates of its value. The purpose of this research was to investigate methods of oat hull percentage determination. Percent hull was measured for progeny populations of three crosses, involving the cultivars Cascade, Robert and AC Marie (30%, 25% and 23% hull respectively). Percent hull was measured by different methods for samples composed of primary and/or secondary kernels. The effect of sample size on percent hull was evaluated using ten samples, ranging in size from 10-100 primary kernels. Correlations among hull percentages determined by different methods, were calculated for F_5 lines of Cascade/AC Marie. Reproducibility of percent hull determination was evaluated for three different methods of measurement. Analysis of variance revealed no significant differences among hull percentages of ten sample sizes. Significant positive correlations, ranging from 0.66-0.99, were observed between hull percentages measured for different sample types. The results showed that random samples of primary kernels provided the most reproducible measurements of percent hull. The effectiveness of near infra red reflectance spectroscopy (NIRS) as a rapid method of measurement for percent hull may be improved with calibration by primary kernel hull percentages.

3.2 INTRODUCTION

An accurate, reproducible method for assessing hull percentage is important to oat breeding programs. Few methods have been available for evaluating seed samples efficiently and accurately for percent hull (Bartley and Weiss, 1951; Forsberg and Reeves, 1992). Large sample sizes of 100 kernels (Love, 1914), 200 kernels (Garber and Arny, 1918), 300 kernels (Stuthman and Granger, 1977) and one thousand kernels (Fore and Woodworth, 1933) were preferred, making measurements tedious.

Time constraints associated with mechanical dehulling of oats necessitate a small sample size. Wesenberg (1968) evaluated percent hull for samples of 5, 10, 15, 20 and 25 primary kernels, and concluded that 15 primary kernels provided an accurate estimate of percent hull. Bunch and Forsberg, (1989) observed a significant positive correlation (0.93) between percent hull of 10 primary kernels taken from an individual plant and mechanically-determined hull percentage for seed samples from larger plots.

Bartley and Weiss (1951) investigated sample composition for the measurement of percent hull. They determined that samples of either primary or secondary kernels were suitable for varietal comparisons. Plourde (1984) concluded that percent hull of primary kernels was effective in revealing differences among progeny lines. In a genetic study of percent hull, samples of primary kernels should be used rather than mixtures of primary and secondary kernels where the proportion of the two kernel types will often vary.

Little research has been done to investigate the reproducibility and relatedness of different methods of determination for percent hull. The objective of this study was to evaluate the effectiveness of different sample types and methods of measurement for percent hull, using progeny populations of three oat crosses.

3.3 MATERIALS AND METHODS

3.3.1 Methods of measurement

Percent hull refers to the ratio of hull weight to total kernel weight, expressed as a percentage (Plourde, 1984). In this study, most measurements of percent hull involved mechanically dehulling a random sample of primary and/or secondary kernels with an electric dehuller similar to the one described by Love and Craig (1944). Random samples were prepared from source seed by removing green and deformed kernels, groats, awns and chaff. Measurements of kernel weight and hull weight were used to calculate hull percentage.

Near infra red reflectance spectroscopy (NIRS) analysis of about 15-20 g of primary and secondary kernels was also used to measure percent hull. Two random sub-samples, prepared as before, were loaded into separate cells and scanned using an NIR Systems model 6500 scanning monochromator. Spectra were recorded at five wavelengths between 400 and 2500 nm. NIRS percent hull was determined based on previous calibration with a representative collection of hull percentages determined for two gram kernel samples. All NIRS data collection and processing were done with Infrasoft International (ISI) software.

3.3.2 Effect of sample size

The effect of sample size on primary kernel percent hull was determined using about 100 g of seed from a sample of AC Marie, grown under disease free conditions at Lacombe, Alberta in 1993. After this seed was separated into mature primary and secondary kernel classes, ten sample sizes, ranging from 10 to 100 primary kernels, were prepared in triplicate and mechanically dehulled. Analysis of variance was conducted to assess any differences in primary kernel percent hull among sample sizes.

3.3.3 Associations among sample types

Associations between hull percentages of five sample types were assessed for 26 F_5 progeny lines of Cascade/AC Marie grown at Lacombe in 1993. These lines showed consistently high or low percent hull across years, locations and generations. Correlation coefficients were calculated between percent hull measured for: (1) two gram kernel mixtures, (2) 15-20 g kernel mixtures, (3) 50 primary kernels, (4) 50 secondary kernels, and (5) equal numbers of primary and secondary kernels (100 kernels) (Table 3.3). Sample types (1), (3), (4) and (5), were mechanically dehulled, but determination of percent hull for type (2) samples was done with NIRS.

The relationships between hull percentages of three different sample types, were assessed for 223 F_5 progeny lines of the cross Cascade/AC Marie grown at Glenlea, Manitoba and Lacombe in 1993. Correlation coefficients were calculated between percent hull measured for: (1) two gram kernel mixtures, (2) 15-20 g kernel mixtures, and (3) samples of 50 primary kernels (Table 3.4).

3.3.4 Reproducibility of measurement

The reproducibility of percent hull, measured for two gram mixtures of primary and secondary kernels, was evaluated for 56 F_2-derived F_4 lines randomly selected from populations of Cascade/AC Marie, Cascade/Robert and AC Marie/Robert grown in 1992. Reproducibility of NIRS-determined percent hull was assessed with 96 measurements of a sample of AC Marie grown at Glenlea in 1993. The reproducibility of primary percent hull, was evaluated for 24 and 47 F_2-derived F_7 lines, randomly selected from Cascade/AC Marie populations grown in 1995 at Portage la Prairie, Manitoba and Glenlea, respectively.

3.4 RESULTS

In the following results and discussion, three measures of percent hull are primarily discussed. Two gram percent hull refers to percent hull determinations on two gram mixtures of primary and secondary kernels. NIRS-determined percent hull refers to the non-destructive measurement of percent hull for 15-20 g mixtures of primary and secondary kernels. Primary kernel percent hull refers to percent hull measured for 50 primary kernels.

3.4.1 Effect of sample size

Analysis of variance revealed no significant differences in primary kernel percent hull among ten sample sizes of AC Marie kernels (Table 3.2). The error mean square was considerably larger than the sample size mean square, suggesting that the method of sampling has a greater effect than sample size on the determination of primary kernel percent hull.

3.4.2 Associations among sample types

An evaluation of hull percentages for 26 Cascade/AC Marie F_5 progeny lines, measured on five different sample types, revealed the largest correlation coefficient (0.99) between primary kernel percent hull and percent hull measured with equal numbers of primary and secondary kernels. The lowest correlation coefficient (0.84) was observed between two gram percent hull and NIRS-determined percent hull (Table 3.2). Hull percentages of all sample types for each line were positively and significantly correlated.

Hull percentages for 223 F_5 progeny lines of Cascade/AC Marie, measured on three different sample types, showed the largest mean correlation coefficient (0.80) between primary kernel and two gram percent hull (Table 3.3). Hull percentages of all sample types for each line were positively and significantly correlated.

Of the five sample types evaluated for hull percentage, the strongest association was observed between primary kernel hull percentage and percent hull measured for a 100 kernel mixture. The 100 kernel mixture, consisting of equal proportions of primary and secondary kernels, was the largest and most representative sample type tested in this study. Although this relationship was evaluated for only 26 progeny lines of the cross Cascade/AC Marie, it underlines the value of primary kernel percent hull as a suitable measurement for the assessment of grain quality in oat cultivars and breeding lines.

Table 3.1. Percent hull and analysis of variance for ten different sub-samples of primary kernels randomly selected from a sample of AC Marie grown at Lacombe, Alberta in 1993.

	Sample size (kernels)									
Observation	10	20	30	40	50	60	70	80	90	100
a	23.27	23.50	24.31	22.76	24.02	23.99	24.57	23.51	23.57	23.65
b	24.07	23.58	23.72	24.41	23.80	23.78	23.56	24.12	24.12	23.40
c	23.23	24.22	23.50	24.06	24.00	23.16	24.09	23.74	23.69	25.03
Mean	23.52	23.76	23.84	23.74	23.94	23.64	24.07	23.79	23.79	24.02
Variance	0.15	0.10	0.12	0.51	0.01	0.12	0.17	0.06	0.06	0.51

Analysis of variance				
Source of variation	Degrees of freedom	Mean square	F value	P-value
sample size	9	0.0839	0.3088	0.96
error	20	0.2717		

Table 3.2. Correlation coefficients between hull percentages measured on five different sample types for 26 F_5 Cascade/AC Marie progeny lines grown at Lacombe, Alberta in 1993.

	Sample type			
Sample type	2 gram [1]	NIRS [2]	50 1° [3]	50 2° [4]
NIRS	0.84**			
50 1°	0.88**	0.88**		
50 2°	0.94**	0.91**	0.94**	
100 1°/2° [5]	0.91**	0.90**	0.99**	0.97**

NS, *, ** Nonsignificant or significant at P < 0.05, 0.01, respectively.

1 a two gram mixture of primary and secondary kernels
2 a 10-15 gram mixture of primary and secondary kernels
3 a sample of 50 primary kernels
4 a sample of 50 secondary kernels
5 a mixture of 50 primary and 50 secondary kernels

Table 3.3. Correlation coefficients between hull percentages measured on three different sample types for 223 F_5 Cascade/AC Marie progeny lines grown at Glenlea, Manitoba (left) and Lacombe, Alberta (right) in 1993.

	2 gram [1]	NIRS [2]
NIRS	0.68**, 0.59**	
50 1° [3]	0.79**, 0.76**	0.74**, 0.69**

NS, *, **Nonsignificant or significant at P < 0.05, 0.01, respectively.

1 a two gram mixture of primary and secondary kernels
2 a 10-15 gram mixture of primary and secondary kernels
3 a sample of 50 primary kernels

3.4.3 Reproducibility of measurement

The reproducibility of two gram percent hull, varied among crosses. For 19 F_4 progeny lines from the Cascade/AC Marie population, the absolute difference between two measurements ranged from 0.1% to 5.5% with a mean of 1.5%. Two gram percent hull was also reassessed for 16 randomly selected F_4 progeny lines from the Cascade/Robert population. Absolute discrepancies between measurements varied from 0% to 7.6% with an average difference of 1.9%. Finally, 19 randomly selected F_4 progeny lines from AC Marie/Robert were evaluated a second time for two gram percent hull. Across the 19 samples, absolute differences between the first and second measurement ranged from 0.1% to 1.7% with a mean of 0.8%.

Reproducibility of NIRS-determined percent hull was assessed using 96 measurements of a single sample of AC Marie grown at Glenlea in 1993. Although a 7% range in percent hull was observed among the 96 measurements, the standard deviation of 1.8% suggested a kurtotic distribution.

The most reproducible method of percent hull measurement involved dehulling 50 primary kernels. Absolute differences between two measurements, for 24 F_7 progeny lines selected from two replicates at Portage la Prairie, ranged from 0% to 1.2%, with a mean of 0.3%. Absolute differences between two measurements, for 47 F_7 progeny lines selected from three replicates at Glenlea, varied from 0% to 1.3%, with a mean of 0.5%.

3.5 DISCUSSION

Analysis of variance revealed no significant differences in primary kernel percent hull among ten sample sizes of AC Marie kernels. However, the analysis of variance showed sampling to be more important than sample size in the determination of primary kernel percent hull. The evaluation of early generation material often requires that measurements be made on small seed samples. Although percent hull can be successfully measured on small samples of primary kernels (Wesenberg, 1968), methods which involve mixtures of primary and secondary kernels probably require larger sample sizes to match the reproducibility of primary kernel hull percentages.

The major difficulty associated with measuring percent hull on a mixture of primary and secondary kernels relates to the relative proportions of the two kernel types within the sample (Plourde, 1984). Primary kernels were characterized by hull percentages 4-6% greater than those of secondary kernels. Unless a constant ratio of the two kernel types can be ensured, reproducible measurements are difficult.

Near infra red reflectance spectroscopy is a desirable method for the assessment of hull percentage because it is rapid and nondestructive to the seed sample. In this study, NIRS proved effective for the classification of lines with extreme hull percentages, supporting its usefulness as a breeding tool to select for low hull percentage. The larger sample size favours reproducibility by increasing the likelihood of primary and secondary kernels in representative

proportions. However, the use of percent hull of smaller two gram samples as the calibration for NIRS-determined percent hull may have adversely affected the quality of data. Although suitable for rapid assessment of percent hull, the described method of NIRS requires an improved calibration, possibly with data from primary kernels, for precise measurements.

Percent hull measured for a sample of 50 primary kernels was highly reproducible. Primary kernel percent hull was strongly correlated to hull percentages of larger samples composed of both primary and secondary kernels. However, the measurement of primary kernel percent hull was time consuming and costly, requiring the separation of primary kernels followed by mechanical dehulling. This method of measurement would only be efficient for small samples of primary kernels (Wesenberg, 1968).

Percent hull continues to be an important indicator of milling and feed quality in oat. A rapid, reproducible method for the measurement of percent hull is desirable in most oat breeding programs. This study has shown that the measurement of hull percentage is less reproducible for mixtures of primary and secondary kernels (two gram, NIRS) than for samples of 50 primary kernels. Moreover, close correlation between hull percentages of primary and secondary kernels suggests that primary kernel percent hull is most suitable for precise comparisons among oat cultivars and breeding lines.

4.0 HERITABILITY OF PERCENT HULL IN OAT, *AVENA SATIVA* L.

4.1 ABSTRACT

Low percent hull has long been recognized as a valuable measure of oat grain quality. However, reliable selection for low hull percentage has been impeded by a lack of understanding of its heritability. The objective of this study was to investigate the heritability of percent hull in three oat crosses, involving the cultivars Cascade, Robert and AC Marie (which are 30%, 25% and 23% hull respectively). Populations of F_2-derived F_4-F_7 lines were grown in replicated and unreplicated trials at several locations in western Canada from 1992-1995. Heritability estimates for each population were calculated based on percent hull determined by mechanically dehulling two gram kernel mixtures or samples of 50 primary kernels. Percent hull data, collected from replicated trials at multiple locations, were used to evaluate the effect of genotype by location interaction on phenotype. Broad-sense heritability of percent hull ranged from 0.54 to 0.90 when estimated by regression, and 0.44 to 0.83 when estimated by correlation over successive generations of three oat crosses. Variance components showed highly significant genotype and location effects for primary kernel percent hull. Genotype effects had a greater influence than did location on primary kernel percent hull, whereas the effect of genotype by location interaction was relatively less important. High broad-sense heritabilities for percent hull, hull weight and groat weight of primary kernels support the use of early generation, multiple trait selection for reduced hull content in oat.

4.2 INTRODUCTION

Percent hull and related measurements such as groat percentage are important indicators of grain quality in oat. The hull encapsulates the groat, protecting it from pathogens and insects. However, the hull has little nutritional value to non-ruminants and thus does not add to the feed energy content of the grain. Although hulless oat cultivars exist, their use has been hindered by problems such as preharvest sprouting and incomplete threshability. Reduced hull content among registered cultivars may be the best means to enhance the production of both feed and milling oats.

Variation in hull percentage is attributed to decreased groat weight, increased hull weight or both, depending on the oat population (Stuthman and Granger, 1977). Variation also exists for hull thickness (Poehlman, 1987), such that cultivars with large kernels may have high percent hull (Love, 1914). The variation in percent hull that exists among oat cultivars under favourable conditions, is often magnified when adverse environmental conditions decrease groat weight (Hunt, 1904).

Studies have implied that percent hull is quantitatively inherited with continuous variation (Hunter, 1935; Wesenberg, 1968). Detailed evaluations of the genetic control of percent hull have suggested that multiple genes with additive (Wesenberg and Shands 1971) or dominant effects (Stuthman and Granger, 1977) account for the observed variation. Others have shown that percent hull exhibits highly significant genotype by location and genotype by

year interactions (Bartley and Weiss, 1951; Gullord and Aastveit, 1987).

The heritability of a trait is an important indicator of the genetic advance expected through phenotypic selection. Percent hull appears to be highly heritable (Zavitz, 1927; Pawlisch, 1959; Wesenberg and Shands, 1971; Bunch and Forsberg, 1989), despite being influenced by environmental factors. Across these studies, broad-sense and narrow-sense heritability estimates generally ranged from 0.5-0.9 and 0.3-0.7, respectively. The heritability of percent hull in oat is affected by parental genotypes and the mode of calculation (Wesenberg and Shands, 1973).

Transgressive segregation, the result of recombinations at multiple genes, is evidenced by progeny plants with phenotypes outside the parental range (Poehlman, 1987). Plant breeders often depend on transgressive segregation to obtain genotypes with characteristics superior to the parental strains. Transgressive segregation for both extremes of percent hull was observed by de Villiers (1935); Wesenberg (1968) and Stuthman and Granger (1977).

The primary objective of this study was to examine the heritability of percent hull within progeny populations of three oat crosses. High heritability estimates and limited genotype by location interaction effects for hull percentage would support an early generation selection strategy with phenotypic data required from only a few locations.

4.3 MATERIALS AND METHODS

Segregating populations of three crosses were used in this study (Table 4.1). These crosses involved the cultivars: Cascade (30%), Robert (25%) and AC Marie (23%). Mean percent hull, hull per kernel, groat per kernel and kernel weight were measured for the three parental oat cultivars, and are summarized in Table 4.2.

All field studies, from 1992-1994, involved progeny populations of three crosses, planted as unreplicated, unrandomized trials. Check plots of the three parental cultivars (eight in 1992, and 16 in 1993 and 1994) were included at equal intervals of 25 plots. Seed samples were planted as 1.5 m rows, with 23 cm row spacings. All plots were separated by rows of fall rye, except for the 1993 field study at Lacombe, Alberta where spacer rows were not included. In the fall, panicles were harvested from several healthy plants within each row and the seed bulked in preparation for the next field study.

In the spring of 1995, the F_7 population of Cascade/AC Marie was planted as a three replicate, randomized complete block design at three locations in Manitoba (Winnipeg, Portage la Prairie and Glenlea). The field design of 240 treatments included 223 F_7 lines, and 17 check plots of the three parental cultivars (eight plots of Cascade and AC Marie, and one plot of Robert). Seed samples were planted as indicated above for 1992-1994. Poor germination, due to limited precipitation, resulted in minimal seed production at the Winnipeg location and these samples were not analysed for hull percentage.

Table 4.1. Progeny populations of three oat (*Avena sativa* L.) crosses grown over a number of years at locations in western Canada, as part of a study of percent hull.

Cross	Year	Location	Number of entries and generation [1]	Experimental design [2]
Cascade/AC Marie	1992	Glenlea	223 F_4	NR
	1993	Glenlea	223 F_5	NR
	1993	Glenlea	95 F_6	NR
	1993	Lacombe	223 F_5	NR
	1993	Lacombe	95 F_6	NR
	1994	Glenlea	223 F_6	NR
	1994	Glenlea	95 F_7	NR
	1995	Winnipeg	223 F_7	RCB
	1995	Glenlea	223 F_7	RCB
	1995	Portage la Prairie	223 F_7	RCB
Cascade/Robert	1992	Glenlea	223 F_4	NR
	1993	Glenlea	223 F_5	NR
	1993	Glenlea	95 F_6	NR
	1993	Lacombe	223 F_5	NR
	1993	Lacombe	95 F_6	NR
	1994	Glenlea	223 F_6	NR
	1994	Glenlea	95 F_7	NR
AC Marie/Robert	1992	Glenlea	223 F_4	NR
	1993	Glenlea	223 F_5	NR
	1993	Glenlea	95 F_6	NR
	1993	Lacombe	223 F_5	NR
	1993	Lacombe	95 F_6	NR
	1994	Glenlea	223 F_6	NR
	1994	Glenlea	95 F_7	NR

[1] F_4-F_7 generations were derived from F_2 plants by bulking seed at each generation

[2] NR = no replication; RCB = randomized complete design with three replicates

Table 4.2. Mean percent hull, hull weight (mg), groat weight (mg) and kernel weight (mg) across a number of years and locations in western Canada of the three parental oat cultivars used in the study of percent hull. (Standard deviations for each trait are given in parentheses.)

Cultivar	Year and location [3]	Percent hull (%)	Hull per kernel [2] (mg)	Groat per kernel [2] (mg)	Kernel weight [2] (mg)
AC Marie	1992 Gln	22.4 (1.0)[1]	----------	----------	----------
	1993 Gln	23.4 (0.4)[2]	10.2 (1.0)	33.3 (2.8)	43.5 (3.8)
	1993 Lac	22.5 (0.3)[2]	11.0 (0.3)	37.9 (1.4)	48.8 (1.7)
	1995 Gln	23.9 (0.4)[2]	11.1 (0.3)	35.4 (1.2)	46.4 (1.5)
	1995 Ptg	23.1 (0.5)[2]	10.2 (0.3)	33.9 (0.8)	44.1 (1.0)
Cascade	1992 Gln	35.4 (2.1)[1]	----------	----------	----------
	1993 Gln	29.7 (0.9)[2]	13.6 (1.4)	32.3 (2.6)	45.9 (3.9)
	1993 Lac	28.3 (0.4)[2]	13.8 (0.5)	34.9 (1.3)	48.7 (1.7)
	1995 Gln	32.3 (0.9)[2]	14.3 (0.5)	30.1 (1.6)	44.5 (2.0)
	1995 Ptg	30.2 (0.8)[2]	13.0 (0.4)	30.2 (1.1)	43.3 (1.3)
Robert	1992 Gln	24.1 (0.9)[1]	----------	----------	----------
	1993 Gln	24.7 (0.7)[2]	13.0 (0.8)	38.0 (2.8)	51.8 (1.8)
	1993 Lac	25.8 (0.4)[2]	14.8 (0.6)	42.5 (1.7)	57.3 (2.3)
	1995 Gln	28.1 (0.5)[2]	15.1 (0.2)	38.6 (1.3)	53.7 (1.4)
	1995 Ptg	26.2 (0.3)[2]	13.5 (0.1)	38.1 (0.7)	51.7 (0.8)

1 percent hull of a two gram mixture of primary and secondary kernels
2 measurements made for a sample of 50 primary kernels
3 Gln = Glenlea, MB., Lac = Lacombe, AB., Ptg = Portage la Praire, MB.

Over the four years of field studies, weeds were controlled by both pre-emergent treatments with a non-selective herbicide (glyphosate) and the application of a selective herbicide (chlorsulfuron) after crop emergence. Herbicides were applied at recommended rates and used only as required. Foliar diseases, in the form of crown rust (*Puccinia coronata* Cda.) and stem rust (*Puccinia graminis* Pers. f. sp. *avenae* Erikss. & E. Henn.) were not controlled in the first three years of field studies. However, in 1995, disease pressure was reduced, throughout the growing season, by three applications of propiconazole (250 g/L, used at the recommended rate of 0.2 L/acre), a non-selective, systemic fungicide.

In the heritability study, percent hull was primarily measured for random samples of 50 primary kernels, dehulled with an electric dehuller similar to the one first described by Love and Craig (1944). A study of hull percentages of five sample types (Ronald et al., 1996) showed that random samples of primary kernels provided the most reproducible estimates of percent hull. Each sample was prepared from source seed by removing green and secondary kernels, groats, awns and chaff. Primary kernels were visually distinguished from secondary kernels by their larger size, elongate shape and attached rachilla. After dehulling, hulls and groats were weighed allowing the calculation of percent hull, hull weight per kernel, groat weight per kernel and kernel weight.

Population characteristics were estimated from data collected for F_5 - F_7 progeny populations of three crosses. Means, parental midpoints, variances and

other descriptive statistics were calculated for kernel characteristics of parental checks and progeny populations using Quattro Pro V. 6.0 (Novell, 1995). Transgressive segregates were defined as those progeny lines with phenotypes greater than one standard deviation above or below individual parental means.

Stepwise regression analysis (Minitab Inc., 1994) was used to determine the effects of hull weight per kernel, groat weight per kernel and kernel weight on primary kernel percent hull for six progeny populations where these four characteristics were measured. Best subsets regression was used to compare all possible combinations of the predictor variables. Regression equations were then developed for percent hull with coefficients for the independent variables.

Phenotypic correlation coefficients (Novell, 1995) were also calculated to define the relationships among kernel weight, groat weight, hull weight, and percent hull of primary kernels. The coefficients of correlation were used to estimate broad-sense heritability.

Analysis of variance and general linear models procedures (SAS, 1988) were used to evaluate the effects of location and genotype on percent hull, hull weight, groat weight, and kernel weight of primary kernels for F_5 and F_6 progeny populations of Cascade/AC Marie. The same procedures were used to evaluate the effects of location, genotype and genotype by location interaction on kernel characteristics of F_7 progeny of Cascade/AC Marie grown in 1995. For analyses of variance all factors were considered random effects. In general, F values were calculated as the fraction of the mean square divided by the error mean square.

Broad-sense heritability of percent hull and related traits was estimated using the regression method of Mahmud and Kramer (1951). Heritability was calculated as $h^2 = (x)(b_{yx})/y$, where x is the mean of the early generation, y is the mean of the derived generation, and b_{yx} is the regression of y on x.

Broad-sense heritability of percent hull and related traits was also estimated in standard units from regression, which is equivalent to correlation coefficients on original data sets (Frey and Horner, 1957). Heritability was calculated as $h^2 = r_{xy}$, where r_{xy} is the correlation coefficient between the early generation, x, and the later generation, y, derived by self pollination from x. This method allows a maximum value for heritability estimates of 100%.

Broad-sense heritability of four primary kernel characteristics was determined using variance components calculated with data from replicated, randomized studies of the F_7 population of Cascade/AC Marie at two locations.

4.4 RESULTS

4.4.1 Statistical Analysis of Progeny Populations

Frequency distributions for percent hull of F_5-F_7 progeny populations were continuous and appeared normal (Figure 4.1, 4.2, 4.3). Similar values for mean, median and mode in all three crosses further supported normal frequency distributions for hull percentage (Table 4.3). The generations mentioned in the results and discussion refer to the plants on which seeds were grown, since kernel characteristics are affected by the environmental conditions encountered by the maternal plant.

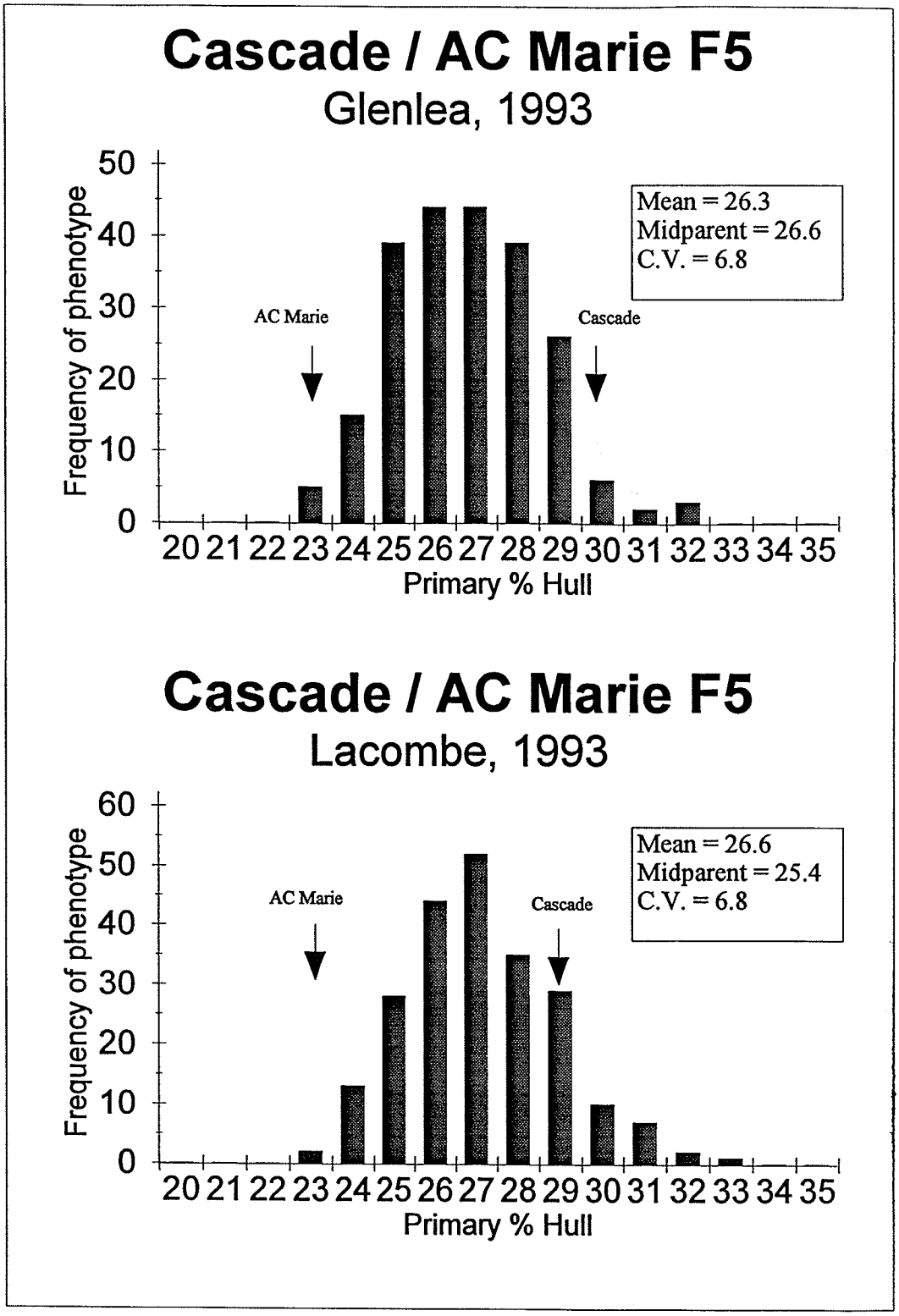

Figure 4.1. Frequency distributions of primary kernel percent hull for F_5 progeny populations of Cascade/AC Marie grown at Glenlea, Manitoba and Lacombe, Alberta in 1993. Arrows indicate hull percentages of parental cultivars.

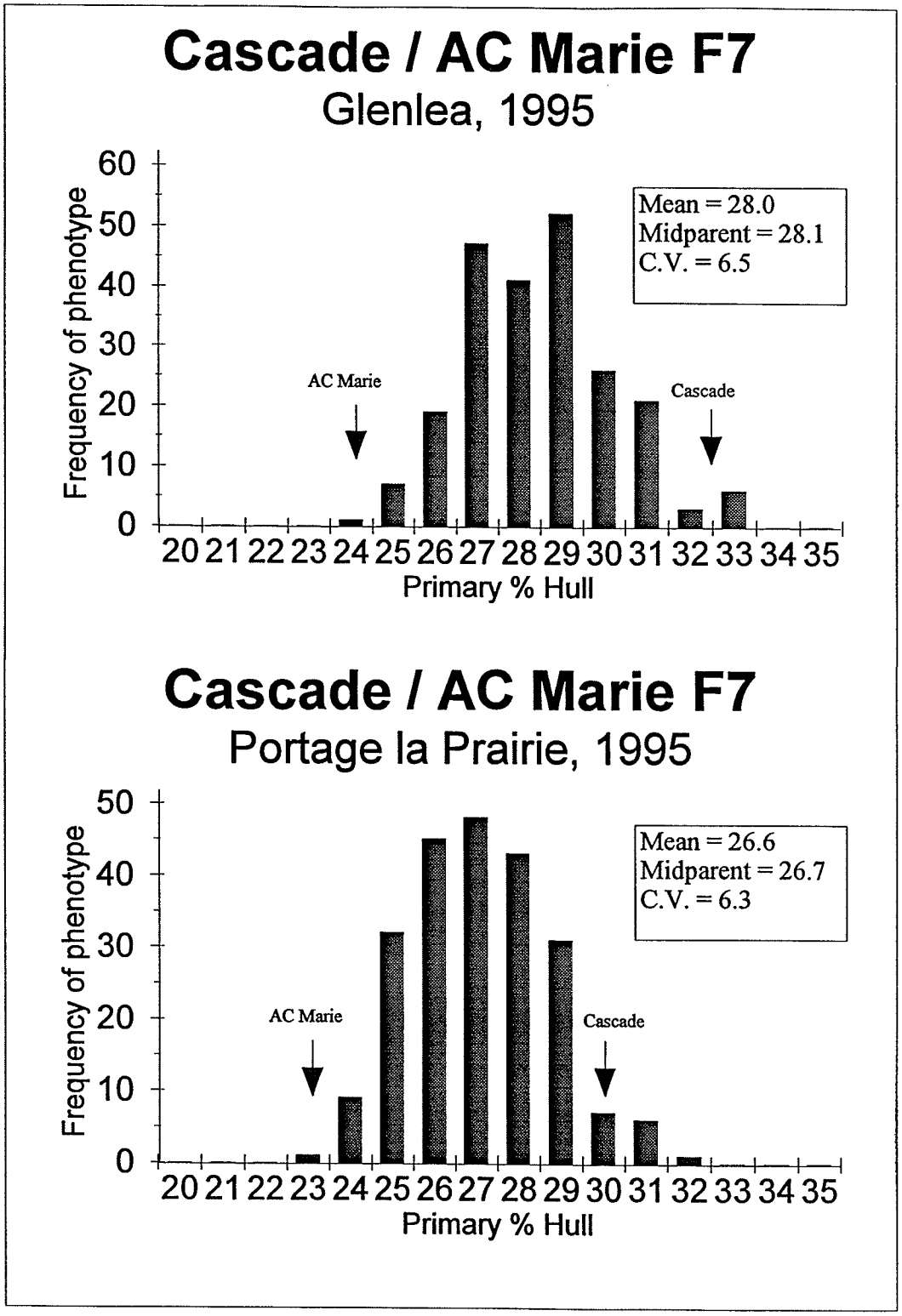

Figure 4.2. Frequency distributions of primary kernel percent hull for F_7 progeny populations of Cascade/AC Marie grown at Glenlea and Portage la Prairie, Manitoba in 1995. Arrows indicate hull percentages of parental cultivars. Hull percentages for progeny lines were means across three replicates.

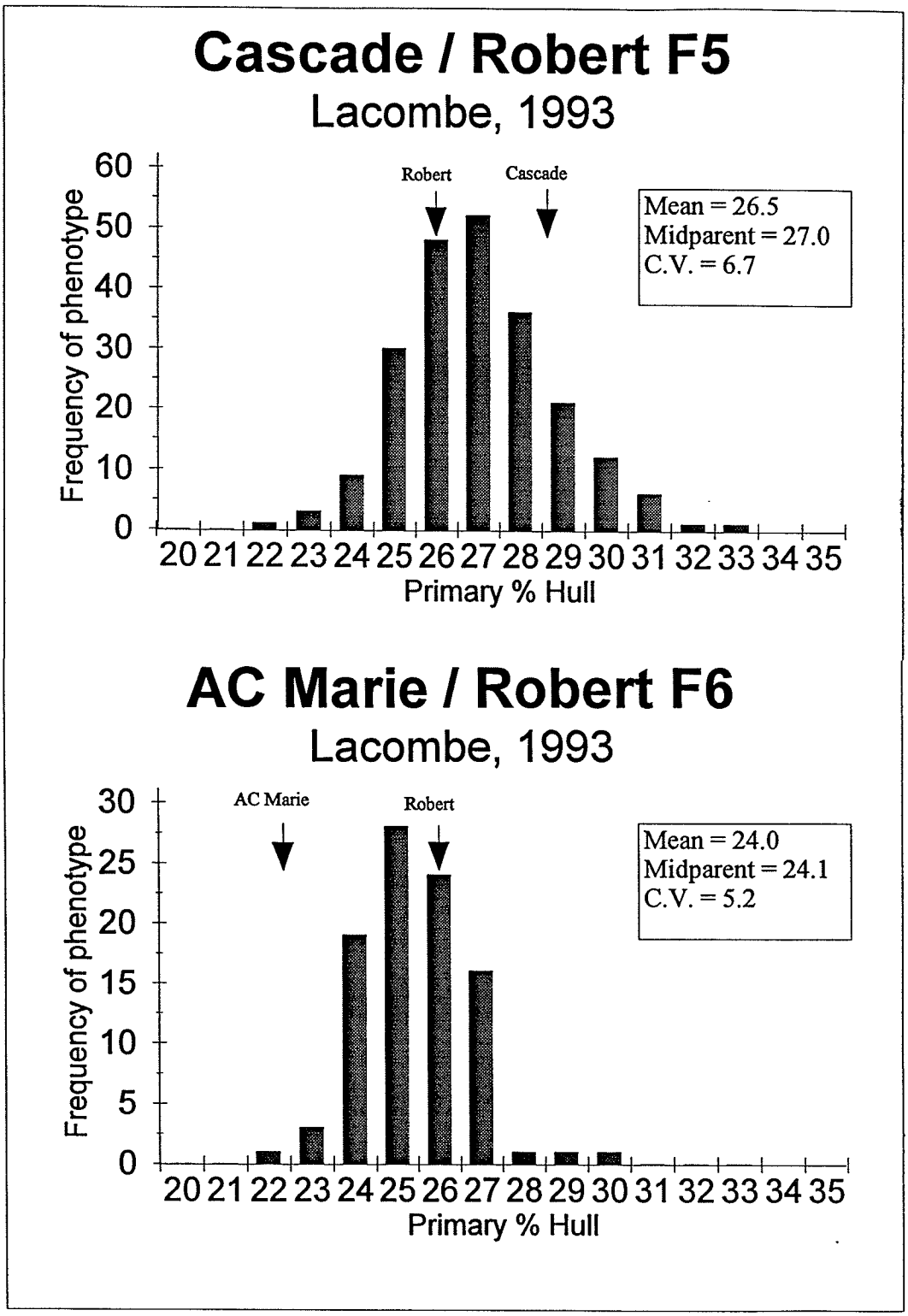

Figure 4.3. Frequency distributions of primary kernel percent hull for F_5 progeny populations of Cascade/Robert and F_6 progeny of AC Marie/Robert grown at Lacombe, Alberta in 1993. Arrows indicate hull percentages of parental cultivars.

Table 4.3. Distribution statistics for primary kernel percent hull measured for F_5-F_7 progeny populations of three oat (*Avena sativa* L.) crosses grown from 1993-1995 at several locations in western Canada.

Population [1]	Year and location [2]	Mean (%)	Median (%)	Modal class (%)
Cascade/AC Marie F_5	1993 Gln	26.3	26.3	26.5
Cascade/AC Marie F_6	1993 Gln	26.1	26.1	26.5
Cascade/AC Marie F_5	1993 Lac	26.6	26.4	27.0
Cascade/AC Marie F_6	1993 Lac	26.2	25.9	26.0
Cascade/AC Marie F_7	1993 Gln	28.0	27.9	29.0
Cascade/AC Marie F_7	1993 Ptg	26.6	26.5	27.0
Cascade/Robert F_5	1993 Lac	26.5	26.4	27.0
Cascade/Robert F_6	1993 Lac	26.5	26.2	26.0
AC Marie/Robert F_6	1993 Lac	24.9	24.8	25.0

[1] F_5-F_7 generations were derived from F_2 plants by bulking seed at each generation

[2] Gln = Glenlea, MB., Lac = Lacombe, AB., Ptg = Portage la Praire, MB.

In general, mean hull percentages for progeny populations were similar to midparent values, with the exception of populations of Cascade/AC Marie and AC Marie/Robert grown at Lacombe in 1993. These populations showed mean percent hull higher than the parental midpoint. Average coefficients of variability for percent hull, across F_5-F_6 generations, were highest for Cascade/Robert and Cascade/AC Marie, and lowest for AC Marie/Robert.

Transgressive segregation for low percent hull was observed in five of the nine data sets evaluated (Table 4.4). Cascade/Robert showed the highest level of transgressive segregation for low percent hull. With the exception of progeny lines grown at Glenlea in 1993, populations of Cascade/AC Marie showed no transgressive segregation for low percent hull.

Transgressive segregation for high percent hull was observed in eight of nine data sets evaluated (Table 4.4). AC Marie/Robert showed the highest level of transgressive segregation for high percent hull, but data for this cross was limited to a 94 F_6 progeny lines. The number of transgressive segregates for high percent hull was lowest in progeny populations of Cascade/AC Marie grown at Glenlea and Portage la Prairie in 1995.

In general, Cascade/AC Marie and Cascade/Robert F_5 progeny lines that exhibited transgressive segregation for primary percent hull, were transgressive segregates in the F_6 generation. For example,19 of 25 Cascade/Robert F_5 lines with F_6 progeny lines also grown in 1993 at Lacombe were designated as transgressive segregates for low percent hull in both generations.

Table 4.4. Summary of transgressive segregation for percent hull of 50 primary kernels for progeny populations of three oat crosses grown at locations in western Canada from 1993 -1995.

Year	Cross	Location	Generation	Extreme parental values		Population		Number of transgressive segregates [1]	
				Low (%)	High (%)	Minimum (%)	Maximum (%)	Low %Hull	High %Hull
1993	Cascade/AC Marie	Glenlea	F5	23.0	30.6	22.6	31.8	5 / 223	4 / 223
			F6	23.0	30.6	22.6	31.4	4 / 95	1 / 95
1993	Cascade/AC Marie	Lacombe	F5	22.2	28.6	22.6	32.4	0 / 223	29 / 223
			F6	22.2	28.6	23.1	31.4	0 / 95	12 / 95
1995	Cascade/AC Marie	Glenlea	F7	23.4	33.2	23.9	32.9	0 / 223	0 / 223
1995	Cascade/AC Marie	Portage	F7	22.6	30.9	22.6	31.2	0 / 223	1 / 223
1993	Cascade/Robert	Lacombe	F5	25.4	28.6	21.9	32.6	64 / 220	29 / 220
			F6	25.4	28.6	22.4	31.3	29 / 94	14 / 94
1993	AC Marie/Robert	Lacombe	F6	22.2	26.1	21.9	29.5	1 / 94	17 / 94

[1] Transgressive segregate defined as a progeny line with percent hull above or below the extreme parental value by more than one standard deviation.

Transgressive segregation for both extremes of hull weight, groat weight and kernel weight was observed in all data sets evaluated, from the three crosses (Tables 4.5, 4.6, 4.7). The highest levels of transgressive segregation for low hull weight per kernel were observed for progeny populations of Cascade/Robert. The highest levels of transgressive segregation for high groat weight and high kernel weight were observed in progeny of Cascade/AC Marie.

In general, progeny lines that exhibited transgressive segregation for hull weight, groat weight and kernel weight had been classified as transgressive segregates in the successive generation. For example, 40 of 53 Cascade/Robert F_5 lines with F_6 progeny lines grown in 1993 at Lacombe were designated as transgressive segregates for low hull weight in both generations.

4.4.2 Inter-year, Intra-location Comparisons

Analyses of variance pooled over the two 1993 sites showed that genotype and location effects were significant for each of the four kernel characteristics evaluated (Table 4.8).

Analyses of variance pooled over the two 1995 sites showed that genotype and location effects were the most significant sources of variation for each of the four traits (Table 4.9). The effect of genotype by location interaction on the four kernel characteristics was significant, but small in relation to the effects of genotype and location. Variance component analysis of 1995 data also showed that the effect of genotype by location was significantly smaller than the effects of genotype, location and error for each of the four traits (Table 4.9).

Table 4.5. Summary of transgressive segregation for hull weight per kernel measured on 50 primary kernels for progeny populations of three oat crosses grown at locations in western Canada from 1993 - 1995.

Year	Cross	Location	Generation	Extreme parental value		Population		Number of [1] transgressive segregates	
				Low (mg)	High (mg)	Minimum (mg)	Maximum (mg)	Low HW	High HW
1993	Cascade/AC Marie	Glenlea	F5	9.2	15.0	8.4	16.5	3 / 223	7 / 223
			F6	9.2	15.0	8.6	15.0	1 / 95	0 / 95
	Cascade/AC Marie	Lacombe	F5	10.7	14.3	9.3	17.2	12 / 223	27 / 223
			F6	10.7	14.3	9.6	16.5	4 / 95	17 / 95
1995	Cascade/AC Marie	Glenlea	F7	10.8	14.8	9.6	18.5	7 / 223	45 / 223
	Cascade/AC Marie	Portage	F7	9.9	13.4	8.6	15.8	7 / 223	36 / 223
1993	Cascade/Robert	Lacombe	F5	13.3	15.4	9.5	19.6	119 / 220	28 / 220
			F6	13.3	15.4	9.6	18.7	41 / 94	17 / 94
1993	AC Marie/Robert	Lacombe	F6	10.7	15.4	9.6	16.2	5 / 94	7 / 94

[1] Transgressive segregate defined as a progeny line with hull weight (HW) above or below the extreme parental value by more than one standard deviation.

Table 4.6. Summary of transgressive segregation for groat weight per kernel measured on 50 primary kernels for progeny populations of three oat crosses grown at locations in western Canada from 1993 - 1995.

Year	Cross	Location	Generation	Extreme parental value		Population		Number of transgressive segregates [1]	
				Low (mg)	High (mg)	Minimum (mg)	Maximum (mg)	Low GW	High GW
1993	Cascade/AC Marie	Glenlea	F5	29.6	36.1	23.8	41.1	18 / 223	42 / 223
			F6	29.6	36.1	26.1	41.0	11 / 95	17 / 95
	Cascade/AC Marie	Lacombe	F5	33.6	39.3	27.6	46.2	60 / 223	22 / 223
			F6	33.6	39.3	28.8	45.0	19 / 95	9 / 95
1995	Cascade/AC Marie	Glenlea	F7	28.5	36.6	27.4	41.9	3 / 223	45 / 223
	Cascade/AC Marie	Portage	F7	29.1	34.7	27.2	41.9	15 / 223	69 / 223
1993	Cascade/Robert	Lacombe	F5	33.6	44.2	27.2	49.6	52 / 220	9 / 220
			F6	33.6	44.2	28.3	47.7	13 / 94	7 / 94
1993	AC Marie/Robert	Lacombe	F6	36.5	44.2	29.6	50.3	23 / 94	10 / 94

[1] Transgressive segregate defined as a progeny line with groat weight (GW) above or below the extreme parental value by more than one standard deviation.

Table 4.7. Summary of transgressive segregation for kernel weight measured on 50 primary kernels for progeny populations of three oat crosses grown at locations in western Canada from 1993 - 1995.

Year	Cross	Location	Generation	Extreme parental value		Population		Number of [1] transgressive segregates	
				Low (mg)	High (mg)	Minimum (mg)	Maximum (mg)	Low KW	High KW
1993	Cascade/AC Marie	Glenlea	F5	40.7	48.3	32.1	56.8	17 / 223	37 / 223
			F6	40.7	48.3	35.2	54.9	8 / 95	11 / 95
	Cascade/AC Marie	Lacombe	F5	47.0	50.5	37.4	61.1	83 / 223	50 / 223
			F6	47.0	50.5	38.4	59.4	28 / 95	29 / 95
1995	Cascade/AC Marie	Glenlea	F7	42.5	47.9	38.0	58.2	18 / 223	102 / 223
	Cascade/AC Marie	Portage	F7	42.0	45.1	36.3	56.3	35 / 223	120 / 223
1993	Cascade/Robert	Lacombe	F5	47.0	59.6	37.4	69.2	68 / 220	8 / 220
			F6	47.0	59.6	38.7	64.8	17 / 94	11 / 94
1993	AC Marie/Robert	Lacombe	F6	47.1	59.6	39.2	65.3	16 / 94	12 / 94

[1] Transgressive segregate defined as a progeny line with kernel weight (KW) above or below the extreme parental value by more than one standard deviation.

Table 4.8. Summary of analyses of variance for percent hull, hull weight (mg), groat weight (mg) and kernel weight (mg) measured on samples of 50 primary kernels for F_5-F_6 progeny lines of Cascade/AC Marie grown at Glenlea, Manitoba and Lacombe, Alberta in 1993.

Population	Trait	Source of variation	Degrees of freedom	Mean square
Cascade/AC Marie F_5	Percent hull (%)	genotype	222	5.67**
		location	1	10.37**
		error	222	0.75
	Hull weight (mg)	genotype	222	3.61**
		location	1	69.34**
		error	222	0.48
	Groat weight (mg)	genotype	222	15.81**
		location	1	327.37**
		error	222	2.44
	Kernel weight (mg)	genotype	222	28.62**
		location	1	700.42**
		error	222	4.27
Cascade/AC Marie F_6	Percent hull (%)	genotype	94	5.89**
		location	1	0.55
		error	94	0.77
	Hull weight (mg)	genotype	94	3.71**
		location	1	51.16**
		error	94	0.38
	Groat weight (mg)	genotype	94	13.85**
		location	1	351.46**
		error	94	3.01
	Kernel weight (mg)	genotype	94	26.28**
		location	1	678.19**
		error	94	4.70

NS, *, **Nonsignificant or significant at $P < 0.05$, 0.01, respectively.

Table 4.9. Summary of analyses of variance and variance components for percent hull, hull weight (mg), groat weight (mg) and kernel weight (mg) measured on samples of 50 primary kernels for F_7 progeny lines of Cascade/AC Marie grown at Portage la Prairie and Glenlea, Manitoba in 1995.

Trait	Source of variation	Degrees of freedom	Mean square	Variance component
Percent hull (%)	location	1	646.1**	0.96
	reps within location	4	5.7**	0.02
	genotype	222	17.5**	2.78
	genotype by location	222	0.8**	0.09
	error	888	0.6	0.53
Hull weight (mg)	location	1	509.8**	0.76
	reps within location	4	1.3**	0.01
	genotype	222	12.4**	1.98
	genotype by location	222	0.5**	0.07
	error	888	0.3	0.26
Groat weight (mg)	location	1	285.7**	0.41
	reps within location	4	12.6**	0.05
	genotype	222	43.8**	6.85
	genotype by location	222	2.7*	0.19
	error	888	2.1	2.07
Kernel weight (mg)	location	1	1561.6**	2.31
	reps within location	4	16.2**	0.06
	genotype	222	86.2**	13.65
	genotype by location	222	4.3*	0.36
	error	888	0.6	3.21

^{NS}, *, **Nonsignificant or significant at $P < 0.01$, 0.001, respectively.

4.4.3 Broad-sense Heritability

Broad-sense heritability estimates of primary percent hull for two crosses varied from 0.80 to 0.90 by regression, and from 0.79 to 0.83 by correlation (Table 4.10). At Lacombe, the heritability of primary percent hull ranged from 0.83 for Cascade/Robert to 0.90 for Cascade/AC Marie. For AC Marie/Robert, primary percent hull was not measured for successive generations. However, broad-sense heritability of two gram percent hull for F_5 and F_6 populations of AC Marie/Robert was 0.49, much lower than that of the other two crosses.

Broad-sense heritability of hull weight per kernel varied from 0.78 to 0.92 by regression, and from 0.79 to 0.89 by correlation (Table 4.10). At Lacombe, the heritability of hull weight ranged from 0.92 for Cascade/Robert populations to 0.82 for Cascade/AC Marie progeny.

Groat weight per kernel was the least heritable characteristic evaluated for Cascade/AC Marie and Cascade/Robert populations. Broad-sense heritability varied from 0.58 to 0.83 by regression, and from 0.64 to 0.84 by correlation (Table 4.10). At Lacombe, the heritability of groat weight per kernel ranged from 0.83 for progeny populations of Cascade/Robert to 0.58 for Cascade/AC Marie.

Broad-sense heritability of kernel weight among populations of two crosses varied from 0.62 to 0.87 by regression, and from 0.68 to 0.86 by correlation (Table 4.10). At Lacombe, the heritability of kernel weight ranged from 0.87 for Cascade/Robert populations to 0.62 for comparable Cascade/AC Marie progeny.

Table 4.10. Estimates of broad-sense heritability by correlation (Corr.) (Frey and Horner, 1957), and the regression method (Regr.) (Mahmud and Kramer, 1951), for percent hull, hull weight (mg), groat weight (mg) and kernel weight (mg) measured for F_5 and F_6 progeny populations of oat crosses grown at both Glenlea, Manitoba and Lacombe, Alberta in 1993.

Characteristic	Cross [1]	Year and location [2]	Number of entries [3]	Heritability Corr.	Regr.
Two gram percent hull (%)	CCD/ACM	1993 Gln	95 F_5/F_6	0.65	0.72
		1993 Lac	95 F_5/F_6	0.57	0.60
	CCD/RBT	1993 Gln	94 F_5/F_6	0.60	0.58
	ACM/RBT	1993 Gln	94 F_5/F_6	0.44	0.54
Primary kernel percent hull (%)	CCD/ACM	1993 Gln	95 F_5/F_6	0.88	0.83
		1993 Lac	95 F_5/F_6	0.79	0.90
	CCD/RBT	1993 Lac	94 F_5/F_6	0.83	0.80
Primary kernel hull weight (mg)	CCD/ACM	1993 Gln	95 F_5/F_6	0.83	0.78
		1993 Lac	95 F_5/F_6	0.79	0.82
	CCD/RBT	1993 Lac	94 F_5/F_6	0.89	0.92
Primary kernel groat weight (mg)	CCD/ACM	1993 Gln	95 F_5/F_6	0.77	0.82
		1993 Lac	95 F_5/F_6	0.64	0.58
	CCD/RBT	1993 Lac	94 F_5/F_6	0.84	0.83
Primary kernel weight (mg)	CCD/ACM	1993 Gln	95 F_5/F_6	0.78	0.80
		1993 Lac	95 F_5/F_6	0.68	0.62
	CCD/RBT	1993 Lac	94 F_5/F_6	0.86	0.87

[1] Cultivar abbreviations: CCD = Cascade, ACM = AC Marie, RBT = Robert

[2] Gln = Glenlea, MB., Lac = Lacombe, AB.

[3] F_5, F_6 generations were derived from F_2 plants by bulking seed at each generation

Broad-sense heritability, estimated by partitioned variance components from replicated studies of the F_7 population of Cascade/AC Marie, was high for all four traits. Broad-sense heritability of percent hull was 0.96 (Table 4.11).

4.4.4 Correlation between Kernel Characteristics

A significant association was observed between hull weight per kernel and groat weight per kernel in all populations. Correlation coefficients ranged from 0.65 - 0.81 across populations of the three crosses (Table 4.12 - 4.14).

Both groat weight per kernel and hull weight per kernel were significantly associated with kernel weight in all populations evaluated. Correlation coefficients ranged from 0.96 - 0.98 for groat weight and 0.84 - 0.89 for hull weight across populations of the three crosses (Table 4.12 - 4.14).

Hull weight per kernel and percent hull showed significant positive correlations in all populations. Correlation coefficients ranged from 0.41 - 0.71 across populations of the three crosses (Table 4.12 - 4.14).

Groat weight per kernel and percent hull showed significant negative correlations in one population and non-significant correlation in the remaining populations. Kernel weight and percent hull showed significant positive correlations in two of three populations (Table 4.12 - 4.14).

4.4.5 Stepwise Regression Analysis

Stepwise regression equations, developed for six progeny populations representing three crosses, revealed that hull weight, groat weight and kernel weight each significantly influence primary kernel percent hull (Table 4.15).

Table 4.11. Estimates of broad-sense heritability by variance components for percent hull, hull weight (mg), groat weight (mg) and kernel weight (mg) measured on samples of 50 primary kernels for F_7 progeny populations of Cascade/AC Marie grown at Glenlea and Portage la Prairie, Manitoba in 1995.

Characteristic	Location[1]	Variance components			Heritability
		error	genotype	phenotype	
Primary kernel percent hull (%)	Ptg/Gln	0.53	2.78	2.91	0.96
	Ptg	0.42	2.66	2.80	0.95
	Gln	0.69	3.06	3.29	0.93
Primary kernel hull weight (mg)	Ptg/Gln	0.26	1.98	2.05	0.97
	Ptg	0.22	1.81	1.88	0.96
	Gln	0.30	2.28	2.38	0.96
Primary kernel groat weight (mg)	Ptg/Gln	2.07	6.85	7.29	0.94
	Ptg	1.68	6.77	7.33	0.92
	Gln	2.56	7.29	8.14	0.90
Primary kernel weight (mg)	Ptg/Gln	3.21	13.64	14.40	0.95
	Ptg	2.68	13.21	14.10	0.94
	Gln	3.85	14.76	16.10	0.92

[1] Gln = Glenlea, MB., Ptg = Portage la Praire, MB.

Table 4.12. Mean phenotypic correlation coefficients between primary kernel percent hull, hull weight (mg), groat weight (mg) and kernel weight (mg) measured for 223 F_7 Cascade/AC Marie progeny lines grown at both Glenlea and Portage la Prairie, Manitoba in 1995.

	Kernel [1] weight (mg)	Groat per [2] kernel(mg)	Hull per [3] kernel (mg)
Groat per kernel	0.96**		
Hull per kernel	0.84**	0.65**	
Primary percent hull [4]	0.20**	-0.10 [NS]	0.71**

[NS], *, **Nonsignificant or significant at P < 0.05, 0.01, respectively.

Table 4.13. Phenotypic correlation coefficients between primary kernel percent hull, hull weight (mg), groat weight (mg) and kernel weight (mg) measured for 222 F_5 Cascade/Robert progeny lines grown at Lacombe, Alberta in 1993.

	Kernel [1] weight (mg)	Groat per [2] kernel(mg)	Hull per [3] kernel (mg)
Groat per kernel	0.97**		
Hull per kernel	0.87**	0.74**	
Primary percent hull [4]	0.18**	-0.05 [NS]	0.64**

[NS], *, **Nonsignificant or significant at P < 0.05, 0.01, respectively.

Table 4.14. Phenotypic correlation coefficients between primary kernel percent hull, hull weight (mg), groat weight (mg) and kernel weight (mg) measured for 94 F_6 AC Marie/Robert progeny lines grown at Lacombe, Alberta in 1993.

	Kernel [1] weight (mg)	Groat per [2] kernel(mg)	Hull per [3] kernel (mg)
Groat per kernel	0.98**		
Hull per kernel	0.89**	0.81**	
Primary percent hull [4]	-0.04 [NS]	-0.20*	0.41**

[NS], *, **Nonsignificant or significant at P < 0.05, 0.01, respectively.

1 average kernel weight of 50 primary kernels
2 average groat weight for 50 primary kernel
3 average hull weight for 50 primary kernels
4 percent hull of 50 primary kernels

Table 4.15. Step wise regression equations describing the effects of hull weight (mg), groat weight (mg) and kernel weight (mg) on primary kernel percent hull for progeny populations of three oat crosses grown in western Canada in 1993 and 1995.

Year and location[1]	Cross [2] and generation [3]	Stepwise regression equation for primary kernel percent hull	R^2
1993 Gln	CCD/ACM F_5	26.12 + 85(KW) - 648(GW) + 1505(HW)	99.2
1993 Gln	CCD/ACM F_6	26.24 + 315(KW) - 893(GW) + 1306(HW)	99.4
1993 Lac	CCD/ACM F_5	26.19 - 113(KW) - 431(GW) + 1644(HW)	99.3
1993 Lac	CCD/ACM F_6	26.36 - 426(KW) - 114(GW) + 1934(HW)	99.4
1995 Gln	CCD/ACM F_7	27.92 + 534(KW) - 1112(GW) + 955(HW)	99.4
1995 Ptg	CCD/ACM F_7	26.69 + 406(KW) - 992(GW) + 1200(HW)	99.3
1993 Lac	CCD/RBT F_5	26.48 + 637(KW) - 1158(GW) + 809(HW)	98.8
1993 Lac	CCD/RBT F_6	26.54 + 251(KW) - 754(GW) + 1140(HW)	98.9
1993 Lac	ACM/RBT F_6	25.01 + 389(KW) - 861(GW) + 1025(HW)	98.9

1 Gln = Glenlea, MB., Lac = Lacombe, AB., Ptg = Portage la Praire, MB.

2 Cultivar abbreviations: CCD = Cascade, ACM = AC Marie, RBT = Robert

3 F_5 - F_7 generations were derived from F_2 plants by bulking seed at each generation

For each of the nine data sets, best subsets regression showed that an equation involving all three independent variables produced in the smallest error mean square (data not shown). Hull weight and kernel weight both had positive effects on percent hull, with the exception of F_5 and F_6 populations of Cascade/AC Marie grown at Lacombe in 1993, which showed negative coefficients for kernel weight, but had the largest positive coefficients for hull weight of all the data sets. Groat weight had a negative influence on percent hull for each of the nine data sets evaluated. Non-linear functions were not explored since scatter diagrams suggested only linear relationships.

4.5 DISCUSSION

The objective of the present study was to investigate the heritability of percent hull in F_5-F_7 generations of three oat crosses. Although the inheritance of three component traits, hull weight, groat weight and kernel weight was also monitored, most of the efforts in data collection focused on percent hull, a valuable indicator of feed and milling quality.

Growing conditions may affect absolute values of percent hull. For example, 1995 growing conditions varied widely across the three Manitoba locations where field studies were conducted. The Glenlea and Winnipeg sites were in close proximity and experienced drought conditions that limited germination resulting in lower grain yields and higher hull percentage. Growing conditions at the Portage la Prairie location in 1995 were more favourable, resulting in vigorous, high yielding plants with kernels of lower hull percentage.

In general, frequency distributions for primary kernel percent hull among F_5 - F_7 progeny populations of three crosses were approximately normal. Normality was suggested by equivalent values for mean, median and mode, and low values for skewness and kurtosis. Normal frequency distributions typically support the quantitative mode of inheritance for hull percentage, first suggested by Wesenberg (1968), with control by multiple additive genetic factors. However, with bulked seed used to advance generations, normal frequency distributions would be expected in later generations regardless of gene effects.

In general, progeny populations of Cascade/AC Marie only showed transgressive segregation for high hull percentage. However, when disease was chemically controlled (1995), no transgressive segregation for percent hull was observed at either extreme. It is possible that some transgressive segregation for high percent hull, observed among progeny of this cross, was confined to progeny lines with susceptibility to disease. Oat plants show decreased groat weight and increased percent hull when damaged by disease.

Disproportionate numbers of high and low transgressive segregates in progeny populations would tend to refute the possibility of additive effects among the genetic factors controlling percent hull. Progeny populations of Cascade/Robert had much higher proportions of transgressive segregates for low percent hull. The progeny population of AC Marie/Robert showed a higher proportion of transgressive segregates for high percent hull. In progeny of these crosses, Robert appears to exert a dominant effect on percent hull.

Analyses of variance, pooled across two locations in both 1993 and 1995, showed that location and genotype had significant effects ($\propto = 0.01$) on four primary kernel characteristics of Cascade/AC Marie progeny populations. In 1995, variance components for genotypic effects were much larger than for location effects, indicating that genetic differences accounted for most of the phenotypic variability among Cascade/AC Marie progeny.

Genotype by location interactions, evaluated for Cascade/AC Marie populations at two locations in 1995, were significant ($\propto = 0.01$) for all traits, but probably have limited biological importance. Although the four primary kernel characteristics varied across locations, the rankings of genotypes across locations showed only minor differences. A lack of biological significance for genotype by location interaction was also supported by its variance component, which was much smaller than those for location and genotype. Small variance components for genotype by location interaction, suggested that the genotypes responded similarly to the different environmental conditions faced at the two locations. Therefore, successful evaluation of hull percentage should be possible with samples from only a few locations. However, large differences in percent hull between locations, necessitate that comparisons should only be made among samples grown under the same conditions. The effect of genotype by location interaction on the four primary kernel characteristics was assessed for a segregating population grown at two locations in one year. The results of such a limited study may not be indicative of the trends that would be

characteristic of larger-scale experimentation. Studies with more extensive replication have shown significant effects of genotype by location interaction on percent hull (Bartley and Weiss, 1951; Gullord and Aastveit, 1987). However, these studies evaluated limited numbers of diverse genotypes, which may have contributed to the significant interaction effects.

Broad-sense heritability estimates represent the total variability due to genetic causes, including additive, dominance, and epistatic variances. The mean broad-sense heritability of primary kernel percent hull ranged from 0.80 to 0.90 for F_5 and F_6 progeny populations of Cascade/Robert and Cascade/AC Marie grown at Lacombe in 1993. Broad-sense heritability of two gram percent hull, evaluated across F_5 and F_6 progeny populations of all three crosses, was observed to be higher for Cascade/AC Marie and Cascade/Robert, than for AC Marie/Robert. This was expected since the latter cross displayed smaller parental differences in percent hull than that of the two wider crosses.

Broad-sense heritability estimates for primary kernel percent hull were much higher than estimates derived from hull percentages of two gram kernel mixtures from the same population. This would be expected due to the lower variability associated with samples consisting wholly of primary kernels. In general, broad-sense heritability estimates by regression were similar to those observed by correlation of data from successive generations of progeny lines.

The highest broad-sense heritability for percent hull was estimated using variance components for F_2-derived F_7 lines of Cascade/AC Marie. Across

replicated experiments at two locations, 96% of the phenotypic variance in hull percentage was due to genotypic differences. This high heritability estimate reflected the broad genetic base of this cross and the extensive replication in 1995, which decreased the phenotypic variance. High heritability estimates support preliminary selection for low hull percentage in early generations. Forsberg and Reeves (1992) suggested that such a practice can be effective in certain crosses.

Selection for component traits can result in increased heritability of the major character when the component traits are genetically determined at different stages of plant development (Murphy and Frey, 1962). Substantial transgressive segregation for low hull weight, as well as high groat and kernel weight, supported multiple trait selection for these components, in addition to percent hull. However, it was also necessary to search for significant, unwanted associations between traits that could prevent their concurrent improvement.

Correlation analysis revealed few associations that would limit simultaneous selection of percent hull and the other kernel characteristics evaluated. However, a positive correlation was observed between kernel weight and percent hull in populations of two crosses, suggesting that simultaneous selection for high kernel weight and low percent hull may not be possible. In agreement with a previous study by Stuthman and Granger (1977), groat weight and percent hull showed little association. Generally, there appears to be little genetic restriction to the simultaneous selection of high groat weight and

reduced percent hull in oat.

Stepwise regression analysis revealed that hull weight, groat weight and kernel weight all significantly affect primary kernel percent hull in oat. Based on coefficients for the three independent variables, an oat breeder will be able to reduce percent hull most efficiently by minimizing hull weight and maximizing groat weight. This observation applied to progeny populations of three different oat crosses and may be universal for this species.

In this study of progeny populations of three oat crosses, disproportionate numbers of high and low transgressive segregates in certain populations suggest that non-additive effects exist among the genetic factors controlling percent hull. In general, percent hull was highly heritable with the highest heritability observed for crosses between parents with large differences in percent hull. Replicated evaluations of a progeny population of Cascade/AC Marie showed limited genotype by location interaction. These observations imply that effective selection for improved milling quality, in the form of lower hull percentage, should be possible in early generations.

5.0 IDENTIFICATION OF RAPD MARKERS FOR PERCENT HULL IN OAT

5.1 ABSTRACT

Percent hull is an important physical parameter of oat grain quality. Hull percentage in oat is affected by environment however, and multiple, time-consuming evaluations of a genotype may be required to assess its true value. This provides a suitable context for the application of marker-assisted selection for the genes involved. Bulked segregant analysis with selected progeny lines from the cross Cascade/AC Marie, was used to identify randomly amplified polymorphic DNA (RAPD) markers linked to quantitative trait loci (QTLs) controlling hull percentage in oat. Twelve polymorphisms, identified between bulks, were tested for linkage to QTLs controlling hull percentage by genotyping 80 randomly selected F_2-derived F_8 lines from the progeny population. These markers were also tested for linkage to QTLs for three other primary kernel characteristics. Three markers showed significant simple interval mapping test statistics for quantitative trait locus effects, when tested with percent hull data from two environments. When combined, the unlinked marker loci $OPC13_{800}$, $OPD20_{600}$ and $OPK7_{1300}$, explained approximately 41% of the genetic variance in percent hull, after accounting for the main effect of environment. Moreover, the unlinked marker loci, $OPC13_{600}$ and $OPK7_{1300}$, together accounted for approximately 36% of the genetic variance in hull weight per kernel across two locations. Multiple genetic factors, with additive effects, influence both percent hull and hull weight per kernel among progeny of Cascade/AC Marie.

5.2 INTRODUCTION

Genetic markers allow the selection of superior plant genotypes from a segregating population (Paterson et al., 1991a). DNA-based markers are best suited for marker-assisted selection because of the large number that exist in most plant populations. DNA markers have been identified that cosegregate with simply inherited, qualitative traits (Hinze et al., 1991; Martin et al., 1991; Michelmore et al., 1991; Yu et al., 1991), as well as complex, quantitative traits (Stuber et al., 1987; Keim et al., 1990; Beavis et al., 1991).

The complex inheritance of quantitative traits often makes their manipulation in breeding programs difficult. Quantitative traits are assumed to be under the control of multiple genetic loci, which produce a continuous distribution of phenotypes when exposed to environmental influences (Nilsson-Ehle, 1909; Johannsen, 1909). Each genetic factor underlying a quantitative trait is designated as a quantitative trait locus (QTL) (Geldermann, 1975). The development of linkage maps of contiguous genetic markers has allowed the characterization of QTLs in many plant species. By determining the map position of an individual QTL, it is possible to quantify its effect on the trait and, more importantly, use linked markers to select for its presence or absence in segregating populations.

Percent hull refers to the ratio of hull weight to total kernel weight expressed as a percentage (Plourde, 1984). It has been long suggested that percent hull is an influential factor affecting oat milling quality (Atkins, 1943;

Root, 1979; Plourde, 1984; Yarrow et al., 1992). The oat hull is primarily composed of fibre, reducing the total energy content of the grain and limiting its market competitiveness with other cereals (Plourde, 1984). Oat grain energy may be increased by reducing hull content, through decreased percent hull or hull weight per kernel. Lower hull contents would not only result in valuable gains in the feed value of oats, but also improve milling efficiency by reducing the proportion of the kernel lost during the dehulling process.

Percent hull, the predominant measure of hull content in oat, is thought to be a quantitatively inherited trait influenced by both genetic and environmental factors (Wesenberg and Shands, 1971, 1973). The significant effect of environment on phenotype supports the application of marker-assisted selection for hull/groat percentage. Although DNA markers associated with groat percentage have been identified in related studies by Siripoonwiwat et al., (1996) and Kianian et al., (1996), these markers are not publicly available.

The objective of this study was to identify RAPD markers linked to QTLs governing percent hull in oat (*Avena sativa* L.). Selection for favourable alleles at QTLs associated with percent hull and hull weight per kernel should identify individuals with low hull content in progeny populations. An indirect selection method for low hull content may represent an effective means to improve feed energy and milling quality in oat.

5.3 MATERIALS AND METHODS

5.3.1 Measurement of Percent Hull

The randomly selected population used to characterize DNA markers consisted of 80 of a possible 223 F_2-derived F_7 lines from the cross Cascade/AC Marie. Seed bulks for each line, previously advanced to the F_6 generation, were grown in randomized, replicated trials at Glenlea, Portage la Prairie and Winnipeg, Manitoba in 1995. Each randomized replicate included the 223 progeny lines and 17 check plots of the three parental cultivars (eight plots of Cascade and AC Marie, and one plot of Robert), with three replications grown at each location. Individual F_7 lines were planted as 1.5 m rows, separated by rows of fall rye. Disease pressure was reduced by three treatments of propiconazole (250 g/L, applied at 0.2 L/acre), a systemic, broad-spectrum fungicide.

An accurate assessment of the hull percentage of harvested oat samples was obtained by mechanically dehulling 50 primary kernels. Percent hull was determined by comparing hull weight to total kernel weight for 223 Cascade/AC Marie F_7 lines grown at Glenlea and Portage la Prairie in 1995. Average hull weight per kernel, groat weight per kernel and kernel weight were also measured for each progeny line. Progeny lines for bulked segregant analysis were selected based on hull percentages measured by: (1) mechanically dehulling a two gram random mixture of primary and secondary kernels, and (2) nondestructive, near infra red reflectance spectroscopy (NIRS) analysis of a 15 - 20 g mixture of primary and secondary kernels (Ronald et al., 1996).

5.3.2 Identification of RAPD markers

Based on two gram- and NIRS-determined percent hull for the F_4-F_6 populations of Cascade/AC Marie, 14 and 12 progeny lines, with consistently high and low percent hull respectively, were selected for bulked segregant analysis. Leaves were harvested from five plants for each of the 26 progeny lines and stored at -80° C until DNA extraction.

DNA was extracted using the CTAB extraction protocol (Kleinhofs et al., 1993), for each progeny line. All DNA samples were treated with ribonuclease (1.5 μl of 10 μg/ml), followed by a one hour incubation period at 37° C. DNA concentration was measured at 260 nm. Finally, DNA samples were visually inspected for concentration and integrity by loading 5 μl samples onto a 1.0% agarose gel in 1x TAE buffer (tris/sodium acetate/EDTA pH 8.0) and exposing the gel to an applied voltage of 210 volt hours.

Equal aliquots of DNA, taken from the seven progeny lines best representing the extremes of percent hull, were combined to form DNA pools for high and low percent hull. These DNA pools were amplified with more than 300 arbitrary primers. PCR reaction mixtures (25 μl) contained about 30 ng of genomic DNA, 0.2 mM of each dNTP (Pharmacia), 1.5 mM $MgCl_2$ (Perkin-Elmer), 20 μM of primer, 1x Taq polymerase buffer (50mM Tris/HCl pH 9.0, 20 mM NaCl, and 1% (v/v) Triton X-100) (Perkin-Elmer), and 1 unit of Taq polymerase. Arbitrary sequence 10-mer primers were purchased from Operon Technology (Alameda, USA) or made in the laboratory using an Applied

Biosystems PCR-mate oligonucleotide synthesiser. DNA amplification was performed using Thermolyne Temptronic thermocyclers with the following temperature conditions: 1 cycle of 94° C for 2 minutes, 36° C for 30 seconds, 72° C for 1 minute; then 34 cycles of 94° C for 5 seconds, 36° C for 30 seconds, 72° C for 1 minute followed by 72° C for 10 minutes. PCR reactions on Thermolyne thermocyclers were overlaid with 20 μl of mineral oil to prevent evaporation.

Amplification products (12 μl) plus 6x loading buffer (4 μl) were then separated electrophoretically in 1.6% agarose gels (1x TAE buffer) using 210 volt hours. Samples showing an abundance of fragments were also separated with 1200 volt hours on 8% acrylamide gels using the temperature sweep gel electrophoresis (TSGE) technique (Penner et al., 1994). TSGE provided improved resolution of polymorphic fragments with separation based on both size and sequence of individual bands. *Hind* III/*Eco* RI Lambda and pGEM DNA markers were used to size amplified fragments. Following ethidium bromide staining [2.5 μl (10mg/ml) /100 ml] fragments were detected under ultra-violet light.

Polymorphic products, identified between the DNA pools, were tested for linkage to genes controlling percent hull by applying the corresponding primers to members of the F_2-derived F_8 population of Cascade/AC Marie. Leaves were harvested and pooled for six plants from each of 80 randomly selected F_8 lines. DNA was extracted and quantified as described above. These DNA samples were scored for genotypes at 12 marker loci, which were polymorphic across

DNA bulks and showed some linkage with percent hull among the 26 F_7 lines with extreme phenotypes. DNA amplification was carried out in microtitre plates (Thermowell-Costar) using the same temperature cycle as above on an MJ PTC-200 thermocycler (DNA Engine). The twelve marker loci, amplified by seven primers, were scored for each of the 80 progeny lines as either parental allele. However, these F_2-derived F_8 lines, consisting of homozygous individuals, may be heterogeneous at any given locus. Therefore, DNA from four single plants within each progeny line, extracted using a modification of the leaf disc DNA extraction protocol of Edwards et al., (1991), was also assessed for marker genotype. Marker data obtained from single plants and the six plant DNA pool, were used to infer the marker genotype of each F_2 plant from which an F_8 line was derived.

5.3.3 Assessing Linkage Between Markers and Percent Hull

Inferred F_2 genotypes at twelve marker loci were tested for linkage using Mapmaker/EXP (Lander et al., 1987). Specified mapping criteria included a logarithm of the odds' ratio of 4.0 and a minimum linkage distance of 35 cM. Kosambi's mapping function was used to convert recombination frequencies to map distances in cM.

Potential QTLs were then identified by two methods: (1) comparison of marker genotype means, and (2) the use of the statistical software package, MQTL (Tinker and Mather, 1995b). Comparison of marker genotype means involved testing for significant differences between mean phenotypes of progeny

lines homogeneous at a marker locus. A two-tailed *t* test was carried out for each pair of marker genotype means using a pooled estimate of variance. A significant difference between genotype means ($P < 0.05$) was interpreted as possible linkage of the marker locus to a QTL influencing the particular trait. Chi-square analysis was also conducted by testing the frequency of the three marker genotypes against the expected 1:2:1 segregation ratio.

For MQTL analysis, percent hull, hull weight, groat weight and kernel weight data averaged over three replicates at each of two locations, were compared with inferred F_2 genotypes at 12 marker loci. Two forms of interval mapping were used to test for the presence of QTL main effects and QTL by environment interactions associated with marker loci. Simple interval mapping (SIM) searched for QTLs linked to individual markers using phenotypic data from both single and multiple locations. The epistasis function of MQTL assessed interactions among the loci defined by the twelve markers. Threshold values for QTL main effects, QTL by environment interactions, and epistatic interactions were calculated by one thousand permutations of the phenotypic data.

Simple composite interval mapping (sCIM) was also used to search the genome for regions contributing to quantitative trait phenotype. Without a complete linkage map from which background markers could be selected, markers explaining the largest portion of the genetic variance in percent hull ($OPC13_{800}$, $OPD20_{600}$ and $OPK7_{1300}$) were reassigned as background markers. Simple composite interval mapping then identified other markers for percent hull,

whose smaller effects were previously masked by the significant markers.

The inference function of MQTL was used to find peak test statistics in SIM and sCIM output, which identified positions where QTL estimates were to be made. Primary QTL inferences were made at markers with large sCIM test statistics corresponding to significant SIM peaks. Secondary inferences were made at marker loci where the sCIM test statistic was much smaller than the SIM test statistic. A multi-locus model was then fitted to the data and estimates of QTL main effects and QTL by environment effects determined at all positions.

Estimates of R^2 {(variance explained)/(total phenotypic variance)} for a single marker locus were made using SIM test statistics, with the formula:

$$\mathbf{R^2 = 1 - 1/exp(TS/n)}$$

where; TS = the SIM test statistic for a single marker, and n = the product of the number of environments and the number of progeny (Tinker and Mather, 1995b). Estimates of genetic variance explained by inferred QTLs were calculated by dividing the proportion of total phenotypic variance explained by the markers, with the broad-sense heritability for each trait.

5.4 RESULTS

5.4.1 Marker Linkage Groups

Of the 300 arbitrary sequence primers screened across the DNA pools, nine produced detectable polymorphisms. These nine primers amplified 17 polymorphic loci, six of which clustered in three separate linkage groups: (1) $OPD20_{600}$ and $OPN3_{900}$ (30.2 cM), (2) $OPE9_{900}$ and $OPM18_{500}$ (7.4 cM) and (3)

$OPC13_{800}$ and $OPO11_{900}$ (unknown). Of the 17 marker loci identified, eight dominant amplification products were produced by Cascade, eight by AC Marie, and one marker locus ($OPE9_{900}$) showed discrete fragments for both parents.

5.4.2 Segregation of Marker Genotypes

Of the twelve marker loci tested for linkage with the measured kernel characteristics of 80 randomly selected F_8 lines, only four fit a 1:2:1 ratio of marker genotypes at $\propto = 0.05$ (Table 5.1). In general, the remaining eight marker loci showed large differences from the expected ratio. The three markers with significant QTL main effects for percent hull, also showed genotypic segregation that fit the expected ratio. However, three of the six markers with significant differences between mean percent hull of lines homogeneous at the marker locus, showed genotypic segregation highly skewed from the expected ratio.

5.4.3 Analysis of Marker Genotype Means for Percent Hull

Of the twelve marker loci analysed, six showed significant differences in percent hull ($\propto = 0.05$), averaged across two locations (Table 5.2). The $OPD20_{600}$ and $OPK7_{1300}$ marker loci showed the most significant differences between genotypic classes ($\propto = 0.001$). The same six marker loci also showed significantly different genotype means for percent hull from single locations.

An examination of mean hull percentage for marker genotypes, revealed that the Cascade allele was associated with high percent hull for four of the six significant marker loci. However, for both $OPC13_{800}$ and $OPG12_{100}$, the AC Marie allele was associated with high percent hull.

Table 5.1. Segregation of inferred F_2 genotypes at 12 marker loci and associated Chi-square values for goodness of fit with 1:2:1 genotypic ratio. Ratio tested across some or all of 80 F_8 progeny lines of Cascade/AC Marie.

Marker	Chi-square variables	Marker genotype CCD	heterozygous	ACM	Total	P value
$OPC13_{800}$	observed	14	36	19	69	
	expected	17.25	34.5	17.25	69	
	Chi-square	0.6	0.1	0.2	0.9	0.5<p<0.9
$OPC13_{600}$	observed	32	18	30	80	
	expected	20	40	20	80	
	Chi-square	7.2	12.1	5.0	24.3	p < 0.005
$OPD20_{600}$	observed	21	35	24	80	
	expected	20	40	20	80	
	Chi-square	0.1	0.6	0.8	1.5	0.1<p<0.5
$OPE9_{900}$	observed	17	27	36	80	
	expected	20	40	20	80	
	Chi-square	0.5	4.2	12.8	17.5	p < 0.005
$OPE9_{600}$	observed	25	26	29	80	
	expected	20	40	20	80	
	Chi-square	1.3	4.9	4.1	10.2	p < 0.01
$OPG12_{500}$	observed	33	30	17	80	
	expected	20	40	20	80	
	Chi-square	8.5	2.5	0.5	11.4	p < 0.005
$OPG12_{100}$	observed	31	22	27	80	
	expected	20	40	20	80	
	Chi-square	6.1	8.1	2.5	16.6	p < 0.005
$OPK7_{1300}$	observed	24	36	20	80	
	expected	20	40	20	80	
	Chi-square	0.8	0.4	0.0	1.2	0.5<p<0.9
$OPK7_{300}$	observed	30	22	28	80	
	expected	20	40	20	80	
	Chi-square	5.0	8.1	3.2	16.3	p < 0.005
$OPM18_{500}$	observed	5	10	13	28	
	expected	7	14	7	28	
	Chi-square	0.6	1.1	5.1	6.9	p < 0.05
$OPM18_{300}$	observed	20	16	18	54	
	expected	13.5	27	13.5	54	
	Chi-square	3.1	3.7	1.5	8.3	p < 0.025
$OPN3_{900}$	observed	19	35	26	80	
	expected	20	40	20	80	
	Chi-square	0.1	0.6	1.8	2.5	0.1<p<0.5

5.4.4 Analysis of Marker Genotype Means for Hull Weight

Five of the six marker loci with significant differences between genotype means for percent hull also showed significant differences between genotype means for hull weight (Table 5.3). The $OPC13_{600}$ and $OPK7_{1300}$ marker loci showed the largest differences in hull weight between genotypic classes, significant at $\propto$ = 0.01 and 0.001 respectively.

5.4.5 MQTL Analysis of Marker-QTL Associations

MQTL was also used to search for significant QTL effects among the twelve RAPD markers. Multi-environment QTL analysis, using data from two locations, established three significant QTL main effects for hull percentage (Table 5.2). SIM test statistics of 19.5, 30.3 and 30.5, for the $OPC13_{800}$, $OPD20_{600}$ and $OPK7_{1300}$ marker loci respectively, exceeded the main effect threshold value of 16.4. None of the twelve marker loci had QTL by environment test statistics for percent hull that exceeded the threshold value.

Markers were also tested for significant QTL main effects using percent hull data from individual locations. Analysis with percent hull data from the Portage la Prairie location revealed four marker loci with SIM test statistics exceeding the threshold for Type I error of 5% (8.2). The $OPC13_{800}$, $OPD20_{600}$, $OPE9_{600}$, and $OPK7_{1300}$ marker loci had test statistics of 11.1, 16.1, 8.3 and 13.3 respectively. However, only the $OPD20_{600}$ and $OPK7_{1300}$ marker loci were significant at the Glenlea location, with SIM test statistics of 14.4 and 17.2 respectively (Table 5.2). When percent hull was averaged across the two

locations, the $OPC13_{800}$, $OPD20_{600}$ and $OPK7_{1300}$ marker loci exceeded the 5% threshold for a Type I error (8.2), with test statistics of 10.2, 15.9 and 16.0.

The twelve marker loci were also tested for association with hull weight per kernel. Multi environment QTL analysis showed $OPC13_{600}$ and $OPK7_{1300}$ to have SIM test statistics of 18.9 and 33.3 respectively, exceeding the threshold value of 16.3 (Table 5.3). Of the twelve marker loci tested, only $OPK7_{1300}$ exceeded the threshold for QTL by environment effect.

Marker loci were also tested for significant QTL main effects using hull weight per kernel data from individual locations (Table 5.3). Analysis of data from the Portage la Prairie location revealed only one marker locus ($OPK7_{1300}$) with a SIM test statistic that exceeded the threshold for Type I error of 5% (8.5). However, both the $OPC13_{600}$ and $OPK7_{1300}$ marker loci were significant at the Glenlea location, with SIM test statistics of 10.7 and 19.6 respectively.

When tested for association with groat weight per kernel and total kernel weight, none of the twelve marker loci had SIM test statistics exceeding predetermined threshold values.

The genetic variance in percent hull across two locations, individually explained by the $OPC13_{800}$, $OPD20_{600}$ and $OPK7_{1300}$ marker loci ranged from 12-18%, after accounting for environmental main effect. The $OPC13_{600}$ and $OPK7_{1300}$ marker loci had the largest effects on hull weight per kernel across locations, explaining 12% and 20% of the total genetic variance, respectively.

Table 5.2. Genotypic means, simple interval mapping test statistics (SIM TS) for QTL main effects (QTL) and QTL by environment interaction (QTL x E), and the proportion of genetic variation (Vg) explained by six marker loci associated with primary kernel percent hull measured at Glenlea and Portage la Prairie, Manitoba in 1995.

Marker locus	Parental Genotype	1995 Multi environment				1995 Glenlea			1995 Portage la Prairie		
		% Hull	SIM TS		Vg	% Hull	SIM TS	Vg	% Hull	SIM TS	Vg
			QTL	QTL x E			QTL			QTL	
OPC13 $_{800}$	Cascade	26.44**	19.5*	0.0	0.12	27.23**	8.6	0.11	25.64**	11.1*	0.14
	AC Marie	28.26**				28.98**			27.54**		
OPC13 $_{600}$	Cascade	27.76**	12.6	0.3	0.08	28.54**	7.7	0.10	26.98*	5.0	0.06
	AC Marie	26.65**				27.27**			26.02*		
OPD20 $_{600}$	Cascade	28.10***	30.3*	0.0	0.18	28.79***	14.4*	0.18	27.41***	16.1*	0.19
	AC Marie	26.14***				26.79***			25.48***		
OPE9 $_{600}$	Cascade	27.75*	12.3	0.2	0.08	28.36*	4.5	0.06	27.14**	8.3*	0.10
	AC Marie	26.58*				27.32*			25.84**		
OPG12 $_{100}$	Cascade	26.84*	11.1	0.1	0.07	27.46*	6.3	0.08	26.21*	4.8	0.06
	AC Marie	27.95*				28.68*			27.22*		
OPK7 $_{1300}$	Cascade	28.22***	30.5*	0.3	0.18	29.00***	17.2*	0.21	27.45***	13.3*	0.16
	AC Marie	26.23***				26.82***			25.64***		

*, **, *** denotes significance at P less than or equal to 0.05, 0.01, or 0.001, respectively

Table 5.3. Genotypic means, simple interval mapping test statistics (SIM TS) for QTL main effects (QTL) and QTL by environment interaction (QTL x E), and the proportion of genetic variation (Vg) explained by five marker loci associated with primary hull weight measured at Glenlea and Portage la Prairie, Manitoba in 1995.

Marker locus	Parental Genotype	1995 Multi environment				1995 Glenlea			1995 Portage la Prairie		
		Hull Wt. (mg)	SIM TS QTL	SIM TS QTL x E	Vg	Hull Wt. (mg)	SIM TS QTL	Vg	Hull Wt. (mg)	SIM TS QTL	Vg
OPC13 $_{600}$	Cascade	13.4**	18.9*	0.4	0.12	14.1***	10.7*	0.14	12.7**	8.1	0.10
	AC Marie	12.4**				12.9***			11.8**		
OPD20 $_{600}$	Cascade	13.2*	10.7	0.0	0.07	13.8*	4.8	0.06	12.5*	6.1	0.08
	AC Marie	12.2*				12.8*			11.7*		
OPE9 $_{600}$	Cascade	13.5*	9.9	0.0	0.06	14.1*	4.5	0.06	12.8*	5.6	0.07
	AC Marie	12.6*				13.2*			12.0*		
OPG12 $_{100}$	Cascade	12.7*	11.4	0.2	0.07	13.3*	6.5	0.08	12.0*	4.9	0.06
	AC Marie	13.5*				14.2*			12.8*		
OPK7 $_{1300}$	Cascade	13.7***	33.3*	0.9*	0.20	14.5***	19.6*	0.24	13.0***	13.8*	0.17
	AC Marie	12.1***				12.7***			11.6***		

*, **, *** denotes significance at P less than or equal to 0.05, 0.01, or 0.001, respectively

Of three possible pairings of the three significant markers for hull percentage, the combination of $OPD20_{600}$ and $OPK7_{1300}$, explained the largest proportion (32%) of genetic variance in percent hull across two locations in 1995. Mean values of percent hull, partitioned according to genotypes at these two marker loci, displayed an additive relationship for the QTLs linked to these markers (Table 5.4, Figure 5.1). No dominance or epistasis was observed between the two marker loci, and none of the remaining twelve marker loci interacted with either $OPD20_{600}$ or $OPK7_{1300}$. The inclusion of $OPC13_{800}$, the other marker with a significant QTL main effect across multiple environments, resulted in a model that explained 41% of the genetic variance in percent hull.

A more comprehensive model, including both primary and secondary inferences explained 55% of the genetic variance in percent hull across two locations in 1995. This model consisted of the three markers significant under SIM analysis ($OPC13_{800}$, $OPD20_{600}$, $OPK7_{1300}$), and four secondary markers that showed larger test statistics under sCIM analysis ($OPC13_{600}$, $OPE9_{900}$, $OPE9_{600}$, $OPG12_{100}$). All these markers also showed significant differences ($\propto = 0.05$) in percent hull between genotype means across the two locations, with the exception of $OPE9_{900}$.

Table 5.4. Mean primary kernel percent hull of combined $OPD20_{600}$ and $OPK7_{1300}$ genotypic classes measured for an F_7 population of Cascade/AC Marie grown in a replicated study at Portage la Prairie, Manitoba in 1995.

$OPD20_{600}$ genotypes	$OPK7_{1300}$ genotypes			Means
	Cascade	heterozygous	AC Marie	
Cascade	29.1	27.7	27.1	28.0
heterozygous	28.2	27.0	26.7	27.3
AC Marie	26.4	26.6	25.3	26.1
Means	27.9	27.1	26.4	27.1

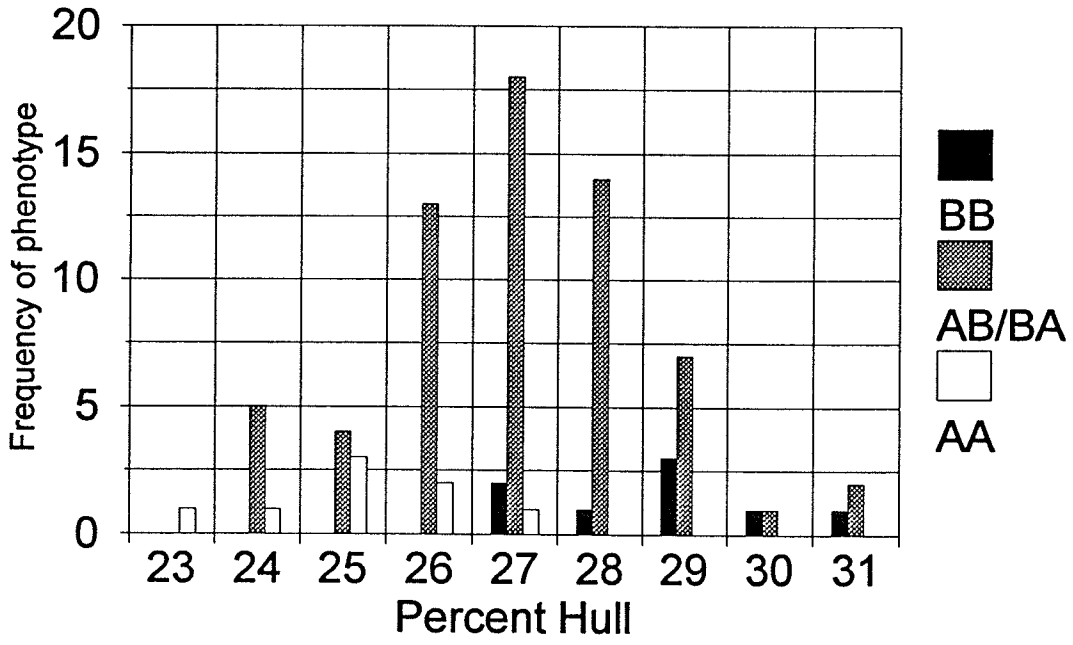

Figure 5.1 The combined effect of $OPD20_{600}$ and $OPK7_{1300}$, as represented by their inferred F_2 genotypes, on mean percent hull of 80 F_7 progeny of Cascade/AC Marie grown at Glenlea and Portage la Prairie, Manitoba in 1995. (BB = duplicate Cascade alleles, AB/BA = different parental alleles at each marker locus, AA = duplicate AC Marie alleles.)

5.5 DISCUSSION

Percent hull is an important measure of both feed and milling quality. However, percent hull is often time consuming and costly to measure, complicating phenotypic selection. The objective of this study was to identify RAPD markers associated with percent hull in oat. The markers $OPC13_{800}$, $OPD20_{600}$, $OPK7_{1300}$, together explained 41% of the genetic variance in percent hull across two locations. The QTLs linked to these markers show additive effects, such that tandem selection for favourable alleles at these loci would identify members of the population with the lowest hull percentage.

The random sample of 80 progeny lines used to quantify marker-QTL associations adequately represented the range of variability in percent hull seen in the larger population. However, the estimates of genetic variance explained by the inferred QTLs for percent hull are likely less than the actual values. Without a linkage map for the Cascade/AC Marie population, interval analysis was impossible and refined estimates of QTL position and effect could not be made. Assuming that the three marker loci with significant QTL main effects are some distance from the respective QTLs, marker loci more closely linked to each gene for percent hull would have larger phenotypic effects.

As expected, none of the markers tested showed associations with groat or kernel weight since polymorphisms were identified by bulked segregant analysis on the basis of percent hull. However, most of the RAPD markers associated with percent hull also showed significant differences in hull weight

per kernel between genotypes. This suggested that the QTLs linked to these markers affect the expression of both traits. Future work should examine the associations between these markers and traits influencing both percent hull and hull weight, such as hull thickness or hull density. It is possible that such characteristics may exhibit even stronger associations with the identified markers.

No attempts were made to infer QTLs in other populations, or to test the cross applicability of these markers for percent hull. Before testing the cross-applicability of the marker loci linked to QTLs for hull percentage, allele specific amplicons must be developed for each marker. This process requires one to: (1) sequence the desired polymorphic fragment for each primer, (2) design locus-specific primers, (3) sequence the resulting monomorphic band, and (4) design allele-specific primers (Penner et al., 1995). Allele-specific primers would then be applied across a collection of oat cultivars with extreme hull percentages to assess the strength of marker-trait associations among diverse germplasm.

Bulked segregant analysis was used to identify marker loci linked to QTLs for percent hull. This supports the use of phenotype-based bulked segregant analysis to identify markers for quantitative traits, providing that accurate assessments of phenotype can be made. Wang and Paterson (1994) examined the utility of phenotype-based DNA pools for tagging QTLs, which are segregating in the presence of other loci and producing environmentally-influenced phenotypes. They concluded that phenotype-based pools are only

effective for the identification of QTLs of large effect. However, bulked segregant analysis was used in this study to identify markers for QTLs that individually explained only small amounts of the variation in percent hull.

The simplest test for putative linkage between unmapped markers and QTLs for percent hull, is a comparison of marker genotype means. Our results indicated that significant differences ($\propto = 0.05$) among marker genotype means did not always agree with significant SIM test statistics for QTL main effect. However, three markers with the most significant differences between genotype means across locations, also showed SIM test statistics exceeding the threshold value for QTL main effect. Genotypes at these marker loci also fit a 1:2:1 ratio, supporting their linkage to discrete genetic factors. Comparison of marker genotype means requires a stringent *a priori* threshold ($\propto = 0.001$) to reduce false positives. This method should not be used to infer QTLs, but rather to support the conclusions of interval analysis.

The assessment of marker genotype for progeny lines, required genotyping of single plants within each line. This method is more time consuming than procedures required to genotype doubled haploids, or progeny lines derived by single seed descent. The bulking procedure used to advance lines to the F_8 generation was the complicating factor in determining the marker genotype of progeny lines. Without single seed descent, genetic loci that were heterozygous in an F_2 plant showed heterogeneity of genotype in the derived F_8 line. Although individuals within F_8 lines were approaching homozygosity, each

seed bulk contained plants of diverse genotype for many loci.

Based on these assumptions, segregation was expected among single plants at each marker locus for about half of the F_8 lines evaluated. Genotypes of the remaining progeny lines should have resembled the parental cultivars, with single plants being both homozygous and homogeneous at the marker locus. An examination of segregation ratios for the twelve marker loci evaluated, showed only four markers fit the expected ratio. It is possible that incorrectly scored genotypes and the limited number of single plants evaluated within each line, could account for the discrepancy. The marker $OPC13_{600}$, associated with hull weight per kernel, showed a genotypic segregation ratio of 2:1:2. Possibly this unexpected segregation ratio was due to a chromosomal rearrangement in the regions flanking this marker locus.

Determining the marker genotype for a particular progeny line was also complicated by the marker system used. RAPD markers are dominant markers, typically scored as the presence or absence of a single band. A codominant marker system would have assessed marker genotypes for each line using only the pooled DNA sample, rather than analysing individual plants. Restriction fragment length polymorphisms may be a more appropriate marker system for the analysis of progeny lines that are heterogeneous in genotype.

Marker-assisted selection refers to the evaluation of segregating plant populations by marker assay, rather than phenotypic assessment (Paterson et al., 1991a). Wesenberg and Shands (1973) suggested that selection for low

percent hull among F_2 plants would be useful if large populations derived from contrasting parents could be screened. The development of cross-applicable, allele-specific amplicons for $OPC13_{800}$, $OPD20_{600}$ and $OPK7_{1300}$ may facilitate the selection of early generation breeding lines with allelic complements for low percent hull. Ultimately, the utility of these markers for percent hull will be revealed by their effectiveness in marker-assisted selection.

6.0 GENERAL DISCUSSION

This study consisted of genetic analyses of percent hull in oat, at both the phenotypic and genotypic levels. Phenotypic data from progeny populations of three oat crosses were used to recommend suitable methods of measurement for percent hull. The inheritance of percent hull in oat was also investigated, including broad-sense heritability and the relative effects of genotype and location. At the genotypic level, DNA markers linked to QTLs controlling hull percentage in progeny of the cross Cascade/AC Marie, were identified.

A rapid, reproducible method of measuring percent hull is desirable in breeding programs seeking to improve milling and feed quality in oat. Among five sample types evaluated, percent hull measured for a sample of primary kernels was most reproducible and highly associated with hull percentages of larger samples. Near infra red reflectance spectroscopy proved effective for the rapid classification of lines with extreme hull percentages, but failed to provide the precise phenotypic measurements required in a genetic study.

In general, estimates of broad-sense heritability for primary kernel percent hull and its component traits were highest for populations of Cascade/AC Marie and Cascade/Robert. Progeny populations of AC Marie/Robert had the lowest heritability for hull percentages of two gram kernel samples. Such a trend was expected due to the limited genetic differences between AC Marie and Robert.

The effect of genotype by environment interaction on percent hull was significant, but small, in relation to the effects of genotype and location,

suggesting that although this trait may vary from location to location, the ranking of genotypes remains similar. Variance component analysis of 1995 data also showed the effect of genotype by environment interaction on percent hull to be much smaller than the individual effects of location and genotype. Based on these results, accurate assessment of hull percentage among early generation breeding lines should be possible with samples from just a few locations.

Stepwise regression analysis revealed significant effects of hull weight, groat weight and kernel weight on primary kernel percent hull in oat. Based on coefficients for the three independent variables, an oat breeder will be able to reduce percent hull most efficiently by minimizing hull weight and maximizing groat weight. Correlation coefficients calculated between these traits revealed few negative associations that would complicate multiple trait selection.

The discovery of associations between marker alleles and quantitative traits provides a diagnostic test for QTLs, and allows estimates of their effect on phenotype, and positions in the genome (Tinker and Mather, 1995a). RAPD markers, $OPC13_{800}$, $OPD20_{600}$ and $OPK7_{1300}$, linked to three QTLs for percent hull in oat, together explained 41% of the genetic variance across two locations. Future confirmation of these associations requires: (1) the Cascade/ AC Marie progeny population to be grown at additional locations, and (2) the remaining 143 progeny lines for the cross to be characterized for marker genotype.

Five of the six markers with significant differences between genotype means for percent hull, also showed significant differences for hull weight. This

agrees with the observation of Paterson et al., (1991b), that correlated traits have some of the same significant marker associations. High correlation coefficients between these two traits (r = 0.85) were observed across all populations and may represent the effects of fundamental genes for hull content in oat, that affect both hull weight and percent hull.

The determination of QTL effect and position would be enhanced by a genomic map for Cascade/AC Marie. Such a map could be constructed by screening the parents for polymorphisms and then scoring these on a progeny population. Mapped markers provide better estimates of: (1) the number of QTLs underlying the trait, (2) chromosomal location of these loci, (3) gene action, (4) phenotypic effect, and (5) sensitivity to environment (Paterson et al., 1991a).

Two markers, $OPD20_{600}$ and $OPK7_{1300}$, explained 32% of the genetic variance in percent hull among progeny of Cascade/AC Marie. Combined marker genotype means for hull percentage suggested that the QTLs linked to these markers displayed an additive relationship. This agrees with the conclusions of Wesenberg and Shands (1971), who suggested that multiple genetic factors with additive gene effects influence percent hull in certain crosses.

Combined selection for favourable alleles at QTLs associated with percent hull and hull weight per kernel should facilitate the identification of individuals with low hull content from progeny populations. An indirect selection method for low hull content is feasible among early generation seedlings and may represent an effective means to improve milling quality in oat.

7.0 LIST OF REFERENCES

Anderson, J. A., M. E. Sorrells, and S. D. Tanksley. 1993. RFLP analysis of genomic regions associated with resistance to preharvest sprouting in wheat. Crop Sci. 33:453-459.

Atkins, R. E. 1943. Factors affecting milling quality in oats. Agron. J. 35:532-539.

Bartley, B. C., and M. G. Weiss. 1951. Evaluation of physical factors affecting oat quality from Bond parentage. Agron. J. 43:22-25.

Beavis, W. D., D. Grant, M. Albertson, and R. Fincher. 1991. Quantitative trait loci for plant height in four maize populations and their associations with qualitative genetic loci. Theor. Appl. Genet. 83:141-145.

Botstein, D., R. L. White, M. Skolnick, and R. W. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32:314-331.

Bunch, R. A., and R. A. Forsberg. 1989. Relationships between groat percentage and productivity in an oat head-row series. Crop Sci. 29:1409-1411.

Burrows, V. D. 1986. Breeding oats for food and feed: Conventional and new techniques and materials. In "Oats: Chemistry and technology." (Webster, F.H., ed.). Am. Assoc. of Cereal Chemists, St. Paul. pp. 13-46.

Canada Grains Council (CGC). Canadian grains industry statistical handbook, 1993 edition. Canada grains council, Winnipeg, Manitoba, 1993, pp. 42-48.

Chalmers, K. J., U. M. Barua, C. A. Hackett, W. T. B. Thomas, R. Waugh, and W. Powell. 1993. Identification of RAPD markers linked to genetic factors controlling the milling energy requirement of barley. Theor. Appl. Genet. 87:314-320.

Christenson, C. M., and R. A. Meronuck. 1986. Quality maintenance in stored grains and seeds. University of Minnesota Press, Minneapolis.

de Villiers, P. J. R. 1935. A genetic study of the inheritance of the various characters in certain *Avena* hybrids. Union of S. Africa Dep. Agr. Sci. Bull. 140. 90 pp.

Doerge, R. W., and G. A. Churchill. 1994. Issues in genetic mapping of quantitative trait loci. In "Analysis of Molecular Marker Data". Proc. Joint Plant Breeding Symposia Series, Corvallis, Oregon. August 1994. pp. 27-32.

Edwards, K., C. Johnstone, and C. Thompson. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res. 19:1349.

Falconer, D. S., and T. F. C. Mackay. 1996. Heritability; Quantitative Trait Loci. In "Introduction to Quantitative Genetics". Longman Group Ltd., Harlow, Essex, England. pp. 123; 359.

Fore, R. E., and C. M. Woodworth. 1933. Analysis of yield in certain oat varieties. J. Amer. Soc. Agron. 25:190-202.

Forsberg, R. A., and D. L. Reeves. 1992. Breeding oat cultivars for improved grain quality. In "Oat Science and Technology". (Marshall, H.G., and Sorrells, M.E., ed.) Amer. Soc. Agron., Madison, Wisconsin, pp. 751-770.

Fulcher, R. G. 1986. Morphology and organization of the oat kernel. In "Oats: Chemistry and technology." (Webster, F.H., ed.). Am. Assoc. of Cereal Chemists, St. Paul. pp. 47-74.

Frey, K. J., and T. Horner. 1957. Heritability in standard units. Agron. J. 49:59-62.

Freyre, R., and D. S. Douches. 1994. Development of a model for marker-assisted selection of specific gravity in diploid potato across environments. Crop Sci. 34:1361-1368.

Garber, R. J., and A. C. Arny. 1918. Relation of size of sample to kernel percentage determinations in oats. J. Amer. Soc. Agron. 10:134-142.

Gebhardt, C. and F. Salamini. 1992. RFLP analysis of plant genomes and its application to plant breeding. Int. Rev. Cytol. 135:201-237.

Geldermann, H. 1975. Investigations on inheritance of quantitative characters in animals by gene markers. I. Methods. Theor. Appl. Genet. 46:319-330.

Greig, R. B., and W. M. Findlay. 1907. The milling properties of oats. J. Bd. Agr. (London) 14:257-268.

Gullord, M., and A. H. Aastveit. 1987. Developmental stability in oats (*Avena sativa* L.). II. Quality characters. Hereditas 107:65-74.

Haley, C. S., and S.A. Knott. 1992. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69:315-324.

Hinze, K., R. D. Thompson, E. Ritter, F. Salamini, and P. Schulze-Lefert. 1991. Restriction fragment length polymorphism-mediated targeting of the *ml-o* resistance locus in barley (*Hordeum vulgare*). Proc. Natl. Acad. Sci. USA. 88:3691-3695.

Hu, J., C. Quiros, P. Arus, D. Struss, and G. Robbelen. 1995. Mapping of a gene determining linolenic acid concentration in rapeseed with DNA-based markers. Theor. Appl. Genet. 90:258-262.

Hunt, T. F. 1904. Oats. In "Cereals of America" pp. 280 - 317.

Hunter, H. 1935. The improvement of winter oats. J. Agr. Sci. 25:419-444.

Johannsen, W. 1909. "*Elemente der exakten Erblichkeitsllehre.*" Fischer, Jena.

Keim, P., B. W. Diers, T. C. Olson, and R.C. Shoemaker. 1990. RFLP mapping in soybean: associations between marker loci and variation in quantitative traits. Genetics 126:735-742.

Kianian, S. F., R. L. Phillips, H. W. Rines, R. G. Fulcher, and D. D. Stuthman. 1996. Analysis of kernel morphology in cultivated oat (*Avena sativa*, 2n=6x=42). In "Nutritional Quality 1 - Food and Industrial" (Slinkard, A., ed.) Proc. 5th Int. Oats Conf., Saskatoon, Canada. August 1996. pp. 74-76.

Kibite, S., and M. J. Edney. 1992. Effects of genotype x environment interaction on the expression of grain quality traits in oat. In "The Changing Role of Oats in Human and Animal Nutrition", (Barr, A. H., ed.) I. Proc. 4th Int. Oats Conf., Adelaide, Australia. 19 October 1992. pp. 101-103.

Kleinhofs, A., A. Kilian, M. A. Maroof Saghai, R. M. Biyahev, P. M. Hayes, F. Chen, N. Lapitan, A. Fenwick, T. K. Blake, V. Kanazin, E. Ananiev, L. Dahleen, D. Bollinger, S. J. Knapp, B. Liu, M. E. Sorrells, M. Heun, J. D. Franckowiak, D. Hoffman, R. Skadsen, and B. J. Steffenson. 1993. A molecular, isozyme, and morphological map of the barley (*Hordeum vulgare*) genome. Theor. Appl. Genet. 86:705-712.

Lander, E. S., P. Green, J. Abrahamson, A. Barlow, M. J. Daly, S. E. Lincoln, and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181.

Lebowitz, R. J., M. Soller, and J. S. Beckmann. 1987. Trait-based analyses for the detection of linkage between marker loci and quantitative trait loci in crosses between inbred lines. Theor. Appl. Genet. 73:556-562.

Love, H. H. 1914. Oats for New York. Cornell Univ. Agr. Exp. Sta. Bul. 343. 55 pp.

Love, H. H., and W. T. Craig. 1944. A machine for dehulling oats. J. Amer. Soc. Agron. 36:264-266.

Mahmud, K., and H. H. Kramer. 1951. Segregation for yield, height, and maturity following a soybean cross. Agron. J. 43:605-609.

Markert, C. L., and F. Moller. 1959. Multiple forms of enzymes: tissue, ontogenetic, and species-specific patterns. Proc. Natl. Acad. Sci. USA. 45:753-763.

Martin, G. B., J. G. K. Williams, and S. D. Tanksley. 1991. Rapid identification of markers linked to *Pseudomonas* resistance in tomato by using random primers and near isogenic lines. Proc. Natl. Acad. Sci. USA. 88:2336-2340.

Michelmore, R. W., I. Paran, and R. V. Kesseli. 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genome regions by using segregating populations. Proc. Natl. Acad. Sci. USA. 88:9828-9832.

Minitab. 1994. Minitab Release 10 for Windows. Minitab Inc., State College, PA.

Morgan, T. H. 1911. Random segregation versus coupling in Mendelian inheritance. Science 34:384.

Murphy, C. F., and K. J. Frey. 1962. Inheritance and heritability of seed weight and its components in oats. Crop Sci. 2:509-512.

Nilsson-Ehle, H. 1909. "*Kreuzunguntersuchungen an Hafer und Weizen.*" Lunds. Univ. Aarskr. NF 5:1-122.

Novell. 1994. Quattro Pro. Version 6.0. Novell Applications, Orem, Utah.

O'Donoughue, L. S., S. F. Kianian, P. J. Rayapati, G. A. Penner, M. E. Sorrells, S. D. Tanksley, R. L. Phillips, H. W. Rines, M. Lee, G. Fedak, S. J. Molnar, D. Hoffman, T. SALAS, B. Wu, E. Autrique, and A. Van Deynze. 1995. A molecular linkage map of cultivated oat (*Avena byzantina* cv. Kanota x *A. sativa* cv. Ogle). Genome 38:368-380.

Paterson, A. H., S. D. Tanksley, and M. E. Sorrells. 1991a. DNA markers in plant improvement. Advances in Agronomy 46:39-91.

Paterson, A. H., S. Damon, J. D. Hewitt, D. Zamir, H. D. Rabinowitch, S. E. Lincoln, E. S. Lander, and S. D. Tanksley. 1991b. Mendelian factors underlying quantitative traits in tomato: comparison across species, generations and environments. Genetics 127:181-197.

Pawlisch, P. E. 1959. Breeding behavior for bushel weight and agronomic characters in early generations of oat crosses. Ph.D. Thesis. University of Wisconsin, Madison.

Peek, J. M., and J. M. Poehlman. 1949. Grain size and hull percentage as factors in the milling quality of oats. Agron. J. 41:462-466.

Penner, G. A., and L. J. Bezte. 1994. Increased detection of polymorphism among randomly amplified wheat DNA fragments using a modified temperature sweep gel electrophoresis (TSGE) technique. Nucleic Acids Research 22:1780-1781.

Penner, G. A., J-A. Stebbing, and B. Legge. 1995. Conversion of an RFLP marker for the barley stem rust resistance gene *Rpg1* to a specific PCR-amplifiable polymorphism. Molecular Breeding 1:349-354.

Plourde, A. 1984. Effect of lemma colour on grain quality in oats, *Avena sativa* L. M. Sc. diss. University of Manitoba, Winnipeg.

Poehlman, J. M. 1987. Breeding barley and oats. In "Breeding Field Crops". (Van Nostrand Reinhold Company Inc., ed.) New York, New York. pp.378-415.

Rafalski, J. A., S. V. Tingey, and J. G. K. Williams. 1991. RAPD markers - a new technology for genetic mapping and plant breeding. AgBiotech News and Information 3:645-648.

Ronald, P. S., P. D. Brown, and G. A. Penner. 1996. Measurement of percent hull in oat, *Avena sativa* L. (to be submitted)

Root, W. R. 1979. The influence of oat (*Avena sativa* L.) kernel and caryopsis morphological traits on grain quality characteristics. Ph. D. diss University of Wisconsin, Madison, Wisconsin.

SAS. 1988. SAS/STAT User's Guide, Release 6.03 Edition. SAS Institute, Cary, N.C.

Simons, M. D., V. L. Youngs, G. D. Booth, and R. A. Forsberg. 1979. Effect of crown rust on protein and groat percentages of oat grain. Crop Sci. 19:703-706.

Siripoonwiwat, W., L. S. O'Donoughue, D. Wesenberg, D. Hoffman, J. F. Barbosa-Neto, and M. E. Sorrells. 1996. Chromosomal regions associated with quantitative traits in oat. JQTL.

Souza, E. J., and M. E. Sorrells. 1988. Mechanical mass selection methods for improvement of oat groat percentage. Crop Sci. 28:618-623.

Stanton, T. R., F. A. Coffman, V. F. Tapke, G. A. Wiebe, R. W. Smith, and B. B. Bayles. 1930. Influence of hulling the caryopsis on covered smut infection and related phenomena in oats. J. Agr. Res. 41:621-633.

Stanton, T. R. 1953. Production, harvesting, processing, utilization and economic importance of oats. Economic Botany 7:43-64.

Statistics Canada. 1995. November estimate of production of principle field crops, 1995. Market Analysis and Statistic Section, Winnipeg, Manitoba.

Stuber, C. W., M. D. Edwards, and J. F. Wendel. 1987. Molecular marker-facilitated investigations of quantitative trait loci in maize: II. Factors influencing yield and its component traits. Crop Sci. 27:639-648.

Stuthman, D. D., and R. M. Granger. 1977. Selection for caryopsis percentage in oats. Crop Sci. 17:411-414.

Teutonico, R. A., and T. C. Osborn. 1995. Mapping loci controlling vernalization requirement in *Brassica rapa*. Theor. Appl. Genet. 91:1279-1283.

Tinker, N. A., and D. E. Mather. 1993. Comparison of selective genotyping vs. pooled DNA for QTL analysis.

Tinker, N. A., and D. E. Mather. 1995a. Methods for QTL analysis with progeny replicated in multiple environments. JQTL.

Tinker, N. A., and D. E. Mather. 1995b. MQTL: software for simplified composite interval mapping of QTL in multiple environments. JQTL.

Wang, G. L., and A. H. Paterson. 1994. Assessment of DNA pooling strategies for mapping of QTLs. Theor. Appl. Genet. 88:355-361.

Waugh, R., and W. Powell. 1992. Using RAPD markers for crop improvement. Tibtech 10:186-191.

Wesenberg, D. M. 1968. Grain quality factors and related plant characters in certain oat crosses. Ph.D. Thesis. University of Wisconsin, Madison.

Wesenberg, D. M., and H. L. Shands. 1971. Caryopsis percentage and related characters in early generations of *Avena sativa* L. Crop Sci. 11:586-588.

Wesenberg, D. M., and H. L. Shands. 1973. Heritability of oat caryopsis percentage and other grain quality components. Crop Sci. 13:481-484.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18:6531-6535.

Yarrow, G. L., R. G. Fulcher, and B. Roskens. 1992. Oat grain quality effects on oat milling efficiency. In "The Changing Role of Oats in Human and Animal Nutrition", (Barr, A.H., ed.) I. Proc. 4th Int. Oats Conf., Adelaide, Australia. 19 October 1992. p. 32.

Yu, Z. H., D. J. Mackill, J. M. Bonman, and S. D. Tanksley. 1991. Tagging genes for blast resistance in rice via linkage to RFLP markers. Theor. Appl. Genet. 81:471-476.

Zavitz, C. A. 1927. Forty years' experience with grain crops. Ont. Dep. Agr. Bull. 332. 98 pp.

Zeng, Z-B. 1993. Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. Proc. Natl. Acad. Sci. USA 90:10972-10976.

Zeng, Z-B. 1994. Precision mapping of quantitative trait loci. Genetics 136:1457-1468.

APPENDIX 1

Table A.1. Distribution statistics for primary kernel hull weight measured for F_5-F_7 progeny populations of three oat (*Avena sativa* L.) crosses grown from 1993-1995 at several locations in western Canada.

Population [1]	Year and location [2]	Mean (mg)	Median (mg)	Modal class (mg)
Cascade/AC Marie F_5	1993 Gln	12.0	11.9	12.0
Cascade/AC Marie F_6	1993 Gln	11.7	11.6	11.5
Cascade/AC Marie F_5	1993 Lac	12.8	12.8	13.5
Cascade/AC Marie F_6	1993 Lac	12.8	12.6	13.0
Cascade/AC Marie F_7	1993 Gln	13.3	13.1	13.0
Cascade/AC Marie F_7	1993 Ptg	12.1	12.0	12.0
Cascade/Robert F_5	1993 Lac	13.2	13.0	14.0
Cascade/Robert F_6	1993 Lac	13.7	13.4	14.0
AC Marie/Robert F_6	1993 Lac	13.2	13.2	13.5

[1] F_5-F_7 generations were derived from F_2 plants by bulking seed at each generation

[2] Gln = Glenlea, MB., Lac = Lacombe, AB., Ptg = Portage la Praire, MB.

Table A.2. Distribution statistics for primary kernel groat weight measured for F_5-F_7 progeny populations of three oat (*Avena sativa* L.) crosses grown from 1993-1995 at several locations in western Canada.

Population [1]	Year and location [2]	Mean (mg)	Median (mg)	Modal class (mg)
Cascade/AC Marie F_5	1993 Gln	33.6	33.6	34.0
Cascade/AC Marie F_6	1993 Gln	33.1	30.0	33.0
Cascade/AC Marie F_5	1993 Lac	35.3	35.2	36.0
Cascade/AC Marie F_6	1993 Lac	35.8	36.0	37.0
Cascade/AC Marie F_7	1993 Gln	34.4	34.1	34.0
Cascade/AC Marie F_7	1993 Ptg	33.5	33.5	34.0
Cascade/Robert F_5	1993 Lac	36.6	36.6	38.0
Cascade/Robert F_6	1993 Lac	37.8	37.1	40.0
AC Marie/Robert F_6	1993 Lac	39.6	39.9	40.0

[1] F_5-F_7 generations were derived from F_2 plants by bulking seed at each generation

[2] Gln = Glenlea, MB., Lac = Lacombe, AB., Ptg = Portage la Praire, MB.

Table A.3. Distribution statistics for primary kernel weight measured for F_5-F_7 progeny populations of three oat (*Avena sativa* L.) crosses grown from 1993-1995 at several locations in western Canada.

Population [1]	Year and location [2]	Mean (mg)	Median (mg)	Modal class (mg)
Cascade/AC Marie F_5	1993 Gln	45.6	45.6	46.0
Cascade/AC Marie F_6	1993 Gln	44.9	44.8	46.0
Cascade/AC Marie F_5	1993 Lac	48.1	48.0	50.0
Cascade/AC Marie F_6	1993 Lac	48.6	48.9	50.0
Cascade/AC Marie F_7	1993 Gln	47.7	47.3	46.0
Cascade/AC Marie F_7	1993 Ptg	45.6	45.5	46.0
Cascade/Robert F_5	1993 Lac	49.8	49.6	50.0
Cascade/Robert F_6	1993 Lac	51.5	50.7	48.0
AC Marie/Robert F_6	1993 Lac	52.8	53.4	56.0

[1] F_5-F_7 generations were derived from F_2 plants by bulking seed at each generation

[2] Gln = Glenlea, MB., Lac = Lacombe, AB., Ptg = Portage la Praire, MB.

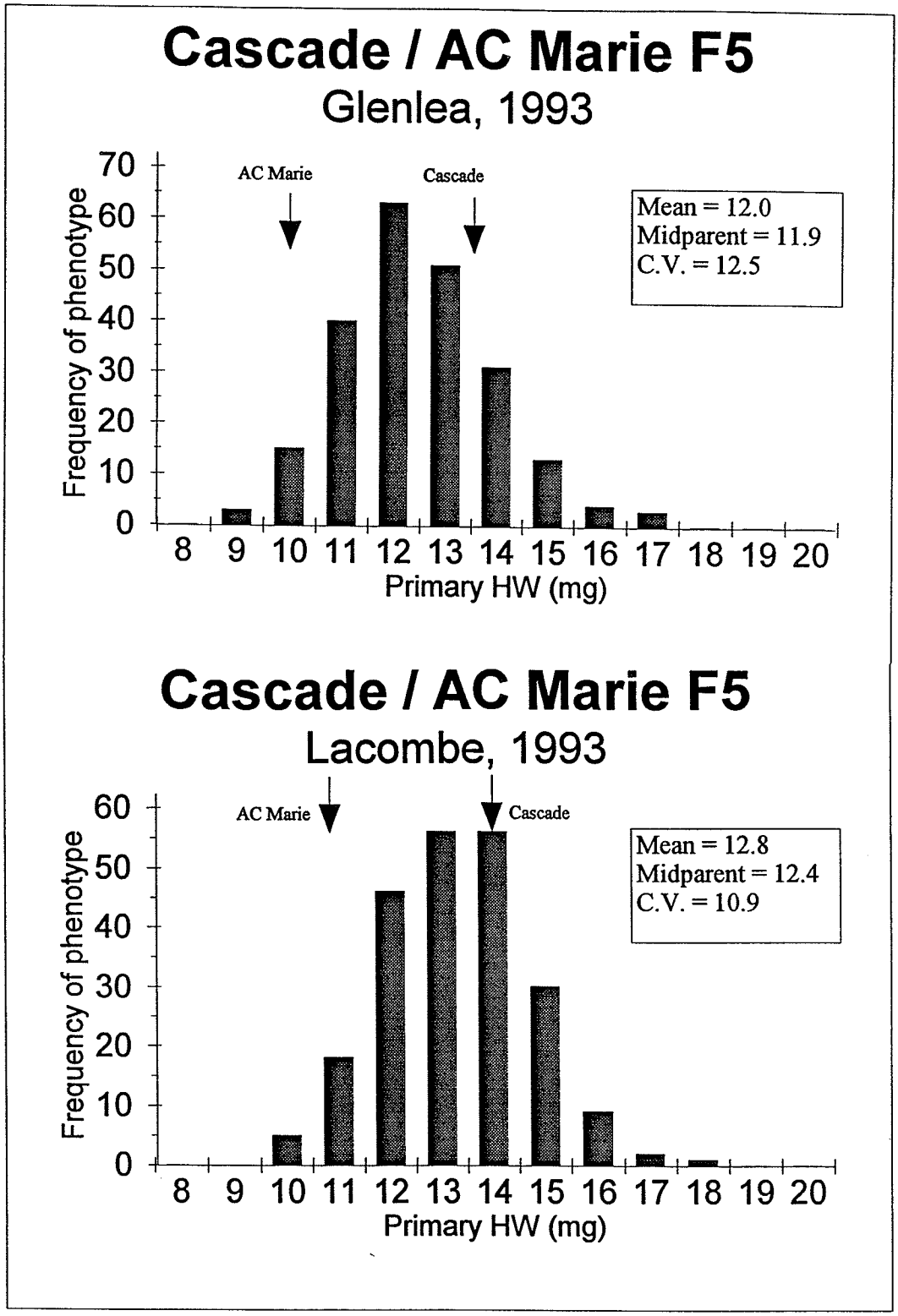

Figure A.1. Frequency distributions of primary kernel hull weight (mg) for F_5 progeny populations of Cascade/AC Marie grown at Glenlea, Manitoba and Lacombe, Alberta in 1993. Arrows indicate hull weight of parental cultivars.

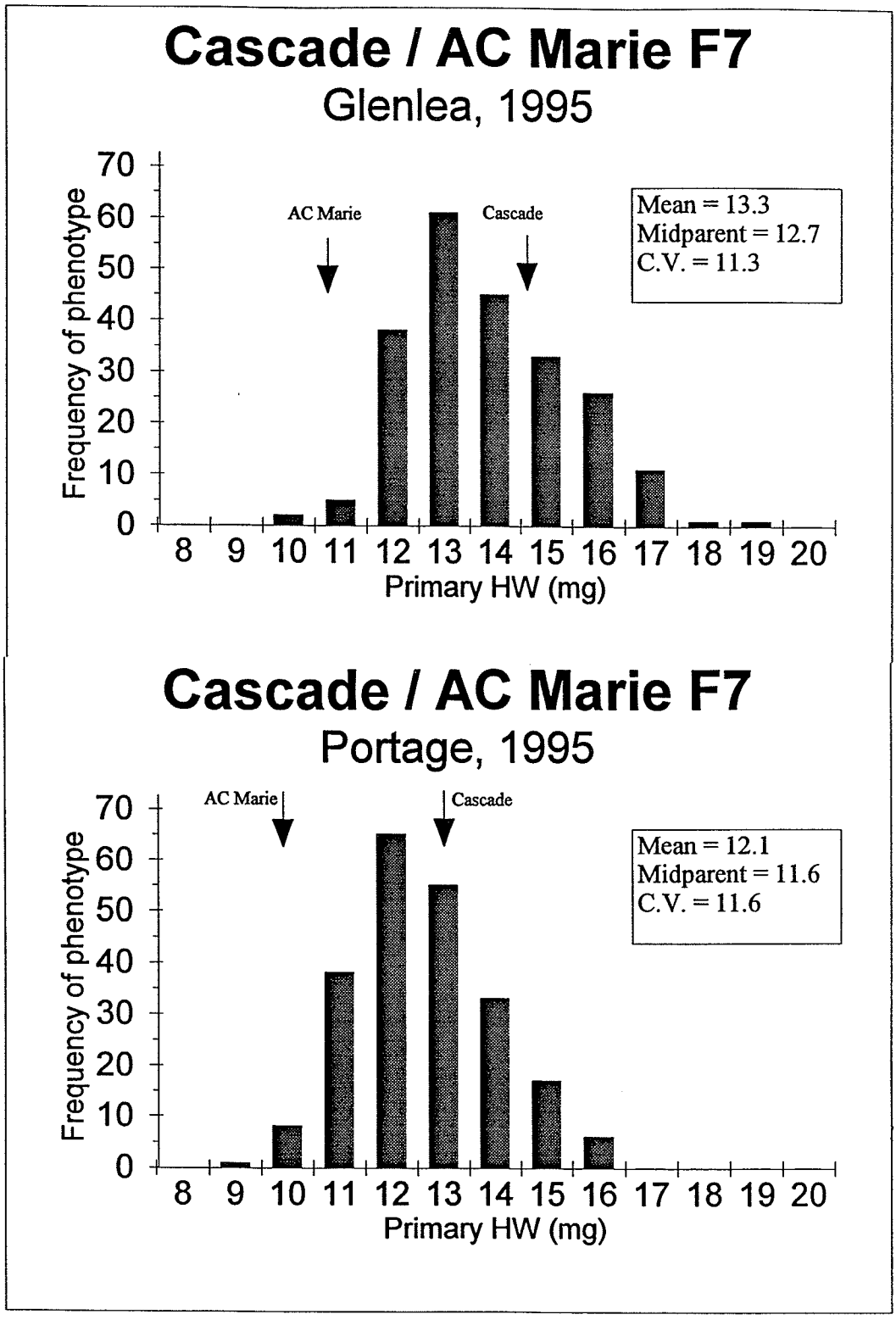

Figure A.2. Frequency distributions of primary kernel hull weight (mg) for F_7 progeny populations of Cascade/AC Marie grown at Glenlea and Portage la Prairie, Manitoba in 1995. Arrows indicate hull weight of parental cultivars.

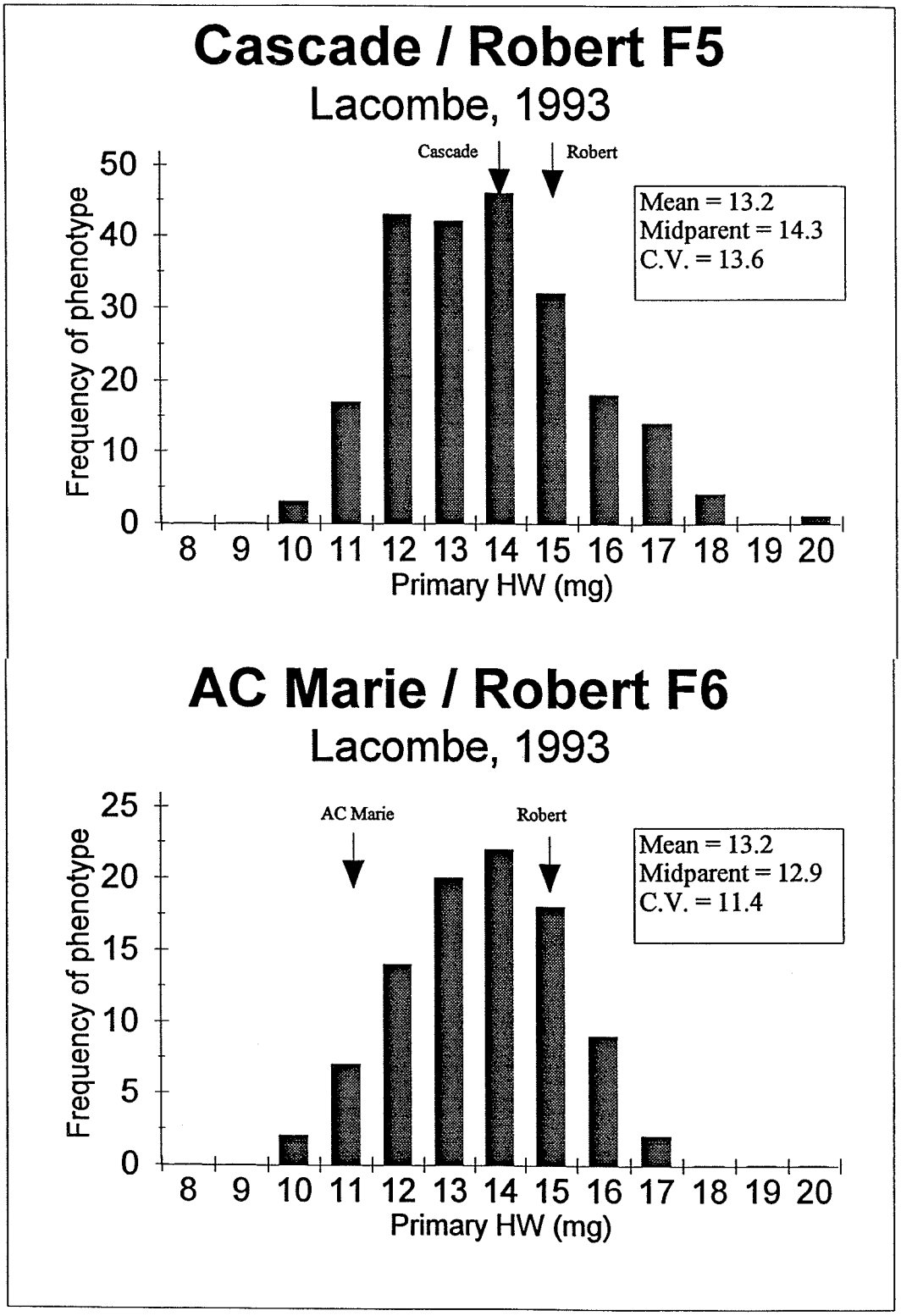

Figure A.3. Frequency distributions of primary kernel hull weight (mg) for F_5 progeny of Cascade/Robert and F_6 progeny of AC Marie/Robert grown at Lacombe, Alberta in 1993. Arrows indicate hull weight of parental cultivars.

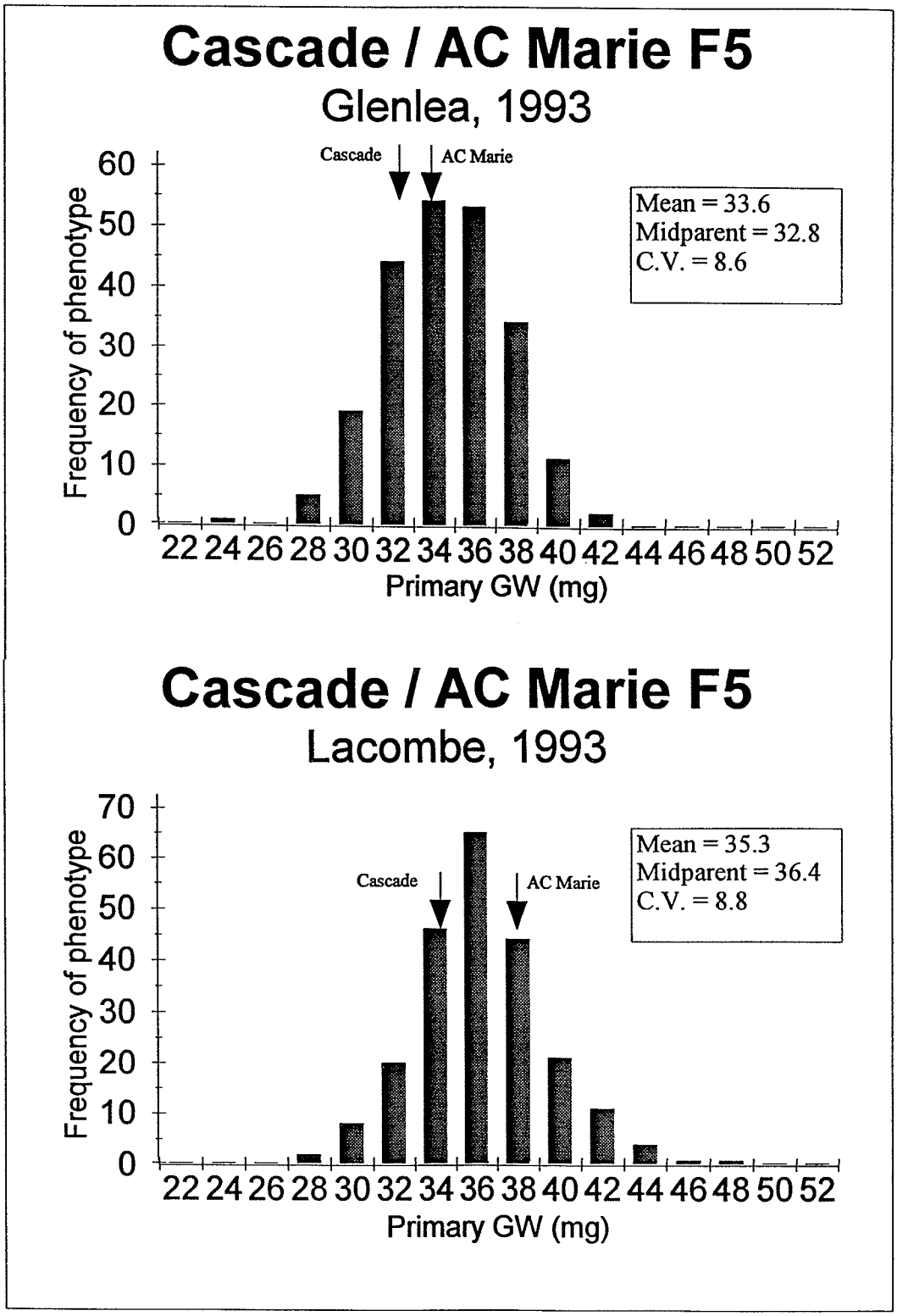

Figure A.4. Frequency distributions of primary kernel groat weight (mg) for F_5 progeny populations of Cascade/AC Marie grown at Glenlea, Manitoba and Lacombe, Alberta in 1993. Arrows indicate groat weight of parental cultivars.

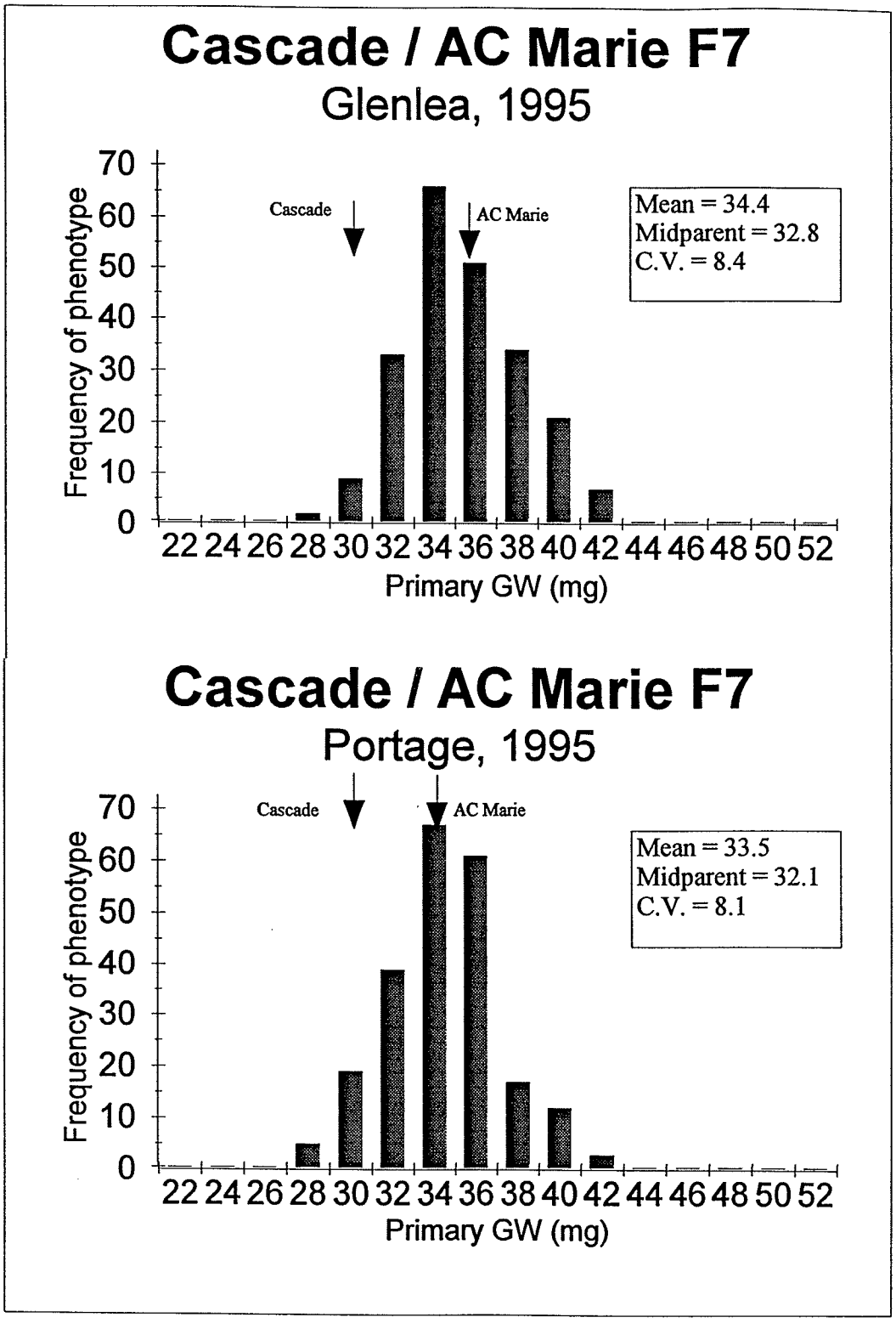

Figure A.5. Frequency distributions of primary kernel groat weight (mg) for F_7 progeny populations of Cascade/AC Marie grown at Glenlea and Portage la Prairie, Manitoba in 1995. Arrows indicate groat weight of parental cultivars.

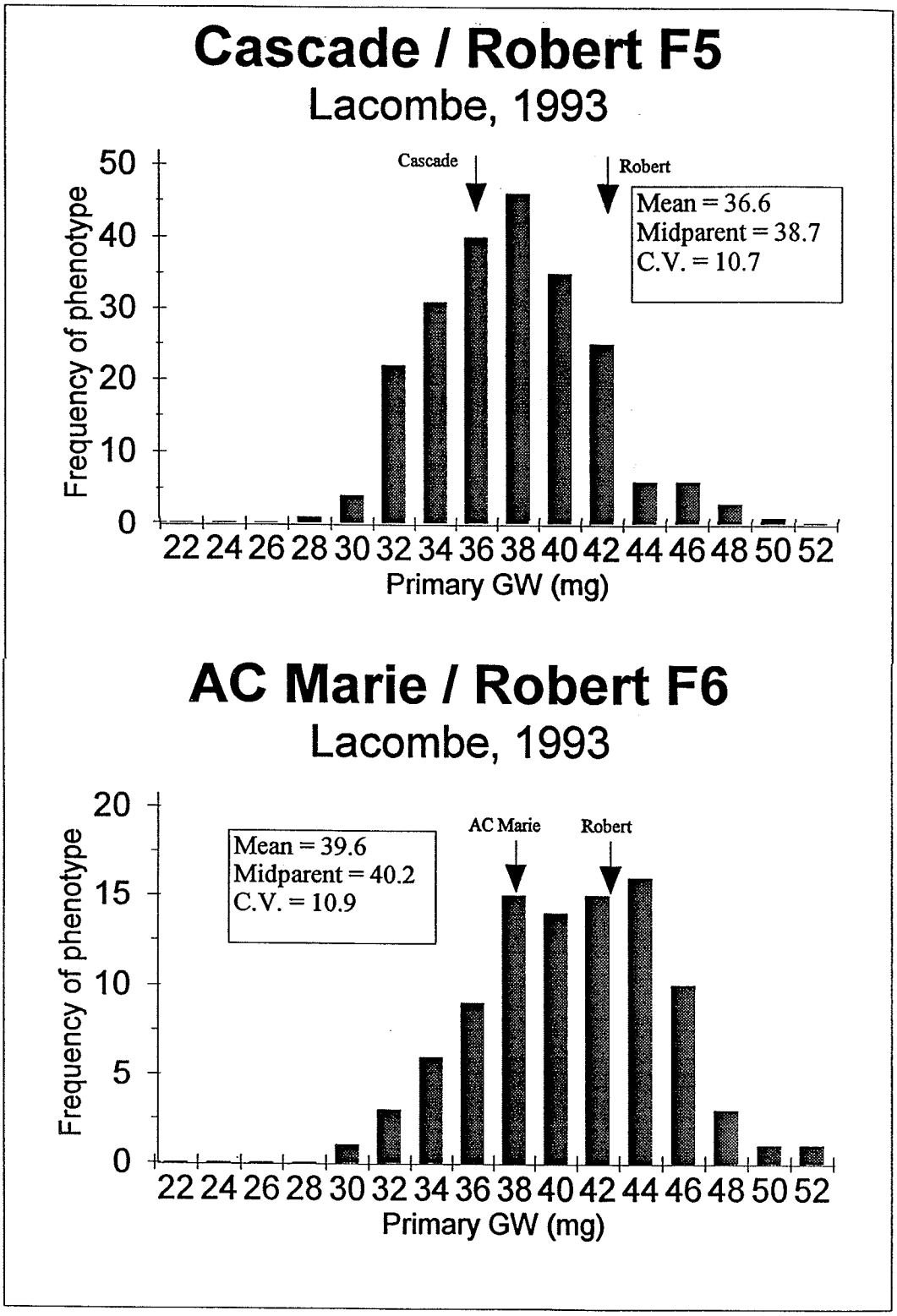

Figure A.6. Frequency distributions of primary kernel groat weight (mg) for F_5 progeny of Cascade/Robert and F_6 progeny of AC Marie/Robert grown at Lacombe, Alberta in 1993. Arrows indicate groat weight of parental cultivars.

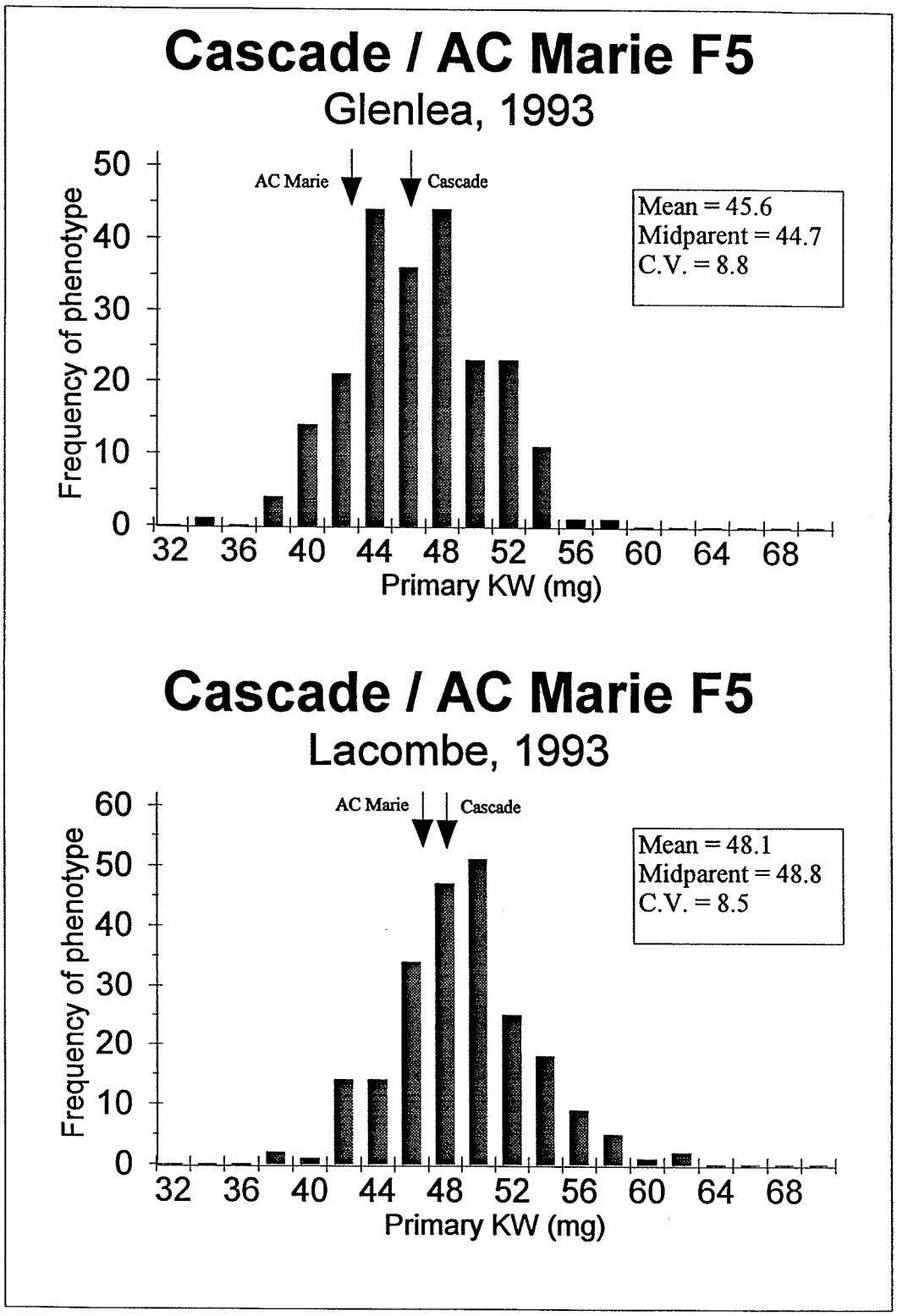

Figure A.7. Frequency distributions of primary kernel weight (mg) for F_5 progeny populations of Cascade/AC Marie grown at Glenlea, Manitoba and Lacombe, Alberta in 1993. Arrows indicate kernel weight of parental cultivars.

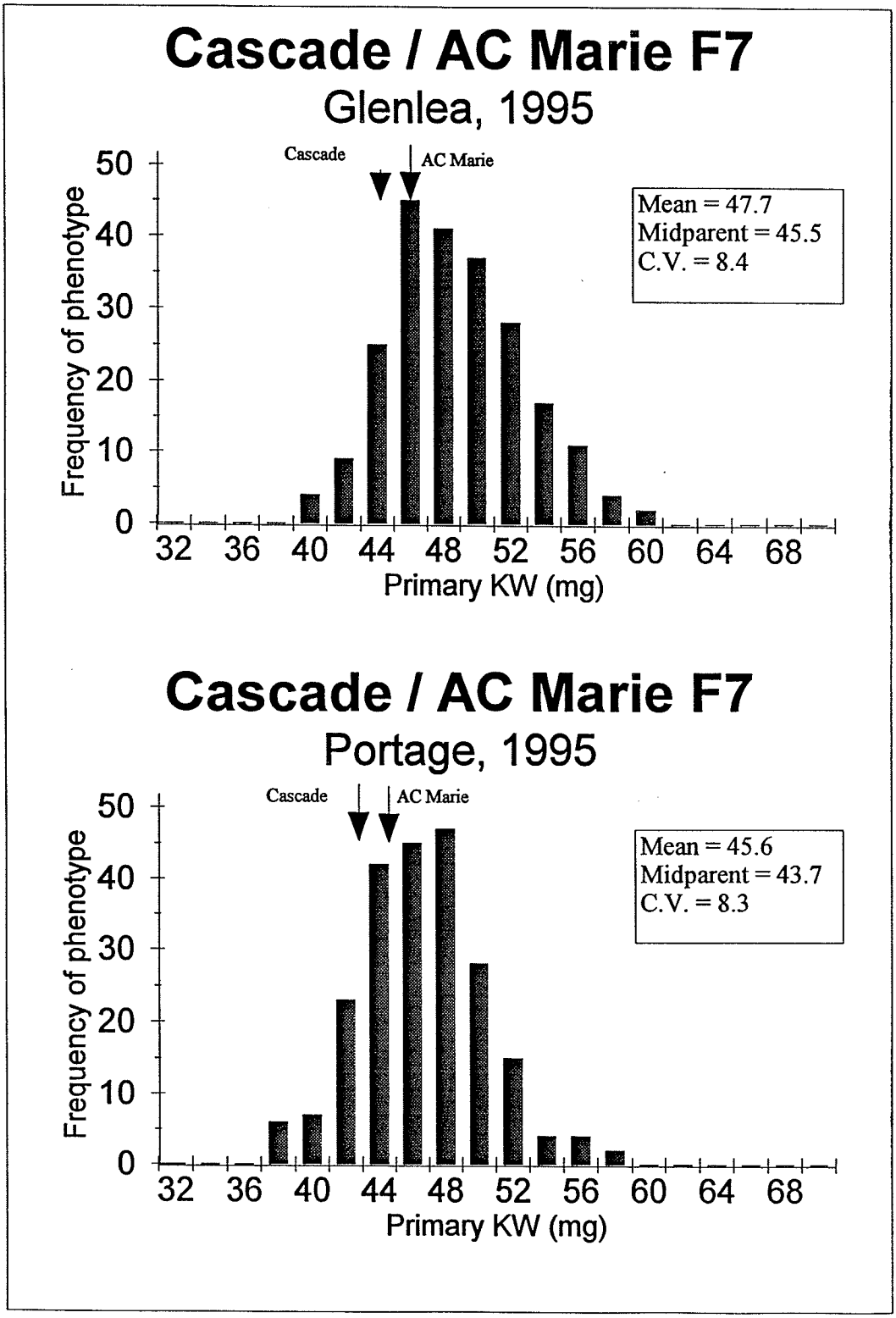

Figure A.8. Frequency distributions of primary kernel weight (mg) for F_7 progeny populations of Cascade/AC Marie grown at Glenlea and Portage la Prairie, Manitoba in 1995. Arrows indicate kernel weight of parental cultivars.

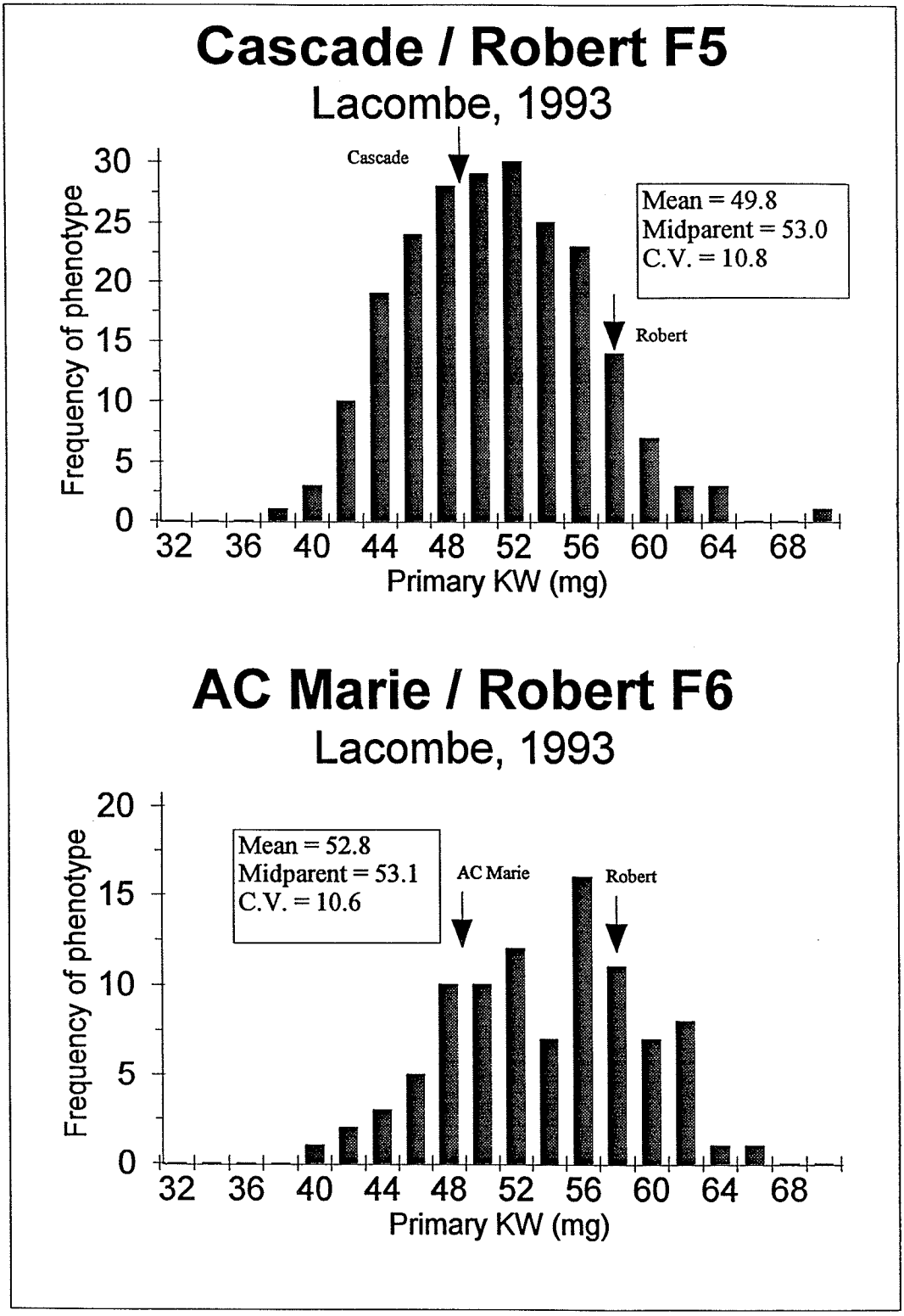

Figure A.9. Frequency distributions of primary kernel weight (mg) for F_5 progeny of Cascade/Robert and F_6 progeny of AC Marie/Robert grown at Lacombe, Alberta in 1993. Arrows indicate kernel weight of parental cultivars.