Quantitative genetics in apple (*Malus* x *domestica* (Borkh.)) breeding:

Fruit shape traits, genetic parameter estimation and breeding strategy development

A thesis presented in partial fulfilment of the requirements
for the degree of
Doctor of Philosophy in Animal Science (Breeding and Genetics)
at
Massey University
Palmerston North
New Zealand

Alastair John Currie

2000

Abstract

The aim of this thesis was to investigate some aspects of breeding for quantitative traits in apple. First, this study explored the measurement and inheritance of a complex quantitative trait (fruit shape). Fourier analysis was used to mathematically describe apple fruit shape in an objective manner and principal component analysis grouped the Fourier descriptors into meaningful shape traits. Heritabilities were estimated to determine genetically inherited shape traits, and genotype by environment interactions were estimated to determine the stability of the trait expression across environments. Fruit aspect ratio accounted for over 76% of the phenotypic variation in shape, fruit conicity for 6% and fruit squareness for only 2%. These traits had moderate to high narrow-sense heritabilities (0.79, 0.38 and 0.38 respectively), which indicated that individual selection would be efficient. High between-site genetic correlation ($r_A > 0.8$), indicated low genotype-by-environment interaction and suggests breeding based at one site would be efficient for altering shape at other sites. A shape chart was constructed to enable these three apple shape traits to be evaluated quickly and accurately in the field, avoiding the need for image capture and Fourier analysis.

Second, heritabilities and genetic correlations were estimated for a range of apple traits from a genetically broad-based apple breeding population of 71 families. Narrow-sense heritability was estimated at each of two sites and across sites for fruit acidity (0.17-0.22), fruit sugars (0.25-0.26), and tree growth habit (0.19-0.41). Higher heritabilities were estimated for fruit squareness (0.32-0.43), fruit conicity (0.32-0.46), powdery mildew incidence (0.40), vigour (0.28-0.62), fruit firmness (0.44-0.53), harvest time (0.66-0.82), leafing (0.60-0.83), fruit aspect ratio (0.74-0.89), flowering (0.92) and fruit size (0.90-1.01). Traits with a high narrow-sense heritability could be selected for efficiently with individual selection. High genetic correlation between sites ($r_A \geq 0.64$) indicated low genotype by environment interaction and that breeding based at one site would be efficient for improving these traits at other sites. High positive genetic correlations between traits at site 1 were estimated for leafing with flowering (0.95), harvest time with fruit firmness (0.92), leafing with harvest time (0.69), and fruit acidity with fruit firmness (0.61). High negative genetic correlations were reported for vigour with fruit squareness (-0.82) and tree growth habit with leafing (-0.53). Rapid gains can

be made with positively correlated traits, but high negatively correlated traits will make simultaneous gain in both traits difficult. Caution must be applied when interpreting genetic correlations between traits due to high standard errors and the possible influence of environmental interaction between traits (dependence) and linkage disequilibrium.

Third, the traditional apple breeding strategy (mass selection) was compared to an option based on recurrent selection for general combining ability (RS-GCA). The excessively long generation interval of the traditional breeding strategy meant negligible gains were made. However, the traditional breeding strategy generated greater gains per generation than the RS-GCA strategy but at a greater cost in terms of eroding genetic diversity, thereby reducing the potential for long-term gain. The slower rate of gain in the RS-GCA was due to the slower rate of gain in the breeding population. However, the RS-GCA strategy made greater gains in the cultivars relative to the breeding population despite similar selection intensities due to better utilisation of the between-family genetic variance as well as the within-family genetic variance.

Breeding strategies based on quantitative genetics have been applied with success to animal, crop and forest tree breeding, but only recently in apple breeding. It is clear that future breeding programmes would benefit from the application of quantitative genetic theory.

Acknowledgements

This thesis could not have been made possible without the patience, assistance and encouragement from a wide range of people.

I am grateful to Prof. Dorian Garrick (Massey University), my chief supervisor, for taking on yet another student in his heavy workload. I have appreciated his encouragement and guidance through the theory of breeding, and his clear, concise criticism for each project.

I am greatly indebted to Dr. Dominique Noiton (HortResearch) who was first my boss, then my supervisor, writing tutor and always a friend. Dominique developed the quantitative genetics programme and set up the scholarship to enable me to pursue full-time study. Throughout the last few years she has been a constant source of encouragement, guidance and motivation. I also appreciate the more recent efforts of Dr. Nnadozie Oraguzie (HortResearch), who supplied valuable advice on the writing and structure of the thesis as well as offering helpful suggestions for each chapter.

I am thankful for Dr. Tony Shelbourne's (Forest Research) invaluable input into every aspect of this thesis. He introduced me to the forest tree breeding theory that this thesis is founded on. I have also benefited from the input of other breeders at Forest Research (Dr. Mike Carson, Dr. Rowland Burdon, Dr. Luis Gea, Dr. Paul Jefferson, Simon Weaver, Dr. Keith Jayawickrama and Dr. Sue Carson). Their input has been educational, motivating and greatly appreciated.

This thesis would not have been possible without access to financial support, facilities and the apple breeding programme offered by The Horticultural and Food Research Institute (HortResearch), New Zealand. In particular I thank Dr Paul Glucina, Dr. Dominique Noiton and Dr. Nnadozie Oraguzie (management) and Madelein Hofstee and Janice Fraser (technical assistance).

Free access to Arthur Gilmour's ASREML program and helpful suggestions were appreciated for the estimation of genetic parameters. The maps were generated with the

map-making program available on the Charles Sturt University web site http://life.csu.edu.au/cgi-bin/gis/Map.

Throughout my time at Massey I have been lucky to have good friends and colleagues that have provided questions, answers, feedback and diversion! Thank you especially to Satish Kumar, Luis Apiolaza, Lisa Watson, Nicolás Villalobos and the rest of the postgraduates in room 2.02.

Although a PhD student has little spare time, I thank members of St Albans Presbyterian Church for stretching my horizons beyond the here and now. They have provided balance in my life and helped me to focus on what is really important.

Lastly I would like to give a special acknowledgement to my family, especially my wife, Deborah, for love and support throughout.

Two are better than one, because they have a good return for their work:

If one falls down, his friend can help him up.

But pity the man who falls and has no one to help him up!

Ecc 4: 9-10

Contents

CHAPTER 5: COMPARISON OF TWO BREEDING STRATEGIES IN APPLE (*M.* ×*DOMESTICA* BORKH.)

CHAPTER 6: GENERAL DISCUSSION

List of figures and tables

Chapter 2: Literature of apple breeding

Chapter 3: Quantitative evaluation of apple (Malus x domestica Borkh.) fruit shape by principal component analysis of Fourier descriptors

Chapter 4: Estimates of heritability and genetic correlation for apple (Malus x domestica Borkh.) traits

Chapter 5: Comparison of two breeding strategies in apple (Malus Xdomestica (Borkh.))

Chapter 1: Introduction

The most common method of apple improvement is to select parents with complementary qualities from the pool of commercial cultivars, cross them, select the best individuals from large progenies and then propagate them onto rootstock for clonal testing (Brown, 1975). This is a type of recurrent mass selection (Janick et al., 1996). With the international export of apples, long-term storage and economies of scale, a few internationally common cultivars hold most of the market share. These few cultivars have been used frequently as parents in breeding programmes, reducing the effective population size and increasing the levels of inbreeding (Noiton and Alspach, 1996).

Reducing the effective population size means fewer genes in the breeding population and a reduction in genetic diversity. Breeding for resistance to apple scab (*Venturia inequalis*) is an example of the outcome of reduced genetic variability in the breeding population. Major genes for resistance to scab have not been found within the breeding population, so interspecific crosses were necessary (Hough et al., 1953; Shay et al., 1953). Unfortunately, many generations of backcrossing to commercial varieties were required to simultaneously recover commercial quality and the disease resistance genes (Crosby et al., 1992). It would be preferable in the long term if a population was developed that had both genetic diversity (including disease resistance genes) and commercial tree and fruit quality traits, so that breeders could incorporate new genes in their breeding programmes within fewer generations.

Deliberately increasing inbreeding is generally avoided in apple breeding to prevent inbreeding depression. One expression of inbreeding depression in apple is a severe reduction of vigour leading to a considerable lengthening of the average juvenile period (Brown, 1973). Inbreeding also decreases the within-family genetic variance, decreasing potential gains and increasing the uniformity within a cross.

To address these concerns Noiton and Shelbourne (1992) proposed a breeding strategy, justified by quantitative genetic principles, to improve the diversity of parents available to apple breeders, to provide estimates of genetic parameters to aid future planning, and

to generate new cultivars. This strategy was based on recurrent selection for general combining ability (RS-GCA) (Allard, 1960).

The principal objectives of this thesis were to:

- review the development of the current apple breeding strategy to establish the composition of the modern apple breeding population
- define and measure a quantitative trait (apple shape)
- estimate genetic parameters for a wide range of agronomically useful traits and use these parameters to gain insight to efficient breeding programme design
- compare short and long-term predicted gains from the traditional apple breeding strategy and a strategy designed using quantitative genetic principles (RS-GCA) to exploit a genetically broad-based population

This thesis draws data from the first generation of an RS-GCA apple breeding programme (Noiton and Shelbourne, 1992) to define quantitative traits for apple shape, estimate genetic parameters for a range of quantitative traits and use these parameters to confirm the basis of this strategy and suggest minor modifications.

Summary of chapters

Chapter 2. Review of literature: Apple breeding strategy and objectives

This chapter is a review of the literature on the development of apple breeding, domestication of apple, current breeding strategies employed by apple breeders, breeding objectives and selection criteria utilised.

The review begins with a presentation of the current theories on the genetic and geographic origins of the Malus genus. Most of the evidence to date suggests that apples have allopolyploid origins and the center of origin was probably the mountains of Central Asia. The modern apple, *Malus* x*domestica* Borkh. arose from both intraspecific

and interspecific hybridisation. The subsequent history of apple development follows apple development from selection within wild forests of apples in Central Asia, through to the breeding strategies utilised by a modern apple breeder. The last sections deal with defining breeding objective traits and selection criteria for apple.

Chapter 3. Apple shape traits

This chapter was an exercise in defining, measuring and estimating genetic parameters for a quantitatively inherited trait. Few quantitative apple shape traits had been defined objectively and little was known about their heritability. Apple shape is one component of fruit quality, with consumers and marketers preferring conical or oval shapes rather than flat shapes (Janick et al., 1996).

Fourier analysis to mathematically describe shape and principal component analysis to group the Fourier descriptors into meaningful shape traits had been successfully applied to representative apple shapes before (Busscher et al., 1995; Paulus and Schrevens, 1999). The new approach taken in this thesis was to apply the analysis to individual apple shapes and to estimate the genetic parameters of heritability and genotype by environment interaction (GxE) for each trait.

The shape traits and the proportion of the total phenotypic variation they accounted for were: fruit aspect ratio (76.8%), asymmetric-crown (7.8%), fruit conicity (6.0%), asymmetric-sides (4.3%), and fruit squareness (2.0%). The asymmetric-crown trait was removed from the analysis due to confounding between the fruit preparation and shape, and the asymmetric-sides trait was excluded from the analysis due to heritability estimates close to zero.

Heritability was high to moderate for fruit aspect ratio (0.79), fruit conicity (0.38) and squareness (0.38), indicating that individual selection was adequate. Low GxE, indicated by high between-site genetic correlation (0.90, 0.82, 0.83 respectively), suggested that breeding at one site would be efficient.

A shape chart was constructed to enable these three apple shape traits to be evaluated quickly and accurately in the field. Utilisation of the shape chart to describe apple shape provides a simple tool for breeders to evaluate and rank apples for fruit aspect ratio, conicity and squareness.

Chapter 4. Estimating genetic parameters

Knowledge of genetic parameters such as heritability and genetic correlation is essential to making successful decisions in breeding strategies. Narrow-sense heritability determines the response to selection, genetic correlation between traits combined with heritability determines the multi-trait response to selection and GxE indicates the stability in ranking across sites.

Few studies with sufficient numbers of families have been undertaken in apple. In order to accurately estimate genetic parameters for the population, a minimum of 50 families (100 preferred) and 20 trees per family are required for estimation of heritabilities (White, 1996). This study used data from a genetically broad-based apple breeding population of 71 families to estimate narrow-sense heritabilities and genetic correlations with restricted maximum likelihood techniques, for mixed full-sib and half-sib analysis of open-pollinated apple progenies.

Heritability estimated from data at two sites and across sites for fruit acidity (0.17-0.22), fruit sugars (0.25-0.26), and tree growth habit (0.19-0.41) indicated high environmental or non-additive effects. Increasing the accuracy of heritability estimation by increasing the accuracy of selection or reducing the environmental variation were discussed. Higher heritabilities were estimated for fruit squareness (0.32-0.43), fruit conicity (0.32-0.46), powdery mildew incidence (0.40), tree vigour (0.28-0.62), fruit firmness (0.44-0.53), harvest time (0.66-0.82), leafing (0.60-0.83), fruit aspect ratio (0.74-0.89), flowering (0.92) and fruit size (0.90-1.01). Traits with a high narrow-sense heritability could be selected efficiently with individual selection. High genetic correlation between sites ($r_A \geq 0.64$) indicated low GxE and that selection at one site would be efficient for these traits. High positive genetic correlations were estimated for leafing day with

flowering day (r_A = 0.95), picking day with penetrometer reading (r_A = 0.92), leafing day with picking day (r_A = 0.69), and fruit acidity with penetrometer reading (r_A = 0.61). High negative genetic correlations were reported for trunk cross section area with fruit squareness shape (r_A = -0.82) and tree habit with leafing day (r_A = -0.53). Rapid gains can be made with positively correlated traits, but high negatively correlated traits will show have slow gains. Care interpreting genetic correlations between traits was advised due to high standard errors (SE ≤ 0.20) and the possible influence of linkage disequilibrium due to non-random mating and dependency between trait expression.

Chapter 5. Apple breeding strategy

Traditional apple breeding strategy, based on mass selection, was compared to a recurrent selection for general combining ability (RS-GCA) to determine the short-term and long-term gains. Population composition, mating design and the selection process in each strategy were examined to determine likely effects on long-term genetic gain, genetic diversity and inbreeding. Gains from 3 generations of each strategy were simulated for a fixed resource of 30,750 trees and at a narrow sense heritability of 0.2 and 0.6 to determine the short-term gain from each strategy. Annual gains for the traditional apple breeding strategy were minimal due to an average of more than 100 years to test cultivars before inclusion in a breeding programme.

Breeding population gain per generation was higher for the traditional strategy due to higher selection intensity. In general cultivar gain per generation was also higher for the traditional strategy (apart from the first generation at h^2 = 0.6) due to higher selection intensity. However, relative gains were higher for the RS-GCA strategy due to balanced use of within-family and between family variation despite similar selection intensities. Reduction in long-term gain is likely for traditional apple breeding strategy due to the erosion of genetic diversity and the increased inbreeding, so a breeding strategy like RS-GCA is required for long-term sustainability of gain in apple breeding.

References

Allard, R. W. 1960. *Principles of plant breeding.* John Wiley & Sons, London.

Brown, A. G. 1973. The effect of inbreeding on vigour and length of juvenile period in apples, p. 30-39. *In* A. G. Brown, R. Watkins and F. Alston (eds.), *Proceedings of Eucarpia Fruit Section Symposium V. Top Fruit Breeding. Canterbury, Sept. 11th-14th 1973.* Eucarpia, Canterbury.

Brown, A. G. 1975. Apples, p. 3-37. *In* J. Janick and J. N. Moore (eds.), *Advances in fruit breeding*, 1st ed., vol. 1. Purdue University Press, West Lafayette, Indiana.

Busscher, R. de, E. Schrevens, and J. de Baerdemaeker. 1995. Automated characterisation of apple shapes using digitised video images. *JSAM 1995 International symposium on automation and robotics in bioproduction and processing. Kobe, Japan.*

Crosby, J. A., J. Janick, P. C. Pecknold, S. S. Korban, P. A. O'Connor, S. M. Ries, J. Goddfreda, and A. Voordeckers. 1992. Breeding apples for scab resistance:1945-1990. *Acta Horticulturae*, 317:43-70.

Hough, L. F., J. R. Shay, and D. F. Dayton. 1953. Apple scab resistance from *Malus floribunda* Sieb. *Proceedings. American Society for Horticultural Science*, 62:341-347.

Janick, J., J. N. Cummins, S. K. Brown, and M. Hemmat. 1996. Apples, p. 1-77. *In* J. Janick and J. N. Moore (eds.), *Fruit Breeding: Tree and Tropical Fruits*, vol. 1. John Wiley & Sons, New York.

Noiton, D. A. M., and P. Alspach. 1996. Founding clones, inbreeding, coancestry and status number of modern apple cultivars. *Journal of the American Society for Horticultural Science*, 121:773-782.

Noiton, D., and C. J. A. Shelbourne. 1992. Quantitative genetics in an apple breeding strategy. *Euphytica*, 60:213-219.

Paulus, I., and E. Schrevens. 1999. Apple shape characterization by Fourier expansion of digitized images. *Journal of Agricultural Engineering Research*, 72:113-118.

Shay, J. R., D. F. Dayton, and L. F. Hough. 1953. Apple scab resistance from a number of *Malus* species. *Proceedings. American Society for Horticultural Science*, 62:348-356.

White, T. L. 1996. Genetic parameter estimates and breeding value predictions: issues and implications in tree improvement programs, p. 110-117. *In* M. J. Dieters, A. C. Matheson, D. G. Nikles, C. E. Harwood and S. M. Walker (eds.), *Tree Improvement for Sustainable Tropical Forestry*, Proc. QFRI-IUFRO Conf. 27th Oct - 1st Nov 1996. IUFRO, Caloundra, Australia.

Chapter 2: Literature review of apple breeding

Introduction

This chapter reviews the literature on the development of apple, the domestication of apple, the breeding strategies, breeding objective traits, and selection criteria currently employed by apple breeders. The review begins with a presentation of the current theories on the genetic and geographic origins of the *Malus* Mill. genus. Although the exact origins are still debated, the evidence to date is presented. The next section outlines the current habitats of the apple species involved in the development of the modern apple, *Malus* ×*domestica* Borkh., providing insight to the diverse genetic and environmental range of the ancestors of *M.* ×*domestica.* The subsequent history of apple development follows through to the strategies utilised by a modern apple breeder. The next section describes the selection criteria and breeding objectives applied to apple and a discussion of selection indices.

Genetic origin of the apple

Taxonomy of apple

Apples belong to the Rosaceae family, which contains many horticulturally important crops ranging from stonefruit, pipfruit, roses and other ornamental shrubs and trees. The Rosaceae family is divided into subfamilies, genera, and species. The Maloideae (originally Pomoideae) subfamily contains the apple genus, *Malus*, as do the genera *Pyrus* (pears), *Cydonia* (quince), and ornamental trees and shrubs such as *Cotoneaster*, *Pyracantha*, *Crataegus*, *Sorbus*, *Aronia*, *Chaenomeles* and *Amelanchier* (Challice, 1974). The Maloideae have a haploid chromosome number of 17, but in contrast the other subfamilies have chromosome numbers ranging from 7 to 9. The ancestors of Maloideae were either autopolyploids, formed by the multiplication of their own chromosomes, or autopolyploids formed from the multiplication of two genetically different sets of chromosomes.

Most of the evidence from cytology and morphology (Dermen, 1949), flavone analysis (Challice, 1974) and isozyme analysis (Chevreau et al., 1985; Chevreau and Laurens,

1987; Weeden and Lamb, 1987) favoured the allopolyploid theory. It was proposed that the Maloideae subfamily developed from hybridisation between a Spiraeoideae ancestor (x=9) and either a Prunoideae ancestor (x=8), or another Spiraeoideae ancestor (x=8), followed by fusion of unreduced gametes to form fertile organisms. A possible scheme for the evolution of the Rosaceae subfamilies, including Maloideae (also called Pomoideae), was presented by Challice (1974) (Figure 1). DNA sequence data has begun to contribute to the understanding of apple taxonomy, but has focussed on elucidating the relationships within the *Malus* genus.

Species within the Malus genus

Species division within the *Malus* genus is not clear, partly due to the wide diversity within a species and partly due to the ease of interspecific hybridisation (Korban, 1986). The classification of apple species frequently changes and species originating from interspecific hybrids are common. The commercial apple, *M.* ×*domestica*, is a species hybrid with a complex history of interspecific and intraspecific hybridisation. The correct nomenclature for the domestic apple includes an '×' immediately before the species name to denote an interspecific origin (Korban and Skirvin, 1984).

The nomenclature used in this thesis is as follows:

Full name	Abbreviation
Malus ×*domestica* Borkh.	*M.* ×*domestica*
Malus ×*floribunda* Sieb. Ex Van Houtte	*M.* ×*floribunda*
Malus ×*robusta* (Carr.) Rehd.	*M.* ×*robusta*
Malus ×*zumi* (Matsum.) Rehd.	*M.* ×*zumi*
Malus baccata (L.) Borkh.	*M. baccata*
Malus cerasifera Spach.	*M. cerasifera*
Malus halliana	*M. halliana*
Malus hupehensis	*M. hupehensis*
Malus mandshurica (Maxim.) Komarov	*M. mandshurica*
Malus micromalus	*M. micromalus*
Malus orientalis Uglitzk. Ex Juz.	*M. orientalis*
Malus prunifolia (Willd.) Borkh.	*M. prunifolia*
Malus pumila Mill.	*M. pumila*
Malus sieboldii (Reg.) Rehd.	*M. sieboldii*
Malus sieversii (Lindl.) Roem.	*M. sieversii*
Malus sylvestris (L.) Mill.	*M. sylvestris*
Malus tschonoskii	*M. tschonoskii*

M. ×*domestica* was considered by some to have originated from *M. pumila*. However, Ponomarenko (1975, 1983) stated that *M. pumila* was a subset of *M. sieversii* brought to ancient Greece and Rome from central Asia, and that since European scientists were unable to study this species in the wild, they erroneously classified them as a separate species, *M. pumila*. Ponomarenko recorded that the majority of *M.* ×*domestica* varieties arose from inter-varietal hybridisation. Later on *M. baccata*, *M. sylvestris*, *M.* ×*floribunda*, *M.* ×*zumi* were involved in the formation of the domestic apple (Ponomarenko, 1983). *M. orientalis* and *M. mandshurica* may also have had a role (Morgan et al., 1993). Spontaneous as well as artificial inter-species hybridisation, mutations and polyploidy have all played a role in the evolution of *M.* ×*domestica* (Way et al., 1989; Janick et al., 1996).

Watkins (1976) suggested that the ease of inter-specific hybridisation in apple may be due to recent geographic isolation of species, and hence not a large genetic differentiation between them. A classification presented by Sax (1931) suggested that, on the basis of genetic and cytological evidence, the existing genera of the Maloideae subfamily could be reclassified as species under a single genera. In contrast Ponomarenko classified 78 wild species and 44 hybrid species with many previously reported species being either hybrids or synonyms of valid wild species (Ponomarenko, 1986). Other classifications were presented by Rehder (1954) (in Watkins, 1976) who listed 25 species of apple and Way *et al.* (1989), who has designated 33 species of apple. Relationships within the *Malus* genus have begun to be elucidated with studies using DNA sequence data (Kato et al., 1993a, b; Dunemann, 1994; Campbell et al., 1995; Matsumoto et al., 1997; Juniper et al., 1999).

Although the exact classification of species of apples has not been finalised, it may be more important for apple breeders to be aware of the great diversity within *Malus* and to recognise that there are few barriers to interspecific hybridisation, rather than be concerned about the exact taxonomy.

Geographic distribution of *M.* × *domestica*

Species of apple can be found in a wide range of environments across the temperate regions of Europe, Asia and North America (Figure 2). A "centre of origin" is the region where the species originated from, usually indicated by increasing diversity. A "centre of diversity" is a region where the species is found in great diversity but may not necessarily indicate the centre of origin.

On the basis of historical, botanical, morphological, and geographical evidence, the centre of origin for *M. sieversii,* the ancestor of the domestic apple, is considered to be the mountainous region of Central Asia (the Tian Shan and Pamir Alai ranges) (Ponomarenko, 1980a; Li, 1989) (Figure 3). Ponomarenko stated that with the distribution of apples by people, secondary centres of diversity have arisen in which new intra- and inter-specific hybrid populations developed. These

centres were the East European (USSR), West European (UK, France and Germany) and more recently a large secondary centre of diversity has arisen in North America. The domestication of apple is charted below (Figure 4).

The origin and a brief description of the species involved in the domestication of apple is listed in Table 1.

Breeding strategies for apple

The history of apple is interwoven with the history of humankind, and so the rise and fall of apple culture closely follows the rise and fall of civilisation. However, in terms of the development of breeding strategy most of this fascinating historical material has little relevance. The purpose of this section is to report on aspects of apple cultural history that have direct impact on the development of apple breeding strategy and to describe the breeding strategies applied to apples.

Initial breeding strategies

Records of human use of apples date to the beginning of civilisation, during the Neolithic period (c8000BC), when people gave up a nomadic lifestyle and started to practice agriculture (Roach, 1985). Roach reported that apple remains dated to 6500BC at Catal Huyuk (Turkey), remains of both large-form apples and crab apples preserved by drying found at a prehistoric Swiss settlement, and impressions of apple pips in stones in England suggested that apples have been used by prehistoric humans for food. These apples were *M. sylvestris*, the European Crab, which was widely distributed throughout Europe, and *M. sieversii*, originating in the mountains of Central Asia but also found in parts of Europe.

The earliest human selection strategy for apple populations was probably simply selecting good phenotypes and saving these trees when forests were cleared for farming, a practice is still observed around the apple forests in central Asia today (Ponomarenko, 1983). Apples were distributed by seed to the Fertile Crescent, Persia and parts of Europe by invading tribes from the Caucasus, a centre of diversity for apple, c4000 BC

(Roach, 1985) (Figure 4). Apples are highly heterozygous and the quality of seedlings would have been extremely variable. The best could be eaten fresh but many would have needed to be cooked or used for cider.

Morgan *et al.* (1993) reported that grafting was discovered c3000 BC, which would have had a major impact on selection and breeding strategies for apple. Fixing the genotype by grafting would have increased the quality of apple orchards, as only the best cultivars would have been propagated rather than the progeny of the best cultivars. Distribution of grafts rather than seed meant a lower overall diversity in the cultivars grown. Propagation by grafting rather than by seed would have lengthened the breeding cycle, as good cultivars would have been repeatedly used for many generations.

Trade developed along routes that stretched from the Aegean Sea to Northern India and from the Caucasus to Egypt spreading the cultivation of apple through much of Europe and into Asia. The Persian (550 - 247 BC), Macedonian (750 - 146 BC) and Roman (146 BC - 476 AD) civilisations embraced the cultivation of apple (Roach, 1985; Morgan et al., 1993; Anon., 1998). The regional differences in environment, the relatively few cultivars reported from that era (Ponomarenko, 1983), the subsequent disruption of distribution networks and loss of grafting skills following the collapse of the West Roman Empire lead to regional differentiation of apple cultivars (Brown, 1975).

Monasteries (developed around 300-500 AD in the West) and later Islamic horticulturists became the preservers, developers and distributors of horticultural knowledge. Grafting skills were rediscovered but little progress was made in the development of new apple cultivars until the 1600s, when America and Canada were settled and apple orchards were established once more by seed. By the late 1800s apples were widely distributed around the world (Figure 4). The Dutch took apples to South Africa in 1654, whereas the English planted apple trees in Australia in 1788 and in New Zealand in 1814. Although China has a wide range of native apple species (Zhang, W. et al., 1993; Tao et al., 1995) western cultivars were planted in the late 1800s and have replaced native species as a commercial crop.

Controlled pollination

A major change in the mating strategy of apple breeding was the introduction of controlled pollination to produce seeds with a known pedigree. Thomas Knight, a West Midlands Squire (UK), erroneously concluded that the poor health in apples in England was due to old age (Bultitude, 1983; Morgan et al., 1993). In fact the decline in vigour was either due to the build-up of viruses (Morgan et al., 1993) or susceptibility to apple canker (*Nectria galligena*) (Bultitude, 1983). However, this hypothesis lead him to the discovery and use of controlled pollination to produce new cultivars.

Knight's discovery should have increased the rate of genetic gain from the selection of both parents and lead to an age of commercial cultivars developed by controlled pollination. In the first half of this century hundreds of cultivars were selected from controlled pollinated progenies in the US, but only two were a commercial success (Janick et al., 1996). Most of the commercial cultivars continued to come from chance seedlings found near orchards (Dorsey, 1917; Janick et al., 1996). Janick *et al.* attributed the poor performance of controlled-pollination to poor selection of parents. Although the relative performance of controlled pollination compared to open pollination or chance seedlings has improved (Janick *et al.* 1996), the poor performance of control-pollinated progeny suggests that either breeders were making poor choices for parents or that breeders lacked the information necessary to make informed choices.

Current breeding strategies

Since Thomas Knight's discovery of controlled pollination apple breeding strategies have multiplied to cover a wide range of strategies. This section briefly reviews the breeding strategies applied to apple breeding.

Recurrent mass selection

Recurrent selection was defined by Allard (1960) as an iterative process of inter-crossing selected parents followed by selection of the next generation's parents from the progeny (Allard, 1960). In recurrent mass selection (also called simple recurrent or mass selection), selection was based on individual phenotype or average of selfed progeny. Because of the long-term nature of apple breeding, recurrent strategies have not been

consciously applied in apple breeding (Bosemark, 1993). However, several researchers have suggested that apple breeders have unconsciously adopted recurrent mass selection (Lyrene, 1981; Bringhurst, 1983; Morgan et al., 1993). The most common breeding strategy used by apple breeders is to select parents from commercial cultivars with the desired complementary characteristics, cross them, and then select individual phenotypes within large full-sib families to test on rootstock for commercial release (Janick *et al.* 1996). This strategy can be justified as "mass selection" because individual selections from the progeny of cultivars become future cultivars, and "recurrent" because, although there is considerable overlap between generations in apple breeding, eventually old cultivars are dropped from the breeding population and replaced by new cultivars.

Recurrent selection for GCA (RS-GCA)

The 3 remaining recurrent selection strategies categorised by Allard (1960) all used selection based on progeny testing for combining ability rather than individual selection as in recurrent mass selection. Combining ability is a measure of the expected performance of a parent. There are two types of combining ability. General combining ability is the average value of progeny for a parent from a large number of random crosses, expressed as a deviation from the population mean. In any particular cross, the expected value of the progeny is the sum of the general combining abilities of the parents, plus the specific combining ability due to that cross. The specific combining ability is the deviation of the average progeny in that cross from the general combining ability of the parents.

The differences between the three classes of recurrent selection, defined by Allard, were in the types of tester used for the progeny test. In recurrent selection for general combining ability (RS-GCA) a genetically broad-based tester was used, in recurrent selection for specific combining ability a genetically narrow-based tester was used and for reciprocal recurrent selection two populations were used to test each other for specific and general combining ability.

Forms of RS-GCA have been widely applied in animal breeding (Falconer, 1989; Roden, 1994), forest tree breeding (Shelbourne, 1969; Namkoong et al., 1988) and

recently in apple breeding (Noiton and Shelbourne, 1992). Selection for GCA, rather than selection for phenotype, increases the frequency of desirable alleles in the breeding population and increases the probability of selecting a superior cultivar. The theoretical limit to selection with RS-GCA is the sum of the desirable alleles in the base population (Allard, 1960; Falconer, 1989). This limit may not be attained due to random fixation of alleles through genetic drift or inbreeding. Maximising genetic diversity through a genetically broad-based population in conjunction with within-family selection would ensure that the limits of selection are maximised (Dempfle, 1975).

Apple breeders (Noiton and Shelbourne, 1992), and other fruit breeders (Bringhurst, 1983; Hansche, 1983; Gallais, 1986; Kronstad, 1986; Bosemark, 1993) have suggested that a long-term breeding strategy with low or moderate selection intensity such as RS-GCA, is essential to maximise long-term gains in a breeding programme. However, Bringhurst (1983) noted that many long-term breeding strategies failed to deliver because of a lack of financial backing to sustain the programme. Watkins (1974) also stated that breeding programmes with long-term objectives were likely to fail due to staff turnover and inflexibility to adapt to changes in the fruit industry requirements. Gallais (1986) suggested that the addition of a short-term strategy provided an answer to these problems by producing output to justify the continuation of the long-term programme, and to provide flexibility in breeding objectives. It is common for RS-GCA strategies to be part of a more complex breeding programme to cater for both long and short-term goals. The breeding strategy proposed for apples by Noiton and Shelbourne (1992) uses RS-GCA to develop a breeding population to ensure long-term gains, and a cultivar production population to exploit opportunities for short-term gains.

Inbreeding

Inbreeding is the mating of related individuals (Allard, 1960). The effect of inbreeding is to increase the likelihood that two alleles at a locus are identical by descent (homozygous). The inbreeding co-efficient (F) measures this likelihood. 'Co-ancestry' is another way to express inbreeding and is defined as the level of inbreeding that will result from planned crosses. Inbreeding is a tool used by some breeders to fix traits, to fix the genotype, eliminate undesirable recessives or to increase total variation. The effects of inbreeding on genetic variance can be partitioned into within-family and

between-family effects. Within-family genetic variation is reduced by a factor of (1-F), and between-family genetic variation is increased by a factor of $2F$ (assuming no dominance or interaction variation) (Falconer, 1989). With no selection, total genetic variation is increased, but inbreeding combined with individual selection (mostly within-family selection) would reduce the overall genetic variation.

Although Bultitude (1983) claimed that inbreeding was unconsciously used as a tool in apple breeding to emulate the success of current commercial cultivars, and Janick *et al.* (1996) also has suggested that mild inbreeding could be used as a tool to reduce tree size and vigour, thereby reducing labour input for picking, thinning and pruning, inbreeding is generally avoided in apple breeding. High levels of inbreeding in apple cause problems with pollen incompatibility and with inbreeding depression. A fruit set of 2% (Brown, 1975) and 1.5% (Janick et al., 1996) for self pollinated apples, down from 7%. Inbreeding depression, the reduction in performance due to the expression of deleterious recessives, is marked in apple. Although seeds from outbred apples have rate of germination close to 100%, Brown (1975) reported that only 30% of the seeds from self-pollinated trees germinated and many died or were weak. Brown (1973) has also shown that inbred apple seedlings have less vigour and a subsequent lengthening of the juvenile period.

Noiton and Alspach (1996) studied the pedigree of modern cultivars to determine the genetic diversity and levels of inbreeding in apple. They found 64% were derived from 5 common founding clones. 23% were derived from 'McIntosh', 20% derived from 'Golden Delicious', 17% from 'Jonathan', 13% from 'Cox's Orange Pippin' and 13% from 'Red Delicious'. Some cultivars featured the same parent more than once in the pedigree. A measure of diversity, the status number (half the inverse of the coancestry), is equivalent to the number of unrelated, non-inbred individuals in an ideal panmictic population that would produce progeny with the same coefficient of inbreeding as the population under study after random mating (Lindgren et al., 1996, 1997; Gea, 1997). Noiton and Alspach found the status number for 50 mainstream apple cultivars was 8, indicating a low genetic diversity in apple breeding populations. Although Noiton and Alspach (1996) found levels of inbreeding were low in apple, the level of coancestry

indicated future levels could be high if current breeding practices were continued, leading to further losses of genetic diversity.

Modified backcross

The modified backcross strategy has been used to introgress genes for disease resistance into commercial apple cultivars. Although there were cultivars with disease resistance within *M.* ×*domestica*, this disease resistance was inherited polygenically, and not simply inherited (Spinks, 1936; Brown, 1959; Williams and Brown, 1960; Visser et al., 1974; Lespinasse and Paulin, 1990). Polygene complexes were easily broken up through sexual recombination, so few progeny are likely to inherit the full complement of resistance genes in a cross between resistant and susceptible parents. In contrast, traits determined by single genes follow Mendelian segregation ratios with potentially higher proportion of the progeny inheriting the trait. Single (major) genes have been found primarily in disease resistance (Hough et al., 1953; Shay et al., 1953; Knight and Alston, 1968), but also for other traits like fruit acidity, russeting, pollen incompatibility, habit etc. (Brown, S., 1992).

A backcross sequence starts with a cross between a recurrent parent and a donor parent. Progeny are screened for the donor gene of interest then back-crossed to the recurrent cultivar. The cycle is repeated until all the recurrent parent genes are recovered, plus the donor gene of interest. The resulting cultivar would be highly inbred, so a modification used in apple breeding has been to use a different commercial cultivar for each cross instead of the same recurrent parent, enabling the progeny to be upgraded without increasing the levels of inbreeding. This strategy has been applied to apple disease resistance programmes (Hough et al., 1953; Alston, 1970a; Watkins, 1974; Baxter and Heaton, 1986; Crosby et al., 1992; Schmidt, 1994; Kruger, 1995; Tancred et al., 1995).

Marker assisted selection

Marker assisted selection (MAS) is selection based on isozyme or DNA markers, linked to traits of interest. Markers can either be linked to qualitative trait loci (major genes) or to quantitative trait loci (QTL). Early research into markers in apple have focussed on qualitative traits, probably because many apple traits of agronomic importance are qualitative (Brown, 1992; Janick *et al.*, 1996).

Isozyme markers have been linked to the self-incompatibility gene (Manganaris and Alston, 1987), pale green lethal gene (Manganaris and Alston, 1988), apple scab resistance gene (V_f) (Manganaris et al., 1994), habit and flowering genes (Lawson et al., 1995). Although isozymes are excellent markers, they are not numerous enough to provide breeders with markers for all traits, nor numerous enough map the genome for geneticists, so recent research has turned to DNA markers. DNA markers have been found for fruit colour, tree habit and sucker formation (Weeden et al., 1994), powdery mildew resistance (Markussen et al., 1995; Dunemann et al., 1999) and apple scab resistance (Gianfranceschi et al., 1996; Cheng et al., 1998; Tartarini et al., 1999).

The European apple genome mapping project was set up to co-ordinate the work on mapping of the apple genome and the search for major gene markers (King et al., 1991; Gardiner et al., 1994; King, 1994, 1996; Maliepaard et al., 1998). Although little work has been reported on quantitative trait loci (QTL) in apple yet, Maliepaard *et al.* (1998) suggested that linkage maps for major genes in apple could also be used to screen for QTL. Research on the use of QTL marker assisted selection (MAS) has been applied in experimental animal breeding programmes (Meuwissen and Arendonk, 1992) and commercial programmes (Spelman and Bovenhuis, 1998). Tomato breeders have also used QTL markers to improve the efficiency of selection (Tanksley and McCouch, 1997). In the near future MAS may become more common place.

There are several applications of MAS that could benefit the breeding of apple:

1. Early selection of traits. Adult traits could be screened during the juvenile phase. Many seedlings could be culled early in the programme so that resources could be focussed on the trees with the desired genotypes. Examples suggested in the literature include components of fruit flavour (Alston et al., 1996), fruit quality traits, harvest maturity, low temperature tolerance (Gardiner et al., 1994), and powdery mildew resistance (Janse et al., 1994; Markussen et al., 1995). Some of these traits are more likely than others to be good candidates for M.A.S. For example the high cost of phenotyping cold tolerance and the complexity of the flavour trait may reduce the feasibility of M.A.S.

2. Selecting multiple genes for one trait. Traditionally the expression of each gene was revealed by progeny testing. An example of the need for multiple genes is apple scab (*Venturia inequalis*) resistance. Many monogenic scab-resistant apple cultivars have shown susceptibility when inoculated by a new race of *V. inaequalis*, showing the vulnerability of monogenic resistance to pathogen mutation (Parisi et al., 1994). Cultivars with additional genes for resistance would have a much lower probability of becoming susceptible to the pathogen through pathogen mutation. Markers for each resistance gene can be used to detect multiple genes without the necessity of expensive test crosses (Gianfranceschi et al., 1996; Cheng et al., 1998; Durel et al., 1999; Tartarini et al., 1999).

3. Selecting traits that are too expensive to measure directly (e.g.: storage).

4. Selecting transformed plants after gene transfer (see Gene transformation, page 20).

5. Improving the efficiency of the backcross strategy (see Modified backcross, page 18), reducing the number of generations required for a backcross strategy (Tanksley, 1983; Tanksley and Nelson, 1996; Tanksley and McCouch, 1997). These authors used quantitative trait loci (QTL) and major gene markers to identify the origin of the DNA in the progeny, and to select seedlings with the donor gene of interest but a minimum of the rest of the donor genome. The pattern or mosaic of DNA due to recombination becomes more complex with each generation so early selection with DNA marker techniques maximised the recovery of the recurrent parent genome and reduced the number of generations required to introgress the donor gene. Apples would be suited to this strategy as the generation interval is 3-5 years, and any strategy that reduced the number of generations required would greatly reduce the cost of the programme.

Gene transformation

Gene transformation or the artificial transfer of genes from one organism to another is another strategy applied to manipulate the genes of apple. Transformation could bypass the need for generations of back-crossing (see Modified backcross, page 18) to introgress genes into a commercial cultivar, and in the case of gene transfer between

different taxa, may be the only option. However, the techniques for transforming apples have not been commonly used and so this strategy has few applications in the literature (Puite and Schaart, 1996). Gene transformation has been successfully applied to transfer the genes for fireblight (*Erwinia amylovora*) resistance into pear cultivars (Chevreau, 1998), rooting performance genes into apple rootstocks (Welander, 1998) and marker genes have been successfully transferred in apple cultivars (Puite and Schaart, 1996; Koller et al., 1998). Although gene transformation has potential in apple breeding strategies, recent consumer resistance to products of genetically modified organisms may reduce the usefulness of gene transformation (Hioux, 1998).

Mutation breeding

Instead of transferring genes from other organisms through gene transformation or back-crossing, another strategy has been to select or induce mutations to change existing traits. Mutations occur naturally, but can also be induced with radiation. Desirable changes such as increased size or increased colouring are selected. Most naturally-occurring sports have been discovered by growers, who find a branch yielding fruit distinct from the original. The plant patent laws in most countries enable distinct, stable and uniform sports to be released as a new cultivar (Whitmore, 1992). The possibility of extracting royalties from the sale of a patented sport has made the selection or inducement of mutations an attractive alternative to the more resource hungry plant breeding.

Types of mutations utilised in apple breeding fall into two groups: changes to one or more traits or changes in ploidy. Mutations that effect only some of the 3 layers of tissue form a chimera, plants that are a combination of genotypes. As the gametes arise from the sub-epidermal layer (L2), mutations arising in the other layers are not passed on to the progeny (Brown, 1975). Mutations of agronomically important traits in apple currently exploited include increased red fruit colouring, russeting of the fruit, spur type or compact tree habit, changes in flowering time or harvesting time. Many apple breeding programmes have incorporated mutation breeding as a strategy to improve existing cultivars (Faedi and Rosati, 1985; Decourtye et al., 1986; White, 1988; Quinlan and Tobutt, 1990; Spiegel-Roy, 1991; Lespinasse, 1992; Paprstein et al., 1994).

Another type of mutation is change in ploidy, or change in the numbers of the base chromosome set (n). Although apples may have evolved from polyploids (see Genetic origin of the apple, page 8), they are mostly functional diploids (2n) with a base set of chromosomes n = 17. Triploids, tetraploids, pentaploids and hexaploids are also found (Way et al., 1989; Janick et al., 1996). Polyploids may arise from fertilisation involving one or two unreduced gametes, from a cross between trees of different ploidy levels, or be artificially induced with mutagenetic chemicals such as colchicine. Some tetraploids produce larger, flatter and more irregularly shaped fruit, with larger, rounder leaves, and the tree has more vigour, thicker shoots, with a wider spreading habit (Brown, 1975). Brown (1975) stated that the irregular fruit shape and thick, short internodes meant that tetraploids had little promise as commercial apple cultivars. In contrast triploids show more promise as they have a more open tree habit and more regular fruit shape than tetraploids and have more vigour and larger fruit than diploids. Triploids have infertile pollen and require a source of diploid pollen to set seed. Despite this few fertile seeds are produced (due to chromosome imbalance following meiosis). Brown (1975) also reported that triploids resulting from unreduced gametes were deemed superior to triploids from crosses between tetraploids and diploids, probably due to the minimal recombination of the genes from the unreduced gamete (which was usually a high quality commercial cultivar). Watkins (1974) suggested that fertilisation of an unreduced gamete from a commercial variety with the haploid pollen from a crabapple species with multiple disease resistance would be a good way to introduce a more robust disease resistance than introgression of single genes, whilst retaining most of the commercial qualities of the diploid gamete parent. Natural triploids occur at a low frequency in apple (1 in 1000) and can be selected by screening for large seeds and large seedling leaf size (Brown 1975).

Although both polyploids and single trait mutations may have a role in enhancing the performance of commercial cultivars, the relatively small genetic changes in single trait mutations and the long juvenile period and complex hybridisation compatibility of polyploids may limit the role of mutation breeding in a multi-generation breeding programme.

Breeding objectives and selection criteria for apple

What are breeding objectives and selection criteria?

Within a breeding programme, trees need to be evaluated and ranked for selection. In order to do this, breeding objectives need to be determined. The overall breeding objective is generally to maximise the profitability of the system, but this objective needs to be partitioned into measurable breeding objective traits. Harris *et al.* (1984) suggested that the first step was to describe the production system, being as numerically specific as possible, and including all the traits that influence the profitability of the system. Selection criterion are the measurements made for each breeding objective trait. In some cases the breeding objective could be the same as the selection criterion (e.g.: fruit weight), but in some cases the selection criteria may be different from the breeding objective. For example, a breeding objective could be to reduce the period between grafting and cropping, but early selection using length of the juvenile period (positively correlated) may be adopted (Brown, 1975).

Multiple trait selection in apple improvement

Apple breeding programmes select for more than one trait by defining independent culling levels for each trait, beyond which all trees are culled (Brown, 1975; Janick et al., 1996). In selection for commercial cultivars this approach is justified as individuals that fall below the threshold in any one trait will not be suitable for commercial production. However, Hazel (1943) showed that selection with the Smith-Hazel multiple trait selection index was more efficient in terms of gain towards the ideal or aggregate genotype. The Smith-Hazel index would be better in a breeding population where improvement is cumulative over several generations, where-as independent culling levels could be used in a short-term cultivar production population.

Development of breeding objectives and selection criteria in apple

A systematic study of breeding objectives for apple has not been carried out. Rather, they have evolved over time, becoming more alike with the internationalisation of the

apple market. For example, American horticultural societies and the American Pomological Society encouraged the selection of cultivars on the basis of commercial qualities such as yield, appearance (Morgan et al., 1993), sweetness and dual purpose (fresh eating and cooking) (Nienhuis et al., 1995). In contrast in the UK the Royal Horticultural Society, estate owners and head gardeners encouraged and pursued the selection of apples on the basis of diversity in flavour, appearance and harvest dates, and largely ignored commercial qualities (Morgan et al., 1993). The commercial thrust of selection in America lead to a brighter coloured, sweeter apple that took the market share from apples grown in England and Europe. In order to compete, English and European apple breeding changed to similar breeding objectives as American breeding programmes (Alston, 1981).

Advances in technology have also impacted on breeding objectives. The development of refrigeration and then controlled-atmosphere refrigeration in the early 1900s meant that crispness became an important quality criteria (Morgan et al., 1993). Morgan reported that grading was introduced in the mid-1900s to increase international competitiveness. Fruit was initially graded on size, appearance and flavour in the UK but later flavour grading was dropped. The consequence of grading and fruit spraying was that consumers came to expect high quality and uniformity in appearance.

Although approximately a third of the world apple production (Fisher and Kitson, 1991), or 45% of the North American apple production (Root, 1996) has been used for processing, few breeding programmes include selection for processing traits. A survey of 42 apple breeders from North American, Europe, Asia, Africa and Oceania revealed that less than 20% of the breeding programs selected for processing apple traits and less than 5% were primarily breeding for processing apples (Laurens, 1999).

Current dessert apple breeding objectives

Recent reviews of apple breeding traits used in breeding programmes around the world list fruit quality as the primary breeding objective, pest and disease resistance as a secondary objective, and a range of other traits of lesser importance such as climatic adaptation, tree habit, and productivity (Janick et al., 1996; Laurens, 1999). In Laurens'

(1999) survey of the objectives for dessert apple breeding from 42 breeders from America, Europe, Asia, and Oceania, breeding objectives were very similar except for breeding programmes in extreme climates. Although there were some regional preferences (e.g.: for low acid fruits in Asia), breeders produced a range of types.

Breeding objectives can be grouped into 3 categories:

a) Fruit quality traits
b) Productivity traits
c) Production cost traits

Fruit quality

Most breeders have defined fruit quality in terms of appearance, flesh texture and taste (Janick et al., 1996; Laurens, 1999).

Fruit appearance

Fruit size

The optimum is large (70-85 mm diameter). Extremely large apples are possible but not favoured except in Japan, smaller apples are popular with consumers for smaller snacks (e.g.: children) but pricing does not reflect this. For processing, larger is better (Janick *et al.*, 1996). Fruit size is polygenically inherited and studies have shown the progeny mean is 34% lower than the mid-parent value (Janick *et al.*, 1996), possibly due to dominance. Janick suggests a good strategy would be to select parents with oversize fruit, not commercially acceptable themselves, to ensure that the majority of offspring fall within a desirable size range. Durel *et al.* (1998), and in chapter 4, estimated narrow-sense heritability of fruit size to be moderate to high (0.33 and 0.82 respectively).

Fruit colour

Janick *et al.* (1996) stated that colour can be bright red, bright green, clear yellow or bicolour (Europeans accept tricolour fruits). Fruit must be free from russet or totally russeted. Dull fruit colours were not acceptable although in some cases brighter

coloured sports improved their marketability. Europe prefers striped colour, while the USA prefers block colour. Japanese growers go to extraordinary lengths to achieve a block colour. They cover developing fruit with paper bags to protect them from disease and to modify the colour. One month before harvest they remove the bags and cover the tree with a muslim tent to prevent sun-scald. Reflective mulch, rotating the fruit by hand and thinning leaves all helps to make an even block colour prized by the Japanese (Yoshida, 1986).

The inheritance of colour in apple is complex. Background or ground colour has two independent mechanisms: a yellow colour range and a green colour range. The yellow colour ranges from pale cream through to yellow and is correlated to flesh colour (Janick *et al.*, 1996). The green scale ranges from none through to green (due to chlorophyll). The overcolour (anthocyanin production in the skin) can be nil through to complete coverage. Combinations of overcolour with background colour change the overall colour. An apple with the same red overcolour is a bright pink when combined with a cream ground colour, a bright crimson with a yellow ground colour or a dark/dull red/brown on a green ground colour. As dull colours are unacceptable, ground colour is of primary importance in fruit with red colouring.

Various modes of inheritance have been proposed for the inheritance of yellow or red colours but although no theory fits all the experimental data, researchers favour one or two major, complementary genes (Lespinasse et al., 1985, 1988; White and Lespinasse, 1986).

The overcolour can be patterned (striped or block) and vary in intensity (intense to pale) and is highly sensitive to the environment. Stripe and blush traits are inherited independently. In a 1992 review of the genetics of apple, Brown found blushed, non-striped parents had only blushed or non-blushed progeny, no striped progeny, and that the presence of stripes was controlled by a major, dominant gene (R_f).

Russeting has been shown to be controlled by a major dominant gene (*Ru*) with minor modifying genes and polygenic effects (Brown, S., 1992).

Shape

A range of shapes are possible but marketers prefer fruits with a tall shape and conical to ovate (oval) profile (Janick et al., 1996). Irregular shapes are not acceptable nor are oblate (flat) shapes currently acceptable. Brown (1960) measured apple aspect (length / width) and found that the progeny mean was close to the parental mid-point. Fruit shape can be further described by conicity (position of the widest point) and squareness (Currie et al., 2000) (chapter 3). All three shape traits are quantitatively inherited and the narrow sense heritabilities were estimated to be 0.74, 0.38, and 0.23 respectively.

Blemish-free

Blemishes on fruit are undesirable and range from partial russet, pest or diseases to mechanical damage. Mechanical damage may be due to birds, bruising, leaf or limb rub, insect damage. For genetic inheritance of russet see section "Fruit colour" page 25, and for pest and diseases see section "Pest and disease resistance" page 30.

Texture or mouth-feel

Texture or mouth-feel breeding objective is a complex of several traits including crispness, firmness (crunch), density, and juiciness. Daillant-Spinnler *et al.* (1996) found texture to be the most important factor in consumer preference of peeled and unpeeled apples. Other researchers have confirmed that consumers are primarily influenced by apple texture (Williams, 1979; Smith, 1984).

Crispness is a major part of flesh texture and ranges from the hard, crisp Braeburn flesh to McIntosh's lower density, crisp-melting flesh. The inheritance of crispness has not been reported. A full range of crispness states exist so one could speculate that this trait is at least partly quantitatively inherited.

Firmness was measured by penetrometer on a range of crosses at two sites in New Zealand (chapter 4). Firmness was a quantitatively inherited trait with a high heritability (0.52 SE ±0.09).

Density is evaluated as fine texture through to an undesirable coarse texture.

Increased juiciness contributes to desirable mouth-feel. Durel *et al.* (1998) estimated the variance components for the juice levels of a range of scab-resistant crosses and found juiciness to be quantitatively inherited with a moderate heritability (0.39 SE ±0.03). Durel *et al.* also found flesh texture to be quantitatively inherited (h^2 = 0.34 SE ±0.02), but did not define "texture".

Taste

Total flavour is a complex of sweetness, acidity and aroma (Yahia, 1994). However, Janick *et al.* (1996) stated that the market has defined flavour more narrowly as the balance between sweetness and acidity irrespective of aroma. Sweetness and acidity are independently inherited but perception of acceptable sweet/sour balance is dependent on the combination of acidity and sugars. Fruit needs sugar to make the fruit palatable but also needs acids to enhance the flavour and prevent the sugars from tasting sickly or insipid. Generally apples with the same sugar:acid ratio, but with more acid, have a stronger flavour. Yahia (1994) state that successful cultivars are either medium acid (pH 3.2-3.5) with medium (11-13%) or high (14-16%) sugar levels, or low acid (pH 3.5-3.7) with low (9-11%) sugar levels. Cooking apples have high acidity (pH 2.8-3.2) and moderate sugar levels.

If texture is within an acceptable range it is largely ignored and flesh flavour is the prime consumer preference (Harker et al., 1997). However, Daillant-Spinnler *et al.* (1996) found texture and flavour combinations are the main preference indicator for apple, with UK consumers preferring either crisp, sweet apples or juicy, acidic apples. Taste preferences differ between regions. The sugar:acid ratio can vary, with Asia preferring a mild to sweet, low acid apple while the US Midwest and Europe prefer a more tart apple (Janick et al., 1996).

Fruit sugars, measured by refractive index and expressed as a percentage of soluble solids in the juice, is polygenically inherited (Brown and Harvey, 1971).

Acidity in apple is due to malic acid and is measured by pH or by total malic acid as a percentage of the juice. Acidity is inherited polygenically and by a single dominant gene (*Ma*). Low acid types are double recessive (*mama*) and are generally discarded in Western apple breeding programmes (Alston, 1981; Brown, S., 1992; Janick et al., 1996). This may be an error as some very successful cultivars are in the low acid class ('Delicious', 'Spartan' and 'Fuji').

Flavour from aroma is complex and many compounds have been isolated which form the different aromas of apple (Yahia, 1994). Terms used to describe apple aroma have included strawberry, raspberry, melon, pineapple, pear, plum, cherry, acid or fruit-drop, winey, aniseed, grassy, nutty, almond, soapy, off-flavour, green or red apple aromas (Morgan et al., 1993; Daillant-Spinnler et al., 1996). Flavours (due to a range of flesh aromas) need to be maximised for processing. Yahia (1994) stated that hundreds of chemicals were responsible for the aroma of apple and that little is known about the inheritance or response to selection for this trait. However, the flavours of parents are inherited and blended, suggesting a quantitative mode of inheritance.

Storage

Apples are produced over a period of approximately three months but are sold throughout the year, often needing a long storage period and shelf-life to reach the target market. Apples that hold their crispness, flavours and juiciness (discussed in previous sections) have increased marketability (Alston, 1988). This complex trait shows a continuous range of expression in progeny, suggesting quantitative inheritance. Due to the expense of screening for storage ability, only 14% of breeders in Laurens (1999) survey of apple breeders selected for storage attributes.

Production costs

Reduction in production costs is a priority for breeding, not just to increase the profitability of growing apples, but also to reduce the chemical input in growing apples, which is an increasing concern for consumers of fresh produce. Bringhurst (1983) cautioned that selection for secondary traits such as pest and disease resistance at the

expense of fruit quality traits will cause the programme to fail, as fruit quality is what ultimately determines the cultivar success.

Pest and disease resistance

Chemicals are used to control pest and diseases, fertilisers enhance growth, and postharvest dips are used to control storage rots and disorders. Reducing the reliance on chemicals would reduce the costs associated with apple production and increase profitability. As well, there is increasing pressure from consumers to reduce the spray residues on fruit, with some markets banning the use of certain chemicals altogether. Genes for resistance to disorders, pests and diseases would satisfy the demand for both residue-free and blemish-free fruit. Although many such genes have been found in apple not many in fruit of high quality.

There are two issues that influence an apple breeders choice of traits:

- ease of transmitting the disease resistance to progeny
- durability of the disease resistance

Traits can be inherited monogenically from one gene with major effect, or polygenically from many genes with small effect. Progeny from a cross between an apple with major gene resistance and a susceptible apple will typically segregate into resistant and susceptible seedlings, with the resistant seedlings having the same or similar level of resistance as the resistant parent. However, progeny from a cross between an apple with polygenic resistance and a susceptible apple will generate seedlings with a range of resistance, of which few may be as good as the resistant parent. This is because the polygene complexes are broken up during meiosis. More than one cross may be necessary to regain or improve on the polygenic resistance. For these reasons apple breeders have favoured the exploitation of major gene resistance traits (Alston, 1970a; Brown, 1975).

However, resistance based on one gene can be easily broken down by a mutation in the pathogen as has happened in the V_f gene for *Venturia inequalis* (scab) resistance (Parisi et al., 1994). For this reason polygenic resistance is likely to be more durable than major

gene resistance. Breeders enhance the durability of major gene resistance by selecting seedlings with more than one resistance gene against the same pathogen by progeny testing or using DNA markers (Dayton et al., 1983; Durel et al., 1999). The probability that a pathogen breaks the resistance of each gene at the same time is the product of the probabilities for each mutation. Incorporating multiple resistance for the same pathogen in one individual, or pyramiding the resistance genes, increases the durability of the resistance. The following sections list the major pests and diseases in apple, the symptoms and, if known, the mode of inheritance.

Scab (*Venturia inequalis*)

A major disease of apples is scab or black spot caused by the fungus *Venturia inequalis*, which forms dark spots on the leaves and fruit. Scab can be controlled effectively by fungicides at this stage, but these chemicals are expensive and may be banned in future due to consumer concerns about spray residues. Polygenic resistance to apple scab is found in 'Freedom', 'Antonovka' (Visser et al., 1974), 'Grieve Rouge' (Zhdanov and Sedov, 1988), and species such as *M. sylvestris*, *M. sargentii*, *M. sieboldii* and *M.* ×*zumi* var calocarpa. Several sources of monogenic resistance have been incorporated into apple breeding programmes e.g.: 'Antonovka' (V_a) and from apple species *M. sieversii* 'R12740-7A' (V_r), *M. baccata* (V_b, V_{bj}), *M.* ×*floribunda* 821 (V_f), and *M. micromalus* (V_m) (Brown, S., 1992; Crosby et al., 1992). Although breeding has focussed on transferring monogenic resistance to scab from crab apple species (Crosby et al., 1992), some breeding programmes utilise polygenic resistance in cultivars (Noiton and Shelbourne, 1992; Lateur et al., 1999).

Powdery mildew (*Podosphaera leucotrica*)

Powdery mildew (*Podosphaera leucotrica*) is a widespread fungal disease that forms a white powdery growth visible on the surface of young leaves. Infected leaves are distorted, in-rolled and become bronzed and fall prematurely. Powdery mildew resistance displays both monogenic and polygenic inheritance. Sources of monogenic resistance include *M.* ×*zumi*, *M.* ×*robusta, M. sargentii, M. baccata jackii*, MA 8,

'David' and 'White Angel' (Way et al., 1989). Polygenic sources of resistance have also been found in cultivars (Visser et al., 1974).

Collar rot (*Phytophthera cactorum*)

Phytophthera is a fungal disease that infects the roots and forms brown lesions that girdle the main trunk at ground level causing the tree to die. Usual symptom is that spring growth collapses. Resistance to Phytophthera has been found in the cultivars 'Northern Spy', 'Mill End', 'Royal Jubilee', Sundog Crab', 'Oporte' and 'Susvorenskoye No. 4' (Alston, 1970b; Utkhede, 1986). Alston (1970) suggested that 'Northern Spy' had monogenic resistance, but Brown (1992) reported that inheritance was more complex than a single dominant gene. Cummins and Aldwinkle (1995) report that breeding for resistance to Collar Rot has been successful.

Other diseases

- Storage rots are caused by a wide range of species primarily from the *Glomerella* genus. A dominant gene for susceptibility to *Glomerella* has been reported in 'Golden Delicious' (Thompson and Taylor, 1971). Brown (1975) reported that some resistance may be polygenic and that most apple cultivars have some degree of susceptibility.
- Silver leaf (*Chondrostereum purpureum*) symptoms are silvery leaves; in severe cases the trees have small leaves and dieback. There is evidence for both polygenic and monogenic resistance (Bus et al., 1996).
- Fireblight is a bacterial disease (*Erwinia amylovora*) that infects blossoms, fruits and shoots. Cankers developing from infected shoots girdle the stem and cause die-back, browning of leaves and eventual death of the tree. A pale brown bacterial ooze is often observed on active cankers. Way *et al.* (1990) report resistance in both cultivars and *Malus* species.
- European canker (*Nectria galligena*) is a disease that enters through pruning cuts, leaf scars and spurs.

Insect pests

- Woolly apple aphid WAA (*Erisoma lanigerum*) has white fluffy material covering the red/purple aphids, which infest both roots and aerial parts of apple trees leading to distorted growth. A single dominant (*Er*) gene that confers resistance to most biotypes of WAA is found in 'Northern Spy' (Knight et al., 1962). Other sources of major genes for resistance include *M.* ×*robusta*, *M. halliana*, *M. hupehensis*, *M. tschonoskii*, 'Ivory's Double Vigour' and 'Kola' (Brown, S., 1992).
- Apple leaf-curling midges (*Dasyneura mali*) are orange-coloured maggots up to 4mm long that cause tightly rolled young apple leaves with no webbing. Damage can be severe on young apple trees. Major genes for resistance have been isolated from 'Cox's Orange Pippin', 'Northern Spy', *M.* ×*robusta*, and *M.* ×*zumi* (Brown, S., 1992).
- Codling moth (*Cydia pomonella*) caterpillars bore into the fruit, pushing droppings out of their entrance holes. Cultivars vary in resistance (Goonewardene, 1987).
- Leaf-roller caterpillars spin web shelters as they feed on the underside of the leaf. Older caterpillars web leaves together, leaves to fruit or roll over the edges of leaves. Leaf-rollers feed on the surface of fruit. Several species of leaf-roller caterpillar exist.
- Scale is caused by a small insect that shelters under a waxy scale on the fruit, shoots and branches. Species of importance in New Zealand include the apple mussel scale (*Lepidosaphes ulmi*), San José scale (*Quadraspidiotus perniciosus*), and Oystershell scale (*Quadraspidiotus ostreaeformis*).

Mites

Mites can cause discoloration of leaves. In New Zealand the European red mite (*Panonychus ulmi*) and two-spotted spider mite (*Tetranychus urticae*) are the important species.

Apple mosaic virus

Symptoms show numerous, small, yellow spots on the leaves. Virus stunts the growth and yield.

Disorders

- Bitter pit consists of dark brown pits on the fruit surface and internally (except for the core) due to calcium deficiency.
- Internal cork has symptoms similar to bitter pit but lesions may occur anywhere in the fruit and are due to boron deficiency.

Productivity

Productivity is another complex trait further subdivided into component traits of yield, juvenile period and tree size.

Yield

Yield in apples is a complex trait that can be defined as heavy, consistent cropping with a high proportion of useable fruit of uniform size, shape and quality. The tree must have a structure or habit that can promote and support heavy crops. Upright branch angles promote vegetative growth, increasing the pruning and training requirement and reducing the potential crop. Wide branch angles (60-90°) promote flower initiation and can bear crop load. Branch angles below the horizontal (>90°) tend to produce poorer quality fruit and the branches get in the way of orchard machinery (weed-sprayers and mowers). Some genes have been isolated for the related traits of dwarfism, branch production, weeping form and spurring but Spinks (1936) found evidence that branch angle was quantitatively inherited.

Heavy crops are a function of flower numbers and flower set (quantitative trait). However, if too many fruit are set then excess fruit need to be removed to prevent over-cropping, which leads to branches breaking and small fruit size. Some trees self-thin, setting only the king fruit in each flower cluster (mode of inheritance unreported) (Laurens, 1999). Another crop related trait is biennial bearing, which is the tendency to set heavy crops one year and a light to nil crop the following year. Biennial bearing can be controlled by careful thinning in the heavy setting year but this increases the labour requirements and so is selected against (mode of inheritance unknown).

The proportion of the crop that has adequate quality is influenced by uniformity of the fruit appearance (fruit size, shape, colour, blemish-free). Productivity is reduced by the number of reject fruit and one criterion for rejection is blemishes. Blemishes for which there is a low tolerance are russeting, superficial insect pest and disease damage, leaf rub. Other blemishes, which have a zero tolerance are bruising, bird damage, extensive ($>1cm^2$) pest or disease damage (O.E.C.D., 1983). Disease and pest resistance is especially important as consumers demand stricter controls on spray residues but demand blemish-free fruits. Resistance to these sources of damage is polygenically and monogenically inherited. Breeding programmes around the world have incorporated disease resistance as a primary aim of their programmes and Janick *et al.* (1996) reviewed the source of genes for simply inherited traits and lists 6 pest resistance genes, 13 disease resistance genes, and 29 fruit and tree attribute genes.

Tree-form or habit for high yield has wide branch angles above the horizontal (Lauri et al., 1997). Upright or vertical branches encourage vegetative growth at the expense of flower induction and branch angles lower than horizontal produce poor quality fruit.

Juvenile period

Juvenile period is the time between germination and flowering. Reducing the juvenile period is essential to breeders to reduce the generation cycle and increase genetic gains per year. Hansche (1983) estimated that 90% of the cost of breeding was associated with the land area the seedlings occupy and the length of time they occupy it. Therefore a long juvenile period not only reduces the genetic gain per unit time, but it also increases the cost. Hansche provided an example of walnut size ($h^2 = 0.86$) which could expect gains of 25% if the top 20% of the population was selected. However, with a juvenile period of 6-8 years the gain in nut size is only 2-3% per year. Long juvenility period is positively correlated to the vegetative phase of grafted nursery stock so reducing the juvenile period is also important to the orchardist (Visser and Vries, 1970; Brown, 1975).

The inheritance of juvenile period is quantitative and mostly additive (Brown, 1975; Visser, 1976; Schmidt, 1985; Gelvonauskis and Gelvonauskiene, 1996). Using high quality apple cultivars Visser (1965) found parents with a long period from flowering to fruiting (or late harvested fruit) also had a long juvenile period and suggested these traits could be used to select precocious parents. Visser also suggested that seedlings with long juvenility could be screened out by selecting against the correlated traits of juvenile character expression (small leaves and thorniness). Wild crab apple species such as *M. coronaria* (L.) Miller, *M. prunifolia, M. sieversii* var. niedzwetzkyana and *M.* ×*zumi* are better sources of precocity than *M.* x *domestica* (Kazakov and Kichina, 1988; Ardelean et al., 1992).

Tree size

Hansche (1983) calculated that reducing the tree size would decrease the cost of a breeding programme, allow a number of propagules to be taken to reduce the environmental error estimate, increasing the heritability estimate and hence increasing the potential genetic gain. Hansche used an example of the heritability of peach yield which was increased from 0.08 to 0.32 by gathering data from 20 propagules rather than one seedling. However the costs of replicating and raising large plants in terms of land and resource use is prohibitive. If plant size was reduced to the level that more than one propagule can be measured cost-effectively, then heritability estimates and hence genetic gains could be increased. Smaller trees would also reduce labour costs (no ladder work) and reduce chemical costs through more efficient spray application. Smaller trees can be planted at a higher density and would take less time to fill the canopy space, reducing the time to maximum yield per unit of land. Lower vigour would also reduce the cost of pruning.

Vigour is a quantitative and mostly additive trait (Watkins and Spangelo, 1970). Brown (1975) discusses the possibility of utilising inbreeding depression to control vigour. An alternative to inbreeding would be the use of several dominant genes found for different forms of dwarfism (Alston, 1976).

Selection index

Selection in apples is on the basis of independent culling levels, in which threshold levels are set for each trait and the selection is made on this basis for each trait independent of the value of other traits. Although Hazel (1943) has shown this to be less effective than the Smith-Hazel index, it remains popular partly because of simplicity (no genetic parameters are needed), and partly because independent culling levels are more suited to commercial cultivar selection.

The most efficient method of selection for multiple traits over more than one generation is a Smith-Hazel index (Hazel, 1943; Falconer, 1989; Hazel et al., 1994). The Smith-Hazel selection index maximises the correlation between the breeding objective and the selection criteria traits using the phenotypic covariance matrix for the selection criteria, and the genetic covariance between the breeding objectives and selection criteria. So selection for one trait is made taking into consideration the effects of selection on all other traits. Use of a multiple trait selection index is standard in animal breeding (Falconer, 1989; Hazel et al., 1994) and forest tree breeding (Falkenhagen, 1988), but has not been adopted in fruit breeding. This is probably because of a lack of information on the heritability, genetic and phenotypic variance components of apple traits (Durel et al., 1998), but may also be due to the rarity of multi-generation apple breeding strategies.

A priority for efficient apple breeding population development would be the definition of a selection index (or indices) for apple to improve the selection efficiency over several generations.

Summary

Apples have had a long history of association with man but are still relatively close to their wild ancestors. Fruits of near-commercial quality can be found in the central Asian mountains and the domestic apple can readily inter-cross with most wild apple species. Most breeding strategies do not utilise this genetic diversity available within apple and risk reducing long-term gains through high levels of inbreeding. However, a strategy recently suggested for apple, based on recurrent selection for general combining ability,

utilises a genetically broad-based population for long-term sustainable gains. Breeding objectives and selection criteria for apple are listed and known modes of inheritance reported.

References

Allard, R. W. 1960. *Principles of plant breeding*. John Wiley & Sons, London.

Alston, F. H. 1970a. Integration of major characters in breeding commercial apples. *Proceedings of the Eucarpia Fruit Breeding Symposium, Angers, p 231-248.*

Alston, F. H. 1970b. Resistance to collar rot, *Phytophthera cactorum* (Leb. and Cohn) Shroet., in apple. *Annual Report of East Malling Research Station*, 1969:143-145.

Alston, F. H. 1976. Dwarfing and lethal genes in apple progenies. *Euphytica*, 25:505-514.

Alston, F. H. 1981. Breeding high quality high yielding apples, p. 93-102. *In* P. W. Goodenough and R. K. Atkin (eds.), *Quality in stored and processed vegetables and fruit*. Academic Press, New York.

Alston, F. H. 1988. Breeding apples for long storage. *Acta Horticulturae*, 224:109-117.

Alston, F. H., K. M. Evans, H. J. H. MacFie, G. J. King, and P. K. Betys. 1996. The potential for improving organoleptic quality in apples through marker assisted breeding related to consumer preference studies, p. 35-38. *In* G. R. Fenwick, C. Hedley, R. L. Richards and S. Khokhar (eds.), *Agri-food quality: an interdisciplinary approach.* Royal Society of Chemistry, Cambridge.

Anon. 1998. Encyclopedia Brittanica CD 99. *Brittanica Online (internet).*

Ardelean, M., R. Sestras, and V. Ghidra. 1992. Precocity of bearing in own-rooted apple hybrids. II. General (GCA) and specific (SCA) combining ability in different types of parental combinations. [Romanian]. *Buletinul Universitatii de Stiinte Agricole Cluj-Napoca. Seria Agricultura si Horticultura*, 46(2):21-25.

Baxter, L. B., and J. B. Heaton. 1986. Breeding apple scab resistant dessert apples for Queensland, p. 220-223. *In* T. A. Williams and G. S. Wratt (eds.), *Plant breeding symposium DSIR*, vol. 5. Agronomy Society of New Zealand, Lincoln. Agron. Soc. N.Z. Special publication 5.

Bosemark, N. O. 1993. The need for a comprehensive plant breeding strategy, p. 525-533. *In* M. D. Hayward, N. O. Bosemark and I. Romagosa (eds.), *Plant Breeding: Principles and Perspectives*, 1st ed. Chapman & Hall, London.

Bringhurst, R. S. 1983. Breeding Stategy, p. 147-153. *In* J. N. Moore and J. Janick (eds.), *Methods in Fruit Breeding*, 1st ed., vol. 1. Purdue University Press, West Lafayette, Indiana.

Brown, A. G. 1959. The inheritance of mildew resistance in progenies of the cultivated apple. *Euphytica*, 8:81-88.

Brown, A. G. 1960. The inheritance of shape, size and season in progenies of the cultivated apple. *Euphytica*, 9:327-337.

Brown, A. G. 1973. The effect of inbreeding on vigour and length of juvenile period in apples, p. 30-39. *In* A. G. Brown, R. Watkins and F. Alston (eds.), *Proceedings of Eucarpia Fruit Section Symposium V. Top Fruit Breeding. Canterbury, Sept. 11th-14th 1973*. Eucarpia, Canterbury.

Brown, A. G. 1975. Apples, p. 3-37. *In* J. Janick and J. N. Moore (eds.), *Advances in fruit breeding*, 1st ed., vol. 1. Purdue University Press, West Lafayette, Indiana.

Brown, A. G., and D. M. Harvey. 1971. The nature and inheritance of sweetness and acidity in the cultivated apple. *Euphytica*, 20:68-80.

Brown, S. 1992. Genetics of apple. *Plant Breeding Reviews*, 9:333-366.

Bultitude, J. 1983. *Apples: A guide to the identification of international varieties.* McMillan Reference Books, London, 323 p.

Bus, V. G., A. G. Spiers, D. T. Brewster, and M. E. Hofstee. 1996. Preliminary screening of apple germplasm for resistance to Silverleaf infection. *New Zealand Journal of Crop and Horticultural Science*, 21:1-6.

Campbell, C. S., M. J. Donoghue, B. G. Baldwin, and M. F. Wojciechowski. 1995. Phylogenetic relationships in Maloideae (Rosaceae): Evidence from sequences of the internal transcribed spacers of nuclear ribosomal DNA and its congruence with morphology. *American Journal of Botany*, 82:903-918.

Challice, J. S. 1974. Rosaceae chemotaxonomy and the origins of the Pomoideae. *Botanical Journal of the Linnean Society*, 69:239-259.

Cheng, F. S., N. F. Weeden, S. K. Brown, H. S. Aldwinckle, S. E. Gardiner, and V. G. Bus. 1998. Development of a DNA marker for Vm, a gene conferring resistance to apple scab. *Genome*, 41:208-214.

Chevreau, E. 1998. Apple and pear biotechnology at INRA Angers, p. 87-93. *In* M. R. Davey, P. G. Alderson, K. C. Lowe and J. B. Power (eds.), *Tree biotechnology: towards the millennium.* Nottingham University Press, Nottingham.

Chevreau, E., and F. Laurens. 1987. The pattern of inheritance in apple (Malus x domestica Borkh.): further results from leaf isozyme analysis. *Theoretical and Applied Genetics*, 75:90-95.

Chevreau, E., Y. Lespinasse, and M. Gallet. 1985. Inheritance of pollen enzymes and polyploid origin of apple (Malus x domestica Borkh.). *Theoretical and Applied Genetics*, 71:268-277.

Crosby, J. A., J. Janick, P. C. Pecknold, S. S. Korban, P. A. O'Connor, S. M. Ries, J. Goddfreda, and A. Voordeckers. 1992. Breeding apples for scab resistance:1945-1990. *Acta Horticulturae*, 317:43-70.

Cummins, J. N., and H. S. Aldwinckle. 1995. Breeding rootstocks for tree fruit crops. *New Zealand Journal of Crop and Horticultural Science*, 23:395-402.

Currie, A. J., S. Ganeshanandam, D. A. M. Noiton, D. J. Garrick, C. J. A. Shelbourne, and N. Oraguzie. 2000. Quantitative evaluation of apple (*Malus X domestica* Borkh.) fruit shape by principal component analysis of Fourier descriptors. *Euphytica*, 111:221-227.

Daillant-Spinnler, B., H. J. H. MacFie, P. K. Beyts, and D. Hedderley. 1996. Relationships between perceived sensory properties and major preference directions of 12 varieties of apples from the southern hemishpere. *Food Quality and Preference*, 7:113-126.

Dayton, D. F., R. L. Bell, and E. B. Williams. 1983. Disease resistance, p. 189-215. *In* J. N. Moore and J. Janick (eds.), *Methods in fruit breeding*, vol. 1. Purdue University Press, West Lafayette.

Decourtye, L., Y. Lespinasse, and M. Duron. 1986. Improvement of fruit varieties by mutagenesis, p. 344-348. *In* T. A. Williams and G. S. Wratt (eds.), *Plant breeding symposium DSIR*, vol. 5. Agronomy Society of New Zealand, Lincoln. Agron. Soc. N.Z. Special publication 5.

Dempfle, L. 1975. A note on increasing the limit of selection through selection within families. *Genetical Research, Cambridge*, 24:127-135.

Dermen, H. 1949. Are the pomes amphidiploids? A note on the origin of the Pomoideae. *Journal of Heredity*, ?:221-222.

Dorsey, M. J. 1917. The inheritance and permanence of clonal varieties. *Proceedings. American Society for Horticultural Science*, 13:41-71.

Dunemann, F. 1994. Molecular classification of Malus with RAPD markers, p. 295-300. *In* H. Schmidt and M. Kellerhals (eds.), *Progress in temperate fruit breeding. Proceedings of the Eucarpia Fruit Breeding Section Meeting, Wadenswil/Einsiedeln, Switzerland, 30 August to 3 September, 1993.* Kluwer Academic Publishers, Dordrecht.

Dunemann, F., G. Bracker, T. Markussen, and P. Roche. 1999. Identification of molecular markers for the major mildew resistance gene Pl2 in apple. *Acta Horticulturae*,

484:411-416. EUCARPIA symposium on fruit breeding and genetics, Oxford, UK, 1-6 September 1996.

Durel, C. E., F. Laurens, A. Fouillet, and Y. Lespinasse. 1998. Utilization of pedigree information to estimate genetic parameters from large, unbalanced data sets in apple. *Theoretical and Applied Genetics*, 96:1077-1085.

Durel, C. E., Y. Lespinasse, M. Chevalier, E. Chevreau, A. P. M. den Nijs, V. Djouvinov, F. Dunemann, K. M. Evans, C. Fischer, C. Gessler, M. Kellerhals, F. Laurens, A. G. Manganaris, L. Parisi, S. Sansavini, H. Schmidt, H. J. Schouten, and H. Schreiber. 1999. Genetic dissection of apple resistance regarding pathogen variability: co-ordination of European research programmes. *Acta Horticulturae*, 484:435-441. EUCARPIA symposium on fruit breeding and genetics, Oxford, UK, 1-6 September 1996.

Faedi, W., and P. Rosati. 1985. First evaluation of apple mutants induced by gamma ray treatments. *Acta Horticulturae*, 159:49-55.

Falconer, D. S. 1989. *Introduction to quantitative genetics.* Longman Group, London.

Falkenhagen, E. R. 1988. Multiple-trait index selection in tree breeding. A critical review. *Report, South African Forestry Research Institute. 1988. No. 5/88, 66 pp. 80 ref.*

Fisher, D. V., and J. A. Kitson. 1991. The Apple, p. 45-66. *In* N. A. M. Eskin (ed.), *Quality and preservation of fruits*. CRC Press, Boca Raton, Florida.

Gallais, A. 1986. A general strategy of plant breeding for varietal development, p. 42-48. *In* T. A. Williams and G. S. Wratt (eds.), *Plant Breeding Symposium D.S.I.R. 1986*, vol. 5. Agronomy Society of New Zealand, Lincoln. Agron. Soc. N.Z. Special publication 5.

Gardiner, S. E., J. M. Zhu, H. C. M. Whitehead, and C. Madie. 1994. The New Zealand apple genome mapping project. *Euphytica*, 77:77-81.

Gea, L. D. 1997. Genetic diversity and gain: the concept of a status number. *Ph.D. Dissertation.* School of Forestry, University of Canterbury, Christchurch, NZ.

Gelvonauskis, B., and D. Gelvonauskiene. 1996. Inheritance of juvenile period in apple. *Biologija*, 3:38-40.

Gianfranceschi, L., B. Koller, N. Seglias, M. Kellerhals, and C. Gessler. 1996. Molecular selection in apple for resistance to scab caused by Venturia inaequalis. *Theoretical and Applied Genetics*, 93:199-204.

Goonewardene, H. F. 1987. E11-24, E14-32 and E36-7 apple germplasm with multiple pest resistance. *HortScience*, 22(6):1346-1348.

Hansche, P. E. 1983. Response to Selection, p. 154-171. *In* J. N. Moore and J. Janick (eds.), *Methods in Fruit Breeding*, 1st ed., vol. 1. Purdue University Press, West Lafayette, Indiana.

Harker, F. R., R. J. Redgwell, I. C. Hallet, S. H. Murray, and G. Carter. 1997. Texture of fresh fruit. *Horticultural Reviews*, 20:121-224.

Harris, D. L., T. S. Stewart, and C. R. Arboleda. 1984. Animal breeding programs: Systematic approach to their design. *Advances in agricultural technology, USDA, Feb 1984. ISSN 0193-3701.*

Hazel, L. N. 1943. The genetic basis for constructing selection indexes. *Genetics*, 28:476-490.

Hazel, L. N., G. E. Dickerson, and A. E. Freeman. 1994. The selection index - then, now, and for the future. *Journal of Dairy Science*, 77:3236-3251.

Hioux, S. 1998. Soya leads the way in GMO debate. *Food Ingredients & Analysis International*,(Mar/Apr):33-34, 36, 39-40.

Hough, L. F., J. R. Shay, and D. F. Dayton. 1953. Apple scab resistance from *Malus floribunda* Sieb. *Proceedings. American Society for Horticultural Science*, 62:341-347.

Janick, J., J. N. Cummins, S. K. Brown, and M. Hemmat. 1996. Apples, p. 1-77. *In* J. Janick and J. N. Moore (eds.), *Fruit Breeding: Tree and Tropical Fruits*, vol. 1. John Wiley & Sons, New York.

Janse, J., J. J. Verhaegh, and A. P. M. den Nijs. 1994. Early selection for partial resistance to powdery mildew, *Podosphaera leucotricha* (Ell. et Ev.) Salm. in apple progenies, p. 13-15. *In* H. Schmidt and M. Kellerhals (eds.), *Progress in Temperate Fruit Breeding*. Kluwer Academic Press, Netherlands.

Juniper, B. E., R. Watkins, S. A. Harris, K. R. Tobutt, and F. H. Alston. 1999. The origin of the apple. *Acta Horticulturae*, 484:27-33. EUCARPIA symposium on fruit breeding and genetics, Oxford, UK, 1-6 September 1996.

Kato, S., S. Ishikawa, S. Imakawa, S. Komori, T. Mikami, and Y. Shimamoto. 1993a. Cytoplasmic relatedness of apple landraces and cultivars: a molecular analysis. *Euphytica*, 66:1-2, 99-102.

Kato, S., S. Ishikawa, S. Imakawa, S. Komori, T. Mikami, and Y. Shimamoto. 1993b. Mitochondrial DNA restriction fragment length polymorphisms in Malus species. *Plant Breeding*, 111:162-165.

Kazakov, I. V., and V. V. Kichina. 1988. Methods of accelerating the cropping of apple trees by reducing the juvenile phase. *Acta Horticulturae*, 224:141-145.

King, G. 1994. Progress in mapping agronomic genes in apple (The European Apple Genome Mapping Project), p. 263-267. *In* H. Schmidt and M. Kellerhals (eds.), *Progress in Temperate Fruit Breeding*. Kluwer Academic Press, Netherlands.

King, G. J. 1996. Progress of apple genetic mapping in Europe. *HortScience*, 31(7):1108-1111.

King, G. J., F. H. Alston, I. Battle, E. Chevreau, C. Gessler, J. Janse, P. Lindhout, A. G. Manganaris, S. Sansavini, H. Schmidt, and K. Tobutt. 1991. The 'European apple genome mapping project' - developing a strategy for mapping genes coding for agronomic characters in tree species. *Euphytica*, 56:89-94.

Knight, R. L., and F. H. Alston. 1968. Sources of field immunity to mildew (*Podosphaera leucotricha*) in apple. *Canadian Journal of Genetics and Cytology*, 10:294-298.

Knight, R. L., J. B. Briggs, A. M. Massee, and H. M. Tydeman. 1962. The inheritance of resistance to woolly aphid, *Eriosoma lanigerum* in the apple. *Journal of Horticultural Science*, 37:207-218.

Koller, B., C. Gessler, L. Bertschinger, and M. Kellerhals. 1998. Assessment of technical impacts of transgenic disease resistant apples and grapevines. *Bulletin Oilb/Srop*, 20(9):150-154. Proceedings of the 4th workshop on Integrated control of pome fruit diseases, Croydon, UK, August 19-23, 1996.

Korban, S. S. 1986. Interspecific hybridisation in *Malus*. *HortScience*, 21(1):41-48.

Korban, S. S., and R. M. Skirvin. 1984. Nomeclature of the cultivated apple. *HortScience*, 19(2):177-180?

Kronstad, W. E. 1986. Genetic diversity and plant improvement, p. 16-20. *In* T. A. Williams and G. S. Wratt (eds.), *Plant breeding symposium DSIR*, vol. 5. Agronomy Society of New Zealand, Lincoln.

Kruger, J. 1995. Breeding for mildew resistance in apples at Ahrensburg - Sources and stability. *Die Gartenbauwissenschaft*, 60(6):269-275.

Lateur, M., C. Wagemans, and C. Populer. 1999. Evaluation of fruit tree genetic resources as sources of polygenic scab resistance in an apple breeding programme. *Acta Horticulturae*, 484:35-42. Proceedings of the EUCARPIA Fruit Breeding and Genetics Symposium.

Laurens, F. 1999. Review on the current apple breeding programmes in the world: breeding objectives for scion-cultivar improvement. *Acta Horticulturae*, 484:163-170. Proceedings of the EUCARPIA Fruit Breeding and Genetics Symposium.

Lauri, P. E., J. M. Lespinasse, and F. Laurens. 1997. What kind of morphological traits should be sought in apple seedling progenies in order to select regular bearing cultivars? *Acta Horticulturae*, 451:725-729.

Lawson, D. M., M. Hemmat, and N. F. Weeden. 1995. The use of molecular markers to analyze the inheritance of morphological and developmental traits in apple. *Journal of the American Society for Horticultural Science*, 120(3):532-537.

Lespinasse, Y. 1992. Breeding apple tree: aims and methods, p. 103-110. *In* F. Rousselle-Bourgeois and P. Rousselle (eds.), *Proceedings of the Joint Conference of the EAPR Breeding & Varietal Assessment Section and the EUCARPIA Potato Section, Landerneau, France, 12-17 January 1992. INRA, Ploudaniel, France: 1992. 103-110. 12 ref.* INRA, Ploudaniel.

Lespinasse, Y., and J. P. Paulin. 1990. Apple breeding program for fire blight resistance: Strategy used and first results. *Acta Horticulturae*, 273:285-295.

Lespinasse, Y., J. M. Lespinasse, and B. Ganne. 1985. Inheritance of two agronomic characters in the apple tree (Malus pumila Mill.): compact type habit and fruit colour. *Acta Horticulturae*, 159:35-47.

Lespinasse, Y., A. Fouillet, J. D. Flick, J. M. Lespinasse, and F. Delort. 1988. Contribution to genetic studies of apple. *Acta Horticulturae*, 224:99-107.

Li, Y. 1988. Identification of the center of *M. pumila* and *Malus* in the world, p. 55. *In International symposium on horticultural germplasm, cultivated and wild (abstracts). Chinese Soc. Hort. Sci. Intern. Acad. Pub., Beijing.* Intern. Acad. Pub., Beijing.

Li, Y. 1989. An investigation of the genetic centre of *M. pumila* and *Malus* in the world [Chinese]. *Acta Horticulturae Sinica*, 16(2):101-108.

Lindgren, D., L. D. Gea, and P. A. Jefferson. 1996. Loss of genetic diversity monitored by status number. *Silvae Genetica*, 45(1):52-59.

Lindgren, D., L. D. Gea, and P. A. Jefferson. 1997. Status number for measuring genetic diversity. *Forest Genetics*, 4:69-76.

Lyrene, P. M. 1981. Recurrent selection in breeding Rabbiteye blueberries (*Vaccinium ashei* Reade). *Euphytica*, 30:505-511.

Maliepaard, C., F. H. Alston, G. Vanarkel, L. M. Brown, E. Chevreau, F. Dunemann, K. M. Evans, S. Gardiner, P. Guilford, A. W. Vanheusden, J. Janse, F. Laurens, J. R. Lynn, A. G. Manganaris, A. P. M. Dennijs, N. Periam, E. Rikkerink, P. Roche, C. Ryder, S. Sansavini, H. Schmidt, S. Tartarini, J. J. Verhaegh, M. Vrielinkvanginkel, and G. J. King. 1998. Aligning male and female linkage maps of apple (Malus pumila Mill.) using multi-allelic markers. *Theoretical and Applied Genetics*, 97:60-73.

Manganaris, A. G., and F. H. Alston. 1987. Inheritance and linkage relationships of glutamate oxaloacetate transaminase isozymes in apple. 1. The GOT-1, a marker for the S incompatability locus. *Theoretical and Applied Genetics*, 74:154-161.

Manganaris, A. G., and F. H. Alston. 1988. The acid phosphatase gene ACP-1 and its linkage with the endopeptidase gene ENP-1 and the pale green lethal gene 1 in apple. *Acta Horticulturae*, 224:177-184.

Manganaris, A. G., F. H. Alston, N. F. Weeden, H. S. Aldwinckle, H. L. Gustafson, and S. K. Brown. 1994. Isozyme locus Pgm-1 is tightly linked to a gene (Vf) for scab resistance in apple. *Journal of the American Society for Horticultural Science*, 119(6):1286-1288.

Markussen, T., J. Kruger, H. Schmidt, and F. Dunemann. 1995. Identification of PCR-based markers linked to the powdery-mildew-resistance gene Pl1 from Malus robusta in cultivated apple. *Plant Breeding*, 114:530-534.

Matsumoto, S., H. Wakita, and J. Soejima. 1997. Chloroplast DNA probes as an aid in the molecular classification of Malus species. *Scientia Horticulturae*, 70:81-86.

Meuwissen, T. H. E., and J. A. M. van Arendonk. 1992. Potential improvements in the rate of genetic gain from marker-assisted selection in dairy cattle breeding schemes. *Journal of Dairy Science*, 75:1651-1659.

Morgan, J., A. Richards, and E. Dowle. 1993. *The Book of Apples.* Ebury Press, London. Published in association with the Brogdale Horticultural Trust.

Namkoong, G., H. C. Kang, and J. S. Brouard. 1988. *Tree breeding : principles and strategies*. Springer-Verlag, New York, viii + 177 p. (Monographs on theoretical and applied genetics; 11).

Nienhuis, J., J. Tivang, P. Skroch, and J. B. dos Santos. 1995. Genetic relationships among cultivars and landraces of Lima bean (Phaseolus lunatus L.) as measured by RAPD markers. *Journal of the American Society for Horticultural Science*, 120(2):300-306.

Noiton, D. A. M., and P. Alspach. 1996. Founding clones, inbreeding, coancestry and status number of modem apple cultivars. *Journal of the American Society for Horticultural Science*, 121:773-782.

Noiton, D., and C. J. A. Shelboume. 1992. Quantitative genetics in an apple breeding strategy. *Euphytica*, 60:213-219.

O.E.C.D. 1983. *Pommes et poires = Apples and pears*. Organisation de coopération et de développement économiques, Paris.

Paprstein, F., J. Blazek, and J. Vondracek. 1994. Results of mutation breeding of apples at RBIP Holovousy, p. 131-133. *In Progress in temperate fruit breeding. Proceedings of the Eucarpia Fruit Breeding Section Meeting, Wadenswil/Einsiedeln, Switzerland, 30 August to 3 September, 1993.* Kluwer Academic Press, Dordrecht.

Parisi, L., Y. Lespinasse, J. Guillaumes, and J. Kruger. 1994. A new race of *Venturia inaequalis* virulent to apples with resistance due to the Vf gene, p. 79. *In* H.

Schmidt and M. Kellerhals (eds.), *Progress in Temperate Fruit Breeding*, Proceedings of the Eucarpia Fruit Breeding Section Meeting, Wadenswil/Einsiedeln, Switzerland, 30 August to 3 September, 1993. Kluwer Academic Press, Dordrecht.

Ponomarenko, V. V. 1972. Siberian crab apple in the Transbaikal region. [Russian]. *Rastitel'n. resursy.*, 8(1):21-28.

Ponomarenko, V. V. 1973. Wild apple trees in Eastern Siberia and the Far East of the USSR. [Russian]. *Trudy po Prikladnoi Botanike, Genetike i Selektsii*, 49:85-94.

Ponomarenko, V. V. 1975a. Apple in Caucasia. [Russian]. *Botanicheskii Zhurnal*, 60:53-68.

Ponomarenko, V. V. 1975b. What is Malus pumila Mill. [Russian]. *Botanicheskii Zhurnal*, 60:1574-1586.

Ponomarenko, V. V. 1980a. Current state of research on problems of the origin of Malus domestica Borkh. [Russian]. *Trudy po Prikladnoi Botanike, Genetike i Selektsii*, 67:11-21.

Ponomarenko, V. V. 1980b. The wild apple of eastern Siberia. [Russian]. *Priroda, USSR*, 8:90-95.

Ponomarenko, V. V. 1982. Origin and distribution of apple, Malus domestica Borkh. [Russian]. *Byulleten' Vsesoyuznogo Ordena Lenina i Ordena Druzhby Narodov Instituta Rastenievodstva Imeni N. I. Vavilova*, 126:7-12.

Ponomarenko, V. V. 1983. History of the origin and evolution of the apple Malus domestica Borkh [Russian]. *Trudy po Prikladnoi Botanike, Genetike i Selektsii*, 76:10-18.

Ponomarenko, V. V. 1986. Review of the species in the genus Malus Mill. [Russian]. *Sbornik Nauchnykh Trudov po Prikladnoi Botanike, Genetike i Selektsii*, 106:16-27.

Ponomarenko, V. V. 1990. Apple varieties in Romania. [Russian]. *Sadovodstvo i Vinogradarstvo*, 4:39-40.

Puite, K. J., and J. G. Schaart. 1996. Genetic modification of the commercial apple cultivars Gala, Golden Delicious and Elstar via an Agrobacterium tumefaciens-mediated transformation method. *Plant Science (Limerick)*, 119:125-133.

Quinlan, J. D., and K. R. Tobutt. 1990. Manipulating fruit tree structure chemically and genetically for improved performance. *HortScience*, 25(1):60-64.

Rehder, A. 1954. *Manual of cultivated tree and shrubs*, 2nd ed., New York.

Roach, F. A. 1985. *Cultivated fruits of Britain: their origin and history*. Basil Blackwell Publisher Ltd., Oxford, 349 p.

Roden, J. A. 1994. Review of the theory of open nucleus breeding systems. *Animal Breeding Abstracts*, 62(3):151-157.

Root, W. H. 1996. Apples and apple processing, p. 1-36. *In* L. P. Somogyi, D. M. Barret and Y. H. Hui (eds.), *Processing fruits: Science and technology. Major processed products*, vol. 2. Technomic Publishing Co., Inc., Lancaster, Pennsylvania.

Sax, K. 1931. The origin and relationship of the Pomoideae. *Journal Arnold Arboretum*, 12:3-22.

Schmidt, H. 1985. Observations on the length of the juvenile period in apple seedlings. *Acta Horticulturae*, 159:31-34.

Schmidt, H. 1994. Progress in combining mildew resistance from *Malus robusta* and *Malus zumi* with fruit quality, p. 3-6. *In* H. Schmidt and M. Kellerhals (eds.), *Progress in Temperate Fruit Breeding*. Kluwer Academic Press, Netherlands.

Shay, J. R., D. F. Dayton, and L. F. Hough. 1953. Apple scab resistance from a number of *Malus* species. *Proceedings. American Society for Horticultural Science*, 62:348-356.

Shelbourne, C. J. A. 1969. *Tree breeding methods*. NZ Forest Service, Wellington, New Zealand. Forest Research Institute, Technical paper No. 55.

Smith, S. M. 1984. Improvement of aroma of Cox's Orange Pippin apples stored in low oxygen atmospheres. *Journal of Horticultural Science*, 59:515-522.

Spelman, R. J., and H. Bovenhuis. 1998. Moving from QTL experimental results to the utilization of QTL in breeding programmes. *Animal Genetics*, 29:77-84.

Spiegel-Roy, P. 1991. Economic impact of mutation breeding in fruit trees, p. 215-235. *In* P. H. Kitto (ed.), *Plant mutation breeding for crop improvement: proceedings of an international symposium on the contribution of plant mutation breeding to crop improvement jointly organized by the International Atomic Energy Agency and the Food and Agriculture Organization of the United Nations and held in Vienna, 18-22 June 1990.*, vol. 1. International Atomic Energy Agency, Vienna, Austria.

Spinks, G. T. 1936. Apple breeding investigations. I. Results obtained from certain families of seedlings. *Annual Report for the Long Ashton Research Station*, 1935:19-49.

Tancred, S. J., J. N. Cummins, S. R. Dullahide, A. G. Zeppa, and J. B. Heaton. 1995. Advancement of the Australian disease resistance apple breeding program by cooperation with USA programs. *Fruit Varieties Journal*, 49:152-157.

Tanksley, S. D. 1983. Introgression of genes from wild species, p. 331-337. *In* S. D. Tanksley and T. J. Orton (eds.), *Isozymes*, vol. Part A. Elsevier, Amsterdam.

Tanksley, S. D., and S. R. McCouch. 1997. Seed banks and molecular maps: Unlocking the genetic potential from the wild. *Science*, 277:1063-1066.

Tanksley, S. D., and J. C. Nelson. 1996. Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theoretical and Applied Genetics*, 92:191-203.

Tao, D. G., L. D. Fu, L. Y. Tang, Z. Y. Shu, W. Q. Chun, L. H. Xiu, and F. L. Zhang. 1995. Distribution and use of *Malus* in the Hengduan mountain region, southwest China. *Plant Genetic Resources Newsletter*, 102:32-36.

Tartarini, S., L. Gianfranceschi, S. Sansavini, and C. Gessler. 1999. Development of reliable PCR markers for the selection of the Vf gene conferring scab resistance in apple. *Plant Breeding*, 118:183-186.

Thompson, J. M., and J. Taylor. 1971. Genetic susceptibility to *Glomerella* leaf blotch in apple. *Journal of Heredity*, 62:303-306.

Utkhede, R. S. 1986. In vitro screening of the world apple germplasm collection for resistance to *Phytophthera cactorum* crown rot. *Scientia Horticulturae*, 29:205-210.

Visser, T. 1965. On the inheritance of juvenile period in apple. *Euphytica*, 14:125-134.

Visser, T. 1976. A comparison of apple and pear seedlings with reference to the juvenile period. II. Mode of inheritance. *Euphytica*, 25:339-342.

Visser, T., and D. P. de Vries. 1970. Precocity and productivity of propagated apple and pear seedlings dependent on the juvenile period. *Euphytica*, 25:339-342.

Visser, T., J. J. Verhaegh, and D. P. de Vries. 1974. Resistance to scab (*Venturia inaequalis*) and mildew (*Podosphaera leucotricha*) and fruit properties of the offspring of the apple cultivar Antonovka. *Euphytica*, 23:353-364.

Watkins, R. 1974. Tree fruit breeding techniques at East Malling. *Indian Journal of Genetics & Plant Breeding*, 34(A):1248-1259.

Watkins, R. 1976. Apple and Pear, p. 247-250. *In* N. Simmonds (ed.), *Evolution of crop plants*, 1st ed. Longman Group Ltd, New York.

Watkins, R., and L. P. S. Spangelo. 1970. Components of genetic variance for plant survival and vigor of apple trees. *Theoretical and Applied Genetics*, 40:195-203.

Way, R. D., H. S. Aldwinckle, R. C. Lamb, A. Rejman, S. Sansavini, T. Shen, R. Watkins, M. N. Westwood, and Y. Yoshida. 1989. Apples (Malus). *Acta Horticulturae*, 290:3-62.

Weeden, N. F., and R. C. Lamb. 1987. Genetics and linkage analysis of 19 isozyme loci in apple. *Journal of the American Society for Horticultural Science*, 112(5):865-872.

Weeden, N. F., M. Hemmat, D. M. Lawson, M. Lodhi, R. L. Bell, A. G. Manganaris, B. I. Reisch, S. K. Brown, and G. N. Ye. 1994. Development and application of molecular marker linkage maps in woody fruit crops, p. 269-273. *In* H. Schmidt and M. Kellerhals (eds.), *Progress in Temperate Fruit Breeding.* Kluwer Academic Press, Netherlands.

Welander, M. 1998. The use of genetic engineering in fruit tree improvement. *Currents (Uppsala),* 17/18:47-50.

White, A. G. 1988. Apple breeding in New Zealand. *Acta Horticulturae,* 224:119-121.

White, A. G., and Y. Lespinasse. 1986. The inheritance of fruit colour in apple (*Malus pumila* Mill.). *Agronomie,* 6:105-108.

Whitmore, F. W. 1992. Plant variety rights testing systems in New Zealand. *Plant Varieties and Seeds,* 5:151-162.

Williams, A. A. 1979. The evaluation of flavour quality in fruit products, p. 287-305. *In* G. Land and H. E. Nursten (eds.), *Progress in Flavour Research.* Applied Sciences Publ. Ltd., London.

Williams, W., and A. G. Brown. 1960. Breeding new varieties of fruit trees. *Endeavour,* 19(75):147-155.

Yahia, E. M. 1994. Apple flavour. *Horticultural Reviews,* 16:197-234.

Yoshida, Y. 1986. Quality improvement in apple breeding in Japan, p. 255-257. *In* T. A. Williams and G. S. Wratt (eds.), *Plant breeding symposium DSIR,* vol. 5. Agronomy Society of New Zealand, Lincoln. Agron. Soc. N.Z. Special publication 5.

Zhang, W., J. Zhang, and X. Hu. 1993. Distribution and diversity of Malus germplasm resources in Yunnan, China. *HortScience,* 28(10):978-980.

Zhang, Y. X., Y. Lespinasse, and E. Chevreau. 1990. Induction of Haploidy in fruit trees. *Acta Horticulturae,* 280:293-305.

Zhdanov, V. V., and E. N. Sedov. 1988. Combining ability of apple varieties for resistance to scab. [Russian]. *Genetika,* 24:1250-1255.

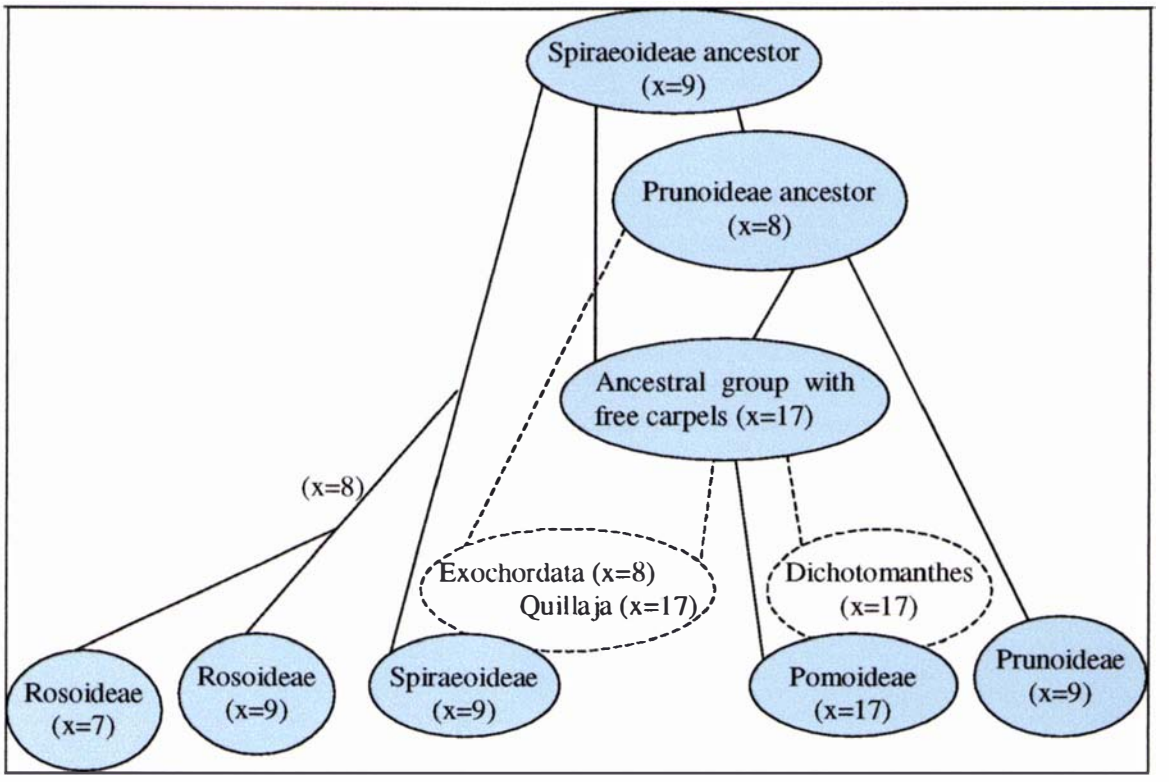

Figure 1. Diagram of the possible evolution of the Rosaceae subfamilies based on morphological, cytological and chemical data (Challice, 1974).

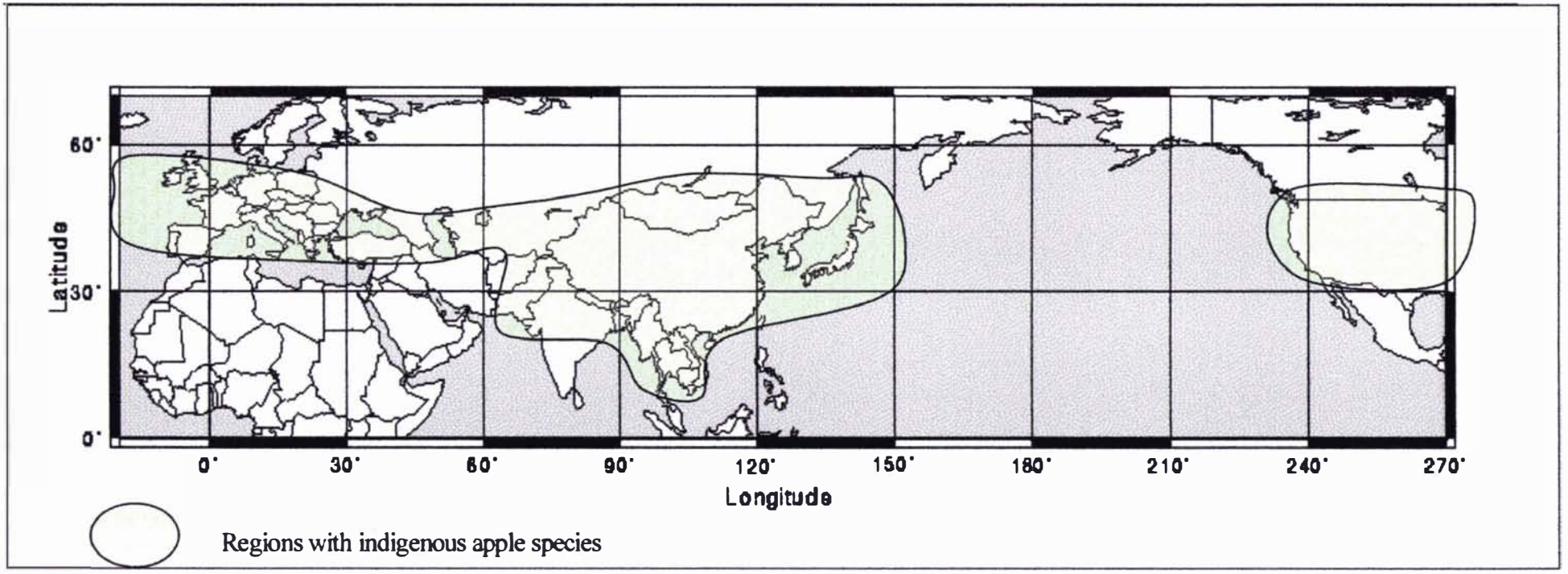

Figure 2. World map of the origins of apple species

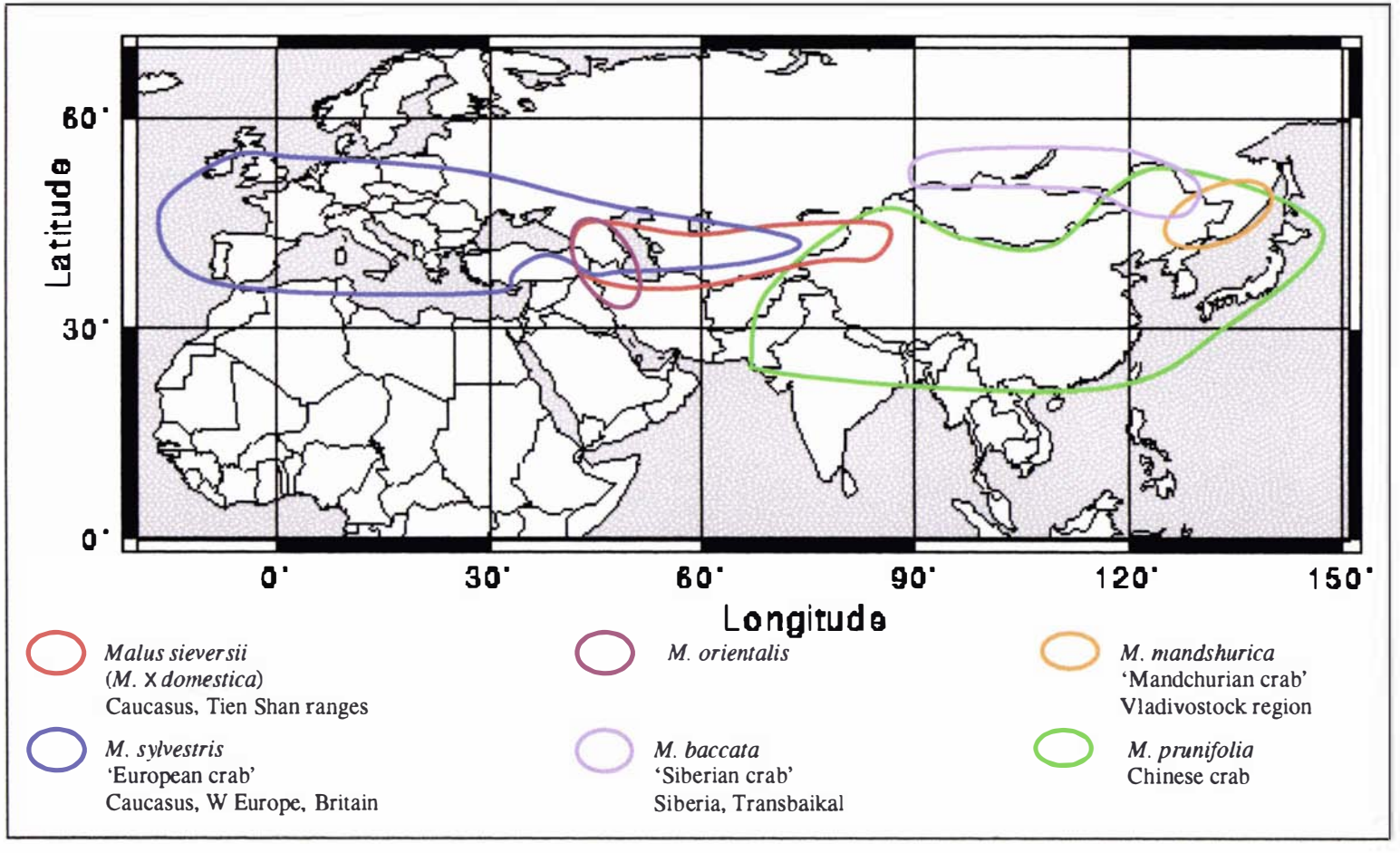

Figure 3. World map of the origins of apple species involved in the ancestry of the domestic apple

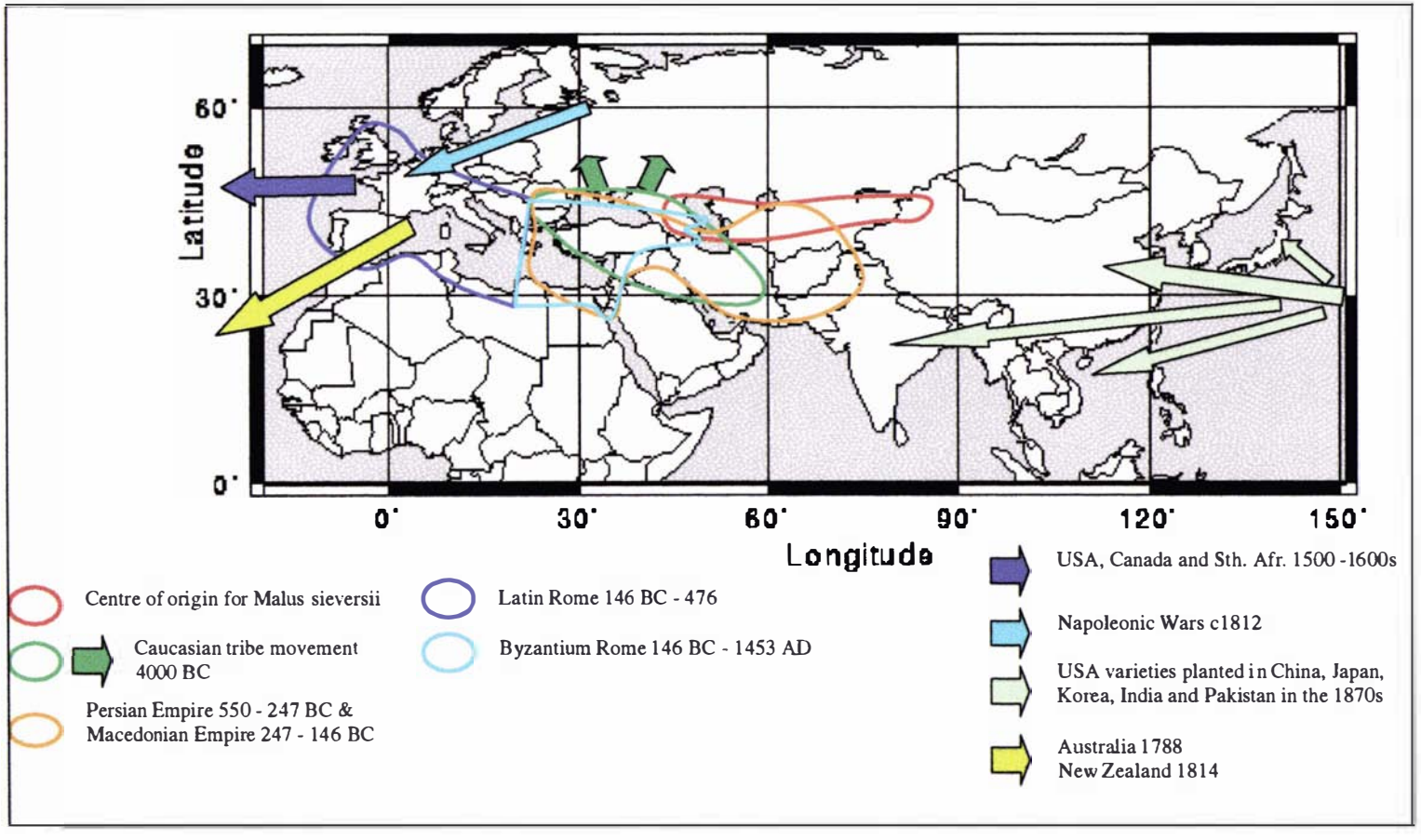

Figure 4. Map of the distribution and domestication of apple (*M.* ×*domestica*)

Table 1. Origin and botanical description of apple species involved in the domestication of apple

Malus species	Origin	Description
M. cerasifera	*M. baccata* x *M.* ×*domestica* (Ponomarenko, 1986)	Fruit size 1cm, deciduous calyx, 5 carpels, fruit hangs on tree when ripe (Way et al., 1989).
M. prunifolia	*M. cerasifera* x *M.* ×*domestica* (Ponomarenko, 1986). Chinese crab (Janick et al., *1996*), Nth & NE China, Korea (Way et al., 1989), and grown extensively in China, Korea, Japan, India and Pakistan (Morgan et al., *1993*)	Larger Chinese crab (Janick et al., *1996*). Small sharp fruit used in preserves (Morgan et al., *1993*).
M. ×*robusta*	*M. baccata* x *M. Prunifolia* (Way et al., 1989)	Source of mildew resistance (Knight and Alston, 1968; Schmidt, 1994).
M. ×*zumi*	*M. mandshurica* x *M. sieboldii* (Way et al., 1989)	Involved (Ponomarenko, 1983, 1990). Resistant to mildew (Schmidt, 1994).
M. ×*floribunda*	Hybrid (Way et al., 1989)	Small fruited crab (Hough et al., 1953). Involved in *M.* ×*domestica* (Ponomarenko, 1983).
M. baccata (L.) Borkh.	Siberia (Morgan et al., *1993*; Janick et al., 1996), *Eas*tern Siberia (Ponomarenko, 1980a), Transbaikal region (Ponomarenko, 1972) and N & NE China, Japan, Korea (Way et al., 1989)	Hardy and small fruited crab (Morgan et al., 1993; Janick et al., 1996) Fruit size 1cm, deciduous calyx, 4-5 carpels, fruit hangs on tree when ripe (Way et al., 1989). Precocious (Ponomarenko, 1980a) Involved (Ponomarenko, 1983)
M. orientalis	Wild in the Caucasus (Ponomarenko, *1975a;* Morgan et al., 1993)	Late keeping bitter fruit (Morgan et al., *1993*; Janick et al., 1996).

Malus species	Origin	Description
M. sieboldii	Japan, Korea (Way et al., 1989)	
M. sieversii	Tian Shan (Heavenly Mountains) and Pamir Alai ranges (Central Asia) (Ponomarenko, 1980a, 1982, 1983). Wild forms are found in Kopetdag, Turkmen, central Tadzhihistan (Ponomarenko, 1980b). Li (1988) indicated that the centre of origin *M. pumila* (*M. sieversii*) includes Yinong, western part of the Autonomous Region of Xingjiang, China (Way et al., 1989). Secondary centres have established in USSR, West Europe (UK, France, Germany), USA (Ponomarenko, 1980b).	Fruit size >2cm, persistant calyx, 5 carpels, ripe fruit drops (Way *et al.*, 1989)
M. sylvestris	Britain across Europe to the Balkans and Northern Turkey (Way et al., 1989; Morgan et al., 1993; Janick et al., 1996).	Smallish, astringent, greenish-yellow fruit (Morgan et al., 1993; Janick et al., 1996). Probably the origin of bitter-sharp cider apples (Morgan et al., 1993). Involved (Ponomarenko, 1983)
M. mandshurica	Wild in the region NE of Vladivostok (Ponomarenko, 1973).	(Morgan et al., 1993; Janick et al., 1996). Fruit size 1cm, deciduous calyx, 5 carpels, fruit hangs on tree when ripe (Way et al., 1989).

Chapter 3: Quantitative evaluation of apple (*Malus* × *domestica* Borkh.) fruit shape by principal component analysis of Fourier descriptors

Accepted for publication by Euphytica 1999. Currie, A. J., S. Ganeshanandam, D. A. M. Noiton, D. J. Garrick, C. J. A. Shelbourne, and N. Oraguzie. 1999. "Quantitative evaluation of apple (*Malus* × *domestica* Borkh.) fruit shape by principal component analysis of Fourier descriptors."

Abstract

Fruits from 1253 apple genotypes representing 82 open-pollinated families planted at three sites were cut along the stem-calyx axis at the widest point to analyse fruit shape. An image analysis program was used to extract calliper measurements of the fruit outline and calculated Fourier descriptors for each fruit outline. Five independent shape traits were identified from a principal component analysis of the Fourier descriptors. The shape traits and the proportion of the total phenotypic variation they accounted for were fruit aspect (76.8%), asymmetric-crown (7.8%), fruit conicity (6.0%), asymmetric-sides (4.3%), and fruit squareness (2.0%). Genetic and residual variance components were estimated with data from two sites using restricted maximum-likelihood techniques and unbiased heritability was estimated to be 0.53 for aspect, 0.41 for conicity and 0.10 for squareness. Multiple regression between calliper measurements and aspect, conicity, and squareness traits showed firstly that aspect was best predicted by fruit length / width ratio (R^2 =0.97), secondly conicity could be predicted by both the distance of the maximum width from the base of the fruit / fruit length ratio and the calyx basin width / fruit width ratio (R^2 =0.44), and finally squareness was predicted by ratio of the product of calyx basin width and distance of the maximum width from the calyx end of the fruit by the product of fruit length and fruit width (R^2 =0.19). A chart based on the aspect, conicity and squareness principal component values was drawn to allow visual assessment of shape.

Abbreviations

Calyx basin width (CBW), fruit length (L), distance of maximum width to calyx end of the fruit (MWD), principal component (PC), principal component analysis (PCA), stem cavity width (SCW), fruit width (W).

Introduction

World-wide consumer demand for fruit is sensitive to fashion and fruit from new cultivars can command a premium (Scott, 1998) creating incentives for breeders to continuously develop new types to maintain premium returns. Fruit shape is one of the traits that consumers use to differentiate cultivars, shape could be manipulated to minimise damage during packing and transport, and fruit shape is also used for describing cultivars when applying for plant variety rights (Anon., 1994) or for cultivar registers.

Shape categories such as oblate, globose, conical, and oblong have been widely used to describe fruit shape in apple cultivar registers (Hedrick, 1938; Smith, 1971; Lane and MacDonald, 1987; Cripps et al., 1993; Morgan et al., 1993). Shape charts developed by the International Board for Plant Genetic Resources (I.B.P.G.R.) (Watkins and Smith, 1982), and by the Union pour la Protection des Obtentions Vegetales (U.P.O.V.) (Anon., 1994) were also based on such shape categories. However, intermediate shapes could not be identified and categories were not ranked which made shape selection difficult. They are therefore of limited use to breeders other than for purely descriptive purposes. To overcome some of the disadvantages of a discrete shape category Smith (1971) sorted the shape categories into groups according to a common shape trait. Aspect shape trait was described with flat, intermediate or tall, conicity shape trait was described with rectangular, truncate-conic or conic and 'roundness' shape trait with convex, straight or waisted.

Fruit aspect ratio was the first quantitative apple shape trait applied by breeders. Spinks (1936) and Brown (1960) both reported that aspect ratio was quantitatively inherited in

apple. Lespinasse (unpublished) expanded the quantitative description of apple shape by proposing a 3x3 shape chart with aspect ratio on the vertical axis and a ratio of the stem cavity-width to the calyx-basin width (conicity) on the horizontal axis. However, the statistical analysis that was used to validate this chart was not reported. Busscher *et al.* (1995) also selected aspect ratio and conicity to describe apple shape based on Fourier and Principle Component analyses of I.B.P.G.R. chart shapes but they did not describe the derivation of conicity and they based their analysis on idealised, symmetrical I.B.P.G.R. shape categories and thus excluded the possibility of other traits found in apples from the field (e.g. asymmetrical shape traits). These researchers did not report on the inheritance of conicity.

Shape traits can be influenced by genetic and environmental factors. Breeders can only select for traits that are genetically inherited, therefore a variance component analysis was needed to select traits with a significant genetic component.

The objective of this study was to comprehensively study apple shape and determine genetically inherited, apple fruit shape traits using data collected from a broad genetic base.

Materials and Methods

Visualisation of principal component (PC) shape traits

Apple genotypes were sampled from a genetically-diverse breeding population consisting of open-pollinated (mixed half and full sib) families planted as single tree plots in incomplete blocks (Noiton and Shelbourne, 1992). Trees were sampled from three sites. The first site was located at the Havelock North Research Centre, Hawkes Bay, New Zealand (39°40'S 176°53'E), the second site was the Nelson Research Centre, Riwaka, New Zealand (41°3'S 173°1'E), and the third site was the Clyde Research Orchard, Central Otago, New Zealand (45°14'S 169°20'E). Two fruit per tree were collected from 1253 genotypes representing 82 families.

Each fruit was cut along the stem-calyx axis through its widest section (Figure 1). One half was photocopied for subsequent image analysis. Images were captured using a Sony colour video CCD camera, model DXC-3000P, linked to a PC Vision Plus frame-grabber card (Imaging Technology Inc., USA) and a multisync monitor. The image analysis software was Visual Image Processing System version 5 (VIPS 5) developed by D. Bailey at Massey University, New Zealand and run on an IBM compatible 486 with Windows 3.1. An image analysis algorithm was developed to extract Fourier descriptors (sine and cosine terms) for the outline and calliper measurements of apple shape. The calliper measurements were fruit length, maximum width, distance from the maximum width to the calyx end of the fruit, stem cavity width and calyx basin width (Figure 1).

Fruit outline was described using Fourier analysis, which measured the radius (mid-point along the stem-calyx axis to the outline) as a function of angle (Titchmarsh, 1967). The function was expressed as the sum of sine and cosine terms of which the first ten pairs fully described the apple outlines. Shape was made scale invariant by dividing the radius by the average radius of the best fitting offset circle (amplitude of the first harmonic).

A covariance matrix of the 20 Fourier descriptors was analysed by SAS principal components analysis (PCA) to extract phenotypically uncorrelated PC shape traits (SAS Institute, 1990; Busscher et al., 1995; Furuta et al., 1995).

Maximum and minimum values for each PC shape trait were converted back into sine and cosine terms (Furuta *et al.*, 1995) using the equation described by Jackson (1991) and the apple outlines were redrawn to visualise each PC shape trait. Visually interpretable PC shape trait traits were selected for further analysis.

$$z = U'(x - \bar{x})$$
$$x = \bar{x} - Uz$$

Where z was the $n * j$ matrix of PC shape trait values, U was the $j * p$ matrix of the eigen vectors of the covariance matrix (j PCs by p variables), x was the $n * p$ observation matrix (n observations by p variables) and $\bar{x}$ was the $p * 1$ vector of the x means.

The PC shape traits were defined in terms of the calliper measurements (Figure 1) by multiple regression. Calliper measurements with the largest regression coefficients were combined then regressed against the PC shape traits to select the best combination.

Genetic analysis

Genetic parameters were estimated to select genetically inherited shape traits and to interpret their application in a breeding program. Due to the extremely unbalanced nature of the data only the Havelock North and Nelson sites were included in the genetic analysis (two fruit per tree were collected from 1008 genotypes representing 71 families). Each family had at least 4 trees per site. A mixed, general linear model with the following model equation was fitted to the data.

$$y_{ijkl} = S_i + B_j(S_i) + P_k + F_l(P_k) + S_i * F_l(P_k) + e_{ijkl}$$

Where S was the i^{th} site, B was the j^{th} incomplete block nested within the i^{th} site, P was the k^{th} genetic group and F was the l^{th} family nested within the k^{th} genetic group. S and P were analysed as fixed effects, $F(P)$, $B(S)$ and $S * F(P)$ were analysed as random effects, and e was the error associated with each observation.

Between-family genetic variation can be inflated by differences in genetic origins. This was adjusted for by assigning families to genetic groups, P_i , by country of origin, era and species. The genetic and residual variance components were estimated with ASREML (Gilmour et al., 1998) to calculate the individual heritability for each PC shape trait. The families were open-pollinated seedlings, so the exact proportion of full-sibs and half-sibs in each cross was unknown. We used a coefficient of relationship (C)

of 0.33 to estimate the individual heritability at each site which is between the *C* for full-sibs (*C* = 0.5) and half-sibs (*C* = 0.25).

$$\hat{h}_{ind}^{\,2} = \frac{\hat{\sigma}_{f(p)}^{\,2}}{C\left(\hat{\sigma}_{f(p)}^{\,2} + \hat{\sigma}_{e}^{\,2}\right)}$$

$$Var\left(\hat{h}_{ind}^{\,2}\right) = Var\left(\frac{\hat{\sigma}_{n}^{\,2}}{\hat{\sigma}_{d}^{\,2}}\right) = \left(\frac{\hat{\sigma}_{n}^{\,2}}{\hat{\sigma}_{d}^{\,2}}\right)^{2}\left(\frac{Var\left(\hat{\sigma}_{n}^{\,2}\right)}{\hat{\sigma}_{n}^{\,4}} + \frac{Var\left(\hat{\sigma}_{d}^{\,2}\right)}{\hat{\sigma}_{d}^{\,4}} - \frac{2Cov\left(\hat{\sigma}_{n}^{\,2},\sigma_{d}^{\,2}\right)}{\hat{\sigma}_{n}^{\,2}\hat{\sigma}_{d}^{\,2}}\right)$$

Where $\sigma_{f(p)}^{2}$ *and* σ_{e}^{2} are the family (nested within genetic groups) and the error variance respectively. For the variance of the heritability, the heritability estimate is expressed in terms variance of the numerator σ_{n}^{2} and the variance of the denominator σ_{d}^{2}. Genetic correlations between the PC shape traits were calculated with ASREML to estimate the genotype by environment interaction (G*E).

$$\hat{r}_{A_{a,b}} = \frac{\hat{\sigma}_{A_{a,b}}}{\sqrt{\hat{\sigma}_{A_a}^{\,2}\hat{\sigma}_{A_b}^{\,2}}}$$

$$Var\left(\hat{r}_{A_{a,b}}\right) = Var\left(\frac{\hat{\sigma}_{a,b}}{\sqrt{\hat{\sigma}_{a}^{\,2}\hat{\sigma}_{b}^{\,2}}}\right)$$

$$= \left(\frac{\hat{\sigma}_{a,b}}{\sqrt{\hat{\sigma}_{a}^{\,2}\hat{\sigma}_{b}^{\,2}}}\right)^{2}\left(\begin{array}{l}\dfrac{Var\left(\hat{\sigma}_{a}^{\,2}\right)}{4\hat{\sigma}_{a}^{\,2^{2}}} + \dfrac{Var\left(\hat{\sigma}_{b}^{\,2}\right)}{4\hat{\sigma}_{b}^{\,2^{2}}} + \dfrac{Var\left(\hat{\sigma}_{a,b}\right)}{\hat{\sigma}_{a,b}^{\,2}} + \dfrac{2Cov\left(\hat{\sigma}_{a}^{\,2},\hat{\sigma}_{b}^{\,2}\right)}{4\hat{\sigma}_{a}^{\,2}\hat{\sigma}_{b}^{\,2}} \\ -\dfrac{2Cov\left(\hat{\sigma}_{a}^{\,2},\hat{\sigma}_{a,b}\right)}{2\hat{\sigma}_{a}^{\,2}\hat{\sigma}_{a,b}} - \dfrac{2Cov\left(\hat{\sigma}_{a,b},\hat{\sigma}_{b}^{\,2}\right)}{2\hat{\sigma}_{a,b}\hat{\sigma}_{b}^{\,2}}\end{array}\right)$$

Where $\hat{r}_{A_{a,b}}$ is the estimate of the genetic correlation between traits *a* and *b*, $\sigma_{A_{a,b}}$ is the covariance between *a* and *b*, $\hat{\sigma}_{a}^{\,2}$ and $\hat{\sigma}_{b}^{\,2}$ are the estimated variance of the traits *a* and *b*, $\hat{\sigma}_{e}^{\,2}$ is the error variance estimate, and *Var* and *Cov* are the variance and covariance.

Shape chart design

Three shape traits were selected and visualised in a chart of apple shapes for scoring fruit shape in the field. The shape traits were displayed as three 5x5 charts with the first two PC traits on each axis of the charts and the minimum, mid and maximum value of

the third PC trait for each of the three charts. Each axis of the chart divided the PC trait into 5 points, which included the minimum, mid and maximum value of the PC trait. Three PC trait values were taken from each chart cell with the remaining PC traits set to zero (zero is the average PC value). The PC values were converted back into Fourier descriptors using the equation from Jackson (1991), then redrawn to produce an apple shape outline for each chart cell.

Results

Visualisation of PC shape traits

The proportion of the total phenotypic variation accounted for by each PC shape trait was calculated from the eigen values of the Fourier data covariance matrix (Table 1). Over 76% of the phenotypic variation in the Fourier descriptors was explained by the first PC shape trait and 96.9% of the variation was explained by the first five PC traits.

A range of values for each PC shape trait were converted into Fourier descriptors and the fruit outline was redrawn for initial visual interpretation (Table 2). The first five PC shape traits were visually interpreted as aspect ratio, asymmetric-crown, conicity, asymmetric-sides, and squareness. The PC shape traits greater than the fifth were removed from further analysis as they were difficult to interpret visually and contributed little to the variation in shape.

Genetic analysis

Heritabilities and their standard errors were calculated from the REML estimates of genetic and residual variance components (Table 3). Aspect PC shape trait was moderate to highly heritable (unbiased h^2 = 0.53). Conicity PC shape trait was moderately heritable (unbiased h^2 = 0.41) and squareness PC shape trait had a low heritability (unbiased h^2 = 0.10).

The asymmetric-crown trait (PC2) was removed from further analysis due to the confounding of the fruit preparation with any real shape trait and the asymmetric-sides trait (PC4) was removed from the analysis due to heritability estimates close to zero (Table 3).

The genetic correlations (Table 4) indicated the degree of genotype by environment (G*E) interaction. High genetic correlation as shown by the conicity shape trait indicated low G*E. The moderate to low genetic correlation shown by aspect and squareness shape traits respectively demonstrated moderate to high G*E.

Interpretation of PC traits

Multiple regression between the calliper measurements of the fruit shape outlines and the aspect, conicity and squareness PC traits revealed which calliper measurements could be used to define each PC trait (Table 5). Aspect PC was dominated by the contrast between length and width. The length / width was a good predictor of aspect PC ($R^2 = 0.97$). Aspect PC was negatively correlated to length / width.

Conicity PC increased with the distance of the maximum width to the base of the fruit (MWD) and fruit width (W), but decreased with both calyx basin width (CBW) and fruit length (L) (Table 5). The position of the maximum with expressed by the ratio MWD/L was the best predictor of conicity PC ($R^2 = 0.39$) although stepwise regression calculated a slight improvement by including the width of the calyx basin relative to the fruit width (CBW/W) in the model ($R^2 = 0.44$). Conicity PC increased with increasing MWD/L and decreasing CBW/W.

Squareness PC was a contrast of MWD and CBW against W (Table 5). Combinations of the calliper measurements selected by stepwise regression lead to the definition of squareness PC as the ratio of the product of MWD and CBW to the product of L and W ($R^2 = 0.19$), which was negatively correlated to squareness PC.

Shape chart

The apple shape chart (Table 6) shows values of aspect on the vertical axis, conicity on the horizontal axis, and the minimum, mid and maximum values of squareness for each 5x5 grid. The chart values were based on the PC traits but the sign was reversed for aspect and squareness PC traits as these were negatively correlated to aspect ratio and squareness. Aspect chart trait exhibits short and wide shapes for the minimum value ranging to tall and narrow apple shapes for the maximum value. The conicity trait chart shapes ranged from pyriform shapes (minimum value) to conical shapes (maximum value). The maximum squareness trait chart values formed the squarest shapes, with the cheek and shoulders of the fruit filling the corners of the square. The minimum squareness trait value drew a shape tending towards a diamond shape, with the maximum width and the ends of the stem-calyx axis filling the corners of the diamond with the shoulders and cheek of the fruit being less developed.

Discussion

In this study, apple fruit shapes from a genetically broad-based population were analysed to determine genetically inherited shape traits. The first five PC shape traits were found to be respectively aspect ratio, asymmetric-crown, conicity, asymmetric-sides, and squareness.

Aspect ratio dominated the observed variation in apple shape accounting for more than 76% of the total apple shape variation. This trait was predicted by the ratio between the length and the width of the fruit. The combination of the importance of the aspect trait and the close fit with length by width ratio supports its past use to measure fruit shape (Spinks, 1936; Brown, 1960; Smith, 1971; Busscher et al., 1995). The heritability of the aspect ratio at each site was found to be high which suggests that individual or phenotypic selection of the next generation's parents for fruit aspect would be efficient. However, the moderate level of genotype by environment interaction for aspect shape

trait, indicated by the genetic correlation between sites, means that the ranking of families would be different at different sites and so selection at each site would increase the genetic gain.

Although asymmetric-crown was the second most important trait in this data set, it was removed from the analysis as it was confounded with the preparation of the apples. Apples either have a smooth crown or a raised crown. Raised crowns are 5-pointed and therefore when an apple with a raised crown was cut in half, both a peak on one side of the fruit and a trough on the other side could be bisected, distorting the symmetry of the crown. Slight variations in the angle of the cut would also increase the variation in the crown asymmetry and reduce the heritability. To confirm the existence of the asymmetric-crown trait, data could be collected from profiles of a whole fruit.

The third PC shape trait was the conicity trait. A conicity trait was also used by Smith (1971) for discrete shape categories, and by both Lespinasse (unpublished) and Busscher et al. (1995) for continuous apple shape traits. In this study, conicity trait best fitted the distance of the maximum width from the calyx end of the fruit standardised by the length. Such a measurement was used by Furuta et al. (1995) who defined conicity of Soya bean leaf shapes as the distance of the widest point along the midrib. Busscher et al. did not define which conicity measurements they used. Some of the variation in conicity PC trait in this study was also explained by the width of the calyx basin relative to the fruit width. Both Smith's (1971) conicity categories of rectangular, truncate-conic, or conic, and Lespinasse's (unpublished) definition of a conicity ratio between the stem cavity width and the calyx basin width included calyx basin width in their definition. Conicity trait had moderate to high heritability, which means that individual selection of the next generation's parents for this trait would be efficient. The high genetic correlation between sites indicates a low genotype by environment interaction for conicity trait. Therefore the family ranking would be similar at different sites and selection of the parents for the next generation could occur at one site.

The fourth PC trait was asymmetric-sides trait which was the least useful shape trait as its heritability was close to zero. This means that most of the variation was due to non-genetic effects. An example of a non-genetic cause of lopsided fruit is poor seed set due to environmental conditions. The tissue next to seedless carpels develops less than tissue next to full carpels. Therefore one side of the fruit is smaller than the other (Heinicke, 1917; Latimer, 1931, 1937a, b; Brittain, 1933; Brault and Oliveira, 1995). There may be other causes of lopsided fruit. Shape traits with heritabilities close to zero would have little use to breeders as the trait is not inherited, nor would it be useful for cultivar description as the shape would vary from environment to environment.

Squareness was the fifth trait found in this study. Smith's (1971) shape categories of convex, straight or concave and Furuta et al. (1995) roundness measure for Soya bean leaf shape both showed similarities with the squareness trait in that they measured the fullness of shape. Squareness was found to be moderately heritable for each site. A low unbiased heritability suggested high genotype by environment interaction, which was confirmed by a low genetic correlation between sites. Moderate heritability indicates that some genetic gains in the squareness shape trait could be made with individual selection but a family selection or progeny testing would increase the accuracy of selection.

In the present study a shape chart was developed to enable breeders to quickly and accurately score trees on aspect, conicity and squareness shape traits, which accounted for 84.8% of the total phenotypic variation in apple shape. Each trait was scored with a continuous scale therefore a meaningful score may be applied to each shape and intermediate shapes may be selected, removing the problems inherent in discrete category charts such as the U.P.O.V. and I.B.P.G.R. charts (Watkins and Smith, 1982; Anon., 1994). Each trait was shown to be genetically inherited, allowing breeders to use the chart to select for a particular shape of interest. This chart contained all the shapes described in the above charts but has the advantage of being based on continuous, genetically-inherited traits. The chart may also prove useful in describing apple shapes in cultivar registers. Small changes in the position of the widest point were observed for

fixed values of conicity and varying values for aspect and squareness traits due to the fit between the calliper measurements and the PC scores on which the chart was based. An alternative design of chart could be designed based on the calliper measurements rather than on the PC traits.

The combination of digital image and statistical analysis has been a useful tool to break down the complex shape traits into components and could be applied to other morphological traits of interest to breeders such as vegetable shape. These shape traits can then be analysed to determine the genetic and environmental components of variation and other genetic parameters for optimising the breeding program. In this case there was significant genotype by environment interaction for aspect and squareness which means that the best genotypes at one site were not the best parents over both sites. If the aim is to maximise genetic gain and adaptability over a range of sites then greater gains could be made with selection at multiple sites rather than selection at one site.

Acknowledgements

The authors would like to thank Dr D. G. Bailey, Image Analysis Unit, Massey University, for helpful suggestions on the image analysis of the apple shapes and colleagues at the Institute of Veterinary, Animal and Biomedical Sciences, Massey University for helpful comments. The analysis was run using the ASREML program developed by Dr. A. R. Gilmour, NSW Agriculture, Australia. This research was made possible by funding with a scholarship from HortResearch, New Zealand.

References

Anon. 1994. Working paper on draft test guidelines for apple (fruit varieties) (*Malus* Mill.). *UPOV. Technical working party for fruit crops, 25th session, Napier/Rotorua, New Zealand, Sept 19-24. TWF/25/3.*

Brault, A. M., and D. de Oliveira. 1995. Seed number and an asymmetry index of 'McIntosh' apples. *HortScience*, 30(1):44-46.

Brittain, W. H. 1933. Apple pollination studies in the Annapolis Valley. *Nova Scotia Can. Dept. Agr. Can. Bul. 162.*

Brown, A. G. 1960. The inheritance of shape, size and season in progenies of the cultivated apple. *Euphytica*, 9:327-337.

Busscher, R. de, E. Schrevens, and J. de Baerdemaeker. 1995. Automated characterisation of apple shapes using digitised video images. *JSAM 1995 International symposium on automation and robotics in bioproduction and processing. Kobe, Japan.*

Cripps, J. E. L., L. A. Richards, and A. M. Mairata. 1993. 'Pink Lady' Apple. *HortScience*, 28(10):1057.

Furuta, N., S. Ninomiya, N. Takahashi, H. Ohmori, and Y. Ukai. 1995. Quantitative evaluation of soybean (Glycine max L Merr) leaflet shape by principal component scores based on elliptic Fourier descriptor. *Breeding Science*, 45:315-320.

Gilmour, A. R., B. R. Cullis, S. J. Welham, and R. Thompson. 1998. *ASREML*, October ed. NSW Agriculture, .

Hedrick, U. P. 1938. *Cyclopediea of hardy fruits*, 2nd ed. The MacMillan Company, New York, 402 p.

Heinicke, A. J. 1917. Factors influencing the abscision of flowers and partially developed fruits of the apple (Pyrus malus L.). *Cornell University Agricultural Experimental Station Bulletin 393.*

Jackson, J. E. 1991. *A User's Guide to Principal Components*. John Wiley & Sons, New York, 569 p.

Lane, W. D., and R. A. MacDonald. 1987. 'Shamrock' apple. *HortScience*, 22(3):515-516.

Latimer, L. P. 1931. Further observations on factors affecting fruit setting of the McIntosh apple in New Hampshire. *Proceedings. American Society for Horticultural Science*, 28:87-92.

Latimer, L. P. 1937a. The effect of reducing the number of functioning stigmas on fruit-setting and characteristics of the McIntosh apple. *Proceedings. American Society for Horticultural Science*, 34:22-25.

Latimer, L. P. 1937b. Self- and cross-pollination in the McIntosh apple and some of its hybrids. *Proceedings. American Society for Horticultural Science*, 34:19-21.

Morgan, J., A. Richards, and E. Dowle. 1993. *The Book of Apples.* Ebury Press, London. Published in association with the Brogdale Horticultural Trust.

Noiton, D., and C. J. A. Shelbourne. 1992. Quantitative genetics in an apple breeding strategy. *Euphytica*, 60:213-219.

SAS Institute, ed. 1990. *SAS/STAT User's Guide Version 6*, 4th ed., Vol. 1-2. SAS Institute Inc., Cary, USA.

Scott, D. 1998. Hortcouture fashion food conference. *Orchardist of New Zealand*,(Feb):37-50.

Smith, M. W. G. 1971. *National Apple Register of the United Kingdom.* Ministry of Agriculture, Fisheries and Food, London, 652 p.

Spinks, G. T. 1936. Apple breeding investigations. I. Results obtained from certain families of seedlings. *Annual Report for the Long Ashton Research Station*, 1935:19-49.

Titchmarsh, E. C. 1967. Convergence and Summability, p. 1-24. *In Introduction to the theory of Fourier integrals*. Oxford University Press, Oxford.

Watkins, R., and R. A. Smith. 1982. *Descriptor list for apple (*Malus*)*. International Board for Plant Genetic Resources. Commission of European Communities: Committee on disease resistance breeding and use of genebanks. C.E.C. Secretariat, Brussels, 46 p.

Table 1. Proportion of the total phenotypic variance accounted for by the first 6 PCs

PC	Percent of total variance	Cumulative percentage of total variance
1	76.8	76.8
2	7.8	84.6
3	6.0	90.6
4	4.3	94.9
5	2.0	96.9
6	0.8	97.7

Table 2. Visualisation of the first six PC shape traits (minimum to maximum value). Apple shapes are drawn with the stem end at the top and the calyx at the bottom.

PC value PC shape trait	Minimum		Mid		Maximum
PC1 Aspect					
PC2 Asymmetric crown					
PC3 Conicity					
PC4 Asymmetric sides					
PC5 Square					
PC6 Asymmetry					

Table 3. Heritability estimates (and standard errors) for PC apple shape traits at the Havelock North and Nelson sites

Site	PC1 Aspect	PC2 Asymmetric -crown	PC3 Conicity	PC4 Asymmetric -sides	PC5 Square
Havelock Nth.	0.85 (0.17)	0.05 (0.08)	0.79 (0.17)	0.11 (0.09)	0.34 (0.12)
Nelson	1.01 (0.18)	0.11 (0.08)	0.51 (0.14)	0.08 (0.08)	0.45 (0.14)
Both sites	0.53 (0.16)	0.06 (0.05)	0.41 (0.14)	0.00 (0.06)	0.10 (0.10)

Table 4. Genetic correlation (r_g) between sites and standard error for apple shape traits

Shape trait	r_g	S.E (r_g)
PC1 Aspect	0.46	0.17
PC3 Conicity	0.67	0.16
PC5 Square	0.17	0.29

Table 5. Regression coefficients between apple PC shape traits and calliper measurements

Calliper measurement	Regression coefficients		
	Aspect PC	Conicity PC	Squareness PC
Length	-0.92	-0.24	0.03
Width	0.79	0.11	0.07
Maximum width distance	-0.05	0.38	-0.11
Stem cavity width	0.00	-0.02	-0.01
Calyx basin width	0.21	-0.27	-0.26

Table 6. Apple shape chart based on aspect, conicity and squareness PC traits. Apple shapes are drawn with the stem end at the top and the calyx at the bottom.

Maximum squareness	Maximum conicity		mid point		Minimum conicity
Maximum aspect					
					
Mid point					
					
Minimum aspect					

Table 6 (continued)

Squareness mid point	Maximum conicity		mid point		Minimum conicity
Maximum aspect					
					
Mid point					
					
Minimum aspect					

Table 6 (continued)

Minimum squareness	Maximum conicity		mid point		Minimum conicity
Maximum aspect					
					
Mid point					
					
Minimum aspect					

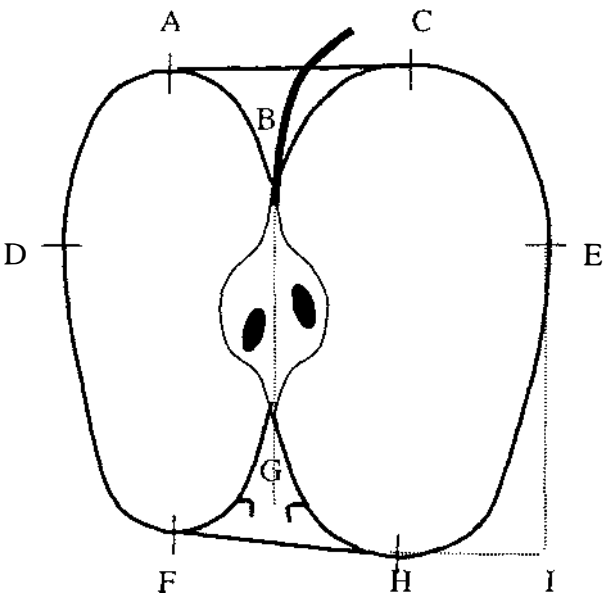

Figure 1. Apple cross-section showing the calliper measurements. B = stem, G = calyx, BG = stem-calyx axis, CH = length (L), DE = width (W), EI = maximum width distance (MWD), AC = stem cavity width, FH = calyx basin width.

Chapter 4: Estimates of heritabilities and genetic correlations for apple (*Malus* × *domestica* Borkh.) traits

Abstract

This study used a genetically broad-based apple breeding population to estimate narrow-sense heritabilities and genetic correlations with restricted maximum likelihood techniques, fitting a model for half-sib analysis of open-pollinated apple progenies. Narrow sense heritability estimated at two sites and across sites for fruit acidity (0.17-0.22), fruit sugars (0.25-0.26), and tree growth habit (0.19-0.41) indicated environmental or non-additive genetic variance. The consequences of these lower heritabilities and methods to increase the heritability estimates were discussed. Higher heritabilities were estimated at two sites for fruit squareness (0.32-0.43), fruit conicity (0.32-0.46), powdery mildew incidence (0.40), vigour (0.28-0.62), fruit firmness (0.44-0.53), harvest time (0.66-0.82), leafing (0.60-0.83), fruit aspect ratio (0.74-0.89), flowering (0.92) and fruit size (0.90-1.01). Traits with a high narrow-sense heritability could be selected for efficiently with individual selection. High genetic correlations were estimated between sites (≥0.64), which indicated low GxE and that selection at one site would be efficient for these traits. High positive genetic correlations were estimated for leafing with flowering (0.95), harvest time with fruit firmness (0.92), leafing with harvest time (0.69), and fruit acidity with fruit firmness. High negative genetic correlations were reported for vigour with fruit squareness (-0.82) and tree growth habit with leafing (-0.53). However, caution must be applied when interpreting estimated genetic correlations between traits due to high standard errors and the possible influence of dependence (non-genetic interaction with other traits) and linkage disequilibrium. Implications for breeding program design are summarised.

Introduction

Estimates of genetic parameters such as heritability and genetic correlation between traits are essential to making successful decisions in choosing among breeding strategies. Narrow sense heritability determines the response to selection based on

family means or individual phenotype. Genetic correlation between sites provides indication of the extent of genotype-by-environment interaction (GxE). High GxE may necessitate selection at each site, while selection at one site for all locations would be efficient in the absence of GxE. Genetic correlation between traits influences the response to multi-trait selection. Little gain will be made if large negative correlations exist. Finally, construction of an index to rank individuals for selection requires knowledge of phenotypic and genetic correlations and heritabilities. Quantitative genetic principles have been widely applied in animal (Falconer, 1989), crop (Hallauer, 1992) and forest tree breeding (Shelbourne, 1969) to enhance the efficiency of short-term and long-term gains.

Historically apple breeders have selected parents from the best commercial cultivars based on phenotypic characteristics, mated them by controlled pollination between selected parents or collected open-pollinated seeds from one selected parent and applied intense phenotypic selection on the resulting large progeny population (Janick et al., 1996). The best individuals were then vegetatively propagated on rootstock for clonal selection based on performance at several sites, and the best were released for commercial production. Such a mode of selection has continued over centuries, with each region around the world developing apples with distinct local fruit characteristics. However, exchange of genetic material from common parents has lead to a reduction in regional differences. Recently only a few cultivars have been used in many crosses, increasing the levels of coancestry and reducing genetic diversity (Noiton and Alspach, 1996).

The introduction of new, agronomically-useful traits into commercial apple breeding lines have mainly been achieved by using two breeding strategies. The first was to introgress major disease resistance genes from wild apple species using a modified backcross strategy, in which a different commercial cultivar was used in each backcross generation to minimise inbreeding (Hough et al., 1953). This strategy has been successful at introducing some major genes from wild crab apple species to a few breeding lines for commercial cultivar production (Crosby et al., 1992; Schmidt, 1994; Goffreda et al., 1996). The second approach was to apply quantitative genetic principles

to a genetically broad-based apple breeding population to improve the genetic diversity and the general combining ability of the apple breeding population with recurrent selection (Noiton and Shelbourne, 1992). Knowledge of genetic parameters is required for efficient design and implementation of such breeding strategies.

Genetic parameters are commonly estimated using restricted maximum likelihood (REML) methods, which has desirable properties in the analysis of data from unbalanced designs (Shaw, 1987). Typical applications of REML assume that the random effects are sampled from normal distributions, although the technique is robust to deviations from normality (Dieters et al., *1995*). REML also assumes that the base population is unrelated, unselected, non-inbred and randomly mated. Pedigree and selection information is required to trace back to an unrelated base population that meets these assumptions. Unfortunately this is impossible for many apple cultivars as little is known about their pedigree. One method used to account for gaps in the pedigree is to use genetic groups (Westell et al., 1988). Genetic groups adjust for differences that would otherwise tend to inflate genetic variance estimates.

Most studies to estimate genetic parameters in apple have been based on a limited number of parents ranging from 3 to 43 (Watkins and Spangelo, 1970; Visser, 1976; Dathe, 1978, 1979; Visser and Verhaegh, 1978; Boicheva-Dancheva, 1980; Fischer et al., 1983; Serova, 1989; Hauagge and Cummins, 1991; Gelvonauskis, 1994; Tancred et al., 1995). However, a sample of 50 families (Hill, 1980) or more (Fins et al., 1992) are needed for accurate estimation of population parameters. Durel *et al.* (1998) were the first to use a large number of families (approx. 213) to estimate genetic parameters.

The purpose of this paper was to estimate genetic parameters in apple from one subline of an apple breeding population started in 1991 in New Zealand (Noiton and Shelbourne, 1992). This study used a genetically broad-based apple breeding population to estimate heritabilities and genetic correlations with REML, using a mother tree model for half-sib analysis. Data were transformed where there was a marked deviation from normality. Genetic groups were fitted to connect separate pedigrees and to account for differences between crab apples and non-crab apples, regional differences and century

of development. The experimental design included blocks and sites to account for environmental variance. Heritability was estimated to quantify the proportion of additive genetic variance in the trait from univariate data from one site. Genetic correlations between sites were estimated from bivariate data of one trait at two sites to indicate the degree of GxE, and to suggest how this information could be used to enhance the program efficiency. Genetic correlations between traits were estimated from bivariate data at the Havelock North site.

Materials and Methods

Experimental design

Apple genotypes were selected from a genetically-diverse breeding population consisting of open-pollinated (mixed half and full sib) families derived from old apple cultivars and wild apple species, and planted as single tree plots in incomplete blocks of 20 trees (Noiton & Shelbourne, 1992). The trees were allocated to blocks using the 'Designer' software (K. Russell, pers. comm.; University of Wollongong, NSW, Australia). Trees were sampled from two sites. The first site was at the Havelock North Research Centre, Hawkes Bay, New Zealand (39°40'S 176°53'E) and the second site was at the Nelson Research Centre, Riwaka, New Zealand (41°3'S 173°1'E). A wide range of traits were measured or scored on each tree as shown in Table 1.

Vegetative traits such as vigour, tree growth habit, and leafing were collected from 4180 genotypes at two sites, and powdery mildew incidence was measured on a subset of the data represented by 2347 genotypes from the Havelock North site. These traits were measured on 73 families with 5 to 90 trees per family. Fruit traits such as flowering, harvest time, fruit size, acidity, sugars, firmness, aspect ratio, conicity, and squareness were measured on 1008 genotypes from 71 families with a minimum of 5 trees per family. Two fruit were randomly selected from the tree, stored at 5°C for at least 24 hours and up to 6 weeks. Fruit were then left at room temperature overnight prior to measuring. Fruit sugars, fruit acidity and fruit firmness measurements were taken from one randomly selected area of the fruit. Fruit aspect ratio, fruit conicity and fruit squareness were recorded from the section through the widest point of the apple along the stem-calyx axis, using principal component analysis of Fourier shape descriptors (Currie et al., 2000).

Model equation

Progeny were from open-pollinated families, where the mother tree was known but the pollen donor was unknown. The numerator relationship matrix (A) was built from the

pedigree and a mixed, general linear mother tree model with the following model equation was fitted to the data. In matrix algebra this can be represented as:

$$y = Xb + Wg + Qk + Zu + e$$

Where y is the vector of observations, b is the vector of fixed effects (site), g is the vector of genetic group effects, k and u are the vectors of block and mother tree family effects (random), and X, W, Q and Z are the incidence matrices for the respective vectors, and e is the matrix of residuals.

It was assumed that:

$$E\begin{bmatrix} y \\ k \\ u \\ e \end{bmatrix} = \begin{bmatrix} Xb + Wg \\ 0 \\ 0 \\ 0 \end{bmatrix}$$

$$\text{var}\begin{bmatrix} y \\ k \\ u \\ e \end{bmatrix} = \begin{bmatrix} V & QK & ZG & R \\ KQ' & K & 0 & 0 \\ GZ' & 0 & G & 0 \\ R & 0 & 0 & R \end{bmatrix}$$

Where

$$V = QKQ' + ZGZ' + R$$

And

$$R = \sum^{\oplus} R_{0_i}$$

$$G = A \otimes G_{\bullet}$$

R_{0_i} = residual variance-covariance matrix for each individual with a pattern of i missing values

A = numerator relationship matrix calculated from the pedigree

$G_{\bullet}$ = additive variance-covariance matrix for the complete set of traits on an individual tree

$\oplus$ = direct sum

$\otimes$ = Kronecker product

Initially the pollen donors were assigned to one genetic group, but a segregation of the fruit sugars into two groups suggested a major effect, either genetic or environmental. Careful examination of the data on a family by family basis showed that progeny from some non-crab mother trees segregated into small fruits with high fruit sugars, or large fruit with lower fruit sugars. This suggested that crab apple and non-crab apple pollen donors contributed to the open-pollinated family. Pollen donors were assigned to crab apple or non-crab apple genetic groups on the basis of their progeny fruit size and fruit sugars. This improved the distribution of the residuals but a bimodal distribution for fruit sugars was still observed, suggesting the existence of some other environmental or major gene effect not accounted for by the model.

Plots of residuals were examined for deviations from normality and homogeneity of variance. Data were transformed if necessary so genetic parameters therefore pertained to the transformed, rather than the raw data. The additive genetic and residual variance components were estimated by restricted maximum likelihood techniques using ASREML software (Gilmour et al., 1998). It was necessary to scale some traits by a factor of ten to increase their variance estimates (tree growth habit, powdery mildew incidence, fruit size, fruit acidity, fruit aspect ratio, fruit conicity, and fruit squareness).

In theory, multivariate analysis using all the traits would increase the accuracy of an estimate, as information from other correlated traits is included in the estimation. However, univariate and bivariate analyses were used in this study, since it was found that including more than two traits did not significantly alter the results but had an enormous effect on computational requirements. Block effects were removed from the model as the estimated block variance was negligible in most cases.

Heritability

Individual narrow-sense heritability is the ratio of additive variance to phenotypic variance. Additive variance (variance of the general combining abilities) was estimated

from univariate data for each site and from across sites ("site" as a fixed effect), with the following formula:

$$\hat{h}^2 = \frac{\hat{\sigma}_{family}^2}{C\left(\hat{\sigma}_{family}^2 + \hat{\sigma}_e^2\right)}$$

Where σ_{family}^2 is the family variance, σ_e^2 is the error variance and C is the coefficient of relationship ($C = 0.25 + 0.25/n$ for n pollen donors, Burdon *et al.*, 1992). Additive variance was estimated by sib analysis (Falconer, 1989). Full-sib family variance overestimates the additive variance (σ_A^2) component as it is confounded with both the common environment variance (σ_{Ec}^2 = non-genetic variance due to belonging to the same family) and one quarter of the dominance variance (σ_D^2).

Full-sibs:

$$\sigma_{family}^2 = \tfrac{1}{2}\sigma_A^2 + \tfrac{1}{4}\sigma_D^2 + \sigma_{Ec}^2$$

therefore $C = 0.5$ and (assuming no dominance and common environmental effects) $2 * \sigma_{family}^2$ is an estimate of σ_A^2

Half-sib analysis is more accurate because the half-sib family variance estimates one quarter of the additive variance, free of the dominance and common environment effect.
Half-sibs:

$$\sigma_{family}^2 = \tfrac{1}{4}\sigma_A^2$$

therefore $C = 0.25$ and $4 * \sigma_{family}^2$ is an estimate of σ_A^2

Neither the identity nor the number of pollen donors were known for this analysis. Heritability estimates assuming a pure half-sib relationship ($C = 0.25$) exceeded 1, so it was likely that the parentage was somewhere between half-sibs and full-sibs. Segregation of some non-crab apple progeny into small fruits with high fruit sugars common to some crab apples, or into larger fruits with lower fruit sugars suggested more than one pollen donor. In this study a coefficient of relationship of 0.33 ($n = 3$

pollen donors) was applied to estimate the narrow-sense heritability. Therefore *3* * σ_{family}^2 was used to estimate σ_A^2.

Genetic correlation

Correlations were calculated with the following formula:

$$\hat{r} = \frac{\hat{\sigma}_{a,b}}{\sqrt{\hat{\sigma}_a^2 \hat{\sigma}_b^2}}$$

Where $\hat{r}$ was the estimate of the correlation between traits *a* and *b*, $\hat{\sigma}_{a,b}$ was the estimated covariance between *a* and *b*, and $\hat{\sigma}_a^2$ and $\hat{\sigma}_b^2$ were the estimated variances of *a* and *b* respectively. Standard error was calculated with Pearson's approximation of the variance of a ratio (Gilmour et al., 1998).

Genetic correlation between sites was estimated from bivariate analysis of a single trait considered as separate traits at each site. Genetic correlation between sites can be used as an indicator of the GxE, where high genetic correlation means low GxE (Falconer, 1989). Genetic correlation between traits was estimated from bivariate analysis of traits taken pair-wise at the Havelock North site. The genetic correlation between traits indicates the potential gains from multi-trait selection.

Results

Estimating variance components

Residuals were examined and data transformed where necessary. The residuals were approximately normal for each trait apart from fruit size, which was log transformed to correct for an increasing variance with increasing family mean. Phenotypic means and standard deviations for each trait were calculated and are presented in Table 2. Vigour was lower at site 2 (8.7) than at site 1 (15.7), otherwise traits at each site were close to the mean value. The means were: tree growth habit between spreading and upright (4), leafing day 20th September (263), powdery mildew incidence (3.7), flowering 14th October (287), harvest time 2nd March (61), fruit size approx. 100g (2.0), fruit acidity

(pH = 3.8), fruit sugars (13.2%), fruit firmness (8.3), and fruit shape traits close to zero (Table 2).

Heritability

Univariate analysis within sites and across both sites was used to estimate variance components for calculation of narrow-sense heritability estimates (Table 3). Flowering and harvest time, fruit size, fruit aspect ratio and leafing all had very high heritabilities (>0.7), moderate heritabilities ($0.2 < h^2 < 0.5$) were estimated for fruit sugars, fruit conicity and fruit squareness and low heritabilities ($h^2 < 0.2$) were found for tree growth habit and fruit acidity. Analyses at each site confound the additive variance estimate with the genotype-by-environment variance, so an analysis pooling data from both sites, and including site as a fixed effect, was needed to produce unbiased results (White, 1996). The pooled-sites heritability estimate was similar to the average heritability estimate for each site, suggesting that GxE was minimal.

Genetic correlation between sites

Genetic correlation between sites was calculated to indicate GxE and was estimated by analysing a trait measured at two sites as separate traits (Table 4). All traits showed high genetic correlation between sites ($r_A \geq 0.64$), indicating low GxE. The estimate of genetic correlation for fruit size, acidity and fruit sugars exceeded the theoretical limit of 1, but were within one standard error of the limit, which supports the validity of this analysis.

Genetic correlation between traits

Phenotypic and genetic correlations between traits were estimated from bivariate analysis of traits across both sites (Table 5).

High positive genetic correlations ($r_A > 0.65$) existed between leafing and flowering ($r_A = 0.95$), harvest time with fruit firmness ($r_A = 0.92$), and leafing with harvest time ($r_A =$

0.69). The largest negative correlations were found between vigour and fruit squareness (r_A = -0.82), and tree growth habit with leafing (r_A = -0.53) (Table 5). A large difference between the phenotypic and genetic correlations was shown for some of the lower heritable traits (e.g.: harvest time and fruit firmness r_A = 0.92 and r_P = 0.16). Standard errors for estimated genetic correlations ranged between 0.02 - 0.22.

The results for vigour, powdery mildew incidence and fruit size traits are detailed below. Vigorous trees produced fruit that was round and was likely to be larger. There was little genetic correlation between vigour and the traits: tree growth habit, leafing, flowering, mildew resistance, harvest time, fruit acidity, fruit sugars, fruit firmness, fruit aspect ratio and fruit conicity (Table 5).

Powdery mildew incidence was not genetically correlated to any trait.

Fruit size was not genetically correlated to any trait apart from a small positive correlation with vigour (r_A = 0.34) and a small negative correlation with firmness (r_A = -0.31).

Discussion

Genetic parameters were estimated to provide insight into the design of apple breeding strategy. Heritability estimates are used to determine whether individual phenotype is an accurate predictor of the parental performance (breeding value). GxE, indicated by genetic correlation between sites, determines whether selection one site is efficient or whether selection is required at each site. Lastly, between-trait genetic correlation indicated response to multiple trait selection.

We found fruit acidity, fruit sugars and tree growth habit had low heritabilities for one or both sites ($h^2 \leq 0.25$). A low heritability estimate indicated the relatively high importance of non-additive genetic variance and/or environmental variance. Low heritability estimates could indicate the need for progeny testing or cloning for accurate selection. The other option would be to increase the heritability estimate by decreasing

the environmental variation. There were several possible sources of environmental variation. There was a lower heritability estimate for tree growth habit at Nelson (h^2 = 0.20) than for Havelock North (h^2 = 0.41). This could be because the main branches were trained onto a post and wire support at Nelson, which could make true branch angle harder to assess. Lower heritability estimates for fruit acidity and fruit sugars may have been due to variations in fruit harvest time, length of storage or storage conditions. Increased care in harvesting and consistent storage conditions may increase these heritabilities. The remaining trait heritabilities were greater than 0.32 and so the individual phenotypic value would be a reasonable estimator of the genotypic value. For those traits with higher heritability estimates, selection based on individual phenotype (forwards / individual selection) would be efficient.

Heritability estimates in this study varied between sites. For example the vigour heritability estimate at Havelock North was 0.62 but at Nelson was 0.28. Differences were also observed in the genetic parameters for apple shape in this study compared to the parameters estimated in the previous apple shape study (chapter 3). The differences in the apple shape parameters were probably due to using a different sample of the population to estimate the genetic parameters (4180 trees from 73 families in this chapter and 1008 trees from 71 families in chapter 3). This confirmed that parameters should be estimated for each breeding population and environment, and that it could be risky to make decisions based on heritabilities derived from other populations (Fins et al., 1992). Despite this, Fins *et al.* noted that it is surprising how often different estimates of parameters for the same trait agree with each other. A review of the narrow-sense heritabilities published previously for apple revealed that many were close to the heritabilities estimated in this study. For example, the combined sites estimate for vigour (0.42) in this thesis was close to the Durel *et al.* (1998) estimate for trunk circumference (0.51). The heritability estimate for fruit maturity was high in this study (h^2 = 0.82, 0.86, and 0.66 at sites 1, 2 and across-sites respectively), and the single-site estimates were similar to the heritability of 0.94 found by Tancred *et al.* (1995). Fruit size heritability estimate was higher in this study (h^2 = 1.01) than the estimate of 0.68 reported by Serova (1989). The low narrow-sense heritability estimates for fruit sugars in this study agreed with a study by Sedov and Sedova (1970), who also estimated a low

heritability for soluble dry matter (fruit sugars). Although genetic parameters should be estimated for each breeding population, cumulative research reporting similar values for particular traits may increase confidence in generalising about probable heritability values.

It was possible that the true heritabilities have been over-estimated due to the unknown number of pollen donors. We used family variance as an estimate of a third of the additive variance. The coefficient of relationship ($C = 0.33$) was calculated assuming 3 pollen donors per family. Increasing the coefficient of relationship has also been used widely in forestry to account for greater relationships between progeny than expected within half-sib families (Squillace, 1974; Potts et al., 1995; White, 1996; Gea et al., 1998). There was unlikely to be more than 3 pollen donors involved in open-pollinated apple families, as this would reduce C and increase the estimates of heritability beyond the limit of 1. However, it was possible that there were less than 3 pollen donors per open-pollinated family. In that case the coefficient of relationship would increase ($C > 0.33$), reducing the heritability estimate. A second consequence of reducing the number of pollen donors would be more full-sibs in the family, increasing the contribution of dominance and common environmental variance to the estimate of additive variance, leading to an overestimation of the additive variance. However, this effect may be negligible as a maximum of a quarter of the dominance effect is included in the full-sib family variance, and Watkins and Spangelo (1970) found dominance variance to be small in comparison to additive variance in some apple traits. A conservative interpretation of the heritabilities would be to take the reported heritabilities as the upper limit (open pollinated family variance * 3) and set the lower limit at two thirds of the reported heritability (using full-sib family variance * 2).

This study was the first to our knowledge that has included a site effect in genetic parameter estimation of apple to estimate the effects of GxE. The large genetic correlation between traits at the Havelock North and Nelson sites indicated a negligible GxE. The low GxE indicated that the ranking of selections would not change if selection was replicated in different environments, and that breeding at only one of these sites would be adequate for these traits. Future analysis of data including a third

site based in Central Otago (45°14'S 169°20'E) and measurement of more traits would increase the certainty of these conclusions. Although ranking may not change, expression of the genotype in each environment may be different and therefore testing in different environments is still required to ensure the right genotype-environment combination is selected. For instance although the ranking for fruit harvest time may not be different between sites, one site may mature later than the other. If date of harvest time was crucial for marketing reasons, then selection at each site may be necessary.

Large positive genetic correlations were estimated between leafing with flowering (r_A = 0.95), fruit firmness with harvest time (r_A = 0.92), leafing with harvest time (r_A = 0.69) and fruit firmness with fruit acidity (r_A = 0.61). Tydeman (1963) also found a positive correlation between leafing and flowering. Aeppli (1984) reported a positive correlation between harvest time and fruit firmness or fruit acidity, which is in agreement with the correlations found in this study. Large positive genetic correlations indicate that selection for one trait will also have a correlated response to selection in the other traits, which enables either indirect selection or rapid gain in multi-trait selection. Subsets of trees within the breeding population, with high between-trait genetic inter-correlations, could be formed to make rapid gains in certain directions. For example rapid gains in this population could be made selecting for late maturing, firm, and low acid fruit cultivars. Large negative genetic correlations such as those found between vigour and fruit squareness (-0.82) and tree growth habit with leafing (-0.53) indicated that gain from these multi-trait combinations could be slow.

Dependence and linkage disequilibrium are two factors that may have influenced these genetic correlations. Linkage disequilibrium is the loss of certain gene combinations through selection so that genetically-independent traits may appear to be correlated. Each generation of random mating halves the linkage disequilibrium between independent traits, quickly returning the population to linkage equilibrium (Falconer 1989). The apple breeding population in this study was founded on open-pollinated families (random mating) so the effect of linkage disequilibrium may be small. To reduce the influence of linkage disequilibrium, correlations could be estimated after further generations of random mating.

Dependence, the non-genetic interaction between traits, may mask true genetic correlations. Powdery mildew incidence was not correlated to any other trait in this study. This agrees with Durel *et al.* (1998) who also found powdery mildew resistance to be independent of other traits. Neither study took precautions to prevent a dependent effect of the disease on other traits, so it was possible that a dependent effect could be masking a genetic correlation. A careful spraying programme should be applied to control disease expression until after vegetative and reproductive traits have been measured.

In conclusion, parameters need to be estimated for each breeding population at a range of locations. For this population the generally high heritabilities for these traits demonstrate that individual selection for these traits would be efficient. Stricter measurement protocols for acidity and fruit sugars may increase their heritability estimates, otherwise progeny testing would be necessary for gain in these traits. Little GxE exists for these traits and so breeding at one site only, combined with testing selections at multiple sites would be efficient for these traits. Further estimates with all three sites would strengthen confidence in these conclusions. Finally, between-trait correlation needs to be monitored to test the reliability of current estimates.

References

Aeppli, A. 1984. Quality characteristics of apple cultivars. I. Effect of cultivar, year and stage of ripening on fruit characteristics. II. Size, form, colour and flesh characteristics of the fruit. *Schweizerische Zeitschrift fur Obst und Weinbau*, 120:456-462; 493-499. Qualitatsmerkmale von Apfelsorten. I. Einfluss von Sorte, Jahr und Reifestadium auf die Fruchteigenschaften. II. Grosse, Form, Farbung und Fleischbeschaffenheit der Frucht.

Anon. 1993. Objective description of variety: Apple (*Malus* Mill.) fruit varieties. *Plant Varieties Office New Zealand.*

Boicheva-Dancheva, R. 1980. Inheritance of some characters and properties in the first generation (Fl) of apple hybrids. IV. Time of flowering and fruit ripening. [Bulgarian]. *Gradinarska i Lozarska Nauk*, 17(2):24-31.

Burdon, R. D., M. H. Bannister, and C. B. Low. 1992. Genetic survey of *Pinus radiata.* 3: Variance structures and narrow sense heritabilities for growth variables and morphological traits in seedlings. *New Zealand Journal of Forestry Science*, 22(2/3):160-186.

Crosby, J. A., J. Janick, P. C. Pecknold, S. S. Korban, P. A. O'Connor, S. M. Ries, J. Goddfreda, and A. Voordeckers. 1992. Breeding apples for scab resistance:1945-1990. *Acta Horticulturae*, 317:43-70.

Currie, A. J., S. Ganeshanandam, D. A. M. Noiton, D. J. Garrick, C. J. A. Shelbourne, and N. Oraguzie. 2000. Quantitative evaluation of apple (*Malus X domestica* Borkh.) fruit shape by principal component analysis of Fourier descriptors. *Euphytica*, 111:221-227.

Dathe, B. 1978. Quantitative genetical investigations on apple seedlings, aimed at developing new selection principles. [German]. *Archiv fur Zuchtungsforschung*, 8(2):79-89.

Dathe, B. 1979. Preliminary results from quantitative genetical studies with apple seedlings with special reference to susceptibility to mildew (Podosphaera leucotricha [Ell. et Ev.] Salm.). [German]. *Tagungsbericht - Akademie der Landwirtschaftswissenschaften der Deutschen Demokratischen Republik*, 174:91-96. Original title: Erste Ergebnisse quantitativ genetischer Untersuchungen an Apfelsamlingen unter besonderer Berucksichtigung der Anfalligkeit gegenuber Mehltau (Podosphaera leucotricha [Ell. et Ev.] Salm.).

Dieters, M. J., T. L. White, R. C. Littel, and G. R. Hodge. 1995. Application of approximate variances of variance components and their ratios in genetic tests. *Theoretical and Applied Genetics*, 91:15-24.

Durel, C. E., F. Laurens, A. Fouillet, and Y. Lespinasse. 1998. Utilization of pedigree information to estimate genetic parameters from large, unbalanced data sets in apple. *Theoretical and Applied Genetics*, 96:1077-1085.

Falconer, D. S. 1989. *Introduction to quantitative genetics.* Longman Group, London.

Fins, L., S. T. Friedman, and J. V. Brotschol, eds. 1992. *Handbook of quantitative forest genetics.* Kluwer Academic Publishers, Dordrecht, Netherlands, xvii + 403 p. (Forestry Sciences; Vol 39.).

Fischer, C., H.-J. Schaefer, M. Fischer, B. Dathe, and W. Ficke. 1983. Preliminary results from breeding for resistance to fireblight, Erwinia amylovora (Burrill) Winslow et al. in pome fruits. 1. Apple varieties. [German]. *Archiv fur Zuchtungsforschung*, 13(2):137-146.

Gea, L. D., R. McConnochie, and N. M. G. Borralho. 1998. Genetic parameters for growth and wood density traits in Eucalyptus nitens in New Zealand. *New Zealand Journal of Forestry Science*, 27(3):237-244.

Gelvonauskis, B. 1994. Inheritance of resistance to fungal diseases, morphological characters and combining ability in juvenile apple seedlings. *Zemes Ukio Mokslai (Lithuanian Academy of Sciences)*, 2:61-66.

Gilmour, A. R., B. R. Cullis, S. J. Welham, and R. Thompson. 1998. *ASREML*, October ed. NSW Agriculture, .

Goffreda, J. C., A. M. Voordeckers, S. S. Korban, S. M. Ries, and J. Janick. 1996. Co-op 39 to 44: six disease resistant apple selections released for advanced testing. *Pennsylvania Fruit News*, 76(4):100-107.

Hallauer, A. R. 1992. Recurrent selection in Maize. *Plant Breeding Reviews*, 9:115-179.

Hauagge, R., and J. N. Cummins. 1991. Genetics of length of dormancy period in Malus vegetative buds. *Journal of the American Society for Horticultural Science*, 116(1):121-126.

Hill, W. G. 1980. Design of quantitative genetic selection experiments, p. 1-13. *In* A. Roberston (ed.), *Selection experiments in laboratory and domestic animals.* Commonwealth Agricultural Bureau, London.

Hough, L. F., J. R. Shay, and D. F. Dayton. 1953. Apple scab resistance from *Malus floribunda* Sieb. *Proceedings. American Society for Horticultural Science*, 62:341-347.

Janick, J., J. N. Cummins, S. K. Brown, and M. Hemmat. 1996. Apples, p. 1-77. *In* J. Janick and J. N. Moore (eds.), *Fruit Breeding: Tree and Tropical Fruits*, vol. 1. John Wiley & Sons, New York.

Noiton, D. A. M., and P. Alspach. 1996. Founding clones, inbreeding, coancestry and status number of modern apple cultivars. *Journal of the American Society for Horticultural Science*, 121:773-782.

Noiton, D., and C. J. A. Shelbourne. 1992. Quantitative genetics in an apple breeding strategy. *Euphytica*, 60:213-219.

Pearce, S. C., and D. A. Holland. 1960. Some applications of multivariate analysis in biology. *Applied Statistics*, 9:1-7.

Potts, B. M., P. W. Volker, G. R. Hodge, N. M. G. Borralho, C. M. Hardner, and J. V. Owen. 1995. Genetic limitations in the exploitation of base populations of *Eucalyptus globulus* ssp. *globulus*, p. 217-221. *In* B. M. Potts, N. M. G. Borralho, J. B. Reid, R. N. Cromer, W. N. Tibbits and C. A. Raymond (eds.), *Eucalypyt Plantations: Improving Fibre Yield and Quality. Proceedings CRCTHF - IUFRO Conference, Hobart, 19-24 Feb (CRC for Temperate Hardwood Forestry: Hobart).* Cooperative Research Centre for Temperate Hardwood Forestry, Sandy Bay, Tasmania.

Schmidt, H. 1994. Progress in combining mildew resistance from *Malus robusta* and *Malus zumi* with fruit quality, p. 3-6. *In* H. Schmidt and M. Kellerhals (eds.), *Progress in Temperate Fruit Breeding*. Kluwer Academic Press, Netherlands.

Sedov, E. N., and Z. A. Sedova. 1970. Breeding apples for an increased content of soluble dry matter in the fruits. [Russian]. *Selektsiya, sortoizuch., agrotekhn. plod. i yagodn. kul'tur. 3. Orel, USSR: 1969. 11-24.*

Serova, Z. M. 1989. Breeding apple for large fruit. *Puti intensifikatsii sadovodstva i selektsii plodovykh i yagodnykh kul'tur. Tula, USSR: 1989. 34-40. 4 ref. (Referativnyi Zhurnal (1990) 3Ya3371).*

Shaw, R. 1987. Maximum-likelihood approaches applied to quantitative genetics of natural populations. *Evolution*, 41(4):812-826.

Shelbourne, C. J. A. 1969. *Tree breeding methods*. NZ Forest Service, Wellington, New Zealand. Forest Research Institute, Technical paper No. 55.

Squillace, A. E. 1974. Average genetic correlations among offspring from open-pollinated forest trees. *Silvae Genetica*, 23(5):149-156.

Tancred, S. J., A. G. Zeppa, M. Cooper, and J. K. Stringer. 1995. Heritability and patterns of inheritance in the ripening date of apples. *HortScience*, 30(2):325-328.

Tydeman, H. M. 1963. The relation between time of leaf break and of flowering in seedling apples. *Annual Report of East Malling Research Station*, 1962:70-72.

Visser, T. 1976. A comparison of apple and pear seedlings with reference to the juvenile period. II. Mode of inheritance. *Euphytica*, 25:339-342.

Visser, T., and J. J. Verhaegh. 1978. Inheritance and selection of some fruit character of apple. 1. Inheritance of low and high acidity. *Euphytica*, 27:753-760.

Watkins, R., and L. P. S. Spangelo. 1970. Components of genetic variance for plant survival and vigor of apple trees. *Theoretical and Applied Genetics*, 40:195-203.

Westell, R. A., R. L. Quaas, and L. D. van Vleck. 1988. Genetic groups in an animal model. *Journal of Dairy Science*, 71:1310-1318.

White, T. L. 1996. Genetic parameter estimates and breeding value predictions: issues and implications in tree improvement programs, p. 110-117. *In* M. J. Dieters, A. C. Matheson, D. G. Nikles, C. E. Harwood and S. M. Walker (eds.), *Tree Improvement for Sustainable Tropical Forestry*, Proc. QFRI-IUFRO Conf. 27th Oct - 1st Nov 1996. IUFRO, Caloundra, Australia.

Table 1. Name and description for each apple trait

Trait	Measurement
Vigour	Tree vigour estimated by trunk cross section area (cm^2) calculated from trunk diameter (Pearce and Holland, 1960) measured 20cm above the ground.
Tree growth habit	Visual score from 1 to 9 where 1= columnar, 3=upright, 5=spreading, 7=drooping and 9 = weeping (Anon., 1993)
Leafing	Day of year when the buds break open to reveal green leaf tips
Powdery mildew incidence	Visual score from 1to 5 where 1 = tree completely covered to 5 = disease free
Flowering	Day of year that the first few flowers open
Harvest time	Day of year that the majority of the fruit are eating ripe
Fruit size	Estimated by fruit weight (g) (log transformed)
Fruit acidity	pH measured with an Orion pH meter with spear-tip probe
Fruit sugars	% soluble solids measured by Atago refractometer
Fruit firmness	Hand-held Efegi penetrometer with an 11mm tip kg/cm
Fruit aspect ratio	Principal component of Fourier descriptors
Fruit conicity	Principal component of Fourier descriptors
Fruit squareness	Principal component of Fourier descriptors

Table 2. Basic statistics for apple traits at Havelock North (site 1), Nelson (site 2) and combined sites of the breeding population

		Vigour	Tree growth habit	Leafing day	Powdery mildew incidence	Flowering	Harvest time	Fruit size (log (g))	Fruit acidity (pH)	Fruit sugars (%SS)	Fruit firmness (kg)	Fruit aspect ratio	Fruit conicity	Fruit squareness
Mean	Site 1	15.7	3.9	262.7	3.7	286.9	63.0	1.9	3.9	12.5	7.9	-0.8	0.0	-0.1
	Site 2	8.7	4.3	265.0	---	---	58.7	2.0	3.8	13.9	8.7	0.6	0.0	0.1
	Both	13.4	4.0	263.4	---	---	60.8	2.0	3.8	13.2	8.3	-0.1	0.0	0.0
Phen SD	Site 1	6.7	1.2	11.1	0.8	10.4	28.3	0.3	0.5	1.6	1.9	4.9	1.3	0.7
	Site 2	4.3	1.5	12.0	---	---	17.8	0.2	0.5	1.5	2.2	5.2	1.3	0.7
	Both	6.0	1.3	11.5	---	---	23.4	0.2	0.5	1.6	2.1	5.0	1.3	0.8
Max	Site 1	39.2	9	312	5	312	120	2.6	5.7	21.0	16.0	19.1	3.9	2.3
	Site 2	32.5	9	321	---	---	127	2.8	5.5	21.0	16.0	19.5	4.2	2.4
	Both	39.2	9	321	---	---	127	2.8	5.7	21.0	16.0	19.5	4.2	2.4
Min	Site 1	0.7	1	235	1	235	15	-0.3	2.8	8.6	2.2	-12.9	-6.0	-2.8
	Site 2	0.5	1	244	---	---	20	0.3	2.7	9.0	3.5	-12.3	-6.3	-3.7
	Both	0.5	1	235	---	---	15	-0.3	2.7	8.6	2.2	-12.9	-6.3	-3.7

Table 3. Univariate narrow-sense heritability (standard error in brackets) for apple traits estimated separately for Havelock North and Nelson, and across both sites.

Trait	Havelock North (S.E.)	Nelson (S.E.)	Combined (S.E.)
Vigour	0.62(0.06)	0.28 (0.05)	0.42 (0.05)
Tree growth habit	0.41 (0.05)	0.20 (0.04)	0.19 (0.03)
Leafing	0.72 (0.07)	0.83 (0.08)	0.60 (0.06)
Powdery mildew incidence[1]	0.40 (0.05)	---	---
Flowering[1]	0.92 (0.09)	---	---
Harvest time	0.82 (0.12)	0.86 (0.11)	0.66 (0.09)
Fruit size	0.90 (0.12)	0.91 (0.12)	1.01 (0.10)
Fruit acidity	0.17 (0.08)	0.22 (0.08)	0.19 (0.06)
Fruit sugars	0.26 (0.08)	0.25 (0.08)	0.26 (0.06)
Fruit firmness	0.44 (0.12)	0.53 (0.10)	0.47 (0.09)
Fruit aspect ratio	0.82 (0.15)	0.89 (0.16)	0.74 (0.12)
Fruit conicity	0.46 (0.13)	0.36 (0.12)	0.32 (0.09)
Fruit squareness	0.43 (0.12)	0.35 (0.12)	0.32 (0.09)

[1] = Measurements taken from only the Havelock North site

Table 4. Bivariate genetic correlations between sites for each apple trait as an indication of GxE

Trait	Genetic correlation between sites (S.E.)
Vigour	0.69 (0.08)
Tree growth habit	0.64 (0.10)
Leafing	0.77 (0.05)
Harvest time	0.96 (0.06)
Fruit size	1.08 (0.05)
Fruit acidity	1.14 (0.36)
Fruit sugars	1.04 (0.24)
Fruit firmness	0.93 (0.11)
Fruit aspect ratio	0.90 (0.09)
Fruit conicity	0.82 (0.20)
Fruit squareness	0.83 (0.21)

As powdery mildew incidence and flowering were measured on only one site there is no genetic correlation between sites for these traits.

Table 5. Bivariate genetic correlations (max S.E. 0.20) in lower triangle and phenotypic correlations above the diagonal between apple traits on individual trees with combined sites data

Trait	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Vigour	1	0.00	-0.13	-0.02	-0.06	0.04	0.17	0.01	-0.06	0.08	-0.04	0.04	-0.16
2. Tree growth habit	-0.11	1	-0.08	0.00	-0.16	0.00	-0.03	-0.06	-0.07	-0.02	0.02	-0.01	-0.03
3. Leafing	0.04	-0.53	1	-0.03	0.64	0.26	0.12	0.05	0.01	0.07	0.00	0.02	0.03
4. Powdery Mildew[1]	-0.07	0.06	0.04	1	-0.01	-0.01	-0.06	0.03	0.05	0.03	0.00	0.00	0.00
5. Flowering[1]	0.18	-0.51	0.95	-0.03	1	0.17	0.16	0.06	-0.03	0.02	0.00	0.02	0.01
6. Harvest time	0.16	-0.01	0.69	-0.01	0.41	1	0.12	0.11	0.18	0.16	0.00	0.00	0.00
7. Fruit size	0.34	-0.11	0.29	-0.13	0.29	0.10	1	0.02	0.00	-0.31	0.00	0.12	0.08
8. Fruit acidity	0.16	-0.23	0.28	-0.07	0.22	0.24	-0.12	1	0.22	0.01	0.02	0.09	0.02
9. Fruit sugars	-0.05	-0.19	0.06	-0.27	0.25	0.06	0.00	0.29	1	0.08	0.07	0.00	-0.02
10. Fruit firmness	0.31	-0.16	0.41	0.04	0.17	0.92	-0.31	0.61	0.37	1	0.03	-0.04	0.00
11. Fruit aspect ratio	-0.03	0.36	0.00	-0.01	0.00	0.00	0.00	0.06	0.09	0.18	1	0.02	0.05
12. Fruit conicity	-0.06	-0.01	0.16	0.00	0.20	0.00	0.09	0.15	0.32	-0.07	0.00	1	-0.05
13. Fruit squareness	-0.82	-0.20	-0.28	0.00	-0.04	-0.28	-0.09	-0.12	0.16	-0.01	0.19	-0.20	1

[1] Powdery Mildew incidence and Flowering were measured only at the Havelock North site.

Chapter 5: Comparison of two breeding strategies in apple (*Malus* × *domestica* Borkh.)

Abstract

The traditional apple breeding strategy, based on mass selection, was compared by simulation to recurrent selection for general combining ability (RS-GCA) to determine the short-term and long-term gains. Gains from three generations of each strategy were simulated for a fixed resource of 30,750 trees with narrow sense heritabilities of 0.2 or 0.6 to determine the short-term gain from each strategy. Population composition, mating design and the selection process in each strategy were examined to determine likely effects on long-term genetic gain, genetic diversity and inbreeding. Gain per year for the traditional apple breeding strategy were minimal because average generation intervals of approximately 100 years have prevailed due to extensive cultivar testing. Gain and rate of gain per generation with a traditional apple breeding strategy were greater than gain from the RS-GCA strategy apart from the first generation gain with a narrow-sense heritability of 0.6. However, a reduction in long-term gain is likely for the traditional apple breeding strategy due to the erosion of genetic diversity and the increased inbreeding.

Introduction

A breeding programme generally aims to optimise gains from a single generation and from several generations. Response to selection (R) is dependent on selection intensity (i), narrow sense heritability (h^2) and the phenotypic standard deviation (σ_P) (Falconer, 1989).

$$R = i\, h^2\, \sigma_P$$

Where selection intensity (i) is the average superiority of the parents standardised by dividing by the phenotypic standard deviation. Narrow sense heritability was defined in the previous chapter, and the phenotypic standard deviation is the standard deviation of the observations. The selection limit is reached when all the genes have been fixed and there is no more genetic variation. Extending the limit to selection would increase the

long-term gains. Robertson (1960) showed that the limit to selection (R_{max}) was set by effective population size or genetic diversity (N_e) as well as response to selection.

$$R_{max} = 2N_e\, i\, h^2\, \sigma_P$$

Gain is made at the expense of genetic diversity so maximising the long-term response to selection is dependent on balancing the trade-off between short-term gain and maintenance of genetic diversity.

Apple breeders have used strategies that are potentially efficient at making genetic gains for traits with high heritability, but do not preserve genetic diversity. The traditional apple breeding strategy is a type of mass selection (Figure 1) (Janick et al., 1996). Allard (1960) defined mass selection as a form of selection in which individual plants were selected and the next generation propagated from their offspring. In the traditional apple breeding strategy, commercial cultivars have been selected, mated, and new cultivars selected from their offspring. There has been considerable generation overlap but eventually older cultivars have been replaced by newer cultivars as parents in the breeding programmes.

There are several concerns over the use of this strategy in apple breeding; the main one is the lack of genetic diversity within the breeding programme, limiting long-term gain. Noiton and Alspach (1996) found that 281 out of 439 cultivars descended from five ancestral cultivars. A recent survey of 42 apple breeders from Europe, America, Asia and Oceania found that these breeders were still using a few common progenitors (Laurens, 1999). There is a wide diversity among apple species available to apple breeders (Way et al., 1989), but it has been utilised in only a limited way to introgress single genes by modified back-crossing (Hough et al., 1953; Shay et al., 1953; Crosby et al., 1992). Although the backcrossing strategy introduced new genes from crab apples to commercial apple breeding population, Noiton and Alspach (1996) found that this strategy had increased co-ancestry and therefore caused an overall loss of genetic diversity. In response to the need for increased genetic diversity in apple breeding Noiton and Shelbourne (1992) proposed a new breeding strategy for apple, based on recurrent selection for general combining ability (RS-GCA) utilising a genetically broad based breeding population.

Repeated use of the same parents will increase inbreeding. Inbreeding combined with truncation selection causes the fixation and loss of alleles and reduces the effective population size. Effective population size can be estimated by $1/(2\Delta F)$, where ΔF is the change in inbreeding coefficient. So using a strategy that increases inbreeding will reduce the effective population size. At small effective population sizes, genetic drift contributes to allele fixation, the corresponding allele loss, and a decrease in genetic diversity. Fixation of deleterious alleles due to inbreeding and genetic drift may cause inbreeding depression. Brown (1973) found inbreeding reduced the vigour in apple seedlings and subsequently lengthened the juvenile period. Although Noiton and Alspach (1996) did not find high levels of inbreeding, coancestry levels indicated that inbreeding would increase if current breeding practices continued. The recurrent selection for general combining ability (RS-GCA) strategy (Allard, 1960) proposed by Noiton and Shelbourne would preserve the genetic diversity in the breeding population, and using sublines would control any build up in inbreeding.

The long generation interval is also a concern. Seedlings have been evaluated on their own roots, and then as grafts (i.e.: clonal testing) before and after commercial release, before an individual has been used as a parent (Alston and Spiegel-Roy, 1985). Parents of 18 apple cultivars released since 1979 (Brooks and Olmo, 1991; Cummins, 1994, 1995; Okie, 1999) had the average period of 98 years between commercial release and use in crosses. The RS-GCA strategy would separate the selection of the next generation's parents from the selection of commercial cultivars, decreasing the generation interval and increasing the rate of generation turnover.

In this chapter the traditional apple breeding strategy is compared with an RS-GCA strategy based on that proposed for apple by Noiton and Shelbourne (1992). The first three sections discuss the proposed structure for a traditional breeding population, an RS-GCA breeding population and an RS-GCA cultivar production population. In each section population composition, mating design and selection strategies are discussed in terms of impact on genetic diversity, genetic gain, and inbreeding. Following the

theoretical discussion a Monte-Carlo simulation of each strategy, with a fixed resource of 30,750 trees, was used to predict genetic gains.

Traditional apple breeding strategy

In the traditional apple breeding strategy (Figure 1) selected parents are mated, progeny selected by individual phenotype, grafted onto clonal rootstock, tested at several sites, and then commercial cultivars are released for commercial testing, distribution and sale.

Traditional breeding population structure

A few crosses with large family sizes ranging from 1,000 to 35,000 are typical for traditional apple breeding populations, although a wide range of average family size has been reported (26 (Alston, 1988), 109 (Lespinasse and Paulin, 1990) 178 (Crosby et al., 1992), 1,000-3,000 (Williams, 1959; Cripps, 1989), up to 35,000 (White, 1988)). Williams (1959) justified large progeny sizes by assuming selection for five independently inherited traits. If the proportion of progeny exceeding the threshold value for each trait was 40%, 20%, 20%, 10%, and 10% respectively, then the probability of an individual with all 5 traits at acceptable levels would be 1 in 6,250. To obtain 5 such plants a family size of 31,250 would be required. Janick *et al.* (1996) suggested that larger progeny sizes may be needed, as selection is based on more than 5 traits and that pre-selection on vegetative characters before fruit evaluation would help to reduce the work required for fruit evaluation.

There are at least two consequences of concentrating resources on a few large progenies with a few commonly used parents. First, there would be a reduction in genetic diversity due to the small number of parents, reducing the potential for genetic gain (Gea, 1997). More unrelated parents would increase the genetic diversity and increase potential genetic gains. Second, there would be an increase in inbreeding due to the common use of parents, reducing the within-family variance by a factor of (1-F), where F is the inbreeding coefficient (Falconer, 1989). As selection is predominantly within-families

in traditional apple breeding, a reduction in the within-family additive variance would greatly impact the potential gains.

Traditional mating design

In traditional apple breeding strategy, mating designs with more or less balanced half-sib and full-sib structure have not been commonly applied (Alston, 1988; White, 1988; Cripps, 1989; Lespinasse and Paulin, 1990; Fischer and Fischer, 1996). Mating designs to pursue gains with no regard for conserving diversity use as many crosses per parent as possible, with diminishing returns for more than 5 crosses per parent (i.e.: each parent in 10 crosses) for high heritability and low specific combining ability (van Buijtenen and Burdon, 1990; Gea, 1997). Diallel, factorial or hierarchical mating designs could be applied. However, if conservation of diversity is a priority then both Buijtenen and Burdon (1990) and Gea (1997) recommended single or double pair mating.

Traditional selection

Selection within the traditional approach follows three stages after mating (Figure 2). The first stage is seedling evaluation. Apples have a juvenile period of 3-10 years (Brown, 1975), followed by 2 or 3 years of crop evaluation during which the best phenotypes are selected by independent culling levels (Janick et al., 1996). For the next stage selections are grafted onto clonal rootstock for testing at different sites. This would be a minimum of 5 years, as apples go through a pseudo-juvenile stage of 2-3 years before fruiting again. Clonal performance as grafts on commercial rootstocks determines which trees are selected for commercial release. In the final stage of selection a cultivar is evaluated for commercial success, so it may be many years before a cultivar is used in a breeding programme.

Separating the process of selecting commercial cultivars from selecting breeding population parents in the traditional breeding strategy would greatly reduce the generation interval and enhance efficiency. Parental performance is determined by

additive genetic effects, which are most accurately assessed by a progeny test, but with high narrow sense heritability this is efficiently assessed by individual phenotype. The generation interval could be reduced to a tenth of the current interval by either selecting on individual phenotype or on a combined index (family mean and individual phenotypic deviation from the family mean weighted by their respective heritabilities (Lush, 1947; Falconer, 1989)).

Mass selection strategies like the traditional apple breeding strategy are efficient at accumulating gains, given medium to high heritability, but do not conserve genetic diversity (Gea, 1997). The gains simulated in this study demonstrate a greater rate of gain for the traditional breeding strategy than in the RS-GCA strategy. However, Gea (1997) showed mass selection had a greater loss in genetic diversity.

In summary, traditional apple breeding appears efficient at realising genetic gain but not at preserving genetic diversity, which is vital for long-term gains. Genetic diversity and gains could be improved in this strategy by increasing the number of parents in each generation, increasing the number of crosses per parent and reducing the generation interval by selecting parents on phenotypic performance. However, an alternative strategy like the RS-GCA strategy may be required to provide parents with a greater genetic diversity.

RS-GCA strategy

In RS-GCA, individuals are selected on the basis of the breeding value or GCA of the parents followed by their individual performance. Selection on GCA increases the frequency of desirable alleles in the population, increasing the performance of parents and increasing the likelihood of selecting commercial cultivars. RS-GCA has been applied to animal (Falconer, 1989), forest tree (Burdon and Shelbourne, 1972; Namkoong et al., 1988; Fins et al., 1992) and crop (Allard, 1960; Hallauer, 1992) breeding programmes. The RS-GCA strategy first proposed for apple by Noiton and Shelbourne (1992) has been re-examined and modified in this comparative study (Figure 2). In this study, the RS-GCA strategy has a breeding population divided into

two sublines and a cultivar production population. The breeding population preserves genetic diversity and provides a low level of improvement, while the cultivar production population utilises the best parents in the population, each generation, to realise genetic gain without affecting the diversity in the breeding population.

RS-GCA breeding population

The primary goal of the breeding population is to protect genetic diversity and the secondary goal is to continually improve the general combining ability of the selected traits in the population. A genetically diverse breeding population would provide novel cultivars, protect against changes in market requirements, and increase the potential gains that can be made in the cultivar production population (Gea, 1997). On the other hand, a new cultivar production population is generated from the breeding population each generation, so increased gain in the breeding population will afford greater gains in the cultivar production population, but with reduced diversity.

RS-GCA breeding population structure

The RS-GCA breeding population used for this simulation (and proposed as a successor to the Noiton and Shelbourne strategy) was divided into 2 unrelated sublines to control the development of inbreeding and allow out-crossing in the cultivar production population (Figure 2). Sublining provides protection against long-term inbreeding in cultivars by restricting mating to within-sublines in the breeding population. Cultivars are formed in the cultivar production population by crossing between unrelated sublines, eliminating inbreeding (McKeand and Beineke, 1980; Burdon and Namkoong, 1983).

The breeding population was generated from 150 parents in each subline, selected from 165 open-pollinated families of 30 trees in the first generation and from 225 full-sib families of 30 trees in subsequent generations. Thirty trees per family was a compromise between the minimum of 10-20 trees per family recommended to accurately estimate the true family mean (Cotterill and James, 1984), and 50 trees per family recommended for a typical forest tree selection programme (Lindgren et al., 1997). The number of parents was maximised given a family size of 30 trees and a

disconnected factorial mating design (for mating design see next section). Sufficient numbers of parents were required to protect against inbreeding and the random fixation of genes by genetic drift, to provide a sufficient sample of the genetic diversity, and to increase the potential for between-family selection without compromising the former requirements.

A relatively small number of unrelated parents would be required if the only goal was to minimise inbreeding. Rosvall (1999) suggested that a census number of 25 unrelated founders in a double-pair mated population (25 families), with balanced, within-family selection was sufficient to keep inbreeding to 1% for 10 generations. However, accurate estimates of heritability and genetic correlation need a minimum of 50 unrelated families, with 100 families preferred (White, T. L., 1996). To sample alleles with a frequency of 5% or more from the population, Lawrence *et al.* (1995) calculated that 172 open-pollinated families were required. Conservation of rare alleles requires larger populations, and population sizes ranging from 500-2,000 unrelated founders have been recommended (Namkoong and Kang, 1990). Recommendations for typical forest tree breeding populations range from 100 unrelated families of 50 progeny (Lindgren et al., 1997) to 500 families (Burdon, 1995). The 300 founders used for the RS-GCA strategy in this study should be sufficient to sample most common alleles, to allow for accurate estimation of genetic parameters, and to provide scope for between-family selection without compromising diversity.

RS-GCA breeding population mating design

The mating design employed in the simulated breeding strategy was a 3x3 disconnected factorial (Table 1). This mating design was a compromise between maximising the number of parents for preserving genetic diversity and optimising the number of crosses per parent for both estimating GCA and generation advancement. Optimum mating designs differ for generation advancement and forwards selection verses estimating GCA and backwards selection. For generation advancement the more crosses per parent the better, with diminishing returns for more than 5 crosses per parent (i.e.: each parent in 10 crosses) if heritability was high and specific combining ability was low. Single or double pair mating provided less genetic gain, but was recommended if conservation of

genetic diversity was a priority (see Traditional mating design, page 108) (van Buijtenen and Burdon, 1990; Gea, 1997).

However, for estimating parental GCA at least 2-3 crosses per parent (parents in 4-6 crosses) were optimum (Burdon and van Buijtenen, 1990; Johnson, 1998). Cross referencing testers was required for maximum efficiency, but given a disconnected design (no cross-referencing) Burdon and van Buijtenen (1990) found that disconnected modified half-diallels (no reciprocals and no selfing) were slightly more efficient than disconnected factorials, but that factorials coped better with flowering constraints such as flowering time and low flower numbers. The use of each parent in only 3 crosses was sub-optimal for both GCA estimation and generation advancement but was a compromise to preserve genetic diversity.

Separate, complementary designs can be used to optimally test for parental GCA and advance the generation. Noiton and Shelbourne (1992) suggested a disconnected 2x2 factorial for generation advancement and a 20-pollen polycross progeny test to estimate the GCA. The trees from the polycross progeny test were not used for generation advancement of the breeding population, as selections would reduce the genetic diversity of the breeding population. Therefore if resources are limiting then complementary designs that divert resources from the breeding population may not be ideal. Although the use of one mating design in the RS-GCA strategy may not be optimal in terms of GCA estimation, more families and trees could be allocated to the breeding population. This increased the genetic diversity and hence the potential for genetic gain (King and Johnson, 1993; Gea, 1997).

Positive assortative mating (PAM) was used for one cross in each 3x3 factorial and random mating was used for the remaining crosses of the RS-GCA breeding population (Table 1). PAM is mating between the first and second ranked trees, the third with the fourth, etc. PAM has been shown to increase the additive variance, leading to an increase in selection response but not markedly changing the level of inbreeding (Smith and Hammond, 1987; Tallis and Leppard, 1987, 1988; Mahalovich, 1990; Shepherd and Kinghorn, 1994; Jorjani, 1995). The increase in additive variance with positive

assortative mating is due to an increase in the covariance between alleles caused by linkage disequilibrium.

Linkage disequilibrium occurs when there is a deviation of genotype frequencies from that expected by their allele frequencies (Falconer, 1989). Linkage disequilibrium caused by PAM would be observed as combinations of desirable alleles occurring more often than expected with random mating. A less desirable expression of linkage disequilibrium likely to be common in the apple breeding population would be due to the wide range of origins of the apples. Apples with disease resistance genes were predominantly small-fruited crab apples, so in initial generations linkage disequilibrium could occur between fruit size and disease resistance. Reducing the undesirable linkage disequilibrium would mix up the alleles and create new genotypes that may be desirable for commercial cultivars. Falconer (1989) showed that random mating reduces linkage disequilibrium between independent genes by half each generation. Hence random mating was used for the remaining crosses in this strategy.

Other forms of non-random mating that could be contemplated would be to:

- rank and mate trees based on combinations of traits that showed favourable genetic correlations in order to promote rapid progress in certain different directions.
- mate better trees more often (see non-random mating and selection designs in next section).

RS-GCA Breeding population selection

Both between-family and within-family selection occurred within the RS-GCA breeding population. Each of the 150 parents were used in 3 crosses (225 crosses in total), the best tree was selected from the best cross, with the added restriction that no full-sibs are selected. Some between-family selection was necessary to maintain the subline size at only 150 parents. Lindgren and Wei (1993) suggested that loss of diversity due to increased selection intensity for large effective population sizes were relatively small. Gea (1997) also suggested that strategies that did not have some element of between-family selection may have unacceptably low gains. Most of the selection was within-family and moderately balanced as each parent contributed. Dempfle (1975) showed

that balanced within-family selection (each parent with an equal contribution) increased the long-term response to selection to more than that expected by unrestricted selection.

There are other non-random mating designs that attempt to optimise the long-term response to selection that may be worth future examination. Nucleus breeding concentrates crossing and selection within a subset of better parents within the breeding population (James, 1977; Cotterill, 1989; Roden, 1994). Different breeding objectives could be applied to the elite subsets to promote gains in different directions. A great deal of research has been undertaken in selection and mating on the basis of breeding value weighted by coancestry to maximise the gains for a given loss of diversity (Toro et al., 1988; Toro and Perez-Enciso, 1990; Wray and Goddard, 1994; Brisbane and Gibson, 1995a, b; Caballero et al., 1996; Lindgren and Mullin, 1997; Meuwissen, 1997; Villanueva and Woolliams, 1997; Grundy et al., 1998; Rosvall, 1999; Sánchez Rodríguez et al., 1999).

In summary, the RS-GCA breeding population maintains genetic diversity, and minimises inbreeding by using a large number of founders, within-family selection, and the minimum number of crosses per parent (low between-family selection). PAM mating was used to enhance covariance between desirable alleles and hence increase gains and random mating was used to break up undesirable linkages and form new combinations of alleles.

RS-GCA cultivar production population

The RS-GCA cultivar production population is intended to exploit genetic diversity in the breeding population for maximum genetic gain. The best selections from the breeding population were mated in a design that balanced between-family selection intensity (large number of parents and crosses per parent) with within-family selection intensity (numbers per cross).

RS-GCA cultivar production population structure

Twelve parents were selected from each subline of the breeding population and were crossed between sublines in a 6x6 parent disconnected factorial to form a cultivar production population of 72 crosses, 226 trees per cross and a total of 16,272 trees (Table 3). The population was structured to maximise genetic gain by:

- utilise many parents, to maximise between-family genetic gain
- utilise many crosses per parent, to increase potential for between-family selection and between-family gain
- balancing the above requirements with the need for many trees per family to optimise within-family gain

RS-GCA cultivar production population mating design

In the RS-GCA cultivar production population a mating design was chosen to generate a high genetic gain from a limited number of highly selected parents. A 6x6 parent disconnected factorial was used to make crosses between selections from different sublines (Table 3). Either a factorial or a diallel mating design is appropriate when several crosses per parent are needed. Although the diallel design is marginally more efficient (van Buijtenen and Burdon, 1990), the factorial design was chosen as it allowed flexibility in assigning the parent as the mother or the pollen donor. The design was disconnected to reduce the number of crosses each parent was involved in from 12 to 6, which is within the range recommended by van Buijtenen and Burdon (1990) for optimal gains. Some reduction in the number of crosses meant that larger progenies could be generated, increasing the within-family selection intensity with fewer resources spent on making different crosses. The mating design could also include commercial cultivars, which would incorporate the qualities of commercial cultivars in the cultivar production population and allow the comparison of commercial cultivar GCAs with both the breeding population parents and the cultivar candidates selected from this population.

RS-GCA cultivar production population selection

The selection strategy for the RS-GCA cultivar production population was chosen to maximise genetic gain. Combined index selection (Lush, 1947) was used to select

intensely between-family and within-family from the breeding population for parents with good combining ability. Selection of relatives was likely, but not a concern for inbreeding as crosses were made between sublines. Selection of a large number of the best phenotypes was used so that a greater intensity of clonal selection could be applied to selections on rootstock. Grafts per selection were kept to a minimum of 6, with 2 at each of 3 sites to allow more selections to be tested. Rosvall *et al.* (1998) reported that within a fixed number of forest trees, the optimum number of ramets were 4 to 28 (for heritability 0.4 and 0.05 respectively). High heritabilities in some apple traits (previous chapter) suggested that the 6 grafts per selection would be adequate.

In summary, the RS-GCA cultivar production population utilises intensive selection of the best parents in the breeding population with a combined index. A balance between increasing the number of parents and number of crosses (between-family selection) and increasing the number of trees per family (within-family selection). Initial phenotypic selection was low to increase the potential for clonal selection.

Simulation

The major requirement of a breeding programme is to make genetic gain, which is determined by selection intensity, heritability and genetic variance. Formulae for deterministic prediction of genetic gain become complex when gain is calculated over more than one generation (Wei et al., 1996), and so Monte-Carlo simulation is often used instead. Monte-Carlo simulation uses a stochastic approach to predict gains, mimicking the random process of recombination with random number generation (Mullin and Park, 1995).

Simulation method

A parameter-based simulation was run on an Microsoft Excel spreadsheet to estimate the gain for each strategy over three generations. A single trait with a mean of 10, a narrow-sense heritability of 0.2 or 0.6 and a phenotypic variance of 1.0 was simulated. The gene action was assumed to be polygenic and additive with no dominance or

epistasis effects. A common base of 330 parents with 30 progeny from open-pollination and a fixed resource of 30,750 trees were used for both strategies to simplify the comparison.

Progeny were initially drawn from a population of open-pollinated progenies and in subsequent generations were drawn from controlled-pollinated crosses. Each tree was the sum of the population mean and independently generated environmental N(0, σ_e^2) and genetic N(0, σ_A^2) effects. The total additive variance can be partitioned into between and within-family additive variance to determine the variance to use for the simulation.

Total	σ_A^2	=	between-family σ_A^2	+	within-family σ_A^2
Half-sibs	σ_A^2	=	$\frac{1}{4}\ \sigma_{Ao}^2$	+	$\frac{3}{4}\ \sigma_{Ao}^2$
Open-pollinated apples	σ_A^2	=	$^1/_3\ \sigma_{Ao}^2$	+	$^2/_3\ \sigma_{Ao}^2$
Full-sibs	σ_A^2	=	$\frac{1}{2}\ \sigma_{Ao}^2$	+	$\frac{1}{2}\ \sigma_{Ao}^2$

The coefficient of relationship for open-pollinated sibs was assumed to be 0.33, which was between that for full-sibs (0.5) and half-sibs (0.25) (see chapter 4 for justification). Sibs were drawn from a distribution with the within-family genetic variance about the average parental breeding value (Falconer, 1989).

Traditional breeding strategy model

The first generation of the traditional apple strategy (Figure 1) consisted of 330 open-pollinated families of 30 progeny. The 20 best trees were phenotypically selected from these 9,900 trees and tested on clonal rootstock, and the best 6 selected as parents of the next generation. The number of years taken to carry out seedling and clonal testing is irrelevant in this simulation study as the gain is expressed in terms of generations rather than years. Subsequent generations were formed by mating these 6 cultivars in a 6-parent modified half diallel design (no selfing and no reciprocals) to form (with 15 crosses of 2,030 progeny each) the total of 30,450 trees. Six cultivars and parents of the

next generation were selected in the same manner as for the first generation, with clonal testing of the best 20 phenotypes.

RS-GCA breeding strategy model

The RS-GCA strategy (Figure 2) started with the same base population as the traditional breeding strategy, but divided the breeding population into 2 sublines of 165 open-pollinated families (4950 trees). From each subline, the best individual within the best 150 families were selected to form the next generation. These 150 trees were mated in a 3x3 disconnected factorial design to generate 225 full-sib families of 30 trees. The best individual from the best cross for each of the 150 parents were selected (150 trees) and the cycle was repeated by mating in a 3x3 disconnect factorial design. This simulation was simplified by not adding the restriction of only one selection per cross that was recommended for this strategy. This would slightly increase the gains for the RS-GCA strategy (and also increase the inbreeding and loss of genetic diversity).

The cultivar production population was formed by using combined index selection to select the best 12 individuals from each subline. The 12 trees from each subline were mated together in a 6x6 disconnected factorial to generate 72 crosses with 226 trees each (16,272 trees). One hundred and sixty three trees were tested on clonal rootstock (6 grafts each) and the best 6 were released as commercial cultivars.

Simulation results

For the breeding populations the response to selection and the rate of gain was always greater for the traditional breeding strategy (Figure 4 and Figure 5) because the selection intensity was much higher (6 / 30,450 verses 300 / (9900 or 13,500)).

Despite the similar selection intensities for cultivars from the breeding populations (RS-GCA strategy = 6 / 29,772 and traditional strategy = 6 / 30,450), the relative gain of the cultivars over the breeding population was always larger for the RS-GCA strategy for both heritabilities. In the first generation this relative advantage meant that the average

gain for the RS-GCA cultivars was greater than the traditional cultivars, but not for subsequent generations (Figure 5) or for lower heritability (Figure 4).

Simulation discussion

The higher gain in the traditional strategy breeding population compared to the RS-GCA strategy breeding population was due to an undivided population structure and fewer parents selected. The role of breeding population and cultivar production population were combined in the traditional breeding strategy, increasing the number of trees to select from. However, by merging the breeding population with the cultivar production population, the genetic diversity is not maintained. Selection of just 6 cultivars to use as parents of the next generation provided large gains but would erode the genetic diversity leading to decreased gain in the long term.

This simulation assumed that the base population was non-inbred with infinite genetic diversity. The RS-GCA could be considered close to these assumptions as it was based on a genetically broad-based breeding population, used low between-family selection intensity and made selections from each parent to preserve genetic diversity. On the other hand, traditional apple breeding strategies have been shown to be based on a few parents with a narrow genetic base (Noiton and Alspach, 1996; Laurens, 1999). Breeding and selection within small effective population sizes increases the rate of inbreeding, reducing the within-family variance, and increases the loss of alleles through genetic drift, reducing the genetic diversity. Hence the gains shown by this simulation for the traditional approach would be biased upwards and would not be sustained over the long term.

The relative gain of the cultivars from the breeding population was always greater in the RS-GCA strategy despite lower selection intensities because the RS-GCA balanced between-family and within-family selection, whereas the traditional strategy utilised mostly within family selection. The RS-GCA strategy increased the number of parents and reduced the number of trees per family to increase the opportunity of utilising the

between-family variation. Half the additive genetic variance is between families (more than half with inbreeding) (Falconer, 1989), so strategies that utilise this source of variation increase the gains.

In conclusion, the undivided population structure of the traditional strategy and the selection of few parents mean that short-term gain can be rapidly accumulated with the traditional apple breeding strategy. However, use of fewer parents would increase inbreeding, decrease within-family variation and decrease gain in the traditional strategy. Better utilisation of between-family variance increased the relative gains from the breeding population for the RS-GCA strategy.

Conclusions

Improvements could be made to the traditional apple breeding strategy by increasing the number of parents, increasing the number of crosses and reducing the generation interval. Given these improvements, higher rates of gain can be made with the traditional apple breeding strategy compared to the RS-GCA strategy, assuming the same base population. However, long-term sustainability of traditional apple breeding strategy is dependent on a strategy, like RS-GCA, that preserves genetic diversity. The apple industry needs strategies like the RS-GCA to ensure the long-term sustainability of genetic gain.

References

Allard, R. W. 1960. *Principles of plant breeding.* John Wiley & Sons, London.

Alston, F. H. 1988. Breeding apples for long storage. *Acta Horticulturae*, 224:109-117.

Alston, F. H., and P. Spiegel-Roy. 1985. Fruit tree breeding: Strategies, achievements and constraints, p. Huntingdon. *In* M. G. R. Cannell and J. E. Jackson (eds.), *Attributes of trees as crop plants.* Inst. Terrestial Ecology, Natural Environ. Res. Council, .

Brisbane, J. R., and J. P. Gibson. 1995a. Balancing selection response and rate of inbreeding by including genetic relationships in selection decisions. *Theoretical and Applied Genetics*, 91:421-431.

Brisbane, J. R., and J. P. Gibson. 1995b. Including genetic relationships in selection decisions: alternative methodologies. *Theoretical and Applied Genetics*, 91:769-775.

Brooks, R. M., and H. P. Olmo. 1991. Register of new fruit and nut varieties. List 35. *HortScience*, 26(8):951-978.

Brown, A. G. 1973. The effect of inbreeding on vigour and length of juvenile period in apples, p. 30-39. *In* A. G. Brown, R. Watkins and F. Alston (eds.), *Proceedings of Eucarpia Fruit Section Symposium V. Top Fruit Breeding. Canterbury, Sept. 11th-14th 1973*. Eucarpia, Canterbury.

Brown, A. G. 1975. Apples, p. 3-37. *In* J. Janick and J. N. Moore (eds.), *Advances in fruit breeding*, 1st ed., vol. 1. Purdue University Press, West Lafayette, Indiana.

van Buijtenen, J. P., and R. D. Burdon. 1990. Expected efficiencies of mating designs for advanced-generation selection. *Canadian Journal of Forestry Research*, 20:1648-1663.

Burdon, R. D. 1995. Strategies for exploitation of base populations, p. 146-151. *In* B. M. Potts, N. M. G. Borralho, J. B. Reid, R. N. Cromer, W. N. Tibbits and C. A. Raymond (eds.), *Eucalypyt Plantations: Improving Fibre Yield and Quality. Proceedings CRCTHF - IUFRO Conference, Hobart, 19-24 Feb (CRC for Temperate Hardwood Forestry: Hobart)*. Cooperative Research Centre for Temperate Hardwood Forestry, Sandy Bay, Tasmania.

Burdon, R. D., and J. P. van Buijtenen. 1990. Expected efficiencies of mating designs for reselection of parents. *Canadian Journal of Forestry Research*, 20:1664-1671.

Burdon, R. D., and G. Namkoong. 1983. Short note: multiple populations and sublines. *Silvae Genetica*, 32:221-222.

Burdon, R. D., and C. J. A. Shelbourne. 1972. Breeding populations for recurrent selection: conflicts and possible solutions. *New Zealand Journal of Forestry Science*, 1(2):174-193.

Caballero, A., E. Santiago, and M. A. Toro. 1996. Systems of mating to reduce inbreeding in selected populations. *Animal Science*, 62:431-442.

Cotterill, P. P. 1989. The nucleus breeding system. *Proc. 20th South Forest Tree Improvement Conference. June 26-30, Charleston, SC.*

Cotterill, P. P., and J. W. James. 1984. Number of offspring and plot sizes required for progeny testing. *Silvae Genetica*, 33(6):203-209.

Cripps, J. E. L. 1989. Experiences in export apple breeding. *Acta Horticulturae*, 240:31-34. Officially missing from Massey library (June 1999).

Crosby, J. A., J. Janick, P. C. Pecknold, S. S. Korban, P. A. O'Connor, S. M. Ries, J. Goddfreda, and A. Voordeckers. 1992. Breeding apples for scab resistance:1945-1990. *Acta Horticulturae*, 317:43-70.

Cummins, J. N. 1994. Register of new fruit and nut varieties: Brooks and Olmo List 36. *HortScience*, 29(9):942-969.

Cummins, J. N. 1995. Register of new fruit and nut varieties: Brooks and Olmo list 37. *HortScience*, 30(6):1135-1150.

Dempfle, L. 1975. A note on increasing the limit of selection through selection within families. *Genetical Research, Cambridge*, 24:127-135.

Falconer, D. S. 1989. *Introduction to quantitative genetics.* Longman Group, London.

Fins, L., S. T. Friedman, and J. V. Brotschol, eds. 1992. *Handbook of quantitative forest genetics.* Kluwer Academic Publishers, Dordrecht, Netherlands, xvii + 403 p. (Forestry Sciences; Vol 39.).

Fischer, C., and M. Fischer. 1996. Results in apple breeding at Dresden-Pillnitz - review. *Die Gartenbauwissenschaft*, 61(3):139-146.

Gea, L. D. 1997. Genetic diversity and gain: the concept of a status number. *Ph.D. Dissertation.* School of Forestry, University of Canterbury, Christchurch, NZ.

Grundy, B., B. Villanueva, and J. A. Woolliams. 1998. Dynamic selection procedures for constrained inbreeding and their consequences for pedigree development. *Genetical Research, Cambridge*, 72:159-168.

Hallauer, A. R. 1992. Recurrent selection in Maize. *Plant Breeding Reviews*, 9:115-179.

Hough, L. F., J. R. Shay, and D. F. Dayton. 1953. Apple scab resistance from *Malus floribunda* Sieb. *Proceedings. American Society for Horticultural Science*, 62:341-347.

James, J. W. 1977. Open nucleus breeding systems. *Animal Production*, 24:287-305.

Janick, J., J. N. Cummins, S. K. Brown, and M. Hemmat. 1996. Apples, p. 1-77. *In* J. Janick and J. N. Moore (eds.), *Fruit Breeding: Tree and Tropical Fruits*, vol. 1. John Wiley & Sons, New York.

Johnson, G. R. 1998. Parental GCA testing: How many crosses per parent? *Canadian Journal of Forestry Research*, 28:540-545.

Jorjani, H. 1995. Genetic studies of assortative mating in selected and unselected populations. *Ph.D. Dissertation.* Swedish University of Agricultural Sciences (Uppsala).

King, J. N., and G. R. Johnson. 1993. Monte Carlo simulation models of breeding-population advancement. *Silvae Genetica*, 42(2-3):68-78.

Laurens, F. 1999. Review on the current apple breeding programmes in the world: breeding objectives for scion-cultivar improvement. *Acta Horticulturae*, 484:163-170. Proceedings of the EUCARPIA Fruit Breeding and Genetics Symposium.

Lawrence, M. J., D. F. Marshall, and P. Davies. 1995. Genetics of conservation. I. Sample size when collecting germplasm. *Euphytica*, 84:89-99.

Lespinasse, Y., and J. P. Paulin. 1990. Apple breeding program for fire blight resistance: Strategy used and first results. *Acta Horticulturae*, 273:285-295.

Lindgren, D., and T. J. Mullin. 1997. Balancing gain and relatedness in selection. *Silvae Genetica*, 46(2/3):124-129.

Lindgren, D., and R. P. Wei. 1993. Gain versus effective number. *Proceedings - Nordic group for tree breeding, Edinburg, Scotland pp 164-177.*

Lindgren, D., R. P. Wei, and S. J. Lee. 1997. How to calculate optimum family number when starting a breeding program. *Forest Science*, 43(2):206-212.

Lush, J. L. 1947. Family merit and individual merit as a basis for selection: Parts I and II. *American Naturalist*, 81:241-261, 362-379.

Mahalovich, M. F. 1990. Modelling positive assortative mating and elite populations in recurrent selection programs for general combining ability. *Ph.D. Dissertation.* Dept. Forestry, North Carolina State University.

McKeand, E., and F. Beineke. 1980. Sublining for half-sib breeding populations of forest trees. *Silvae Genetica*, 29(1):14-17.

Meuwissen, T. H. E. 1997. Maximising the response of selection with a predifined rate of inbreeding. *Journal of Animal Science*, 75:934-940.

Mullin, T. J., and Y. S. Park. 1995. Stochastic simulation of population management strategies for tree breeding: A new decision-support tool for personal computers. *Silvae Genetica*, 44(2-3):132-141.

Namkoong, G., and H. Kang. 1990. Quantitative genetics of forest trees. *Plant Breeding Reviews*, 8:139-188.

Namkoong, G., H. C. Kang, and J. S. Brouard. 1988. *Tree breeding : principles and strategies.* Springer-Verlag, New York, viii + 177 p. (Monographs on theoretical and applied genetics; 11).

Noiton, D. A. M., and P. Alspach. 1996. Founding clones, inbreeding, coancestry and status number of modern apple cultivars. *Journal of the American Society for Horticultural Science*, 121:773-782.

Noiton, D., and C. J. A. Shelbourne. 1992. Quantitative genetics in an apple breeding strategy. *Euphytica*, 60:213-219.

Okie, W. R. 1999. Register of new fruit and nut varieties. List 39. *HortScience*, 34(2):181-205.

Robertson, A. 1960. A theory of limits in artificial selection. *Proceedings of the Royal Society of London*, Series B, Biological Sciences, 153:234-249.

Roden, J. A. 1994. Review of the theory of open nucleus breeding systems. *Animal Breeding Abstracts*, 62(3):151-157.

Rosvall, O. 1999. Enhancing gain from long-term forest tree breeding while conserving genetic diversity. *Ph.D. Dissertation.* Swedish University of Agricultural Sciences, Umeå.

Rosvall, O., D. Lindgren, and T. J. Mullin. 1998. Sustainability robustness and efficiency of a multi-generation breeding strategy based on within-family clonal selection. *Silvae Genetica*, 47(5-6):307-321.

Sánchez Rodríguez, L., M. A. Toro, and C. García. 1999. Improving efficiency of artificial selection: More selection pressure with less inbreeding. *Genetics*, 151:1103-1114.

Shay, J. R., D. F. Dayton, and L. F. Hough. 1953. Apple scab resistance from a number of *Malus* species. *Proceedings. American Society for Horticultural Science*, 62:348-356.

Shepherd, R. K., and B. P. Kinghorn. 1994. A deterministic multi-tier model of assortive mating following selection. *Genetics, Selection, Evolution*, 26:495-516.

Smith, S. P., and K. Hammond. 1987. Assortative mating and artificial selection: a second appraisal. *Genetics, Selection, Evolution*, 19:181-196.

Tallis, G. M., and P. Leppard. 1987. The joint effect of selection and assortative mating on a single polygenic character. *Theoretical and Applied Genetics*, 75:41-45.

Tallis, G. M., and P. Leppard. 1988. The joint effect of selection and assortative mating on multiple polygenic character. *Theoretical and Applied Genetics*, 75:278-281.

Toro, M. A., and M. Perez-Enciso. 1990. Optimisation of selection response under restricted inbreeding. *Genetics, Selection, Evolution*, 22:93-107.

Toro, M. A., B. Nieto, and C. Salgado. 1988. A note on minimization of inbreeding in small-scale selection programmes. *Livestock Production Science*, 20:317-323.

Villanueva, B., and J. A. Woolliams. 1997. Optimization of breeding programs under index selection and constrained inbreeding. *Genetical Research, Cambridge*, 69:145-158.

Way, R. D., H. S. Aldwinckle, R. C. Lamb, A. Rejman, S. Sansavini, T. Shen, R. Watkins, M. N. Westwood, and Y. Yoshida. 1989. Apples (Malus). *Acta Horticulturae*, 290:3-62.

Wei, M., A. Caballero, and W. G. Hill. 1996. Selection response in finite populations. *Genetics*, 144:1961-1974.

White, A. G. 1988. Apple breeding in New Zealand. *Acta Horticulturae*, 224:119-121.

White, T. L. 1996. Genetic parameter estimates and breeding value predictions: issues and implications in tree improvement programs, p. 110-117. *In* M. J. Dieters, A. C. Matheson, D. G. Nikles, C. E. Harwood and S. M. Walker (eds.), *Tree Improvement for Sustainable Tropical Forestry*, Proc. QFRI-IUFRO Conf. 27th Oct - 1st Nov 1996. IUFRO, Caloundra, Australia.

Williams, W. 1959. Selection of parents and family size in the breeding of top fruits, p. 211-213. *In Second Congress of the European Association for Research on Plant Breeding*. Eucarpia, .

Wray, N. R., and M. E. Goddard. 1994. Increasing long term selection response. *Genetics, Selection, Evolution*, 26:431-451.

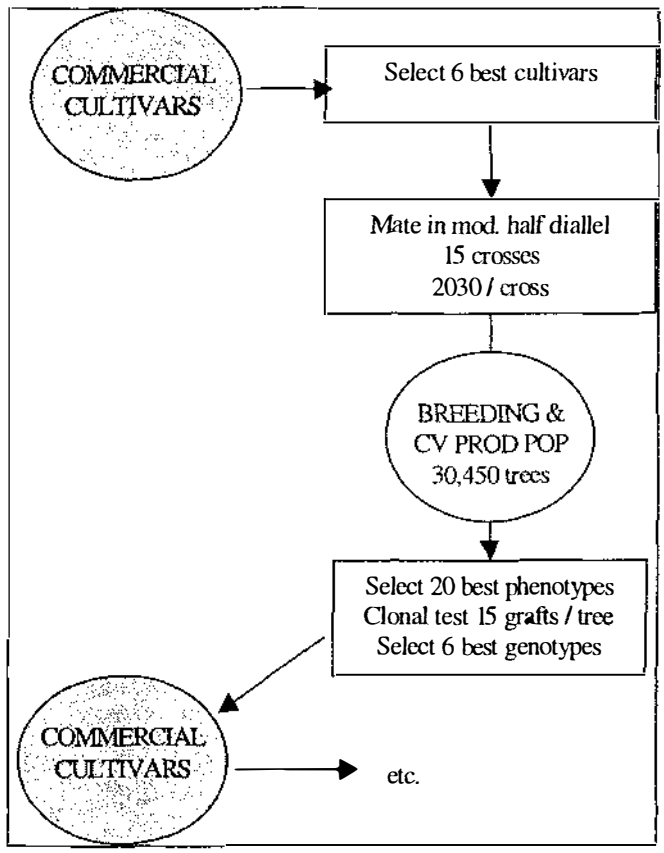

Figure 1. Task flow chart for a typical traditional apple breeding strategy (numbers indicated for a simulation of a fixed resource of 30,750 trees indicated).

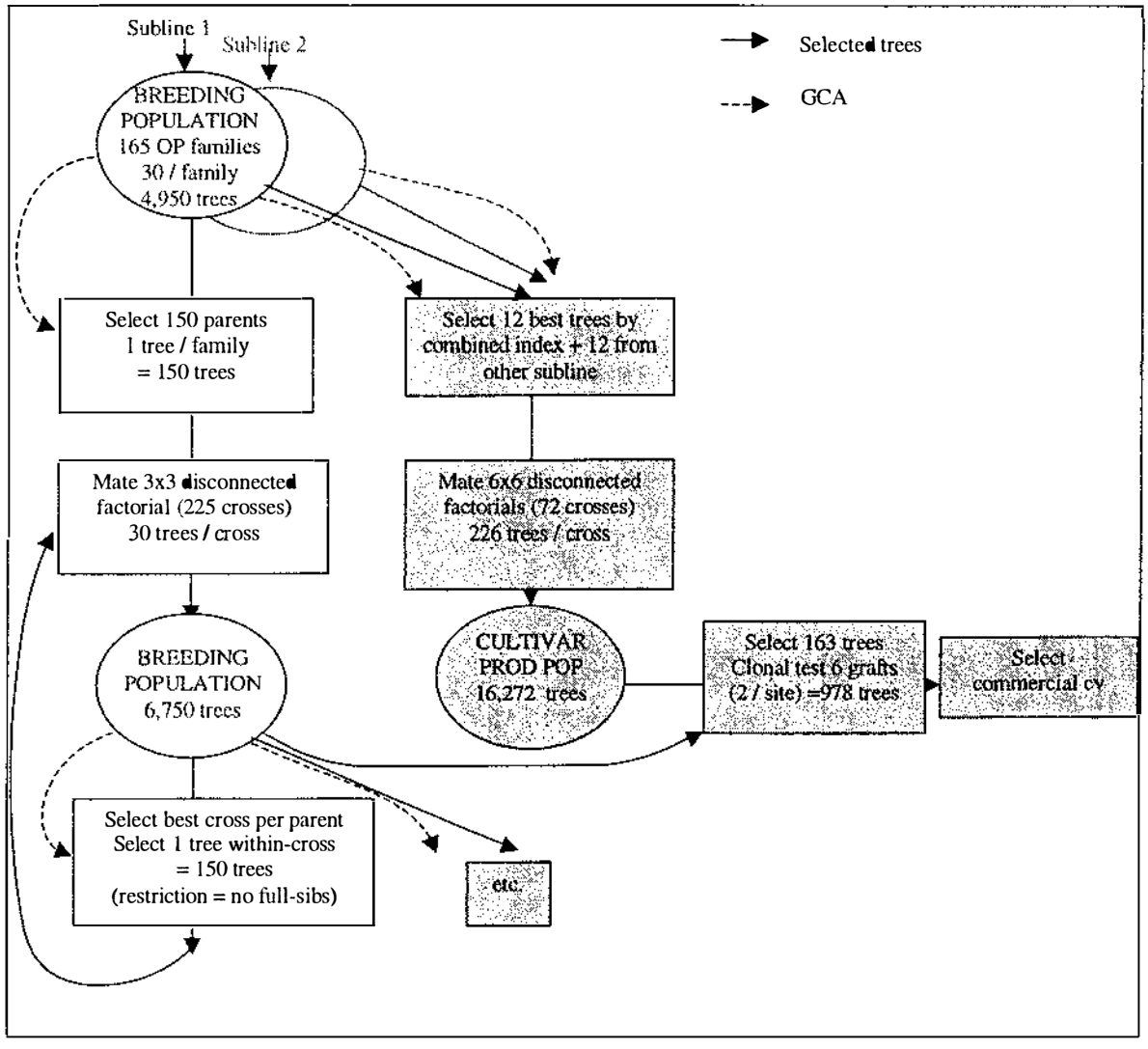

Figure 2. Task flow chart of recurrent selection for general combining ability (RS-GCA) strategy based on a fixed resource of approximately 30,750 trees per generation. Only one of two sublines is shown.

Table 1. 3x3 disconnected factorial mating design for the RS-GCA breeding population. The parents of the first cross in each of the 25 sets (P^1 to P^{25}) were positively assortative mated, the remaining parents were randomly mated (R).

		Selection						
		1	2	3	4	5	6	etc
Selection	76	P^1	R	R				
	77	R	R	R				
	78	R	R	R				
	79				P^2	R	R	
	80				R	R	R	
	81				R	R	R	
	etc.							etc.

Table 3. RS-GCA cultivar production population mating design. Two 6x6 disconnected factorials.

		Breeding population selections: Subline 1											
		1	2	3	4	5	6	7	8	9	10	11	12
Breeding population selections: Subline 2	13	■	■	■	■	■	■						
	14	■	■	■	■	■	■						
	15	■	■	■	■	■	■						
	16	■	■	■	■	■	■						
	17	■	■	■	■	■	■						
	18	■	■	■	■	■	■						
	19							■	■	■	■	■	■
	20							■	■	■	■	■	■
	21							■	■	■	■	■	■
	22							■	■	■	■	■	■
	23							■	■	■	■	■	■
	24							■	■	■	■	■	■

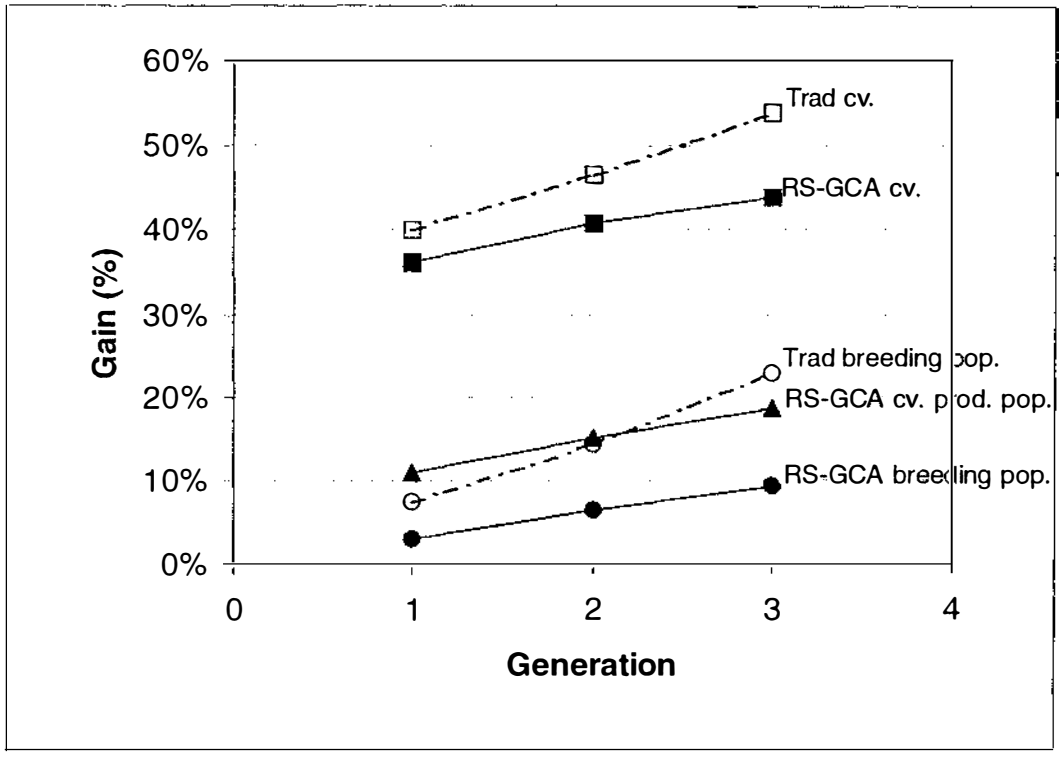

Figure 3. Genetic gain for a trait with initial values for $h^2 = 0.2$, $\mu = 10$, and $\sigma_P^2 = 1.0$ for both the traditional apple breeding strategy and the RS-GCA breeding strategy.

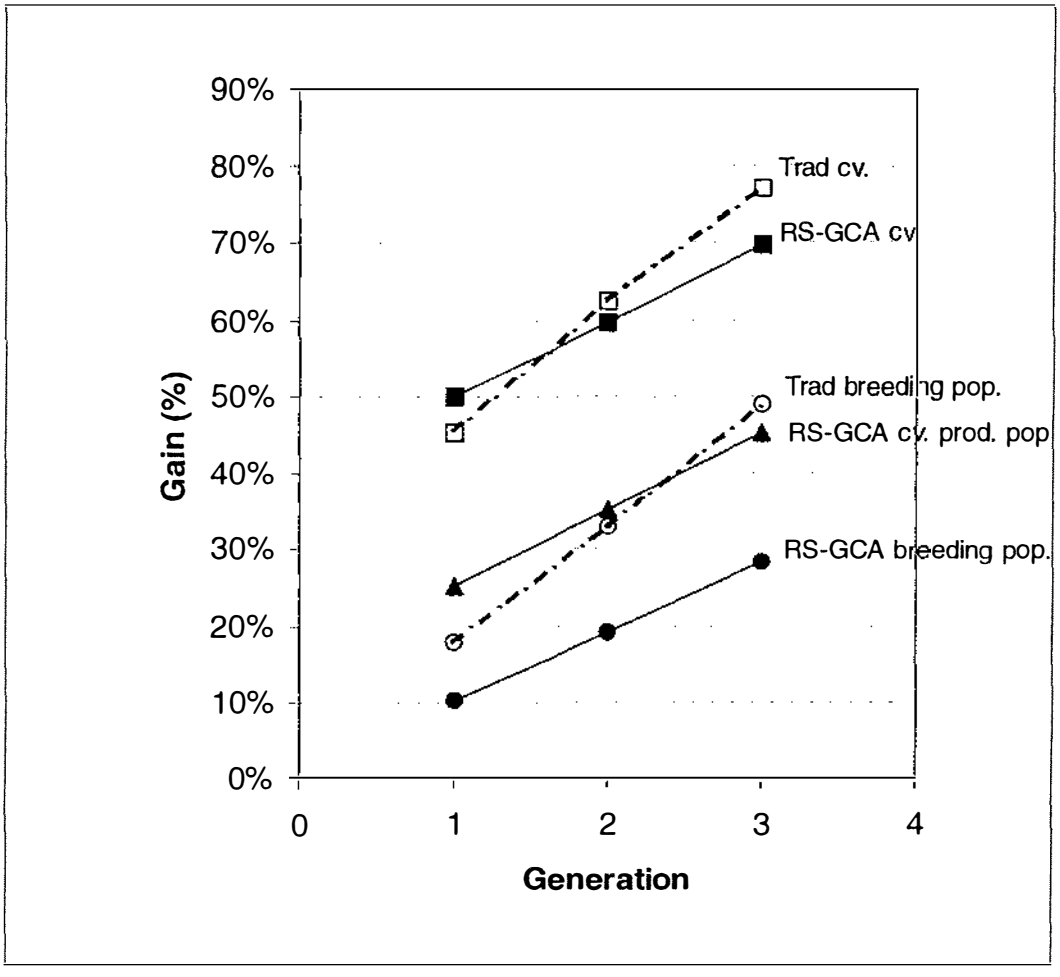

Figure 4. Genetic gain for a trait with initial values for $h^2 = 0.6$, $\mu = 10$, and $\sigma_P^2 = 1.0$ for both the traditional apple breeding strategy and the RS-GCA breeding strategy.

Chapter 6: General discussion

Many agronomically important traits in apple are quantitatively inherited (chapter 2), so an understanding of quantitative genetic principles could enhance the efficiency of apple breeding programmes. This thesis examined aspects of breeding and selection for quantitative traits in apple. First, the evolution of the domestic apple (*M.* ×*domestica*), modern apple breeding strategies and breeding objectives were reviewed to determine what genetic resources are available to apple breeders, how they have utilised them and what the future direction of breeding is (chapter 2). Second, apple shape was analysed to develop quantitative measurements for use in breeding (chapter 3). Third, narrow-sense heritability, between-site and between-trait genetic correlations were estimated for a wide range of agronomically important traits to provide some insight into efficient breeding strategies for these traits. Finally, simulation was used to predict short-term gain and the literature reviewed to determine long-term gain for both the traditional breeding strategy and a strategy using recurrent selection for general combining ability (RS-GCA) (chapter 5). This section discusses the key points covered in the thesis and looks to some future directions for apple breeding utilising quantitative genetics.

In chapter 2 the evolution of *M.* ×*domestica* was reviewed. Interspecific and intraspecific hybridisations formed a species with a wide reserve of genetic diversity (Ponomarenko, 1983; Way et al., 1989; Morgan et al., 1993; Janick et al., 1996). As well, *M.* ×*domestica* can readily mate with most of the apple species providing an even broader source of genes for the apple breeder. However, international exchange of breeding stock, utilisation of fewer, common parents and the use of breeding strategies that maximise gain but erode genetic diversity has lead to an increase in coancestry within the modern apple breeding population (Noiton and Alspach, 1996). Genetic erosion will lead to a reduction in genetic diversity and a decline in long-term gain. As well, many of the strategies developed this century involve the manipulation of only a few genes at a time through mutagenesis, gene transformation, or backcrossing and do not really address the concern about genetic diversity (see chapter 2). However, Noiton and Shelbourne (1992) proposed a new strategy for apple, based on recurrent selection for general combining ability (RS-GCA) as used in forest tree breeding (chapter 5), which maintains genetic diversity and is the basis of the RS-GCA strategy proposed in this thesis.

Strategies that utilise quantitative variation require traits to be measured with a continuous or an approximately continuous scale. Apple shape was chosen to study quantitative trait measurement (chapter 3) as no comprehensive genetic analysis of apple shape traits had been done, despite a preference for conical and oval apple shape (Janick et al., *1996*). Paulus and Schrevens (1999) were the first to apply principal component analysis of Fourier descriptors to apple shapes. They defined fruit aspect ratio and fruit conicity as the primary shape traits for International Plant Genetic Resources Institute apple shape categories. This thesis took the analysis two steps further by applying the analysis to real apple outlines and then by estimating the variance components to select genetically inherited shape traits. This study found that both fruit aspect ratio and fruit conicity traits identified by Paulus and Shrevens (1999) were under genetic control and a third trait, fruit squareness, was found to be genetically inherited.

Narrow-sense heritability, genetic correlation between sites and genetic correlation between traits were estimated in chapter 4 for a range of agronomically important traits (tree vigour, powdery mildew incidence, leafing date, flowering date, harvest time, fruit size, fruit sugars, fruit acidity, fruit firmness, fruit aspect ratio, fruit conicity and fruit squareness). Accurate estimation of genetic parameters requires over 50 unrelated parents (Hill, 1980) or more (Fins et al., 1992). Durel *et al.* (1998) were the first to use a sufficient population size to provide accurate parameter estimates for apple. Despite the possible inaccuracies in other reported parameter estimates, and the differences in apple populations used, heritability estimates from this study were in line with similar traits previously reported (Sedov and Sedova, 1970; Serova, 1989; Tancred et al., 1995; Durel et al., 1998). The possibilities were discussed of increasing estimates of heritability for tree growth habit, fruit acidity, and fruit sugars ($h^2 \leq 0.26$) by reducing the environmental variance with stricter and consistent tree-training, fruit harvesting and fruit storage protocols, or by using clonal averages or progeny testing. Heritability for the remaining traits ($h^2 \geq 0.32$) indicated that individual phenotypic selection would be efficient.

Between-site genetic correlations were high ($r_A \geq 0.64$), which indicated GxE was low and that family rank was not different between the Havelock North and Nelson sites for the traits measured. The implication for breeding strategy design is that breeding at one site to improve performance at other sites would be efficient for these traits. This simplifies breeding programme design as only one breeding population would be required. However, although ranking may not alter for different environments, testing genotypes at each environment would still be required to determine individual performance.

Between-trait genetic correlations, estimated in chapter 4, indicated the likely response to selection with multiple traits. Reliability of these estimates due to large standard errors (SE $\leq$ 0.2), linkage disequilibrium and possible dependency between trait expressions was discussed. The possibility of making rapid gains in certain directions by exploiting groups of traits with positive genetic inter-correlations between traits was explored. Application of between-trait genetic correlations in a multi-trait selection strategy was mentioned in chapters 2, 4, and 5 but not covered in detail.

In chapter 5, the recurrent selection for general combining ability (RS-GCA) strategy proposed by Noiton and Shelbourne (1992) was examined and redefined using the information from the genetic parameter study in chapter 4 and information from the literature. Short-term gain was simulated for both strategies, using a fixed resource of 30,750 trees, and the likely long-term gain was estimated from published studies.

A generation interval exceeding 100 years meant that annual genetic gain would be extremely small for the traditional breeding strategy. The long generation interval was due to extensive cultivar testing both in the breeding programme and by commercial growers before inclusion in a breeding programme. Separation of the selection of parents from cultivar testing would greatly enhance gains in the traditional strategy. A comparison of genetic gain per generation showed that the traditional breeding strategy made greater short-term gains than the RS-GCA strategy due to higher selection intensity. However, the use of fewer parents in the traditional strategy would mean an increase in inbreeding and a greater loss of genetic diversity for the traditional breeding strategy (Gea, 1997), risking loss in long-term gain (Robertson, 1960). On the other

hand, relative gain between the breeding population and the released cultivars was greater for the RS-GCA strategy, despite similar selection intensities. Half the additive genetic variance being between full-sib families (more with inbreeding), which was better exploited by the RS-GCA strategy.

A future development for apple breeding strategy would be to develop a selection index to enable efficient multiple trait selection in the breeding population. Selection for more than one trait in apple is currently based on independent culling levels (Janick et al., 1996), which has been shown to be less efficient than the Hazel-Smith multiple trait selection index in parent selection (Hazel, 1943; Hazel et al., 1994). The Hazel-Smith index is a linear function of the selection traits weighted by their economic value that best predicts the genetic merit of the individual. More than one index could be defined. For the RS-GCA breeding population the index should include only the traits that are common to all apples. Common traits could include tree health (disease resistance), yield (uniformity, quality, tree habit, volume/unit area, etc.), efficiency (precocity, compact tree size) and fruit qualities (size, blemish-free, bright colour, high soluble solids, storage ability, texture, disorder-free). More detailed selection indices could be applied to subsets of the breeding population or to the cultivar production populations to select for different end-uses e.g.: high tannins for cider apples. Independent thresholds may continue to be of use for cultivar selection, where efficiency in gain is less important than selection for phenotypes that pass a certain quality threshold. Also, defining a selection index may be difficult due to the non-linearity of many traits.

Differentiating selection for subsets of the breeding population either to enhance gains in clusters of traits with high genetic correlation (suggested in chapter 4), or to form cultivars for specialist end-uses, may require further division of the breeding population. In this case nucleus breeding strategies (James, 1977; Cotterill, 1989; Roden, 1994) or other unbalanced mating or selection designs (see chapter 5) may be worth investigating.

In conclusion, both long-term gains and short-term gains are required for sustainable apple breeding programmes. Continued application of the traditional strategy with a genetically narrow-based breeding population would lead to further erosion of the

genetic diversity and a decline in long-term gains. However, application of a strategy like the RS-GCA strategy to a genetically broad-based breeding population would unlock the largely untapped genetic diversity within germplasm repositories. As well as breeding population improvement, use of a population like the cultivar production population provides a means to convert the genetic diversity to gain for commercial release. Application of strategies RS-GCA may be the start of a promising future for apple breeding with a greater diversity of new apple cultivars to tempt the consumer.

References

Cotterill, P. P. 1989. The nucleus breeding system. *Proc. 20th South Forest Tree Improvement Conference. June 26-30, Charleston, SC.*

Durel, C. E., F. Laurens, A. Fouillet, and Y. Lespinasse. 1998. Utilization of pedigree information to estimate genetic parameters from large, unbalanced data sets in apple. *Theoretical and Applied Genetics*, 96:1077-1085.

Fins, L., S. T. Friedman, and J. V. Brotschol, eds. 1992. *Handbook of quantitative forest genetics.* Kluwer Academic Publishers, Dordrecht, Netherlands, xvii + 403 p. (Forestry Sciences; Vol 39.).

Gea, L. D. 1997. Genetic diversity and gain: the concept of a status number. *Ph.D. Dissertation*. School of Forestry, University of Canterbury, Christchurch, NZ.

Hazel, L. N. 1943. The genetic basis for constructing selection indexes. *Genetics*, 28:476-490.

Hazel, L. N., G. E. Dickerson, and A. E. Freeman. 1994. The selection index - then, now, and for the future. *Journal of Dairy Science*, 77:3236-3251.

Hill, W. G. 1980. Design of quantitative genetic selection experiments, p. 1-13. *In* A. Roberston (ed.), *Selection experiments in laboratory and domestic animals.* Commonwealth Agricultural Bureau, London.

James, J. W. 1977. Open nucleus breeding systems. *Animal Production*, 24:287-305.

Janick, J., J. N. Cummins, S. K. Brown, and M. Hemmat. 1996. Apples, p. 1-77. *In* J. Janick and J. N. Moore (eds.), *Fruit Breeding: Tree and Tropical Fruits*, vol. 1. John Wiley & Sons, New York.

Morgan, J., A. Richards, and E. Dowle. 1993. *The Book of Apples.* Ebury Press, London. Published in association with the Brogdale Horticultural Trust.

Noiton, D. A. M., and P. Alspach. 1996. Founding clones, inbreeding, coancestry and status number of modern apple cultivars. *Journal of the American Society for Horticultural Science*, 121:773-782.

Noiton, D., and C. J. A. Shelbourne. 1992. Quantitative genetics in an apple breeding strategy. *Euphytica*, 60:213-219.

Paulus, I., and E. Schrevens. 1999. Apple shape characterization by Fourier expansion of digitized images. *Journal of Agricultural Engineering Research*, 72:113-118.

Ponomarenko, V. V. 1983. History of the origin and evolution of the apple Malus domestica Borkh [Russian]. *Trudy po Prikladnoi Botanike, Genetike i Selektsii*, 76:10-18.

Robertson, A. 1960. A theory of limits in artificial selection. *Proceedings of the Royal Society of London*, Series B, Biological Sciences, 153:234-249.

Roden, J. A. 1994. Review of the theory of open nucleus breeding systems. *Animal Breeding Abstracts*, 62(3):151-157.

Sedov, E. N., and Z. A. Sedova. 1970. Breeding apples for an increased content of soluble dry matter in the fruits. [Russian]. *Selektsiya, sortoizuch., agrotekhn. plod. i yagodn. kul'tur. 3. Orel, USSR: 1969. 11-24.*

Serova, Z. M. 1989. Breeding apple for large fruit. *Puti intensifikatsii sadovodstva i selektsii plodovykh i yagodnykh kul'tur. Tula, USSR: 1989. 34-40. 4 ref. (Referativnyi Zhurnal (1990) 3Ya3371).*

Tancred, S. J., A. G. Zeppa, M. Cooper, and J. K. Stringer. 1995. Heritability and patterns of inheritance in the ripening date of apples. *HortScience*, 30(2):325-328.

Way, R. D., H. S. Aldwinckle, R. C. Lamb, A. Rejman, S. Sansavini, T. Shen, R. Watkins, M. N. Westwood, and Y. Yoshida. 1989. Apples (Malus). *Acta Horticulturae*, 290:3-62.

~ END ~