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Sustainable Population Management of *Actinidia* (Kiwifruit) for Multiple Products

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ABSTRACT

The maintenance of genetic variance is the key issue in breeding and conservation. To increase the productivity of kiwifruit, kiwifruit breeders have been exploring methods of selecting the best genotypes. The future success of this programme depends on the availability of sufficient genetic variance in the population. It is feared that continued selection will deplete genetic variance of commercial traits. Increasing the total genetic variance is possible through recombination, mutation and breeding. Methods to generate inter-population variance and its subsequent release through advanced generation 'hybrids' are reviewed, to gather knowledge and focus on breeding opportunities from germplasm management relevant to kiwifruit (as well as any other) breeding programme. The use of molecular information and molecular tools has started to transform the approach to conservation and breeding with *Actinidia*, from allelic diversity analysis link to provenance variation to the implementation of molecular markers for commercially important traits. Increasing the opportunities for selecting new cultivars with traits highly associated with consumer and industry demands are the major drivers of Plant and Food Research strategy; a robust breeding and conservation strategy will guarantee enough sources of variation for the future.

Keywords: *Actinidia*, *ex situ* population management, breeding strategy, conservation strategy, genetic variance

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INTRODUCTION

The New Zealand Kiwifruit Journal (January/February 2009) reported that one hundred million trays of ZESPRI Kiwifruit were exported from New Zealand by the end of 2008. That would be enough to fill 92 Olympic-sized swimming pools and if placed end-to-end would wrap around the world five times. For a crop almost unknown in international markets until 1970 (Ferguson 1997), that is an impressive figure.

However, New Zealand's kiwifruit industry is currently facing uncertainty caused by the increasing pressure from overseas competitors and fluctuating market returns dominated mainly by a single kiwifruit cultivar, 'Hayward', in the species *Actinidia deliciosa* (A. Chev.) C.F.Liang et A.R.Ferguson var. *deliciosa* (Ferguson 1990).

In order to increase profitability and market share, the New Zealand industry marketed an additional variant of yellow-fleshed kiwifruit known as 'Hort16A' (marketed as ZESPRI® GOLD Kiwifruit). This cultivar of *A. chinensis* represents approximately 23% of the total New Zealand production (The New Zealand Kiwifruit Journal 2009). More recently, *A. chinensis* 'Hongyang' ("Red Sun") became the first red-fleshed kiwifruit cultivar to be grown on a commercial scale (Comeskey *et al.* 2009) and a few mutants and other cultivars of *A. deliciosa* and *A. chinensis* are currently grown in Italy and China for the same purpose (Ferguson and Seal 2008). A few other cultivated *Actinidia* species grown around the world (*A. arguta*, *A. eriantha*, *A. kolomikta* and *A. polygama)* are of very minor commercial importance (Huang and Ferguson 2006).

To confirm New Zealand as the leader in the international kiwifruit market, it is therefore essential that the kiwifruit industry continues to diversify into new cultivars. To enable diversification and cultivar selection, steps should be taken to ensure maintenance and enhancement of genetic variance through appropriate germplasm conservation and breeding programmes.

In recent years a number of reports have documented the development of *Actinidia* genetic resources and breed-

Received: 24 September, 2009. Accepted: 23 March, 2011.

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ing programmes (Ferguson 1997; Ferguson and McNeilage 1999; MacRae 2006; Beatson *et al.* 2007). *Actinidia* requires diversity for all purposes. Variation is required to respond to market changes and genetic variance is a key parameter that determines the rate of response to selection forces.

Increasing the total genetic variance is possible through recombination, mutation and breeding. Methods to generate inter-population variance and its subsequent release through advanced generation 'hybrids' are reviewed, to gather knowledge and focus on breeding opportunities from germplasm management and new cultivar selection in *Actinidia*.

GENETIC VARIANCE AND SOURCES OF VARIATION IN *ACTINIDIA*

The effective utilisation of genetic variance requires an understanding of the process that regulates genetic variance within and between populations, its scientific management and selected breeding strategy. The present forms of variation may be neither optimal nor even valuable. Managing populations to maintain or maximise genetic variability in which rarity will be a factor will not necessarily increase the inherent value of a rare gene. Thus a structure is needed where genetic variability and breeding opportunities are matched and are consistent with the paramount principle of maximising genetic gain per unit of time. The goal is focused on how to structure the germplasm and breeding populations to increase, secure and utilise genetic variance, with minimal loss of genetic diversity, with a long-term programme in mind. This objective is crucial given current market forces and competitive pressures. Public plant breeding has increasingly become concentrated around core crops where there is strong partnership with industry. Most programmes have achieved genetic gains in excess of 1% of the mean per year for a variety of target traits, and these gains are likely to continue, given the high genetic variation present within these species (Woodfield *et al.* 2006). Balancing genetic diversity and ensuring genetic gain will ensure the success of breeding programmes and the robust longevity of new cultivars.

Although the approaches vary, the development of a breeding objective aligned to genetic resource management is a crucial step in the design of a breeding programme. The underlying principle is that the breeding objective traits are the ends, whereas the characters used as selection criteria are the means used to achieve the ends (Ponzoni and Newman 1989). Those characters are present in the breeding or in the germplasm population and are the subject of anthropomorphic selection to suit new markets. The profit function that drives breeding objectives is usually non-linear, but for simplicity and convenience, linear profit functions are used (Dekkers *et al.* 1995). These linear approximations are usually considered good approximations for *Actinidia* because the rates of genetic change per generation are assumed small.

There is little information on the genetic variability and heritability of important new traits in *Actinidia.* In most cases, research is reduced to commercial traits from small trials and samples subjected to the effects of truncated selection from advanced elite lines (Beatson 1991; Ferguson and McNeilage 1999; Marsh *et al.* 2003; Cheng *et al.* 2004, 2006; Beatson *et al*. 2007).

Variation is assumed to be reduced by selection (Falconer 1989; Lynch and Walsh 1998). Restrictions in population size aimed at productivity increases and uniform crop productions have recently been a focus of numerous studies in evolutionary genetics regarding population subdivision and inbreeding (Briggs and Goldman 2006; Saccheri *et al*. 2001), founder effects (Goodnight 1988), and domestication (Whitt *et al*. 2002). Following a bottleneck, additive genetic variance may increase because of contributions from dominance (Willis and Orr 1993) and epistatic variances (Naciri-Graven and Goudet 2003), in particular for traits closely related to fitness (Zhang *et al*. 2004). This has special relevance to breeders, as the trait of interest is inextricably tied to fitness through selection. The increases in additive variance observed for some traits following a restriction in population size are in contrast to a purely additive model, which predicts a decline in additive variance parallel to the loss of genetic diversity caused by the bottleneck (Nei *et al*. 1975; Davies *et al* 1999).

The earlier breeding carried out with the small number of *A. deliciosa* accessions introduced to New Zealand in 1904 increased inbreeding and moved the population into a definite bottleneck. Variability for some traits, such as storage, was reduced and could not be restored in elite lines until crosses with new accessions were made during the 1990s.

A recent publication from Beatson *et al.* (2009) demonstrates the large variability for most traits observed even in a small (2x7 factorial) population of *A. arguta* (**Fig. 1**). However, if selection indices with no family restrictions were to be applied for next generation breeding, 21 out of the 25 cultivars would belonged to a single cross. The consequent response in additive variance from the F_2 population is expected to be reduced for most traits and the corresponding population will follow the predicted bottleneck trends, with a reduction in additive genetic variance and an increase in dominance and epistatic variances.

Additive variance is the only part of the genetic variance that is heritable and contributes to selection response. Thus, domestication and breeding share a common feature of population bottlenecks followed by significant genetic gain. To date, no crop models have been developed to investigate the evolution of genetic variance, selection response, and population diversity following bottlenecks.

If new variation is needed to increase adaptability of productivity part of it can be restored by mutation; the rate at which such variation accumulates in *Actinidia* is unknown although is common.

Bud mutations of diploid *A. chinensis* have been identified by occasional shoots carrying fruit that are, for example, much larger than usual (Martin 2005). Plants derived by grafting these shoots onto rootstocks have been tested by flow cytometry: some have proved to be mixoploid (2*x*, 4*x*), others tetraploid.

Spontaneous mutations happen in nature at a relatively frequent rate. In most cases they are deleterious. However, mutations can result in valuable new traits. Discovery of spontaneous mutants or sports by observant growers has been an important means of cultivar improvement. Most of *A. deliciosa* new cultivars in Italy are mutants of 'Hayward' (Ferguson and Seal 2008). Commercially successful bud mutations of 'Hayward' have been described by Testolin and Ferguson (2009) and include Bo-Erica®, Earligreen®, Top Star®, Green Light® and other variants of 'Hayward'.

Theoretical simulations have shown the possibility of maintaining existing levels of genetic variance based on mutation-selection balance models (Lynch and Walsh 1998). Continued response to selection is likely to be due to new mutations. How soon a new mutant allele will contribute to genetic variance and will respond to selection will depend on the selection pressure on the allele and the population size. Spontaneous *4x* mutants like the ones from "Hort16 A" have been incorporated into tetraploid lines to increase population sizes; however, the stability of the identifiable mutant trait has not been properly evaluated.

Mutation rates on a per trait basis can be quite high; hence, new allelic effects are constantly being added to the population even as some alleles become fixed. Keightley and Hill (1990) show that in long-term selection experiments, a steady state genetic variance can be created as the result of the effects of selection, population size and mutation rates.

There is a dynamic to the genetic variance, some factors increase while others decrease the total variance in natural as well as bred populations and the levels predicted depend on the assumptions of the model. The genetic variance is thus an emergent property of genetic dynamics, subject to

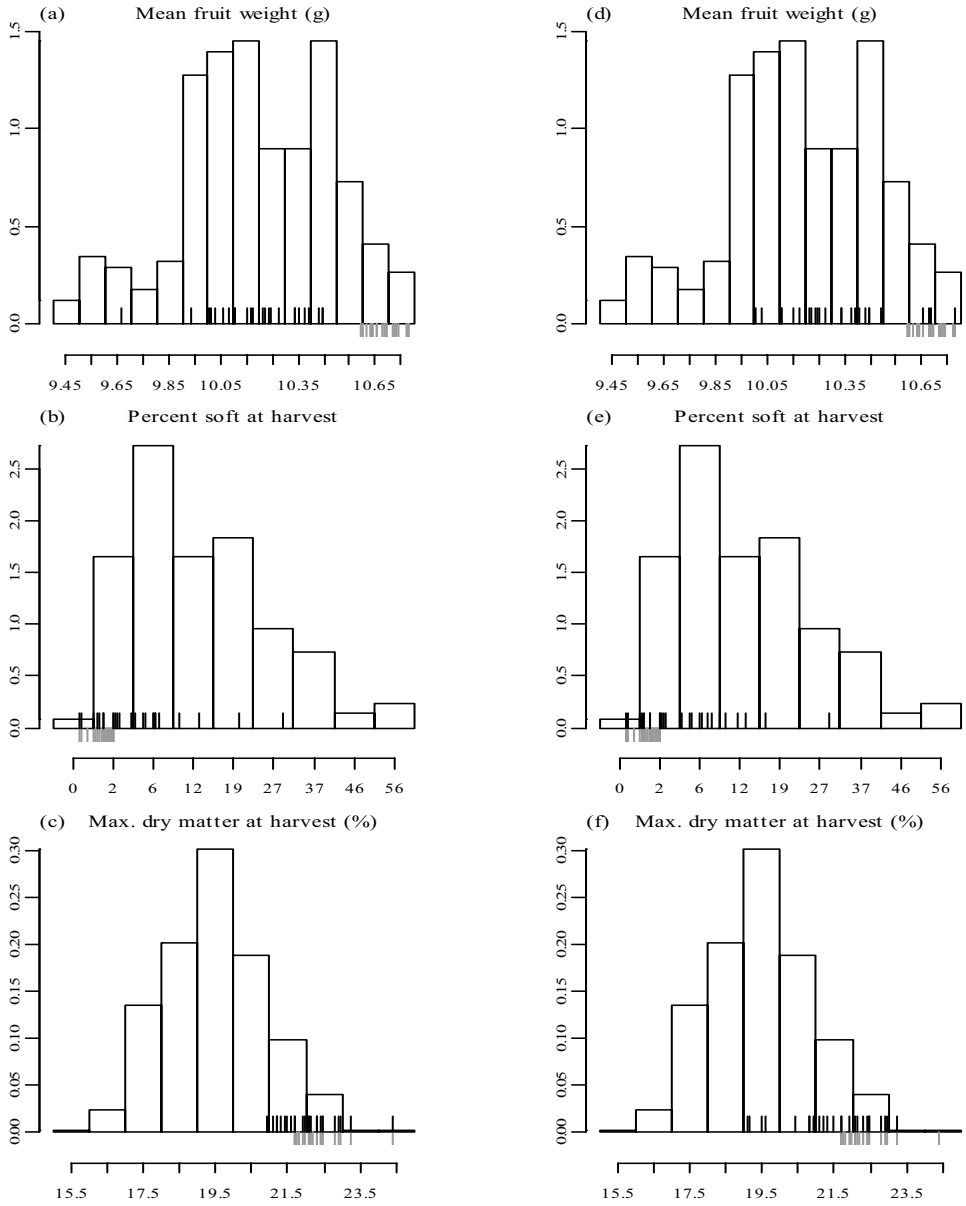

Fig. 1 Frequency histograms for *Actinidia arguta* fruit weight, percent soft at harvest and maximum dry matter content at harvest under two different selection indices. The best 25 values for each trait superimposed as the grey rug. The black rug shows the top 25 genotypes. Source: Beatson *et al*. 2009.

selection, mutation and population size and is, for *Actinidia*, a cause and effect of anthropomorphic evolution and management.

UTILISING GENETIC VARIANCE UNDER DIFFERENT SELECTION SCHEMES

Actinidia is still in the early stages of domestication and most of the variation is still under natural evolutionary process (MacRae 2006). It is important to understand the dynamics of genetic variance within natural and breeding populations under simple gene action models. More complex models of factors that regulate the dynamics of within- and between-population genetic variance should also be considered.

Under genetic management options, some ways are implied/suggested by which these dynamics can be managed so that populations can respond to pressures for higher gain and adaptive response.

Stabilisation selection

One of the paradigms for species with reasonably large populations under relatively constant environmental conditions is that stabilising selection for an intermediate 'optimum' directs evolution. Aside from occasional heterotic loci or frequency dependent selection for rare alleles, genetic variance is not expected to be maintained under stabilisation selection. However, mutation can maintain variation and diverse mutation-selection models have indicated the possibility of maintaining genetic variability (Briggs and Goldman 2005). Somatic polyploids resulting from spontaneous doubling of the chromosome number might have contributed to the formation of ploidy races (Ferguson *et al.* unpublished data) and contribute to the maintenance of genetic variance in *Actinidia*.

The simplest models assume large numbers of alleles per locus and a Gaussian distribution of allelic effects. The models put forward by Lande (1976) and Fleming (1979) are extensions of the model described earlier by Kimura (1965), and reviewed by Bürger (1986). These models describe equilibrium genetic variance at low selection intensities, higher mutation rates and low mutational variance. Their derivation requires mutational variance $m^2 << \sigma^2 g$, where m^2 is the variance due to mutational effects and $\sigma^2 g$ is the genetic variance.

In contrast, for higher selection intensities, low mutational rates and higher mutational variance, Turelli (1984),

based on the 'house of cards' model put forward by Kingman (1978), describes mutation-selection equilibrium, assuming that allelic effects after mutation are independent of allelic effects before mutation. In numerical calculations he found that the Gaussian approximations gave better predictions when mutational variance is low and mutational rates are high, while 'house of cards' approximation gave more accurate predictions with lower mutational rates, higher mutational variance and stronger selection (Mahdi and Lessard 2000). A scenario where selection intensity and mutational variance are high, and mutation rate is low, has not been investigated.

Directional selection on breeding programmes

In contrast to models of stabilising selection, breeding populations are mainly subject to directional selection. In many cases, continued response has been observed through many generations of selections. Mutations during the course of the experiment are thought to cause this continued response. In selection over 50 generations for oil content in corn (Dudley *et al.* 1977; Briggs and Goldman 2005), continued response with no clear indication of a plateau was observed, indicating that point mutations, unequal crossing-over or other sources could maintain substantial genetic variance in the population. Theoretically, continuous directional selection can eventually achieve a steady state of genetic variance and account for continued response (Keightley and Hill 1987). This equilibrium variance was shown to be a function of mutation rate and population size.

The dynamics of multi-locus systems introduce other mechanisms for considering the genetic variance to be in continual flux. The equilibrium structure of multi-locus systems is very complex (Karlin 1975). In order to understand these systems, the nature of epistasis, linkage and other factors needs to be explored.

Based on a 'house of cards' model, Barton (1999) has shown that fitness surfaces may contain many local adaptive peaks separated by adaptive valleys; these valleys prevent deterministic evolution from carrying populations from one peak to another. Wright (1952) suggested that these multiple fitness peaks are generated by epistatic interactions between genes. Wright's Shifting Balance Theory hypothesises that populations can move from one peak to another by random genetic drift caused by periods of small population size. Populations will then be carried to new peaks by migration effects. What this means when trying to applying the results to population management, in particular with *Actinidia*, is that some peaks may have similar fitness values, even though they can vary greatly in allele and genotypic frequencies. Small populations originated from intense selection ratios for flavour and storage on a *A. chinensis* elite line may show similar fitness values to those of another population originated from an unrelated *A. chinensis* elite line, but their allele and genotypic frequencies are expected to be substantially different from one other. In subdivided populations with a number of fitness peaks, there is potential for rapid evolution through recurrent directional migration (Crow *et al.* 1990). If properly structured (i.e. accounting for relatedness and similar selection pressures on mothers and fathers), factorial crosses commonly used in progeny tests in *Actinidia* have offered the opportunity to create populations with similar fitness values and quite possibly great variability on allele frequencies.

One of the causes of random drift shifting a population between alternative equilibria is the mutation rate. Barton and Rouhani (1987) derived formulae for that frequency shift. "When the mutation rate is low, the probability of shift reduces to the product of mutation rates and probability of fixation of a mutation. However, when the mutation rate is higher, the joint probability is higher than what would be expected if the mutations are dealt with independently".

Population shifts between alternative fitness peaks can be facilitated if the troughs between peaks are bridged. Whitlock and McCauley (1999) have shown that increases in phenotypic variance, even with a constant individual fitness function with multiple peaks, can cause the fitness to change from bimodal to unimodal. This will allow a change of phenotypic mean of the population by selection. When the phenotypic variances revert to the equilibrium state, the multiple peaks will re-emerge. In the meantime the population will have the opportunity to move from one adaptive peak to another. Once populations reach different peaks, they eventually establish equilibrium variance within populations, but also generate a between-population genetic variation component.

Inter-population dynamics also affect the genetic variance. With *Actinidia* populations subdivisions exist naturally to some extent and mating barriers in our breeding programmes have been constructed by the use of controlled pollination and pedigree management. Thus, the genetic variance between the populations may increase, decrease or may fluctuate as differential selection and migration rise and fall. If there is some continuous inter-population gametic migration, the level of the total genetic variance will depend on divergence and directional selection as well as on the migration rate (Bulmer 1980). The analysis is complex because of the effects of different forms of selection between populations and whether migration effects are confounded with selection differences. Selection can be stabilising or directional within-populations and may converge or diverge between-populations. For example, early *Actinidia* breeding programmes emphasized selection on fruit size, dry matter and ripe soluble solids content (SSC). Changes in the breeding objectives have shifted the pressure to unrelated lines with new traits generating divergent populations. When these populations are intercrossed, a larger genetic variance is obtained, increasing opportunities for cultivar selection.

Results from a diallel cross between two divergent

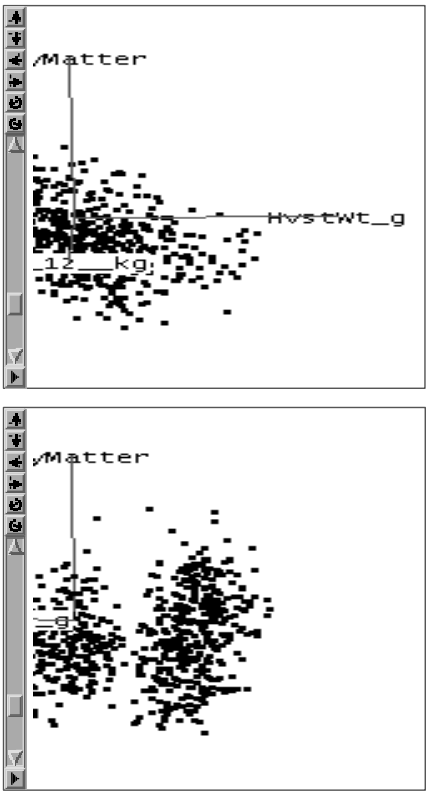

Fig. 2 Relationships between dry matter (DM), harvest weight (Hvstwt_g) and storage (FF12) resulting from a cross between two divergent populations in *Actinidia chinensis*. Figure shows an increased size of the genetic variance for storage without disruption of the genetic relationships between other known traits (DM and Hvstwt_g), increasing the opportunities for selecting new cultivars with good storage and high DM.

tetraploid (4x) populations of *A. chinensis*, one selected for storage and the other for dry matter (DM), have clearly shown (**Fig. 2**) an increased size of the genetic variance for storage without disruption of the genetic relationships between other known traits, increasing the opportunities for selecting new cultivars with good storage and high DM (Gea and Lowe unpublished data.).

Continuous geographical clinal trends in selection effects may be associated with models of isolation by distance as described by Slatkin (1978), but most of these cases have not been thoroughly investigated theoretically or experimentally in *Actinidia*, and clinal variation studies and genetic correlations between selection traits have not been thoroughly reported.

While complex epistatic models are likely to shift populations among different adaptive peaks or allelic combinations, simple gene action models can also generate complex dynamics. When many loci are involved in a trait, under stabilisation selection individual loci are free to drift and can result in selection for different allelic combinations among populations. Different isolated populations under stabilisation selection with the same mean may thus reach different allelic combinations. In directional selection also, such varying allelic combinations are possible by drift when population size is small. The potential segregational variance can then be utilised through breeding. Thus, genetic variance is not fixed either within or between populations, and hence "the total genetic variance remains a variable factor that itself is subject to various evolutionary or other pressures" (Namkoong and Koshy 1995).

The possibility of managing the genetic variance by controlling between-population divergence, and the consequent release of inter-population variation through advanced generation inter-population hybrids is worth discussing. Slatkin and Lande (1994) have shown a significant advantage in crossing populations that are allowed to drift for a period of time. The genetic variance within the parental population may remain the same, but the magnitude of segregation variance in the F_2 population may increase. The magnitude of the segregation variance will depend on the kind of genetic variance underlying a trait. Namkoong and Koshy (1995) reported that when variation is maintained by numerous alleles of moderately small effect, segregation variance will increase linearly with the time of separation of the lines, but when variation is maintained by low frequency alleles, the increase in segregation variance is likely to be small.

When populations are selected divergently and crossed to produce an F_2 generation, genetic variance is found to increase as the divergence between the parental populations increases (Koshy *et al.* 1996). Based on a forwards-backwards mutation-selection model, numerical calculations showed higher variances than for crosses where unselected parental populations were used (Koshy *et al.* 1996). The magnitude of the F_2 genetic variance expressed when hybridising divergent populations varies depending on the initial frequency distribution of gene frequencies in the parental populations. Uniform and U-shaped initial distribution cases showed increases in F_2 genetic variance with increases in population size, while a skewed initial distribution showed a decrease with an increase in population size. When selectively neutral alleles prevail in the population for a long period of time, a U-shaped distribution is probable, while a recent mutant allele will have a skewed distribution with higher probabilities at lower gene frequencies. Once a mutation event has occurred, the newly arriving alleles contribute to the genetic variance sooner, when population size is smaller. Compared with larger population sizes, the initial gene frequency of the new allele will be higher in smaller populations, causing higher F_2 genetic variance (Koshy *et al*. 1996). These results indicate that the outcome of genetic management options will depend on the selection history of the traits under consideration.

For practical purposes, however, the question is about the usefulness of any enhanced variation. In an operational *Actinidia* breeding programme it may not be economically practicable to maintain divergent populations. Furthermore, simulation studies showed that if gene action is entirely additive, then an undivided population has a better advantage than divided populations (Madalena and Hill 1972).

On the other hand, it is well known that several populations of small size can more completely search an adaptive surface for multiple peaks of adaptation than can a single population of any size. Wright (1952) concluded that more rapid improvement is possible if a population is subdivided into small lines with regular crossing and selection among lines. Controlling inbreeding could be cumbersome (given the dioecious nature of *Actinidia*) and costly (managing multiple populations v. a single population) and the effects of inbreeding on vine vitality, pests and disease resistance and fruit productivity traits have yet to be understood for *Actinidia*. Recent studies from Popowski (pers. comm.) suggest that pests and disease resistance in *Actinidia deliciosa* are highly associated with inbreeding levels.

Populations within a species that are adapted to divergent climatic conditions over many years may be utilised for such crosses. Such provenance hybrids may be considered as an option for preconditioning populations to future traits related to climate change. Species with short generations also may have advantages when environments are rapidly changing in a consistent direction. To design effective breeding and gene conservation programmes, one must first understand present population structures and the factors that influence them. Whether current patterns of variation provide something near maximum fitness for present and future conditions must be considered.

Furthermore, the results of simulation studies by Enfield and Ankelsaria (1986) have conclusively shown that a subdivide-merge breeding scheme can be most effective, when multiple peaks epistasis is the overriding kind of gene action. Even if gene action is independent and additive at individual locus, a multiple trait value function can create allelic fitness values such that multiple epistatic value peaks exist. To explore such complex surfaces by breeding, multiple populations can use different gene and trait combinations to achieve ultimately higher values than are possible with any single population. While on one hand it will help to reach the higher values than that possible by a single population, it will also make it possible to generate higher variance by raising F_2s from parental populations that are at different peaks.

With *Actinidia*, where improvement involves a complex of multiple traits, a total value epistatic can be expected even if individual trait gene actions are largely additive between loci. Just as non-additivity between traits can cause an interaction in a composite value function, the non-additivity demonstrated for various traits can create a complex surface (**Fig. 3**). For some traits the expected trend of large SCA at early stages is followed by increased GCA as the vine matures; other traits are showing opposite trends and more complex breeding strategy models are required to account for such situations (Gea and Currie 2008).

Simultaneous improvement of the population in the direction of the interest of the breeder and maintenance of higher values of potential for genetic variance are thus simultaneously possible by a multiple population strategy.

Wild relatives of kiwifruit are a potential source of genetic diversity for characters that are absent from the cultivated species. Interspecific hybridization now forms part of the breeding strategy of *Actinidia* in New Zealand to combine novel characters such as a range of skin and flesh colours, ripening indicators, and the unique flavour of wild species with the large fruit size and postharvest storage of cultivated kiwifruit species (Beatson *et al.* 2007). Many of the wild species of interest are tetraploid.

Inter- and intra-specific hybrid populations of *Actinidia* with different ploidy numbers have been created with the objective to introduce novel traits. Sterility of the F_1 interspecific populations has occurred; Seal (pers. comm.) has suggested a breeding strategy to circumvent this barrier,

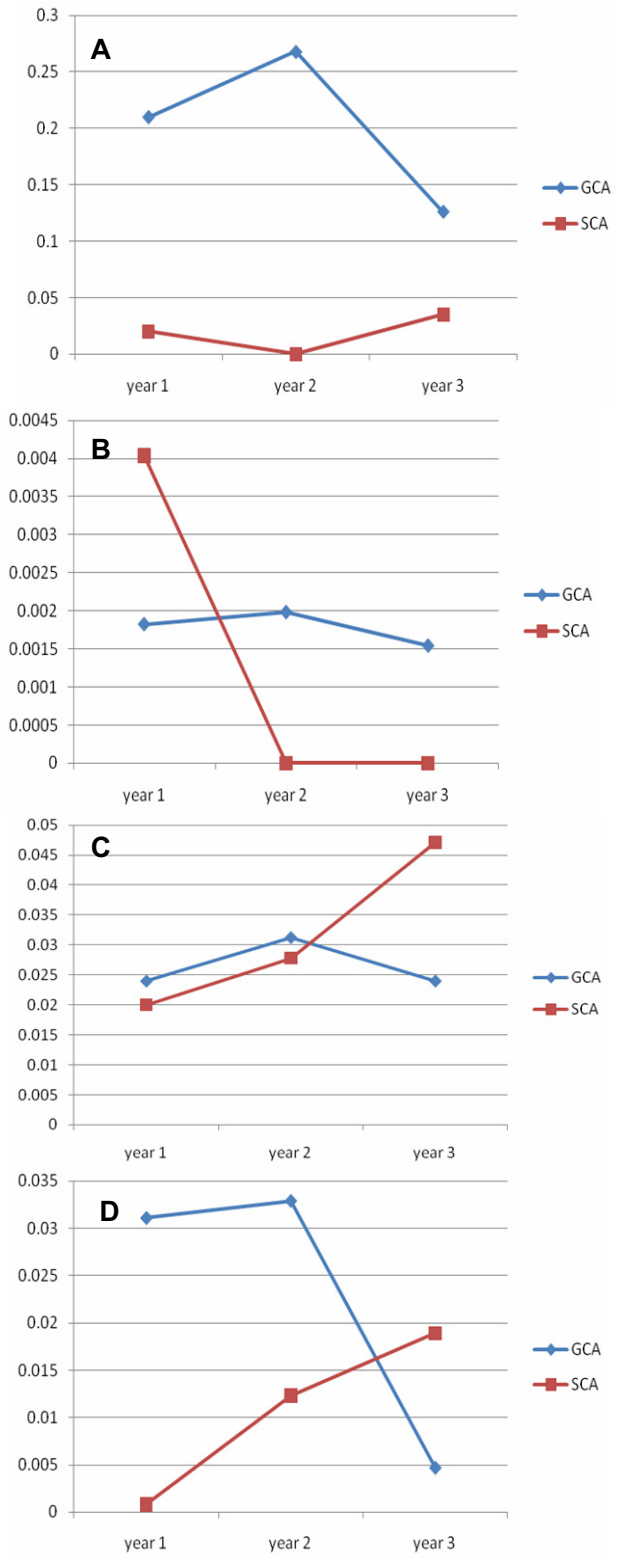

Fig. 3 Complex General Combining Ability (GCA) and Specific Combining Ability (SCA) relationship for Brix (A), colour (Hue Angle) (B), Storage at 12 weeks (Storage 12) (C)and Woody Core (WCore) (D). Expected trends would suggest a reduction on the SCA contribution to the total variance over the assessment years, as reflected for Hue Angle and Brix. Results shown here for Storage and Woody Core contradict expectations and suggest caution when selecting vines and parents early, given the increased importance of SCA, particularly for very important commercial traits. Source: Gea and Currie 2008.

with a reduced gamete strategy to avoid the problem of working with hybrid populations of different ploidy levels. However, despite the sterility problems, promising novel hybrid genotypes have been produced that have a kaleidoscope of colours, flavours, fruit sizes and fruit shapes. While none has commercial potential in its own right, together they form the germplasm base from which further novel interspecific hybrid populations are being developed.

A multiple population breeding system will allow the development of different sets of genes and gene complexes that will facilitate greater potential for reaching higher levels of adaptive or economic values and also generate potential for increased genetic variance in the future through advanced generation hybrids. By managing different populations through size and selection schemes, breeders may have greater options for improving economic and adaptive value than are possible by conventional methods.

With such complex dynamics affecting the structure and levels of genetic variance, there appear to be several ways that breeders may influence the levels and availability of the genetic variance. Both between- and within-population management options exist for breeders. Furthermore, since *Actinidia* breeding is so recent, there are often relatively wild population structures with a large diversity that may be used.

The most efficient and cost-effective way of managing existing *ex situ* populations and breeding lines of *Actinidia* with minimal loss of genetic diversity appears to follow closely Namkoong's Multiple Breeding Population System (Namkoong *et al.* 1980) (in contrast to hierarchical populations) emphasizing interpopulational diversity. Within each population, separate trait combinations can be selected for using in simple recurrent selection programmes. Diversity among populations can be enhanced by excluding intermating among populations and by selecting for divergent characteristics. Commercial populations can consequently be addressed by inter-population crosses among selected cultivars. This kind of controlled 'evolution' is similar to breeding for economic objectives in multiple selection regimes. The, use of multiple populations is arguably the best method for gene conservation for coping with future uncertainties regarding environmental conditions and economic values of traits (Eriksson *et al.* 1993) and for breeding, given the efficient use of small factorials in breeding lines, the opportunities for introgressing new traits and increased group connections useful to increase Linkage Disequilibrium (LD) studies.

In vitro induction of polyploidy for *Actinidia*

To manage within-population variance, population size, selection intensity and mutation rates may be manipulated. Attempts to increase available variation through artificial mutation induction have been employed recently in *Actinidia*.

Doubling the chromosome number appears to be a good way of increasing the fruit size of selections whose fruit have many valuable characteristics but are of inadequate size for commercialization, especially for some species or lines of kiwifruit (Wu *et al.* 2007) (**Fig. 4**). Autotetraploids of some selections may have commercial potential in their own right. They may allow introgression of desirable cha-

Fig. 4 Fruit size, shape and section from autotetraploid and diploid *Actinidia chinensis* 'Hort16A'. (**A**) Autotetraploid large 'Hort16A'; (**B**) Autotetraploid squashed "Hort16A'; (**C**) Benefit®-enhanced diploid 'Hort16A'; (**D**) Normal diploid 'Hort16A'. Source: Wu *et al.* 2007.

racters into species at different ploidy levels through cross breeding, solve some problems (embryo abortion and hybrid sterilization) faced in traditional breeding, and also increase the gene pool for kiwifruit improvement. However, somatic doubling, whether spontaneous or induced, does not increase heterozygosity. Autotetraploids may suffer from chromosome pairing irregularities at meiosis (multivalent formation), leading to partial infertility and the breakup of favourable gene combinations through recombination (de Silva *et al*. 2005; Ferguson *et al*. unpublished data).

New allelic variation generated by copy number differences for multi-gene families as well as transposable element-induced mutations have been considered (Wu *et al.* 2007). Though many artificially induced mutations are reported, the majority are undesirable. Even in the absence of induced mutations, based on the reported levels of spontaneous mutation rates (Martin 2005), it is possible to maintain substantial levels of genetic variance in the populations.

To summarise, effective management of genetic variance within- populations depends on the methods used to capture these new mutations and increase their frequency to levels where they can contribute substantially to the expressed genetic variance.

GENOMICS FOR GENETIC RESOURCE MANAGEMENT AND BREEDING

Quantitative genetics theory is often considered a fully developed science with no new questions and no new insights, so it is interesting to review some of the basic assumptions in the light of new developments from molecular sciences and breeding as an international business. Some of the assumptions underlying those theoretical concepts have recently been stretched. The most obvious has been the undermining of the infinitesimal concept.

By and large, the early optimism about exploiting the new technologies as expressed in Smith *et al.* (1986) has still to be realised, but molecular tools can complement and enhance population structure opportunities in different ways so as to quantify diversity present within and between populations, to assess how diversity is partitioned (within and between), to establish evolutionary history (through genetic linkages), to measure genetic distances, estimate relatedness, and control identity as well as monitoring gene flow and linkage disequilibrium.

Diversity and variation within *Actinidia* have been discussed quite thoroughly by several authors (Huang and Ferguson 2006; Li *et al.* 2006; MacRae 2006). Approximately 76 species and another 50 infraspecific taxa have been des-

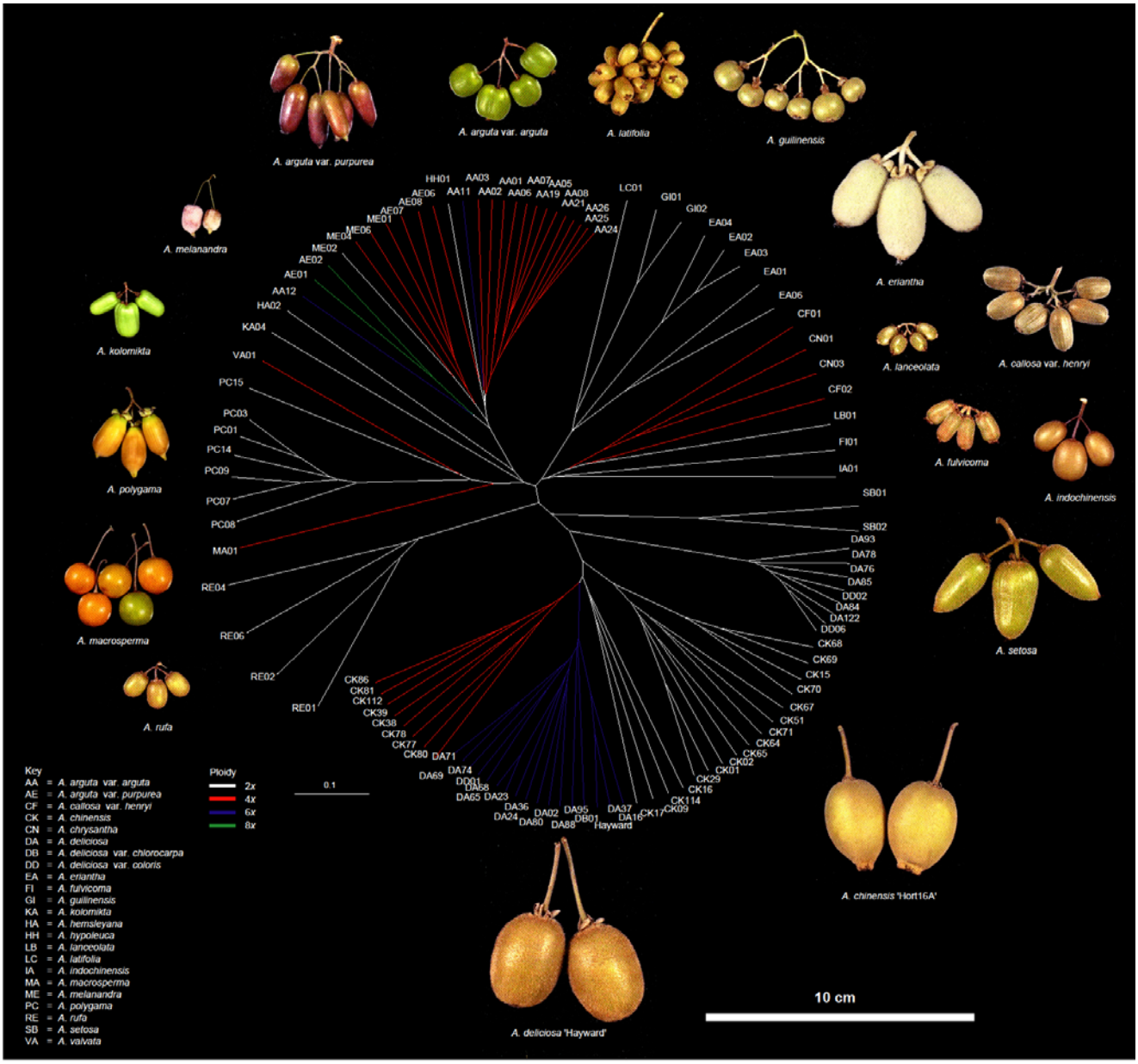

Fig. 5 Microsatellite studies to characterise *Actinidia* populations in a germplasm collection. Evolutionary relatedness could conduce to breeding populations reaching higher heterozygosity levels. Source: Datson *et al.* 2008.

cribed. *Actinidia* can be diploid, tetraploid, hexaploid or octaploid depending on the species or even on particular genotypes within a species. Plant & Food Research in New Zealand has the single most comprehensive *ex situ* collection of *Actinidia* germplasm, with more than 310 separate accessions of budwood and seed comprising representatives of 23 species (Ferguson 2007). There is a collection of about 80 *Actinidia* cultivars and named selections from both New Zealand and other countries. The collection provides the genetic resources for kiwifruit breeding. This incredible variation in the genus is reflected in a large diversity in vine and fruit attributes. Where gaps are identified in the collections, targeted exploration and collection of germplasm should be prioritised.

Lee *et al.* (2007) argued that with parental selection making use of genotypic as well as phenotypic differences, greater heterozygosity in the progeny is to be expected, generating potentially superior genotypes. Datson *et al.* (2008) (**Fig. 5**) showed the power of microsatellite studies to characterise *Actinidia* populations and the usefulness for structuring populations, as well as inferring evolutionary relatedness that could conduce to breeding populations with higher heterozygosity levels.

Microsatellite markers can be used to estimate relationships and genetic distances between different plant populations. However, their use for the estimation of genetic diversity among populations that differ in ploidy levels is difficult, as most of the statistical methods available are inappropriate for polyploidy species.

Other opportunities exist to exploit the diversity within kiwifruit germplasm, by focusing on genes that coordinate expression (Hellens *et al.* 2007). These regulatory genes are called transcription factors (TFs) and are DNA-binding proteins that initiate and control the level of mRNA transcription. Transcription factors coordinate complex biochemical processes. Using the model species *Arabidopsis thaliana* Hellens' group have identified, through sequence homology to the transcription factors (TFs), genes present in the kiwifruit sequence database. To complement the collection of cloned regulatory genes, they have developed a functional test to identify candidate TFs that initiate transcription of genes involved in the processes of interest. In kiwifruit, these include input traits such as budbreak and output traits of health, taste and convenience. By understanding the genes that regulate horticultural traits, molecular biology will be able to explain some of the diversity within our germplasm. In some cases, this will be because of the alteration in the regulatory genes; in other cases, small changes in the way these regulatory proteins interact with their target genes will be sufficient to explain an alteration in phenotype. In this way, it will be possible to identify allelic variants in germplasm that are not currently being exploited, but can contribute significantly to breeding efforts in the future.

Breeding for pests and disease resistance

A number of pests and diseases in New Zealand have been reported for kiwifruit. The value proposition of incorporating pests and disease resistance into the mainstream selection traits comes from two different sources. The first one relates to market access through the presence of pests or the presence of residues and the second relates to loss of productivity.

Scolypopa australis known as Passionvine hopper (PVH) cause losses due to sooty moulds on the skin substrate estimated at NZ$20M in the 2010 season alone (C. McKenna, pers. comm.). Control of *Latania* spp. requires three sprays per season at an average of NZ$150/ha plus monitoring and post harvest disinfestations costs. Similar costs have been reported for *Sclerotinia spp.* and *Botrytis*, while in extreme conditions up to 30% of the orchard production can be damage by leaf roller.

Over the last 5 years, the development of several bioassay techniques has enabled the screening of a range of *Actinidia* germplasm and breeding lines against several pests and diseases. This has revealed large variations in susceptibility to most of the major pests and diseases.

There is evidence that pest and disease resistance is moderately heritable (Hill *et al*. 2007). Patterns of resistance against some species (e.g. scale, leafroller) within screened *Actinidia* germplasm showed hypersensitive-like responses and variable distribution patterns of resistance. So far, there is little evidence to suggest strong cross-resistance to several pests or diseases and prioritisation based on value proposition may be needed. For example "Hort16 A" shows reasonable to strong resistance to latania scale, leafrollers and botrytis; but it is very susceptible to white peach scale, and greedy scale (G Hill *pers. comm*.). Positive genetic correlations between resistance and traditional breeding traits like Brix are encouraging, suggesting that no negative selection pressures have been applied to the breeding lines. Furthermore, divergent indirect selection pressures have been established in both main *Actinidia* species and recently established large hybrid populations are offering the prospect for successful combined selection through a very similar strategy as the one discussed above.

On November 2010 an outbreak of *Pseudomonas syringae* pv *actinidiae* (PSA), a bacterial canker, was reported in New Zealand. A similar outbreak has decimated kiwifruit orchards in Italy and in a number of countries around the world. The advent of PSA has highlighted the need to increase the speed of development of a robust strategy; the highly diverse breeding and commercial lines are offering a unique opportunity for selection for tolerance and resistance.

Genomic breeding

Various schemes and approaches to genetic improvement should be tested to make kiwifruit breeding more efficient and to strengthen overall breeding efforts in order to increase genetic gain per unit of time. Selection of superior cultivars for economically important quantitative traits is traditionally based on phenotypic records of the individual and its relatives. Recent papers (Gea *et al.* 2006; El-Kassaby and Lstiburek 2009) summarised opportunities of what they call 'breeding without breeding'. This method combines the use of genotypic or phenotypic pre-selection of superior individuals, informative DNA markers and pedigree reconstruction of offspring to assemble naturally created full and half-sib families, with the same concept applicable to cultivar selection.

The expectation is that information at the DNA level will lead to faster genetic gain than that achieved based on phenotypic data only. The availability of a sparse map of genetic markers with 644 microsatellite markers from three genetic libraries published by Plant & Food Research recently resulted in a genetic linkage map of kiwifruit (Crowhurst *et al.* 2009; Fraser *et al* 2009) with the detection of some QTLs. The map showed 29 linkage groups, representing the expression of 587 genes and revealed that sex-linked sequence characterised amplified region (SCAR) markers and the flower sex genotype mapped to a subtelomeric region that bears the hallmarks of an early sex-determining locus. As the genus *Actinidia* is dioecious, an obvious application of marker assisted selection (MAS) in a breeding programme is for gender. As only the females bear fruit, there are obvious cost-efficiencies in reducing the number of males in a breeding progeny that are planted in the research orchard, to the minimum required for effective pollination. Currently a high throughput MAS for gender takes place in our commercial programme, with several thousand seedlings tested for the sex marker every year (Gardiner *et al.* unpublished data).

This gene-rich map will be a valuable resource for quantitative trait loci analysis to identify markers related to traits of importance for novel kiwifruit cultivars (Tsang *et al.* 2007). The inclusion of marker information into Best Liner Unbiased Prediction (BLUP) of breeding values was demonstrated by Fernando and Grossman (1989) and predic-

ted to yield 8–38% extra genetic gain. However, the usefulness of information from a sparse marker map in outbreeding species like *Actinidia* is limited because the linkage phase between a marker and QTL must be established for every family in which the marker is to be used for selection. Quantitative traits are usually affected by many genes and consequently the benefit from MAS is limited by the proportion of the genetic variance explained by the QTL. Thus, the question is 'where is the most profitable use of this information in a high throughput breeding and commercial programme that focuses on fast selection of new cultivars?' Utilising Bayesian statistics, Meuwissen *et al.* (2001) concluded that by using a dense marker map covering all chromosomes, it is possible to estimate accurately the breeding value of individuals that have no phenotypic record of their own and no progeny. However, this implies a larger amount of available data from several generations that is not found yet in the *Actinidia* breeding programme. However, the theory and practicality of such a method is showing the pathway, where existing processes for progeny testing may face a fundamental transformation and may even become redundant. The current process of crossing-planting-evaluation-selection-progeny testing may actually be severely reduced and allow the total number of seedlings produced per cross to increase drastically, allowing new methodologies to take place. Whole genome selection (WGS) can be utilised to minimise the drag of undesirable genes during introgression of genes from wild populations (an opportunity for breeders to search for alternative crossing designs that can exploit and or maximise linkage disequilibrium), and to produce bridges of connectivity with existing mapping populations.

Whole Genomic Selection (WGS) or Whole Genomic Profiling (WGP) has been applied on several breeding programmes (Konig *et al.* 2009; Volz *et al.* 2009). Introgression of novel fruit traits and new resistances to known diseases has been possible through several generations of backcrossing. With WGS, allelic contributions from low and high quality grandparents can easily be checked, increasing the rate at which inferior alleles are eliminated, speeding up the breeding cycle and increasing the genetic gain per unit of time.

Genomic Selection (GS) and WGP are not possible yet with *Actinidia*, given the difficulties exposed by different ploidy levels. The allele frequency-based analysis will be biased because of the co-dominant nature of microsatellite markers (Datson *et al.* 2008). Recent work (D. Chagné pers. comm.) has been focusing on developing an assay based on the high resolution melting (HRM) technique. This is much faster and easier to perform than the gel-based assay and offers an opportunity to overcome the ploidy issue with encouraging results from di-, tetra- and hexaploid kiwifruit from *A. chinensis* and *A. deliciosa*.

Breeding for the consumer

As with all food crop research, one of the aims is to achieve a cost-effective way to continue producing convenient kiwifruit with consumer appeal. A considerable amount of research has taken place to identify the most important attributes that promote or inhibit kiwifruit consumption. Consumer beliefs, attitudes and perceptions will affect the willingness to buy kiwifruit (Jaeger *et al.* 2003, 2006; Roty and Christy 2008). The breeder is faced with the question of how to breed for traits with very low heritability or at least how to ensure that trait associations with novel flavours are not detrimentally selected in recurrent breeding programmes.

Special and unique flavours have the ability to create new niches, while novel traits can be an advantage or a disadvantage depending on the attitude of consumers (Harker *et al.* 2006). While novelty might be associated with traits of moderate to high heritability (e.g. peelability, eatable skin, shelf life, colour), flavours are usually reported to be of very low heritability and highly associated with errors on the sensory panel scores. Several techniques have been trialled to increase the accuracy for taste selections, including the use of a highly selected panel, artificial tongues, artificial noses and metabolite associations.

Efforts aimed at increasing the genetic parameter estimates of taste often encounter problems with ripening levels and confounded flavours because of different levels of sugars and acids. Tilling technology (MacRae 2006) is one of the tools that may possibly provide the most accurate way of selecting better tasting kiwifruit from among runner-up candidates, but will offer very small advantages to the breeder, since phenotyping large number of individuals from progeny tests still is an expensive proposition.

In summary, selecting cultivars is a relatively easy task, but breeding the right tasting cultivar has to date been an art associated with serendipity more than the result of a purposely built programme.

Functional food through nanotechnology and breeding

A major growth area recently has been the development of so-called 'functional foods' - nutritionally engineered foods that are marketed with nutrient or health claims. Non-traditional genetic modifications and nanotechnology provide a range of approaches to the cost-effective production of foods with modified nutrient profiles and novel traits. Consumer acceptance of cultivars purposely built by means of unconventional genetic modifications is a topic that is not covered here.

Nanotechnology commonly refers to any engineered materials, structures and systems that operate at a scale of 100 nanometres or smaller (one nanometre is one billionth of a metre) (Moraru *et al.* 2003). Nano-biotechnology refers to the use of nanotechnology to manipulate living organisms, as well as to enable the merging of biological and non-biological materials. This includes the use of nanotechnology to facilitate genetic engineering in breeding programmes, the incorporation of synthetic materials into biological organisms, and ultimately the '*creation*' of new life forms (Kuzma 2007).

Nanotechnology promises to enable the DNA of seeds to be rearranged in order to obtain different plant properties, including colour, growth season, and yield. Although at a very early stage and not yet in *Actinidia*, researchers have already succeeded in 'drilling' holes through the membrane of rice cells to enable the insertion of a nitrogen atom, to stimulate rearrangement of the rice DNA for colour changes (Gardener 2002). A range of nano-techniques and materials are being developed in an attempt to assert greater control over food character traits, and to enhance processing functionalities, such as flavour, texture, speed of processing, heat tolerance, shelf life, and the bioavailability of nutrients. The stability of these new compounds is yet to be proven, and opportunities for breeders and the potential for this new tool to expand genetic variation to new levels are still a few years in the future.

Nano-encapsulation techniques may make it possible to alter the nutritional composition, flavour and other attributes of food to match consumers' personal tastes and physiological requirements, and to utilise 'smart' food packaging able to detect the presence of pathogens. These and other applications of nanotechnology across the agri-food system are now emerging (Joseph and Morrison 2006). This new technology will certainly shape the research trajectory of breeding and genetic resource management. However, its applicability should currently be focused on functional biochips for breeding purposes, speeding up and increasing the opportunities for WGP, in order to make large data evaluations affordable, to reduce evaluation time.

CONCLUDING REMARKS

A key issue in breeding and conservation is the maintenance of genetic variance. It has been suggested that continued selection will deplete genetic variance of commercial traits,

adding fears to the success of selecting new cultivars in the future. Methods to generate inter-population variance and its subsequent release through advanced generation 'hybrids' have been discussed, and the use of small groups and multiple population management suggested as a practical and theoretically sound option to overcome practical restraints and commercial demands.

Increasing the opportunities for selecting new cultivars with traits highly associated with consumer and industry demands are the major drivers of the current strategy. In the near future, molecular and nano-tools will be able to facilitate the introgression of desirable and new traits into breeding lines, offering extra options to the management and conservation of existing genetic resources. However, structured elite breeding lines will be required to facilitate the use of those new traits across species and ploidies.

ACKNOWLEDGEMENTS

To Ross Ferguson, Alastair Currie, Garry Hill and Peter Minchin for their useful notes on earlier versions of this paper. To Paul Datson for facilitating his Poster information..

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