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Statistical Methods for Plant Breeding

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Abstract

In this chapter we highlight differences in the application of quantitative methods to plant breeding compared to their application in animals and humans. These originate from the very different and diverse mating systems, life histories and genome organisations found in plants compared to animals. In addition, it is common to replicate plant genotypes as clones, inbred lines, or F1 hybrids. This gives the breeder greater control experimentally over the precision with which genotypic values are estimated and reduces gain from incorporating information from relatives, as common in animal breeding. Plants show great plasticity in their response to environmental challenges, and genotype–environment interactions are typically larger than in animals. Consequently, there has been considerable emphasis on improving experimental design for field testing. The implementation of genomic selection and prediction in plants is becoming common, and there are opportunities for its incorporation into plant breeding programmes which differ from those in animals. Ultimately, however, the link between applications of statistical methods to plant and animal breeding remains the breeder's equation.

17.1 Introduction

Much of the current advance in statistical genetics and the resurgence of its application in plant breeding originated with work in animal and human genetics. This is particularly so for genomic prediction and selection (see **Chapter 28**), which is revolutionising the approach to animal breeding, and of genome-wide association mapping, which originated in human genetics (Bodmer, 1986; Risch and Merikangas, 1996) but has become routine for trait mapping in plants too (Huang and Han, 2014; Barabaschi *et al*., 2016). Any delay in uptake of methods of statistical genetics prevalent elsewhere is not due to ignorance or lack of skills among plant researchers. Rather, plant breeding has a proprietary set of strengths and weaknesses, often not recognised outside the field, which influence the application of statistical methods, and in some cases have resulted in the development of different and novel approaches. These are sometimes specific to individual species: there is much greater variation in genetic systems in domesticated plants than in animals, many more species are domesticated, and research tends to be divided into small communities working on each.

Handbook of Statistical Genomics, Fourth Edition. Edited by David J. Balding, Ida Moltke and John Marioni.

This chapter reviews the application of statistical and quantitative genetics to plant breeding, highlighting differences from applications in animals and humans. We start on common ground by describing the central place of the breeder's equation in plant breeding and its role in assessing and optimising programme design and selection strategy. This is followed by descriptions and consequences of complications in plant breeding arising from, in turn, the diverse breeding systems of plants, the prevalence of polyploidy, and of polymorphic genomic rearrangements. A particular feature of plants compared to most domesticated animals is their plastic response to the environment and the importance of genotype–environment interactions. Methods used by plant researchers to study and model these are presented. Genomic selection will have greater impact on animal and plant breeding than any other area of quantitative genetics in the next decade. This is discussed comprehensively in **Chapter 28**. Here we discuss eight areas in which its application in plant breeding can vary from animal breeding. These are: the use of genomic selection to accommodate genotype–environment interaction; the incorporation of major genes and quantitative trait loci (QTLs) into genomic prediction; the prediction of the merit other than the breeding or trait value of an individual; the use of genomic prediction to avoid cost in phenotyping; mate selection; the development of sequential selection schemes; the prediction of hybrid performance; and heterosis and marker imputation.

We end the chapter with a discussion of experimental design and analysis for the phenotypic assessment of new genotypes and varieties, an area in which plant breeding has a long history of innovation.

17.2 Heritability and the Breeder's Equation in Plant Breeding

For most of the major plant species, the crop in a farmer's field is a single genotype: an inbred line, an F1 hybrid, or a vegetatively propagated clone, though minor crops are often genetically heterogeneous. In contrast, animals raised by farmers are most commonly genetically distinct. This difference has influenced the development and uptake of methods. The focus of plant breeding theory has been on 'transgressive segregation', that is, identifying individuals in crosses with trait values which fall outside the range of the parents and may therefore be developed as improved varieties. Animal breeding considers genetic improvement as a process of increasing the frequency of favourable alleles in a population. The distinction is not perfect; for example, pasture grasses and population varieties of rye are often genetically heterogeneous. Although this distinction characterises a broad difference in philosophy between much of plant and animal breeding, the two approaches are merely different ways of viewing genetic progress, and the inviolable link between all breeding methods remains the 'breeder's equation' (Lush, 1943, Section 2.6),

$$R = \frac{\sigma_g \times i \times r}{L},$$

where R is the change in trait mean per unit of time, σ_g is the amount of genetic variation within the population, i is the selection intensity, r is the accuracy of selection, and L is the interval between successive cycles of selection. The accuracy of selection is the square root of heritability if selection is on an individual's phenotype, or more generally it is the correlation between the mean of the progeny and the criterion on which selection was based, which can include information from other individuals and other traits.

The breeder's equation makes explicit that there are only four ways to improve response to selection: increase accuracy of selection (i.e. increase heritability for direct selection), increase genetic variability, increase intensity of selection, and reduce the time required for a single breeding cycle. Plant breeders have more opportunities to influence this equation than animal breeders. Generation times can be reduced through out-of-season nurseries by moving plant

material between hemispheres, or for example by shuttle breeding, as practised at the International Maize and Wheat Improvement Center (CIMMYT), in which complete wheat and maize breeding programmes are moved between two regions of Mexico to achieve two generations per year (Borlaug, 2007). Controlled environment facilities are used, for example the 'speed breeding' platform (Watson *et al.*, 2018) in Australia in which up to six generations per year are possible for some crops. To date, no equivalent approach is possible routinely in animals. This may be one reason why genomic selection (GS; **Chapter 28**) has been so readily adopted in animal breeding but not in plants: although the decoupling of selection from phenotyping in GS can greatly reduce cycle time, for several crop species a cycle of selection is already fast. There is still a delay, however, often of several years, in which the number of seed or clones of a new variety must be increased before release to growers. This has led to the proposal for a two-stage selection process in which cycles of GS proceed quickly, but product development is slower (Gaynor *et al.*, 2017). This is similar to approaches based solely on phenotypic selection, developed as recurrent selection schemes (Hallauer, 1981).

Plant breeders have greater control over accuracy of selection than animal breeders. Individual genotypes (inbred lines, F1 hybrids, clones) can be replicated at any scale and tested in multiple environments, for multiple traits over several years. As a result, heritability is under the control of the breeder and can range from extremely low values to near 1 if a genotype is raised in a sufficient number of replicate plots over multiple sites and years, with each plot containing several hundred plants. In its simplest form the heritability of a replicated genotype in a single experiment laid out in complete blocks is

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2/n},$$

where σ_g^2 is the genetic variance, σ_e^2 is the residual error variance and n is the number of replicates. The heritability can be interpreted as the proportion of phenotypic variance, $\sigma_p^2 = \sigma_g^2 + \sigma_e^2/n$, that is due to genetic variance. It is also the squared correlation between the observed phenotype (the genotype's mean over replicates, corresponding to the best linear unbiased estimator (BLUE) of the genotypic value) and the unobserved genotypic value. When trials are conducted across multiple environments, the phenotypic variance also comprises variances due to genotype–environment interaction, because the 'phenotype' is a mean over environments. For a trial series replicated over years and locations, the phenotypic variance is

$$\sigma_p^2 = \sigma_g^2 + \frac{\sigma_{gy}^2}{y} + \frac{\sigma_{gl}^2}{l} + \frac{\sigma_{gyl}^2}{yl} + \frac{\sigma_e^2}{yln},$$

where subscripts *gy*, *gl* and *gyl* on the variances indicate genotype–year, genotype–site and genotype–year–site interactions, respectively. The phenotypic variance can be manipulated by the choice of trial design (here we have assumed a randomised complete block design), particularly the number of replicates per trial, n, and the number of years, y, and sites, l (Talbot, 1997).

The most common use of heritability is as a measure of accuracy of selection in the breeder's equation. Heritability is also often used as a measure of the accuracy of a trial or series of trials. It must be realised, however, that both the breeder's equation and the above simple equation for heritability are based on a number of simplifying assumptions, for example, (i) the trial design has equal replication for each genotype and the design is either a completely randomised design or a randomised complete block design; (ii) the genotypic value is identically and independently normally distributed, which precludes usage of pedigree or kinship information; (iii) only a single trait is considered and no information from other traits is involved; and (iv) all trials have the same error variance. Most of the time, at least one of these assumptions is violated and hence the definition of heritability needs to be modified (Oakey *et al.*, 2006; Piepho and Möhring,

2007). Most importantly, in many cases selection will be based on best linear unbiased prediction (BLUP) rather than BLUE of breeding values or genotypic values, and hence definitions of heritability/reliability as used in animal breeding are required (Mrode and Thompson, 2005; Cullis *et al.*, 2006).

An additional means of increasing heritability is through improved trial design and analysis. Historically, research in experimental design was initiated and driven by the requirement to reduce environmental error in large-scale field trials (Gosset, 1931; Fisher, 1935), coupled with the requirement for ease of analysis with little or no electronic assistance. With the restraint of simplicity of analysis now greatly reduced, improvements in the design of field trials continue to be made. This is an area where innovation in plant breeding can be spread elsewhere, for example in the similarity between agricultural field trials and design for microarrays (Kerr and Churchill, 2001).

17.3 The Breeding System of Plants

In contrast to the vast majority of higher animals, which are diploid and dioecious (males and females are separate individuals), flowering plants (angiosperms) have very diverse mating systems. As a consequence of their inability to change location or choose their mates, plants depend on external agents (such as animals, wind and water) to transfer gametes (pollen) between individuals. Most plants have hermaphroditic flowers, which can result in self-pollination. The presence of multiple reproductive structures results in mating systems with considerable complexity. A consequence is a more complicated distribution of gametes within and between plants in populations. Individuals can mate with themselves and with numerous related and unrelated partners (Barrett and Crowson, 2016).

It is useful to distinguish two types of breeding systems: sex systems (hermaphroditic, separate sexes and others), and the mating system of hermaphroditic populations (inbreeding, outcrossing or intermediate) (Charlesworth, 2006). The second classification, together with the possibility of asexual reproduction, is commonly used in plant breeding (Bernardo, 2010). Species that predominantly self from within-flower pollination are called autogamous, and those with predominantly between-flower pollination are described as allogamous or panmictic. Examples of asexually propagated species are sugar cane, potato, and cassava, while maize and rubber tree are allogamous and soybean, wheat and rice are autogamous. Asexually propagated species can also frequently reproduce sexually (e.g. sugar cane, strawberry).

Sexual reproduction generates variability through meiosis and recombination. For asexual reproduction, the whole genome behaves like a single linkage group, and linkage disequilibrium (LD) is total (Richards, 1996). Asexual reproduction involves two main mechanisms: vegetative reproduction through stems, tubers and other structures, and the less common production of seeds without sex, termed agamospermy or apomixy. Species such as maize and squash have populations with unisexual flowers but with male and female flowers present on the same individual (termed monoecy), preventing self-pollination within flowers and favouring outcrossing. Although not common (Renner and Ricklefs, 1995), other species, such as kiwifruit and hops, have unisexual flowers on separated individuals (dioecy). Other combinations of individuals are gynodioecious (females and hermaphrodites) and androdioecious (males and hermaphrodites). To make things even more complicated, these sex phenotypes can sometimes coexist (Barrett and Crowson, 2016).

The mating systems of flowering plants have varied extensively during their evolution, in response to changes in life history, ecology and availability of pollinators. Autogamy has evolved multiple times from outcrossing species. As a result, there is considerable variation in mating systems both within and between species, though with a predominance of hermaphroditic

sex expression (cosexuality). There are some common patterns; for example, perennial tree species with stable communities tend to be predominantly outcrossing, whereas weedy plants in ephemeral colonies tend to be selfing (Barrett and Crowson, 2016). There is an important number of species with mixed mating, a mixture of outcrossing and selfing. Cosexual individuals are not necessarily self-compatible and outcrossing rates are normally inferred using data from molecular markers (Charlesworth, 2006). In natural plant populations, a mixed reproductive strategy has evolved, with habitual selfers occasionally becoming outcrossers, and perennial plant species having some asexual reproduction (Richards, 1996).

The reproductive system influences genetic variability. Selfing leads to reproductive isolation, restricted gene flow, reduced rate of recombination and small effective population size, whereas outcrossing populations maintain high levels of diversity. Outcrossing tends to break up LD. This has positive and negative effects. It can increase the rate at which favourable coadapted alleles at linked loci are selected in coupling, building up adaptive linkage groups and reducing hitchhiking of deleterious alleles, but it also acts to break up existing favourable linkages (Richards, 1996). A population of homozygotes has half of the effective size of an outcrossing population with the same number of individuals (Charlesworth, 2006). Outcrossing species have mechanisms to prevent the occurrence of selfing or crosses between related individuals, most notably homomorphic self-incompatibility systems (Lawrence, 2000; Castric and Vekemans, 2004). When forced to inbreed, these species commonly show strong inbreeding depression. Theoretical panmixia assumes a sexual population of infinite size and random distribution of male and female gametes; this is not achieved in real populations. A common parameter used to study departures from panmixia is F, the fixation index, derived from the observed and expected frequencies of heterozygous individuals for a given locus; t, the outcrossing rate, and its complement s (selfing rate, $t = 1 - s$) are based on comparison of these frequencies.

The mode of reproduction also influences breeding strategy, the types of cultivar and the procedures used in their development. In autogamous species individuals are homozygous, and historically breeding has consisted of making crosses between divergent individuals and then making selections during several generations of selfing until near homozygous cultivars or inbred lines are produced. For allogamous species many loci are heterozygous and breeding can be by recurrent selection in populations, or by obtaining hybrids by crossing inbred lines obtained by selfing or other methods, such as double haploid production. These distinctions are becoming blurred, however; systems to create F1 hybrid cultivars in inbreeding species like barley, wheat and rice are increasingly being used commercially and the breeding processes by which inbred parents of hybrid cultivars are developed are very similar to those used to produce inbred cultivars in other species. Asexual reproduction does not increase genetic variability, but allows the cloning of superior individuals with many heterozygous loci. Schnell (1982) classified plant breeding systems broadly into the four categories described in Table 17.1, depending on the degree of heterozygosity and homogeneity within the resulting varieties, the propagation system for the variety and the importance of non-additive variation in determining yield. The difference in variety type and their heterogeneity and homozygosity are illustrated in Figure 17.1.

17.4 Polyploidy in Plants and Its Genetic Consequences

Another feature peculiar to plants is the large number of polyploid species (having more than two sets of chromosomes). Excepting some fish and amphibians, this condition is not common for animals, but it is widespread among plants, including economically important species such as potato, strawberry, wheat and sugar cane. The most common number of sets

Table 17.1 Four categories of variety (after Schnell, 1982)

	Line breeding	Population breeding	Hybrid breeding	Clonal breeding
Is dominance a major factor determining yield in resulting varieties?	no	yes	yes	yes
Are genetically homogeneous varieties feasible?	yes	no	yes	yes
Can the variety be propagated from itself?	yes	yes	no	yes
Are varieties propagated by seed?	yes	yes	yes	no

of chromosomes (the ploidy level) is four (tetraploidy), and the majority of methodological developments are for species in this category. Even numbers of chromosome sets are more common than odd numbers. Polyploids can have some problems during mitosis and meiosis, but polyploidy has been associated with a number of advantages for plants, including heterosis, gene redundancy and a tendency for asexual reproduction with apomixy (Comai, 2005).

Polyploids are classified into two categories: allopolyploids (with sets of chromosomes from different origin, e.g. wheat) and autopolyploids (having sets of chromosomes of same origin and type, e.g. potato). Meiotic pairing is different for these categories. Allopolyploids can exhibit preferential pairing between the chromosomes from the same ancestral species, resulting in what is named disomic inheritance. In practice, this implies that they will segregate as diploids, and so genetic analyses can be based on standard models for diploids. In contrast, autopolyploids typically have multisomic inheritance, where all chromosomes from the same set (homology group) can be associated in pairs in meiosis, forming bivalents. Multivalents (more than two homologous chromosomes pairing) can also be formed, and in this case recombination can take place between the locus and centromere, with sister chromatids migrating to the same pole, causing what is named double reduction, in which a gamete can be formed which contains copies of a single parental allele (Figure 17.2). Double reduction

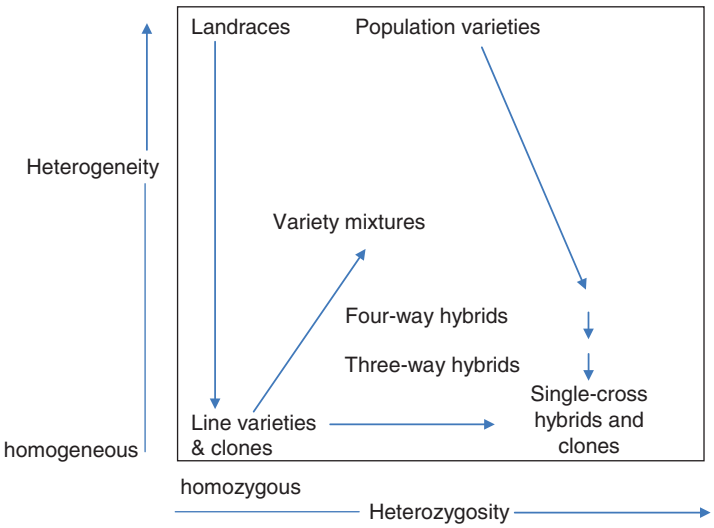

Figure 17.1 Variety types classified by their heterogeneity and heterozygosity (after Schnell, 1982).

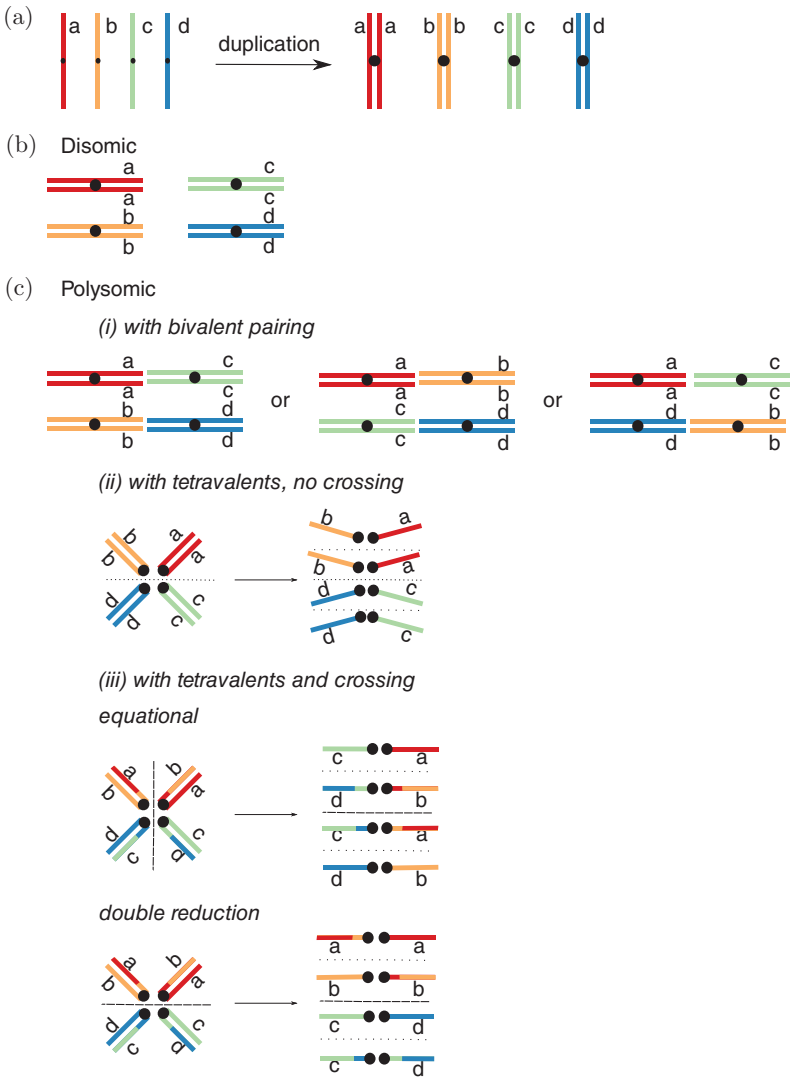

Figure 17.2 A schematic illustration of the meiotic behaviour of polyploids with four sets of chromosomes (tetraploid). (a) Four chromosomes with different alleles, showing DNA duplication for meiosis. (b) If, for example, chromosomes with allele *a* always pair with chromosomes with allele *b*, and the same happens for *c* and *d*, the inheritance is disomic and the species is an allotetraploid, behaving like a diploid. (c) With polysomic inheritance, it is possible to have bivalents and tetravalents. (i) shows all possible bivalents when there is no exclusive pairing. Notice that gametes will have allelic combinations not present in allotetraploids (*ab* and *cd*). (ii) illustrates a possible tetravalent configuration and the gametes; others are possible as well. (iii) shows that the occurrence of crossing-over between the loci and the centromere can result in gametes with two copies of the same allele when the gametes that recombine migrate to the pole of the cell during the division. Therefore, autopolyploids with multivalents can have inbreeding even without mating between relatives.

can be regarded as the result of random chromatid assortment in meiosis rather than random chromosome assortment. For example, in a tetraploid of composition *AAaa*, random chromosome assortment would produce gametes with frequencies $\frac{1}{6}$ *AA*, $\frac{2}{3}$ *Aa*, $\frac{1}{6}$ *aa*, whereas with random chromatid assortment (double reduction) the frequencies are $\frac{1}{4}$ *AA*, $\frac{1}{2}$ *Aa*, $\frac{1}{4}$ *aa*. Gamete frequencies are different for disomic and multisomic inheritance, although Mendel's

rules still apply and segregation patterns can be predicted. For example, an autohexaploid individual *AAAaaa* carries three *A* alleles. It will produce gametes with frequencies of $\frac{1}{2}$ for each allele; gametes with all three *A* alleles (*AAA*) will then have frequency $\frac{1}{8}$, since they will have independent segregation. An autohexaploid *AAAaaa* with bivalent formation will have gametes *aaa*, *Aaa*, *AAa* and *AAA*, with expected frequencies $\frac{1}{20}$, $\frac{9}{20}$, $\frac{9}{20}$ and $\frac{1}{20}$, respectively. Therefore models developed for diploids cannot be directly used for autopolyploids with polysomic inheritance. Another complication arises from the possibility of populations that can deviate from exclusive disomic or polysomic inheritance.

Even with the enormous contemporary advances in genotyping and sequencing technologies, polyploids are lagging behind diploids in terms of available genomic information, mainly because of the technical and methodological challenges caused by their complexity. One of the most important of these is the difficulty in resolving the allelic dosage of individual loci (Dufresne *et al.*, 2014). For example, it is important to distinguish an *AAAAaa* individual from *AAAaaa* (for di-allelic loci), or $A_1A_2A_2A_3$ from $A_1A_2A_3A_4$ (for multi-allelic loci). It is difficult to distinguish fully heterozygous individuals (all alleles are different) from partial heterozygotes (at least one allele has two or more copies). Traditional markers such as microsatellites have codominant behaviour only when ploidy level is four or less. SNPs, although abundant in the genome and very promising for studies in polyploids (Garcia *et al.*, 2013), are di-allelic and so not fully informative. There are statistical methods to infer the genotype of polyploids (for example, Voorrips *et al.*, 2011; Serang *et al.*, 2012; Bourke *et al.*, 2018; Gerard *et al.*, 2018), but further advances are needed, because the consequences of assumptions made by these methods demand detailed verification.

A detailed review of the population genetics of polyploids can be found in Dufresne *et al.* (2014). Using allelic and genotypic frequencies, the same principles developed for diploids can be used in principle, but the presence of polysomic inheritance adds complexity. There are more genotypic categories, since the allele dosage varies from zero up to the ploidy level. In the absence of double reduction, expected genotype frequencies under Hardy–Weinberg equilibrium are given by the expansion of $(p_1A_1 + p_2A_2 + p_3A_3 + \cdots + p_kA_k)^n$ for a locus with alleles A_i, corresponding frequencies $p_i (i = 1, 2, \ldots, k)$ and ploidy level n. On expansion, $A_2A_3^3$ would, for example, represent the tetraploid genotype $A_2A_3A_3A_3$. Equilibrium frequencies are reached slowly in comparison to diploids. Double reduction adds further complexity, and raises the expected frequencies of homozygous genotypes. *F*-statistics used for measuring population differentiation (Weir, 1996) can be adapted to polyploids, but with difficulties caused by uncertainty in estimation of allelic dosage. The same problems affect methods for studying population structure, exacerbated by the tendency of many polyploids to reproduce asexually. Model-free methods (such as principal components analysis) can avoid these problems.

Measuring genetic relatedness for autopolyploids, based on pedigrees or molecular data, demands a number of modifications. In diploids, it is possible to calculate the probabilities that two individuals share 0, 1 or 2 alleles identical by descent (IBD) within a given pedigree, which requires consideration of nine possible IBD configurations among the four alleles. For autotetraploids, autohexaploids and autooctoploids, the corresponding numbers of configurations are 109, 1043 and 8405, respectively (Huang *et al.*, 2015). Since marker systems cannot reveal all possible alleles when the ploidy level is higher than four, measures of relatedness for autopolyploids with high ploidy level suffer from incomplete information, although haplotype prediction might help. Genotype ambiguity, the necessity to separate identity in state from IBD, and the presence of double reduction bring yet another layer of complexity. There are methods and software for estimating relatedness from pedigree and molecular data (Kerr *et al.*, 2012; Huang *et al.*, 2015; Amadeu *et al.*, 2016), but there is room for improvement and this will be the subject of research in upcoming years.

For polyploids, building linkage maps, mapping QTLs in biparental crosses or through association mapping, and performing genomic selection are not as advanced as for diploids, due to the complications mentioned above. For tetraploids, Voorrips *et al.* (2011) developed a method based on fitting mixture models with five components to call genotypes for di-allelic markers (allelic dosages). Schmitz Carley *et al.* (2017) developed a clustering method for the same purpose. A similar method has been developed to call SNPs in crosses of F1 offspring of two autotetraploid parents, prior to creating a linkage map and QTL detection (Hackett *et al.*, 2013). For more complex polyploids, Serang *et al.* (2012) have developed a graphical Bayesian model for genotyping which has been applied successfully to sugarcane (Garcia *et al.*, 2013). The decay of LD is also slower in autopolyploids, since alleles in different homology groups have the opportunity to recombine only when they pair in meiosis. Asexual reproduction is also common in these species, which can lead to large LD blocks. Estimating LD also depends on inferring the correct genotypes. Mapping approaches for polyploids (Hackett *et al.*, 2017; Grandke *et al.*, 2017; Bourke *et al.*, 2018) assume that the estimated allelic dosages used as input, usually inferred from finite mixture models fitted to quantitative data (Voorrips *et al.*, 2011), are error-free, which they are not. There is room for improvement by developing methods that can take allelic dosage uncertainty into account (Piepho, 2001).

17.5 Genomic Rearrangements in Plants

Many plant species tolerate gross genomic rearrangements. In part, this is due to recent allopolyploid and autopolyploid ancestry of many species. Large tracts of genome, including whole chromosomes, can often be deleted without noticeable disadvantage. Cultivated sugar cane, for example, is a cross between *Saccharum officinarum* and *S. spontaneum*, and clonal varieties will typically have between 100 and 120 chromosomes. Given the magnitude of variation in plants, the concept of the pangenome, the total set of genes present in a species, is becoming of increasing practical importance (Morgante *et al.*, 2007; Hurgobin and Edwards, 2017). In maize, the reference genome of line B73 was estimated to represent only 70% of the pangenome (Gore *et al.*, 2009).

Historically, this tolerance of chromosome loss and rearrangements has enabled deletion mapping and substitution mapping. For example, in wheat, whole chromosome deletion and substitution stocks were developed through classical cytogenetics and used to locate trait effects to individual chromosomes (Law *et al.*, 1987). More recently, stocks with smaller overlapping chromosome tracts have been used in bin mapping to locate markers and QTLs to shorter deleted tracts of chromosome (Endo and Gill, 1996). At the final extreme, polyploids are more tolerant of mutations, permitting the development of stocks in which an individual plant may carry very many induced mutations. Targeting Induced Local Lesions in Genomes (TILLING: McCallum *et al.*, 2000) is a method that creates mutagenised populations carrying very high numbers of mutations per line; over 5000 per line in hexaploid wheat, and 23–24 missense and truncation alleles per gene across the population (Uauy, 2017). This gives a very high probability of detecting a desired knockout in a specific gene or of identifying a desired mutant phenotype by screening a modest number of lines. In both cases, however, a backcrossing or crossing programme may be required to isolate a mutant or to combine mutations in homoeologous genes. For targeting individual loci, gene editing (Song *et al.*, 2016) may soon supersede tilling, but the very large number of mutations carried by lines in tilling populations will remain an advantage.

More problematically, this great variability in the genome makes the creation of genetic maps and subsequent QTL mapping more complex. In a population derived from a cross between two inbred lines, chromosome rearrangements cause fewer problems: they may result

in segregation distortion or in large non-recombining regions, but genetic maps can be created without problem. However, other populations may have quite different maps. A practical result is that trait mapping in plants is usually accompanied by the creation of a genetic map *de novo* for each population. In humans, in contrast, a single genetic map has generally been used across multiple studies, for example that based on CEPH families (Dib *et al*. 1996). The more recent uptake of multi-founder populations such as NAM (Yu *et al*., 2008; McMullen *et al*., 2009), MAGIC (Cavanagh *et al*., 2008; Kover *et al*., 2009) and AMPRIL (Huang *et al*., 2011) and QTL mapping across multiple populations (Blanc *et al*., 2006) has increased the problem since the effects of suppression of recombination and viability distortion are not uniform across all recombinant individuals. There remains limited software to create consensus genetic maps in these populations (Stam, 1993; Huang and George, 2011). Shah *et al*. (2014) developed a method that accounts for segregation distortion among tens of thousands of markers in a single linkage group and which can result in a considerable reduction in map length in the presence of a segregating tract of alien chromosome. However, problems remain, as evidenced by the extended map length of most consensus genetic maps compared to that typical of biparental crosses. The presence of high marker density and segregation distortion can be used as a method of detecting new chromosome rearrangements, however, though these should also be confirmed cytogenetically.

17.6 Genetic Architecture of Traits in Plants

The major plant species were domesticated ~10,000 years BP (Doebley *et al*., 2006), though the process was protracted and may have started much earlier (Allaby *et al*., 2017). There is a domestication syndrome of genetic changes, which are common across several crops, most notably for those grown for seed. These changes include loss of seed dispersal mechanisms, loss of seed dormancy, increase in seed or fruit size and changes in photoperiod response (Meyer and Purugganan, 2013). Some species have been domesticated much more recently, for example sugar beet within the last 250 years and oil palm which remains very close to the wild form. Other species have come into cultivation through mimicry of previously domesticated species; for example, 'false flax' (*Camelina sativa* subsp. *linicola*), and rye as a mimic of wheat and barley. The population bottleneck of domestication resulted in a major loss of genetic variation in all species, with additional loss occurring with the advent of scientific plant breeding and subsequent very small effective population sizes. Effective population sizes (N_e) among collections of elite cultivars are commonly regarded as low, though we know of only one published, based on 30 microsatellite loci: the wild ancestor of durum wheat has $N_e = 32{,}500$, falling to 6000 for landraces and 1300 among recent improved varieties (Thuillet *et al*., 2005).

An interesting case is that of bread wheat. This came into cultivation from crosses between an allotetraploid and a diploid ancestral species to create a new allohexaploid. Few diploid individuals must have been involved in this process as the genetic diversity of the ancestral diploid genome within hexaploid bread wheat is much lower than that of the tetraploid genome (Akhunov *et al*., 2010). Maize is the most studied crop. It was domesticated from its wild form, teosinte, about 9000 years ago and bears less resemblance to its ancestor than any other edible plant (Kingsbury, 2009). Much of this difference maps to only six major domestication loci (Doebley *et al*., 1990) though Hufford *et al*. (2012) detected 484 genomic regions with strong evidence of selection during domestication.

Due to small effective population sizes, LD decays at a slower rate in domesticated plants than in most domesticated animals. As a result, genome-wide association study (GWAS) panels can in principle be smaller than in animals and humans yet maintain adequate power, though without the precision possible in humans and animals. However, creating GWAS panels of even

a few hundred elite lines for many crops is difficult: there may not be that number in existence. The use of breeders' early generation lines could augment the number, but in general, to increase size, lines must be included from a greater geographical area or chronological range, though both these processes introduce population structure which acts to reduce power. There is also an additional risk that QTLs are identified that are not relevant to the target environment or for which the favourable allele is already fixed. Consequently, there is a considerable danger that GWASs in crops are too small and are underpowered, leading to many false positives (MacArthur, 2012). Unfortunately, replication studies in plant genetics are rare, though they have been firmly advocated (Ingvarsson and Street, 2011). QTL mapping in biparental populations has already gained a poor reputation (Bernardo, 2008) and there is a risk that GWASs in plants will also be viewed as lacking utility unless the problem of inadequate power and the consequent need for replication studies becomes more widely appreciated.

Much of the genetic improvement seen in plants is attributable to a small number of major genes. Common examples are major genes for dwarf plant habit introduced into cereals during the Green Revolution of the 1960s and 1970s, genes for photoperiod sensitivity in several crops, and genes for disease resistance. The importance of major genes in plant breeding is greater than in animals. In many cases the variant responsible for domestication is known to be present in the ancestral species, but in others the responsible mutations have arisen post-domestication (Meyer and Purugganan, 2013). For highly polygenic traits, such as yield, knowledge of the origins of the variants responsible for the phenomenal improvements that have been made would assist in plant conservation strategies and knowledge of how best to exploit variation stored in genebanks.

17.7 Response to the Environment and Plasticity

Genotype–environment interaction (G × E) in crops is of greater importance than in animals and humans. Plants cannot avoid environmental challenge by moving; they must respond through phenotypic plasticity. While plants are homeostatic to some extent, for example they are able to regulate their gas and water supply, this is not on the same scale as in mammals. Genetic variation in response to environmental challenges, is therefore important. The G × E variance component is often larger than that for genetic variance for traits like yield (Talbot 1997; Laidig *et al.*, 2008).

Philosophically, G × E can be treated either as a source of noise which reduces heritability and hence response to selection; or as a manifestation of variation in the adaptation of genotypes to the environment, the drivers of which should be identified and incorporated into selection decisions and variety recommendations. Analytical approaches are available to address each of these objectives. For the first, partitioning trait variance into components for G and G × E, which may be further partitioned into terms for G × sites, G × years and G × sites × years, allows optimisation of testing programmes to maximise average response to selection across all environments. Of these components, G × sites is largely reproducible whereas the other two components are not (Annicchiarico, 1997). By treating the same trait scored in different environments as separate, classical multi-trait selection indices can be built to maximise response to selection in any individual environment (Curnow, 1988; Piepho & Möhring, 2005). Using information on all sites, the best estimate of the genetic value g_{ij} of variety i at site j can be expressed as a selection index given by

$$\hat{g}_{ij} = b_1 y_{i1} + b_2 y_{i2} + \cdots + b_m y_{im},$$

where $\hat{g}$ is a weighted mean of the m individual site means (y) and the b are regression coefficients, estimated as

$$\mathbf{b} = \mathbf{aGP}^{-1},$$

where **G** and **P** are the genetic and phenotypic variance–covariance matrices of variety means across sites. Generally, the covariance terms, but not the variances, of **P** are the same as **G** (since usually the only cause of correlation between sites is genetic). The vector **a** is the 'economic value' of each site, in the terminology of selection indices. Here **a** would be 1 for the site of interest and 0 for all other sites. However, rather than focusing on individual sites, breeders usually select for a target population of environments, TPE (Comstock & Moll, 1963). If the TPE can be divided into sub-regions, the selection index can be set up for a specific sub-region, considering the phenotypic means in each of the sub-regions as different traits (Atlin *et al*., 2000; Piepho & Möhring, 2005).

To identify individual drivers of adaptation, traits scored in each environment can be regressed on quantitative and qualitative measures of environment using factorial regression (Malosetti *et al*., 2013). Environments may be controlled, allowing specific components of the environmental to be varied, or uncontrolled. In the latter case, which typically involves experiments conducted over multiple locations and years, there is a degree of luck as to how large the environmental variance turns out to be and what are the drivers of that variation (e.g. rainfall, temperature). Until recently, data on environmental variables has been hard to collect, but with the advent of cheap and frequent means of recording data such as rainfall, temperature and light intensity, the opportunity to model G × E as a function of underlying environmental measurements has improved.

Historically, the absence of adequate environmental data and limited understanding of the physiology of adaptation and plasticity in most crops favoured approaches to quantifying G × E that used trait data alone. The simplest model for a set of varieties grown in multiple environments is

$$y_{ij} = m + g_i + e_j + (ge)_{ij},$$

where y_{ij} is the trait value (henceforth yield) of the ith variety in the jth environment, m is the mean, g_i is the effect of the ith variety, e_j the effect of the jth environment and $(ge)_{ij}$ is the interaction term (here we ignore within-environment error for simplicity).

The $(ge)_{ij}$ terms can be further modelled and interpreted. The simplest approach is to treat $\sum_j (ge)_{ij}^2$ for each variety as a measure of stability. This is the ecovalence (Wricke, 1962). High values indicate varieties which are sensitive (or responsive) to changes in environment. The ecovalence can be partitioned into a component that is linearly dependent on e_j and a remainder as popularised by Finlay and Wilkinson (1963) and first proposed by Yates and Cochran (1938). The model is

$$y_{ij} = m + g_i + b_i e_j + d_{ij}.$$

The linear regression coefficient b_i, with an expected value of 1, is a measure of stability or sensitivity. This model is nonlinear due to the multiplicative term $b_i e_j$ and there are complications in its fitting, but the approximate methods in common use work well (Bulmer, 1980). The interpretation of the regression coefficients is subjective. A coefficient $b_i > 1$ indicates a responsive variety that performs better at high yielding sites or a sensitive variety whose performance falls away at poor sites. Equally, a coefficient $b_i < 1$ could be a stable variety (good) or an unresponsive variety (bad). In practice, varieties must be judged by both g_i and b_j. The remainders, d_{ij}, can be squared and summed over environments for each variety and are also a measure of stability. Early work (Mather and Jinks, 2013) established that g_i and b_j could be selected for independently. More recently, g_i and b_j have been treated as traits and mapped. For example, using the maize NAM population (McMullen *et al*., 2009), QTLs for these were found to be largely non-overlapping (Kusmec *et al*., 2017).

Singular value decomposition of the $(ge)_{ij}$ matrix is now more common than Finlay–Wilkinson regression to summarise G × E. Here, the $(ge)_{ij}$ terms are decomposed into one or more (most frequently two) genotype and environmental scores and their respective eigenvalues. The model, generally referred to as AMMI (additive main effects, multiplicative interaction; Gauch, 2013), is

$$y_{ij} = m + g_i + e_j + \sum_{n=1}^{N} u_{ni} w_n v_{nj} + d_{ij},$$

where n indexes the first N dimensions of the decomposition of $(ge)_{ij}$, $\mathbf{u}_n = (u_{n1}, u_{n2}, \ldots)$ is the nth eigenvector of genotype scores, $\mathbf{v}_n = (v_{n1}, v_{n2}, \ldots)$ is the nth eigenvector for scores for environments, w_n is the nth singular value, and d_{ij} is the residual G × E terms not accounted for by inclusion of the first N dimensions.

The first two **u** and **v** terms are usually depicted in biplots, and clustering within and between genotypes and environments can sometimes be interpreted in terms of underlying genetic relationships and environmental variables (Kempton, 1984). The d_{ij} terms, squared and summed across environments for each variety, can also be considered as measures of stability. The AMMI model has also been advocated for use in animal breeding (Meyer, 2009).

As described earlier, the **u** scores can also be used in QTL analyses (Romagosa *et al.*, 1996; Rodrigues *et al.*, 2015).

AMMI has been developed further. Firstly, rather than decomposing the matrix of $(ge)_{ij}$, the matrix of $g_i + (ge)_{ij}$ can be decomposed, which is called a GGE analysis. This may provide a better visual summary and easier interpretation of the performance of each line at each site (Yan, 2014).

Secondly, the AMMI model is suitable for fixed effects only, which is restrictive in many plant breeding contexts and the standard method of fitting also requires a balanced data set (but see Gauch and Zobel, 1990; Paderewski and Rodriguez, 2014). AMMI has been extended in a mixed model framework in which either genotypes or environments are treated as random effects, giving rise to factor-analytic variance–covariance structures for genotype–environment effects (Piepho, 1998; Smith *et al.*, 2001), and QTL and environmental covariates are incorporated (Boer *et al.*, 2007; Crossa, 2013). These models are also directly applicable to animal breeding (Meyer, 2009). Factor-analytic variance–covariance structures can be viewed as low-rank approximations to unstructured variance–covariance matrices. At the same time, these models provide more flexibility than simple random effects analysis-of-variance models. For example, if the effects g_i and $(ge)_{ij}$ are modelled as independent with constant variances σ^2_g and σ^2_{ge}, the random vector $y_i = (y_{i1}, y_{i2}, \ldots)$, comprising all responses of the ith genotype, has variance–covariance structure with variances $\sigma^2_g + \sigma^2_{ge}$ on all diagonal positions and covariances σ^2_g on all off-diagonal positions. This 'compound symmetry' structure is not usually a realistic model, however, as genetic variance may depend on the environment, giving rise to heterogeneity of variance, and covariances between environments may depend on the pair of environment being considered. It is therefore natural to assume a completely unstructured model for the variance of y_i, but this comes at the cost of a drastic proliferation of the number of parameters, as each environment has its own variance parameter and each pair of environments has its own covariance parameter. Factor-analytic models cover the range between these two extremes and allow the right balance to be struck between parsimony on the one side and model realism on the other (Gauch, 1992). For example, a factor-analytic model with a single factor (akin to Finlay–Wilkinson regression) assumes a variance of $\lambda^2_j + \sigma^2_j$ for the jth environment and a covariance of $\lambda_j \lambda_h$ for environments j and h, thus allowing for heterogeneity of both variances and covariances. Adding more factors (λ) further increases flexibility, but this needs to be balanced against

the cost of extra parameters to be estimated. Using model selection criteria such as the Akaike information criterion helps to identify a factor-analytic structure of sufficient complexity.

The availability of more detailed environmental measures, routine genotyping and better statistical techniques is changing the analysis of G × E from a descriptive to a predictive process, at least for interaction of G with locations rather than across years (Jarquín *et al.*, 2014). Nevertheless, the greatest cause of variability in yields in crops is variation in the environment, and the major cause of this, the weather, cannot be controlled. Improved long-range weather forecasts would help growers avoid some problems through better planning, including selecting varieties to match predicted conditions. Better long-range weather forecasting is important for many reasons and progress is being made (Scaife *et al.*, 2014; Ossó *et al.*, 2017).

In plants, variation between repeating units within a single plant, or between plants of the same genotype, has a long history of being treated as a phenotype. One of the benefits of hybrid cultivars over inbred cultivars has long been recognised as the uniformity of the product – particularly important in vegetable breeding. This can be due to increased repeatability of a trait within individuals, such as fruit size or leaf veining, or reduced phenotypic response to micro-environment variability in the same field or glasshouse. This accords with Lerner's (1954) proposal that increased stability was associated with high heterozygosity. Although experimental evidence generally shows F1s to be less variable than their parents (Oettler *et al.*, 2005), residual heterozygosity among the inbred parents accompanied by dominant gene action of the trait can generate the same effect (Lynch and Walsh, 1997). In principle, developmental stability should be more easily and accurately measured and modelled in plants than in animals, because of the wider availability of genotypes fixed as clones, doubled haploids, fully inbred lines or F1 hybrids which can be replicated to give any desired level of precision. In practice, most work has been on animals, at least partly driven by greater interest in evolutionary aspects of homeostasis and canalisation. There is a broad distinction between response to the environment in plants compared to animals. In animals the essential body plan is laid down during embryogenesis, with scope for some subsequent physiological and behavioural adaptation to the environment. In plants, development is much less fixed at embryogenesis and there is greater opportunity for a plastic response, to environmental challenges and opportunities, during subsequent growth and development (Leyser, 2011).

At their simplest, methods of trait mapping and modelling for plasticity could treat plant to plant variation among replicated genotypes as a trait and map the standard deviation or the coefficient of variation directly. However, since trait means and variances are often correlated, and variances are not themselves normally distributed, this can cause false positives and confusion over interpretation. Ordas *et al.* (2008) mapped QTLs for several morphological traits in a maize biparental mapping population by comparing variances within homozygous marker classes using an *F*-statistic and assessing significance through a permutation test. Methods for study of environmental variation and for detecting loci affecting phenotypic variability have been reviewed by Hill and Mulder (2010) and Rönnegård and Valdar (2012) who had previously introduced a method (Rönnegård and Valdar, 2011) to detect QTLs that affect trait mean and/or variance simultaneously based on a double generalised linear model.

17.8 Genomic Selection

17.8.1 Genotype–Environment Interaction

Other than for multinational organisations working on the major crop species, it is difficult for breeding programmes to switch wholly to genomic selection (Hickey *et al.*, 2017). However,

the incorporation of genomic prediction into the later stages of cultivar testing and release is more feasible. Multi-environment trial series in most crop breeding programmes are based on a rolling process of between two and five seasons (for annual crops), in which candidate varieties are first tested in a single year at only one or two locations, with those selected for retesting in subsequent years tested at an increasing number of locations. Varieties can be dropped at any stage of testing if, for new varieties, they offer no improvement or, for old varieties, they are outclassed. The optimisation of such sequential testing processes has a long history in plant breeding (Curnow, 1961; Patterson and Silvey, 1980). Candidate varieties entering the trial series are commonly closely related to each other (as full sibs or half sibs) and to the varieties that have been in trial for several years (as parent–offspring and grandparent–offspring). These close relationships enable genomic prediction with relatively small numbers of training population individuals and markers. The prediction of the performance of new candidates from the historical trials data may save a season of testing or be used to increase precision. Since the training population will have been tested over several seasons, predictions are buffered against large variety–season interactions. Year-to-year correlations in phenotypic performance are often low, and selection on predicted performance can be more accurate than selecting on results from a single year of testing. A small number of candidates can therefore be selected first on predicted performance for inclusion in trials at a greater number of locations. Similarly, the number of locations at which a variety needs to be tested can be reduced, with loss of precision compensated for by incorporating information from relatives. The incorporation of genomic prediction into multi-environment trial series is an area of much current research (Malosetti *et al.*, 2016; Oakey *et al.*, 2016; Crossa *et al.*, 2017).

The size of the training set is one of the key determinants of accuracy in genomic prediction. Enhancing the training set size in breeding programmes requires integrating data across multiple years and cycles, and such integration has been demonstrated to improve predictive accuracy (Auinger *et al.*, 2016). For many annual crop species, the same genotype will not be phenotyped in more than one year, as segregation progresses from year to year. Thus, a major challenge is to suitably connect phenotypic data across trials with little or no connectivity and to disentangle genomic estimates of breeding values (GEBVs) from genotype–year interaction effects. Whereas there may be little replication across years for genotypes in a breeding programme, there is always ample replication at the level of alleles, and this can be exploited by modelling both GEBV and genotype–year interaction using marker information, thus permitting these effects to be dissected (Bernal-Vasquez *et al.*, 2017). However, Brandariz and Bernardo (2018) showed empirically in maize that if the training population for the next generation is a selected set of lines from the current cycle of selection, then response to selection and prediction accuracy are considerably reduced compared to an equal sized unselected set of lines. Inclusion of a small number of lines with poor performance in the training population compensated for this loss. In their examples, training population sizes (after selection) ranged from 224 to 2543, with restoration of response coming from the inclusion of the five lines with the poorest response.

Predictive accuracy in genomic selection can be enhanced by making use of biological knowledge as available, for example, in the form of crop growth models (Bustos-Korts *et al.*, 2013). One option is to introduce genetic variation in component traits as modelled by markers into the crop growth model, thus obtaining predictions for the target trait via genomic prediction for the component traits (Cooper *et al.*, 2016; Messina *et al.*, 2018). The approach can be implemented using approximate Bayesian computation or linear mixed models (Bustos-Korts *et al.*, 2013).

17.8.2 Quantitative Trait Loci and Major Genes

For the majority of domesticated plants, biparental mapping populations are easily created and QTLs are routinely detected. Unfortunately, this has not been effectively translated into marker-assisted breeding programmes except for a small number of large and consistent effects (Bernardo, 2008, 2016), often responsible for major gene disease resistance or major changes in plant phenology. However, genomic selection (**Chapter 28**, this volume) will become routine in most crops as the appropriate infrastructure is developed and there is better understanding of how best to incorporate it into breeding programmes. Although this will overcome the historical restriction of marker-assisted selection to genes of major effect, the major loci already detected should not be discarded nor simply included as part of a genome-wide marker set. In these circumstances, use of genomic best linear unbiased prediction (GBLUP, in which DNA-based estimates of the relationship among individuals are incorporated into the prediction of trait values), which commonly works well and gives accuracies close to those of more sophisticated methods (Heslot *et al.*, 2012; Ogutu *et al.*, 2012), can perform poorly. For a hypothetical quantitative trait where the majority of the genetic variation is determined by a single major gene, inclusion of increasing numbers of genome-wide markers could reduce rather than increase accuracy as the effect of the major gene is over-penalised. The simplest means of accommodating known QTLs is to include them as fixed effects in the prediction model. This works well provided each individually accounts for less than 10% of the genetic variance (Bernardo, 2014a). Alternatively, known QTLs can be penalised independently of the genome-wide marker set. More sophisticated approaches from the Bayesian alphabet (Juevas *et al.*, 2017) may work better in these circumstances, as will methods that take into account prior knowledge that specific subsets of markers are likely to behave differently; for example, MultiBLUP (Speed and Balding, 2014) in which multiple sets of markers can be penalised independently. Classes of markers could include candidate genes, markers tagging known functional genes or QTLs, and separate classes for each genome in allopolyploid species. With only two classes of predictors, standard software for ridge regression can be used by scaling the variances of markers in the two sets independently, then maximising cross validation accuracy for the variance scaling factor. For example, 10 probable QTLs were penalised independently of a background set of 3046 makers to predict seven traits in 376 wheat varieties with improvements in cross-validation accuracy made using standard ridge regression software (Bentley *et al.*, 2014).

Genomic selection will not replace all forms of marker-assisted selection in plants. There remain traits and objectives where tracking one or a few major genes is more efficient. Successes of marker-assisted backcrossing are described by Hospital (2009) who also reports this to be routine but unpublished in the private sector. There are also many reported successes for marker-assisted gene pyramiding to fix multiple QTLs in one line or variety (Hospital, 2009), most notably of disease resistance genes. The advantage of stacking multiple QTL for disease resistance is that the pathogen must evolve to overcome two or more host resistance loci, which outcome is less likely than overcoming a single resistance locus. Pyramiding of disease resistance cannot easily be accomplished through phenotypic selection alone, since increasing dosages of favourable alleles cannot be distinguished phenotypically: this is duplicate epistasis in which a favourable genotype at one locus masks the effect of favourable genotypes at others. The probability of successful pyramiding depends on recombination fractions between loci, number of generations of crossing, and population sizes in each generation (Servin *et al.*, 2004). The optimum strategy will depend on cost and time. In addition, each QTL must be accurately tagged by a marker or by flanking markers. Identification of multiple QTLs by association mapping in populations of elite lines could provide candidates for immediate gene pyramiding in the same genetic background. This approach might complete with GS for traits such as disease

resistances in many species, in which a large proportion of the variation is determined by a modest number of loci. We are not aware that this has been tested.

Although a consensus is emerging in animal breeding that GBLUP predicts GEBVs with accuracies close to those of more sophisticated methods, and with benefits in ease of implementation and speed of analysis, other methods may be more appropriate for plants. This is particularly so if the training population and the candidates for selection are members of a population with little genetic structure. An extreme example of this would be an F2, or lines derived from an F2, but populations undergoing recurrent selection with little or no immigration are also common. In these cases there is relatively little variation in kinship among individuals, and so GBLUP, which is known to strongly exploit variation in kinship (Habier *et al*., 2007; Hayes *et al*., 2009), is less likely to perform well. Methods such as the LASSO and Bayesian LASSO can work better (Heslot *et al*., 2012; Pasanen *et al*., 2015). Empirical testing of methods in these circumstances has been limited, partly because of the absence of suitable datasets.

17.8.3 Genomic Selection and Cross Prediction

For plant breeders, rather than directly selecting on GEBV, methods to predict the probability of transgressive segregation in the descendants of an individual would be attractive. These are, in effect, genomically updated versions of older methods to predict probabilities of transgressive segregation from estimates of the mean and variance of crosses (Jinks and Pooni, 1976). To date, this has received little attention in comparison with approaches to predict GEBVs. A simple method is to simulate progeny from a cross and predict the GEBVs of the simulated individuals. The distributions of the simulated crosses are then used as selection criteria (Bernardo, 2014a; Tiede *et al*., 2015). A similar approach is to select on the optimal haploid value: the predicted performance of the best doubled line which an individual could produce (Daetwyler *et al*., 2015).

17.8.4 Genomic Selection and Phenotyping Cost

A major use of genomic prediction in plants is to reduce the cost of phenotyping in multi-environment series of trials. These are generally used in the later stages of a breeding programme to identify lines for release to growers, are expensive to run and require investment in seed production (or clonal propagation) to generate enough individuals to test. Substituting GEBVs for direct phenotyping eliminates the requirement for all individuals to be tested at all sites (Heffner *et al*., 2009), which reduces the scale of trials and also requires less seed. Alternatively, the number of lines tested could be increased for the same cost of phenotyping. Optimal designs for multi-environment trial series are required. This approach is likely to be integrated with the improved modelling of G × E described earlier.

17.8.5 Mate Selection

The intensity of selection in a breeding programme can be restricted by concern over of loss of variation due to small effective population size, or equivalently for outbreeding species by concerns about inbreeding. One way of mitigating the adverse effects of small population size is to move away from simple truncation selection, in which each selected individual has an equal probability of contributing to the next generation (or equal contributions are forced through controlled mating). Schemes can be found which, for the same intensity of selection as truncation selection, have a greater effective population size or (equivalently) rate inbreeding per generation. Most research in this area has focused on animal breeding and is described as

optimal contribution theory (Woolliams *et al.*, 2015). In inbreeding species there has been little interest in these methods, since the equivalent of truncation selection in a closed population is rarely applied, though it has been considered in plants, most notably in tree breeding (Lindgren and Mullin, 1997). Optimal contribution theory has received new impetus from the adoption of genomic selection by animal breeders and accompanying concerns about accelerated rates of inbreeding (Woolliams *et al.*, 2015). As genomic selection is adapted to plant breeding and schemes of rapid population cycling in closed populations are incorporated, including for inbreeding species, application of optimal contribution theory and related methods will become more important (Hickey *et al.*, 2017; Lin *et al.*, 2017).

17.8.6 Sequential Selection

Substitution of GEBVs for direct phenotyping can be done sequentially. First, a small number of individuals from a population are genotyped and phenotyped. Then, a second batch of individuals is genotyped, selection made on GEBVs, and the selections phenotyped. The phenotyped individuals are used to augment the training population and the process is repeated on the next batch. The process stops when a target trait value is reached or a predefined number of individuals tested. This approach is most suitable for traits such as brewing quality in barley, or bread-making quality in wheat, which are expensive, but relatively quick, to measure (assuming seed is available). Tanaka and Iwata (2018) proposed this system as a means of reducing the phenotyping required to screen germplasm collections. They proposed that, rather than selecting on GEBVs alone, selection should be on the probability that an untested individual is better than any with known phenotype. This requires the GEBV of the individual and an estimate of the variance of that GEBV; the probability of improvement can be higher for an individual with higher variance but lower GEBV than another. In practice, this translates into giving additional weight to individuals with lower kinship to others in the current training set.

17.8.7 Genomic Prediction of Hybrid Performance and Heterosis

Hybrid breeding to exploit heterosis is typically based on the development of parental lines in different heterotic pools. A heterotic pool is a set of lines or individuals which tend to show similar levels of heterosis when crossed to members of other pools. The number of possible crosses is usually very large, often too large to permit field-testing of all possible hybrids. Thus, prediction of hybrids based on a subset of tested hybrids is a very promising strategy. This has been pioneered by Bernardo (1992) in maize, first making use of pedigree data for the parental inbred lines and derived matrices of coancestry coefficients, which can be used to model random effects for general combining ability (g.c.a., the average effect of a line in hybrid combination) and specific combining ability (s.c.a., the deviation in effect of a cross from that predicted by the parental g.c.a.). Later, the pedigree-based matrices were replaced by restriction fragment length polymorphism marker-based equivalents (Bernardo, 1994), employing BLUP for prediction. Schrag *et al.* (2010) used amplified fragment length polymorphism and simple sequence repeat markers for predicting hybrid performance in a large unbalanced multi-site, multi-year data set and demonstrated that BLUP outperforms simpler regression-based approaches. Maenhout *et al.* (2010) showed that support vector machine regression is a viable alternative to BLUP for hybrid prediction. Predictive performance can be substantially enhanced by using omics data (Xu *et al.*, 2012; Riedelsheimer *et al.*, 2012), which are closer physiologically to the quantitative traits of interest than genomic markers. There is by now ample evidence from genomic prediction studies that heterosis is governed by additive, (over-)dominance and epistatic effects, but as yet no consensus has emerged as to the relative importance of these types of effect (Li *et al.*, 2008; Larièpe *et al.*, 2012; Zhou *et al.*, 2012; Mäki-Tanila and Hill, 2014; Jiang *et al.*, 2017).

17.8.8 Marker Imputation

Marker imputation is required in plant breeding, as in animal genetics, to reduce the cost of genotyping in genomic selection. Methods developed for imputation of marker data are described in **Chapter 3**. Highly heterozygous autopolyploids are a problem for imputation methods in plants. In contrast, for inbreeding species, the preponderance of homozygous markers should make imputation easier. Methods are now being developed specifically for plants; Swarts *et al.* (2014) developed a method for populations of inbred lines which exploit the reduced requirement for phasing, and the method of Hickey *et al.* (2015) works on basic plant pedigrees of biparental crosses, selfs, backcrosses and top crosses. However, as yet there are no accurate imputation methods for autopolyploids.

17.9 Experimental Design and Analysis

Agricultural science has always been at the forefront of experimental design. In plant breeding and crop genetics, the scale of variety trials continues to grow, with the testing of more than 1000 lines now common. The historical development of methods has been reviewed, for example, by Edmondson (2005), Smith *et al.* (2005) and Mead *et al.* (2012). The design and analysis of trials were initially restricted by the necessity for simple analysis, often carried out by hand. The classic textbook of Cochran and Cox (1957) describes and catalogues such designs. These placed severe restrictions on the number of genotypes that could be tested, for example to perfect squares. Access to cheap powerful computers and to good algorithms and software means that current designs are much more flexible. Alpha designs, developed in the 1970s (Patterson and Williams, 1976), are in common use. These are circulant partially balanced resolvable incomplete block designs (John and Williams, 1995) with only modest restrictions, primarily that the number of entries per block must not be greater than the square root of the number of varieties, which is never a problem in practice. Resolvable row–column designs (John and Williams, 1995) are used too, and different software packages are available for generating such designs. Unfortunately, many plant breeders and plant researchers continue to use classical designs inappropriately, overcoming limitations, for example, by assigning large numbers of candidate varieties to multiple designs for small numbers of entries, linked by a common set of control varieties. This is demonstrably less efficient than testing all candidates in a single experiment (Piepho *et al.*, 2006). This process of forcing experimental material into inappropriate and inefficient designs has been described as 'Procrustean design' by Mead *et al.* (2012) and is no longer necessary.

Typical replicated designs, such as alpha designs, rely on incomplete blocking, in one or two dimensions, with block sizes of the order 10, tempered by knowledge of local field variability. A recommended starting block size is the square root of the number of varieties. The basic model for analysis of a single trial with blocking in two dimensions, in which blocks are arranged physically into complete replicates in the field (the design is said to be resolvable), is

$$y_{ijhk} = \mu + g_i + w_j + r_{jh} + c_{jk} + e_{ijhk},$$

where w_j is the effect of the jth replicate, r_{jh} is the effect of the hth row within the jth replicate, c_{jk} is the effect of the kth column within the jth replicate and e_{ijhk} is a residual error. This model is commonly fitted by restricted maximum likelihood (which was originally published as a method for trials analysis; Patterson and Thompson, 1971). Blocks (rows and columns) are treated as random effects, allowing 'recovery of interblock information', giving modest improvements in accuracy of variety differences. If variation among blocks is large, there is little

difference between treating them as fixed or random (Piepho *et al*., 2013). Varieties are commonly treated as fixed (but see below).

A plot typically contains multiple plants of the same genotype or family. The number of plants per plot varies greatly with species, for example 16 oil palms might occupy about 1000 m^2 while 500 cereal plants require 12 m^2. Size of plot is generally determined by dimensions of specialist planting and harvesting equipment, but the requirement to avoid inter-genotype competition effects places a lower limit on plot size while absence of intra-genotype competition places an upper limit on plant spacing. In practice, experimental technique places an implicit balance between bias and precision from these sources. More complex modelling of direct and indirect genetic effects (David *et al*., 2000, 2001), as used to take account of interactions between animals in herds, or of siblings raised in litters (Bijma, 2014), is known but not generally used; avoidance of problems through adequate plot construction is simpler. Exceptions involve restricted randomisation in some species (e.g. oil-seed rape) in which lines in plots are grouped by height, flowering time or variety type (e.g. hybrid or inbred) in a split-plot type of design with groups as main plots. These factors are known to contribute to inter- and intra-genotype competitive effects. Simple adjustment through regression of plot yields on covariates, such as height of neighbouring plots, has also been used (David *et al*., 2001), but genetic and environmental competitive effects are confounded, and it is difficult to adjust plot yield to a value expected in the presence in intra-genotype competitive effects but the absence of inter-genotype competition. Designs balanced so that all genotypes are adjacent to all other genotypes an equal number of times have been proposed (Azais *et al*., 1993), but the number of genotypes that can be included is small.

As breeding programmes and experiments have increased in scale there has been greater interest in designs with variable replication. For traits of high heritability, and where seed or clone numbers are limiting, the greatest response to selection can come from experiments in which each entry occurs only once in a single plot (see below). Precision can be increased if a proportion of the entries are replicated, allowing for adjustment for field fertility effects and also providing an estimate of error. Two common approaches are the augmented design, in which a small number of entries, usually standards or controls, are replicated many times (Federer, 1956, 1961) and partially replicated designs (p-rep) designs (Smith *et al*., 2006; Williams *et al*., 2014) in which a larger number of experimental entries are present in two replicates. With p-rep designs, there is scope for optimisation by making sure that each entry is replicated about the same number of times across locations of a trial series.

Inter-plot competition aside, generally, the closer two plots are, the more closely correlated their performance, since their local environments tend to be more similar. Knowledge of the autocorrelation between adjacent plots has been incorporated into trial design (Cullis *et al*. 2006; Williams and Piepho, 2013). Analysis of trials data to take into account the spatial relationships has a long history, dating back to Papadakis (1937), in which regression of a plot yield on that of neighbouring plots is used in an analysis of covariance to improve precision. More recently, autoregressive methods in one and two dimensions have been developed (Gilmour *et al*., 1997) and adopted, most notably in Australia. Software to fit these is not readily available or free. The most commonly used programs are ASReml, GenStat and SAS. A recent R package allowing analysis of trials and series of trials by mixed model procedures is sommer (Covarrubias-Pazaran, 2016). Methods of modelling and fitting fertility trends continue to be developed, for example by fitting a two-dimensional spline in the R package SpATS (Rodriguez-Alvarez *et al*., 2018).

When integrating data across trials (years, sites), there are two basic options. Either the data are analysed in a single stage using a model for the plot data across trials, or analysis is performed in several stages, starting with the analysis of individual trials in the first stage to obtain

genotype means. These means are then analysed across trials using information in the precision of means from the first stage as weights (Smith *et al.*, 2005; Damesa *et al.*, 2017). Analysis can also proceed in more than two stages, for example, with GBLUP or GWAS performed in a third stage after integrating the phenotypic data across trials in the second stage. In stage-wise analysis, it is crucial to model genotypes by fixed effects throughout all stages except the last where genotypes are fixed or random depending on the objective of the analysis (e.g., random for GBLUP, fixed for obtaining variety means, fixed and random for GWAS) (Piepho *et al.*, 2012).

Research on trial design is now taking into account the availability of information from pedigree or realised relationships among entries. For very high (single-plot) heritabilities, trial design is irrelevant, since any layout will give equivalent high response to selection. However, at lower heritabilities, the importance of information from related lines increases and designs that do not take into account these relationships will not be optimal (Bueno Filho and Gilmour, 2003). More recent work (Butler *et al.*, 2014; Feoktistov *et al.*, 2017) describes methods for the optimal spatial arrangement of related varieties in trials, though as far as we are aware software to implement these approaches is not available.

17.10 Conclusions

While heeding the warning of Bernardo (2016) to beware of bandwagons, it is probable that most innovation in plant breeding in the next ten years will be driven by adoption of genomic selection. It now seems tautological that if a trait is heritable, then it can be predicted from a genome-wide set of markers used, most simply, to estimate genetic relationships. Research in crops is increasingly focused on to how best to implement genomic selection in breeding programmes rather than to make improvements in prediction accuracy. This can range from the complete redesign of a breeding programme (Gaynor *et al.*, 2017) to a simple substitution of trait prediction for direct phenotyping of traits which are expensive and time-consuming to measure. The requirement for statistical genetics input in plant breeding will become more important, and this will align plant and animal breeding more closely (Hickey *et al.*, 2017). There remain areas in which approaches differ, however. Plant breeders focus more on major genes, particularly for phenology and disease resistance, have to work with greater genotype–environment interactions, and regard success as identifying transgressive segregants rather than increasing frequencies of favourable alleles.

There is a strong need for better training of plant breeders in basic statistics and quantitative genetics to exploit ever cheaper marker and sequencing platforms (van Eeuwijk *et al.*, 2016). Underpowered studies remain common and carry a risk that researchers can apply sophisticated analyses without sufficient understanding, leading to false positives (MacArthur, 2012). A hypothetical example, closely modelled on published studies, is a 'genome-wide' study which used 48 markers on 33 accessions tested for 15 morphological and agronomic traits. This reported 59 significant marker–trait associations involving 30 markers. This improbably high success rate from a small study exemplifies a widespread lack of understanding of statistical power in experimental studies (Kanehman, 2011).

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