

A theoretical and practical analysis of the optimum breeding system for perennial ryegrass

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The goal of plant breeding is to effectively and efficiently select for the best phenotypes leading to the development of improved cultivars. The objectives for this review are to describe and critically evaluate breeding methods appropriate to the improvement of perennial ryegrass (*Lolium perenne* L.) in a long-term breeding programme. The optimum breeding system is dependent on the traits for improvement, and the available physical and human resources. Forage dry matter yield, persistency, disease resistance, nutritional value and seed yield are considered among the most important traits for improvement. Careful consideration should be given to the expression of the trait under the management regime imposed in the breeding programme and under real-world sward conditions in the target sowing region. Recurrent selection programmes for intra-population improvement are most appropriate for breeding perennial ryegrass. Three distinct types of recurrent selection may be implemented: (i) phenotypic recurrent selection, (ii) genotypic recurrent selection and (iii) marker-assisted selection. Genotypic recurrent selection will be a necessary part of the breeding system if forage yield is a trait for improvement. Genotypic recurrent selection may be practiced using full-sib or half-sib families, each with their own advantages and disadvantages. Phenotypic recurrent selection in tandem (i.e., within-family selection) or in succession with genotypic recurrent selection should be used to improve traits that have a high-correlation between performance from spaced plants and from sward plots. Genome-wide selection represents the most interesting and exciting potential application of marker-assisted selection, although it remains to be seen how beneficial it will be in practice.

Keywords: breeding method; efficiency; gain; *Lolium perenne* L.; traits for improvement

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Introduction

The goal of the plant breeder is to create new phenotypes, improved in one or more important characteristics, in the most efficient manner possible. New phenotypes created by the breeder are a function of changes in genotype associated with selection and the environmental conditions under which the new cultivar will be utilized and which the breeder has replicated to the greatest extent possible.

Perennial ryegrass (*Lolium perenne* L.) is the main forage grass species sown in northwest Europe, New Zealand, and in the temperate regions of Japan, Australia, South Africa and South America (Humphreys *et al.* 2010). This species is the major, and arguably primary, concern in forage grass breeding programmes in these areas. Forage grasses have a relatively short history of formal breeding. Genetic variation among and within populations is still extremely high, offering significant scope for genetic improvement (Casler *et al.* 1996). The target traits for improvement are largely determined by the market requirements as dictated by the official cultivar evaluation trials and/or farmers in each area.

The objectives in this review are to describe and critically evaluate breeding methods appropriate to the improvement of perennial ryegrass in a long-term breeding programme, and to consider theory and practice in designing the optimum breeding system for a range of the most important agronomic traits in this species.

Traits for improvement

The goal of the breeder is to select for the minimum number of only the most important traits, thus maximising the gain per individual trait. The more traits selected for improvement the slower the rate of gain for each individual trait, and the greater

the difficulty and cost. The key traits for improvement in perennial ryegrass, as indicated by previous reviews (Wilkins and Humphreys 2003; Casler and van Santen 2010), are discussed below. The appropriate breeding strategy is a product of the trait(s) heritability, genotypic and phenotypic variances, and expression under the management schemes imposed in the breeding centre compared to real-world swards on farms.

Forage dry matter yield

Dry matter (DM) yield is one of the most important traits of perennial ryegrass and is measured in nearly every cultivar evaluation trial. Estimates of narrow-sense heritability in sward plots are highly variable but typically low to moderate (0.20 to 0.50) (Frandsen 1986; Jafari 1998). The trait is subject to substantial genotype \times environment (G \times E) interaction (Conaghan *et al.* 2008a). Therefore, the response to selection can be considerably improved through the judicious use of environmental replication, or by developing cultivars for specifically defined environments.

There is generally a zero to low (≤ 0.20) phenotypic correlation between the yield of sward plots and spaced plants (Hayward and Vivero 1984; Jafari 1998). Thus, genetic progress for increased forage yield requires yield measurement and selection on sward plots. Genotypes may also rank differently on annual and seasonal yield depending on the frequency of cutting (Wilkins 1989). The seasonal pattern of production is more important than annual production as the monetary value of grass at different points in the growing season can vary markedly (Doyle and Elliott 1983). Accordingly, harvest frequency and timing in the breeding programme should be designed to reflect real-world practices in the target sowing region (Casler and van Santen 2010).

Ryegrass yield under cutting and grazing are highly correlated (Camlin and Stewart 1975; Aldrich 1987) facilitating indirect selection for grazing yield. The use of animal grazing trials for yield assessment is discouraged as larger plot sizes and more replicates are required than for cutting trials because of the additional within-plot variability introduced by livestock (Casler and van Santen 2010), thereby increasing the total breeding programme costs. However, costs may be reduced by indirect selection for DM yield using fresh matter yield as the selection criterion (Conaghan *et al.* 2008b).

Persistency

Persistency is an economically important trait for perennial forages because of the costs involved in sward establishment. Persistency may be defined as sustained forage yield and ground cover over several years. It is dependent on the vigour of a plant and its ability to survive and contribute to yield and ground cover. Persistency is not a single trait but rather a complex of traits that are each dependent on the environment and management of the crop (Casler and van Santen 2010). Environmental (e.g., disease, temperature, drought etc.) and management (e.g., cutting and grazing) stresses may play an important role in limiting persistency. Ryegrass ground cover under cutting and grazing are highly correlated (Camlin and Stewart 1975; Aldrich 1987). Thus, persistency under grazing can be indirectly selected and improved by measuring persistency under cutting.

Persistence is moderately heritable (Novy, Casler and Hill 1995; Ravel and Charmet 1996). However, selection should be practiced in the target sowing location as differences among locations in abiotic and biotic stresses can lead to large G×E effects (Ravel and Charmet 1996).

Disease resistance

Disease is one of the most important factors limiting the yield, persistency and nutritional value of perennial ryegrass. Crown rust (*Puccinia coronata* f. sp. *lolii*) and leaf spot (*Drechslera* or *Rhynchosporium*) are probably the most widespread and damaging diseases in ryegrass (Carr *et al.* 1975). O'Kiely (1991) found that under the low levels of disease pressure in Ireland the application of a fungicide to ryegrass swards, managed for 1st-cut silage, only increased the DM yield by proportionally 0.06.

The heritability of disease resistance to the common fungal pathogens is generally moderate to high (*ca.* 0.50 to 0.75) (Bonos, Clarke and Meyer 2006). Selection may be practiced on spaced plants or swards plots, as these exhibit a high phenotypic correlation (≥ 0.75) for disease resistance (Easton *et al.* 1989).

Nutritional value

Digestibility is the most important selection criterion for improving the nutritional value of grasses (Smith, Reed and Foot 1997). *In vitro* DM digestibility (IVDMD) may be increased by selection for IVDMD *per se* or other correlated traits (e.g., concentrations of water-soluble carbohydrate, neutral detergent fibre, acid detergent fibre or lignin), or changes in reproductive development affecting the ratio of leaf to stem (i.e., the timing and intensity of primary heading and the frequency and intensity of aftermath heading) (Casler 2001).

The digestibility of leaf and stem are largely under independent genetic control (Buxton and Marten 1989) and selection may be practiced on either or both plant parts, measured separately or pooled. If selecting on the whole plant or sward, cognisance should be taken of maturity stage, otherwise the primary selection

pressure may be on heading date rather than IVDMD *per se* (Casler 2001).

Leaf digestibility and stem digestibility measured in sward plots and spaced plants have a high phenotypic correlation (Casler and van Santen 2010). However, the correlation between the whole-plant IVDMD of a spaced plant, which includes all herbage above a fixed cutting height, and that of sward plots cut to a similar height (Beerepoot and Agnew 1997; Jafari 1998) is often low due to morphological differences in plants grown as spaced plants and swards (Cooper and Breese 1980). The IVDMD of grazed and mechanically-cut swards are highly correlated (Casler and van Santen 2010).

The heritability of laboratory indicators of nutritional value of perennial ryegrass are typically low to moderate (Frandsen 1986; Posselt 1994). In contrast, the heritability of traits related to reproductive development is generally high (Cooper 1960; Ravel and Charmet 1996). Both sets of traits tend to be far less sensitive to G×E effects than traits such as forage yield (Casler and van Santen 2010), reducing the need for the same extent of environmental replication as required to accurately estimate forage yield.

The relationship, for perennial ryegrass genotypes, between IVDMD at different harvests within years is highly inconsistent (Frandsen 1986), primarily due to the extreme variability between genotypes in reproductive development (Casler 2001). Selection should focus on improving IVDMD during the periods when nutritional value is most limiting for animal production potential, using the dominant plant morphological characteristic (i.e., leaf or stem) during this period as the selection criterion.

Seed yield

Seed yield is one of the more contentious selection criteria. Many seed production

traits are negatively correlated with agronomic performance. However, the ability of a cultivar to produce reasonable seed yield is essential to its commercial success.

Selecting for seed yield is difficult because it has low to moderate heritability, is considerably influenced by the environment (Elgersma 1990; Elgersma, Winkelhorst and den Nijs 1994), and the location of the breeding trials may be far removed from the main areas used for commercial seed production. Furthermore, there is generally a low phenotypic correlation between the seed yield and individual seed yield components (e.g., number of reproductive tillers, number of spikelets per inflorescence etc.) of spaced plants with that of drilled plots (Elgersma 1990; Elgersma *et al.* 1994) meaning selection on spaced plants is largely ineffective, other than to indicate extremes in performance in drilled swards.

Breeding systems

Recurrent selection for intra-population improvement is most appropriate for breeding perennial ryegrass (Breese and Hayward 1972). In general, three distinct breeding systems may be applied: (i) phenotypic recurrent selection (PRS) based on the phenotypic value of individuals, (ii) genotypic recurrent selection (GRS) based on the phenotypic value of the progeny of an individual under evaluation and (iii) marker-assisted selection (MAS) based on molecular (DNA) marker scores. The efficiency of alternative breeding strategies is typically judged in terms of the genetic gain (ΔG) per cycle or per year. The breeding methods and strategies discussed below are valid for diploid and tetraploid ryegrass (Gallais 2003).

Phenotypic recurrent selection

Phenotypic recurrent selection is the oldest and simplest breeding method (Figure 1).

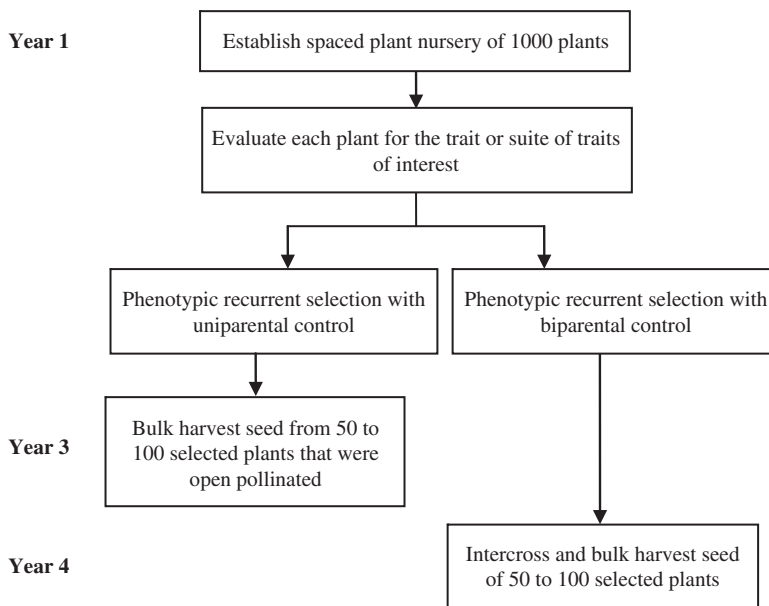


Figure 1. Schematic flow diagram of 1 cycle of phenotypic recurrent selection.

Uniparental or biparental pollen control may be practiced. Biparental control offers greater theoretical ΔG on a per cycle basis than uniparental control (Fehr 1987), but also increases cycle time and costs, if the intercrossing must be done the following year. The fewer parents selected to form the next generation, the greater the superiority of the selected individuals, but the higher the level of inbreeding and the lower the limits of selection (Posselt 2010). When selection is based on only a subset of the target traits for improvement, 100 parents should be chosen to reduce the probability of fixing undesirable genes for the non-selected traits through linkage with genes influencing the trait under selection (Wilkins and Humphreys 2003). Separate broad-based and narrow-based populations may be produced for use in recurrent selection and release as a synthetic cultivar, respectively. As few as four parents may be used in the construction of a synthetic cultivar (Hill 1971).

The advantages of PRS are that it makes full use of all additive genetic variance, offers the shortest possible breeding cycle and is easy to implement. It facilitates the evaluation and selection of large numbers of individual plants offering the potential for high selection intensity, low inbreeding and the maintenance of genetic variability.

One of the major weaknesses of PRS is that selection is practiced on individual spaced plants. Depending on the trait, selection on spaced plants may be of limited use in improving performance in real-world swards on farm. Furthermore, selection is generally based on unrepeated, individuals in a single location and is not appropriate for traits of low heritability (Fehr 1987).

Genotypic recurrent selection

Genotypic recurrent selection is based on assessing genetic merit of individuals from the performance of their progeny (Figures 2 and 3). In an out-crossing species, such

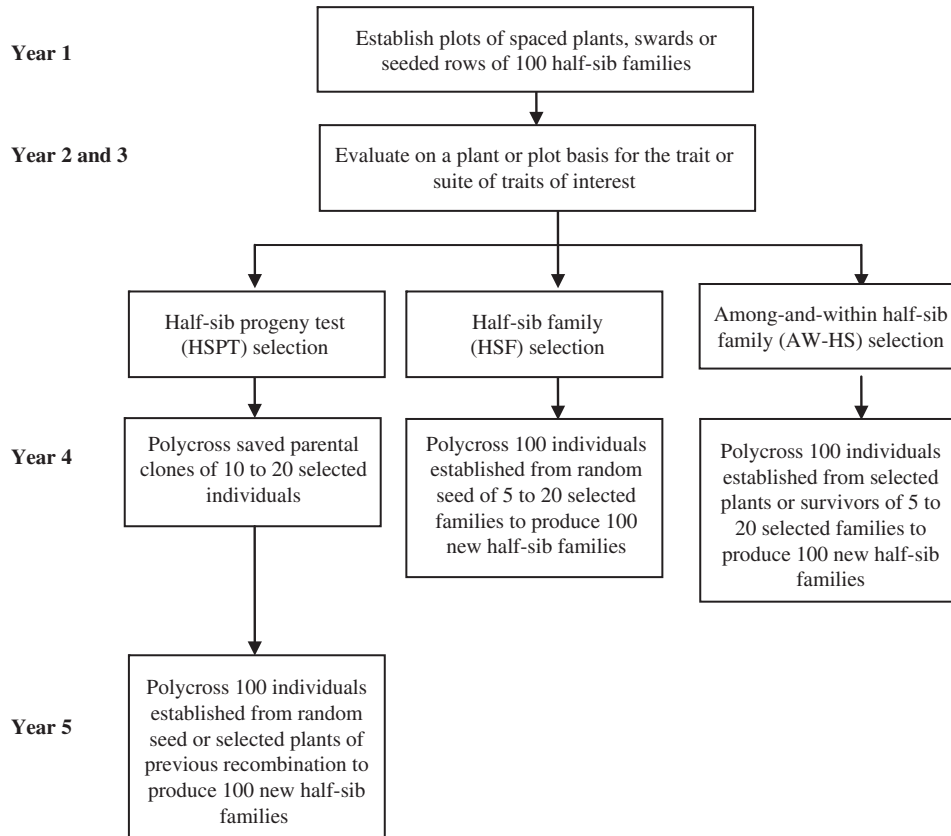


Figure 2. Schematic flow diagram of one cycle of genotypic recurrent selection using half-sib families (adapted from Casler and Brummer 2008).

as perennial ryegrass, progeny testing may be based on either half-sib or full-sib families.

Full-sib families facilitate the evaluation of twice as many parents as with half-sibs for the same number of families produced. However, the production of full-sib families may require greater labour and cost than half-sib families as controlled pollination is involved compared with half-sib families, which are typically produced using an open-pollinated polycross. Furthermore, F_1 full-sib families may not give enough seed for sowing replicated sward plots unless they are multiplied in isolation to produce F_2 seed, but this

would increase the cost and cycle time by 1 year compared with GRS using half-sib families. Full-sib families facilitate crosses among plants of different maturity as the heading date of each parent can be readily manipulated under controlled pollination conditions. In contrast, half-sib families tend to be useful only for assessing breeding value of parents of similar heading date as pollination is generally conducted under natural conditions.

Three different units of intercrossing may be used for each mating system to form a new set of families for the next selection cycle: random plants grown from remnant seed from the original cross of

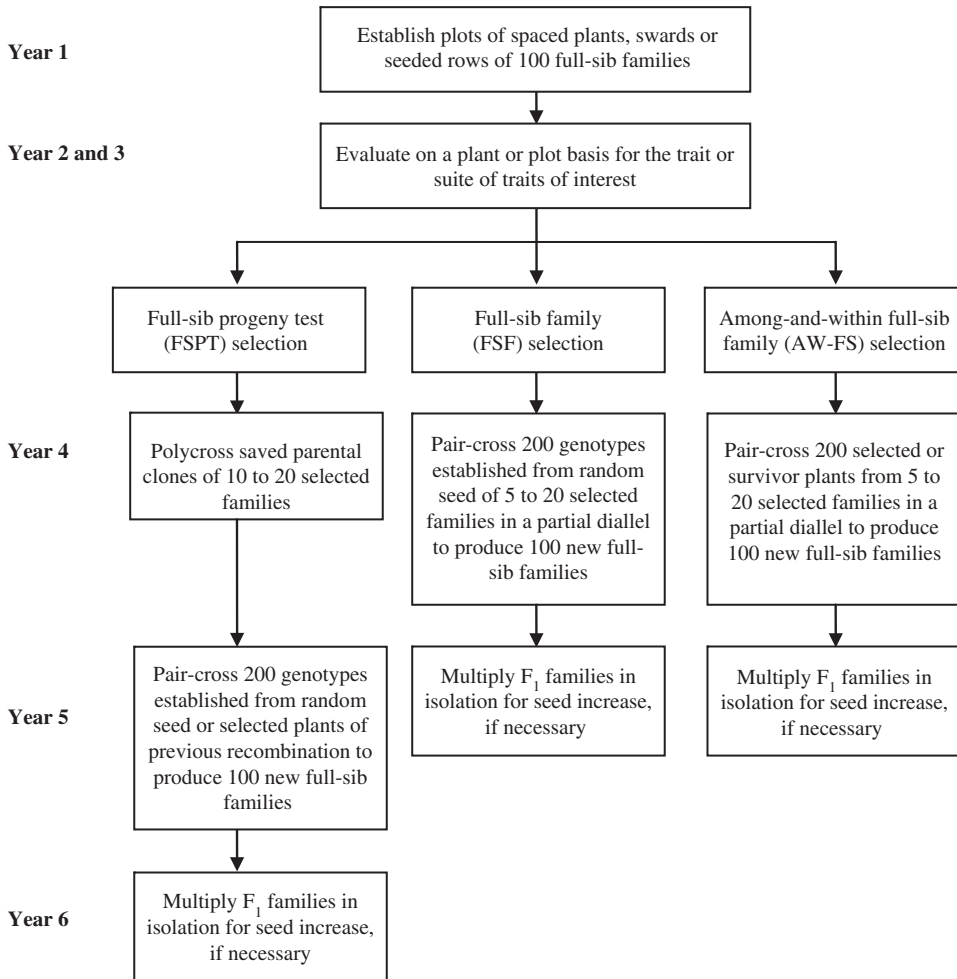


Figure 3. Schematic flow diagram of one cycle of genotypic recurrent selection using full-sib families (adapted from Casler and Brummer 2008).

the selected family, saved maternal plants of the selected families or selected plants within the selected families. The recombination unit used can have a considerable effect on ΔG (Fehr 1987).

Progeny testing requires a double-crossing event which adds 1 extra year to the cycle time. The full-sib progeny test involves greater cost and labour than full-sib family (FSF) selection, because the parental plants must be saved, or cloned, and kept alive until the selection decisions

are made, but yields the same theoretical ΔG (Hallauer and Miranda 1988). Therefore, in practice, full-sib progeny test selection is not undertaken and will no longer be discussed as part of this review. The half-sib progeny test (HSPT) may offer greater short-term gain than either half-sib family (HSF) or FSF selection but if the number of parents intercrossed at each cycle is low inbreeding depression may affect long-term selection limits. New individuals from outside the population

may have to be introduced after a number of generations to counteract the depletion of genetic variance.

Genotypic recurrent selection enables evaluation of progeny from each family using replicated, multi-location testing. This allows G×E interaction to be taken into account, resulting in higher heritability and typically greater ΔG for traits with low heritability compared with PRS (Brummer and Casler 2009). The generation of seed also facilitates evaluation of the progeny in sward plots, which is essential for improving traits, such as forage yield, that have a poor correlation between measurements on spaced plants and on swards.

The greatest disadvantage of among-family selection is that it utilises only a fraction of the additive genetic variation and is less successful at increasing ΔG than PRS for traits with high heritability (Casler and Brummer 2008). Compared with PRS, family selection methods may require a longer cycle time, and certainly greater cost, as the crossing events must be highly controlled and managed and the seed of each family individually managed. There is less flexibility and scope to increase the selection intensity with GRS than with PRS as the production of extra families requires significant additional work and cost.

Marker-assisted selection

There are three principal methods by which MAS may be used for population improvement, namely, (i) marker-assisted introgression, (ii) marker-assisted recurrent selection (MARS) and (iii) genome-wide selection (GWS) – also referred to as genomic selection.

The goal of marker-assisted introgression is to incorporate one or several major quantitative trait loci (QTL) into individual plants or populations by selecting for the specific QTL. In this instance, the QTL

and their effects need to be clearly identified, and those QTL with strong statistical support and large effects will be most useful (Brummer and Casler 2009). A major limitation of marker-assisted introgression is that incorporating desirable QTL into a single cultivar becomes increasingly difficult as the number of QTL increases. In practice, only a limited number of QTL (e.g., 2 to 5) can be introgressed at any one time so as to avoid prohibitively large population sizes in the breeding programme (Brummer and Casler 2009).

The MARS approach can target a larger number of QTL (typically 20 to 35) for selection at any one time through the use of a weighted selection index which includes QTL with small effects and only marginal statistical support. The MARS approach requires less precision for pinpointing QTL than marker-assisted introgression but the selected genotype may not have the favourable allele across all QTL included in the selection index (Bernardo 2008).

Genome-wide selection, first proposed by Meuwissen, Hayes and Goddard (2001), focuses purely on predicting performance based on estimating and then summing the joint effects of all markers across the entire genome to give the “genomic estimated breeding value” on which selection is practiced. In GWS selection is practiced without significance testing and without identifying the subset of QTL associated with any trait. Estimates of the breeding value can be continually re-calculated and improved upon over time as more phenotypic and genotypic data become available. Simulation studies have shown that GWS is considerably more effective than marker-assisted introgression and MARS in increasing genetic gain, especially for complex traits controlled by many QTL and with low heritability (Bernardo 2008).

Markers will not explain more of the genotypic variation than the phenotypic

data. However, MAS may still increase the ΔG per unit time, cost and cycle in breeding programmes, particularly when phenotypic evaluation for the traits of interest is time-consuming, expensive, inconsistent and dependent on specific environments or developmental stages. If MAS can be applied more easily or cheaply than phenotypic selection it allows greater selection intensity for a given level of resources. The attractiveness of MAS increases as the cost of genotyping decreases and the cost of phenotypic evaluation increases.

The development and application of MAS can be effectively integrated into a recurrent selection system (Brummer and Casler 2009; Casler 2010) (Figure 4). The essential part of this proposed system is the establishment of a marker selection index by using the DNA marker data of the parents and robust phenotypic data of their progeny collected from replicated, multi-environment trials. A molecular marker index created on this basis should be sufficiently robust to allow up to 3 cycles of selection and recombination before the DNA of the parents must be analysed again and the index recalibrated.

Applying MAS to select the best plants within families (among-and-within-family selection) as opposed to recombining random (HSF selection) or parental (HSPT selection) plants to form a new set of families for the next cycle of selection could increase the ΔG per cycle by up to 4 fold for a modest number of plants analysed (Figure 5). A marker index that explains even a small proportion of the genetic variation could offer considerable improvements in ΔG compared with HSF and HSPT selection. Using MAS in PRS (cycles 2 and 3 in Figure 4) could increase the ΔG per cycle by up to 2 fold compared with phenotypic selection, for the same number of plants evaluated (Figure 6), by halving the cycle

time. Marker-assisted selection is only as good as the phenotypic data on which the markers are based. If the phenotypic data do not accurately describe the trait, no or few true QTL will be identified and their effects will be incorrectly estimated resulting in negligible breeding gain using MAS.

Identifying real and consistent QTL effects, and by extension molecular marker effects, is difficult (Bernardo 2008). Markers need to be considered in the context of the population in which they are being used. The effect of a desirable allele at a QTL on the deviation of an individual from the population mean will depend on the frequency of the allele in the population. Over generations of MAS, the marker-trait associations will be continually eroded by recombination so that the efficiency of MAS will decline and the cost per unit ΔG will increase unless the marker-trait associations are recalibrated.

Despite predictions for more than 2 decades that MAS would reshape breeding programmes and facilitate rapid gains from selection (Heffner, Sorrells and Jannink 2009), its use has been limited in practical breeding, especially with low-value crops such as perennial ryegrass. Past work on the detection of QTL for use in marker-assisted introgression and MARS was based on the analysis of biparental mapping populations. This has proved impractical, costly and of limited effectiveness in a breeding programme (Bernardo 2008; Brummer and Casler 2009). The emphasis has now shifted towards the use of association mapping strategies. Experiments investigating its potential use and application are ongoing. It remains to be seen whether association mapping approaches will significantly accelerate the use of MAS in commercial grass breeding programmes (Roldán-Ruiz and Kölliker 2010). However, current

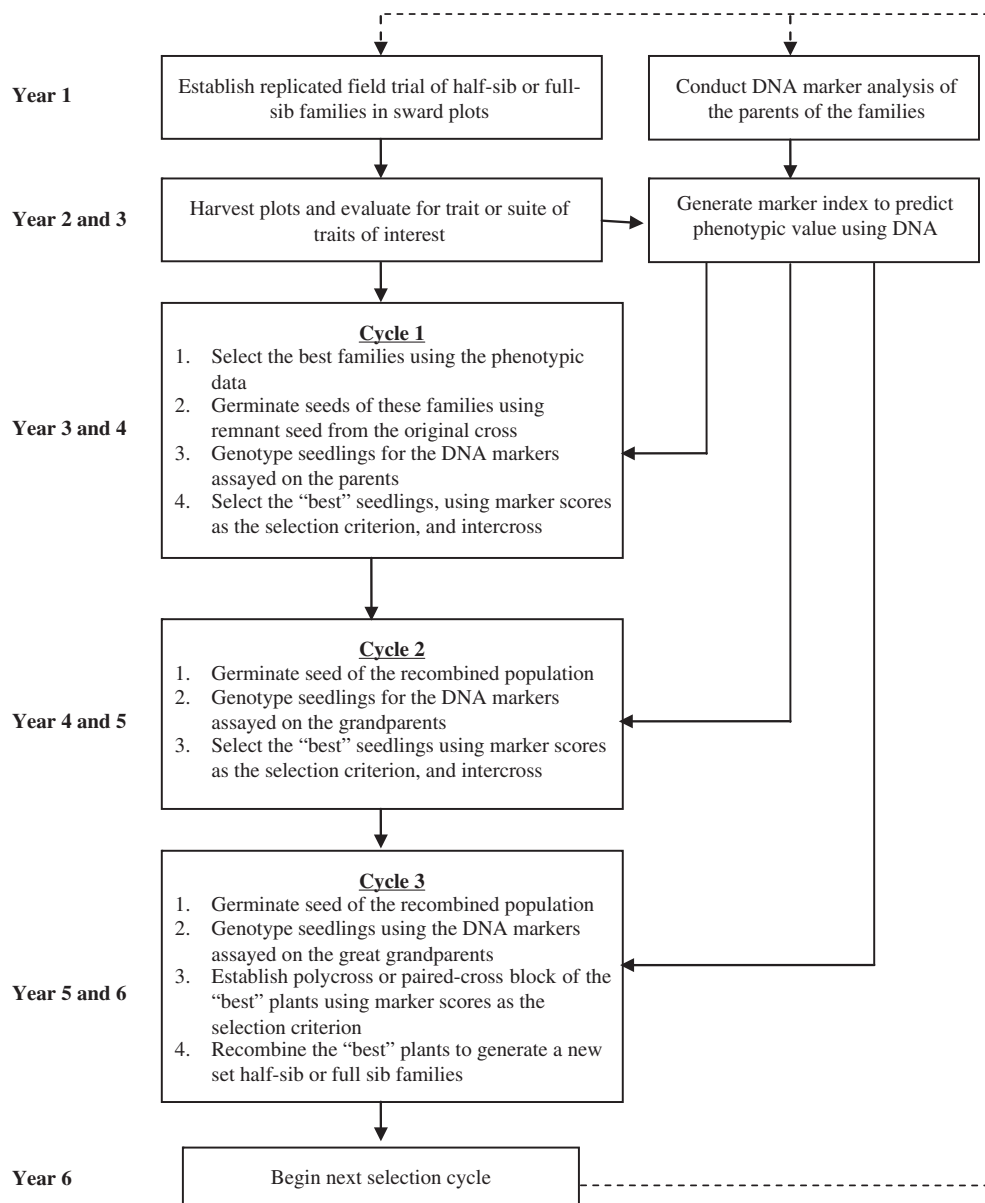


Figure 4. Schematic flow diagram of the development and application of three cycles of selection and recombination using molecular markers (adapted from Casler 2010).

association mapping efforts allow identification of only a few QTL with overestimated effects (as reviewed by Heffner *et al.* 2009).

The proposed solution, GWS, lies in harnessing (i) the developing capacity for scoring many markers at low cost and (ii) statistical methods that enable the simultaneous

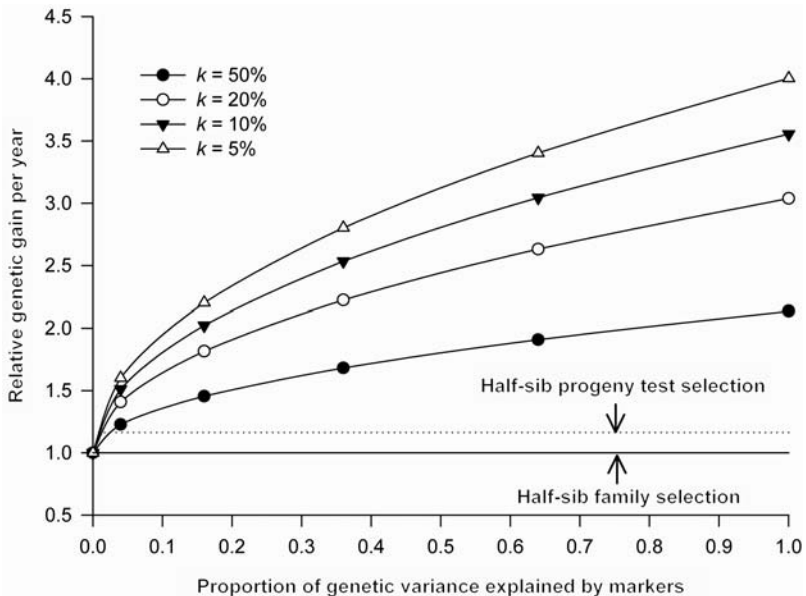


Figure 5. Expected genetic gain per year from among-and-within half-sib family (AW-HS) selection using phenotypic data and molecular markers as selection criteria, and half-sib progeny test (HSPT) selection and half-sib family (HSF) selection using phenotypic data as the sole selection criterion. The AW-HS selection is conducted as per cycle 1 of Figure 4, with phenotypic data used to select the best 5/100 families and molecular marker scores used to select 20 individuals within each selected family. Four different within-family selection intensities (k) are considered: 50%, 20%, 10% and 5%, requiring DNA analysis of 200, 500, 1,000 and 2,000 plants, respectively. Gain is expressed as a function of the proportion of genetic variance explained by the markers. The HSPT selection is based on an among-family k of 10% and intercrossing parental plants. All expected genetic gains are expressed relative to gains from HSF selection with phenotypic data as the selection criterion, an among-family k of 5% and recombining remnant seed. Narrow-sense heritability of the phenotypic values (family means and individual plants) is assumed to be 0.20. The heritability of the molecular marker is 1.0, assuming there are no errors in scoring. The phenotypic variance for all methods is 1.0.

estimation of all marker effects. The GWS approach represents the most interesting and exciting application of MAS, although its application to breeding gain has almost exclusively been tested through simulation. On these grounds, Heffner *et al.* (2009) and Jannink, Lorenz and Iwata (2010) advise that its potential value should be assessed with “cautious optimism”. The GWS approach is currently the subject of intense scientific study but practice currently outpaces theory

and many questions remain unanswered (Jannink *et al.* 2010).

Ultimately, a cost-benefit analysis is needed to determine whether the cost of applying MAS in a commercial grass breeding programme is worth the gain. This analysis would depend on the cost of applying MAS to achieve a given level of gain *vs.* the cost of applying different approaches to increase the response to phenotypic selection to a similar level.

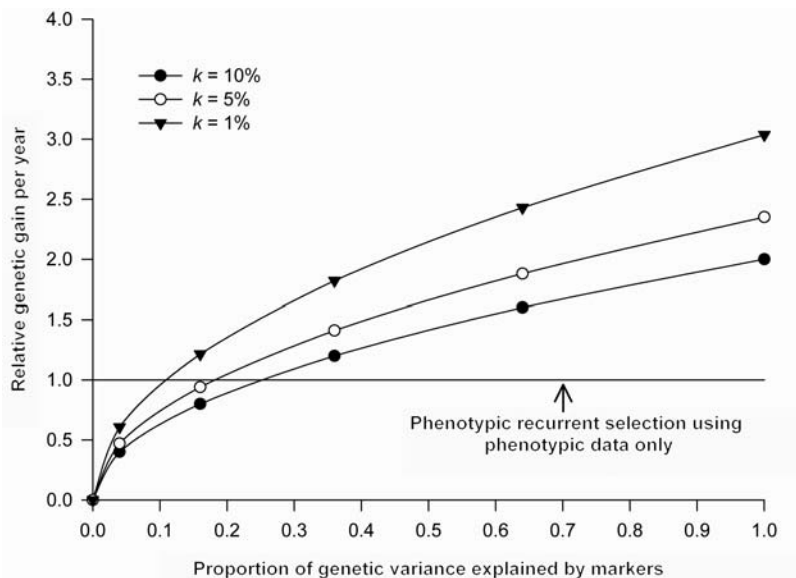


Figure 6. Expected genetic gain per year from biparental phenotypic recurrent selection (PRS) using molecular marker scores as the selection criterion. Three different selection intensities (k) with molecular markers are considered: 10%, 5% and 1%, requiring DNA analysis of 1,000, 2,000, and 10,000 plants, respectively, assuming 100 individuals are selected to form the next generation. Gain is expressed as a function of the proportion of genetic variance explained by the markers. All expected gains for PRS using markers are expressed relative to gains from biparental PRS with phenotypic data as the sole selection criterion and a selection intensity of 10%. The cycle time using molecular markers is 2 years compared to 4 years using phenotypic data. Narrow-sense heritability on the individual plant is assumed to be 0.20. The heritability of the molecular marker is 1.0, assuming there are no errors in scoring. The phenotypic variance for all methods is 1.0.

Breeding gain

The most relevant breeding methods for perennial ryegrass are summarised in Table 1. In this review, phenotypic evaluation is assumed to be conducted for 2 consecutive harvest years (excluding the establishment year) for all breeding methods. It was assumed that the individuals to be used in the next selection cycle are determined at the end of each cutting season (around October), allowing plant establishment and vernalisation during the autumn and winter and intercrossing in the following year. The predicted genetic gain per cycle for the different breeding systems was

calculated from the formulas presented by Fehr (1987). Each breeding system was compared across a number of scenarios spanning the potential extremes of each variance component using $\Delta G/\text{year}$ as the index of efficiency (Table 2).

Biparental vs. uniparental phenotypic recurrent selection

The genetic gain per year with biparental control is 1.5 times that with uniparental control if uniparental control shortens the cycle time by 1 year, and 2.0 times that with uniparental control if the cycle time is the same for both methods. The utility of PRS

Table 1. Summary of the key characteristics of the intra-population breeding methods relevant to perennial ryegrass (adapted from Posselt 2010)

Method of selection	Selection unit	Test unit	Recombination unit	Parental control	Cycle time (years)
Phenotypic recurrent selection					
Uniparental control	Plant	Plant	Plant	0.5	3
Biparental control	Plant	Plant	Plant	1	4
Half-sib (HS) family structure					
HS family selection	Family	Plot	Seed	1	4
HS progeny test	Family	Plot	Maternal parent	2	4
Among- and within-HS family selection	Family and plant	Plot and plant	Plant	1	5
Full-sib (FS) family structure [†]					
FS family selection	Family	Plot of F ₁ seed	Seed	1	4
FS progeny test	Family	Plot of F ₁ seed	Parent	1	4
Among- and within-FS family selection	Family and plant	Plot and plants of F ₁ seed	Plant	1	4

[†] Cycle time = 5 years if evaluation is conducted using F₂ seed.

with uniparental control tends to be low in perennial ryegrass breeding programmes.

Half-sib family selection vs. half-sib progeny test selection

Half-sib progeny test selection yields 1.6 times the annual genetic gain from HSF selection, on average across the scenarios in Table 2, assuming equal selection intensity and level of replication. This is due to a combination of the longer cycle time but greater parental control with the HSPT. Although HSF selection allows a higher selection intensity than HSPT selection for the same level of inbreeding, HSPT selection, at a selection intensity of 10%, still yields 1.4 times the genetic gain from HSF selection at a selection intensity of 5%. The greater efficiency of HSPT selection should more than compensate for its greater cost compared to HSF selection owing to an additional crossing event and the maintenance of the parental clones.

Full-sib family selection vs. half-sib family selection

Compared with HSF selection, FSF selection utilises twice as much additive genetic

variance, and requires a longer cycle time if F₂ seed must be produced for evaluation. Overall, FSF selection tends to be more efficient than HSF selection (Hallauer and Miranda 1988). For all variance scenarios considered in Table 2, FSF selection using F₂ seed for evaluation was more efficient than HSF selection by, on average, 30%, for equal selection intensity and level of replication.

Full-sib family selection vs. half-sib progeny test selection

Compared with HSPT selection, FSF selection utilises twice as much additive genetic variance, half the level of parental control and greater phenotypic variation. Overall, HSPT selection tends to be more efficient than FSF selection. Across the range of variance scenarios considered in Table 2, the genetic gain per year with HSPT selection was, on average, 1.2 times that from FSF selection, using F₂ seed for evaluation, for equal selection intensity and level of replication.

However, HSF selection allows higher selection intensity than HSPT selection for the same level of inbreeding. The advantage of HSPT selection, at a

Table 2. Predicted genetic gain per year (as a percentage of additive genetic variance) for alternative breeding methods across different levels of selection intensity, phenotypic and genetic variances, and environmental replication

Selection method [†]	Selection intensity among families and variance components model [‡]														
	20% Selection intensity					10% Selection intensity					5% Selection intensity				
	Lo _e	Me _{AE}	Hi _{AE}	Me _D	Hi _D	Lo _e	Me _{AE}	Hi _{AE}	Me _D	Hi _D	Lo _e	Me _{AE}	Hi _{AE}	Me _D	Hi _D
<i>One replicate in one environment</i>															
Biparental PRS	23	13	7	11	6	29	16	9	14	8	34	19	10	16	9
Uniparental PRS	16	9	5	7	4	20	11	6	9	5	23	13	7	11	6
HSF	12	7	3	7	3	16	9	3	9	3	18	11	4	11	4
HSPT	20	11	4	11	4	25	14	5	14	5	29	17	6	17	6
FSF (F ₁)	20	12	5	11	5	25	16	6	14	6	30	18	7	16	7
FSF (F ₂)	16	10	4	9	4	20	12	5	11	5	24	15	6	13	6
AW-HS ₂₀ (F ₂)	31	19	8	16	7	33	20	8	17	7	36	22	9	18	8
AW-HS ₅₀ (F ₂)	22	13	5	11	5	24	15	6	13	5	27	16	6	14	6
AW-FS ₂₀ (F ₂)	32	20	8	17	7	36	22	9	20	9	40	24	10	22	9
AW-FS ₅₀ (F ₂)	25	16	6	14	6	29	18	7	17	7	33	20	8	19	8
<i>Two replicates in one environment</i>															
HSF	14	9	4	9	4	18	11	4	11	4	21	13	5	13	5
HSPT	23	14	6	14	6	29	18	7	18	7	34	21	8	21	8
FSF (F ₁)	22	14	7	12	6	28	18	8	16	8	33	21	10	18	9
FSF (F ₂)	18	11	5	10	5	22	14	7	12	6	26	17	8	15	7
AW-HS ₂₀ (F ₂)	32	20	9	17	8	35	22	9	19	8	38	24	10	20	9
AW-HS ₅₀ (F ₂)	23	15	6	13	6	26	16	7	14	6	29	18	8	16	7
AW-FS ₂₀ (F ₂)	34	21	9	19	9	38	24	11	22	10	42	27	12	24	11
AW-FS ₅₀ (F ₂)	27	17	8	16	7	31	20	9	19	9	35	22	10	21	10
<i>Two replicates in each of two environments</i>															
HSF	16	11	5	11	5	20	14	6	14	6	23	16	7	16	7
HSPT	25	18	8	18	8	31	22	10	22	10	37	26	12	26	12
FSF (F ₁)	23	18	9	15	8	29	22	11	19	10	34	26	13	22	12
FSF (F ₂)	19	14	7	12	6	23	18	9	15	8	28	21	11	18	9
AW-HS ₂₀ (F ₂)	34	22	10	19	9	37	24	11	21	10	39	26	12	23	11
AW-HS ₅₀ (F ₂)	24	16	7	14	7	28	19	8	17	8	30	21	9	19	9
AW-FS ₂₀ (F ₂)	35	24	11	21	11	40	27	13	25	13	44	31	15	28	14
AW-FS ₅₀ (F ₂)	28	20	10	18	9	33	23	11	22	11	37	26	13	25	13

[†] PRS = phenotypic recurrent selection; HSF and FSF = half-sib and full-sib family selection, respectively; (F₁) and (F₂) = evaluation on F₁ and F₂ seed; AW-HS₂₀ and AW-HS₅₀ = among-and-within-half-sib-family selection with selection intensities within families of 20% and 50%, respectively; AW-FS₂₀ and AW-FS₅₀ = among-and-within-full-sib-family selection with selection intensities within families of 20% and 50%, respectively.

[‡] Lo_e = ($\sigma_A^2 = 1$, σ_{AE}^2 , σ_D^2 and $\sigma_{DE}^2 = 0$, and $\sigma_e^2 = 0.25$); Me_{AE} = ($\sigma_A^2 = 1$, $\sigma_{AE}^2 = 1$, σ_D^2 and $\sigma_{DE}^2 = 0$, and $\sigma_e^2 = 1$); Hi_{AE} = ($\sigma_A^2 = 1$, $\sigma_{AE}^2 = 3$, σ_D^2 and $\sigma_{DE}^2 = 0$, and $\sigma_e^2 = 10$); Me_D = (σ_A^2 , σ_{AE}^2 , σ_D^2 , σ_{DE}^2 and $\sigma_e^2 = 1$); Hi_D = ($\sigma_A^2 = 1$, σ_{AE}^2 , σ_D^2 and $\sigma_{DE}^2 = 3$, and $\sigma_e^2 = 10$) where σ_A^2 , σ_{AE}^2 , σ_D^2 , σ_{DE}^2 and σ_e^2 correspond to the additive genetic, additive-genetic × environment interaction, dominance genetic, dominance-genetic × environment interaction and within-experiment error variances, respectively. For PRS, the plot-to-plot and within-plot environmental variance are assumed to sum to σ_e^2 .

selection intensity of 10%, over FSF selection, using F₂ seed for evaluation, at a selection intensity of 5%, was negligible. Therefore, the choice between FSF and HSPT selection largely comes down to practicality and cost.

Among-and-within-family selection vs. family selection

Among-and-within-half-sib-family (AW-HS) and -full-sib-family (AW-FS) selection will always be more efficient than HSF and FSF selection, respectively

(Casler and Brummer 2008). Among-and-within-family (AWF) selection utilises all additive genetic variation, allows greater selection intensity within families, and has relatively low within-family phenotypic variance (i.e., high individual-plant heritability). There is no additional cost of implementing AWF selection if phenotypic data are routinely collected on individual plants. However, the cost of AWF selection may be high if family selection is based solely on plot values and within-family selection requires the additional establishment and/or evaluation of spaced plants. The cost of AWF may be reduced by conducting within-family evaluation on only a proportion of the best families identified after 1 or 2 evaluation years. The $\Delta G/\text{year}$ with AW-FS selection (using F_2 seed for evaluation), where family selection is postponed until the best families are selected, is 1.1 times that from the multistep FSF plus biparental PRS approach (Table 2). For comparable within- and among-family selection intensities, AW-FS selection is more efficient than AW-HS selection and the advantage increases as the within-family selection intensity increases (Table 2).

An alternative to using a dedicated spaced-plant nursery is to base within-family selection on survivorship, by taking a random sample of surviving plants from the sward plots of the selected families. The amount of realised gain achievable, if any, using survivorship as the selection criterion within perennial ryegrass sward plots is unknown (Casler and Brummer 2008).

Among-and-within-family selection vs. half-sib progeny test selection

The advantage of AWF selection over HSPT selection is less than its advantage over HSF or FSF selection, for equal selection intensity and level of replication, as HSPT selection offers double the level

of parental control. The AW-FS selection (using F_2 seed for evaluation), at a selection intensity of 5% among families and 20% within families, offered, on average, 1.2 times the $\Delta G/\text{year}$ from HSPT selection with a selection intensity of 10% (Table 2). This assumes that the within-family selection was not conducted until evaluation of the best families had been completed using 2 replicates in one environment per family, and individual plants were evaluated for 1 replicate in one environment. The higher selection intensity that may be imposed using family selection methods compared with HSPT selection for a given level of inbreeding would reduce the advantage of AWF selection over HSPT selection compared with HSF or FSF selection.

Phenotypic recurrent selection vs. genotypic recurrent selection

Genotypic recurrent selection in replicated plots may increase $\Delta G/\text{year}$ compared with unreplicated biparental PRS, particularly when the error variance and $G \times E$ interaction variance are large. However, the considerable extra cost of implementing GRS rarely justifies the extra $\Delta G/\text{year}$ unless that the trait of interest cannot be efficiently improved using spaced plants, in which case GRS is the only viable option. The average advantage (across the variance scenarios considered in Table 2) of GRS [HSF, HSPT and FSF (F_2) selection], based on 2 replicates in each of 2 environments at a selection intensity of 5%, over biparental PRS, based on 1 replicate in one environment at a selection intensity of 10%, was 20%. The cost of this extra $\Delta G/\text{year}$ is considerable. For the typical range of heritability (≥ 0.10) reported for perennial ryegrass traits, biparental PRS is the most cost efficient and cost effective breeding method, provided there is a good correlation between the trait as measured in spaced plants and in swards.

Conclusions

The optimum breeding system for perennial ryegrass depends on the traits to be improved, and the resources and skills available. Careful consideration should be given to the expression of the trait under the management regime imposed in the breeding programme and under real-world sward conditions in the target region. Genotypic recurrent selection will be a necessary part of the breeding system if forage yield is a trait for improvement and may be practiced using full-sib or half-sib families, each with their own advantages/disadvantages. Phenotypic recurrent selection in tandem (i.e., within-family selection) or in succession with GRS should be used to improve traits that have a high correlation between measurements on spaced plants and those on sward plots. Genome-wide selection represents the most interesting and exciting potential application of MAS, although it remains to be seen how beneficial it will be in practice.

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