Evaluation of genotypes of subterranean clover *(t. subterraneum* l.) in a Mediterranean environment: a personal and historical account

R.C. Rossiter

CSIRO, Perth, WA

Introduction

My interest in sub-clover began in the mid-1930s, when as an undergraduate I spent a summer season certifying clover seed at P.D. Forrest's property "Dwalganup" at Boyup Brook. At this time the area of sown annual pasture -mainly sub-clover - in the south-west of Western Australia was only *c.* 0.2×10^6 ha; thereafter the area began to take off, and reached over 6×10^8 ha by the start of the 1970s. (Figure1). As you can see, I seem to have grown up with sub-clover!

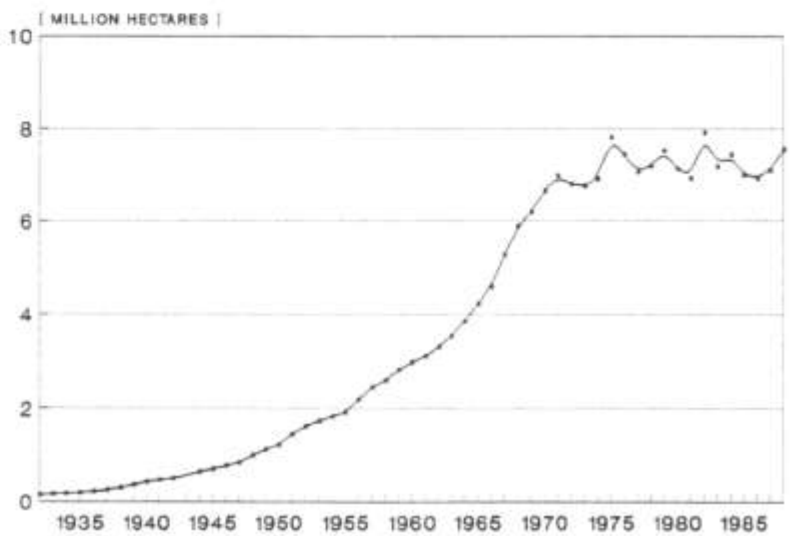

Figure1. Area of sown pasture in the south-west of W.A. Sub-clover sowings began in the first two decades of the 1900s. Most of the total area is sub-clover pasture, but from the 1970s medics have increased substantially in wheatbelt areas.

Although my first main research project concerned perennial veld grass *(Ehrharta calycina),* I began some work on sub-clover in the mid-1940s, and my principal interest gradually moved from mineral nutrition to pasture ecology, including physiological ecology. Evaluation studies of clover strains (and other cognate work) was always an important part of the research program. What follows is a developmental account of my own work and thoughts in this area. Over the years I have been helped and influenced by many colleagues and associates, to whom I am deeply indebted, and I need hardly remind you in passing that the phrase "my own work and thoughts" is very loose talk.

Early years (to c. 1962)

Growing out single-plant rows of sub-clover strains in the 1940s and '50s seemed part of the conventional wisdom. For me, it probably stemmed from the old 1937 paper by Colin Donald and Cedric Neal-Smith (9), reporting results from 14 strains (grown as spaced plants) during 1935-36 at the Waite Institute, Adelaide, S.A.

Our own early work (15), done from 1949-55 mainly at Kojonup, W.A., was more extensive, and included swards (ungrazed) as well as single plants, and many more genotypes. For single plants, total tops yield always increased linearly with maturity grading, or lateness of flowering (MG), as was the case in the

Donald paper. However, in our experiments, seed yields also increased linearly with MG, but this was not so at the Waite where there was an increase followed by a decrease except for the anomalous late strain Tallarook.

For swards, on the other hand, our results were markedly different from those for single plants. Total yields changed little (a slight decline in fact) with MG, whereas seed yields always fell rapidly. This was the case at Perth as well as at Kojonup. The sward/single plant contrasts were interpreted essentially in terms of available soil moisture supply.

Some of Donald's discussion on seed yields now seems inappropriate, though two of his conclusions are interesting and worth quoting in full. The first of these was almost correct at the time, while the second came of age much later.

"There is a sufficient number of good types already available to ensure that at least one of them will be adapted to any set of environmental conditions within the range tolerated by the species and the development of these types offers abundant scope for production of commercial varieties. Should hybridisation be adopted, an effective procedure, which, though slow, involves little labour, would be to plant a large F_2 under sward conditions and to allow natural selection to isolate over a number of years those types (through their increased seed production) suited to the environment."

At best, information from 1-year experiments can give pointers on those plant characteristics likely to be important for long-term adaptation, ie. success or failure. By 1950, there were few clues on what were the important characteristics. From 1950-55 three field experiments were begun at Kojonup, two of which extended over a 9-year period and one over 6 years; there was a smaller experiment at Perth over 9 years. The Kojonup experiments were grazed and the one at Perth mown. Success or failure of strains was assessed on a relative basis and in three competitive situations, in each of which the usual associated annual volunteer species were present. The situations were: (i) single-strain swards; (ii) single-strain swards in association with an indicator strain (Dwalganup) at low initial density; (iii) mixed swards of several strains.

The results in my 1966 paper (16) supported a claim already made by Donald in 1960 (10) that the main determinant of success in a mixed pasture was seed-producing capacity (SPC) in ungrazed pure swards. But it appeared that the form of the relationship between relative success and seed yield differed for the three ecologically separate situations: increased SPC was evidently needed for success as ecological complexity increased.

No evidence was found for the importance of seed size, petiole length or hardseededness, except for hard-seededness under low rainfall. The need for further studies of factors associated with success of closely related genotypes was emphasised.

Although the main thrust of the studies so far described was evaluation of genotypes as individual genotypes, I was aware of the possible importance of mixtures *per se.* These were discussed briefly at the end of the 1966 paper where reference was made to the co-existence of Dwalganup/Mt Barker mixtures.

While these experiments were in progress (1950-62) an exploratory trial was done to obtain some information on live-weight gain in sheep over the dry summer period on 3 pastures sown to Dwalganup, Yarloop and Bacchus Marsh sub-clovers. The pastures were stocked moderately at 7 wethers per ha., and new sheep were introduced in early December each year (equalised for mean live-weight). The average live-weight gains over the first three summers were +1.5 kg for Bacchus Marsh, -2.1 kg for Yarloop and -2.7 kg for Dwalganup, with the rankings consistent between years. Further work on sub-clover strains in relation to animal performance was planned and then deferred because of a recurrence of the "clover disease" problem.

Clover disease: a digression (1945-52 and 1961-72)

A sheep problem characterised by several abnormalities, the most important practically of which was infertility, first became prominent in the early 1940s (4). The problem was active for a decade or so, i.e. until the early 1950s, then quiescent for a decade, then active again from about 1960 for almost another decade (my so-called 'second phase'). From the beginning, the problem was observed to be associated with dominant (> 80%) Dwalganup sub-clover pastures, hence the name "Clover Disease" (C.D.); and it seemed to be caused by phyto-oestrogens. The C.D. problem was always much more serious in Western Australian than in the Eastern States.

My own involvement in the first phase was fairly minor, and included the collection of large numbers of samples of pasture plants, in particular of sub-clovers. From 1946-51 these were tested for oestrogenic activity by the then standard small animal bio-assay techniques. David Curnow did most of these tests, using mice. The results, unpublished, were much as we expected: no activity in any of the common annual grasses, or in capeweed, erodium, and small-seeded naturalised clovers; but high and consistent activity in sub-clovers. It all seemed to add up!

By 1951 Bradbury and White had isolated the isoflavone genistein, the oestrogenic compound *thought* to be responsible for C.D. Curnow and Rossiter (8) examined 120 genotypes of sub-clover and all without exception contained appreciable levels of genistein in the leaf fraction. A solution to C.D. along this selection route thus seemed remote. In view of what follows below this is a paper that David Curnow and I would like to forget!

During the 1950s, pastures generally in the affected areas lost their Dwalganup clover dominance, and farmers lost interest in C.D., as did the research workers.

The resurgence of C.D. was presumably due largely to Yarloop-dominant clover pastures in the Esperance region from sowings in the late 1950s during the development of that region. Two "breakthroughs", by Lloyd Davies and Bennett (14) using sheep uterus weight bioassays, and by Beck (3) using thin-layer chromatography for isoflavone analyses laid the foundation for many experiments and also for many research papers. It was fairly soon evident that the isoflavone formononetin (F) was the main culprit in the ruminant (sheep); that strains such as Mt Barker (low-F) were non-potent or of low potency compared with Dwalganup and Yarloop (high-F); and that strains varied substantially in leaf isoflavone concentrations, most importantly in F (eg. Rossiter (17); see Table 1).

Table 1: Average Values for Percentage Isoflavones in Expanded Leaves of Subterranean Clover Strains

Strain	% Dry Weight			
	F*	G	BA	Total
Yarloop	1.5	2.8	0.5	4.8
Dwalganup	1.3	1.6	0.8	3.7
Dinninup	1.2	1.1	1.5	3.8
Geraldton	0.9	0.6	0.9	2.4
Tallarook	0.8	0.7	0.6	2.1
Daliak	0.23	0.4	0.15	0.8
Clare	0.15	2.8	0.10	3.0
Woogenellup	0.15	2.0	0.6	2.7
Seaton Park	0.12	0.8	1.6	2.5
Northam A	0.12	1.5	0.4	2.0
Bacchus Marsh	0.11	1.0	2.3	3.4
Mt Barker	0.06	0.9	2.0	3.0
Uniwager	0.06	0.01	0.01	<0.1

*F [a] formononetin; G = genistein; BA = biochanin A

Field experiments (1965 to 1973) showing the effects of clover strain on percent ewes lambing and also the tie-up between infertility and F began at Kojonup by Lloyd Davies *et al. (13).* These were followed by experiments at Esperance, Badgingarra, Bakers Hill and elsewhere. Generally, but not without exception, the high-F strains Dwalganup, Yarloop and Dinninup caused serious infertility when dominant; Geraldton frequently did so; but moderately low F strains such as Daliak, Woogenellup, Seaton Park, Northam, and especially Mt Barker, gave much less trouble. More recent studies, however, especially by Norm Adams *et al.,* suggest that there is no safe threshold level for F.

One of the issues during the second phase of C.D. was whether or not "good" (low-F) genotypes could be introduced successfully into pastures dominated by "bad" (high-F) strains. Fortunately, our earlier Kojonup studies on mixtures had indicated that this was possible. Indeed, the two strains Daliak and Seaton Park "surfaced" in this way. My 1974 study on binary mixtures (18) further supported the notion that Dwalganup and Yarloop could be successfully kept in check; and more recently, Little and Beale (12), working on Kangaroo Island, showed that Trikkala (low-F) could successfully check Yarloop. However, the high-F strain Dinninup has usually proved intractable to control in this way, and for reasons which are not always clear.

So much then for evaluation in terms of one specific trait, viz. oestrogenicity. The C.D. problem, incidentally, provides what I regard as one of the best examples of co-operative research - in this case between State Departments of Agriculture, Universities and CSIRO.

Later years (from *c.* 1967)

More on "Success"

My 1977 paper (19) was a sequel to my earlier 1966 paper on success and failure of clover strains which has already been discussed in Section 2. The same three ecological-competitive situations, with emphasis on mixed-strain swards, were re-examined in the light of data from the intervening period.

The term success (E) was defined as follows:

"When 'success' is used without qualification it refers to biological success. Biological success signifies the long-term mean quantity of dry matter (D.M.) produced per annum, by a given strain, as a pasture component. For single-strain swards a biased estimate is the amount of residual D.M. (clover herbage + clover burr) at the end of the growing season. This could be improved to a rough estimate by including an animal intake term, itself an approximation. For mixed-strain pastures the same definitions apply, but measurement is much more difficult. A simple and rather imprecise measure is mostly used, viz. the long-term mean percentage frequency of seed (on a number basis) in the seed pool at the end of the growing season.

Agronomic success involves additional considerations. These include ability to increase soil N (not just legume D.M.) in rotations where cereal cropping occurs; and also pasture quantity and quality. Thus for pastures total D.M. (not just clover D.M.) becomes important - and in particular, available D.M. -while clover strains containing high levels of the oestrogenic isoflavone formononetin could have low agronomic success in sheep pastures (e.g. Davies et *al.* 1970)."

Broadly, for single-strain swards the earlier claim was considered valid, viz. that beyond some critical or threshold SPC (seed-producing capacity in pure swards) there is no major increase in E; but, in contrast to earlier conclusions, I suggested that this claim holds also for strain mixtures. Beyond the critical SPC, however, the probability of success for any strain or genotype would almost certainly be greater in single-strain than in mixed-strain swards. Contrast Figures 2 and 3. Frequency of cropping would of course influence the critical SPC in ley systems.

Clearly, and principally in mixed-strain swards, characteristics other than SPC must influence Z. Some of these, including phyto-oestrogen content, have been mentioned already. Others are considered in the

1977 paper. However, the point is made that some strains have proved successful in the field, and for these we have no satisfactory explanation. Two examples from the old "Heinz Trial" at Kojonup are Gingin and the plant introduction 434A (see Figure4).

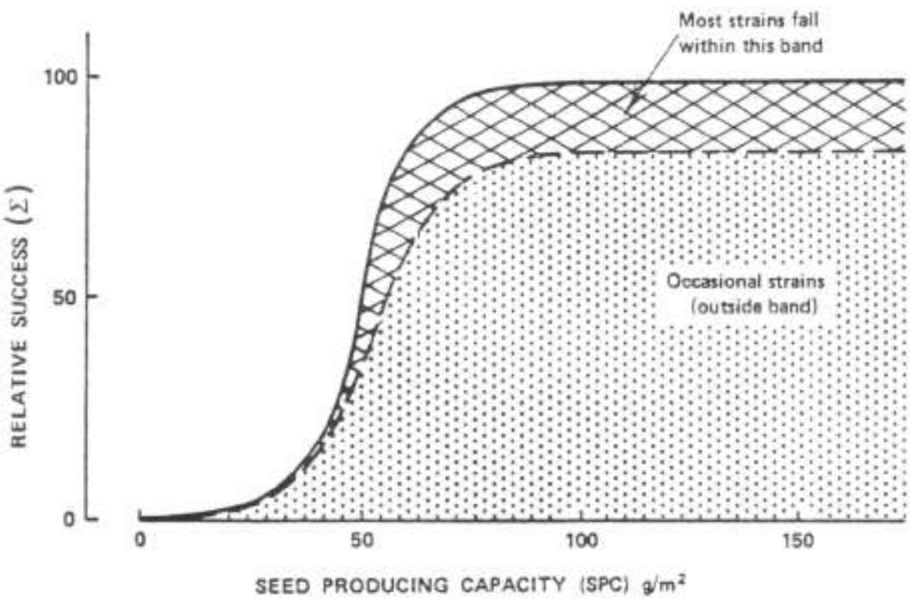

Figure2. Proposed schematic relationship between Σ and SPC for single strain swards. The scale for SPC applies to >500mm rainfall zone (for the 300-500mm zone the SPC values would be considerably lower).

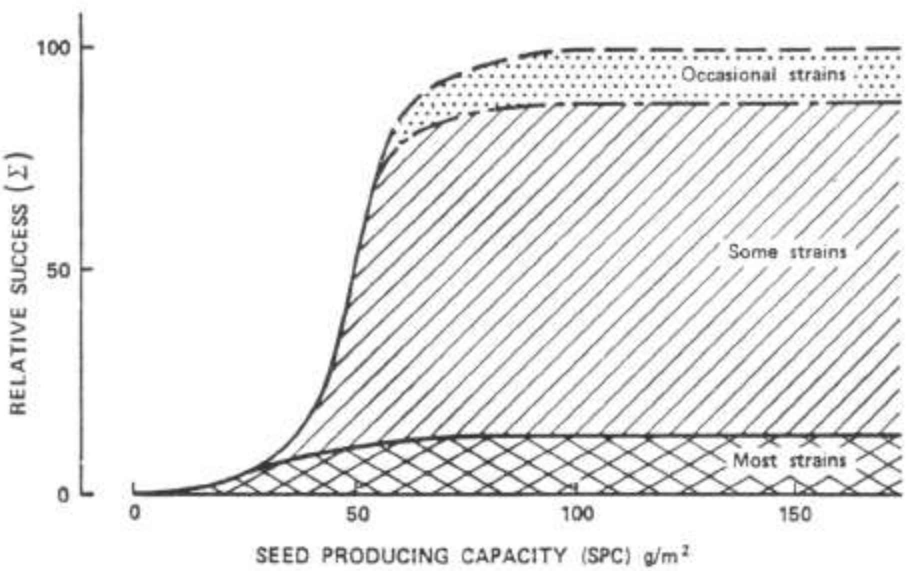

Figure3. Proposed schematic relationship between Σ and SPC for mixed-strain swards. Scale for SPC as in Fig.2.

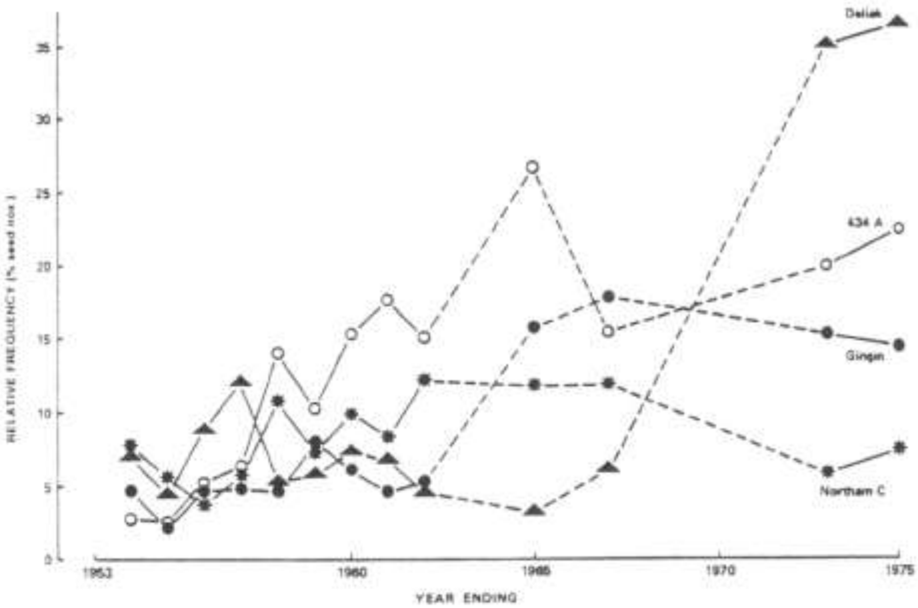

Figure4. Long-term time trends in relative frequency (seed no. basis) for dominant strains in P.491 experiment at "Glen Lossie", Kojonup. Experimental area open to farm grazing after 1962. In 1970 the area (and surrounding paddocks) cropped to rye grass, together with 3kg/ha each of Daliak and Seaton Park sub-clovers. To what extent the additional Daliak seed accounts for the high post-1970 figures for Daliak is not known; the effect may well have been slight.

A speculation on the possible importance of variant genotypes was raised at the end of the 1977 paper, but more on this point later.

I now turn, with some reluctance, to my first modelling effort. For several reasons, including C.D., and so-called "competition" effects, and the classical *Tribolium* (flour beetle) models, I was interested in the long-term outcome of binary mixtures of sub-clover strains. By the early 1980s we had collected enough field data to consider a model. Ross Maller (Mathematics and Statistics, CSIRO, now UWA) and Tony Pakes (UWA) were co-authors for the 1985 paper(20).

We produced a fairly simple deterministic model, combining life-cycle parameters based on measurements in monocultures, together with "k" parameters of the de Wit replacement series model, for mixtures during the seed production phase.

Results, for the Bakers Hill environment, indicated that Daliak rapidly dominated Dwalganup/Daliak mixtures; that Seaton Park dominated Yarloop/Seaton Park, but more slowly; and that the two strains Seaton Park and Midland B in admixture may co-exist for long periods *(c.* 100 years). For other mixtures, Dinninup usually dominated Geraldton and also Daliak - largely due to overtopping effects in the seed development phase. However, on a lateritic site with relatively poor water relations, a mixture of Dinninup and Daliak has co-existed for at least 5 years, usually with more than 50% Daliak.

Of the life-cycle parameters, SPC was of course important, but from sensitivity tests on the model *over-summer seed survival* was clearly the most important and the one with least field data. Other parameters of significance were juvenile plant establishment, and "k" values (for seed production) which can be management-dependent (7).

In principle, more complex mixtures could be modelled along similar lines, but for reasons which will soon be apparent, the task may eventually require a new approach.

Genetic Diversity

Until recently, and probably even today, the common view has been that the sub-clover component of pastures usually consists of one or two or at most only a few commercial strains. What caused us (I myself anyway) to question this view in the mid-1970s? First, the speculation that within the widespread Dwalganup areas considerable natural selection (e.g. for flowering time) may have occurred, but gone unnoticed. Second, the actual discovery in 1977 of plants from a pasture at Kojonup, sown in 1962 to commercial Dwalganup, with more leaf flecking than in Dwalganup itself (much more obvious in single plants than in swards) and which were about two weeks later flowering - in other words, Dwalganup-like plants or variants of Dwalganup. And third, the Californian studies by Allard and his colleagues on inbreeding annuals, e.g. (2) which suggested to us the possibility of considerable genetic diversity in old subterranean clover populations.

Thus began a study by Rossiter and Collins (21) of "Genetic diversity in old sub-clover (*T. subterraneum,* L.) populations in Western Australia", firstly in pastures initially sown to Dwalganup, and then in high rainfall pastures initially sown to Mt Barker. In this study, and in the present paper also, we use 'strain' as synonymous with 'variety', and to indicate separate genotypes which generally are named, and occur as substantial components of clover populations. 'Variants' or 'variant genotypes' are genotypes which usually are neither named nor common. Also, we use 'commercial strain' as synonymous with 'cultivar'.

The main finding was that populations from sites with <500 mm average rainfall usually consisted of one or two strains (Dwalganup with or without Geraldton), whereas from sites with >500 mm rainfall, whether old Dwalganup or old Mt Barker pastures, the clover populations on average consisted of *c.* 50% of a few known strains - including of course Dwalganup and Mt. Barker and 50% of numerous variant (or "unknown") genotypes. Possible reasons for the absence of variants from the lower rainfall sites are discussed in the 1988 paper. We suggest that the main source of diversity in old Dwalganup pastures is from occasional out-crossing of Dwalganup with Mt Barker, and possibly with Daliak and Dinninup; and, in old Mt Barker pastures, from out-crossing of Mt Barker with Woogenellup and Dwalganup, and possibly with Bacchus Marsh and Tallarook.

Seed isozyme patterns as well as morphological characters provided the basis for the above statements. It is worth noting that some Dwalganup-like variants and many Mt Barker-like and Woogenellup-like variants were *visually* indistinguishable from the standard strains.

There is one point of particular interest for the C.D. problem already discussed. We tested for F content throughout these studies and our evidence does *not* support the notion of selection in the field for high-F variants. A fortunate finding!

We recognised four basic types of population structure (Figure5) and we speculate that, given sufficient time, Type IV will predominate, at least for sites with >500 mm rainfall.

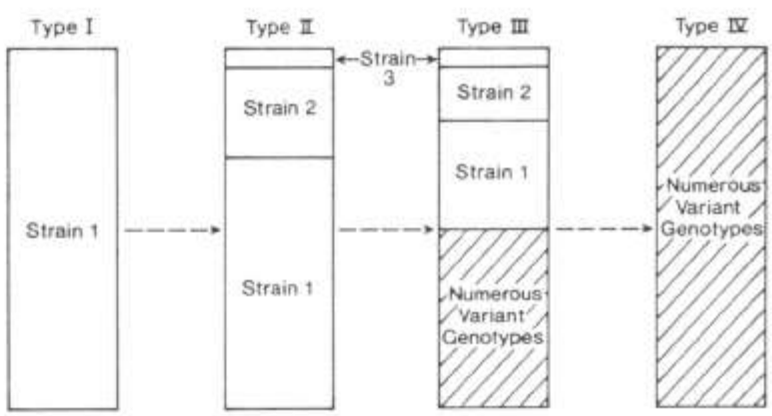

Figure5. Diagrammatic representation of proportionate distribution (no. basis) of genotypes in the four main types of population structure.

In a quite independent study in S.A., Phil Cocks (6) also found large numbers of variant genotypes (or "divergent strains"). Later, Cocks *et al.* (5) from a strain mixture experiment in a 700 mm rainfall area observed that variants constituted 30% of the population after a 19-year period. This result is closely similar to that from our "Heinz Trial" at Kojonup (550 mm rainfall), where in 1973, after 21 years, we found 38% of variant genotypes. As Phil Cocks pointed out, the large numbers of variant genotypes have serious implications for seed certification. The Mt Barker-like plants, Woogenelluplike plants, and so on - already referred to - seem bad news for certification. Perhaps we should consider relaxing the pure line (or single-genotype) concept for commercial strains of sub-clover.

Following on Allard's earlier Californian studies we suggest that substantial genetic diversity in sub-clover may be important if not essential for its long-term success. And it may well be that many genotypes within the population function as "Sisyphean" genotypes (24) among which dominance varies according to changes in the local environment. Furthermore, as Allard (1) suggested more than 25 years ago from work on lima beans, mixed populations of *numerous* genotypes may both stabilise and also increase overall production. Thus the earlier concept of success, centred around single (or very few) genotypes may eventually be abandoned.

If these ideas are anywhere near correct - and I believe they are, at least in >500 mm rainfall areas - there are clear implications for genotype testing and evaluation. Here I mention two of these which seem to me paramount: first, the need to test potential new cultivars in admixture with several, and if possible many, already adapted genotypes; and second, that the eventual success of any new cultivar may depend on performance in an integrated and interacting system of genotypes. Should this latter be the case, some difficulties would be expected in attempting to relate success to plant traits *measured from monoculture* (especially of single plants).

Evaluation for the Grazing Animal

To conclude this section I return briefly to the question of evaluation of genotypes in *animal terms* - one of the features of agronomic success. Earlier in this paper an instance was cited where the feeding value of dry clover pasture was greater for Bacchus Marsh than for Yarloop or Dwalganup. Differences in voluntary intake may have contributed to this, but differences in digestibility seem more likely, with the mid-season Bacchus Marsh senescing before physiological maturity, ie. "haying off". My colleagues (Graham Taylor and Louis Klein) have strong evidence for this phenomenon based on *in vitro* digestibility studies.

Apart from anti-quality constituents, eg. phyto-oestrogens, we know that clover genotypes can differ in voluntary intake, relative palatability, %N content, % non-structural carbohydrates, digestibility, etc. We also know that even within the one genotype differences of the order of 15-20 percentage units of digestibility can occur, as a consequence of location and/or year effects.

The extent of genotype differences - independent of differences in maturity or flowering time - for the characteristics mentioned remains for exploration. The general importance of some of these for breeding programs has been emphasised recently by Wheeler and Corbett (23). However, even if superior genotypes of sub-clover can be developed their role in self-regenerating clover pasture systems of the type envisaged could possibly be significant, but is probably not great. This follows from the "dilution effects" of non-clover components in the pasture and of other sub-clover genotypes already established.

The future?

In my life-time there has been little change in the key annual pasture plants at the *species* (or species-complex) level. This is despite a welter of introductions over many years. I believe this situation will continue unless some drastic modification to the pasture ecosystem intervenes. Nevertheless, it is now evident that changes have occurred at the *infra-specific* level - in sub-clover surely and quite likely in capeweed (Biddiscombe, Arnold, unpublished) and also in silvergrass (I have in mind Californian work on a closely-related species). Such changes may be expected to continue.

As my favourite philosopher of science Ian Hacking (11) says, there is more than one way to do science. Likewise for trying to improve sub-clover. In addition to the use of traditional plant breeding methods (and even genetic engineering) for tackling difficulties such as insect pests, plant diseases, etc., selection in the field - especially diversifying selection -may well have a steady and continuing role to play. This role could be facilitated by

the use of hybrid populations as originally suggested by Colin Donald in his 1937 paper, and indeed as was begun in 1978 at Geraldton and Mt Barker by my colleagues in the Department of Agriculture, and myself.

The days of "sub and super" have long since gone and those of clearing one million acres per year almost forgotten. Whether or not the areas of sub-clover pasture in W.A. - or indeed in southern Australia - will substantially increase in future years is problematic. In any case, I believe that our main job as pasture technologists and research scientists will be to build, maintain, and even improve our clover pastures, based on a better understanding of the world of pastures, including its structure as well as its mechanisms. Despite almost 2,000 research papers since as late as 1960 (22) we are still some distance from "knowing all about sub-clover".

1. Allard, R.W. (1961) - Crop Sci. 1:127.

2. Allard, R.W., Jain, S.K. and Workman, P.L. (1968) - Adv. Genet. 14:55.

3. Beck, A.B. (1964) - Aust. J. Agric. Res. 15:223.

4. Bennetts, H.W. (1944) - J. Dept. Agric. W. Aust. 21:104.

5. Cocks, P.S., Craig, P.S. and Kenyon, R.V. (1982) - Aust. J. Agric. Res. 33:679.

6. Cocks, P.S. and Phillips, J.R. (1979) - Aust. J. Agric. Res. 30:1035.

7. Collins, W.H., Rhodes, I., Rossiter, R.C. and Palmer, M.J. (1983) - Aust. J. Agric. Res. 34:671.

8. Curnow, D.H. and Rossiter, R.C. (1955) - Aust. J. Exp. Biol. and Med. Sci. 33:243.

9. Donald, C.M. and Neal-Smith, C.A. (1937) - J. Coun. Sci. Industr. Res. Aust. 10:277.

10. Donald, C.M. (1960) - Herb. Abst. 30:81.

11. Hacking, I. (1983) "Representing and Intervening" (Cambridge University Press).

12. Little, D.L. and Beale, P.E. (1988) - Aust. J. Exper. Agric. 28:737.

13. Lloyd Davies, H., Rossiter, R.C. and Mailer, R. (1970) - Aust. J. Agric. Res. 21:359.

14. Lloyd Davies, H. and Bennett, D. (1962) - Aust. J. Agric. Res. 13:1030.

15. Rossiter, R.C. (1959) - Aust. J. Agric. Res. 10:305.

16. Rossiter, R.C. (1966) - Aust. J. Agric. Res. 17:425.

17. Rossiter, R.C. (1970) - Aust. Veter. J. 46:141.

18. Rossiter, R.C. (1974) - Aust. J. Agric. Res. 25:757.

19. Rossiter, R.C. (1977) - Proc. Ecol. Soc. Aust. 10:76.

20. Rossiter, R.C., Mailer, R.A. and Fakes, A.G. (1985) - Aust. J. Agric. Res. 36:119.

21. Rossiter, R.C. and Collins, W.J. (1988) - Aust. J. Agric. Res. 39:1051.

22. Symon, D.E. (1986) - Dept. Agri. South Aust. Tech. paper No. 15.

23. Wheeler, J.L. and Corbett, J.L. (1989) - Grass and Forage Sci. 44:77.

24. Williams, G.C. (1975) - "Sex and Evolution" (Princeton Uni. Press).