

THE POTENTIAL VALUE OF CLONED RAMS FOR USE IN COMMERCIAL MERINO FLOCKS

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INTRODUCTION

It is already possible, by embryo splitting and nuclear transplantation, to produce a small group of genetically identical animals. This process is called cloning. In the future, by using embryonic stem cells as nuclear donors, it may be possible to produce very large clones. How might animal breeders use this technology and how much would the extra genetic improvement be worth?

One potential use of cloning is to clone embryos from the very best rams and ewes in the elite stud (nucleus) tier of a Merino population. Initially small numbers could be generated from each clone and these could be tested for characteristics which may be of importance to the breeder. The best clones could then be chosen and used to generate a large number of rams for use as sires in commercial flocks. The rate at which elite stud flocks improve is unaffected by this procedure but the genetic lag between these elite flocks and the commercial flocks may be lessened. In this paper the results of using a scheme such as this are compared with more usual procedures involving natural mating and artificial insemination.

METHOD

The basic reference point in this study is a traditional three-tiered hierarchical structure; the nucleus tier (which is closed) is analogous to the elite flock within a parent stud; the daughter or multiplier tier multiplies the genes of the elite flock and transfers these, via flock rams, to the commercial flocks, which are the base tier in the scheme. In this study, natural mating, artificial insemination, and four different uses of cloning are compared for their effectiveness in terms of the extent to which rams and ewes born in commercial flocks lag behind the nucleus tier when the population has reached equilibrium. The lag was calculated using the discounted gene flow method of Hill (1974).

Rams and ewes are assumed to be mated in their second year. There are five age groups of ewes (including one maiden group) for all tiers and all schemes. Rams are used once in the nucleus, twice in the daughter tier, and four times in the base tier. Mortality is 5% per annum in both sexes. Ewe fertility varies with age. The fertility in age groups two to five is respectively: 0.65, 0.82, 1.02, 1.08, resulting in a mean fertility of 0.883 when mortality is taken into account. The proportion of rams selected varies according to the mating ratio for each tier, which varies between breeding schemes. The proportions of rams and ewes retained are sufficient to maintain breeding flock sizes and provide rams to the next tier down whilst still allowing a low level of culling. Ratios between tiers approximate those in the industry as a whole if only the "special" parent stud flocks are included in the nucleus and other parent stud sheep are included in the daughter tier.

Three different selection objectives are considered: a highly heritable trait alone (fibre diameter; heritability = 0.5), a lowly heritable trait alone (heritability = 0.1), and the Woolplan objective 1 (Ponzoni 1988), which includes clean fleece weight, fibre diameter, number of lambs weaned, hogget weight and mature weight. For the first two objectives,

the selection criteria are the same as the objectives, while for the Woolplan index, the selection criteria include only clean fleece weight, fibre diameter and hogget weight. All criteria are measured on both sexes prior to breeding age. Phenotypic variances and covariances are those used in Woolplan. Genetic progress resulting from selection on the Woolplan index is expressed in terms of dollars per ewe-year.

Natural mating scheme (N)

The number of ewes in the three tiers is in the ratio 1:20:640 for nucleus:daughter:base respectively. The mating ratio is 1.5% rams in the nucleus and 3% in other tiers. The best 3.6% of nucleus-born ram lambs are retained in the nucleus and the next 70.4% are used as sires in the daughter level. 64.3% of ram lambs born in the daughter tier are used as flock sires in the base and the remainder are culled. No base-born rams are used as sires.

Artificial Insemination Scheme (AI)

There are many ways in which AI can be used in a hierarchical breeding structure. In this study, we consider just one use of AI, that is, to enable the use of the same sires in the nucleus and daughter tiers. The use of AI in commercial flocks is not considered. Identical sires are used in both nucleus and daughter tiers. Rams are used for one year only in the nucleus and twice in the daughter tier. Because fewer sires are needed to service the daughter tier, the number of breeding ewes in the nucleus need not be as large as that in the Natural mating scheme (N). The ratios of breeding ewe numbers are: 1:40:1280 for nucleus:daughter:base. The daughter to base ratio is unaltered from the previous case. The mating ratio is 1.5% rams in the nucleus, 0.076% in the daughter tier, where artificial insemination by nucleus level rams is utilised, and 3% in the base. Again 64.3% of rams born in the daughter tier are used as flock sires and the rest culled.

Clone Schemes

Four mating schemes which generate cloned flock rams are modelled. In every case, dams chosen for providing embryos for the generation of clones are assumed to be selected on their own performance. Selected dams are superovulated, inseminated, and the embryos collected to establish embryonic stem cell lines. These are used to donate nuclei to create the cloned embryos. In schemes requiring the evaluation of clone lines prior to use, ten individuals from each clone are tested. Selection is in effect a two-stage process, the first stage involving the selection of dams, and the second of clones. Selection differentials are calculated accordingly. The tested individuals from selected clone lines are able to be used immediately, but it is necessary to multiply the stem cell (clone) lines, produce embryos, and grow these embryos into mature rams, to supply enough rams to service the entire base. This process takes three years. Tested clones which do not meet the requirements for selection are discarded. Since embryos of the selected clones are used directly to generate flock rams, no daughter tier is required to provide rams for the base.

It is arbitrarily assumed that 15 clone lines will be required to service a base population of 1,280,000 ewes. This number is equivalent to the number of rams used in the nucleus of the artificial insemination scheme. The number of clones tested determines the selection intensity in the second stage of selection. Thus, if 100 clones are tested, then the best 15% will be selected. The number of ewes required in the first stage of selection, and therefore the final selection intensity, rely upon the success rate of establishing embryonic cell lines. Since large-scale cloning schemes are not currently operational, it is difficult to estimate a success rate for clone establishment. In the absence of concrete evidence, it is assumed that the proportion of lines surviving per superovulated ewe equals the proportion of ram lambs weaned per naturally mated ewe, i.e. 0.44. Clonally derived rams are used for four years in the commercial flocks, but clones are used to generate rams in one year only. As

in other schemes, the mating ratio in the nucleus is 1.5% rams. The nucleus to base ratio for breeding ewes is 1:1280, as for the AI scheme.

(i) Scheme C100: No evaluation of clones

Dams to generate clones are chosen on individual performance and the clones are not evaluated. This reduces the genetic merit of sires used in the base tier, since they are selected with lower accuracy, but allows the cloned rams to be used three years in advance of those undergoing evaluation (one year and a portion of the next is allowed to multiply the clones and get the required number of ram lambs on the ground, and the remaining period for the rams to reach breeding age). Rams are available for their initial mating three years after embryos are collected from the dams as some time is allowed to establish and multiply the cell lines.

(ii) Evaluated clone schemes (schemes C5, C10, C30)

Three different schemes are considered, involving the selection of different proportions of clones. The same number of lines is retained in each clone scheme but a different number of clone lines is tested for each clone retained. The identifiers for the alternative strategies reflect this difference in proportion selected at stage two. In scheme C5, for example, 20 clones are tested for each clone retained, giving a proportion selected of 5%.

The accuracy of selection, when n individuals from each clone are tested ($n=10$), and selection is for a single trait, will be r_{yz} (Equation 1), where y is the clone phenotypic average, z is the breeding objective, and where h^2 and H^2 are the narrow- and broad-sense heritabilities of the selection criterion respectively. If selection is on an index, the phenotypic variances and covariances of the selection criteria must be individually adjusted to reflect clone mean, and the correlation between the selection index and breeding objective recalculated accordingly. When there is no non-additive genetic variance, $H^2 = h^2$. All calculations here assumed no non-additive genetic variance.

$$r_{yz} = \sqrt{\frac{nh^2}{1+H^2(n-1)}} \quad (1)$$

RESULTS AND DISCUSSION

In all schemes considered, the genetic merit of the elite nucleus tier is the same. The base tier lags behind the nucleus by different amounts depending upon the efficiency with which genes are transmitted through the hierarchy. The lags (in years) and the difference in lag between each scheme and the natural mating scheme are presented in Table 1.

Perhaps the most surprising feature of the results is the high efficiency of the conservative AI scheme represented. When selection is on a trait of moderate to high heritability, AI is almost as good as clone schemes C100 and C30, and offers 67% of the improvement afforded by scheme C5. When the trait considered has low heritability, the benefits of cloning are more pronounced, in one case allowing the base tier to surpass the nucleus (which does not utilise cloning).

The results suggest that if the proportion of clones selected from those evaluated is high, evaluation will offer little additional benefit. Although unevaluated clone schemes use flock sires selected with lower accuracy, the reduction in generation interval relative to the evaluated clone schemes more than compensates for this. As the selection intensity among evaluated clones is increased, the increases in selection differential and accuracy override the long generation interval to increase the relative value of the evaluated clone schemes.

Wade et al. (1991) used the gene flow method to calculate the value of improving the

Table 1 Comparison of schemes in terms of genetic lag

Breeding scheme	Low Heritability		High Heritability		Woolplan Index	
	Lag*	Superiority [#]	Lag	Superiority	Lag	Superiority
natural (N)	8.9	0	8.9	0	8.9	0
artificial insemination (AI)	5.2	3.7	5.2	3.7	5.2	3.7
clone (C100)	4.6	4.3	4.6	4.3	4.6	4.3
clone (C30)	2.8	6.1	5.1	3.8	4.8	4.1
clone (C10)	0.7	8.2	4.2	4.8	3.7	5.2
clone (C5)	-0.3	9.2	3.8	5.1	3.4	5.5

*Lag indicates the difference in years between age group 1 in the nucleus and age group 1 in the base.

[#]Superiority indicates the advantage shown by the scheme (in years) over Scheme N.

genetic merit of flock rams. They found that each 1 unit increase in Woolplan index score increased ram value by \$29. In this study, the annual rate of improvement for index score was 5.51 units. Thus, each year rams bred with Woolplan breeding objective option 1 increase in value by \$158 (5.51 units x \$29). Using this value it is possible to estimate that a flock ram bred in the AI scheme for the Woolplan objective will be worth \$585 (\$158 x 3.7 years) more than similar rams from the natural mating scheme, and cloned rams from scheme C5 are worth \$869 (\$158 x 5.5 years) more than in scheme N and \$284 more than those from the AI scheme. Therefore, the costs of establishing embryo cell lines and producing rams from these lines would need to be less than \$869 for scheme C5 to be profitable. Dollar values for other traits will depend upon the economic value of one year's improvement and the genetic improvement which is shown. An increase in either will increase dollar value of the schemes considered.

There is a danger that once clone lines are established and evaluated, operators will be tempted to use the same clones over a number of years. The cost of producing each ram is reduced as more rams are generated from any clone, since common costs are shared among more individuals. The temptation to re-use clone lines will be exacerbated by producers wishing to use clones that others have tried and which have been proven in a commercial operation. Each additional year that a clone is used to generate flock sires forgoes one year of potential progress. Consequently, in the third year in which a clone in the C5 scheme is used, the rams produced will be no better than rams produced in an AI breeding scheme.

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