

# Minimising inbreeding in small populations by rotational mating with frozen semen

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## Summary

Mating plans are investigated in order to minimize inbreeding in small populations when frozen semen is available. For a single dam line it was found that specific sire rotations minimized the asymptotic level of inbreeding when semen is used repeatedly from certain generations. When semen of  $N$  foundation ( $G_0$ ) sires is used rotationally over generations it is shown that the inbreeding level asymptotes to  $1/(2^{N+1}-2)$ . However, if only  $G_0$  sires are used then all genes will eventually descend from the founder sires. Inbreeding can be reduced further by using sires from generation one ( $G_1$ ) and later as this retains genes from the founder dams in the long-term gene pool. If semen from  $N G_0$  sires and  $N$  unrelated  $G_1$  sons is used rotationally then inbreeding asymptotes to  $(2^{N-1}+1)/(2^{2N+1}-2)$ . When there are more founder dams than sires, the asymptotic inbreeding can be reduced even further by using the semen of half-sib  $G_1$  sires in rotation. Optimal rotations using full-sib  $G_1$  sires or generation 2 (or later) sires will lower the asymptotic inbreeding also, but generally not by much. It was found that when unlimited frozen semen from a specified group of sires was available, the optimal mating plan was achieved by selecting each generation the sire with the least co-ancestry with the current female of the dam line.

## 1. Introduction

Genetic conservation schemes often involve the management of small breeding populations that are prone to problems associated with inbreeding and loss of genetic diversity. The problems are usually fitness-related and include increased risk of extinction (Frankham, 1999) and inbreeding depression affecting not only reproductive performance but also many other characters (Lynch & Walsh, 1998).

Smith (1977) discussed the idea of freezing semen from the founder sires and using it repeatedly in a control line to avoid inbreeding and genetic drift for many generations when estimating genetic trend. Sonesson *et al.* (2002) showed that by repeatedly using semen from  $N$  foundation (generation 0) sires, the level of inbreeding asymptotes to  $1/(2N)$  with random mating. However, if only sires of generation 0 ( $G_0$ ) are used then all genes will eventually descend from the founder sires. They show that the level of inbreeding can be reduced further by using sires from generation

1 ( $G_1$ ) as this retains genes from the founder dams in the long-term gene pool. They propose a scheme where the semen from  $N G_0$  sires and  $N G_1$  sires is used alternatively for dams of odd and even generation numbers respectively. With this scheme, the level of inbreeding asymptotes to  $1/(3N)$  with random mating each generation. It was concluded using computer simulation that no further reduction in inbreeding is possible using semen from later generations.

Sonesson *et al.* (2002) recommend mating sires and dams randomly each generation. However, selecting mates at random each generation is unlikely to minimize long-term inbreeding as it does not optimally manage mating patterns across generations to produce the best possible genomes in the long-term. Kinghorn & Shepherd (1999) call this optimal management mate selection, and implement mate selection for the next generation only; but it has to be performed across multiple generations to minimize long-term inbreeding (Sanchez-Rodriguez *et al.*, 2003), with the result being a breeding programme structured across generations.

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When frozen semen of  $N$  foundation sires is available Smith (1977) suggested using the semen from each sire on the daughters of other sires in rotation in order to avoid inbreeding for the initial  $N$  generations. Sonesson *et al.* (2002) use a restricted form of rotational mating by recommending that  $G_0$  and  $G_1$  sire groups be used in odd and even generation numbers respectively. The minimum achievable rate of inbreeding for a normal breeding population with discrete generations, developed by Sanchez-Rodriguez *et al.* (2003), also involves an intrinsic rotation of purpose over generations.

Rotational mating is a structured breeding programme which is commonly practised in cross-breeding systems (Bourdon, 1997). Sires of different breed compositions are mated rotationally in order to maximize heterozygosity and thus hybrid vigour in the progeny. The analogy here is that each foundation sire (and dam) is similar to a different breed, while the generation 1 sons are similar to crossbred sires. This paper extends the suggestion of Smith (1977) by mating beyond the initial  $N$  generations to study the asymptotic properties of rotational mating plans when frozen semen of various sire groups is available, and thereby optimize mating plans to manage long-term contributions, and thus minimize inbreeding, in small populations.

**2. Theory and methods**

Let  $C_{sd}$  denote the co-ancestry between sire  $s$  and dam  $d$  which by definition is identical to the inbreeding coefficient of their progeny. We shall use often the following identities:

$$C_{xy} = \frac{1}{2}(C_{xsy} + C_{xdy}),$$

where  $s_y$  and  $d_y$  are the sire and dam of individual  $y$ ;

$$C_{xx} = \frac{1}{2}(1 + F_x),$$

where  $F_x$  is the inbreeding coefficient of individual  $x$ .

(i) *Inbreeding when rotationally mating only  $G_0$  sires*

Consider a scheme involving only sires of the founder generation ( $G_0$ ) mated in rotation over generations and initially restrict attention to two  $G_0$  sires. We assume the  $G_0$  sires are unrelated and non-inbred for simplicity although the derivation could incorporate modifications to these assumptions. Figure 1a illustrates the breeding plan in which the two founder  $G_0$  sires ( $s_1, s_2$ ) are mated in rotation to a single dam line.

Consider the inbreeding coefficient  $F_{t+1}$  of dam  $d_{t+1}$ . Using her ancestral line shown in Fig. 1a, the following equation for  $F_{t+1}$  can be derived:

$$F_{t+1} = \frac{1}{2}(C_{s_1s_2} + \frac{1}{2}(C_{s_1s_1} + C_{s_1d_{t-2}})).$$

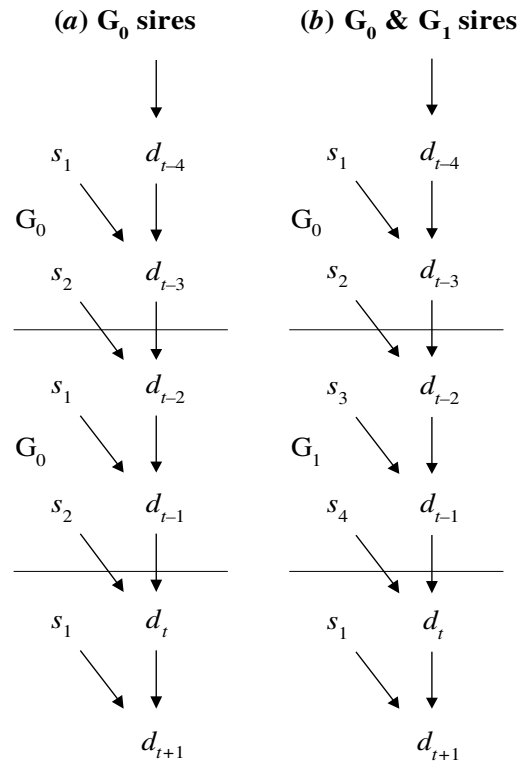


Fig. 1. Rotational mating plan for a dam line using either (a) semen from generation 0 sires ( $G_0$  sires), or (b) semen from generation 0 and generation 1 sires ( $G_0$  &  $G_1$  sires).  $G_1$  sires  $s_3$  and  $s_4$  have different dams and are the sons of  $G_0$  sires  $s_1$  and  $s_2$  respectively. Dam  $d_{j+1}$  is the daughter of dam  $d_j$ .

Now as  $C_{s_1s_2} = 0$ ,  $C_{s_1s_1} = \frac{1}{2}$  and  $C_{s_1d_{t-2}} = F_{t-1}$ , the equation becomes

$$F_{t+1} = \frac{1}{2}(\frac{1}{2} + F_{t-1}).$$

It can be shown that this equation holds for progeny of any sire. Generalizing this in an obvious manner it can be shown that for  $N$  sires used in a similar rotational mating plan,  $F_{t+1} = \frac{1}{2^N}(\frac{1}{2} + F_{t-N+1})$ . This difference equation can be solved (Mickens, 1990) to show the existence of an asymptotic level of inbreeding  $F_{eq}^0$  which, by substitution, is found to be

$$F_{eq}^0 = \frac{1}{2(2^N - 1)}. \tag{1}$$

This is the asymptotic level of inbreeding in the dam line when the  $N$  founder  $G_0$  sires are mated in rotation. This will also be the asymptotic level of inbreeding for multiple dam lines as each dam line has the same pattern of inheritance over generations.

(ii) *Inbreeding when rotationally mating  $G_0$  and unrelated  $G_1$  sires*

Consider a rotational mating scheme which uses two unrelated and non-inbred  $G_0$  sires ( $s_1, s_2$ ) and two

unrelated  $G_1$  sires ( $s_3, s_4$ ).  $s_3$  is the son of  $s_1$  and  $s_4$  is the son of  $s_2$ , and  $s_3$  and  $s_4$  have different  $G_0$  dams. Fig. 1b illustrates the breeding plan in which the four sires are mated in the rotation  $(s_1, s_2, s_3, s_4) = (G_0, G_1)$  to a single dam line such that  $G_0$  sires are mated in turn in the first phase of the rotation, and each  $G_1$  son replaces his sire in the second phase. This cycle of length four ( $2N$ ) is then repeated.

Consider the inbreeding coefficient  $F_{t+1}$  of dam  $d_{t+1}$ . Using her ancestral line shown in Fig. 1b, we can derive the following equation for  $F_{t+1}$ :

$$F_{t+1} = \frac{1}{2}(C_{s_1s_4} + \frac{1}{2}(C_{s_1s_3} + \frac{1}{2}(C_{s_1s_2} + \frac{1}{2}(C_{s_1s_1} + C_{s_1d_{t-4}}))))$$

Now as  $C_{s_1s_4} = C_{s_1s_2} = 0$ ,  $C_{s_1s_3} = \frac{1}{4}$ ,  $C_{s_1s_1} = \frac{1}{2}$ , and  $C_{s_1d_{t-4}} = F_{t-3}$ , we have that

$$F_{t+1} = \frac{1}{2^2}(\frac{1}{4} + \frac{1}{2}(\frac{1}{2} + F_{t-3}))$$

This equation holds for progeny of any sire. By analogy it can also be shown that for a similar rotational mating of  $2N$  sires, comprised of  $N$  unrelated and non-inbred  $G_0$  sires and  $N$  unrelated  $G_1$  sons, that  $F_{t+1} = \frac{1}{2^N}(\frac{1}{4} + \frac{1}{2^N}(\frac{1}{2} + F_{t-2N+1}))$ . The asymptotic level of inbreeding  $F_{eq}^{01}$  is found to be

$$F_{eq}^{01} = \frac{2^N + 2}{4(2^{2N} - 1)} \tag{2}$$

(iii) Asymptotic inbreeding for full-sib  $G_1$ , half-sib  $G_1$  and  $G_2$  sire rotations

Full derivations of the equations in this section can be found in Shepherd & Woolliams (2004). Consider a mating plan which uses  $(k+1)N$  sires, comprising  $N$   $G_0$  foundation sires, each with  $k$  full-sib  $G_1$  sons, and otherwise unrelated. The sons are grouped into  $k$  groups, denoted  $G_{1(1)}, G_{1(2)}, \dots, G_{1(k)}$ , with each group containing a full-sib from each of the sire families. Using the sire rotation  $(G_0, G_{1(1)}, G_{1(2)}, \dots, G_{1(k)})$ , the asymptotic level of inbreeding  $F_{eq}^{01(kf)}$  is

$$F_{eq}^{01(kf)} = \frac{2^{N-1}(2^{kN} - 1) + 2^N - 1}{2(2^N - 1)(2^{(k+1)N} - 1)} \tag{3}$$

Figure 2a1 shows the full-sib rotational mating plan for  $N=2$  and  $k=2$ . As  $k \rightarrow \infty$ ,  $F_{eq}^{01(kf)} \rightarrow \frac{1}{4(2^N - 1)} = \frac{1}{2}F_{eq}^0$ .

When there are many more foundation dams than sires a sound breeding strategy is to use half-sib  $G_1$  sires in rotation. Assume that each of the  $N$   $G_0$  sires has  $k$  half-sib  $G_1$  sons and that each  $G_1$  sire has a different  $G_0$  dam. If the sons are grouped into  $k$  groups, denoted  $G_{1(1)}, G_{1(2)}, \dots, G_{1(k)}$ , with each group containing a half-sib from each of the sire families, and mated in the rotation  $(G_{1(1)}, G_{1(2)}, \dots, G_{1(k)})$ , the asymptotic level of inbreeding  $F_{eq}^{01(kh)}$  is

$$F_{eq}^{01(kh)} = \frac{2^{kN} + 3 \times 2^N - 4}{8(2^N - 1)(2^{kN} - 1)} \tag{4}$$

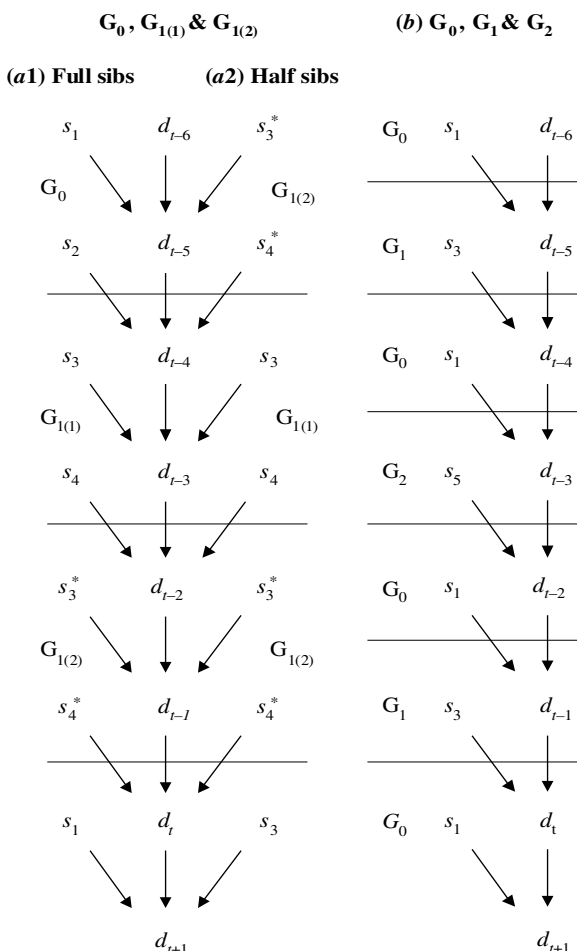


Fig. 2. Rotational mating plan for a dam line using either (a) semen from generation 0 and generation 1 sires ( $G_0, G_{1(1)}$  &  $G_{1(2)}$ ), or (b) semen from generation 0, generation 1 and generation 2 sires ( $G_0, G_1$  &  $G_2$ ).  $G_1$  sires  $s_3$  and  $s_4$  (denoted as a group by  $G_{1(1)}$ ) have different dams and are the sons of  $G_0$  sires  $s_1$  and  $s_2$  respectively.  $G_1$  sires  $s_3^*$  and  $s_4^*$  (denoted as a group by  $G_{1(2)}$ ) are either full-sibs (a1) or half-sibs (a2) of  $s_3$  and  $s_4$  respectively.  $G_2$  sire  $s_5$  is the son of  $G_1$  sire  $s_3$ . The sire and dam of  $G_2$  sire  $s_5$  are full-sibs. Dam  $d_{j+1}$  is the daughter of dam  $d_j$ .

Figure 2a2 displays the half-sib rotational mating plan for  $N=2$  and  $k=2$ . As  $k \rightarrow \infty$ ,  $F_{eq}^{01(kh)} \rightarrow \frac{1}{8(2^N - 1)} = \frac{1}{4}F_{eq}^0$ .

Consider a mating plan using  $G_0, G_1$  and  $G_2$  sires. Assume the parents of the  $G_2$  sires are full-sibs. For  $3N$  sires, comprised of  $N$   $G_0$  sires,  $N$  unrelated  $G_1$  sons and  $N$  unrelated  $G_2$  grandsons, mated in the rotation  $(G_0, G_1, G_0, G_2)$ , it was found that the asymptotic level of inbreeding depended on the progeny's sire. Hence the asymptotic level of inbreeding, averaged over the progeny of sires in a complete cycle of the rotation, *ave*  $F_{eq}^{012}$ , is given by

$$ave F_{eq}^{012} = \frac{2^{3N} + 7 \times 2^{2N-2} + 2^N + 17 \times 2^{-3}}{4(2^{4N} - 1)} \tag{5}$$

Figure 2b shows the rotational mating plan for  $N=1$ .

(iv) *Optimization algorithm*

Computer software was written to simulate a breeding scheme conducted over a number of discrete generations. The matings over all generations were simultaneously allocated to optimize a specific objective function (e.g. average inbreeding over the last  $T$  generations). The optimization algorithm used was an extension of the evolutionary algorithm (i.e. Differential Evolution) described by Kinghorn & Shepherd (1999) which performs mate selection for a single mating occasion. Details of this extended algorithm will be published in a separate paper. Here a simplified breeding scenario was assumed in which a single dam line was bred by each generation, mating the dam to a sire selected from a specific group of sires. Two strategies of sire selection were evaluated. In the multiple-generation breeding strategy (OPT $\infty$ ) sires were selected simultaneously over all generations such that the average asymptotic level of inbreeding in the dam line was minimized. The one-generation breeding strategy (OPT1) focused only on the current mating and each generation the sire with the least co-ancestry was selected for mating with the current female candidate of the dam line.

### 3. Results and discussion

In most scenarios investigated, the mating plan produced by OPT1 was identical to that produced by OPT $\infty$ . Thus selecting the sire with the least co-ancestry with the current female candidate minimized the average asymptotic level of inbreeding in the dam line. This equivalence was produced because an initial breeding structure was set up in which unlimited frozen semen of a specific sire group was available for mating to the dam line(s). However, OPT1 will not always produce the optimal design as it does not allow the preferential mating of relatives which is often required in an optimal design (Sanchez-Rodriguez *et al.*, 2003). For example, OPT1 will not allow the full-sib mating required to produce a  $G_2$  sire and thus would not find a benefit in using  $G_2$  sires unless they existed *a priori*. Basically OPT1 does not allow look-ahead mate selection (Shepherd & Kinghorn, 1998) which is implicit in a multiple-generation breeding strategy.

Results obtained from the optimization software were as follows, and agreed with those derived analytically. When  $G_0$  males were the only available male candidates for mating each generation, the rotational mating plan where each  $G_0$  sire was used in turn (as in Fig. 1*a*) produced the smallest asymptotic level of inbreeding (as in eqn (1), which can be shown to be the true lower bound). When  $N$   $G_0$  males and  $N$  unrelated  $G_1$  sons were available for mating each generation, the rotational mating plan where each  $G_0$  sire was first used in turn, then in the next phase each

$G_1$  son replaced his sire and so on (as in Fig. 1*b*), produced the smallest asymptotic level of inbreeding (as in eqn (2)). When  $N$   $G_0$  males,  $N$  unrelated  $G_1$  sons and  $N$  unrelated  $G_2$  grandsons (whose parents are full-sibs) were available for mating each generation, the rotational mating plan where each  $G_0$  sire was first used in turn, then in the next phase each  $G_1$  son replaced his sire, then each  $G_0$  sire was again used in turn, then in the last phase each  $G_2$  grandson replaced his grandsire, and so on (as in Fig. 2*b*), produced the smallest average asymptotic level of inbreeding (as in eqn (5)).

Table 1 shows the asymptotic level of inbreeding achieved for different numbers of  $G_0$ ,  $G_1$  and  $G_2$  sires either with rotational mating or with random mating plans. The formulae for random mating are derived by Sonesson *et al.* (2002). Although the random mating formula assumes unrestricted random mating for the scheme using only  $G_0$  sires, when  $G_0$  and  $G_1$  sires are used the mating plan is a restricted form of random mating (denoted by *RandomR* in Table 1). In this case Sonesson *et al.* (2002) recommend mating  $G_0$  and  $G_1$  sires randomly at odd and at even generation numbers respectively. From Table 1 it can be seen that the asymptotic level of inbreeding is decreased by rotational mating compared with either restricted or unrestricted random mating. The asymptote with rotational mating decreases exponentially with  $N$ , rather than  $1/N$  as found by Sonesson *et al.* (2002) for random mating.

An interesting observation in Table 1 is that if  $F_N^0$  is used to denote the asymptotic level of inbreeding in a rotational scheme using  $N$  founder  $G_0$  sires only (i.e.  $F_{eq}^0$ ) and  $F_N^{01}$  is defined similarly, then  $F_N^{01} = \frac{1}{2}(F_N^0 + F_{2N}^0)$ . That is, rotational mating with  $N$  sires from each of generations 1 and 2 produces an asymptotic level of inbreeding which is halfway between that achieved with rotational schemes using  $N$  and  $2N$  founder sires.

Contrary to the claim of Sonesson *et al.* (2002), the asymptotic level of inbreeding can be lowered if semen from  $G_2$  sires is used in a rotational mating plan (Table 1). For example, for  $N=2$ , using  $G_0$ ,  $G_1$  and  $G_2$  sires in a rotation as in Fig. 2*b* produces an asymptotic level of inbreeding of 0.096 compared with 0.1 which results from a rotation as in Fig. 1*b* using only  $G_0$  and  $G_1$  sires. However, the decrease in the asymptotic level of inbreeding is only small and would not be of much value in practice. The decrease is a result of using more genes from each founder dam by optimally managing the contributions through the  $G_2$  sires. As a result of the full-sib mating, we expect a  $G_2$  sire to have 50% of his genes from his founder dam, but as half of these genes are transmitted through his  $G_1$  sire, only 25% of his genes are of founder dam origin and passed independent of his  $G_1$  sire. Hence to optimally manage this independent 25% of founder dam genes



Table 1. Asymptotic level of inbreeding achieved using semen from sires of either generation 0 ( $F_{eq}^0$  using  $G_0$  sires), generations 0 and 1 ( $F_{eq}^{01}$  using  $G_0$  &  $G_1$  sires) or generations 0, 1 and 2 (ave  $F_{eq}^{012}$  using  $G_0$ ,  $G_1$  &  $G_2$  sires). The  $N$  sires used from each generation are mated to dams either completely at random (Random) or in a rotation (Rotation) as in Fig. 1 or Fig. 2b. For RandomR,  $G_0$  sires are mated randomly at odd generation numbers and  $G_1$  sires are mated randomly at even generation numbers

Mating plan	No. sires ( $N$ ) used from each generation						
	1	2	3	4	5	...	$N$
$F_{eq}^0$ using $G_0$ sires							
Random <sup>a</sup>	0.5	0.25	0.167	0.125	0.1	...	$\frac{1}{2N}$
Rotation	0.5	0.167	0.071	0.033	0.016	...	$\frac{1}{2(2^N - 1)}$
$F_{eq}^{01}$ using $G_0$ & $G_1$ sires							
RandomR <sup>a</sup>	0.333	0.167	0.111	0.083	0.067	...	$\frac{1}{3N}$
Rotation	0.333	0.1	0.04	0.018	0.008	...	$\frac{2^N + 2}{4(2^{2N} - 1)}$
ave $F_{eq}^{012}$ using $G_0$ , $G_1$ & $G_2$ sires							
Rotation	0.319	0.096	0.039	0.017	0.008	...	eqn (5) <sup>b</sup>

<sup>a</sup> Formulae for Random and RandomR derived by Sonesson *et al.* (2002).

<sup>b</sup> Formula for ave  $F_{eq}^{012}$  given in eqn (5).

we replace the  $G_1$  sire in the next phase of the rotation ( $G_0, G_1$ ) (Fig. 1b) with the  $G_2$  sire to produce the optimal rotation ( $G_0, G_1, G_0, G_2$ ) (Fig. 2b) so that  $G_2$  sires contribute only 25% to the long-term gene pool.

Full-sib  $G_1$  sires have not only 50% of their genes from their founder dams but also all founder dam genes are transmitted independent of their full-sib brothers. Hence we find that using full-sib  $G_1$  brothers produces even lower asymptotic levels of inbreeding than using  $G_2$  sires if mated optimally (Table 2). For example, when  $N=2$ , the average asymptotic level of inbreeding, ave  $F_{eq}^{012}$ , using  $G_0, G_1$  and  $G_2$  sires is 0.096 (Table 1), whereas for two groups ( $k=2$ ) of full-sib  $G_1$  sires in an optimal rotation  $F_{eq}^{01(2f)}$  is 0.087 (Table 2). Using full-sibs gives more certain access to each allele carried by each founder dam, since a  $G_1$  sire will carry only one of the pair. Thus when managed optimally over generations, a lower asymptotic level of inbreeding is obtained. There is only a small decrease in the asymptotic inbreeding by using more than two or three ( $k > 2$  or 3) full-sib  $G_1$  groups (Table 2).

If there are many more foundation dams than sires then a strategy which utilizes the large pool of foundation dam genes (and thus lowers inbreeding further) is to use groups of half-sib  $G_1$  sires in rotation. However, unlike the optimal rotation for full-sib  $G_1$  sires, the optimal rotation for half-sib  $G_1$  sires does not include any  $G_0$  sires. For example when  $N=2$  and  $k=2$ , sire rotations ( $G_0, G_{1(1)}, G_{1(2)}$ ) and ( $G_{1(1)}, G_{1(2)}$ )

(Fig. 2a1 and a2) produce asymptotic inbreeding levels of 0.087 and 0.1 respectively for full-sib  $G_1$  sires, and 0.074 and 0.067 for half-sib  $G_1$  sires. However, as the number ( $k$ ) of  $G_1$  sons increases, the genes from  $G_0$  sires become redundant and so the benefit from using  $G_0$  semen in the optimal full-sib rotation decreases.

As expected the asymptotic level of inbreeding is lower for half-sib than for full-sib  $G_1$  sires due to the greater number, and optimal use, of foundation dam genes in half-sib  $G_1$  sire rotations (Table 2). Just two groups of half-sib  $G_1$  sires produce a lower optimal asymptotic level of inbreeding than a large (infinite) number of full-sib  $G_1$  sire groups. This decrease in asymptotic inbreeding continues as more groups of half-sib  $G_1$  sires are included in the rotation such that with a large (infinite) number of half-sib  $G_1$  sire groups the asymptotic level of inbreeding is one-quarter of that achieved using the optimal rotation with only  $G_0$  sires (Table 2). However, there is only a small benefit in using more than two or three half-sib  $G_1$  sire groups (Table 2).

If each generation sires are bred by full-sib mating, the average asymptotic level of inbreeding can be lowered in optimal rotations using sires from generation 2, lowered further again by using sires from generation 3, and again for generation 4 sires (e.g. for  $N=2$ , ave  $F_{eq}^{012} = 0.096201$ , ave  $F_{eq}^{0123} = 0.0960790$ , ave  $F_{eq}^{01234} = 0.0960789$ , respectively), but the decreases are only small. They result from the fact that there are still founder dam genes available in these sires which

Table 2. Asymptotic level of inbreeding achieved using semen of  $G_0$  and full-sib  $G_1$  sires ( $F_{eq}^{01(kf)}$  using  $G_0$  & full-sib  $G_1$  sires) or half-sib  $G_1$  sires ( $F_{eq}^{01(kh)}$  using half-sib  $G_1$  sires). Both use  $kN$   $G_1$  sires which consist of  $k$  (either full- or half-sib) sons of each of the  $N$   $G_0$  sires. The sons are grouped into  $k$  groups, with each group containing one son from each sire family, in the rotational mating plan ( $G_0, G_{1(1)}, G_{1(2)}, G_{1(3)}, \dots, G_{1(k)}$ ) for  $F_{eq}^{01(kf)}$  using  $G_0$  & full-sib  $G_1$  sires and ( $G_{1(1)}, G_{1(2)}, G_{1(3)}, \dots, G_{1(k)}$ ) for  $F_{eq}^{01(kh)}$  using half-sib  $G_1$  sires. Figure 2 shows the rotational mating plan for full-sib (a1) or half-sib (a2)  $G_1$  sires when  $N = 2$  and  $k = 2$

No. $G_1$ sire groups ( $k$ )	No. $G_0$ sires					
	1	2	3	4	...	$N$
1	0.333	0.1	0.04	0.018	...	$\frac{2^N + 2}{4(2^{2N} - 1)}$
$F_{eq}^{01(kf)}$ using $G_0$ & full-sib $G_1$ sires						
2	0.286	0.087	0.036	0.017	...	eqn (3) <sup>a</sup>
3	0.267	0.084	0.036	0.017	...	eqn (3) <sup>a</sup>
⋮	⋮	⋮	⋮	⋮	⋮	⋮
∞	0.25	0.083	0.036	0.017	...	$\frac{1}{4(2^N - 1)} = \frac{1}{2}F_{eq}^0$
$F_{eq}^{01(kh)}$ using half-sib $G_1$ sires						
2	0.25	0.067	0.024	0.010	...	eqn (4) <sup>b</sup>
3	0.179	0.048	0.019	0.008	...	eqn (4) <sup>b</sup>
⋮	⋮	⋮	⋮	⋮	⋮	⋮
∞	0.125	0.042	0.018	0.008	...	$\frac{1}{8(2^N - 1)} = \frac{1}{4}F_{eq}^0$

<sup>a</sup> Formula for  $F_{eq}^{01(kf)}$  given in eqn (3).

<sup>b</sup> Formula for  $F_{eq}^{01(kh)}$  given in eqn (4).

are independent in origin from the dam founder genes in sires of earlier generations. For example, 12.5% of the genes of  $G_3$  sires are of both founder dam origin and passed independent of  $G_0, G_1$  and  $G_2$  sires, and so  $G_3$  sires are only mated every  $8N$  generations in the optimal rotation.

In general to minimize the asymptotic level of inbreeding using semen from sires of  $t$  generations the optimal sire rotation is  $R_t = (R_{t-1}, R_{t-2}, \dots, R_2, R_1, R_0, G_t)$ , which is defined recursively for  $t > 0$ , while for  $t = 0, R_0 = G_0$  as in Figs 1 and 2b. The importance of this result is that if semen from earlier generations becomes exhausted then those generations are simply deleted from the optimal rotation. For example, if  $G_0$  semen is depleted then for  $N = 2$  the asymptotic inbreeding can be reduced from 0.167 using a  $G_1$  sire rotation to 0.1375 using the sire rotation ( $G_1, G_2$ ). Hence including semen from sires of generation 2 or later in a rotation will also lower the asymptotic inbreeding but is of no practical use unless frozen semen from earlier generations is exhausted.

The analogy with rotational crossbreeding discussed in Section 1 can be demonstrated quantitatively when semen of  $G_0$  sires is used rotationally. The

proportion of F1 heterosis retained at equilibrium in a rotational crossbreeding system that uses purebred sires ( $H_p$ ) is given by

$$H_p = \frac{2^N - 2}{2^N - 1} \tag{6}$$

where  $N$  is the number of breeds in the system (Bourdon, 1997). The  $N$  founder  $G_0$  sires in the rotational purebreeding mating plan correspond to the  $N$  breeds in the crossbreeding system. This correspondence is confirmed by noting that using eqns (1) and (6) it can be shown that  $H_p = 1 - 2F_{eq}^0$  where  $1 - 2F_{eq}^0$  would be the asymptotic level of heterozygosity in the rotational purebreeding mating plan.

In practice there may be many other issues which are important when planning a breeding programme for a small population. For example, if there are known relationships between the founder parents then these should be taken into account in the mating plan together with resource limitations or mating constraints. If the issues can be quantified and put into an appropriate objective function then an optimization algorithm like that discussed in Kinghorn &

Shepherd (1999) or a multiple-generation strategy like that in this paper needs to be used in order to appropriately and simultaneously account for all the issues involved. However, as noted by Sonesson *et al.* (2002) a drawback of conservation breeding programmes using only frozen semen is that no further evolution (genetic adaptation or accumulation of mutations) is allowed in the conserved population. Hence as in any breeding programme the breeding objective needs to be given careful consideration.

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