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Fisher's Reticulate Mating System for Immigration into an Improved Stock

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Summary. A reticulate mating system is described which was designed by the late R.A. Fisher to permit the introduction of new genetic variability into an improved stock by immigration. Analysis of part of a long-term experiment to alter the degree of dominance of the mutant *Sd* in mice using the system demonstrates a rapid response. Its applicability to stocks of animals of economic value is considered.

Key words: Mating system – Selection – Mice

Introduction

The problem of what to do with a population which, owing to inbreeding or other causes, fails to respond to artificial selection is far from being solved. The introduction of new genetic stock to such plateau'd, or indeed any selected, populations is fraught with difficulties (Webb 1976). The late R.A. Fisher proposed a solution to this problem in his report on an experiment to alter the dominance relationship between the mutant Danforth's Short Tail (*Sd*) and its normal allele in the mouse (Fisher and Holt 1944). The main part of this paper reported a straightforward selection experiment on the tail lengths of *Sd+* mice. But in a brief appendix, Fisher outlined the mating system which is described here as reticulate, and was able to show by means of histograms of *Sd+* tail lengths that the method did generate variability and could produce animals of sufficient merit to be suitable for incorporation into the selected stock. It was used extensively in this same experiment after 1944 with apparent success, but the results have not been published. The purpose of the present paper is to analyse these results over a crucial period of the experiment and to consider whether the method may not have relevance to economic selection programmes.

Mating System

An idealised version of the reticulate mating system designed by Fisher is shown diagrammatically in Figure 1. Each cycle of the system takes two generations. 'E' represents the elite improved stock, which is derived from the strain originally selected by Fisher and Holt (1944). In each cycle the opportunity exists for new stock to be incorporated into the elite from the offspring of 'A'-type matings of the previous cycle if these prove to be of sufficient merit. The 'A' matings are between elite stock and selected offspring of 'B' matings which also supply mates for the outcross stock in 'C' matings. The 'B' matings themselves are between selected offspring of the 'A' and 'C' matings of the previous cycle. As Fisher pointed out the relative proportion of elite stock genes in the three mating types, 'A', 'B', 'C' are 75%, 50% and 25%, and the system will equilibrate to these proportions no matter how it is started. But an outcross of elite stock to form mating 'B_0', as used by Fisher, will ensure immediate

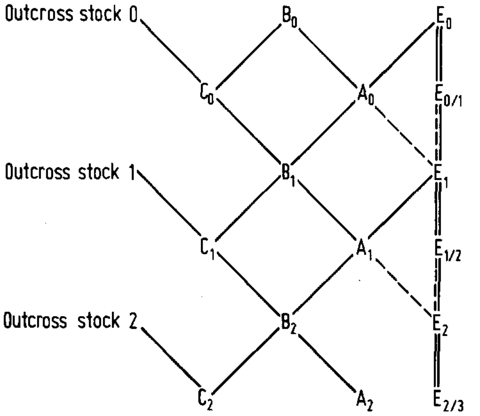

Fig. 1. The reticulate mating system. 'E' indicates the elite selected stock. The dotted lines represent alternatives whereby an 'A'-type animal is only used if up to the elite standard

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equilibrium in this particular example of the system. However the choice of three stages between the outcrosses and the elite stock appears arbitrary and nowhere does Fisher justify this choice.

Selection was thus within generation/type in the mating types 'A', 'B' and 'C', but animals from 'A' matings were only to be incorporated into the elite stock if of sufficiently high standard on the selection criterion. Selection at the 'A-E' boundary is therefore necessarily over mating types.

Figure 1 is idealised because in the elite stock of the experiment to be described 'no attempt was made to separate the generations, as these would not be separate in a wild colony' but instead the animals were 'kept unmated until, at intervals of two months, the stock available for selection could be surveyed' (Fisher and Holt 1944, p. 104).

Experimental Data

The purpose of the experiment was so to modify the phenotype of the genotype *Sd+* that mice of this genotype should appear indistinguishable from normal homozygotes, the *Sd* gene should be rendered recessive and support given to the theory of the evolution of dominance (Fisher 1928, 1931). To this end, at each selection of available *Sd+* heterozygotes those with the longest tails at 4 weeks of age were mated inter se. (In its original form the experiment also had a negative selection line which succeeded to the point that *Sd+* heterozygotes were infertile as normally are *Sd Sd* homozygotes).

The period of the experiment to be analysed covers the six and a half years from June 1 1951 to the end of 1957. Between Fisher and Holt's (1944) report and this time, the stock had been through at least two serious bottlenecks; the first, in the mid-1940s, caused by disease, and the second, early in 1951, when it was suspected that the gene fuzed (*Fu*) might accidentally have been introduced into the stock, imperilling the whole experiment. At this stage the experiment had to be restarted with a few mice which could be demonstrated to be clear of *fuzed*, and whose offspring are represented by generations 'A_{-1}' and '$\mathrm{E}_{-2/-1}$' in Table 1. It would appear from the results for generations '$\mathrm{E}_{-1/0}$' and '$\mathrm{E}_{0/1}$' that further progress was not possible within this restricted elite stock. By the end of 1957, the tails of some *Sd+* mice were as long as their normal contemporaries. Long tailed *Sd+* mice are usually distinguished by a bluntness of the end of the tail caused by the absence of the finely graded minute terminal vertebrae of normals. But by this time classification of these heterozygotes had become difficult and an *Sd Sd* homozygote, usually a lethal genotype, had bred (Wallace and Herbertson 1969). From the point of view of the objective the experiment was by then successful, and from our present viewpoint further analysis becomes difficult.

During this period the mice were housed in what had been a bedroom of Fisher's residence with normal domestic central heating and light during working hours in the winter. Various technicians and students had charge of the

Table 1. The number (n) of *Sd+* heterozygotes in each generation/mating type, the mean ($\bar{x}$ in mm.) and variance (σ^2 in mm^2) of their tail lengths, together with the mean variance of each mating type weighted by the number of degrees of freedom available in each generation, with its 95% confidence limits

Generation	Mating type	C			B			A			E integer			E non-integer			Mating type
		n	$\bar{x}$	σ^2	n	$\bar{x}$	σ^2	n	$\bar{x}$	σ^2	n	$\bar{x}$	σ^2	n	$\bar{x}$	σ^2	Generation[a]
−1								9	38.3	104				26	38.2	61	−2/−1
0		15	41.9	104	5	40.8	104	11	45.8	31				38	33.5	235	−1/0
1		12	39.0	44	30	49.0	59	18	43.7	29	18	49.3	27	10	39.1	122	0/1
2		35	45.5	77	12	48.8	89	7	47.6	33				35	47.6	45	1/2
3		11	48.4	213	40	50.5	68	43	48.0	73	17	55.8	43	13	50.1	35	2/3
4		29	24.0	237	38	53.3	79	31	52.4	71				29	52.8	92	3/4
5		33	35.7	76	35	50.2	74	9	45.4	61	11	53.9	12	15	57.7	20	4/5
6		32	41.2	60	36	50.8	124	27	53.5	46	26	50.0	79	30	52.0	120	5/6
7		12	51.3	31	25	46.2	52	26	53.2	29	37	51.5	58				
8		21	28.3	61	57	47.8	49	46	52.0	17				34	51.1	34	7/8
9		82	35.5	77	149	46.4	109	110	56.6	48	47	57.0	38	16	47.9	36	8/9
10		8	35.4	135	44	51.0	71	23	56.9	38	27	59.0	20	31	60.5	57	9/10
Total d.f./mean σ^2		279		94.5	460		84.2	348		46.5	176		43.3	266		86.5	
(95% limits)			(80.6-112.3)			(73.4-96.3)			(40.4-54.3)			(35.5-53.9)			(74.5-106.0)		

[a] The E non-integer mating type was not bred in discrete generations: see text page 281.

experiment, but using the same techniques as Fisher and Holt (1944) and supervised throughout by Dr. Margaret E. Wallace.

Analysis

The tails of adult male laboratory mice are usually longer than those of adult females (Butler 1952). Although the sex differential in body size becomes clearly apparent between 20 and 30 days of age (Korkman 1957) or 21 and 29 days (Bakker 1974), and increases thereafter, the differential in body size but not tail length is demonstrable at 28 days (Morton 1970). This is related to a lower correlation between tail length and body weight at earlier than later ages (Bakker 1974).

In the present data on *Sd+* mice the females had insignificantly longer tails by 0.52 ± .46 mm, as estimated by the mean weighted difference of *Sd+* mice between sexes within litters, based on a weighting of 2,755 effective comparisons. As these figures imply, negative sex differentials were approximately as common as positive, and the data from males and females were pooled for all subsequent analysis.

In the data reported by Fisher and Holt (1944) the mice were kept in garden sheds and an attempt was made by these authors to adjust for the known effects of temperature on tail length (see, for example, Harrison, Morton and Wiener 1959). By the time that the present data were collected the conditions for housing the mice had improved, as described above, although accurate temperature control was still not possible. The data were therefore analysed to see whether there was evidence for seasonal effects on the tail lengths of *Sd+* mice. To discover what constituted a season under these conditions the change of tail length in consecutive months within each generation/mating type was calculated and a weighted mean for all generation/mating types derived. The result is shown in Figure 2, but the pattern shows little relation to the known climatic variation in Cambridge. Three of the 12 monthly changes were significant at the 5% level, from January to February, June to July and July to August, of which only the first would seem to relate possibly to a change in temperature. Since no sensible method of adjusting for season appeared from this analysis, it was decided to continue the main analysis without adjustment.

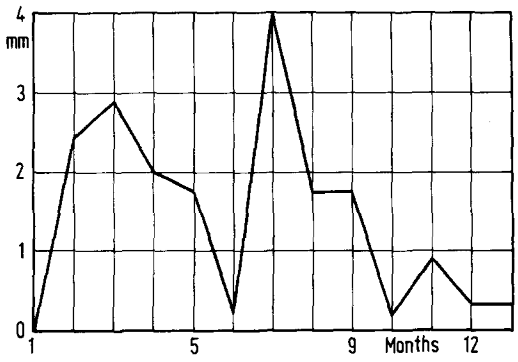

Fig. 2. Monthly variation in the tail-length of *Sd+* mice

The results are shown in Table 1, in the form of the number of heterozygous mice measured in each generation/mating type and the means and variances of their tail lengths. It was noted earlier that Fisher had decreed that the 'E' type was not to be mated in discrete generations, and that mice of type 'A' were only to be introduced into the elite 'E' stock when they proved themselves superior to the existing 'E' line. The data for the 'E' type have been divided into two sets, those deriving from an 'A x E' mating which are described as 'E integer' and the remainder called 'E non-integer' (c.f. Fig. 1). The 'E non-integer' data are grouped into 'generations' by which, for example, all 'E x E' matings made up between the 'E_1' and 'E_2' sets of matings are described as '$E_{1/2}$'. The means of both types of 'E' mating thus represent the progress of the experiment, but while the variances of the 'E integer' type are within generation variances as are those of the 'A', 'B' and 'C' mating types, the 'E non-integer' variances contain between, as well as within, generation effects and are therefore not comparable with the remainder.

As 'E non-integer' matings provided one parent to many of the 'A' and 'E' integer matings, calculation of the response to selection in any of the 'E' and 'A' matings is not straight-forward. In the report of Fisher and Holt (1944) this problem could be solved because the 'E' matings were made at regular two month intervals and an exact comparison could be made between the mice selected and those available for selection. But in the present data matings were made less regularly and it was not possible to define the population available, nor to present the response to selection in the elite stock in the manner of Fisher and Holt (1944). The 'C' type matings derived from outcrosses to laboratory strains of various degrees of inbreeding which had not been selected for traits of body size or conformation and, in particular, did not contain the *Sd* gene. Thus only 'B' type matings can be used to estimate the response. The ratio of the response to the selection differential in each mating in 'B' was calculated and weighted by the number of offspring measured to give an overall weighted mean realised heritability of 0.82, based on 456 offspring. There is some consistency between this estimate and results from other parts of the data. A realised heritability of 0.82 suggests that the variance due to causes other than additive genetic was 15 mm^2. No generation/mating type showed a variance substantially below this figure. Of those ('A_8', 'E_5' and '$E_{4/5}$') whose overall variance was similar to 15 mm^2, two were involved in one of the few sequences of selection ('$E_{4/5}$' → 'E_5' → '$E_{5/6}$') that showed no progress, once the reticulate system was operative.

If this variation of 15 mm^2 is taken to be environmental in origin and equally applicable to all mating types, the additive genetic variation within a generation was, in round figures, 79, 69, 31 and 28, mm^2 in types 'C', 'B', 'A' and 'E integer' respectively. If we make the extreme opposite assumption that the variation is due to non-additive effects and that it is absent from types 'A' and 'E integer', the additive genetic variation in type 'B' still remains half as great again as the total variation in types 'A' and 'E integer'.

The genetic variation thus generated was sufficient to overcome the mostly adverse effects of the outcrosses to unselected stock. And the remarkable progress in tail length in the experiment from 'A_0' onwards, compared to that in '$E_{2/-1}$' to '$E_{0/1}$' before the system had taken effect, suggests that the system is capable of the continuous accumulation of advantageous modifers into the elite stock.

Discussion

Evidence from the main analysis that in the 'B'-type matings the great majority (82%) of the variation in the tail length of *Sd+* mice was additive genetic in origin demonstrated that at least no great harm accrued from ignoring seasonal effects. It also contrasts with the earlier reports of this experiment where Fisher calculated that about 30% of the variation in 'E'-type matings, that is about 23 mm^2, was due to additive genetic and about 52 mm^2 to other effects (Fisher and Holt 1944). But the value of 23 mm^2 for the additive genetic variance is in close agreement with that of 28 mm^2 proposed above for the E integer mating type on the assumption that there was 15 mm^2 of environmental variation common to all mating types in the present data. The much greater value for other variations in Fisher's analysis may simply be the result of the cruder housing of the mice at that time, or it may be that Fisher's attempt to correct for seasonal effects by adding or subtracting a proportion of the known monthly variation in the tail lengths of homozygous normal mice contributed as much variation as it removed. Baker and Cockrem (1970) showed that the response of tail length to increased temperature is dependent on the genetic background control of tail length. It may be that since all *Sd+* mice had tails shorter than the optimum for the temperature in which they were reared there were in fact no seasonal affects in the original data to be corrected.

As noted in the introduction, the reason for analysing these data after the passage of some 20 years was to see whether it could indicate a useful method of immigration of new material into improved commercial strains of outbreeding organisms. The experiment was not designed for this purpose in that, for example, we do not know what level the 'E'-type would have reached in the absence of the reticulate mating immigration system. But it is clear from Table 1 that little progress was made before and much after the system was introduced, so that the system may be thought to have proved itself in general. But it is less clear that the particular form of the system chosen by Fisher, with four mating types, 'E', 'A', 'B' and 'C' was necessarily the best.

Fisher gives no justification for this particular choice, noting only that types 'A', 'B' and 'C' will have ¼, ½ and ¼ of the 'E' stock genotype respectively. By the same reasoning in any reticulate mating system of this kind in which there are *m* mating types, they will have $\frac{m}{m}$('E'), $\frac{m-1}{m}$ ('A'), $\frac{m-2}{m}$ ('B') etc. of the 'E' genotype. But this is only strictly true if the selection practised does not alter the ratio of 'E' to 'non-E' genes which, assuming effective selection intensity and heritability, implies that the outcross is of equal genetic merit to the 'E' stock; and in these circumstances the only value of reticulate mating system is in allowing for the otherwise confusing effects of heterosis (Webb 1976). It has been suggested (M.E. Wallace, personal communication) that Fisher may have used as many as four mating types to be more certain of selecting any recessive modifiers.

In fact, in the experiment heritability was very high, precluding important effects from heterosis, or from recessive genes, and the performance of the offspring of the outcross ('C') matings usually was much below those of the 'E' stock. Wallace (1972, 1976) has demonstrated a single gene locus with large modifying effects on the tail length of *Sd+* mice, so that the total number of loci involved in the selection experiment may not have been large. Taking these points together it seems reasonable to assume that the selection was powerful enough to allow only the best genotypes to be selected, and the following conclusions can be drawn. Inferior homozygotes in the stock used for the outcross will produce genetic segregation in the 'B' mating type; heterozygotes in the outcross stock with one allele identical to that in the 'E' stock and the other inferior will produce genetic segregation in the 'C'-type; and heterozygotes both of whose alleles are inferior to the fixed allele in the 'E' stock will produce segregation in both 'B' and 'C' mating types; only superior selected alleles will pass to the 'A' stock and will inflate the 'A' and 'E' genetic variances equally. The variances shown for 'C', 'B', 'A' and 'E' integer types in Table 1 suggest that this is in fact what happened. If this is so the experiment would have been simpler and as effective with one less stage in the reticulate mating system; as shown by the fact that the 'A'-type differs little in variance or mean from the 'E' integer. But it is only right to point out that Fisher believed the heritability to be very much lower than in fact transpired.

The system described here for introducing further genetic variation into a selected line is in marked contrast to those reported by Osman and Robertson (1968). These authors crossed a selected strain of *Drosophila* to the original unselected stock from which it arose, or to briefly selected sub-strains of this stock. They were therefore attempting to select for those genes which, for reasons of small effect or low frequency, had been missed in the original selection. In the *Sd* experiment, diverse strains of different origin to the selected line were used for outcrosses; few outcross animals were used at each stage, and it appears that genes of relatively large effect were selected. The reticulate mating system is designed to select those genes which have the largest beneficial effect from outcross strains in which they are not rare, but which were absent or rare in the stock from which the original selection was made. Thus unfortunatly the selection theory developed by Osman and Robertson (1968) to explain the results of their experiment is inapplicable to the present situation, being based on the premises of small selective effect or low gene frequency.

In applying the reticulate mating system to animals of economic value, the selection theory developed by Dickerson (1973) is relevant. He showed that, where combined family plus individual selection is practised, alternating generations of inbreeding and outbreeding can considerably increase the rate of response to selection, by as much as 15% in the growth characters of pigs, for example. Dickerson envisages his system within a closed population, which would need to be large for the cycle of inbreeding and outbreeding to be achieved without either a loss of selection intesity or accumulation of excess inbreeding. His subsequent recognition of this difficulty led Dickerson to doubt the usefulness of the method (Dickerson and Lindhe 1977). But slight modification of the reticulate mating system as shown in Figure 1 could obviate the problem: for example those selected from generation 'E', which were closely related could be mated to produce '$E_{1/2}$', while the remainder were used for 'A_1', providing the inbreeding phase; while 'E_2' could be produced by mating selections from 'A_1' and '$E_{1/2}$' for the outbreeding phase.

The above argument is an example of a general use of the reticulate mating system for circumstances, which are common in competitive situations of animal breeding, in which it can be predicted by calculations such as those given in Smith (1969) that a selection plateau is imminent. The improvement in long term gain due to avoidence of inbreeding can be calculated by Smith's method and compared to any loss resulting from lowered selection intensities through having several mating types per generation.

Finally, the reticulate mating system can clearly be used for introducing a new attribute, previously not selected for or pre-eminent in a strain which is inferior in other economic traits, without lowering the merit of the elite line.

In general, the system described can be used in any situation in which immigration would be desirable, were it not that it would reduce the performance of the selected stock. The problem remaining to be solved is the number of mating types required to protect the elite stock from deterioration. Although, a rigorous mathematical demonstration seems difficult the following general conclusion appears reasonable. Where heritability in outbred stocks is high, heterosis unimportant and available stocks of fair genetic merit exist, a reticulate mating system similar to that showed in Figure 1 but with only two stages between the outcross and the Elite stock should be sufficient. To the extent that these desiderata are not met, more stages may be necessary. Where the level of performance of stocks available for outcrossing is known, a reasonable rule of thumb would be to allow half as many stages as the number of generations that the elite stock needed to rise from that to its present level. If the number of stages necessary makes the method impractical, this is but another way of saying that immigration is no solution to the problem unless other stocks of reasonable performance can be obtained.

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