Selection for resistance to myxomatosis in domestic rabbits (*Oryctolagus cuniculus*)

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(Received 4 June 1969)

The course followed by a disease resulting from the infection of an animal by a pathogen invariably displays some variation, and selection in a population for resistance or susceptibility generally results in an advance in the direction of selection (Gowen, 1951; Hutt, 1958). A build up of innate resistance to myxomatosis in the wild rabbit population after the release and spread of myxoma virus in Australia in 1950 was both predicted and found (Fenner, 1953; Marshall & Fenner, 1958; Marshall & Douglas, 1961). A programme of selection for resistance to myxoma virus in domestic rabbits was begun in this laboratory in 1954. It was planned to obtain estimates of the genetic factors underlying resistance, and in time, to build up a strain of genetically resistant rabbits for evaluating the usefulness of new virus strains, collected from the field, or produced as a result of laboratory manipulation. The present paper describes the progress made by selection.

MATERIALS AND METHODS

Rabbits

Domestic rabbits donated by, or purchased from, other scientific institutions made up the initial population. Complete breeding records were kept, each animal being identified by a number tattooed in the ear. Animals were housed in wire cages in a brick building, and fed on a dry pellet diet and water *ad lib.*, supplemented by fresh green feed. Heating was provided to give a minimum temperature of 70° F in winter, but no cooling was available and during hot spells in summer the temperature often reached 95° F. during the day. In July 1963 a temperature controlled room for housing animals under test became available. The temperature régime adopted was a constant $72 \pm 4^\circ$ F. raised to $85 \pm 3^\circ$ F. for 24 hr. 3–4 days after infection (Sobey, Menzies, Connolly & Adams, 1968). Rabbits were weaned at 8 weeks of age and tattooed. Rabbits were infected with the appropriate virus after the age of 16 weeks; this was considered a sufficient interval from birth to obviate the effects of maternal antibodies (Fenner & Marshall, 1954).

Virus strains

Four strains of virus were used, the highly virulent Standard laboratory strain (SS) Aust/Corowa/12-52, Uriarra (U) (Aust/Corowa/2-53-1, Fenner & Marshall, 1957) and two strains of KM 13 which will be referred to as KM 13/1 (Aust/

Corowa/12-52/2, Fenner & Marshall, 1957) and KM13/2 (Aust/Corowa/12-52 A, Marshall, 1959). Although a standard inoculation dose of 500 lesion-forming units, (L.F.U., Sobey *et al.* 1966) was aimed at, this was found on titration to vary between 100 and 1000 L.F.U. in different tests.

Grade

The time interval between infection and when an animal could be used for breeding varied widely in animals which recovered from infection. Bucks were often sterile, or became fertile only after extended periods of time (Sobey & Turnbull, 1956). For this reason it was found inconvenient to record data on a generation basis and a system of grading was introduced. Unselected laboratory rabbits were graded 0. If a rabbit of grade 0 (or any other grade) recovered from infection it was allotted a grade of 1 (or its previous grade plus 1). The offspring of a 0×1 mating were graded 0.5, and 0.5 grade rabbits which recovered from infection became grade 1.5, and so on. A grade was thus a generation equivalent. It was possible for a rabbit to have two gradings one before and one after it had been exposed to infection. The pre-inoculation grade will be given in the text.

Selection was based in the first instance on an animal's ability to survive virus infection. While the percentage mortality was high it was necessary to breed, where possible, from all surviving rabbits and from unchallenged selected does. When the percentage mortality was low, as during 1960-1, criteria other than just survival were resorted to. The range of symptoms increased with decreased mortality, from rabbits which showed only a clearly demarcated lesion at the site of injection to rabbits which were very severely diseased. On the basis of symptoms the following classes were devised and used as a basis for selection:

- (a) Reaction at the site of injection only.
- (b) Mild eye reaction usually in the form of small and isolated inflamed areas.
- (c) Eyelids red and swollen but without exudate.
- (d) Eyes swollen and closed with exudate.

The survival time (s.t.) of all rabbits which died was recorded. For the purposes of statistical analysis, each rabbit was given a score equal to the time it lived in days after injection with virus: animals which survived infection with SS were given a score of 30 and those which survived any of the more attenuated virus strains a score of 60.

EXPERIMENTAL RESULTS

Response to selection

The response to selection, using each of the four strains of virus is illustrated in Fig. 1 in which recovery is plotted against grade. The number of animals tested with each strain at each grade is given in Table 1. SS/1 represents Standard strain before temperature control for testing and SS/2 Standard strain with temperature controlled and an elevated temperature 3-4 days after infection. Environmental variation was evident even during the period 1963-8 when temperature control was available for testing animals. This is illustrated in Fig. 2 where the percentage recovery from infection with the four strains of virus is plotted

against time and the average annual mean grade. These data also show the times during which the different strains were used. During 1956 and 1957 a severe outbreak of *Pasteurella multocida* infections depleted rabbit numbers and also adversely affected the ability of rabbits to recover from myxomatosis. The data for 1956 and 1957 have, for this reason, been omitted from Fig. 1. No explanation can be offered for the trough in 1965; unselected animals also showed a depressed s.t. during this period.

Fig. 1. The percentage recovery plotted against grade for the four virus strains used during selection. SS/1 denotes animals tested before and SS/2 those tested after temperature control for testing became available.

Table 1. *The number of animals tested with each virus in the different grades*

(SS/1 denotes the animals tested before and SS/2 those after temperature control for testing to Standard laboratory strain of virus.)

Virus strain	No. of animals tested in each grade													
	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	5.4	5.0	5.5	6.0	6.5
U	297	207	261	250	41	—	—	—	—	—	—	—	—	—
KM13/2	—	—	28	107	313	213	78	24	—	—	—	—	—	—
KM13/1	178	264	578	509	201	46	—	54	76	—	—	—	—	—
SS/1	—	—	—	—	—	48	118	293	164	—	—	—	—	—
SS/2	138	—	—	—	—	—	—	67	297	316	331	256	153	34

Because of overlapping generations any one grade was tested over about a 3-year period and, as seen in Fig. 1, most of the environmental variation has been ironed out, and response to selection appears to be linear for all four virus strains. Clearly selection in the earlier stages with attenuated viruses raised the level of resistance of the animals to the fully virulent SS virus.

Maternal antibodies

The consequences of passive immunity were examined by Fenner & Marshall (1954) up to the age of 47 days. The offspring from does with maternal antibodies were found to have a higher percentage recovery, an extended survival time and

Fig. 2. Percentage survival of the animals and their mean annual grade, illustrating the viruses used for selection in time, and the large variation due to environmental factors. Each point represents the mean of 100–300 animals tested in two to four experiments. Between 1958 and 1967 there was an average annual increment of 0.5 of a grade.

Table 2. *The percentage recovery and mean survival times of offspring from does which had recovered from KM 13/1 with a post-inoculation grade of 1 and offspring from does not exposed to virus with a pre-inoculation grade of 1.*

(Data collected between 1957 and 1960).

Offspring from	No. of animals	% recovery	$\bar{x}$ s.t.
Recovered does	268	13.1	26.3
Uninfected does	204	13.7	25.7

a lowered rate of infection by mosquito bite when compared with the progeny of animals without maternal antibodies. To ensure that passive immunity was not influencing the recovery rate in selected animals challenged between 16 and 23 weeks of age the offspring from does which had recovered from KM 13/1 with a post-inoculation grade 1 were compared with the offspring from unchallenged does with a pre-inoculation grade 1, with respect to percentage survival and mean survival time. The results, shown in Table 2, demonstrate that by the age of 16–23 weeks, maternal antibodies no longer influenced the course of the disease.

Fig. 3. Changes in the distribution of survival time with selection, illustrating the extended survival times of animals with increased genetic resistance. All animals were infected with the standard laboratory strain of myxoma virus.

Heritability analysis

Intra-sire correlation

The basis of selection was quantal (death or recovery) and heritability (h^2) could not be estimated by direct parent-offspring comparison. Survival time (s.t.) within virus strains is probably a valid index of genetic resistance; as shown in Fig. 3

the mean s.t. increases with increased selection. It is probably valid to compute the h^2 of resistance using s.t. as an index where the percentage recovery is low, and recoveries are allotted an arbitrary s.t. However, the bimodality of the s.t. distribution is exaggerated as the percentage recovery increases, and at some point the assumption of a normal distribution must become untenable. On the assumption that a percentage recovery of 10 % was not excessive, h^2 was estimated by intra-sire correlation (Lerner, 1950) on data collected between 1957 and 1960, where KM 13/1 was used. Each buck was mated to five does selected at random and the s.t.'s of four of her offspring selected at random recorded for h^2 analysis. The data were grouped into two lots, each consisting of the results from 320 offspring of 16 bucks and 80 does; the first lot had a 5 % and the second a 10 % recovery rate. The average grade rose from 0.3 for females and 1.0 for males in the first lot to 1.2 and 1.7 in lot 2. The results are presented in Table 3.

Table 3. *Estimates of heritability (h^2) of resistance to myxomatosis. Intra-sire correlations based on $\log_{10}$ survival times as an index of resistance after infection with KM 13/1.*

Lot no.	Estimated h^2	Estimated 95 % limits	D.F.	% recovery within lot
1	Sire 0.41	0-1	15	5
	Dam 0.37	0.13-0.53	64	—
2	Sire 0.22	0-1	15	10
	Dam 0.83	0.75-0.88	64	—
Total	Sire 0.33	0-0.62	31	7.5
1 and 2	Dam 0.64	0.40-0.70	128	—

In the first lot both sire and dam components of h^2 are about 40 %, whereas in lot 2 there is an elevated dam component 83 % and a lowered sire component 22 %. The sire components of h^2 are based on relatively few animals and the difference is well within the limits of error. The elevated dam h^2 in lot 2, however, appears to be outside the limits of experimental error, suggesting an increase in the dam component with time, i.e. with increased selection. This apparent maternal effect cannot be accounted for by maternal antibodies (see Table 2). A possible explanation could be that the apparent maternal effect is a litter effect due to seasonal variation, more pronounced in lot 2 than in lot 1 because of the time of year at which litters were tested. Individuals within litters were invariably infected at the same time so that any variation affecting survival, such as seasonal effect, would increase between-litter variation or spuriously decrease within-litter variation. This would, in estimates of intra-sire correlations, tend to elevate the dam component of h^2 . The frequency distribution of the time at which litters were tested is shown in Fig. 4. It will be seen that in lot 1 the majority of litters were tested during the warmer months of the year, 42 % during the summer months of December, January and February and only 14 % during the winter months June, July and August; whereas in lot 2 23 % were tested in the summer and 30 % in the winter. The differential effect of the times of testing litters is reflected in the range of mean survival times over the year; 18.2-25.6 days for lot 1 and 16.0-34.0 days for lot 2.

Achieved heritability measured by probit analysis

Resistance is an all-or-none character and in the absence of some quantitative index of resistance does not lend itself to parent/offspring comparison. A method of determining the heritability of all-or-none characters has been given by Robertson & Lerner (1949) using the heterogeneity chi-squared in determining the genetic

Fig. 4. The number of litters tested at different months of the year for two lots each of 80 litters. 42 % of litters were tested during the summer months (D.J.F.) and 14 % during the winter months (J.J.A.) in lot 1 and 23 and 30 % respectively in lot 2.

variance. The subclass numbers in the present data were too small for the above method to be used. Using the same basic proposition

$$\text{heritability} = \frac{\text{genetic improvement}}{\text{phenotypic selection differential}}$$

and the probit transformation, the following method was used to calculate heritability.

$$h^2 = \frac{\Delta P_0}{\frac{1}{2}(Is + Id)},$$

where Is and Id are the selection differentials of sire and dam respectively, and ΔP_0 is the difference in mean phenotype between the parental and filial generations. Is and Id are estimated from the percentage of survivors in the population of

which the parent was a member when it was infected with virus to test its resistance. These tests were done in batches of 30–150 animals and the selection differential of an individual is measured from the percentage survival of the batch in which it was tested. The percentage is expressed as the amount in standard deviations by which the mean of that fraction of a normal distribution would exceed the mean of the whole distribution, on the assumption that whatever is responsible for survival is a normally distributed quality with a sharp cut-off

Fig. 5. An assumed normally distributed factor determining survival indicating the basis of the probit value and selection differential.

separating values adequate for survival from those leading to death (see Fig. 5). ΔP_0 is estimated by subtracting the mean survival of the parental population from the mean survival value of the filial populations. The survival value of each parent, estimated by converting the percentage of survivors in the population in which it was tested into probits, is weighted for the number of offspring each parent had for the purpose of estimating mean survival value. The filial survival value is the percentage surviving in a test batch converted into probits. The probit values indicate where the mean of the two populations falls relative to the cut-off between life and death and so the difference between the two in probits.

All offspring were tested with SS virus but as not all parents were tested with the same virus it was necessary for calculating ΔP_0 to equate the different viruses used, as shown in Fig. 6. Table 4 shows the results of 30 tests. Columns 5 and 6 are the selection differentials, columns (8–4) is the estimate of ΔP_0 . Columns 2 and 3 are not comparable to 5 and 6 because they have been adjusted for the virus used to test the different sires and dams. Fig. 7 is a histogram of the values of h^2 estimated from each of the 30 tests, involving 1228 animals, with a mean h^2 of 0.36.

It is of interest to note that most of the negative values of heritability in Table 4 occurred in tests made in 1964/5 when the whole level of recovery was lowered by some unknown environmental effect.

Fig. 6. The distributions of the assumed factor determining survival for the different strains of virus relative to the cut-off point between death and survival, determining the relation of KM 13/1, KM 13/2 and U to SS.

Fig. 7. The distribution of the heritabilities calculated from 30 different tests by probit analysis with a mean of h^2 of 0.36.

Table 4. *Estimate of heritability (h^2) using an all-or-none probit analysis*

Date of expt.	Sire probit	Dam probit	$\bar{x}$	Sire S.D.	Dam S.D.	$\bar{x}$	Offspring probit	h^2
17. i. 61	3.20	3.54	3.37	1.25	1.57	1.41	3.92	0.39
16. ii. 61	3.48	3.91	3.70	1.48	0.87	1.18	4.33	0.53
22. iii. 61	3.38	3.55	3.47	1.40	0.95	1.17	4.42	0.81
13. ix. 61	3.38	3.34	3.28	0.71	0.96	0.84	4.07	0.94
1. xi. 61	3.30	3.48	3.39	0.75	0.61	0.68	3.84	0.66
22. xi. 61	3.15	3.55	3.35	0.72	0.69	0.71	4.03	0.96
3. i. 62	3.25	3.58	3.42	0.67	0.74	0.71	3.92	0.71
21. ii. 62	3.31	3.44	3.38	0.58	0.58	0.58	3.80	0.72
14. iii. 62	3.29	3.30	3.30	0.58	0.67	0.63	3.59	0.46
14. xi. 62	3.44	3.59	3.52	0.95	0.98	0.97	3.96	0.45
30. i. 63	3.63	3.58	3.61	0.91	0.95	0.93	3.87	0.28
6. iii. 63	3.51	3.47	3.49	0.96	1.11	1.04	4.13	0.62
31. vii. 63	3.54	3.68	3.61	1.03	1.07	1.05	4.16	0.52
1. xi. 63	3.74	3.64	3.69	1.27	1.24	1.25	3.51	-0.14
9. xii. 63	3.55	3.35	3.45	1.01	1.16	1.09	4.05	0.55
10. xii. 63	3.79	3.46	3.58	1.08	1.71	1.40	5.18	1.14
11. xii. 63	3.53	3.62	3.58	1.16	1.29	1.18	4.28	0.59
13. i. 64	3.66	3.42	3.54	1.26	1.23	1.24	3.72	0.15
13. iii. 64	3.60	3.56	3.58	1.26	1.37	1.32	3.98	0.30
10. iv. 64	3.58	3.72	3.65	1.20	1.50	1.35	4.15	0.37
15. vi. 64	3.60	3.47	3.60	1.33	1.24	1.28	3.87	0.21
17. vii. 64	3.55	3.86	3.71	1.33	1.64	1.49	3.69	-0.01
14. viii. 64	3.65	3.79	3.72	1.70	1.20	1.48	3.85	0.08
16. x. 64	3.51	3.85	3.68	1.12	1.25	1.18	3.24	-0.37
20. xi. 64	3.59	3.98	3.79	1.24	1.54	1.39	3.31	-0.35
11. xii. 64	3.60	3.76	3.68	1.30	1.70	1.50	4.07	0.26
5. ii. 65	3.79	3.86	3.83	1.26	1.51	1.38	3.63	-0.14
2. iv. 65	3.96	4.13	4.04	1.52	1.39	1.45	3.59	-0.31
18. vi. 65	4.08	3.83	3.96	1.36	1.45	1.41	4.38	0.02
								$\bar{x}$ 0.36

DISCUSSION

Selection for resistance resulted in a steady increase in the percentage of animals able to survive myxomatosis. The inoculation dose of 500 L.F.U. was high, and it is probable that at a lower dose rate, of say 5 L.F.U., the resistance achieved would be reflected by a much higher percentage recovery. The generation increment of 0.5 of a grade per year was low for an animal such as the rabbit, capable of achieving a generation turnover in 6–12 months. The reduced turnover was caused by the necessity to wait until an animal reached 4 months of age before it could be tested, the further interval while it recovered sufficiently from the disease to be capable of breeding, and the necessity of including in the breeding stock unchallenged selected does.

Determining the heritability of resistance with any degree of accuracy proved impossible. The use of survival time as an index of resistance and its utilization in intra-sire correlation is severely limited by the bimodality of the distribution even at low recovery rates. The method does however reveal an elevated dam component of heritability. This has been interpreted as a 'litter effect' because members of a litter were invariably tested at the same time, and within-litter

variation would be decreased relative to between-litter variation by environmental fluctuations. The limited data available, with overlapping generations, did not allow existing techniques for dealing with an all-or-none character to be used. The estimation of realized heritability by converting percentage survivors into probits used in the present work, while not precise, at least gives an acceptably valid estimate of heritability. The method is affected by the vicissitudes of the environment in that the selection differential is calculated on the basis of animals surviving in one year and the genetic improvement, in part-at-least, on animals surviving in following years. As has been shown (Fig. 2) year-to-year variation in survival is often marked. The accuracy of the above estimate of heritability will increase with the number of tests on which the mean is based and the number of years over which the tests extend.

The degree of genetic resistance to myxomatosis attained by wild rabbits in Australia will vary considerably depending mainly on the number of epizootics experienced. In measuring the genetic resistance the many factors affecting the course of the disease such as strain of virus, dose, route of inoculation, temperature conditions etc. (see Fenner & Ratcliffe (1965) for a detailed review) must be taken into account, particularly if results from different laboratories are to be compared. A recent finding that the age at which rabbits with some genetic resistance are infected is of considerable importance to their chance of recovery (Sobey, unpublished data) adds to the above list of variables. Further, the effects of the variables listed appear to be exaggerated in rabbits with increased genetic resistance. Current investigations are aimed at developing a suitable procedure for comparative studies in assessing the genetic resistance of wild rabbit populations.

SUMMARY

1. Response to selection was achieved with all strains of Myxoma virus used.
2. Heritability of resistance to myxomatosis was determined by intra-sire correlation using survival time as an index and by an all-or-none probit analysis. Both resulted in an estimate of heritability of about 35–40 %.
3. The ability of animals to survive myxomatosis varied widely with environmental variation in time.

I am indebted to W. Menzies for his very able assistance in all aspects of the work; to Dorothy Conolly for her critical assistance with data analysis, and to Dr J. M. Rendel for suggesting the probit analysis for determining heritability.

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