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Inheritance of reaction to halothane anaesthesia in pigs

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SUMMARY

The inheritance of reaction to halothane anaesthesia (so-called malignant hyperthermia syndrome) in pigs was studied in a series of 61 litters by 18 sires from an F_3–F_4 Pietrain–Hampshire crossbred population. A single locus two allele model allowing for reactors from all three genotypes, was fitted by maximum likelihood. It was concluded that the reaction is due to a single autosomal recessive gene with a frequency of 0·46 (0·44–0·48) and penetrance of 0·95 (0·92–0·98) in this stock.

Two disorders of some economic importance in the pig, porcine stress syndrome (PSS) and pale soft exudative pork (PSE), have been shown to be closely associated with halothane-induced malignant hyperthermia syndrome (MHS) (Christian, 1972; Eikelenboom & Minkema, 1974), and the halothane reaction is being used in some pig populations as a diagnostic test for individuals liable to these syndromes. The induced metabolic reaction is likely to be more simply inherited than either of the syndromes since the stress stimulus will be variable in practice. Ollivier, Sellier & Monin (1975) fitted an autosomal recessive Mendelian model to data on halothane reaction in French Pietrain pigs. Here in an analysis of similar data we were interested in trying other Mendelian models, with varying proportions of different genotypes reacting (to allow for false positives and false negatives). The methods also take account of the joint probabilities for sires and dams and use a maximum-likelihood method to derive the parameter estimates. In man there is a similar halothane induced malignant hyperthermia, but it is rather rare (1 in 10000) and is inherited as an autosomal dominant (Wingard, 1974).

MATERIAL

In 1975 a population of F_3 Pietrain–Hampshire crosses were tested for reaction to halothane anaesthesia (Webb & Smith, 1976). The frequency of reaction in this parental population was $0{\cdot}20 \pm 0{\cdot}025$. Two lines were then formed, mating reactors or doubtful reactors with reactors ($R \times R$) and non-reactors with non-reactors ($N \times N$). The progeny, born in 1976, were also tested with halothane and the data obtained are given in Table 1.

METHODS

For a single locus model and two alleles, the genotypes, their frequencies (with Hardy–Weinberg equilibrium) and the proportions reacting (the penetrance) are:

Genotype ...	AA	Aa	aa
Code	1	2	3
Genotype frequency	p^2	$2pq$	q^2
Proportion reacting (Penetrance)	f_1	f_2	f_3

This general specification allows for non-genetic cases and for errors in classification (false positives and false negatives) and would correspond to the strict autosomal recessive case only if $f_1 = f_2 = 0$ and $f_3 = 1$. The frequency of reaction in the population for the general case is

$$F = p^2 f_1 + 2pqf_2 + q^2 f_3.$$

If F is given there are three unknown parameters, p, f_2 and f_3 since q and f_1 can be derived given the others. Hardy–Weinberg frequencies were assumed in the *parental* generation which was randomly bred before selection.

The conditional probabilities of the genotype for an individual given its halothane reaction can be derived. For example, the probability that an individual is Aa is:

$$2pqf_2/F = Q_2 \qquad \text{for reactors (R)},$$
$$2pq(1-f_2)/(1-F) = Q_2' \qquad \text{for non-reactors (N)},$$

and similarly Q_1, Q_1' and Q_3, Q_3' for the other genotypes. Where the reaction status of the individual is not known, the conditional probability is taken as the genotype frequency.

If the parental genotypes are known, then the expected distribution of offspring genotypes and so of offspring halothane reaction phenotypes can be specified. For example, with an $Aa \times Aa$ mating (2×2), the proportion of offspring reacting is

$$PR_{22} = \tfrac{1}{4}f_1 + \tfrac{1}{2}f_2 + \tfrac{1}{4}f_3$$

and the proportion not reacting is $(1 - PR_{22}) = PN_{22}$.

The joint likelihood for a set of observed results can now be written down, taking into account the fact that a sire is mated to several (d_k) dams. The joint likelihood is (L)

$$\Pi_s \sum_1^3 Q_i \Pi_{d_k} \sum_1^3 Q_j \frac{(NR+NN)!}{NR!\,NN!} (PR_{ij})^{NR} (PN_{ij})^{NN},$$

omitting subscripts for sires (s) and dams (d_k per sire). This derives the probability of NR reacting and NN non-reacting offspring in a litter by sire genotype (i) and dam genotype (j). These probabilities are then summed over the three possible dam genotypes (j) weighting each by the prior probability Q_j calculated, as shown above, using the dam's halothane phenotype. The terms for all dams within a sire genotype are then multiplied together. This is repeated for each of the three possible sire genotypes (i) and the terms summed. Finally, the probabilities for

all sires are multiplied together to give the overall likelihood. A computer program was written to derive the likelihood from a set of data and the maximum likelihood was quickly found by a trial and error procedure. A trial set of data with $f_1, f_2, f_3 \neq 0$ returned the input parameters as the maximum likelihood estimates, thus providing a check on the program and on the methods. The 95 % confidence intervals of the parameter estimates were derived by finding parameter estimates which reduced the likelihood such that

$$-2(\ln L_\theta - \ln L_\theta^*) \doteq \chi_1^2 = 3{\cdot}84,$$

where θ refers to the maximum likelihood estimates and θ^* to the 95 % confidence level estimates.

RESULTS

In the offspring generation the frequency of reaction to halothane was $0{\cdot}78 \pm 0{\cdot}033$ in the (R × R) line and $0{\cdot}12 \pm 0{\cdot}025$ in the (N × N) line, compared with $0{\cdot}20 \pm 0{\cdot}025$ in the initial unselected population (Webb & Smith, 1976). The maximum-likelihood estimates of the parameters, with their 95 % confidence limits, for the data presented in Table 1 are:

$$q = 0{\cdot}46 \quad (0{\cdot}44 - 0{\cdot}48),$$
$$f_3 = 0{\cdot}95 \quad (0{\cdot}92 - 0{\cdot}98),$$
$$f_1 = f_2 = 0.$$

No advantage was gained by fitting non-zero values for f_1 and f_2. The maximum likelihood was thus very well defined. This was partly due to the volume of data available and to use of the prior information on parents. As shown by the very small 95 % confidence limits of the estimates, any deviations from the maximum likelihood parameters reduced the likelihood appreciably. In general, the data in Table 1 seem to fit well with the recessive model. However, a goodness of fit test (using $2 \ln (L) \sim \chi_{61}^2$ D.F.) was highly significant showing that, in fact, the parameters did not provide a good fit to the whole data. Some of the parents and offspring (marked with an asterisk in Table 1) had anomalous phenotypes and these, and the assumption of Hardy–Weinberg in the parental population, may contribute to the lack of fit for the whole data.

The data of Ollivier *et al.* (1975) were reanalysed using the above methods. Again the maximum likelihood was obtained at $f_1 = f_2 = 0$. The parameter estimates and the 95 % confidence limits are:

$$q = 0{\cdot}57 \quad (0{\cdot}53\text{–}0{\cdot}72),$$
$$f_3 = 0{\cdot}85 \quad (0{\cdot}54\text{–}0{\cdot}99).$$

Note the much wider confidence levels than with the British data. The parameter estimates found by Ollivier *et al.* (1975) on the same data using the method of moments (Lefort, Ollivier & Sellier, 1975) were

$$q = 0{\cdot}64 \pm 0{\cdot}13, \quad f_3 = 0{\cdot}69 \pm 0{\cdot}25.$$

The difference in the estimates and the smaller confidence limits are probably due to considering the joint parental distribution and in using a maximum-likelihood method. Through the joint distribution, each litter gives information on the genotype of the sire which is used in deriving the likelihood for other litters by the same sire.

Table 1. *Halothane reaction results from 61 litters by 18 sires*

		Offspring				Offspring	
Sire	Dam	No. reacting (R)	No. not reacting (N)	Sire	Dam	No. reacting (R)	No. not reacting (N)
—	R	6	0	N*	R	8	0
	R	1	0		N	4	3
	—	3	2		N	0	9
	R	7	0	—	—	0	8
	N	3	4		—	0	6
N*	R	9	2*		N	0	8
	R	6	0		N	0	6
R	—	4	6	—	—	2	2
	—	6	0		N	0	6
	R	8	0		N	4	5
	R	6	0		N	0	7
	—	2	4	N	N	1	7
	N*	8	0		N	2	4
—	—	9	0		N	0	9
	—	10	0	N	N	1	6
	—	4	5		N	0	6
R	R	6	1*		N	0	2
	R	7	0	N	N	0	6
	R	5	3		N	0	6
R	—	2	2		N	1	4
	R	3	0	N	N	0	2
	R	1	0		N	2	5
	R	4	0	N	N	0	6
	—	3	1		N	0	4
R	R	6	0		N	2	4
	N	0	10	N	N	0	9
	N	3	4		N	0	7
	R	8	0	N	N	1	5
	N	0	8		N	2	7
	N	0	7	N	N	3	6
	R	3	0				

R, Reactors; N, not-reacting; —, not known.
* Anomalous.

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DISCUSSION

The analysis of the present data, and that of Ollivier *et al.* (1975) support an autosomal recessive mode of inheritance. The penetrance (f_3) of the recessive homozygote is high, indicating a low misclassification rate. Because both likelihoods were maximum when f_1 and f_2 were equal to zero, this indicates that very few or none of the other two genotypes are being classed as reactors. This is confirmed by finding no reactors in some breeds, for example Durocs (Webb & Smith, 1976) or in French Large White (Ollivier, Sellier & Monin, 1976). In a repeatability trial, the probability of classifying a genetically liable individual as a non-reactor was estimated as $25 \pm 10\%$ and $6 \pm 3\%$ in 1975 and 1976 respectively, with a pooled estimate of $8 \pm 3\%$. The corresponding estimate in this analysis was $5 \pm 2\%$. This is confirmed by the frequency of 2% for non-reactors from the strict ($R \times R$) ratings in the table.

The gene frequency in a crossbred population should be the average of those in the two parental breeds. Some data on the two breeds in different countries is given below:

Country	Reaction frequency (%)	Penetrance	Estimated gene frequency
Netherlands			
Pietrain	100	100	1·0
Hampshire	1·7	100	0·13
France			
Pietrain	40	64	0·8
Britain			
Hampshire	0·9	95	0·10

The reaction frequencies and gene frequencies are rather different for Pietrains in the Netherlands (Cöp & Buiting, 1976) and in France (Ollivier *et al.* 1976), showing the importance of different sources and samples of stocks. Hampshire crosses with these stocks would have gene frequencies of 0·55 and 0·45 respectively, compared with the estimate of 0·46 found. The British Pietrains were imported from Belgium in 1964.

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