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Genetics of warfarin-resistance in house mice of three separate localities

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SUMMARY

Mice from Loughborough and Nottingham were obtained in order to compare the inheritance of warfarin resistance in these populations with that established for a Cambridge population (Wallace & MacSwiney, 1976). Using the same breeding programme and warfarin testing technique, it is established that resistance in the new areas is, as in the Cambridge area, controlled by the major resistance gene, *War*, in chromosome 7, with penetrance affected by sex and modifiers. In addition, survival differences in males of different ages strongly suggests that *War*+ has less penetrance with age. Penetrance differences between the experiments establishes that wild populations differ in their modifier complex and that more than one modifier, probably several, exist. Questions are posed as to the adaptive significance of the phenomena, and the way in which they work, in the patchwork of warfarin baited and unbaited areas in this country.

INTRODUCTION

It has been established (Wallace & MacSwiney, 1976) that there is in the wild house mouse a major dominant warfarin-resistance gene, *War*. It is located in chromosome 7 in approximately the same position as is the resistance gene Rw^2 in Linkage Group I of the rat (*Rattus norvegicus*). The recombination percentage in the analogous chromosomes in the two species are (in round figures):

Mouse: *War* – 0 – *fr* – 17 – *sh*-1 – 6 – c^{ch} – 15 – *p*
Rat: Rw^2 – – – – – 22 – – – – – *c* – – 16 – *p*

Until recently it has been thought that penetrance in the rat is complete. However, a Scottish resistance gene, Rw^3, allelic with the better-known Welsh resistance gene, Rw^2, has now been shown to have incomplete penetrance controlled by modifiers (Greaves & Ayers, 1976). In contrast to the rat situation, *War* has penetrance varying greatly between male and female individuals.

The wild mice under study in the 1976 paper were a colony derived from mice trapped in Cambridge. The present work seeks to establish whether or not *War* occurs in wild mice in other parts of the country, and if so, whether penetrance in the sexes varies in different genomes. A genome effect is thought likely since in the first study, penetrance in males, though not in females, varied according to the 'dosage' of a susceptible genome. Male penetrance with age is also studied.

MATERIALS

In 1975, six males were received from Mr F. P. Rowe, three trapped in a pet shop in Loughborough and three from Carlton-on-Trent near Nottingham. Standard tests for warfarin-resistance (Rowe & Redfern, 1964) on samples of the populations they came from killed 15% of the Loughborough mice (0/6 males, 5/28 females) and 28% of the Nottingham (7/20 males and 10/41 females), indicating that the colony in Loughborough was more resistant as a whole than that in Nottingham.

METHODS

Breeding programme

This was the same for the 1976 Cambridge wild mice (known as PBI *CC*). The Loughborough and Nottingham males were mated to the Cambridge laboratory fourfold recessive fully susceptible stock. Of the F_1, 8 females and 8 males in each case, were backcrossed to the marker stock and then tested at 4 months for resistance. From subsequent F_1 litters, 8 females and 8 males were tested at 4 months for resistance. A backcross progeny numbering about 100 in each case was raised to 4 months, the females and half of the males were warfarin tested, and the remaining males were warfarin tested at 8 months. In addition a new single male PBI *CC* mouse was crossed to the susceptible stock, and a backcross progeny numbering 44 (males only) was raised; in contrast to the rest, whose tests were done mainly at 4 months, these F_1 and backcross progeny were all tested at 8 months.

Finally all the males used in the outcross to the susceptible stock were tested when they were no longer wanted for breeding (10 months for the new PBI *CC*, 15 months for the Loughborough mice and at an uncertain age exceeding 6 months for the Nottingham mice).

The 100% susceptible stock common to all these crosses was marked by frizzy (*fr*), shaker-1 (*sh*-1), chinchilla (c^{ch}) and pink-eyed dilution (*p*).

Warfarin tests

These were carried out exactly as in the Cambridge 1976 study with the PBI *CC* mice.

RESULTS

All the wild mice used in the outcrosses survived the warfarin tests, but the new PBI *CC* male died on the eighth day after the test.

Data pertaining to the 1976 Cambridge study are given for comparison in the tables now presented: they are referred to as 'old PBI *CC*'. Data for the present Cambridge study are referred to as 'new PBI *CC*'. Data for the present Loughborough mice are referred to as 'Loughb', and those for the Nottingham mice as 'Notts'.

Table 1 shows the results of warfarin testing the F_1 of the four wild types crossed to the susceptible stock. Table 2 shows the results of warfarin testing the backcross progeny of the three types in the present study. Table 3 shows,

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Table 1. *Response to warfarin testing of the* F_1 *of crosses of resistant wild mice* × *susceptible fr sh-1* $c^{ch}p$*/fr sh-1* c^{ch} *p mice*

	War/fr sh-1 $c^{ch}p$						
	Females			Males			
	Lived	Died	Impene-trance %	Lived	Died	Impene-trance %	Sex diff. sig.
Old PBI *CC*	38	0	0	8	19	70	Yes
New PBI *CC*	—	—	—	0	8	100	—
Loughb.	16	0	0	16	0	0	No
Notts.	14	2	12	10	6	37	No

for all four types, the recombination values between *War* and *fr*, and a measure of the penetrance of *War*, estimated from the backcross data. Explanations of items in the tables unfamiliar to non-geneticists follow, and interpretation of the data is given in the Discussion.

Penetrance is the percentage of a given genotype which shows a mutant phenotype. Thus, all F_1 progeny (Table 1) are *War*+ and the percentage that survive warfarin testing express the resistant (mutant) phenotype. In the backcross data, the progeny are expected to be *War*+ and + + in equal numbers. When observed numbers that lived and that died agree with this expectation, penetrance is 100 %. But when there are fewer than 50 % survivors, some *War*+ genotypes are not expressing the resistant phenotype: the estimated percentage of *War*+ which express is known as 'penetrance percent' and the remainder of *War*+ that do not express is commonly known as the 'percentage misclassified' and given the symbol λ. Estimates of λ in these data are calculated from Bailey's formula (1961, p. 75) and called, for clarity in the present context, 'impenetrance percent'. Estimates of recombination values, commonly symbolized y, have to take impenetrance into account: the formula used is also from Bailey (1961, p. 75).

The labelling of all F_1 in Table 1 as heterozygous for *War*+ (*War/fr sh-1* c^{ch} *p*) requires some justification. It is correct if the wild animal used in the outcross is *WarWar*. The alternative is that this animal is *War*+, in which case there would be 50 % or less survivors in the F_1; this is not the case in the various female F_1, where penetrance is high – as expected from the old PBI *CC* data. The lower survival rate in the male F_1 must therefore reflect the (also expected) lower penetrance of *War*+ in this sex. The very low survival for the male F_1 from the new PBI *CC* male, and the latter's death so soon after warfarin testing, may suggest that he was + + at the *War* locus; however, the segregation of both mice that lived and mice that died in the backcross data can only be explained if the F_1 are *War*+, and so the new PBI *CC* male was probably *War*+ or *WarWar*, not fully expressing the resistant phenotype.

Interpretation of the present study will be based largely on comparisons between the new backcross data (Table 2) and those in the 1976 study, i.e. on the analysis given in Table 3. It should therefore be noted that it has been

Table 2. *Response to warfarin testing of the resistant* F_1 *+ + + +/fr sh-1* $c^{ch}p$ *mated to susceptible fr sh-1* $c^{ch}p$*/fr sh-1* $c^{ch}p$ *mice*

		(0)*		(1)		(2)		(3)		(1, 2)		(1, 3)		(2, 3)		(1, 2, 3)		Totals	
		fr *sh-1* c^{ch} *p*	+ + + +	*fr* + + +	+ *sh-1* c^{ch} *p*	*fr* *sh-1* + +	+ + c^{ch} *p*	*fr* *sh-1* c^{ch} +	+ + + *p*	*fr* + c^{ch} *p*	+ *sh-1* + +	*fr* + + *p*	+ *sh-1* c^{ch} +	*fr* *sh-1* + +	+ + c^{ch} *p*	*fr* + c^{ch} +	+ *sh-1* + *p*		
New PBI *CC*																			
Males	Lived	0	4	0	1	0	0	0	1	0	0	0	0	0	0	0	0	6	44
	Died	17	11	2	2	1	1	1	3	0	0	0	0	0	0	0	0	38	
Loughb.																			
Females	Lived	0	9	0	1	0	1	0	4	0	0	0	0	0	0	0	0	15	31
	Died	8	0	1	0	3	0	3	0	0	0	1	0	0	0	0	0	16	
Males	Lived	0	13	0	6	0	1	0	7	0	0	0	0	0	0	0	0	27	70
	Died	23	7	3	1	2	0	6	1	0	0	0	0	0	0	0	0	43	
Notts.																			
Females	Lived	0	12	0	1	0	0	0	1	0	0	0	0	0	0	0	0	14	32
	Died	10	0	4	1	0	0	2	1	0	0	0	0	0	0	0	0	18	
Males	Lived	0	14	1	3	0	0	0	9	0	0	0	0	0	0	0	0	27	80
	Died	24	9	9	2	0	2	5	1	0	0	0	0	1	0	0	0	53	

* These headings assume adjacent segments of chromosome 7 to be labelled as follows: *fr*–1–*sh*-1–2–c^{ch}–3–*p* and indicate recombination in successively 0 segments, segment 1 only, segment 2 only, segment 3 only, segments 1 and 2 only, etc.

Table 3. *Recombination values War/fr and impenetrance percent for War, from all the backcross data*

	Females				Males			
	Recombination		Impenetrance		Recombination		Impenetrance	
	%	S.E.	%	S.E.	%	S.E.	%	S.E.
Old PBI *CC*	0·00	+1·41	10·68	±3·46	0·00	+3·00	66·67	±4·47
New PBI *CC*	—	—	—	—	0·00	+14·28	73·91	±9·38
Loughb.	0·00	+5·74	3·03	±4·35	0·00	+3·60	25·00	±7·34
Notts.	0·00	+2·23	12·50	±8·24	3·70	±3·60	32·50	±7·93

Within sexes, the only significant differences are in the impenetrance percent: between Old PBI *CC* and Loughb. in males ($P < 0{\cdot}01$), and between Old PBI *CC* and Notts. in males ($P < 0{\cdot}02$).

checked that the single-factor ratios (non-frizzy:frizzy, etc.) and the recombination values (frizzy/shaker-1 etc.), pertinent to chromosome 7, are normal and comply with those in the 1976 study and earlier published data, and they do not therefore affect the interpretation.

The feature of the backcross data in the 1976 study which unequivocally established the existence of a major resistance gene, is the close linkage between resistance and frizzy (recombination is 0%). The estimate of this recombination value, i.e. *War/fr*, in the new data must therefore provide the basis for claiming the existence of *War* here too, and so estimates of this value, rather than of other relations between *War* and the chromosome 7 markers are given in Table 3.

Finally, the feature of the backcross data in the 1976 study which posed the question of genome effect on the sex-limitation of *War*, was the apparent immunity in females to a change in 'dosage' of the susceptible genome, compared with the big response in males. (F_1 have a half dose of the wild genome, and backcross progeny one quarter.) Estimates of impenetrance percent, obtained from the segregation of *War* with *fr* in all the backcross data, are therefore given in Table 3.*

DISCUSSION

Table 3 shows that penetrance of *War* in 8-month-old backcross progeny (the new PBI *CC* data) is insignificantly decreased as compared with the 4-month progeny (the old PBI *CC* data). This and the high death rate of the 8-month F_1 (Table 1, new PBI *CC* data) suggests that penetrance at ages exceeding 4-months decreases. There is a similar change, of the same size and in the same direction, within the Loughborough and Nottingham backcross data. (This is not shown, because it is not significant, in Table 3, which pools the 4-month and 8-month data for the interpretation below.) These age trends probably deserve study on

* The penetrance percent in the 1976 paper, p. 179, for the old PBI *CC* backcross female progeny, is given as 99%; this is an error and should have been 89%, so as to correspond with the impenetrance percent of 10·68% given for these data in Table 3.

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a large scale. Sexual maturity is also important (Rowe & Redfern, 1967): 4 months is thus an optimal age for detecting the segregation of *War* and for future comparative work on penetrance.

Table 3 also shows agreement in both sexes of the Loughborough and Nottingham data, with the old PBI *CC* data, in the recombination value between resistance and frizzy (0%): it may be concluded that resistance in the wild mice of the new areas is also controlled by the major gene *War* established in the 1976 study.

The significant differences in male impenetrance percent in Table 3 between the old PBI *CC* and Loughborough backcross data and the old PBI *CC* and the Nottingham backcross data reflect the difference in the quarter-wild genome contribution of the two populations to these data. The observation in the 1976 data that different 'dosages' of susceptible stock affect penetrance indicates that one or more minor modifying genes (other than sex itself) affect penetrance in one wild population. The present data thus show that the new wild genomes differ in their effect on penetrance, and thus again that more than one minor modifier exists. The fact that the Loughborough impenetrance is (insignificantly) greater than the Nottingham, in both sexes in Table 3, coupled with a similar disparity in both sexes in the F_1 data (Table 1: half-wild genome contribution) suggests that the pure wild mice in these two areas differ between each other by at least one modifier. The single major gene, *War*, and varying modifier complexes may well be a sufficient explanation of the observed variation in resistance in all populations, i.e. that no other major gene need be sought.

In the recent rat study (Greaves & Ayres, 1976), it was disclosed that the Welsh resistant strain is similar to the Scottish resistant strain in its response to coumatetralyl, but unique in its high resistance to warfarin and diphacinone. The difference in resistance spectra is the main reason for concluding that the genes responsible are different alleles. It would be interesting to have different resistant populations of mice studied in this way; until different spectra are shown, however, there is no reason to propose different *War* alleles in the mouse.

The fact that all the female backcross progeny (Table 3) agree in their impenetrance percent, whereas the male progeny from the different wild sources disagree, indicates that the female sex is a stronger modifier than the male sex. The fact that all the female backcross progeny have a very low impenetrance percent compared with the males, suggests a ceiling effect: as impenetrance percent approaches nought, due to modifiers, the addition of further modifiers has little effect.

The sex difference in penetrance in the overall data poses the problem: what is the function of this in terms of survival in the wild environment – which includes warfarin baited areas and unbaited areas throughout the country? It was suggested in the 1976 study that *War* may have some disadvantage as does Rw^2 in the rat (which increases the requirement of vitamin K), and that the flexibility of penetrance of *War* in males allows a balance to be struck, even in an almost fully homozygous colony, between the advantages and disadvantages in

the species as a whole. It is now suggested that this balance is one of the factors which make mice infestations more difficult to control than those in rats. It may work as follows: given polygamy and emigration of unmated males, in a long-standing warfarin baited environment, where it may be supposed that *War* has become homozygous, some of the bait-shy emigrating males are *WarWar* unexpressed and therefore not suffering the diet disadvantage proposed above. These then survive food shortages in the new area (which we shall suppose is not baited), encounter females homozygous for the normal allele of *War*, and so set up a new colony where both *War* and modifiers segregate, and flexibility for adaptation in one direction or the other is re-established.

This of course is speculation; but it suggests that a study of the physiological expression of the *War* gene, and the effects of sex, together with field studies, could provide understanding of what promises to be an interesting ecological problem.

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