A major gene controlling warfarin-resistance in the house mouse

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SUMMARY

The spread of a 'cream' mutant in a wild population of house mice is reported. The hypothesis that the gene responsible for the colour, extreme chinchilla, c^e , has spread because of linkage with a major gene for warfarin-resistance, is tested by a linkage backcross.

The results prove that a major gene does exist, that it is very closely linked with frizzy, fr , in chromosome 7, which in turn is linked with c^e , that it is fully dominant in females at 4 months of age, and that its partial dominance in males is under the control of modifiers.

The symbol *War* is proposed for the gene. Its position in chromosome 7 is analagous with the position of the resistant gene, Rw^2 , in the rat in the analagous chromosome.

The adaptive significance of this finding is discussed, as also are reports of certain other mutants in wild populations of mice.

INTRODUCTION

Blood anticoagulants have been used as rodenticides for the last twenty years, both in Europe and the U.S.A.; the most widely used of these has been warfarin [3-(α -acetylbenzyl)-4-hydroxycoumarin]. In the early 1960s infestations of the house mouse (*Mus musculus*) became increasingly difficult to control in Britain, even after continuous warfarin treatment (Dodsworth, 1961), and, in urban areas, mice began to replace rats as the major pests. A heritable warfarin resistance trait was proposed (Rowe & Redfern, 1965), but the genetic basis of this has been difficult to establish.

Warfarin resistance in the rat (*Rattus norvegicus*) is inherited as a single dominant autosomal gene at the *Rw* locus in Linkage Group I (Greaves & Ayres, 1969). In the house mouse, Linkage Group I (chromosome 7) shares certain loci with Group I in the rat. The present paper presents evidence that a major resistance gene occurs in the mouse, in an analogous linkage position to that in the rat; it also describes features of this gene's expression which explain why the hereditary basis of resistance has been more difficult to establish than in the rat.

MATERIALS

Wild-derived 'PBI' colonies

In December 1970 one author (M.E.W.) was informed by Mr Whitehouse of the Plant Breeding Institute (P.B.I.), Cambridge, that 'cream' mice were appearing among mice in a recently infested grain store in the Institute. Wild mice had been common in outbuildings for at least two years, despite prolonged warfarin baiting, and for one year cream mice had been increasing in frequency in nests found there; their genotype was established by breeding tests as extreme chinchilla, $c^e c^e$.

On enquiry it transpired that, over the past few years, artificial substances which mice living in outbuildings (presumably on treated grain or cereal plants) might have ingested, were: Rogor, Karathene, Nicotine, Lindane, D.D.T., Oxytril P., Actril C., Avadex BW, Metasystox, Agrosan, Murganic RPB, Calixin, Milstem, Gramoxone, Benlate T.M., Zinc Phosphide, and warfarin. It was suspected that one of the latter substances had acted as a mutagen, producing the extreme chinchilla gene.

A further hypothesis suggested that the spread of the c^e gene is due to its linkage with an important resistance gene, and that, using crossing-over between the resistance gene and its marker, c^e , two completely resistant colonies could be made, one cream and one wild type. From specimens trapped in the grain store, and from an outbuilding nest containing a cream, a colony was set up, and tested for resistance; the test indicated, as predicted, that more creams than normals were resistant (Wallace, 1971, 1972). Using the survivors, the colony was then expanded and split into $c^e c^e$ and CC (wild type) sections; the former traced to two trapped Cc^e females and the one cream male nestling, and the latter to two female and one male CC trapped mice. Each section was selected through two further generations for resistance and became virtually 100% resistant (Wallace & MacSwiney, 1974a).

The PBI colonies, as they are known, are becoming a standard reference resistant stock (Lush, 1975; Rowe, 1975).

Susceptible marker stock

A stock, 100% susceptible, was made up as follows. On the assumption that a wild stock untreated with warfarin would be susceptible, our wild-derived Skokholm Island inbred line SK/Cam was expanded and tested for resistance; all mice tested died. A cross was then made with SK/Cam females and a male homozygous for the chromosome 7 markers frizzy, *fr*, shaker-1, *sh*-1, chinchilla, c^h , and pink-eyed dilution, *p*, obtained by courtesy of Dr A. G. Searle of M.R.C. Radiobiological Research Unit, Harwell. Through further generations of breeding, a marker colony was established of which a further 50 tested animals all died (Wallace & MacSwiney, 1974a). The colony is maintained with the marker genes in a permanent coupling backcross so that the wild type or the marker genes may be used as required.

METHODS

Breeding programme

Survivors of the second generation of selection in the PBI *CC* stock were presumed to be homozygous for resistance; they were mated with fourfold recessive mice of the susceptible marker stock. The second litters of the F1 (65 mice) were tested for resistance, and the first and third litters (39 females and 8 males) were backcrossed to the marker stock. A backcross progeny numbering 457 was raised, of which 10 died before testing and 447 were warfarin tested. Of the backcross progeny, a female homozygous for frizzy, and a female homozygous for frizzy, shaker-1 and chinchilla, were backcrossed to the marker stock and their progeny (41 mice) tested.

Warfarin tests

At weaning, the sexes were separated, and all animals held in single-sex cages until they were 4 months old, i.e. until the males were sexually mature. This is necessary because response to warfarin varies with sex and age, females being more resistant than males, and immature males behaving like females, i.e. being more resistant than mature males (Rowe & Redfern, 1967).

Warfarin testing was conducted as follows (based on Rowe, 1964). Mature mice were weighed and isolated in separate cages for 3 days; they were provided with an unrestricted diet of 95 % coarse pinhead oatmeal and 5 % corn oil, and water *ad lib*. On the fourth day, this diet, with 0.025 % warfarin added, was fed for 21 days. The diet was weighed daily; the mice were weighed weekly, and when they looked ill, and on death. The survivors were then caged (sexes singly) for a month on Dixon's diet FFG(M), to note any long-term effect of warfarin poisoning; they were then autopsied. Any animals that died during the test were dissected to discern whether or no they had died from warfarin poisoning (as seen by massive internal haemorrhages).

Throughout both breeding and testing programmes, the mice were housed at 20° C. room temperature, in the Cambridge cage (Wallace, 1963) which has proved a very successful breeding microenvironment for wild mice (Wallace & Hudson, 1969).

RESULTS

The response, to selection, of the parental PBI colonies can be seen from Table 1; this shows the results of warfarin tests in the two selection generations preceding the outcross to the susceptible marker stock.

On the whole, females are more resistant than males, as expected from observations on wild mice taken in the field (Rowe & Redfern, 1965). As predicted on the hypothesis of a single major resistance gene linked to the *c^e* gene, the cream mice reached 100 % survival more quickly than did the wild type.

Response to the introduction of an unrelated susceptible genotype can be seen from Table 2; this shows the results of warfarin testing the F1 of the PBI *CC* outcross to the susceptible stock.

Females have maintained their 100 % survival, but survival in males is between that of the male parental PBI stock and the 100 % mortality of the susceptible stock. On the assumption of a single major gene for resistance and its heterozygosity in F1, these results indicate that the gene is fully dominant in females and partially dominant in males, i.e. its dominance is sex-limited. The symbol *War* is proposed (Wallace & MacSwiney, 1974b).

Table 1. *Response of PBI colonies to two generations of selection for resistance*

	Wild type			Cream (<i>c^cc^e</i>)		
	Lived	Died	Survival (%)	Lived	Died	Survival (%)
First generation (one colony of mixed <i>CC</i> , <i>Cc^e</i> and <i>c^cc^e</i> mice)						
Females	11	3	78.6	23	0	100.0
Males	3	6	33.3	8	5	61.5
Second generation (separate <i>CC</i> , and <i>c^cc^e</i> colonies)						
Females	23	0	100.0	25	0	100.0
Males	18	6	75.0	23	0	100.0

Table 2. *Response to warfarin testing of the F1 of a cross of resistant PBI CC × susceptible fr sh-1 c^{ch}p/fr sh-1 c^{ch}p mice*

	All wild type <i>Cc^{ch}</i>		
	Lived	Died	Survival (%)
Females	38	0	100.0
Males	8	19	29.6

Response to a second dose of the unrelated susceptible genotype can be seen from Table 3; this shows the results of warfarin testing the progeny of the F1 backcrossed to the susceptible marker stock.

On the assumption that the major gene *War* is segregating in backcross fashion, *War* + × +, equal numbers of resistant and susceptible mice are expected. This is so for females (118 and 110 respectively). Thus females have maintained the 100 % survival rate in heterozygotes, and 100 % mortality of homozygous recessives. That is, penetrance of *War* has remained at 100 % despite the second dose of the susceptible genotype. It may now be assumed that backcross males consist of *War* + and + + mice in equal numbers. The survival rate of *War* + is then estimated as $43/(2 \times 219) = 9.8\%$. This is significantly lower than the 29.6 % observed in F1 *War* +; thus the second dose of the susceptible genotype has lowered the penetrance of the *War* gene in heterozygotes, i.e. the expression of *War* in males is controlled by modifiers other than sex.

Before considering the linkage relations of the proposed *War* gene, it is as well to check that the between-marker relations are undisturbed. Disturbance of the linkage estimate occurs when two or more single-factor ratios depart significantly from the 1:1 segregation expected; the estimate, in turn, can disagree with

Table 3. Response to warfarin testing of the resistant $F1 + + + + /fr\ sh-1\ c^{ch}p$ 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	$sh-1$	fr	$sh-1$	fr	$sh-1$	fr	$sh-1$	fr	$sh-1$	fr	$sh-1$	fr	$sh-1$	fr	$sh-1$	
		c^{ch}	p	c^{ch}	p	c^{ch}	p	c^{ch}	p	c^{ch}	p	c^{ch}	p	c^{ch}	p	c^{ch}	p	
Females	{Lived	0	75	1	15	0	7	1	17	0	0	2	0	0	0	0	0	118
	{Died	56	5	15	10	8	1	14	0	0	0	0	1	0	0	0	0	
Males	{Lived	0	27	0	8	0	2	0	6	0	0	0	0	0	0	0	0	43
	{Died	57	61	12	10	6	3	15	11	0	0	0	1	0	0	0	0	
																		228
																		110
																		43
																		176
																		447

* 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, segment 3 only, segments 1 and 2 only, etc.

published figures because of some genetic phenomenon like a deletion in the wild-type chromosome (Wallace, 1972).

Table 4 shows the single-factor observations as derived from the data in Table 3.

The two sexes agree very closely in their single-factor segregations. In each sex the only significant departure from 1:1 is for +:fr ($\chi^2 = 6.33$ for females and 6.95 for males, $P = 0.01$). The insignificant disturbance of the other three single-factor segregations clearly derives from their linkage with frizzy. With only one single-factor disturbed, reliance may be placed upon the recombination values as derived from the addition formula (observed recombinants divided by total). Table 5 shows how these are obtained; the sexes are combined for this estimate as they agree closely. Each of the estimated recombination values is well within the 95% fiducial limits of the published values for data from heterozygous females (Robinson, 1972, pp. 226-7). There is thus no detectable abnormality in the wild-derived chromosome.

Table 4. *Single factor ratios obtained from the backcross data in Table 3*

	fr	+	sh-1	+	c ^h	+	p	+	Totals
Females	95	133	107	121	107	121	106	122	228
Males	90	129	97	122	97	122	96	123	219

Table 5. *Recombination between markers, from the data in Table 3*

	Segments			Total
	fr--sh-1	sh-1--c ^h	c ^h --p	
Nonrecombinants:recombinants	372:75	420:27	379:68	447
Recombination value (%)	16.8	6.0	15.2	

Table 6. *Recombination values (%) for War in relation to the four chromosome 7 markers, from data in Table 3*

	Segments			
	War -- fr	War -- sh-1	War -- c ^h	War -- p
Females	2.4 ± 1.5	16.9 ± 3.0	23.3 ± 3.2	36.2 ± 3.3
Males	3.2 ± 2.8	22.3 ± 6.1	28.0 ± 6.5	42.7 ± 7.3

Turning to the recombination relations between the proposed *War* gene and the four chromosome 7 markers: It is very clear that there is linkage between a resistance gene and the markers, because seven of the eight $2 \times 2 \chi^2$ testing contingency of the survived:died ratio on each of the single-factor ratios, in each sex of progeny, are significant at the 0.01 or 0.001 level of probability (not shown). The relevant recombination values are shown in table 6; they are derived from the data in table 3, using the formulae appropriate to imperfect penetrance (Bailey, 1961, p. 75).

There is good agreement between the sexes for all recombination values. This is as it should be, despite the great difference in penetrance of *War* as between the

sexes, and shows that they are valid. (Penetrance, as calculated from Bailey's formulae, is about 99 % for females and 36 % for males.)

These values, and the values between markers (table 5) agree with a linear arrangement of the proposed *War* with the markers:

$$War -- fr -- sh-1 -- c^{ch} -- p;$$

but *War* is clearly close to frizzy and a position for *War* inside the *fr*--*sh*-1 segment cannot be ruled out.

It might be argued on these data that *War* is in fact simply the normal allele of frizzy. The test of the two apparent recombinants, the two surviving frizzy females backcrossed to the susceptible fourfold recessive, does nothing to disprove this; for, from two litters from each female tested with warfarin, only one mouse out of a total of 41 survived. These females are therefore genetically susceptible, i.e. normal for *War*, and there is no recombination between the supposed *War* and the non-frizzy allele. However, if resistance is merely a pleiotropic effect of a non-frizzy allele, there has to be another non-frizzy allele, not resistant, in the fourfold recessive colony; this is a less likely situation than the existence of a separate *War* gene close to the frizzy locus. Moreover, the latter view is in agreement with the findings in the rat, for our data place *War* in the same position in the mouse as is *Rw*² in the rat, where Linkage Group I is:

$$Rw -- 22 \% -- c -- 16 \% -- p.$$

It is therefore concluded that the major resistance gene, *War*, does exist, and that it recombines very rarely if at all with frizzy.

DISCUSSION

The finding of a major dominant gene for warfarin resistance in the mouse establishes a simple mode of inheritance in this species. The finding that dominance is both sex-limited, and modifiable in males by the residual genotype, accounts for the greater difficulty in discovering the mode of inheritance as compared with that in the rat: without a fully penetrant marker closely linked to it (here, frizzy), the discernment of genotype underlying phenotype through the generations is very difficult. Rowe & Redfern's suggestion of a single major gene influenced by modifiers was a shrewd one.

The evolutionary significance of the sex difference in penetrance and dominance is a matter for speculation. If *War* is entirely of advantage to the species – that is, advantageous at times of warfarin-baiting and at least neutral in the absence of warfarin – one would expect both sexes to have full penetrance and full dominance. Possibly *War* has some disadvantage, in both sexes or mainly in males, and the flexibility of penetrance 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. This is more plausible where selection is strongest in the male sex, i.e. in the presence of polygamy, a supposition which can only be established by field studies. However, it is possible that the male sex, where baiting is prolonged, is protected either by modifiers or by a fuller penetrance of *War*, at the most usual

age for siring the next generation. This question can be answered by an extension of the present work, which studies response to warfarin at only one age, 4 months.

It is of interest that, without the present study, a report of the gene for 'cream' spreading in the population could have been made by someone in support of the commonly held view that colour mutants are not uncommon in wild populations. The only supporting evidence so far concerns light-bellied agouti, A^w , and pink-eyed dilution, p (Brown, 1965). However, the notion that A^w is an alternative wild-type gene to dark-bellied agouti, A , has been called in question (Wallace, 1954, 1965): breeding tests were not reported for the light-bellied animals found, except in one case where the belly-colour was shown to be due to modifiers in the presence of A (Falconer, 1947).

The evidence is best summarized by Deol (1970) who states 'Considering the large number of loci involved in the determination of coat colour and their relatively high rates of spontaneous mutation, the degree of coat colour polymorphism observed in wild populations is surprisingly low.' He concludes 'It is conceivable that one of the reasons for the absence of extensive polymorphism in wild populations with regard to coat colour is that the vast majority of coat colour mutations have adverse effects on the physiological balance of the animal.' The report of the spread of the p gene suggests that a reason therefore needs to be found when colour genes are found in the wild. It also indicates the nature of the reason; for L. N. Brown (personal communication) has replied in the affirmative to the author's query as to whether the population under study was under intensive warfarin-baiting. It may well be that other reports of mutants in the wild have a similar explanation, i.e. that they appear in sufficient numbers to be observed because they are linked to unusual genes advantageous temporarily in the presence of pollutants, or because of man's use of pesticides. Thus F. P. Rowe (personal communication) agrees that the 'slate-grey' and 'white' mice he has observed in a warfarin resistant population were of a similar colour to that produced by chinchilla, c^{ch} , and albino, c , respectively, and may therefore have persisted for the same reason that 'cream' persisted in the present study, namely linkage with *War*.

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