

GENETIC PREDISPOSITION TO *BACILLUS PILI- FORMIS* INFECTION AMONG MICE.

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TYZZER (1917), who described an epidemic that had appeared in his mouse colony, named the causative organism *Bacillus piliformis*, giving an inclusive description of its behaviour and morphology. Both natural and experimental infections were studied. The high susceptibility of the Japanese waltzers as contrasted with the relative immunity of the common mice suggests that the disease susceptibility may be linked with the waltzing factor.

Hagedoorn and Hagedoorn (1920) observed among laboratory mice an epidemic reaction similar in some respects to that reported by Tyzzer. The Hagedoorn colony was made up of common albino mice, a small Japanese strain, F_1 and F_2 hybrids, and back-crosses of F_1 's to Japanese. Some of his Japanese mice carried the waltzing factor. This was apparently present to such a small extent that he made no report as to the specific relation of the factor to the epidemic. The disease was attributed to a staphylococcus infection. The Japanese mice and a portion of the F_2 and back-cross individuals rapidly succumbed to the epidemic, while the albino and F_1 groups were entirely immune. The proportions dead in the F_2 and back-cross groups suggested a single factor segregation for susceptibility, resistance in the albinos being dominant. The fact that the Japanese mice were susceptible although they did not waltz, and the possibility that waltzing mice are derived from the small Oriental species *Mus bactrianus* (Hicks and Little, 1931), again suggested that linkage might exist between the susceptibility to the epidemic disease and the waltzing gene.

A laboratory epidemic of the *B. piliformis* disease was observed by Dr Ten Broeck among mice in Peiping, China. The original infective material for the tests reported here came from that source. On arrival in Princeton Dr Ten Broeck turned over this material to us. Throughout the experiments the mice which were to be tested were bred and raised entirely apart from those which were exposed to the disease, the breeding room and the workers caring for the same having no contact with the disease testing room and the workers making the disease resistance tests. We are indebted to Dr Ten Broeck for making the early disease resistance tests and for his advice and counsel throughout the investigation.

The identity of the disease with that observed by Tyzzer was quite evident. The Japanese waltzing mice were highly susceptible. In fatal cases the characteristic roughing of the fur and diarrhoea appeared before death. The multiple yellow-gray lesions of the liver were most striking. Histological preparations showed the slender bacilli lying parallel to one another within the liver cells peripheral to the necrotic areas. Their presence also was noted in cells of the intestinal mucosa. Films of infected liver material often showed the thread-like organisms staining in a characteristically banded fashion.

Infected liver was preserved by drying over phosphorus pentoxide. The ampules containing the dried material were sealed under vacuum and stored in the ice box.

The technique used for infecting the test animals was as follows. Waltzing mice were fed; the pulverised dry liver, described above, mixed with a small amount of the regular cooked cereal ration. After the customary 10-day incubation period the mice generally came down with disease and died. Fresh livers with active lesions from dead or moribund mice were ground in a mortar

Table I. *Parental stocks, percentage positive cases, and uniformity in the groups fed with B. piliformis*¹.

Stock mice	+	-	Per-centage takes	No. of test groups	χ^2	P
Waltzing	80	19	80.1	30	39.2	0.10
Black-eyed white	0	18	0.0	4	—	—
Silver brown	0	19	0.0	6	—	—
Chinese white	14	42	25.0	13	7.9	0.80
Chinese black	18	42	30.0	10	29.0	<0.01

and mixed with a small quantity of cereal. This fresh mixture formed the test material for the experimental mice listed in this paper. Each test mouse was isolated and fed a small quantity of infective material, no other food being given until this material was completely consumed. One infected liver was generally fed to five to eight mice. Several waltzing mice were fed portions of the infective material in each test group. Throughout this investigation every effort was made to determine whether each animal was susceptible or resistant. In line with this purpose, three or four mice were combined in one cage after each had consumed its liver portion, so that they would be exposed to cross-infection.

At 21 days after feeding, all survivors were chloroformed and examined for liver and intestinal lesions. Mice showing no lesions were classified as resistant; mice showing healed pits or scars were classified as doubtful; mice which died and showed characteristic liver lesions were classified as susceptible.

A list of the parental lines of mice is given in Table I along with the

¹ The numbers of animals in this table and the tables to follow are not as large as are desirable. Circumstances beyond the writers' control are such that this work must be brought to a close at this stage. While in some cases the data are meagre they do indicate definite segregation of the genetic complex favouring *B. piliformis* infection and the other factors studied, and suggest random assortment of the factors involved.

percentage of positive cases, and the χ^2 test for homogeneity within the test groups.

The waltzing mice came from an inbred line. Dr Gates¹ had bred this line brother by sister for 34 generations before we received it. In the 25th generation the line was continued through but a single pair of mice. Theoretically this stock should be homogeneous and form a good standard by which to judge the uniformity of the tests. The 30 tests in which the waltzers fell were distributed over the year and a half during which tests were being made. The P of 0.10 indicates that the variation observed was well within that of random sampling. If the waltzers are uniformly genotypically susceptible, then the 20 per cent. failures of positive infection presumably had their origin in the technique or in the variability of the pathogen itself. However, the statistic of $P = 0.10$ indicates that the series was not disturbed throughout the test period and that the 80 per cent. mortality of the waltzers can be used as a standard of comparison.

The black-eyed white and silver brown mice showed no evidence of susceptibility to the disease.

The Chinese white stock was imported from Peiping, China, through the courtesy of Dr Fortuyn, as 10 females and 4 males. It was the intention to have these animals come from pure stock of the small Oriental species, *M. bactrianus*, and of the strain susceptible to the *piliformis* disease. Experience with the imported stock and their progeny showed that this was not the case. The parent stock animals became nearly as large as the common laboratory mice, *M. musculus*, and they and their progeny displayed but little susceptibility to *B. piliformis*. These mice were also heterogeneous for coat colour genes.

The Chinese black stock was brought over from China and was believed to be a susceptible stock. The exigencies of the boat trip were such that only two males survived to reach Princeton. One of these males was sterile and not susceptible to *piliformis*. The other male was bred to our waltzing stock and these progeny bred back to this black Chinese male for six generations. The total percentage of positives in this line is given as 30 per cent., but these positives came from specific matings as suggested by the low value of P , < 0.01 . The eight test groups made during the earlier part of the experiment show only 5 positive cases to 36 negative, a percentage of 15.2 takes, with a P of 0.05 for homogeneity. The families in which the positives appeared were retained and those showing complete negatives were discarded. From the families showing positives two test groups were fed near the close of the experiment. Of the 19 mice in these groups 13 gave positive reaction to 6 negative, a percentage of 68.4. The P for uniformity between the two groups was 0.20. This tendency towards susceptibility is of interest from the standpoint of selection within a group.

No other group of animals was subject to selection during the experiment. The parental stock strains were continued by brother-sister mating.

¹ We are indebted to Dr W. H. Gates for our initial stock of waltzers.

Hybrids between various lines were produced. The results of tests on the F_1 groups are given in Table II.

Table II. *Percentage of positive cases in the F_1 groups fed with B. piliformis.*

Stock F_1 from	+	-	Per-centage takes	No. of test groups	χ^2	P
Waltzer \times black-eyed white	14	47	22.9	7	5.4	0.50
Waltzer \times silver brown	0	28	0.0	6	—	—
Waltzer \times Chinese white	2	12	14.3	4	0.4	0.95
Chinese white \times black-eyed white	0	4	0.0	2	—	—
Chinese white \times silver brown	0	14	0.0	4	—	—

There were 61 F_1 hybrids from the waltzers mated to black-eyed whites that were classed as positive or negative. The black-eyed white parental strain showed no susceptibility. However, the threshold of infectibility was expressed in the hybrids. They showed 22.9 per cent. of positives. In this group 9 cases appeared that were difficult to classify. At autopsy the liver showed scar pits as if lesions had started and rapidly healed. These 9 cases were classed as doubtful.

In the waltzer \times Chinese white F_1 susceptible animals appeared. Neither parental line was immune, and hybrid vigour, if such were expressed in the cross, did not prevent infection.

The F_1 from waltzer \times silver brown remained free from infection, as did the F_1 between the Chinese white \times silver brown. The four F_1 's from Chinese white \times black-eyed white parents also showed no positives.

The F_1 hybrid groups are interesting in that they show one parental line, the silver brown, giving resistance to their hybrid progeny from the waltzers and also from the less susceptible Chinese white. On the other hand the black-eyed whites, themselves apparently immune, when crossed with waltzers have some susceptible offspring. This difference is brought out further in the back-cross groups.

Table III shows the results obtained from the back-cross generations. The

Table III. *Reaction to B. piliformis in back-cross mouse groups.*

Stock back-crosses F_1 to parental lines	+	-	Per-centage positive	No. of test groups	χ^2	P
Black-eyed white $\times F_1$ waltzer black-eyed white	4	61	6.2	3	0.6	0.8
Waltzer $\times F_1$ waltzer black-eyed white	22	17	56.4	6	7.7	0.2
Silver brown $\times F_1$ waltzer silver brown	0	10	0.0	2	—	—
Waltzer $\times F_1$ waltzer silver brown	16	13	55.2	5	2.5	0.7
Silver brown $\times F_1$ Chinese white silver brown	0	5	0.0	1	—	—
Chinese white $\times F_1$ Chinese white silver brown	5	10	33.3	2	1.9	0.2
Chinese white $\times F_1$ Chinese white black-eyed white	3	14	17.6	4	2.8	0.4

test for homogeneity within each line indicates that they are uniform. And the percentage of positives is more or less characteristic of the parental lines into which the back-crosses were made.

The back-cross generation from black-eyed white $\times F_1$ waltzer black-eyed white showed 4 positives to 61 negatives and 3 classed as questionable. This

ratio of 4 to 61 against 0 to 18 of the black-eyed white is not statistically significant as χ^2 equals 1.2 and P equals 0.20. Nevertheless, the fact that 4 positives were observed is of interest in that the factors carried by the waltzers against those carried by the black-eyed whites may possibly tend toward some intermediate and positive expression of the disease.

The back-cross of F_1 waltzer silver brown and F_1 Chinese white silver brown to the silver brown parental line showed no positives. This follows the tendency of the silver brown to cover all susceptibility, as was seen in the waltzer \times silver brown F_1 group. On the other hand the two groups from back-crosses into the Chinese white show some susceptibility, as might be expected from the reaction of the parental line which gave 25 per cent. positive.

The two groups of animals produced by back-cross into the waltzers showed practically the same percentage of positives, waltzer $\times F_1$ waltzer black-eyed white 56.4 per cent. and the waltzer $\times F_1$ waltzer silver brown 55.2 per cent.

These percentages suggest that a simple factor difference may be responsible for the susceptibility of the waltzers and the resistance of the black-eyed white or silver brown strains to this disease. A single major factor prescribes a back-cross ratio for the F_1 waltzer \times black-eyed white or the F_1 waltzer \times silver brown mated back to waltzer of approximately 1 susceptible to 1 resistant. Testing the 22 : 17 of the waltzer $\times F_1$ waltzer black-eyed white against this ratio gives a P of 0.4. The 16 : 13 ratio of the waltzer $\times F_1$ waltzer silver brown gives a P of 0.5. The direct interpretation of the data suggests that a single major factor difference is the inheritance mechanism operating in this cross. Facts brought out in the previous crosses must be taken into consideration, however, before these data can be so interpreted. Table I showed that 80 per cent. of the all-susceptible waltzers died of this disease when exposed to it. Test for the homogeneity of the waltzers by litters showed them to be alike genetically. Such being the case, the 20 per cent. of waltzers which did not take the disease would be attributable to variations in the infective material fed. This fact is borne out by the waltzers which were re-tested.

On the basis of these data only four-fifths of the susceptible die on test. The percentages noted should consequently be increased by this amount to note the animals which should be listed as genetically susceptible in the F_2 . On this basis the 56.4 per cent. becomes 70.4 per cent. and the 55.2 per cent. becomes 69.0 per cent. as the proportion of susceptibles in the back-crossed population. The F_1 results show that in the waltzer \times black-eyed white cross 22.9 per cent. of these animals reacted to the disease. As half of these back-crossed progeny are of this F_1 constitution, it follows that about 12 per cent. should be deducted from the 70.4 per cent. to account for this lack of complete dominance and to obtain the genetic ratio. The silver brown F_1 crosses do not show this lack of complete dominance. The percentages for the back-crosses remain high for the expectation on the basis of a single Mendelian factor difference accounting for susceptibility and resistance in the two strains. Considering the numbers, however, the difference is not significant. The most

likely tentative hypothesis for these data seems to be that of a single major factor difference accounting for the resistance of the *M. musculus* strains and the susceptibility of the *M. bactrianus* strains.

TESTS FOR LINKAGE.

Waltzing factor. As indicated previously early investigations suggest linkage between the locus for the factor pair making for susceptibility or resistance to *B. piliformis* and the locus for the waltzing factor pair.

The groups from waltzer back-crosses also showed segregation of the waltzing factor. The proportion of waltzing to non-waltzing segregates with the incidence of positives to negatives was as follows:

		+	-	χ^2	P
Waltzer \times F_1 waltzer black-eyed white:	Waltzing	10	8	—	—
	Non-waltzing	12	9	0.0	0.95
Waltzer \times F_1 waltzer silver brown:	Waltzing	6	6	—	—
	Non-waltzing	10	7	0.2	0.60

The proportionate numbers of positives to negatives are almost equal in the waltzing and non-waltzing classes in both groups of animals. There is no indication in the data to suggest that the waltzing factor is closely linked with the factor pair for *piliformis* susceptibility or resistance.

Another group of animals was tested that indicated independence of susceptibility from the waltzing factor. They were from non-waltzing parents of the waltzer \times F_1 waltzer black-eyed white back-cross generation. These animals are designated as F_3 (waltzer \times black-eyed white). As a group in test they gave 24 positives, 17 negatives and 3 waltzing segregates classed as questionable. Grouping the positive and negative cases as waltzing and non-waltzing we have:

	+	-	χ^2	P
Waltzing	4	2	—	—
Non-waltzing	20	15	1.0	0.30

Here again we have a group of animals in the non-waltzing class that gave a large number of positives.

The waltzing factor thus seems to be independent of the factorial basis for susceptibility or resistance to this disease.

These data also suggest that since F_2 segregates which waltz are equally likely to be resistant or susceptible, any physiological effects of waltzing as such play no part in the susceptibility or resistance of the mouse to the *piliformis* disease.

SEX LINKAGE.

That the disease has no relation to the physiological differences of the sexes, as pointed out by Tyzzer, is evident also from the observations in these tests. Tests for sex linkage indicate that the locus of the susceptibility-producing factor is not in the sex chromosome. Table IV gives the classification by sex in several of the larger groups.

Table IV. *Reaction to B. piliformis in the sex classes and comparison of the proportions in each.*

Stock		+	-	χ^2	P
Waltzers	$\sigma\sigma$	41	10		
Chinese white	$\sigma\sigma$	39	9	0.0	0.80
Chinese black	$\sigma\sigma$	7	19		
	$\sigma\sigma$	7	23	0.1	0.70
	$\sigma\sigma$	6	12		
F_1 (σ waltzer \times σ black-eyed white)	$\sigma\sigma$	12	30	0.1	0.70
	$\sigma\sigma$	2	12		
F_1 (σ black-eyed white \times σ waltzer)	$\sigma\sigma$	11	29	1.0	0.40
	$\sigma\sigma$	1	6	—	—

In no case do the proportions in the sex classes depart from approximate equality. The F_1 of waltzer $\sigma\sigma \times$ black-eyed white $\sigma\sigma$ where, on a sex-linked hypothesis, the males should be negative shows that both male and female classes have positive and negative individuals in them. From the reciprocal cross no males came to test. One positive case was observed in the seven females.

These data indicate that the factor for susceptibility is not sex-linked. The susceptibility is distributed equally between the sexes, showing that the physiological differences of the sexes play no part in the susceptibility.

DOMINANT WHITE SPOTTING.

The data from these crosses may be arranged to test for the presence of linkage between the loci for the susceptibility factor and the factor for dominant white spotting. This test comes from the data of the F_1 waltzer \times black-eyed white carrying the dominant white factor back-crossed to waltzers. In the progeny of this cross the dominant white factor should be linked with the factor for resistance if the factors are close together in the same chromosome. The mice could be accurately classified for the presence or absence of the dominant white spotting factor, and no linkage between susceptibility and dominant white spotting was observed. The data are as follows:

Parents: Waltzer \times F_1 waltzer \times black-eyed white.

Progeny	+	-	χ^2	P
Dominant white spotted	8	3	—	—
Coloured	6	3	1.1	0.30

The χ^2 of 1.1 with a P of 0.3 suggests that the two groups of data are from the same population.

It was noted in the F_1 crosses from waltzer by dominant white spotted parents that a certain proportion of these animals were susceptible. The physiological effects of the black-eyed white gene are profound. All black-eyed white animals are heterozygous for the dominant white factor because the homozygous condition produces an anaemia that causes death shortly after birth [De Aberle (1925), Gowen and Gay (1932)]. The mating of black-eyed white animals to waltzers gives, in the F_1 , a segregation of the dominant white

factor and its normal allelomorph. This segregation furnishes two groups of animals, one dominant white in colour and having a genetic constitution heterozygous for the dominant white factor and the waltzing factor, the other coloured and having a genetic constitution homozygous for the normal allelomorph of the dominant white factor and heterozygous for the waltzing factor. If susceptibility is influenced by the physiological action of the black-eyed white factor the progeny which are dominant white in coat colour should be more susceptible to the *piliformis* disease than the coloured mice. The data are indicated below:

Parents: *Waltzer* × *dominant white spotting*.

Progeny	+	-	χ^2	P
Dominant white	5	15	—	—
Normal coloured	9	32	0.7	0.40

The identical proportions of the susceptible and resistant mice in the two classes show that the dominant white spotting factor, heterozygous, does not influence the animal's reaction to the *piliformis* disease.

DISCUSSION.

The data herein presented lead to the view that susceptibility to the disease caused by *B. piliformis* may be dependent on a single major factor difference with modifying factors playing a small part in the expression of the disease. Dominance of the resistant allelomorph appears to be complete in one cross but incomplete in another. The genetic factor complex is apparently not linked to the sex chromosome or the chromosomes containing the loci of the dominant white spotting gene or the waltzing gene. The physiological changes in the mice due to sex or the dominant white spotting gene or the waltzing gene seem to play no part in the susceptibility. Susceptibility or resistance to *B. piliformis*, while characteristically found in a species of mice, *M. bactrianus*, is shown to be capable of transfer from this species to another species, *M. musculus*, when the proper genetic factors are introduced by the species crossed, a fact which strongly suggests that in immunity differences of species which do not cross these too may be due to differences distinguishing the gene constitutions of the species compared.

The inheritance of the resistance to this disease is of significance to comparative genetics of the host and disease relationships which are found in nature. Our data confirm Tyzzer's in showing that resistance is dominant to susceptibility. This relationship of dominance is almost universally found to be true of the host inheritance mechanism for warding off a disease. Rich (1923) has shown that it holds for the resistance of two strains of guinea-pigs to both *B. suis* and *B. cholera suis*, Irwin (1929) for resistance of the rat to inoculation of Danysz bacillus, Lambert (1932) for the domestic fowl to *Salmonella gallinarum*, Frateur (1924) for the domestic fowl to the diphtheria bacillus, Roberts and Card (1926) for the domestic fowl to *S. pullorum*,

Manresa (1927) for the rabbit to inoculated *B. abortus*, Schott (1932) for the mouse to inoculated *S. aertrycke*, and Webster (in a paper in press which he kindly allows us to quote) for the mouse to stomach tube inoculation of *B. enteritidis*. This relation of dominance in resistance to pathogenic organisms is furthermore as wellnigh universal in plants as it is in animals. One exception to this general rule has been noted in some work of the writers' in a virus disease, pseudorabies, of mice produced by inoculation, where resistance is recessive.

The apparently simple nature of the inheritance of resistance to the disease produced by this *piliformis* organism is not necessarily common to all diseases. This simple genetic mechanism is known for a few other cases of disease resistance in animals and many in plants. Rich noted a simple Mendelian difference between two strains of guinea-pigs in their complement. These strains tested by inoculation with a standard dose of *B. cholera suis* showed that 77 per cent. of the low complement strain of pigs died while but 20 per cent. of the full complement pigs succumbed. Similar differences were also noted in a natural outbreak of *B. suis* within the colony. Manresa, working with abortions produced in the rabbit by inoculation with *B. abortus*, likewise comes to the view that it is quite possible that the susceptible and resistant strains developed are due to a single Mendelian pair of genes. Other cases are quite obviously more complex.

CONCLUSION.

Susceptibility to *B. piliformis* apparently segregates independently of the waltzing factor, the dominant white factor, and sex.

Resistance seems best interpreted as depending on a major dominant factor the expression of which is modified in certain crosses.

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