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Non-Mendelian Inheritance of Mosquito Susceptibility to Infection with *Brugia malayi* and *Brugia pahangi*

Abstract. *The mode of inheritance of susceptibility or refractoriness of insect vectors to medically important pathogens such as those causing malaria or filariasis is usually believed to follow normal Mendelian laws and to involve a single pair of alleles. In this report, experiments are described that demonstrate another mode of inheritance of mosquito susceptibility to filarial parasites. Crosses were made between susceptible and refractory species of the* Aedes scutellaris *complex, and the hybrid and backcross progeny were tested for susceptibility to infection by* Brugia malayi *and* Brugia pahangi. *The data indicate that inheritance follows a non-Mendelian pattern indicative of extrachromosomal factors inherited through the maternal parent.*

The interaction between medically important pathogens and their vector hosts is vital to the survival of both. The vector host must possess certain defense mechanisms against the parasite and the parasite must cope with the insect host defenses. Through coevolution, pathogens become increasingly specialized with respect to their vector hosts. Variations in vector susceptibility to infection with either filarial worms or malaria parasites have been observed among diverse populations of vectors, as well as among individuals of the same population. The genetic basis of the relation between vectors and pathogens is poorly understood. Most reports on the genetics of vector susceptibility to organisms pathogenic to man or domestic animals deal with susceptibility of *Culex pipiens* or *Aedes aegypti* to *Plasmodium cathemerium* (*1*, *2*), *P. gallinaceum* (*3*), *Dirofilaria immitis*(*4*-*7*), *Brugia malayi* (*8*), *B. pahangi* (*9*, *10*), and *Waltonella flexicanda* (*11*). In all of these studies, the susceptibility of the vectors to the pathogens appeared to be controlled by a single gene system.

In this report we demonstrate another mode of inheritance of mosquito susceptibility to filarial parasites. It is maternal, non-Mendelian inheritance controlled by cytoplasmic factors. Our genetic studies were done on species of the *Aedes scutellaris* complex of mosquitoes. The *A. scutellaris* complex consists of about 30 species that are widely distributed throughout the Pacific and Oriental regions. The Oriental and the western Pacific groups of the *A. scutellaris* complex that we have tested (*A. alcasidi*, *A. riversi*, *A. seatoi*, and *A. malayensis*) are refractory to infection with both *B. malayi* and *B. pahangi* (*12*). In contrast, all species of the Polynesian group (*A. polynesiensis*, *A. cooki*, *A. tabu*, *A. kesseli*, and *A. pseudoscutellaris*) are highly susceptible (*13*-*15*). All species of the Polynesian group are vectors of subperiodic *W. bancrofti* in the South Pacific (*16*). Backhouse and Woodhill (*17*) tested *A. scutellaris* and *A. pseudoscutellaris* for susceptibility to infection with the New Caledonian strain of *W. bancrofti* and found that *A. scutellaris* was refractory and *A. pseudoscutellaris* was susceptible. Since all tests for susceptibility of different members of the *A. scutellaris* complex to infection with *Brugia* were in agreement with susceptibility or refractoriness to the subperiodic *W. bancrofti*, we could use *B. malayi* and *B. pahangi* as laboratory models for vector transmission studies.

In our experiments, mosquito larvae were reared in an insectary at 27° ± 1°C with a relative humidity of 80 ± 5 percent. Larvae were reared in white enamel pans, approximately 100 larvae per pan containing 2 liters of water. Larvae were fed with 2 ml of a suspension of liver powder in water daily. Pupae were collected from the pans, their sexes were determined, and those of the same sex were placed together in 50-ml cups until emergence of the adults. The sexes of the newly emerged adults were rechecked and appropriate crosses were made by placing the adults in cylindrical cages (180 mm in diameter and 180 mm high). All adult mosquitoes were provided with a cup of water and with honey mixed with cellulose fibers. After 5 days the females were fed on gerbils infected with *B. malayi* or *B. pahangi*. The blood-fed mosquitoes were individually collected from the feeding cage and placed into a new cage provided with honey food and a 50-ml paper cup lined with paper towel and filled with water for oviposition. Ten days after the infective blood meal, all surviving females were checked for infective third-stage larvae of *B. malayi* or *B. pahangi*. Individual females were placed on a microscope slide with four drops of insect saline and dissected into four parts: the proboscis, head, thorax, and abdomen. These parts were opened and the tissue teased in order to release active *Brugia* larvae into the saline. The number of larvae found in each body part was recorded.

Table 1. Inheritance of mosquito susceptibility to infection with *B. malayi* in crosses of susceptible females with refractory males. Po = *A. polynesiensis*, which is susceptible; Al = *A. alcasidi*, which is refractory.

Cross	Parents ♀	Parents ♂	Number dissected	Susceptible Number	Susceptible Percentage	Refractory Number	Refractory Percentage
P	Po/Po	Po/Po	87	87	100	0	0
P	Al/Al	Al/Al	92	0	0	92	100
F_1	Po/Po	Al/Al	42	42	100	0	0
F_1	Al/Al	Po/Po*					
F_2	Po/Al	Po/Al	22	22	100	0	0
F_3	Po/Al	Po/Al	38	38	100	0	0
F_6	Po/Al	Po/Al	68	68	100	0	0
Bx_1	Po/Al	Po/Po	17	17	100	0	0
	Po/Po	Po/Al	73	73	100	0	0
Bx_2	Po/Al	Al/Al	8	8	100	0	0
	Al/Al	Po/Al*					

*Incompatible cross.

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Table 2. Inheritance of mosquito susceptibility to infection with *B. malayi*, in crosses of refractory females with susceptible males. Al = *A. alcasidi*, which is refractory; Co = *A. cooki*, which is susceptible.

Cross	Parents		Number infected	Susceptible		Refractory	
	♀	♂		Number	Percentage	Number	Percentage
P_1	Al/Al × Al/Al		103	0	0	103	100
P_2	Co/Co × Co/Co		176	176	100	0	0
F_1	Al/Al × Co/Co		50	0	0	50	100
F_1	Co/Co × Al/Al*						
F_2	Al/Co × Al/Co†		4	0	0	4	100
Bx_1	Al/Co × Al/Al		15	0	0	15	100
	Al/Al × Al/Co†		2	0	0	2	100
Bx_2	Al/Co × Co/Co		43	0	0	43	100
	Co/Co × Al/Co*						

*Cross incompatible. †Partial hybrid sterility in males.

Interspecific hybridization resulting in various degrees of compatibility within the *A. scutellaris* complex indicates a close genetic relation among geographically isolated island populations of various forms (*18, 19*). Crosses between the western and eastern Pacific groups of *A. scutellaris* complex result usually in unidirectional compatibility (*12*). The species included in this study were *A. polynesiensis* from American Samoa and Western Samoa, *A. cooki* from Niue Island, *A. alcasidi* from Taiwan, and *A. malayensis* from Bangkok. Whereas the first two species were fully (100 percent) susceptible to infection with *B. malayi* and *B. pahangi*, the second two were fully (100 percent) refractory.

The cross between *A. polynesiensis* (Po) female (susceptible) and *A. alcasidi* (Al) male (refractory) was successful (Table 1). The hybrid offspring Po/Al females were susceptible to *B. malayi*, and the reciprocal cross Al/Al ♀ × Po/Po ♂ did not give any progeny. Thus the F_2 generation and backcrosses were possible only with the F_1 Po/Al hybrids. The F_2 offspring as well as the subsequent generations F_3 to F_6 were viable and normal. All of them were as susceptible as the original maternal stock. The backcross of the F_1 Po/Al ♀ to the Po/Po ♂ was successful and the progeny were susceptible. The reciprocal backcross (Po/Po ♀ × Po/Al ♂) was successful and susceptible. The second possible backcross of the F_1 Po/Al worked with the refractory male parent (Po/Al ♀ × Al/Al ♂). The offspring of this backcross were again susceptible. If susceptibility is controlled by nuclear genes, according to the Mendelian segregations we should obtain a 3:1 ratio of susceptible to refractory females in the F_2 progeny provided that refractoriness is the recessive trait. Under the same conditions we would receive a 1:1 ratio of susceptible to refractory females in the B_2 backcross (Table 1).

The second series of crosses was done with *A. cooki* as a susceptible parent and *A. alcasidi* as a refractory one (Table 2). This crossing type is also unidirectional, but it is substantially different from the first type in terms of parent susceptibility and the direction of compatibility. While in the first type (Po/Po ♀ × Al/Al ♂) the female parent was susceptible, in the second type (Al/Al ♀ × Co/Co ♂) the female parent was refractory. The F_1 hybrid females Al/Co were refractory to infection with *B. malayi*. The F_1 hybrid males were partially sterile, so that a reduced number of F_2 offspring could be obtained. Fertility in the female hybrid Al/Co was normal. Four females of the F_2 offspring were dissected and were refractory. The Bx_1 backcross Al/Al ♀ × Al/Co ♂ gave reduced number of offspring because of the reduced fertility in the male parents. The second backcross Bx_2 (Al/Co ♀ × Co/Co ♂), which was a cross between a refractory hybrid female and a susceptible male, yielded refractory offspring. The reciprocal cross Bx_2 was incompatible. The second crossing type did not obey the Mendelian law of segregation of phenotypic traits, neither in the F_2 offspring nor in the backcross of the Al/Co hybrid to a recessive parent. If we suppose that susceptibility is a recessive trait, then 25 percent of the F_2 female offspring should be susceptible, and 50 percent of the backcross Bx_2 offspring females should be susceptible. As shown in Table 2, the susceptibility or refractoriness of the offspring is determined by the female parent.

The cross between *A. malayensis* and *A. polynesiensis* has been shown repeatedly to be unidirectional (*13, 19*). The viable offspring can be obtained from the cross *A. malayensis* (Ma) female to *A. polynesiensis* (Po) male. Most of the offspring from the crosses included in Table 3 were tested for infection with *B. malayi* and *B. pahangi*. The compatible cross Ma/Ma ♀ × Po/Po ♂ resulted in hybrids that were refractory to infection with both *Brugia* species. It is interesting that some crosses (marked with asterisks in Tables 2 and 3) gave in some replicates a low percentage of offspring (*19*). The F_2 offspring was also refractory. In the backcross Ma/Po ♀ × Po/Po ♂ the prog-

Table 3. Inheritance of mosquito susceptibility to infection with *B. malayi* and *B. pahangi* in crosses of *A. malayensis* (Ma, refractory) with *A. polynesiensis* (Po, susceptible).

Cross	Parents		*B. malayi*					*B. pahangi*				
	♀	♂	Number dissected	Susceptible		Refractory		Number dissected	Susceptible		Refractory	
				Number	Percentage	Number	Percentage		Number	Percentage	Number	Percentage
P_1	Po/Po × Po/Po		38	38	100	0	0	35	35	100	0	0
P_2	Ma/Ma × Ma/Ma		46	0	0	46	100	44	0	0	44	100
F_1	Ma/Ma × Po/Po		139	0	0	139	100	111	0	0	111	100
F_1	Po/Po × Ma/Ma*											
F_2	Ma/Po × Ma/Po		123	0	0	123	100	124	0	0	124	100
Bx_1	Ma/Po × Po/Po		5	0	0	5	100	25	0	0	25	100
	Po/Po × Ma/Po*											
Bx_2	Ma/Po × Ma/Ma		88	0	0	88	100	75	0	0	75	100
	Ma/Ma × Ma/Po		60	0	0	60	100	60	0	0	60	100

*Incompatible cross.

eny females were refractory. The reciprocal cross Bx_1 was not compatible. The backcross Bx_2 (Ma/Po ♀ × Ma/Ma ♂ and Ma/Ma ♀ × Ma/Po ♂) gave refractory offspring. The crossing type including *A. malayensis* and *A. polynesiensis* is similar to that of *A. alcasidi* and *A. cooki*. This third series of crosses again indicates a maternal type of inheritance of susceptibility to brugian filariasis in the *A. scutellaris* complex of mosquitoes. It also indicates that the mode of inheritance of susceptibility to *B. pahangi* is similar to *B. malayi*. On the basis of these results we suppose that the inheritance of susceptibility of the *A. scutellaris* complex of mosquitoes to infection with the subperiodic *Wuchereria bancrofti* will be of the same mode.

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20. A generally incompatible cross Co/Co ♀ × Al/Al ♂ (Table 2) produced, in three of five replicates, offspring in the F_1 generation (1.0 to 6.4 percent). Seven females of this progeny (Co/Al) were dissected and all were susceptible to infection with *B. malayi*. Similarly, the cross Po/Po ♀ × Ma/Ma ♂ (see first asterisk in Table 3) gave a low percentage of F_1 and F_2 (Po/Ma) progeny. Eight females dissected for *B. pahangi* were all susceptible. Ten F_2 Po/Ma females dissected for *B. malayi* were also susceptible. These results provide additional evidence supporting the maternal type of inheritance.
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Ultrasensitive Stain for Proteins in Polyacrylamide Gels Shows Regional Variation in Cerebrospinal Fluid Proteins

Abstract. *A new silver stain for electrophoretically separated polypeptides can be rapidly and easily used and can detect as little as 0.01 nanogram of protein per square millimeter. When employed with two-dimensional electrophoresis, it should permit qualitative and quantitative characterization of protein distributions in body fluids and tissues. It has been used to demonstrate regional variations in cerebrospinal fluid proteins.*

Many biological studies require the detection and characterization of trace quantities of proteins. Developments in two-dimensional electrophoresis have made it possible to resolve thousands of proteins from complex biological mixtures (*1*). However, inability to detect proteins present in low concentration has limited the application of this method, particularly in clinical screening for pathological states, endocrinology, mammalian metabolism, developmental biology, and immunology.

The most common nonradioactive polypeptide detection methods employ intense organic stains such as Coomassie blue. These stains lack the sensitivity to detect proteins present in low or trace concentrations. Body fluids, such as cerebrospinal and amniotic fluids, are often difficult to obtain in quantity and frequently contain abundant proteins, which cause distortion of electrophoretic patterns when sufficient sample is analyzed to observe trace proteins.

This sensitivity problem has been overcome by the application of techniques employing a histologically derived silver stain to proteins in acrylamide gels. A hundredfold increase in sensitivity was achieved over Coomassie blue stain (*2, 3*). These techniques had three main drawbacks: (i) they took 3 to 4 hours, (ii) they consumed large quantities of silver, and (iii) it was necessary to prepare several solutions just before use.

In this report we describe a new, photochemically derived silver stain. The method rquires three relatively stable solutions, takes less than 1 hour to perform, and uses 2 percent of the silver needed for the histological stain.

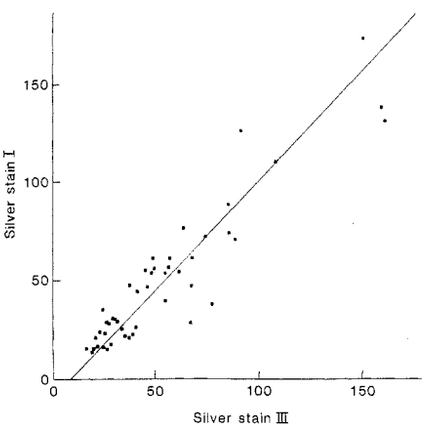

Fig. 1. Comparison of original histochemically derived stain (*Silver stain I*) with new photochemically derived stain (*Silver stain III*). This is a density versus density plot of all polypeptide spots within a small subregion of an *E. coli* lysate gel pattern. The slope was 1.08, the *Y* intercept −8.1, and the correlation coefficient .94. Gels were positioned next to a National Bureau of Standards calibrated photographic density standard and photographed with Tri-X 120-mm film (Kodak). These photographic images were then scanned at a resolution of 100 μm with an Optronics (Chelmsford, Mass.) 1000 HS scanning densitometer. Image densities were converted to optical density units by using the calibrated density standard. This conversion normalized gel images for the significant variations in photography and scanning densitometry. Measurements were made with a IP5000 image processor (DeAnza Systems Inc., San Jose, Calif.) and PDP 11/60 computer (Digital Equipment Corp., Maynard, Mass.); background was subtracted and identical measurement windows were used. The original gel pattern was produced by subjecting 10 g of *E. coli* lysate proteins to two-dimensional gel electrophoresis by the method of O'Farrell (*1*).

Proteins were separated by the two-dimensional electrophoretic method of O'Farrell (*1*). The second-dimension gels were 10 percent acrylamide, 16 by 12 cm by 0.8 mm thick. Proteins were fixed in a solution of 50 percent methanol and 12 percent acetic acid for a minimum of 20 minutes, and excess sodium lauryl sulfate was removed from the gels by three 200-ml, 10-minute rinses containing 10 percent ethanol and 5 percent acetic acid.

Gels were then soaked for 5 minutes in a 200-ml solution of 0.0034*M* potassium dichromate and 0.0032*N* nitric acid. They were washed four times, for 30 seconds in 200 ml of deionized water, and placed in 200 ml of 0.012*M* silver nitrate for 30 minutes. This was followed by rapid rinsing with two 300-ml portions of the image developer solution, which contained 0.28*M* sodium carbonate and 0.5 ml of commercial Formalin per liter. The gels were gently agitated in a third portion of this solution until the image had reached the desired intensity. Development was stopped by discarding the developer and adding 100 ml of 1 percent

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