

2020 CED #1

In many countries, *Anopheles gambiae* mosquitoes are responsible for transmitting the parasite that causes malaria to people through their bites. A primary tool for mosquito control is the use of insecticidal nets sprayed with chemicals known as pyrethroids, which are relatively safe for people but toxic to mosquitoes. However, mosquito resistance to pyrethroids has now become widespread. Pyrethroids interfere with the function of a transmembrane sodium channel found in cells of the mosquitoes (Figure 1). In one common mutation to the channel protein, a phenylalanine is substituted for a leucine at amino acid position 1014. Scientists hypothesize that this mutation is responsible for some cases of pyrethroid resistance.

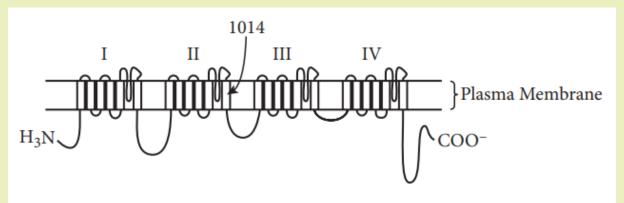


Figure 1. Schematic drawing of the transmembrane sodium channel targeted by pyrethroids and other insecticides. The arrow points to the position of amino acid 1014.



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To investigate pyrethroid resistance, mosquitoes were collected four times over a two-year period from the following two regions.

- Region A: a southern vegetable-growing region where large amounts of insecticide are applied for crop protection
- Region B: a northern rice-growing region where very little insecticide is applied for rice protection

Scientists exposed the collected mosquitoes to filter papers soaked in two different pyrethroid insecticides, deltamethrin and permethrin, and the percent mortality of the mosquitoes was determined after 24 hours (Figure 2). The scientists simultaneously determined whether leucine or phenylalanine was encoded at position 1014 by each of the two copies of the sodium channel gene (Table 1).



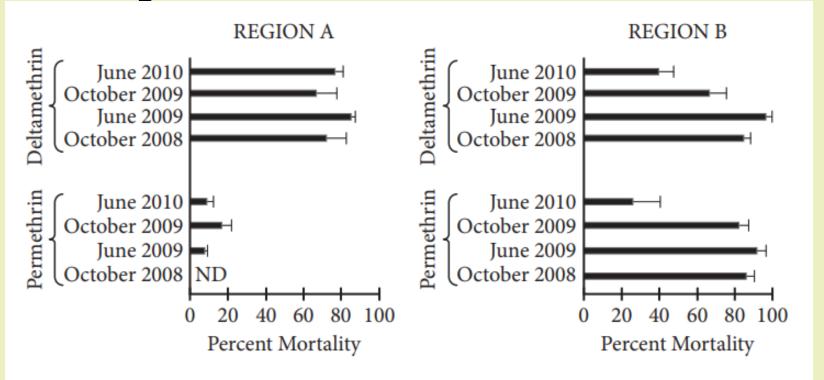


Figure 2. Susceptibility of *A. gambiae* mosquitoes from two regions to the pyrethroids deltamethrin and permethrin. A mosquito strain that is susceptible to the insecticides displayed at least 95% mortality in all experiments, and mosquitoes exposed to untreated filter paper displayed less than 10% mortality. Error bars represent standard deviation. "ND" means no data are available.



Table 1. Frequencies of leucine and phenylalanine at position 1014 of the sodium channel

Region	Date	Total Mosquitoes Tested	Homozygous for Leucine	Heterozygous for Leucine and Phenylalanine	Homozygous for Phenylalanine
Α	October 2008	39	3	5	31
Α	June 2009	29	-	5	24
Α	October 2009	28	-	1	27
Α	June 2010	46	-	9	37
В	October 2008	27	20	5	2
В	June 2009	26	18	7	1
В	October 2009	34	20	8	6
В	June 2010	44	12	20	12



- (a) **Describe** the most likely cause of the amino acid substitution in the sodium channel protein. **Explain** how the substitution of a single amino acid in the channel protein could cause pyrethroid resistance in mosquitoes.
- A change occurred in the DNA sequence encoding the sodium channel polypeptide.
- The substitution could change the shape of the protein channel so that pyrethroids can no longer affect it / bind to it / interfere with its function.



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(b) **Identify** the dependent variable in the experiment whose data are graphed in Figure 2. **Identify** the positive control in the experiment. **Justify** exposing some mosquitoes to untreated filter paper each time the experiment was performed.

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Accept one of the following:

- Percent mortality
- Susceptibility to insecticide

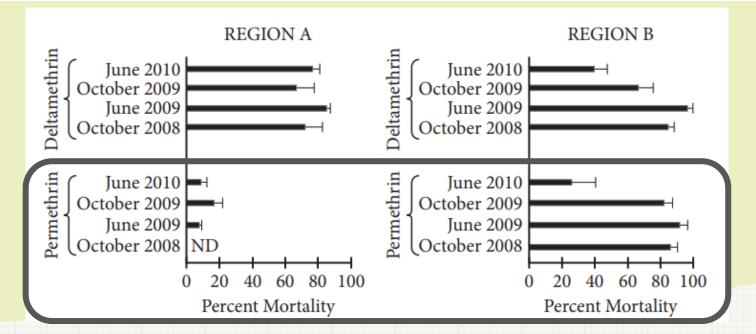


- (b) **Identify** the dependent variable in the experiment whose data are graphed in Figure 2. **Identify** the positive control in the experiment. **Justify** exposing some mosquitoes to untreated filter paper each time the experiment was performed.
- Testing the strain that is susceptible to the insecticides
- Exposing mosquitoes to untreated filter paper confirms that any observed mortality is from the insecticides rather than from the filter paper itself or any other experimental conditions.



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(c) Based on the data in Figure 2, **describe** whether mosquitoes from region A or from region B are more likely to exhibit greater evolutionary fitness if exposed to permethrin in their native environment over the time period of the experiment. Based on the data in Figure 2, **describe** any significant change in the susceptibility of mosquitoes from region B to <u>each</u> of the two insecticides over the two-year period. Use the data in Table 1 to **calculate** the frequency of the allele coding for phenylalanine in each population of mosquitoes in October 2008. Round your answers to two decimal places.



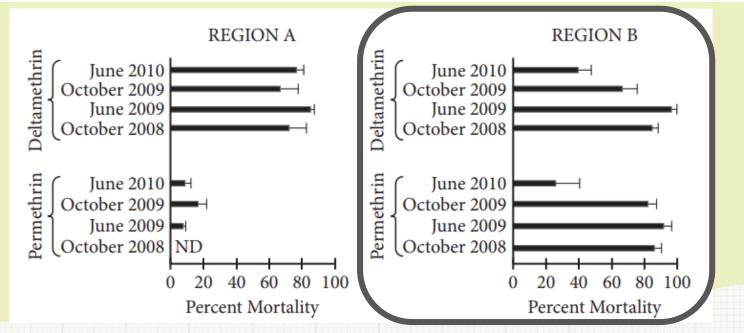


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- Mosquitoes from region A are much more likely to survive to reproduce, so the region A mosquitoes will have greater evolutionary fitness.



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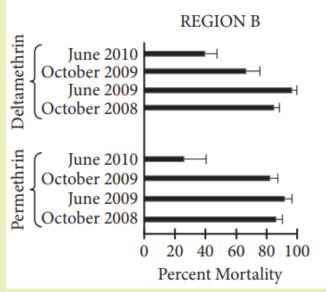
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For permethrin, there was little significant change in susceptibility until the June 2010 test, when the
mosquitoes were significantly less susceptible than they had been for the previous three tests. For
deltamethrin, there was a significant decrease in susceptibility from June 2009 to October 2009 and
then a further significant decrease from October 2009 to June 2010.



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$$\frac{\#Phe \; allele}{total \; alleles} = \frac{2(homozgyous \; Phe) + 1(heterozygous)}{2(total \; mosquitos)}$$

$$\frac{\#Phe \ allele}{total \ alleles} = \frac{2(31) + 1(5)}{2(39)}$$

Mosquitoes from region A:
$$5 + 2(31) = 67$$
 and $\frac{67}{78} = 86$.

Acceptable range is .85 - .86

$$\frac{\text{#Phe allele}}{\text{total alleles}} = \frac{62 + 5}{78}$$

$$\frac{\text{#Phe allele}}{\text{total alleles}} = \frac{67}{78} = 0.859$$

Region A: 0.86



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В	October 2008	27	20	5	2

$$\frac{\#Phe \; allele}{total \; alleles} = \frac{2(homozgyous \; Phe) + 1(heterozygous)}{2(total \; mosquitos)}$$

$$\frac{\#Phe \ allele}{total \ alleles} = \frac{2(2) + 1(5)}{2(27)}$$

$$\frac{\#Phe \ allele}{total \ alleles} = \frac{4+5}{54}$$

$$\frac{\text{#Phe allele}}{\text{total alleles}} = \frac{9}{54} = 0.167$$

Mosquitoes from region B: 5 + 2(2) = 9 and $\frac{9}{54} = .17$.

Acceptable range is .16 – .17

Region B: 0.17



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(d) Using mosquitoes from insecticide-free areas, the scientists developed mosquito strains with amino acid substitutions at other positions in the sodium channel protein. They exposed the mosquito strains to nonpyrethroid insecticides. **Predict** the susceptibility of the mosquitoes to the insecticides. The scientists claim that the mosquito population of region B evolved resistance over the period of the experiment and that resistance arose as a result of the immigration of resistant mosquitoes from other regions. Based on the data in Table 1 and the information provided, **provide evidence** to support the scientists' claim.

Accept one of the following:

- The mosquitoes will all die.
- The mosquitoes will be 100% susceptible



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Predict the susceptibility of the mosquitoes to the insecticides. The scientists claim that the mosquito population of region B evolved resistance over the period of the experiment and that resistance arose as a result of the immigration of resistant mosquitoes from other regions. Based on the data in Table 1 and the information provided, **provide evidence** to support the scientists' claim.

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October '08: 0.17

June '10: 0.5

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The frequency of the phenylalanine allele increased from very low to much higher (OR: from 0.17 to 0.5)
for population B mosquitoes that come from an area with low insecticide use. Thus insecticide use
is not selecting for those mosquitoes with the phenylalanine allele; it is more likely that pyrethroidresistant mosquitoes with the phenylalanine allele are immigrating to the area, thus increasing the
frequency of the allele in the population.

