Spiess, E.B. and R. Ritzlin. University of Illinois, Chicago, Illinois. Bacterial infection and cultures of D. persimilis tested for mating propensity.

Fluctuation of apparent random nature in mating speed performance of D. persimilis strains has been bothersome in the past. In addition, when two or more samples are tested for mating from the same culture, there is far more variation between cultures than between replicates from the same culture (Spiess, Sherwin, & Yacher, in press, Genetics). These facts have tended to indicate that microorganism contamination may be detrimental to stability of flies' behavior. It was apparent some years ago that poorly mating flies from fast-mating strains often had a yellow discoloration in the crop. During the summer of 1969, microorganisms from the crops of poorly mating females were isolated and cultured on nutrient agar. The most consistent organism found was tested for diagnostic traits in the laboratories of Drs. E.E. Vicher (University of Illinois at the Medical Center) and M. Silverman (V.A. Research Hospital) with the tentative identification as Achromobacter sp. Its traits are as follows: 1) short Gram-negative rod which forms mucoid colonies on nutrient agar (BHI); mucus growth is greater at low temperature (15°C) than at warm (25°C). 2) Best growth at 25°C, with no growth at 37°C, but good growth at 15°C. 3) Aerobic, tending to overgrow yeast on the surface. 4) Catalase and dextrose positive; negative for oxydase, citrate, indole, methyl red, acetoin; odorless. 5) No flagella visible on electron micrograph after fixation with 10% formalin and uranyl acetate. 6) Sensitive to tetracycline, novobiocin, streptomycin and kananycin, but resistant to penicillin, erythromycin and neomycin.

Flies were cultured on food to which tetracycline and streptomycin had been added in solution to the surface (with about the concentrations listed by Hendrix and Ehrlich, 1965), in three different ways: first, on the assumption that larvae would not ingest much antibiotic unless it was mixed with yeast, food made without yeast was inoculated on the surface with an antibiotic-yeast mixture. This technique suffered from low nutrition to the larvae since the yeast did not grow well and was insufficient to produce normal-sized adults. Second, antibiotics were added in surface solution to food on which parent females were depositing eggs; but females tended to lay eggs on food sources lacking the antibiotics when given a choice, and too few adults were raised to be tested. Finally, the treatment adopted was to add antibiotics to the food after first instar larvae had hatched (on plastic spoons with yeasted food which were then inserted into regular culture food plus 10 drops of antibiotic solution added to the surface). All strains benefitted by that treatment in that productivity increased over untreated, and mating speeds became more consistent and reliable, though they were uniformly lower in propensity (mating index) than the controls. Consequently the antibiotic treatment not only had lowered the bacterial growth, but also depressed the flies' activity.

To test for detrimental effects on preadult stages of the life cycle, eggs and larvae were grown on nutrient (BHI) agar in which there was a two-day growth of bacteria, using the following techniques: parent flies of two strains (D. persimilis), selected for either fast (F2) or slow (S1) mating (KL homokaryotypes from the redwoods population) in their 20th generation of selection, laid eggs on food in plastic spoons. Eggs were washed in 70% ethanol for 15 minutes and planted in lots of 50 on 1) BHI agar with 2-day growth of Achromobacter sp. or 2) on BHI sterile (control). Egg hatch results are as follows with 4 replicates per treatment per strain:

	Control		Bacteria	
Strain	Total Hatch	Range	Total Hatch	Range
F2	173/200=86.5%	79.6%-94.0%	179/200=89.5%	86.0%-96.0%
S1	158/200=79.0%	74.0%-86.0%	172/200=86.0%	84.0%-88.0%

It was surprising to see no real difference between treatments; in fact, there was a slight benefit to hatching when the bacteria were growing on both strains.

For larval-pupal survival, first instar larvae were collected from spoons of food, washed in sterile water on a black cloth which had been sterilized with a strong disinfectant (Microquat) and then rinsed a second time in sterile water. Larvae were then placed in vials on slanted food, 25 per vial, either with a 2-day growth of bacteria or sterile, then were given yeast suspension and sterile paper strips for pupation. Adult emergent results were as follows with 4 replicates per treatment per strain: