Cytoplasmically Inherited Reproductive Incompatibility in Tribolium Flour Beetles: The Rate of Spread and Effect on Population Size

Lori Stevens[1] and Michael J. Wade

Department of Ecology and Evolution, University of Chicago, Chicago, Illinois 60637

Manuscript received May 19, 1989
Accepted for publication November 4, 1989

ABSTRACT

This paper reports on the effects of a cytoplasmically inherited reproductive incompatibility in different genetic strains of the flour beetle, *Tribolium confusum*. We measured the rate of spread and the effect of host population size using different initial frequencies of infection with a cytoplasmic factor that mediates reproductive incompatibility. There were two experiments, in one the infected and uninfected lines were from the same genetic strain, b-Yugoslavia. In the other, the infected line was from the "high cannibalism" bIV strain and the uninfected line from the "low cannibalism" bI strain. We estimate that the fitness ratio of infected to uninfected in b-Yugoslavia is 0.63 and the observed rate of spread for this strain corresponds to a model of cytoplasmic inheritance that takes into account the productivity differences between the infected and cured lines. In the bI-bIV experiment, because the uninfected and infected lines are from different genetic strains, we cannot partition the effects of the cytoplasmic factor from other factors. The rate of spread in the bI-bIV experiment is faster in males and slower in females than predicted from a model of cytoplasmic inheritance. In both experiments, productivity varies with initial infection frequency; however, the relationship is not explained by a simple model that predicts lower population size at intermediate infection frequencies.

POPULATIONS of the flour beetle, *Tribolium confusum*, from different geographic locations may exhibit partial reproductive isolation owing to infection with a cytoplasmic factor in some localities and its absence in others (WADE and STEVENS 1985). The reproductive incompatibility is inherited maternally through the cytoplasm and a cross between infected males and uninfected females never produces offspring. All other crosses are fertile.

A similar pattern of reproductive incompatibility has been reported in 13 species spanning five orders of insects [Coleoptera: *Tribolium confusum* (STANLEY 1961; WADE and STEVENS 1985), *Hypera postica* (HSAIO and HSAIO 1985a, b); Lepidoptera: *Ephestia cautella*, *Cadra figulilella* (KELLEN, HOFFMANN and KWOCK 1981); Diptera: *Drosophila simulans* (HOFFMANN, TURELLI and SIMMONS 1986), *Drosophila melanogaster* (HOFFMANN 1988), *Culex pipiens* (LAVEN 1967), *Rhagoletis cerasi* (BOLLER *et al.* 1976); Hymenoptera: *Nasonia* (=*Mormoniella*) *vitripennis* (SAUL 1961); RICHARDSON, HOLMES and SAUL 1987); and Homoptera: *Laodelphas striatellus* (NODA 1984)], a species of acarina (spider mites), *Tetranychus urticae* (OVERMEER and VAN ZON 1976) and a species of isopod, *Porcellio dilatatus* (LEGRAND *et al.* 1986). In addition a similar pattern of reproductive incompatibility exists among members of the *Aedes scutellaris* complex (SMITH-WHITE and WOODHILL 1954) and among species of bark beetles; *Ips integer* is partially reproductively isolated from *I. plastographus plastographus* and *I. plastographus maritimus* (LANIER 1971). Treatment of presumably infected strains with tetracycline can make them compatible with uninfected strains. It is hypothesized that rickettsia-like microorganisms mediate reproductive incompatibility in these species. Recently it has been reported that rickettsia-like microorganisms are found in the ovaries of *T. confusum* and that antibiotic treatment with tetracycline hydrochloride eliminates the symbiont from the ovaries (O'NEILL 1989).

As a result of maternal transmission and complete infertility of infected males × uninfected females, the cytoplasmic factor is expected to spread through a population, the rate of spread accelerating with positive frequency dependence (CASPARI and WATSON 1959; FINE 1978). If all compatible crosses are equally fertile and the cytoplasmic factor has no effect on host fitness, the expected infection frequency in generation $t + 1$ is:

$$p_{t+1} = \frac{p_t}{[1 - p_t(1 - p_t)]} \qquad (1)$$

where p_t is the infection frequency in generation t (CASPARI and WATSON 1959; FINE 1978). As $t \rightarrow \infty$,

[1] Present address: Department of Ecology, University of Vermont, Burlington, Vermont 05452.

The publication costs of this article were partly defrayed by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

$p_t \rightarrow 1$ for $p > 0$; *i.e.*, the populations will be completely infected.

In contrast to this expectation, polymorphisms of infectedness have been reported both within (HOFFMANN and TURELLI 1988; BARR 1980) and between populations (WADE and STEVENS 1985; HSAIO and HSAIO 1985b; KLOSTERMEYER and MANGLITZ 1979; HOFFMANN, TURELLI and SIMMONS 1986; BOLLER *et al.* 1976; LEGRAND *et al.* 1986; OVERMEER and VAN ZON 1976; SAUL 1961; NODA 1984; LAVEN 1967). Explanations for such polymorphisms include: lowered fitness of infected individuals (CASPARI and WATSON 1959), incomplete maternal transmission of the microorganism (SUBBARAO *et al.* 1977; FINE 1978; BARR 1982), loss of incompatibility with male age (SINGH, CURTIS and KRISHNAMURTY 1976; HOFFMANN, TURELLI and SIMMONS 1986), nonrandom mating (HOFFMANN and TURELLI 1988) and environmental curing agents (HOFFMANN and TURELLI 1988, STEVENS 1989a). We have not observed either loss of incompatibility with male age in *Tribolium* or any offspring from crosses between infected males and uninfected females maintained as pairs for over one year (WADE and STEVENS 1985; STEVENS 1989a; M. J. WADE and L. STEVENS unpublished results). The ability of some of these mechanisms to maintain a stable polymorphism within a single population will depend on the parameter values (*e.g.*, rate of environmental curing or degree of nonrandom mating); however, others produce unstable polymorphic equilibria (*e.g.*, lowered fitness) and hence require population structure to explain persistent polymorphisms.

This study uses various initial infection frequencies of the cytoplasmic factor in the flour beetle, *T. confusum* to examine its rate of spread and effect on population size. We measured population size in populations fixed for either the presence or absence of the cytoplasmic factor to estimate the effect of the factor on host fitness. We measure population size in polymorphic populations to determine how segregation for the cytoplasmic factor affects a population's productivity. We compared the observed rate of spread in a single generation to that expected on the basis of the model described above (Equation 1). We did this for two experiments: (1) the uninfected and infected beetles are from the same genetic strain and thus the presence or absence of the cytoplasmic factor is presumably the only variable; and, (2) the uninfected and infected individuals are from different genetic strains. Two strains in the second experiment have similar fitness components, except for their cannibalism rates. Thus these experiments provide us with information on the rate of spread and effect on population size in a genetically homogeneous population (experiment 1) and in populations in which there is a correlation between the cytoplasmic factor and other genetic factors (experiment 2).

MATERIALS AND METHODS

We established populations at different initial frequencies of infection with the cytoplasmic factor and scored the productivity and infection frequencies in the offspring generation. Productivity is the number of individuals (larvae + pupae + adults) in a vial. Initial infection frequencies ranged from completely uninfected ($p = 0$) to completely infected ($p = 1.0$) with replicate populations of each treatment. Populations were initiated with young adults and the initial infection frequency was the same in both sexes. In one experiment, all adults were from the same genetic strain b-Yugoslavia (bY) which is infected with the cytoplasmic factor responsible for reproductive incompatibility (STEVENS 1989a). An uninfected strain of bY was obtained by placing about 400 premated bY adults on tetracycline medium (WADE and STEVENS 1985; STEVENS 1989a). By using a large number of adults, we eliminate the possibility of genetic drift creating genetically based differences between the cured and infected strains. This strain was created prior to the start of the experiment and is maintained in the laboratory as a cured counterpart of the infected bY strain. Eight adults of each sex were used to initiate the populations and initial infection frequencies were 0.0, 0.125, 0.25, 0.5, 0.75 and 1.0 with four replicates per treatment (6 treatments × 4 replicates/treatment = 24 populations). There was an additional treatment which simulated an infected female migrating into an uninfected population with eight uninfected males, seven uninfected females and one infected female, for a total of 28 populations.

In the second experiment, the adults from different genetic strains, the infected adults were from strain bIV and uninfected from bI. bI has been shown to produce equilibrium populations (larvae + pupae + adults) ten times larger than bIV (PARK, MERTZ and PETRUSEWICZ 1961). Extensive assays have shown that the strains have remarkably similar birth and death rates; however, bI has low cannibalism rates while bIV has high cannibalism tendencies (PARK, MERTZ and PETRUSEWICZ 1961; PARK *et al.* 1965; MERTZ, PARK and YOUDEN 1965; STEVENS and MERTZ 1985; STEVENS 1989b). The between strain cannibalism differences are polygenic (STEVENS 1989b) and contribute to the observed differences in population size (PARK *et al.* 1965; PARK *et al.* 1970; LLOYD, 1968). bI and bIV were derived from the same parental strain by three to four generations of inbreeding in the late 1950s. bIV and the parental strain are infected with the microorganism, yet bI is uninfected (CAWTHON and MERTZ 1975; WADE and STEVENS 1985). bI was not cured artificially by tetracycline and presumably is the result of a population level cure (*e.g.*, heat shock, STEVENS 1989a) sometime during its laboratory tenure. In the bI-bIV experiment 10 adults of each sex were used to initiate the populations. Initial infection frequencies ranged from 0.0 to 1.0 in increments of 0.1 with three replicate populations per treatment for a total of 33 populations.

In both experiments, the adults were placed in an 8-dram shell vial containing 8 g of standard laboratory media, 95% (by weight) fine sifted whole wheat flour and 5% dried powdered brewer's yeast. The vials were maintained in a darkened incubator regulated at 29° and 70% relative humidity. The adults were permitted to lay eggs for one week and were then discarded. When the populations began to produce pupae they were censused and pupae separated by sex to obtain virgin adults. The adults were tested for

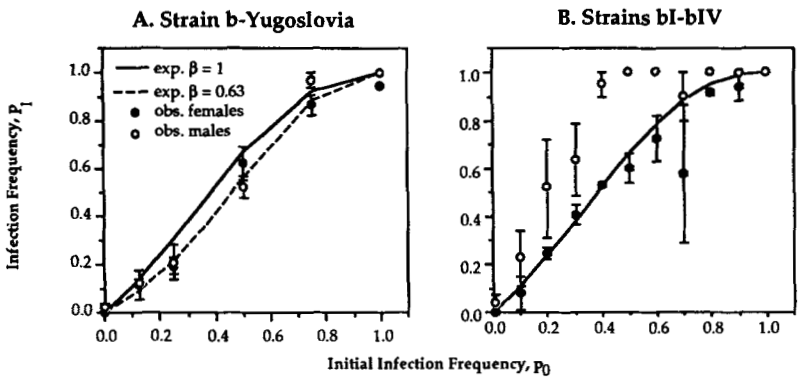

FIGURE 1.—Rate of spread as a function of initial infection frequency.

state of infection by mating females to infected males and the males to uninfected females (WADE and STEVENS 1985; STEVENS 1989a). In the bY experiment, we attempted to test 25 adults of each sex from each population (the average number tested per population and standard deviations are: $x_m = 22.0$, $s = 3.1$; $x_f = 21.7$, $s = 4.1$). In the bI-bIV experiment we attempted to test 10 males and all of the females from each population ($x_m = 9.6$, $s = 0.8$; $x_f = 35.2$, $s = 19.4$). In the bI-bIV experiment, we remated 42 males that did not produce progeny in the testor crosses with an infected female in order to distinguish infection from male infertility. All but four of these retested males produced offspring with an infected (compatible) female, giving an overall rate of male sterility of 3% (4/120) which is typical of these laboratory strains. The rate of female sterility was not estimated because earlier work (M. J. WADE, unpublished results) indicates that the incidence of female sterility is an order of magnitude lower; however, our results indicate that male and female sterility are of the same magnitude (2–3%, see RESULTS). In each experiment, the populations fixed for the presence or absence of the cytoplasmic factor served as controls to verify the state of infection.

RESULTS

Estimates of the rate of spread of the cytoplasmic factor are shown in Figure 1. In both sexes of bY the rate of spread is significantly different from that expected by the cytoplasmic model with equal fitness (Equation 1; females: $\chi^2_{38} = 82.17$, $P < 0.001$ [an independent test based on normal approximation of the binomial distribution was performed for each replicate (BROWNLEE 1965, pp. 140–143) and the probabilities from these independent tests of significance were combined (SOKAL and ROHLF 1981 p. 780)] and males: $\chi^2_{38} = 75.11$, $P < 0.001$). For females, when $p_0 = 0$, $p_1 > 0$ as a result of female sterility, *i.e.*, $p_1 > 0$ is an artifact of the scoring procedure, p_1 includes sterile and infected beetles. Although previous results (M. J. WADE, unpublished results) estimated this to be on the order of 0.3% this experiment suggests a value between 2 (bY) and 3% (bI-bIV). This difference in sterility could result from either small sample sizes or strain differences in sterility. In this experiment the uninfected and infected beetles are from the same genetic strain; thus, using the populations fixed for the presence or absence of the cytoplasmic factor, we estimated the effect of the cytoplasmic factor mediating reproductive incompatibility (as well as other tetracycline sensitive microorganisms and organelles) on host productivity. The ratio of productivity of infected/uninfected, $\beta = 0.63$, demonstrating that the reproductive incompatibility reduced the productivity of the infected cultures. When the cytoplasmic factor has an effect on the host population, equation 1 is modified to:

$$p_{t+1} = \frac{\beta p_t}{[1 - p_t (1 - p_t) - p_t (1 - \beta)]} \qquad (2)$$

(see also CASPARI and WATSON 1959 and FINE 1978), where β is the productivity ratio of infected to uninfected. The observed rate of spread in both sexes of bY is not significantly different from that predicted by this modified model (females: $\chi^2_{38} = 47.86$, $P > 0.10$ and males: $\chi^2_{38} = 40.51$, $P > 0.10$).

For the bI-bIV experiment, because the uninfected and infected individuals are from different genetic strains the productivity ratio of the infected/uninfected populations is not a measure of the effect of the cytoplasmic factor on its host; thus, we only tested the rate of spread using Equation 1. In fact, previous work (PARK, MERTZ and PETRUSEWICZ 1961) has shown that bI and bIV are remarkably similar demographically except for cannibalism, supporting our use of this model. Statistical analysis using a χ^2 test indicates that neither the male nor female component of the population behaves as predicted by this model. Figure 1 suggests that the rate of spread in females is significantly slower than expected ($\chi^2_{46} = 87.53$, $P < 0.001$) and in males was significantly faster than expected ($\chi^2_{46} = 110.7$, $P < 0.001$).

The results of the productivity assay are shown in Figure 2. Based on the incompatibility one would predict that productivity would be highest in the strains fixed for the presence or absence of the cytoplasmic factor and lower in polymorphic populations; however, in both experiments the productivity is highest at low frequencies of infection. We tested the fit of the polymorphic populations to a model that assumes that the frequency of incompatible matings is responsible for the between population differences in

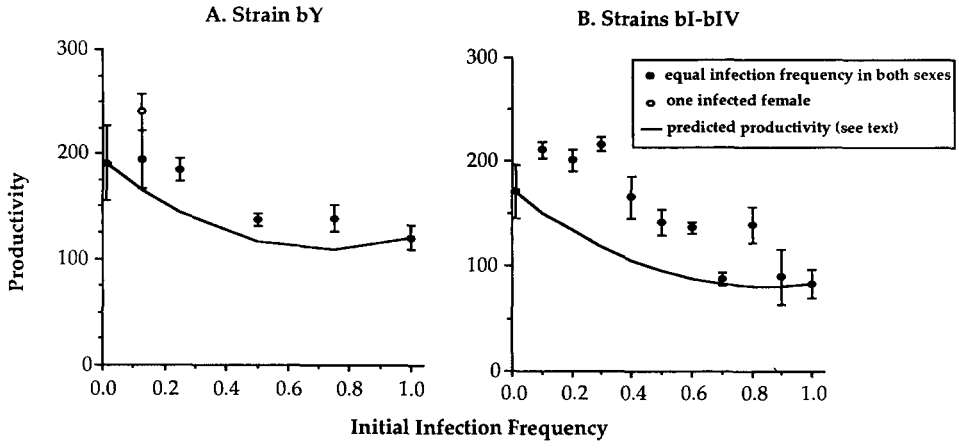

FIGURE 2.—Productivity as a function of initial infection frequency. Populations were initiated with equal infection frequencies in the two sexes.

productivity:

$$\bar{W}_{exp} = (1 - p)^2u + p(1 - p)m + p^2i,$$

where $\bar{W}_{exp}$ is the population fitness or productivity; $(1 - p)^2$, $p(1 - p)$ and p^2 represent the frequencies of the three types of compatible matings; and u, m, and i represent the productivities from each of these types of crosses, respectively. The parameters u and i are estimated from the fixed populations. The model predicts that in a random mating population with intermediate infection frequencies, productivity will be lowered as a result of decreased egg laying resulting from incompatible matings; *i.e.*, imagine a term $(1 - p)p \times 0$ representing the incompatible mating. Because u and i are parameterized independently, the model also allows for the differences in productivity among infected and uninfected individuals described above. Our experiment measured or controlled all the parameters in the model.

We examined three assumptions about the productivity of the mixed (i female $\times$ u male) matings: productivity is determined by the female, $m = i$, productivity is determined by the male, $m = u$; and the productivity is intermediate $m = (u + i)/2$. Previous work with different genetic strains (M. J. WADE, unpublished results) indicates that crosses between infected females and uninfected males will have intermediate productivity. Five replicate populations, each in 8 g of standard acclimatized medium were established with virgin adults (<1-week old) of the following types. There were ten males and ten females of strain bI, homozygous for the wild body color (+/+) and ten males and ten females of the strain b-Mgblk (*T. confusum* McGill black), homozygous for a semidominant black body color mutant (b/b). The bI strain was uninfected (u) and the b-Mgblk strain was infected (i). The resulting progeny in each population were censused and scored for body color genotype into three categories, +/+, +/b, and b/b. The number of +/+ offspring divided by 10 is the per head productivity of a +/+ female when mated with a +/+ male; *i.e.*, the per head productivity of a $u \times u$ cross. The number of heterozygous offspring, +/b, divided by 10 is the per head productivity of b/b females mated with +/+ males; *i.e.*, the per head productivity of the $u \times i$ cross. Lastly, the numbers of b/b offspring divided by 10 is the per head productivity of the $i \times i$ cross. Note that the genetic marker permits us to determine the productivity of each of the three types of crosses, $u \times u$, $u \times i$ and $i \times i$ (the $i \times u$ cross is completely infertile), within the population. We can, thus, compare the observed productivity of $u \times i$ matings with that of the additive expectation $([u \times u] + [i \times i])/2$. The means across all five replicates and their standard errors are: $u \times u$ (or +/+) 10.24 ± 2.59, $i \times i$ (or b/b) 6.95 ± 1.60 and $u \times i$ (or +/b) 8.54 ± 2.28. The observed average per head productivity of $u \times i$ crosses is 8.54 offspring per female whereas the additive expectation is 8.60 (=[10.24 + 6.95]/2), $\chi^2_5 = 2.06$, $P < 0.50$).

The productivity data do not fit this model for either experiment. For each of the three assumptions about the productivity of the mixed matings, the fit of the data to the model was determined by nonlinear regression comparing the populations polymorphic for the infection with predictions based on the average performance of the populations fixed for the presence or absence of the cytoplasmic factor (SAS 1985). The nonlinear regression estimates the parameters of the model, u and i, and constructs 95% confidence intervals for these estimates. In neither experiment did the estimates ±95% confidence intervals from the polymoprhic populations include the mean values of both the uninfected and infected populations (Table 1).

DISCUSSION

We examined three aspects of the interaction between a cytoplasmic factor mediating reproductive incompatibility and its flour beetle host: the rate of spread of the cytoplasmic factor, the effect of a polymorphism on productivity, and the effect of the cytoplasmic factor on host fitness.

The rate of spread fits a model of cytoplasmic

TABLE 1

Observed and predicted productivities (± 95% confidence intervals)

Strains	Observed	Predicted, m =		
		$(u + i)/2$	u	i
Strain b-Yugoslovia				
u	191.23	229.4	222.3	234.9
		(195.3, 263.4)	(193.1, 251.5)	(196.4, 273.4)
i	120.8	153.8	142.0	165.1
		(104.4, 203.3)	(81.6, 202.5)	(124.9, 205.2)
Strains bI-bIV				
u (bI)	170.7	266.4	250.4	278.4
		(239.9, 292.9)	(228.9, 271.9)	(245.9, 311.0)
i (bIV)	83.3	111.7	92.1	132.7
		(83.5, 139.9)	(60.5, 123.8)	(107.6, 157.8)

inheritance that considers the negative effect of the cytoplasmic factor on host fitness in a genetically homogeneous population (experiment 1). In the second experiment with a genetically heterogeneous population, the rate of spread is different from that predicted, being slower than expected in females and faster than expected in the males. At present, we have no explanation for the observed differences in the rate of spread between males and females, it could be because of genetic differences between the strains used in the two experiments or because of the correlation between the cytoplasmic factors and other genetic factors. For example, genetic heterogeneity may result in selection on nuclear genes within the host population affecting the rate of spread of the microorganism. The observed pattern, higher infection frequency in males than females, will increase the frequency of incompatible matings in a randomly mating population. STEVENS (1989a) reported that females were more sensitive to environmental cures than males. Thus both reported sex differences in infection frequency increase the frequency of incompatible matings.

The productivity assay indicates that the affect of the cytoplasmic factor cannot be predicted from the performance of the populations fixed for the presence or absence of the infection. In both of the experiments, the productivity was highest in populations with low frequencies of infection. Previous work has shown that productivity in *Tribolium* is dependent on biological attributes other than fecundity, particularly cannibalism (CHAPMAN 1928; PARK *et al.* 1965; LLOYD 1968; PARK *et al.* 1970; STEVENS and MERTZ 1985; HASTINGS and COSTANTINO 1987; DESHARNAIS and LIU 1987; DENNIS and COSTANTINO 1988; STEVENS 1989b). PARK *et al.* (1965) showed that strains bI and bIV of *T. confusum* had similar birth and death rates yet previous studies had shown they produced tenfold differences in population size (larvae + pupae + adults) as a result of cannibalism (PARK, MERTZ and PETRUSEWICZ 1961).

It can be shown from Equation 2 that when $(1 - p_0) < \beta$ the cytoplasmic factor will not be able to successfully invade a population. Comparing the uninfected and infected populations in Experiment 1 demonstrates that the cytoplasmic factor has a negative effect on productivity for the bY strain. When the infection frequency is less than 0.37 the cytoplasmic factor will be lost from a population. A negative effect of the reproductive incompatibility on host fitness has also been reported in *D. simulans* (HOFFMANN and TURELLI 1988). Such negative effects of the cytoplasmic factor on host fitness may contribute to the observed polymorphisms of infectedness between populations. Because there is no stable intermediate equilibrium point (CASPARI and WATSON 1959), negative effects of the cytoplasmic factor will contribute to polymorphisms within populations when there is migration between infected and uninfected populations or colonization by both uninfected and infected individuals.

In summary, we have shown that the rate of spread is as predicted by a cytoplasmic model of inheritance in a genetically homogeneous populations (Experiment 1). In a second experiment, using infected and uninfected strains with different genetic backgrounds, the rate of spread is different from expected. We observed a higher than expected infection frequency in males and lower than expected in females. It may be that the deviation from the expected rate of spread is due to: (1) the different genetic background of the infected and uninfected strains or (2) due to genetic differences between the strains used in the two experiments. We demonstrated that the cytoplasmic factor has a negative effect on host productivity; such a negative effect on host productivity may contribute to polymorphisms among populations. Finally, we observed polymorphic populations with low infection frequencies have higher than expected productivity.

We thank C. A. HECKMAN and O. L. M. M. LUCAS for their assistance in the laboratory, C. GOODNIGHT for comments on the

manuscript and C. JOHNSON and J. SEXTON for technical assistance. This research was supported by National Institute of Health grant GM222523 to M.J.W. and National Science Foundation grant BSR-86-05672 to D. MERTZ and C. GOODNIGHT; BSR-89-06347 to L. S.

LITERATURE CITED

BARR, A. R., 1980 Cytoplasmic incompatibility in natural populations of a mosquito, *Culex pipiens* L. Nature **283:** 71–72.

BARR, A. R., 1982 Symbiont control of reproduction in *Culex pipiens*, pp. 153–158 in *Recent Developments in the Genetics of Insect Disease Vectors*, edited by W. W. STEINER, W. J. TABACHNICK, K. S. RAI and S. NARANG. Stipes, Champaign, Ill.

BOLLER, E. F., K. RUSS, V. VALLO and G. L. BUSH, 1976 Incompatible races of European cherry fruit fly, *Rhagoletis cerasi* (Diptera: Tephritidae), their origin and potential use in biological control. Entomol. Exp. Appl. **20:** 237–247.

BROWNLEE, K. A., 1965 *Statistical Theory and Methodology in Science and Engineering*, Ed. 2. John Wiley & Sons, New York.

CASPARI, E., and G. S. WATSON, 1959 On the evolutionary importance of cytoplasmic sterility in mosquitoes. Evolution **13:** 568–570.

CAWTHON, D. A., and D. B. MERTZ, 1975 Reproductive failure of females of genetic strain bI of *Tribolium confusum* in crosses with other strains. Tribolium Inform. Bull. **18:** 82–83.

CHAPMAN, R. N., 1928 Quantitative analysis of environmental factors. Ecology **9:** 111–122.

DENNIS, B., and R. F. COSTANTINO, 1988 Analysis of steady-state populations with the gamma abundance model: Application to *Tribolium*. Ecology **69:** 1200–1213.

DESHARNAIS, R. A., AND L. LIU, 1987 Stable demographic limit cycles in laboratory populations of Tribolium castaneum. J. Anim. Ecol. **56:** 885–906.

FINE, P. E. M., 1978 On the dynamics of symbiote-dependent cytoplasmic incompatibility in Culicine mosquitoes. J. Invert. Pathol. **30:** 10–18.

HASTINGS, A., and R. F. COSTANTINO, 1987 Cannibalistic egg-larva interactions in Tribolium: an explanation for the oscillations in population numbers. Am. Nat. **130:** 36–52.

HOFFMANN, A. A., 1988 Partial cytoplasmic incompatibility between two Australian populations of *Drosophila melanogaster*. Entomol. Exp. Appl. **48:** 61–67.

HOFFMANN, A. A., and A. TURELLI, 1988 Unidirectional incompatibility in *Drosophila simulans*: inheritance, geographic variation and fitness effects. Genetics **119:** 435–444.

HOFFMANN, A. A., M. TURELLI and G. M. SIMMONS, 1986 Unidirectional incompatibility between populations of *Drosophila simulans*. Evolution **40:** 692–701.

HSAIO, C., and T. H. HSAIO, 1985a Rickettsia as the cause of cytoplasmic incompatibility in the alfalfa weevil, *Hypera postica*. J. Invert. Pathol. **45:** 244–246.

HSAIO, T. H., and C. HSAIO, 1985b Hybridization and cytoplasmic incompatibility among alfalfa weevil strains. Entomol. Exp. Appl. **37:** 155–159.

KELLEN, W. R., D. F. HOFFMANN and R. A. KWOCK, 1981 *Wolbachia* sp. (Rickettsiales: Rickettsiaceae) a symbiont of the almond moth, *Ephestia cautella*: ultrastructure and influence on host fertility. J. Invert. Pathol. **37:** 273–283.

KLOSTERMEYER, L. E., and G. R. MANGLITZ, 1979 Distribution of eastern and western alfalfa weevil in Nebraska determined by cross-matings (Coleoptera: Curculionidae). J. Kansas Ent. Soc. **52:** 209–214.

LANIER, G. N., 1971 Cytoplasmic incompatibility and breeding isolation in bark beetles (Coleoptera: Scolytidae). Can. J. Genet. Cytol. **13:** 160–163.

LAVEN, H., 1967 Speciation and evolution in *Culex pipiens*, in *Genetics of Insect Vectors of Disease*, edited by J. W. WRIGHT and R. PAL. Elsevier, North Holland, Amsterdam.

LEGRAND, J. J., P. JUCHAULT, D. MORAGA and E. LEGRAND-HAMELIN, 1986 Microorganisms symbiotiques et speciation. Bull. Soc. Zool. Fr. **111:** 135–147.

LLOYD, M., 1968 Self-regulation of adult numbers by cannibalism in two laboratory strains of flour beetles (*Tribolium castaneum*). Ecology **49:** 245–259.

MERTZ, D. B., T. PARK and W. J. YOUDEN, 1965 Mortality patterns in eight strains of flour beetle. Biometrics **21:** 99–114.

NODA, H., 1984 Cytoplasmic incompatibility in allopatric field populations of the small brown planthopper, *Laodelphax striatellus*, in Japan. Entomol. Exp. Appl. **35:** 263–267.

O'NEILL, S. L., 1989 Cytoplasmic symbionts in *Tribolium confusum*. J. Invert. Pathol. **53:** 132–134.

OVERMEER, W. P. J., and A. Q. VAN ZON, 1976 Partial reproductive incompatibility between populations of spider mites (Acarina: Tetranychidae). Entomol. Exp. Appl. **20:** 225–236.

PARK, T., D. B. MERTZ and K. PETRUSEWICZ, 1961 Genetic strains of *Tribolium*: their primary characteristics. Physiol. Zool. **38:** 289–321.

PARK, T., D. B. MERTZ, W. GRODZINSKI and T. PRUS, 1965 Cannibalistic predation in populations of flour beetles. Physiol. Zool. **38:** 289–321.

PARK, T., M. R. NATHANSON, J. R. ZIEGLER and D. B. MERTZ, 1970 Cannibalism of pupae by mixed species populations of adult *Tribolium*. Physiol. Zool. **43:** 166–184.

RICHARDSON, P. M., W. P. HOLMES and G. B. SAUL, 1987 The effect of tetracycline on nonreciprocal cross incompatibility in *Mormoniella* [=*Nasonia*] *vitripennis*. J. Invert. Pathol. **50:** 176–183.

SAS User's Guide: Statistics, Version 5 Edition, 1985 SAS Institute, Inc., Cary, N.C. 956 pp.

SAUL, G. B., 1961 An analysis of non-reciprocal cross incompatibility in *Mormoniella vitripennis* (Walker). Z. Vererbungsl. **92:** 28–33.

SOKAL, R. R., and F. J. ROHLF, 1981 *Biometry*, Ed. 2. W. H. Freeman, San Francisco.

SINGH, K. R. P., C. F. CURTIS and B. S. KRISHNAMURTY, 1976 Partial loss of cytoplasmic incompatibility with age in males of *Culex fatigans*. Ann. Trop. Med. Parasitol. **70:** 463–466.

SMITH-WHITE, S., and A. R. WOODHILL, 1954 The nature and significance of non-reciprocal fertility in Aedes scutellaris and other mosquitoes. Proc. Linn. Soc. NSW **79:** 163–176.

STANLEY, J., 1961 Sterile crosses between mutations of *Tribolium confusum*. Duv. Nature **191:** 934.

STEVENS, L., 1989a Environmental factors affecting reproductive incompatibility in flour beetles, genus *Tribolium*. J. Invert. Pathol. **53:** 78–84.

STEVENS, L., 1989b The genetics and evolution of cannibalism in flour beetles, genus *Tribolium*. Evolution **43:** 169–179.

STEVENS, L., and D. B. MERTZ, 1985 Genetic stability of cannibalism in *Tribolium confusum*. Behav. Genet. **15:** 549–559.

SUBBARO, S. K., B. S. KRISHNAMURTY, C. F. CURTIS, T. ADAK and R. K. CHANDRAKAS, 1977 Segregation of cytoplasmic incompatibility properties in *Culex pipiens fatigans*. Genetics **87:** 381–390.

WADE, M. J., and L. STEVENS, 1985 Microorganism mediated reproductive isolation in flour beetles (genus *Tribolium*). Science **227:** 527–528.

Communicating editor: M. TURELLI