The Incidences and Evolution of Cytoplasmic Male Killers

Laurence D. Hurst

Proceedings: Biological Sciences, Vol. 244, No. 1310 (May 22, 1991), 91-99.

Stable URL:
http://links.jstor.org/sici?sici=0962-8452%2819910522%29244%3A1310%3C91%3ATIAEOC%3E2.0.CO%3B2-Y

Proceedings: Biological Sciences is currently published by The Royal Society.

The incidences and evolution of cytoplasmic male killers

LAURENCE D. HURST

Department of Zoology, South Parks Road, Oxford OX1 3PS, U.K.

SUMMARY

Two types of male-killing cytoplasmic gene are distinguished by their time of action. Early male killers strike embryos, whereas late killers, so far only observed in mosquitoes, kill fourth instar larvae. Maternal inheritance of cytoplasmic factors underlies the sex bias in mortality in both instances. However, the evolutionary logic of early and late killing is, it is argued, otherwise different. Early male killing is interpreted in terms of kin selection. The death of male embryos early on allows resources (food or space) to be redistributed to the surviving females. A simple model relying on the redirection of resources after male death indicates that so long as a more than critical proportion of 'freed' resources goes to females containing clonal relatives of the male-killing cytoplasmic gene, then that gene can invade and go to an equilibrium. This equilibrium is significant in that it allows a population to avoid extinction prior to the evolution of a nuclear repressor gene. The distribution of male killing is discussed in the light of these findings. The late male-killing microsporidians of mosquitoes differ from early male killing factors in possessing horizontal, as well as vertical, transmission between generations. It is argued that to maximize the efficiency of horizontal transmission it is in the interests of the microsporidians to maximize their number by waiting for the host to grow before killing it. Not all microsporidians kill only the male mosquito, some also kill the female. The variety of mosquito–microsporidian pathologies is interpreted by considering a trade-off between the efficiency of vertical against horizontal transmission. A review of possible incidences of cytoplasmic male killing is presented.

1. INTRODUCTION

Cytoplasmic genes typically gain no transmission to the next generation when in a male. Thus the male is an evolutionary dead end for a cytoplasmic gene (Lewis 1941; Howard 1942; Hamilton 1967). Cytoplasmic genes employ several mechanisms to bias the sex ratio towards females and thus increase their fitness (Stouthamer *et al.* 1990; Hurst *et al.* 1990; Legrand *et al.* 1987). One such mechanism is the killing of males. The incidences of male killing can be divided into two types: those in which males are killed early in the development of their host (embryonically) and those in which killing is late (typically fourth instar larvae). This distinction has not been discussed previously and the instances of late killing have typically been cast in the same breath as early male killing. I shall argue that the evolutionary logic underlying these two types of action is fundamentally different.

2. EARLY MALE KILLING

Table 1 is a list of possible instances of cytoplasmic early male killing. Here I argue that the success of a cytoplasmic gene that kills male embryos can be understood in terms of kin selection. Central to the spread of the male-killing cytoplasmic gene is the fact that neither it nor its opposing allele (the lack of the cytoplasmic male killer) are transmissible through males. As a consequence there is no cost to a cytoplasmic gene in killing males (it is the nuclear genes in the male that suffer all of the costs). However, for the cytoplasmic gene to spread it must satisfy two conditions. First, if it is assumed that the cytoplasmic gene has no direct fitness advantage to the females possessing it, then male killing must advantage females containing clonal relatives of the male-killer cytoplasmic gene more than it advantages females without the gene. Secondly, infected females must be able to leave a high enough proportion of their offspring infected with male-killer gene. It is argued that these conditions are linked in that the death of males releases resources that the males would otherwise have used, and the number of males in a brood that die (and hence the degree of fitness advantage) is a function of the efficiency of vertical transmission. What follows is a simple population-genetic model to attempt to clarify the problem.

Consider a population of sexual, dioecious, univoltine insects (e.g. flies, butterflies, etc.). There exist two forms of adult female in the population: those with the male-killing cytoplasmic gene at a frequency p and those without at frequency $1-p$. If we assume that the fecundity of a female is unaltered by the presence of male killers (i.e. she lays the same number of male and female eggs as an uninfected female) than the frequency of infected (p_e) and uninfected female eggs (q_e) will be:

$$p_e = p\alpha; \quad q_e = 1-p\alpha,$$

Table 1. *Possible incidences of early male killing by cytoplasmic genes*

host	cytoplasmic agent	reference	comments
Diptera			
Drosophila willistoni	spiroplasma (WSRO)	Malogolowkin (1958)	Koch's postulates fulfilled
D. equinoxalis	spiroplasma (ESRO)	Malogolowkin (1959)	Koch's postulates fulfilled
D. nebulosa	spiroplasma (NSRO)	Poulson & Sakaguchi (1960)	Koch's postulates fulfilled
D. paulistorum	spiroplasma (PSRO)	Malogolowkin (1958)	Koch's postulates fulfilled
D. prosaltans	—	Cavalcanti & Falcao (1954)	—
D. borealis	—	Carson (1956)	—
D. bifasciata	virus(?)	Leventhal (1968); Buzzati-Traverso (1941)	temperature shock cures condition
D. robusta	—	Poulson (1966, 1968)	found in single female, stable in laboratory, not temperature sensitive
Hymenoptera			
Nasonia vitripennis	*Androcidium nasoniae*	Werren *et al.* (1986)	*A. nasoniae* is a Proteus vulgaris-like Enterobacteria
Lophyrus pini	—	Munro (1928)	all female brood, males die as larvae
Coleoptera			
Coccinellidae			
Adalia bipunctata	—	Lus (1947)	male killing suspected
Harmonia axyridis	bacteria?	Matsuka *et al.* (1975); Gotoh & Niijima (1986); Hu (1979); Niijima (1983)	agent susceptible to tetracyclines
Hippodamia quinquesignata	—	Shull (1948)	male killing probable
Menochilus sexmaculatus	bacteria?	Gotoh & Niijima (1986); Niijima & Nakajima (1981); Niijima (1983)	agent susceptible to tetracyclines
Scolytidae			
Orthotomicus latidens	—	Lanier & Oliver (1966)	—
Pithyophtorous sp.	—	Lanier & Oliver (1966)	male killing suspected
Dendroctonus jefferyi	—	Lanier & Wood (1968)	male killing suspected
Xyleborus sp.	—	Chamberlin (1939)	male killing possible
Dermestidae			
Attagenus anicolor japonica	—	Nakamoto (1984)	—
Chrysomelidae			
Calligrapha philadelphica	—	Robertson (1964)	male killing possible
Lepidoptera			
Pieridae			
Pieris napi	—	Bowden (1966, 1987)	male killing possible
Noctuidae			
Spodoptera littoralis	—	Brimacombe (1980)	—
Nymphaliidae			
Hypolimnas bolina	—	Clarke *et al.* (1975, 1983); Simmonds (1923*a*, *b*, 1926, 1928, 1930)	no virus or spirochaete found
Arctiidae			
Estigmene acrea	—	Earle & MacFarlane (1968)	—
Notodontidae			
Pygaera pigra	—	Federley (1911, 1936)	—
Torticidae			
Epiphyas postvittana	—	Geier *et al.* (1978), Geier & Briese (1977)	—
Pycitinae			
Cadra cautella	—	Takahasi & Kuwahara (1970)	high male mortality cured at high temp.
Gracillariidae			
Phyllonorycter sorbicola	—	Ujiye (1981)	male killing suspected
Hemiptera			
Oncopeltus fasciatus	—	Leslie (1984)	—

Table 1. (*cont.*)

host	cytoplasmic agent	reference	comments
Oncometopia nigricans	spiroplasma	McCoy *et al.* (1978)	cited by Fitz-Earl & Sakaguchi (1986) (McCoy *et al.* (1978) do not mention male killing)
Acari			
Tetranychidae			
Tetranychus urticae	—	Mitchell (1972); Overmeer (1981); Overmeer *et al.* (1972); Overmeer & Harrison (1969)	cytoplasm implicated in sex-ratio control, male mortality is possible mechanism
Trombiculidae			
Leptotrombidium fletcheri	*Rickettsia tsutsugamushi*	Roberts *et al.* (1977)	—
L. arenicola	*Rickettsia tsutsugamushi*	Roberts *et al.* (1977); Hastriter *et al.* (1987)	—

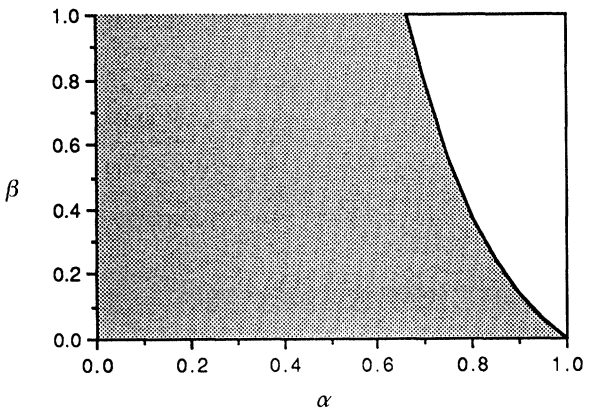

Figure 1. Plot of the invasion criteria for a male killing cytoplasmic gene as a function of the efficiency of vertical transmission through females (α) and the proportion (β) of the 'freed' resources which are used by members of the same brood as that which produces the 'free' resources. The hatched area is that in which the gene cannot invade.

where α is the probability than an egg of an infected female contains the male-killing cytoplasmic gene. Notably, inefficient transmission of the cytoplasmic gene acts against the invasion of the cytoplasmic gene in two ways. First, because some offspring of infected mothers do not receive the cytoplasmic gene, the absolute number of infected individuals is less than it could otherwise have been; second these same offspring represent an increase in the absolute number of uninfected individuals (the alternative cytoplasmic allele). To put it another way, the model considers the frequency of infected individuals in the population as a function of the total number of individuals and not in comparison with a similar benign cytoplasmic gene with equally inefficient vertical transmission.

The value of α is dependent upon the system. In *Nasonia vitripennis* it is around 0.95 (Skinner 1985) as it is for various *Drosophila* male-killing spiroplasmas (see Williamson & Poulson 1979), although it might be significantly lower in ladybirds (see Shull 1948; Matsuka *et al.* 1975) and some lepidopterans (Brimacombe 1980).

Males infected by the male-killing cytoplasmic gene die. This death has fitness consequences for the survivors. For instance, the resources that would have gone to males will now go to the survivors (Lewis 1941; Werren *et al.* 1986). The survivors in broods of the ladybird *Adalia bipunctata* consume the dead eggs (Lus 1947). Equally, a reduction in crowding might benefit the survivors. Less immediately, a reduced probability of inbreeding will result (Poulton 1928; Charlesworth & Ganders 1979; Delannay *et al.* 1981; Werren 1987) although this advantage will not be considered here (and probably is less relevant to inbreeders such as *Nasonia*) and has been modelled (Werren 1987).

Per brood there are T units of resource available. The average brood size is of $2N$ individuals, so that with no killing every individual receives $T/2N$ resources. Assuming a 1:1 sex ratio, αN males die in any given infected brood. This means that $T\alpha/2$ resources are available for redistribution. A fraction of this (β) is used by members of the brood and all of the remainder goes unused.

There are $N(2-\alpha)$ survivors in an infected brood, thus in addition to their normal allowance each of them receives $\beta T\alpha/(2N(2-\alpha))$ resources because of the death of males within their own brood. If fitness is a linear function of total resource obtained then the frequency of the females in the second generation with and without the male-killing gene will be respectively

$$p' = Tp\alpha(1+\beta\alpha/(2-\alpha))/2NW \quad (1)$$

and

$$q' = T((1-p)+p(1-\alpha)\,((1+\beta\alpha/(2-\alpha))/2NW \quad (2)$$

where

$$W = T(2-\alpha+p\alpha\beta)/2N(2-\alpha). \quad (3)$$

This gives

$$p' = p\alpha(2-\alpha+\beta\alpha)/(2-\alpha+p\alpha\beta). \quad (4)$$

Invasion criteria are found at $dp'/dp > 1$ when $p = 0$, which gives

$$\beta > (2-\alpha)\,(1-\alpha)/\alpha^2. \quad (5)$$

Figure 1 is a plot of the bounds (for α and β) within which the male-killing cytoplasmic gene can invade.

The equilibrium frequency (p^*) of the cytoplasmic male killer is obtained from letting $p' = p$, which gives

$$p^* = (\alpha^2(\beta-1)+3\alpha-2)/\alpha\beta. \quad (6)$$

Real equilibria exist when $p^* > 0$, which gives that

$$\beta > (2-\alpha)(1-\alpha)/\alpha^2 \quad (7)$$

must be true. This is, however, the very same condition as for the cytoplasmic gene to invade. Hence, if a cytoplasmic gene can invade it will find an equilibrium. This equilibrium is unique in the domain in which p^* is positive and less than unity, thus it must be stable.

When will this equilibrium frequency be polymorphic, i.e. $0 < p^* < 1$? (n.b. if $p^* = 1$, not only is the population monomorphic, but it is also liable to go extinct if males do not migrate in from other populations). Letting $p^* = 1$ gives that $\alpha = 1$ and β at any value is consistent with $p^* = 1$. Away from these marginal conditions of fixation a polymorphic equilibrium can exist if the values of α and β are high enough. Specifically, it can exist if vertical transmission is greater than 66.7% but less than 100% and if there is special benefit to siblings when males die. If transmission is less than 66.7% the infection always dies out even if only sibs benefit. This minimum value of α at 66.7% is sensitive to changes in the model and specifically to alterations in the relation between male death and its effects on increased female fitness.

If it is assumed that a proportion of resources β is kept within the brood but the proportion $(1-\beta)$ that leaks out is available for general consumption (including possibly by the brood which produced the extra resources), then invasion conditions are unaltered but equilibria conditions are given by:

$$p^* = 1.5-((\beta(\alpha^2\beta-24\alpha+8\alpha^2+16))^{0.5}/2\alpha\beta). \quad (8)$$

As with the 'patchy' population, fixation of the cytoplasmic gene in such a panmictic population is possible only if vertical transmission is 100% efficient.

3. LATE MALE KILLING

One prediction of the kin selective theory of male killing is that if cytoplasmic genes do kill males then they should do so early so as to maximize the benefit to the suicide gene's clonal relatives. However, male-killing microsporidians of mosquitoes (see table 2 for incidences) kill fourth instar larvae and have no appreciable effects on egg mortality; hence any kin selective advantage is likely to be minimal. The all-important difference between the two situations is that the microsporidians responsible for late male killing gain horizontal transmission after having killed the male (Andreadis 1985*a*; Sweeney *et al.* 1985, 1988, 1990; Bechnel 1986; Avery 1989; Avery and Undeen 1990). The haploid spores released by the dead mosquito larvae are incapable of reinfecting larval mosquitoes (but see Toguebaye & Marchand 1986) but do orally infect female copepods of various species (see table 2). In the copepod the microsporidian enters a new phase of development, killing the host and releasing spores that are infective to mosquito larvae (see Sweeney *et al.* 1988).

This discovery of horizontal transmission resolved the enigma of the maintenance of the microsporidians in mosquito populations (infection frequencies in the wild are typically anywhere from 2 to 40%). Andreadis & Hall (1979), using a mathematical model derived by Fine (1975), showed that, owing to the inefficiency of transovarial transmission (see table 2), microsporidians infecting *Culex salinarius* could not be maintained solely by transovarial transmission for more than a few generations. The same conclusion was reached by other workers (Andreadis 1983; Lord *et al.* 1981).

The potential for horizontal transmission allows an explanation for why larval killing is so late. After the fourth stadium mosquitoes enter pupation and remain in the water. After pupation the adult mosquito is only briefly associated with the water. Thus the fourth stadium can be understood as the last stage during which the microsporidians can escape from their host to water and hence to the intermediate host (assuming the pupal case prevents microsporidian release). Hence the killing of larvae at the fourth stadium can be seen as an optimizing strategy. Given that vertical transmission is not possible in males the parasites wait until the host is as large as possible then enter the full blown infectious state, thereby maximizing spore number.

Mosquito larvae acquire the protists *per os*. In such newly infected hosts the microsporidians typically develop in a benign manner in both sexes (although see Andreadis 1988 and Sweeney *et al.* 1990) and are vertically transmitted to the next generation through the eggs. The subsequent possible relationships between host and protist have been classified by Kellen *et al.* (1965) who define four basic types, depending on the differential mortality of sexes, the tissues infected and the occurrence of sporogeny (incidences of infection by type are given in table 2).

Type I are those that have been discussed above, i.e. interactions involving males dying at the fourth stadium but females surviving. Death is due to progressive infection after sporogeny, which occurs in males only. Females maintain the protists as schizogonts. The females transmit the parasites transovarially with no reduction in fecundity or egg hatch. Infections are limited to the mosquitoes oenocytes which in males tend to become hypertrophied.

Type II infections are superficially similar to type I in that more males die as larvae than do females. However, sporogeny in these instances occurs in both sexes but males appear to be more susceptible to progressive infections. Adipose tissue and oenocytes are affected. It is assumed that females can acquire inapparent infections. Kellen *et al.* (1965) make no mention of the ability of females with inapparent infections to transmit the microsporidians transovarially.

Type III relationships represent what Kellen *et al.* (1965) argue to be a relatively primitive state. Both male and female larvae succumb to infections during the fourth stadium, but a few females survive sporogeny and transmit the protist through eggs. Oenocyte or fat body, or both, can be affected. In laboratory studies fewer than 2% of infected larvae of *Culex incidens* and *C. inornata* survive to adulthood. This figure is variable between species and can be selected within species (Sweeney *et al.* 1989).

Type IV parasitisms are non-lethal sporogenic

Table 2. *Incidences of late male killing*

host	microsporidian	efficiency of vertical transmission	copepod intermediary host	reference
type I				
Aedes caspius caspius	*Thelohania opacita*	—	—	Alikhanov (1973)
Culex tarsalis	*T. californica*	—	*Macrocyclops albidus, M. leukarti*	Kellen & Wills (1962); Kellen & Lip (1960)
Anopheles quadrimaculatus	*T. legeri*	—	—	Hazard & Weiser (1968)
A. quadrimaculatus	*Parathelohania anophelis*	—	*Microcyclops varicans*	Avery & Undeen 1990
C. salinarius	*Amblyospora* sp.	90%	—	Andreadis & Hall (1979)
Aedes stimulans	*A.* sp.	50% (range 0–100%)	horizontal transmission shown, agent unknown	Andreadis (1985*a*)
A. cantator	*A. connecticus*	—	*Acanthocyclops vernalis*	Andreadis (1985*b*, 1988, 1989)
C. erythrothorax	*T. gigantea*	—	—	Kellen *et al.* (1965)
A. squamiger	*T. bolinasae*	—	—	Kellen *et al.* (1965)
A. cataphylla	*T.* sp.	—	—	Kellen *et al.* (1965)
A. hexodontus	*T.* sp.	—	—	Kellen *et al.* (1965)
A. ventrovittus	*T.* sp.	—	—	Kellen *et al.* (1965)
A. increpitus	*T.* sp.	—	—	Kellen *et al.* (1965)
type II				
A. melanimon	*T. unica*	—	—	Kellen *et al.* (1965)
C. apicalis	*T.* sp.	—	—	Kellen *et al.* (1965)
type III				
C. thriambus	*T. noxia*	—	—	Kellen *et al.* (1965)
A. dorsalis	*T.* sp.	—	—	Kellen *et al.* (1965)
Culiseta incidens	*T. campbelli*	—	—	Kellen *et al.* (1965)
C. inornata	*T. inimica*	—	—	Kellen *et al.* (1965)
C. particeps	*T.* sp.	—	—	Kellen *et al.* (1965)
Culex annulirostris	*A. dyxenoides*	60%	*Mesocyclops albicans*	Sweeney *et al.* (1985, 1988, 1989)
C. sitiens	*A. indicola*	—	*Apocyclops* sp.	*Sweeney et al.* 1990
Anopheles quadrimaculatus	*Parathelohania* sp.	—	horizontal transmission shown, agent unknown	Avery (1989)
Aedes taeniorhynchus	*A. polykara*	—	—	Lord *et al.* (1981)
A. aegypi	*Edhazardia aedis*	—	—	Bechnel *et al.* (1989)
type IV				
C. apicalis	*T. benigna*	—	—	Kellen *et al.* (1965)

infections of a few small areas of the thorax and abdomen and are limited to adipose tissue. The infection is transovarially transmissible. Type IV relationships are the only ones where sporogenic infections are regularly benign in both sexes.

How can the variety of host–parasite interactions be accounted for? The key parameters are probably the relative efficiency of vertical and horizontal transmission. The protist in males have no incentive not to kill their host. However, if vertical transmission through eggs is poor, as it often is, and adequate copepod vectors are available, then killing females as well as males can be the optimal strategy (type III). If, however, protists in females are assured better chances of transfer to the next generation by vertical rather than horizontal transmission, then type I infections are to be expected. Hence one might predict that type I organisms should be found where vertical transmission is efficient and copepods are not abundant at the appropriate time and type III transmission where the opposite conditions abound. Sweeney *et al.* (1989) have suggested that type IV benign infections might be associated with a sparsity of intermediate hosts, thus forcing vertical transmission as being the best route to the next generation, although why males are not killed is an enigma. As it is not known whether the microsporidians involved in type II infections can be horizontally transmitted it is impossible to predict under which ecological circumstances it will be found.

Although late male killing is restricted to mosquitoes, sex-dependent activity of vertically transmitted parasites is not. Some helminths of mammals have specialized so as to be capable of vertical transmission through milk and across the placenta (Shoop 1991). In females, the helminths typically remain latent until pregnancy at which point they reactivate and migrate to the uterus and mammaries. In males, however, they undergo somatic migration, infection and mature to adulthood. As adults they shed eggs that can horizontally transmit.

4. DISCUSSION

The model presented here does make predictions about the occurrence of early male killing. For early male-killing cytoplasmic genes to invade, the 'freed' resources must be preferentially redirected to females containing clonal relatives of the male killing gene. It is perhaps by considering the potential for the redirection of resources that some of the phylogenetic pattern of the incidences of early male killing or sterility can be understood. Do all the affected organisms have a system of producing eggs such that the death of males preferentially redirects resources towards infected females? In a hermaphroditic plant the genes that are advantaged by the sterility of male tissue are all but guaranteed to be clonal relatives of the suicide genes. In dioecious organisms this it not necessarily true and β might well be less than unity. It is perhaps this difference that underlies the relative abundance of instances of cytoplasmic male sterility (CMS) in plants above male killing in animals. As of 1972 CMS had been described in 140 species from 47 genera and 20 families (Laser & Lersten 1972) whereas, as table 1 indicates, early cytoplasmic male killing is probably less abundant (approximately 30 species from 6 different orders of arthropods).

Can the phylogenetic distribution of early cytoplasmic male killing be understood in terms of the potential for preferential distribution of resources? The parasitoid *Nasonia vitripennis* lays its eggs in larvae (Skinner 1982, 1985). In these close confines the redirection of resources towards brood mates can be guaranteed except under conditions of superparasitism. If superparasitism were common enough then the cytoplasmic gene could be prevented from invading. In the case of *Nasonia* this problem is avoided by the female parent provisioning the body cavity of the host cyclorraphan larvae with the male killing bacteria (a proteus-like Enterobacteria Werren *et al.* 1990). The young wasps ingest the bacteria regardless of their parentage (Skinner 1982, 1985; Werren *et al.* 1986). Thus even the eggs of the superparasitizing females become infected and hence the death of male eggs allows the redirection of resources towards females, most of which are infected, although not necessarily related by parentage. The evolution of this mode of infection would tend to indicate that the redistribution of resources is a fundamental factor in understanding the evolution of cytoplasmic male killing and that the dual parameters α and β are central to an understanding of the process.

Do all the possible instances of early cytoplasmic male killing occur in conditions of high β, i.e. typically where sib–sib competition is stronger than sib–non-sib competition? The bark beetles (Scolytidae) lay their eggs in chambers dug in bark. Under these conditions sib–sib competition is a dominant force. Similarly, the coccinelids all lay eggs in clusters and the early hatched larvae tend to eat undeveloped or late-hatching sibs (Lus 1947). Within the remaining groups, including the neotropical drosophilids, the story is less clear in that the degree of sib–sib competition is unknown and not easy to predict, though in *D. bifasciata* larval competition is known to be an important force (Ikeda 1970). The degree of sib–sib competition in the wild is one parameter that deserves examination in these groups as an attempt to confirm the kin-selective resource diversion hypothesis. If the diversion of resources were the key parameter in the equation, then replacing dead eggs with viable ones should act to prevent the invasion of male-killing cytoplasmic genes. Equally, removing the females from a brood in which male killing has occurred and switching them with females from an unaffected brood should have the same effect. Were inbreeding avoidance the dominant advantage in male killing the invasion of cytoplasmic male killers would be unaffected by the first manipulation.

If inbreeding avoidance is the sole advantage to male killing it is hard to explain why male killing is done early on and not just before reproduction. There are, however, instances in which the cytoplasmic gene does act late and sterilizes males only. In *Drosophila paulistorum* sterility of males is induced by the proliferation of vertically transmitted Staphylococci. The adaptive significance of this phenomenon is uncertain as this sterilization is only found in hybrid males (Ehrman *et al.* 1989; Ehrman & Kernaghan 1971). Similarly, in hybrid crosses between *Heliothis* species (Lepidoptera) male-specific sterility due to maternally inherited genes has been shown (Lansman *et al.* 1983). The possibility of the Y imprinting the X while in the female cannot be ruled out as the cause of such effects (Roehrdanz 1990) although cytoplasmic viruses have been observed in the testes of the sterile males (Degrugillier 1989). Cytoplasmic genes have also been implicated in male sterility in hybrid tse-tse flies (*Glossina morsitans*) (Gooding 1990).

It would be nonsense to pretend that the above model for early male killing is in any way an accurate predictor of the equilibrium frequency of cytoplasmic male killers (which is typically at around 10% (Ikeda 1970; Fitz-Earle & Sakaguchi 1986)). There is no allowance made for either any potential affect on fitness associated with the infection in females or the effect of the distortion of sex ratio. As the sex ratio tends towards either zero or unity so selection against the majority sex will reduce the mean fitness of individuals of the majority sex (Werren 1987; Charlesworth & Ganders 1979; Delannay *et al.* 1981; Frank 1989; Uyenoyama & Feldman 1978). This in turn will provide the selective environment for the evolution of controller genes. It is probably a balance between autosomal repressors and cytoplasmic factors that determines the equilibrium frequency of male killers (Frank 1989; Uyenoyama & Feldman 1978; Hurst & Pomiankowski 1991). The importance of the existence of the equilibrium frequencies found in this paper is that in those instances where the cytoplasmic gene does not go to fixation the population can be saved from extinction. That is to say, the population can be held stably in polymorphism until a nuclear modifier of the cytoplasmic gene evolves. This conclusion appears to be valid for both panmictic and patchy populations.

The model presented here predicts that a cytoplasmic gene that does not kill males and does not have

100% efficient transmission will be lost from the population. This prediction appears to be contradicted by the finding in *Drosophila hydei* of a non-male-killing spiroplasma that is a relative of the male killing spiroplasmas of other *Drosophila* species (Ota *et al.* 1979; Oishi *et al.* 1984). How this is maintained in the population is uncertain.

The model for early male killing presented here is superficially similar to previous attempts but differs in explicitly modelling the redirection of resources. Uyenoyama & Feldman (1978) and Werren (1987) define the fitness of females simply in terms of whether they possess the cytoplasmic gene or not. Watson (1960), writing before Hamilton's (1964) seminal paper, defines the fitness of females as a function of both the frequency of infected females and the possession of the cytoplasmic gene. It is contended that explicitly modelling the redistribution of resources allows a clearer understanding of the nature of the cause of evolutionary change, clarifies problems of invasion and allows a clear delineation of the two processes of late and early killing. Bull (1983), in arguing that male killers are more or less equivalent to sex transformers if net brood size is not greatly affected by male death, misses the kin-selective components and the problems of invasion (which are different from feminizing genes where the viability of *YY* individuals is of central importance (see Taylor 1990)).

Some might describe the uptake of bacteria from the surroundings, as evidenced by *Nasonia*, as a form of horizontal transmission. There are, however, crucial differences between this form of transmission and that identified for the microsporidians of mosquitoes. The central distinction between the two forms is that microsporidians can apparently gain transmission after killing, whereas *Nasonia*'s bacteria gain no transmission from the death of males, and to gain access into the next generation they appear to require that the mother survives to provision the developing young's larval host. The hypothesis presented here to account for the variety of mosquito–microsporidian infection is testable when more data on the relative efficiency of horizontal and vertical transmission in natural populations are available. Manipulation of these parameters could potentially be a longer-term experimental means of testing the notion.

REFERENCES

Alikhanov, S. G. 1973 Izmeneniya v sootnoshenii polov u komarov *Aedes caspius* caspius (Pall) Edw. pri zarazhenii yestestvennykh populyatziyakh mikrosporidiyami *Thelohania opacita* Kudo 1922. *Parazitologiya* **7**, 175–179.

Andreadis, T. G. 1983 Life cycle and epizootiology of *Amblyospora* sp (Microspora: Amblyosporidae) in the mosquito *Aedes cantator*. *J. Protozool.* **30**, 509–518.

Andreadis, T. G. 1985*a* Life cycle and epizootiology and horizontal transmission of Amblyospora (Microspora: Amblyosporidae) in a univoltine mosquito *Aedes stimulans*. *J. Invert. Pathol.* **46**, 31–46.

Andreadis, T. G. 1985*b* Experimental transmission of a microsporidian pathogen from mosquitoes to an alternate copepod host. *Proc. natn. Acad. Sci. U.S.A.* **82**, 5574–5577.

Andreadis, T. G. 1988 *Amblyospora connecticus* sp. nov. (Microsporidae: Amblyosporidae): horizontal transmission studies in the mosquito *Aedes cantator* and formal description. *J. Invert. Pathol.* **52**, 90–101.

Andreadis, T. G. 1989 Infection of a field population of *Aedes cantator* with a polymorphic microsporidium, *Amblyospora connecticus* via release of the intermediate copepod host, *Acanthocyclops vernalis*. *J. Am. Mosq. Contr. Assoc.* **5**, 81–85.

Andreadis, T. G. & Hall, D. W. 1979 Significance of transovarial infections of *Amblyospora* sp. (Microspora: Thelohaniidae) in relation to parasite maintenance in the mosquito *Culex salinarius*. *J. Invert. Pathol.* **34**, 152–157.

Avery, S. W. 1989 Horizontal transmission of *Parathelohania* (Protozoa: Microsporida) to *Anopheles quadrimaculatus* (Diptera: Culicidae). *J. Invert. Pathol.* **53**, 424–426.

Avery, S. W. & Undeen, A. H. 1990 Horizontal transmission of *Parathelohania anophelis* to the copepod, *Microcyclops varicans*, and the mosquito *Anopheleo quadrimaculatus*. *J. Invert. Pathol.* **56**, 98–105.

Bechnel, J. J. 1986 Microsporidian sexuality in culicine mosquitoes. In *Fundamental and applied aspects of invertebrate pathology* (ed. R. A. Samson, J. M. Vlak & D. Peters) (*IV Int. Coll. Invert. Pathol.*), pp. 331–334. The Netherlands: Veldhoven.

Bechnel, J. J., Sprague, V., Fukuda, T. & Hazard, E. I. 1989 Development of *Edhazardia aedis* (Kudo, 1930) N.G., N. Comb. (Microsporida: Amblyosporidae) in the mosquito *Aedes aegypti* (l) (Diptera: Culicidae). *J. Protozool.* **36**, 119–130.

Bowden, S. R. 1966 Sex ratio in *Pieris* hybrids. *J. lepidop. Soc.* **20**, 189–196.

Bowden, S. R. 1987 Sex ratio anomaly in an English pierid butterfly. *Ent. Gaz.* **38**, 167–174.

Brimacombe, L. C. 1980 All-female broods in field and laboratory populations of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). *Bull. ent. Res.* **70**, 475–481.

Bull, J. J. 1983 *The evolution of sex determining mechanisms*. Menlo Park, California: Benjamin Cummings.

Buzzati-Traverso, A. 1941 An extreme case of sex ratio in *Drosophila bilineata*. *Dros. Info. Serv.* **14**, 49.

Carson, H. L. 1956 A female producing strain of *D. borealis* Patterson. *Dros. Info. Serv.* **30**, 109–110.

Cavalcanti, A. L. G. & Falcao, D. N. 1954 A new type of sex ratio of *Drosophila prosaltans* Duda. In *Proc. IX int. Congr. Genet.*, vol. 2, pp. 1233–1235.

Chamberlin, W. J. 1939 *The bark and timber beetles of North America*, pp. 1–513. Corvallis: Oregan State College Cooperative Association.

Charlesworth, D. & Ganders, F. R. 1979 The population genetics of gynodioecy with cytoplasmic-genic male-sterility. *Heredity, Lond.* **43**, 213–218.

Clarke, C., Johnston, G. & Johnston, B. 1983 All-female broods in *Hypolimnas bolina* (L). A resurvey of West Fiji after 60 years. *Biol. J. Linn. Soc.* **19**, 221–235.

Clarke, C., Sheppard, P. M. & Scali, V. 1975 All female broods in the butterfly *Hypolimnas bolina* (L). *Proc. R. Soc. Lond.* B **189**, 29–37.

Degrugillier, M. E. 1989 Virus-like particles in testes of *Heliothis virescens*, *H. subflexa* and backcross males derived from the hybridization of *H. virescens* males x *H. subflexa* females. *J. Invert. Pathol.* **54**, 281–289.

Delannay, X., Gouyon, P. H. & Valdeyron, G. 1981 Mathematical study of the evolution of gynodieocy with cytoplasmic inheritance under the effect of a nuclear restorer gene. *Genetics* **99**, 169–181.

Earle, N. W. & MacFarlane, J. 1968 A unisexual strain of the salt marsh caterpillar, *Estigme acrea*. *Ann. ent. Soc. Am.* **61**, 949–953.

Ehrman, L., Factor, J. R., Somerson, N. & Manzo, P. 1989 The *Drosophila paulistorum* endosymbiont in an alternative species. *Am. Nat.* **134**, 890–896.

Ehrman, L. & Kernaghan, R. P. 1971 The micro-organismal basis of the infectious hybrid sterility in *Drosophila paulistorum*. *J. Hered.* **62**, 66–71.

Federley, H. 1911 Sur un cas d'heredite gynephore dans une espece de papillon. *IV Conf. Int. de Genetique, Paris.* 469–477.

Federley, H. 1936 Sex limited hereditary cancer in Lepidopterus larvae. *Hereditas* **22**, 193–216.

Fine, P. E. M. 1975 Vectors and vertical transmission: an epidemiologic perspective. *Ann. N.Y. Acad. Sci.* **266**, 173–194.

Fitz-Earle, M. & Sakaguchi, B. 1986 Sex ratio distortion in populations and its possible role in insect suppression: experimental studies with strains of *D. melanogaster* carrying cytoplasmically-inherited male killing spiroplasmas. *Jap. J. Genet.* **61**, 447–460.

Frank, S. A. 1989 The evolutionary dynamics of cytoplasmic male sterility. *Am. Nat.* **133**, 345–376.

Geier, P. W., Briese, D. T. & Lewis, T. 1978 The light-brown apple moth *Epiphyas postvittana* (Walker) 2. Uneven sex ratios and a condition contributing to them in the field. *Aust. J. Ecol.* **3**, 467–488.

Geier, P. W. & Briese, D. T. 1977 Predominantly female progeny in the light brown apple moth. *Search* **8**, 83–85.

Gooding, R. H. 1990 Postmating barriers to gene flow among species and subspecies of tsetse flies (Diptera: Glossinindae). *Can. J. Zool.* **68**, 1727–1734.

Gotoh, T. & Niijima, K. 1986 Characteristics and agent(s) of abnormal sex ratio (SR) in two aphidophagous coccinellid species. In *Ecology of Apidophaga* (*Proc. II Symp. Zvikovske Podhradi* (ed. I. Hodek), pp. 545–550. Dordrect: W. Junk.

Hamilton, W. D. 1964 The genetical evolution of social behaviour I and II. *J. theor. Biol.* **7**, 1–52.

Hamilton, W. D. 1967 Extraordinary sex ratios. *Science, Wash.* **156**, 477–488.

Hastriter, M. W., Kelly, D. L., Chan, T. C., Phang, O. W. & Lewis, G. E. Jr 1987 Evaluation of *Leptotrombidium* (*Leptotrombidium*) *fletcheri* (Acari: Trombiculidae) as a potential vector of *Ehrlichia sennetsu*. *J. med. Entomol.* **24**, 542–546.

Hazard, E. I. & Weiser, J. 1968 Spores of thelohania in adult female Anopheles: development and transovarial transmission of these protozoan parasites. *J. Protozool.* **15**, 817–824.

Howard, H. W. 1942 The genetics of *Armadillidium vulgare* Latr. II. Studies on the inheritance of monogeny and amphogeny. *J. Genet.* **44**, 143–159.

Hu, K. 1979 Maternally inherited 'sonless' abnormal sex-ratio (SR) condition in the lady-beetle (Harmonia axyridis). *Acta Genetica Sinica* **6**, 296–304.

Huger, A., Skinner, S. W. & Werren, J. H. 1985 Bacterial infections associated with the son killer trait in the parasitoid wasp *Nasonia* (= *Mormoniella*) *vitripennis*. *J. Invert. Pathol.* **46**, 272–285.

Hurst, L. D., Godfray, H. C. J. & Harvey, P. H. 1990 Antibiotics cure asexuality *Nature, Lond.* **346**, 510–511.

Hurst, L. D. & Pomiankowski, A. 1991 Causes of sex ratio bias may account for unisexual sterility in hybrids: a new explanation of Haldane's rule and related phenomena. *Genetics* (In the press.)

Ikeda, H. 1970 The cytoplasmically inherited sex ratio condition in natural and experimental populations of *Drosophila bifasciata*. *Genetics* **65**, 311–333.

Kellen, W. R., Chapman, H. C., Clark, T. B. & Lindegren, J. E. 1965 Host-parasite relationships of some Thelohania from mosquitoes (Nosematidae: Microsporidia). *J. Invert. Pathol.* **7**, 161–166.

Kellen, W. R. & Lipa, J. J. 1960 *Thelohania californica* n. Sp. a microsporidian parasite of *Culex tarsalis* Coquillet. *J. Insect Pathol.* **2**, 1–12.

Kellen, W. R. & Wills, W. 1962 The transovarian transmission of *Thelohania californica* Kellen and Lipa in *Culex tarsalis* Coquillet. *J. Insect Pathol.* **4**, 321–326.

Lanier, G. N. & Oliver, J. H. 1966 Sex ratio condition: unusual mechanism in bark beetles. *Science, Wash.* **153**, 208–209.

Lanier, G. N. & Wood, D. L. 1968 Controlled mating, karyology morphology and sex ratio in *Dendroctonus ponderosae* complex. *Ann. ent. Soc. Am.* **61**, 517–526.

Lansman, R. A., Avise, J. C. & Huettal, M. D. 1983 Critical experimental test of the possibility of paternal leakage of mitochondrial DNA. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1969–1971.

Laser, K. D. & Lersten, N. R. 1972 Anatomy and cytology of microsporogenesis in cytoplasmic male sterile angiosperms. *Bot. Rev.* **38**, 425–454.

Legrand, J. J., Legrand-Hamelin, E. & Juchault, P. 1987 Sex determination in Crustacea. *Biol. Rev.* **62**, 439–470.

Leslie, T. F. 1984 A sex ratio condition in *Oncopeltus fasciatus*. *J. Hered.* **75**, 260–264.

Leventhal, E. 1968 The sex ratio condition in *Drosophila bifasciata*; its experimental transmission to several species of Drosophila. *J. Invert. Pathol.* **11**, 170–183.

Lewis, D. 1941 Male sterility in natural populations of hermaphroditic plants. *New Phytol.* **40**, 158–160.

Lord, J. C., Hall, D. W. & Ellis, E. A. 1981 Life cycle of a new species of Amblyospora (Microspora: Amblyosporidae) in the mosquito *Aedes taeniorhynchus*. *J. Invert. Pathol.* **37**, 66–72.

Lus, Y. Y. 1947 Some aspects of the population increase in *Adalia bipunctata* L. 2 The strains without males. *Dokl. Akad. Nauk. SSSR* **57**, 951–954.

Magni, G. 1952 Sex ratio in *Drosophila bifasciata Rend. 1st Lombardo Sci. Lettere* **85**, 391–414.

Malogolowkin, C. 1958 Maternally inherited 'sex ratio' conditions in *Drosophila willistoni* and *Drosophila paulistorum*. *Genetics* **43**, 274–286.

Malogolowkin, C. 1959 Temperature effects on maternally inherited abnormal sex ratio in *Drosophila willistoni* and *Drosophila equinoxalis*. *Am. Nat.* **93**, 365–368.

Matsuka, M., Hashi, H. & Okada, I. 1975 Abnormal sex-ratio found in the lady beetle *Harmonia axyridis* Palla (Coleoptera: Coccinellidae). *Appl. Entomol. Zool.* **10**, 84–89.

McCoy, R. E., Tsai, J. H. & Thomas, D. L. 1978 Occurrence of a spiroplasm in natural populations of the sharpshooter *Oncometopia nigricans*. *Phytopathology News* **12**, 217.

Mitchell, R. 1972 The sex ratio of the spider mite *Tetranychus urticae*. *Entomol. Exp. Appl.* **15**, 299–304.

Munro, J. W. 1928 Notes bearing on unisexuality in insects. *Proc. R. ent. Soc. Lond.* **3**, 33–34.

Nakamoto, N. 1984 Analysis of the damage by dermestid beetles (Coleoptera: Dermestidae) in the sericultural industry and its control. Ph.D. thesis. Japan: Kyushu University.

Niijima, K. 1983 Experimental transfer of abnormal sex ratio in two lady-bird species. *Bull. Fac. Agric. Tamagawa Univ.* **23**, 11–17.

Niijima, K. & Nakajima, K. 1981 Abnormal sex ratio in Menochilus sexmaculatus (Fabricius). *Bull. Fac. Agric. Tamagawa Univ.* **21**, 59–67.

Oishi, K., Poulson, D. F. & Williamson, D. L. 1984 Virus-mediated change in clumping properties of Drosophila S.R. spiroplasmas. *Curr. Microbiol.* **10**, 153–158.

Ota, T., Kawabe, M., Oishi, K. & Poulson, D. F. 1979 Non-male killing spiroplasmas in *Drosophila hydei*. *J. Hered.* **70**, 211–213.

Overmeer, W. P. J. 1981 Distorted sex ratio control in the two-spotted spider mite *Tetranychus urticae*. *Z. angew. Entomol.* **91**, 124–130.

Overmeer, W. P. J. & Harrison, R. A. 1969 Notes on the control of the sex ratio in populations of the two spotted spider mite *Tetranychus urticae* Koch (Acarina: Tetranychidae). *N.Z. Jl Sci.* **12**, 920–928.

Overmeer, W. P. J., Van Zon, A. Q. & Helle, W. 1972 Androgenesis in spider mites. *Entomol. Exp. Appl.* **15**, 256–257.

Poulson, D. F. 1966 Further cases of maternal SR in Drosophila species. *Dros. Info. Serv.* **41**, 77.

Poulson, D. F. 1968 Nature, stability and expression of hereditary SR infections in Drosophila. *Proc. Int. Cong. Genet. XII* **2**, 91–92.

Poulson, D. F. & Sakaguchi, B. 1960 Evidence concerning the nature of the sex ratio agent in Drosophila. *Anat. Rec.* **138**, 376.

Roehrdanz, R. L. 1990 Maternal inheritance of a Y chromosome in Heliothis could play a role in backcross sterility. *J. Hered.* **81**, 66–68.

Roberts, L. W., Rapmund, G. & Cardigan, F. C. 1977 Sex ratios in *Rickettsia tsutsugamushi* – infected and noninfected colonies of *Leptotrombidium* (Acari: Trombiculidae). *J. med. Entomol.* **14**, 89–92.

Robertson, J. G. 1964 Effect of supernumary chromosomes on sex ratio in *Calligrapha philadelphica* L. (Coleoptera: Chyrysomelidae). *Nature, Lond.* **204**, 605.

Shoop, W. L. 1991 Vertical transmission of Helminths: hypobiosis and amphiparatenesis. *Parasit. Today* **7**, 51–54.

Shull, A. F. 1948 An all-female strain of lady beetles with reversions to normal sex ratios. *Am. Nat.* **82**, 241–251.

Simmonds, H. W. 1923*a* Families of *Hypolimnas bolina* L., bred from known female parents from different Fijian islands. *Trans. R. ent. Soc. Lond.* **1923**, 651–662.

Simmonds, H. W. 1923*b* All female families of *Hypolimnas bolina* L., bred in Fiji by H. W. Simmonds. *Proc. R. ent. Soc. Lond.* ix–xii.

Simmonds, H. W. 1926 Sex ratio of *Hypolimnas bolina* L. in Viti Levu, Fiji. *Proc. R. ent. Soc. Lond.* **1**, 29–31.

Simmonds, H. W. 1928 Mr. Simmonds' conclusion that all-female-producing females form a persistent strain in Suva, Fiji. *Proc. R. ent. Soc. Lond.* **3**, 43–44.

Simmonds, H. W. 1930 Further notes on *Hypolimnas bolina* L. in Fiji. *Proc. R. ent. Soc. Lond.* **5**, 75–77.

Skinner, S. W. 1982 Maternally inherited sex ratio in the parasitoid wasp *Nasonia vitripennis*. *Science, Wash.* **215**, 1133–1134.

Skinner, S. W. 1985 Son-killer: a third extrachromosomal factor affecting sex ratio in the parasitoid wasp *Nasonia* (= *Mormoniella*) *vitripennis*. *Genetics* **109**, 745–754.

Stouthamer, R., Luck, R. F. & Hamilton, W. D. 1990 Antibiotics cause parthenogenetic Trichogramma (Hymenoptera: Trichogrammatidae) to revert to sex. *Proc. natn. Acad. Sci. U.S.A.* **87**, 2424–2427.

Sweeney, A. W., Doggett, S. L. & Gullick, G. 1989 Laboratory experiments on infection rates of *Amblyospora dyxenoides* (Microsporidia: Amblyosporidae) in the mosquito *Culex annulirostris*. *J. Invert. Pathol.* **53**, 85–92.

Sweeney, A. W., Doggett, S. L. & Piper, R. G. 1990 Life cycle of *Amblyospora indicola* (Microspora: Amblyosporidae), a parasite of the mosquito *Culex sitiens* and of *Apocyclops* sp. copepods. *J. Invert. Pathol.* **55**, 428–434.

Sweeney, A. W., Graham, M. F. & Hazard, E. I. 1988 Life cycle of *Amblyospora dyxenoides* sp. nov. in mosquito *Culex annulirostris* and the copepod *Mesocyclops albicans*. *J. Invert. Pathol.* **51**, 46–57.

Sweeney, A. W., Hazard, E. I. & Graham, M. F. 1985 Intermediate host for an *Amblyospora* sp. (Microspora) infecting the mosquito *Culex annulirostris*. *J. Invert. Pathol.* **46**, 98–102.

Takahasi, F. & Kuwahara, Y. 1970 Studies on sex pheromones of Pyralididae. III. The inheritance of the abnormal sex ratio condition in a strain of the almond moth *Cadra cautella* Walker (Pycitinae). *Botyu-Kagaku* **35**, 11–21.

Taylor, D. R. 1990 Evolutionary consequences of cytoplasmic sex ratio distorters. *Evol. Ecol.* **4**, 235–248.

Toguebaye, B. S. & Marchand, B. 1986 Fusion entre deux cellules uninucléées au cours du cycle de développement sexuelle d'*Amblyospora culicus* (Protozoa: Microspora); un arguement en faveur d'une fecondation chex les microsporidies. *Protistologica* **22**, 359–367.

Ujiye, Y. 1981 Abnormal sex ratio in the population of *Phyllonorycter sorbicola* Kumata (Lepidoptera: Gracillariidae). *Jap. J. appl. Entomol. Zool.* **25**, 244–248.

Uyenoyama, M. K. & Feldman, M. W. 1978 The genetics of sex ratio distortion by cytoplasmic infection under maternal and contagious transmission: an epidemiological study. *Theor. Popul. Biol.* **14**, 471–497.

Watson, G. S. 1960 The cytoplasmic 'sex ratio' condition in *Drosophila*. *Evolution* **14**, 256–265.

Werren, J. H. 1987 The coevolution of autosomal and cytoplasmic sex ratio factors. *J. theor. Biol.* **124**, 317–334.

Werren, J. H., Gherna, R. L., Weisburg, W., Jensen, R. A., Ahmed, S., Woese, C. R., Mandelco, L. & Brenner, D. J. 1990 *Androcidium nasoniae*, gen. nov. sp. nov. causative agent of the 'Son–Killer trait' in the parasitic wasp *Nasonia vitripennis*. *Int. J. Syst. Bacter.* (In the press.)

Werren, J. H., Skinner, S. W. & Huger, A. M. 1986 Male killing bacteria in a parasitic wasp. *Science, Wash.* **231**, 990–992.

Williamson, D. L. & Poulson, D. F. 1979 Sex ratio organisms (Spiroplasmas) of Drosophila. In *The Mycoplasmas*, vol. III (ed. R. F. Whitcomb & J. G. Tully), pp. 175–208. New York: Academic Press.

Received 21 November 1990; revised and accepted 25 February 1991