J. theor. Biol. (1991) **148,** 269–277

The Evolution of Cytoplasmic Incompatibility or When Spite can be Successful

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(*Received on* 30 *April* 1990, *Accepted in revised form on* 3 *July* 1990)

It is proposed that the phenomena of cytoplasmic incompatibility is explicable in terms of the selfish interests of the prokaryotic symbionts associated with the phenomena. It is hypothesized that in males the symbionts produce a product, termed wolbachin, which is carried in sperm and has the capability of inhibiting zygotic development if not neutralized. Symbionts are capable of neutralizing wolbachin. If this is the correct mechanism then the symbionts by killing eggs incapable of neutralizing wolbachin are acting spitefully. A simple model demonstrates that spiteful symbionts can invade a population of non-spiteful symbionts. The resulting population of spiteful symbionts is capable of resisting invasion by other spiteful symbionts even if the invaders have more efficient vertical transmission. Spite is successful in this system because all of the costs of being spiteful are inflicted on the host and not on the symbionts. This is in contrast to other systems of spite.

1. Introduction

Within any given population of *Culex pipiens* there are two sorts of individuals: those harbouring cytoplasmic symbionts of the genus *Wolbachia* and those which do not. *Wolbachia is* a Gram-negative prokaryote sometimes referred to as a rickettsid. These symbionts are vertically transmitted within eggs but not in sperm (or at least if they are transmitted through sperm it is very infrequent). The symbionts are responsible for a system of reproductive compatibility known as cytoplasmic incompatibility (Fig. 1). A female mosquito with the symbionts is compatible with males regardless of whether the male is infected. Similarly, an uninfected female is compatible with uninfected males (symbionts are not necessary for the development of the embryos). However, an uninfected female is incompatible with infected males (the female lays eggs which do not hatch). Experimental evidence for this scheme is detailed in the work of Yen & Barr (1973). This brief paper proposes two possible explanations for the evolution of this phenomenon and discusses this evolution in terms of spite.

1.1. SPITE

An organism which harms another organism but in so doing reduces its own fitness as well, is said to be spiteful. Consider for instance, a population of birds each with its own territory. The birds nest and mate in these territories but feed in

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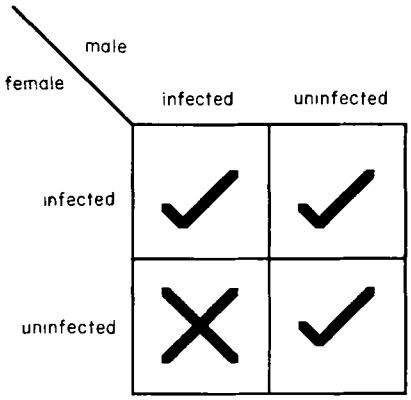

FIG. 1. Intra-populational cytoplasmic incompatibility. Cross implies incompatibility (i.e. eggs are laid but do not hatch, a tick implies compatibility, i.e. eggs are fertilized and develop as "normal".

their own territory and that of their neighbours. Resources are limited and competition is intense. Would it not be to a bird's advantage to be spiteful and kill its neighbour? Were the bird to annihilate its neighbour it would reduce the competition which it, the spiteful bird, would then have to face. However, the spiteful party as well as the recipient of the spite both incur some cost but the non-spiteful neighbours of the dead bird receive the benefit of the spiteful action (the reduced competition) without the cost of the spiteful action. Thus, spite is not selected at the individual level (Rothstein, 1979; Knowlton & Parker, 1979). By considering the evolution of cytoplasmic incompatibility this paper describes a possible system in which spite can evolve and indeed might have done.

2. Cytoplasmic Incompatibility

The phenomenon of cytoplasmic incompatibility has been described for crosses between different races and crosses within populations of numerous insects [e.g. in *Drosophila simulans* (Diptera), Binnington & Hoffman, 1989; Hoffmann & Turelli, 1988; *D. melanogaster* (Diptera) Hoffman, 1988; *Rhagoletis cerasi* (Diptera) Boller *et al.*, 1976; *Aedes* sp. (Diptera) Yen, 1975; Dev, 1986; Taylor & Craig, 1985, Wright & Barr, 1981; weevils (Coleoptera), Hsiao & Hsiao, 1985; *Tribolium* (Coleoptera) Wade & Stevens, 1985; Stevens, 1989; *Ephestia cautella* (Lepidoptera) Kellen *et al.*, 1981; Scolytid beetles (Coleoptera), Lanier, 1971; spider mites (Acari) Overmeer & Van Zon, 1976; *Laedodelphax striatellus* (Homoptera) Noda, 1984; *Nasonia vitripennis* (Hymenoptera) Richardson *et al.*, 1987]. This paper considers the dynamics of the evolution of incompatibility between members of the same population so as to illustrate the notion that spite can evolve. For a more detailed analysis of the dynamics see Fine (1978). The example of the mosquito *C. pipiens* will be discussed as typifying the situation (Laven, 1956; Yen & Barr, 1974).

Attempts to comprehend cytoplasmic incompatibility have concentrated on the advantage of incompatibility to the mosquito. Here, I propose that this reasoning

may be misguided and that the situation might be best understood as a form of manipulation by the symbiont. The fact that selfish cytoplasmic elements can distort sex ratios implies that this balance of power is not implausible (Werren *et al.*, 1988). If sperm were not so small as to prevent the elimination of the symbionts from its cytoplasm, then the best thing for the symbiont to do would be to colonize any symbiont free egg itself by vertical transmission through sperm. This is not however possible and hence from the symbionts point of view any mosquito egg without a population of its clonal relatives ($r = 1$) is a potential competitor.

2.1. MODEL 1

Consider a population with no incompatibility and a mutant symbiont which programmed sperm to kill all eggs without symbionts, but left all symbiont infected eggs to develop normally (Fig. 2). Under certain circumstances, the decline in competition due to mortality of the symbiont free eggs might more effectively promote the fitness of mosquitoes harbouring the mutant symbionts than those which did not. Hence, the elimination of a proportion of the future competion could spread the mutant symbiont through the population, i.e. incompatibility between infected males and uninfected females is kin selected as regards the symbiont.

The circumstances which could benefit the mutant might possibly be found in mosquito populations. Consider if the mutant symbiont developed in an egg which developed into a female. This female in turn produced males and females infected with the mutant symbiont. If the population were to have a degree of inbreeding then one such male mating with his sister would fertilize the eggs which contained

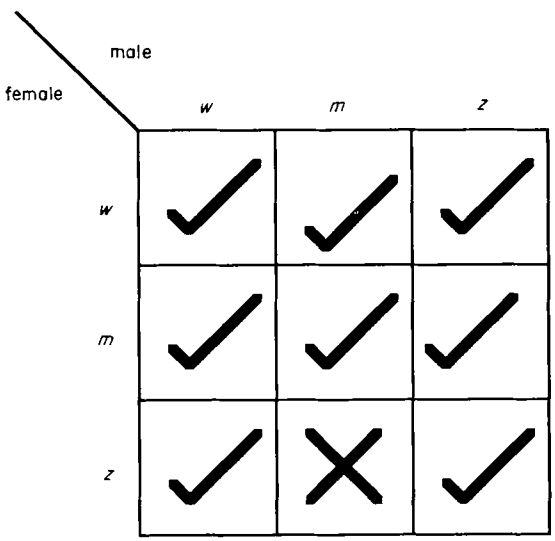

FIG. 2. The system of incompatibility for model 1. Males with the mutant (m) symbiont are incompatible with eggs which have no symbiont (z). All other sperm/egg pairings including those involving wildtype symbionts (w) are compatible.

the symbiont but kill her eggs which were symbiont free. Mosquitoes lay their eggs in rafts and it is not unlikely that there would be competition between eggs on the raft for resources. Hence, by killing symbiont free eggs, the symbionts in the male increase the fitness of females with the clonal relative of the symbiont and hence it is conceivable that the mutant symbiont might be able to invade the population.

2.2. MODEL 2

Consider a system much the same as the one above, only in this case mutant symbiont sperm can kill all eggs which do not have a clonal population of the *same* mutant (Fig. 3; Breeuwer & Werren, 1990; O'Neill & Karr, 1990).

The system can be modelled as follows:

There are three types of individuals in the population: those with wildtype symbionts which have no incompatibility (frequency W) those with the mutant symbionts (frequency M) and those with no symbionts (frequency Z). It is assumed that the mosquitoes are dioecious and that the sex ratio is 1 : 1 within the population and within the separate classes of infection. The probability that a female with symbionts lays eggs which are infected with the symbiont is ϕ_w for the wildtype symbiont and ϕ_m for the mutant type. It is trivial that for the wildtype symbiont to remain in the population without any horizontal transmission and without any fitness advantage over the symbiont free organisms that $\phi_w = 1$. Hence, this is assumed to be the value of ϕ_w in the population that the mutant must invade. ϕ_m is believed to be less than unity (Fine, 1978). French (1970) has demonstrated the segregation of cytoplasmic genes in *C. pipiens*.

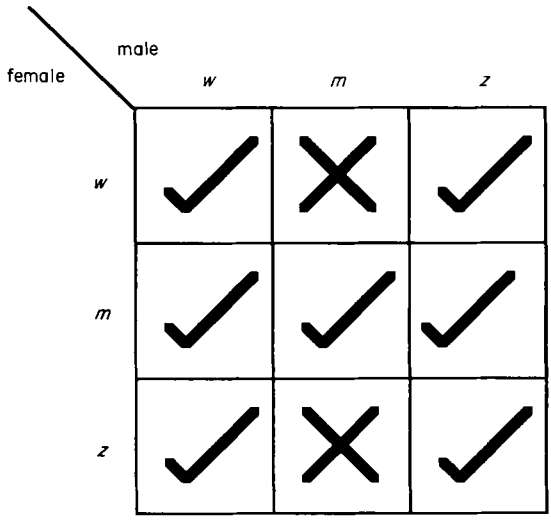

FIG. 3. The system of incompatibility for model 2. The males with the mutant (m) symbionts have sperm which can kill both uninfected (z) type eggs and eggs infected with wildtype (w) symbionts.

Mating is assumed to be random (not inbreeding) hence taking the initial frequencies of the three types of mosquito (W, M, Z) it follows that the resulting frequency of mosquitoes with the mutant symbiont will be given by:

$$M' = \phi_m . M/[\phi_m . M + W(W+Z) + Z^2 + Z . W + (1-\phi_m) . M(W+Z)].$$

Using $W+Z = 1-M$ this simplifies to

$$M' = \phi_m . M/(\phi_m . M^2 - M + 1).$$

Equilibria exist when $M' = M$, i.e. at

$$M = 0 \quad \text{and} \quad \phi_m . M^2 - M + 1 - \phi_m = 0$$

$$\rightarrow M = \frac{1 +/- \sqrt{[(1 - 4 . \phi_m . (1-\phi_m)]}}{2 . \phi}$$

$$\rightarrow M = 1 \quad \text{or} \quad M = (1-\phi_m)/\phi_m.$$

The equilibrium values of M as a function of ϕ are plotted in Fig. 4.

How resilient is a population with every mosquito infected with the mutant type? Consider the introduction of a new mutant (type 2) which behaves as the type 1 mutant as regards incompatibility (i.e. its sperm will kill the eggs of type 1 mutants and symbiont free eggs). What are the conditions for the invasion of this second mutant?

Let the degree of vertical transmission of the type 2 symbionts in mosquitoes be ϕ_b and that of the type 1 mutant be ϕ_a (it was previously ϕ_m). If M is the initial frequency of type 1 mutants and m is the initial frequency of type 2 mutants then:

$$m' = \phi_b m^2/(\phi_b m^2 + \phi_a M^2),$$

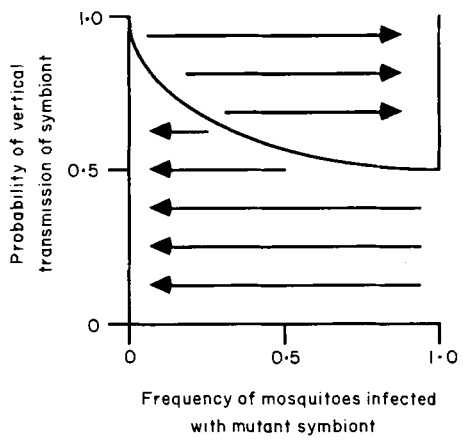

FIG. 4. The frequency of mutant symbiont infected mosquitoes as a function of ϕ (the probability of vertical transmission). The model has two stable equilibria ($M = 0$ and $M = 1$) and one unstable equilibria [$M = (1-\phi_m)/\phi_m$]. The arrows indicate the direction of departure. This is a simplified version of Fine's (1978) result.

equilibria exist when

$$m' = m$$

which resolves to

$$m(1-m) = \phi_a M^2 / \phi_b$$

substituting $M = 1 - m$ gives

$$m = 1 \quad \text{or} \quad m = \phi_a / \phi_a + \phi_b,$$

These equilibria conditions are plotted in Fig. 5. For the first type of symbiont to be in equilibria with no invading second mutant, ϕ_a must be greater than 0·5 (see Fig. 4). Hence, the maximum value of r ($r = \phi_b / \phi_a$) is 2 and the minimum is 0. Invasion of a small population of mutant symbionts is not possible.

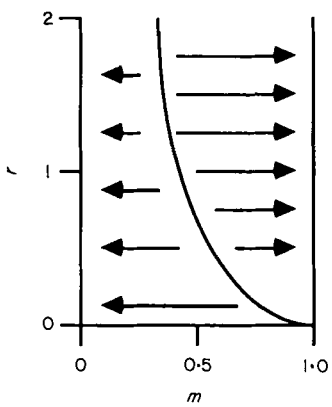

FIG. 5. Plot of the equilibria conditions of m (the frequency of the second mutant symbiont type) when invading a population of mosquitoes all of whom have the original type of mutant. r is the ratio of the probability of vertical transmission of the second mutant symbiont and the probability of vertical transmission of the first type of mutant symbiont ($r = \phi_b / \phi_a$).

3. Discussion

3.1. A NOVEL MECHANISM

How can the symbiont in the male, but not in sperm, inhibit development in the zygote? One possibility is that during the development of the sperm in the infected male, the symbiont places in the cytoplasm of that sperm some substance, which if not neutralized upon introduction into the egg will prevent the development of the egg. For convenience sake the product will be referred to as *wolbachin*. This substance will have to be in low quantities (sperm volume is small). Wolbachin can be neutralized by the clonal relatives of the symbiont which produced it (model 2) or any symbiont (model 1). Two possible mechanisms are worth mentioning.

First, wolbachin might be a colicin like agent, i.e. the symbionts produce a toxin (the colicin) which is coded for by a plasmid and either the same plasmid codes

for the resistance gene (model 2) or symbiont non-plasmid DNA confers immunity (model 1). Plasmid ColE1 of *Escherichia coli* codes for two products: a colicin which kills all *E. coli* without a protector molecule and the protector molecule (discussed in Maynard Smith, 1989: 193). *Paramecium aurelia* killer strains behave in a similar fashion to ColE1, i.e. they produce a substance (paramecin) which kills all those without the same cytoplasmic killer particle but not those with the killer inclusions (Sonneborn, 1965). Bevan and his coworkers (Bevan & Somers, 1969; Somers & Bevan 1969; Wood & Bevan, 1968) have described a killer system in *Saccharomyces cerevisiae* which is analogous to the first model. Yeasts have cytoplasmic factors which render them either killer type (k), neutral (n) or sensitive (s). S types lack the cytoplasmic particles of both k and n type. S types are susceptible to the action of killer strains which release into the medium a protein which both k and n types are resistant to. Puhalla (1968) has described a similar condition in *Ustilago maydis*.

Alternatively wolbachin might be a virus which can replicate in and kill mosquito embryo cells. If this virus stays dormant in/in the presence of symbionts with the same virus (model 2) or appropriate symbionts (model 1) (e.g. numerous bacteriophages) then it would behave appropriately. *SpV* viruses of *Drosophila* sex-ratio spiroplasmas can attach and lyse related sex ratio spiroplasmas which lack the same virus, but are not hostile to spiroplasmas with the same virus (Cohen *et al.*, 1987; Williamson *et al.*, 1977). These viruses might be responsible for death of *Drosophila* embryos (Cohen *et al.*, 1987). Low doses of bacterial viruses have been shown to be capable of killing eukaryotic cells (Preer, 1975). This viral scenario is compatible with the evidence of intra-populational incompatibility.

An alternative mechanism suggested by Barr (1982) perceives the mosquito as being in control of compatibility rather than the symbiont. Barr argues that the egg evicts the sperm which contain "foreign" bacterial matter but allows both sperm contaminated with non-foreign bacterial matter and uncontaminated sperm to fertilize and develop. For some reason the eviction of sperm terminates development and kills the egg. The evolutionary advantage of such an action as far as the mosquitoes are concerned is unclear.

The production of colicins and viruses by "infected" bacteria usually involves the suicidal death of the producer bacteria. If this is true for wolbachin production then death of symbionts during spermatogenesis of infected insects would be predicted. If wolbachin is only produced by suicidal symbionts and if it is possible to artificially administer bacteria to mosquito zygotes, then the wolbachin theory would predict that artificially killed bacteria should illicit no response from the zygote (Barr would predict the opposite) but an extract of suicidal bacteria would cause inhibition of development. If symbionts can be grown in culture and can be stimulated to produce wolbachin (*E. coli* start producing colicins during stationary phase) then the properties of this substance can be tested (if it exists).

3.2. SPITE

If the mechanism presented above, namely that the death of the eggs is due to the action of the symbionts on the sperm and thence on the zygote, is correct, and

incompatibility is not due to the egg rejecting the sperm, then the symbiont is being spiteful, i.e. it is annihilating its competitors. The examples given above of killer particles, etc, can also be interpreted as spite. What makes cytoplasmic incompatibility different from other systems where spite could not evolve? In the other cases there is a cost both to being spiteful and to receive the spite, in this case the symbiont in the male will receive no vertical transmission and hence in evolutionary terms its own fitness is at a minimum, i.e. it incurs no cost in being spiteful. All of the costs of the spiteful act inflict the hosts not the spiteful symbiont. Under these conditions, as the simple models demonstrate, spite can evolve and can be stable. Cytoplasmic incompatibility can thus be seen as a special case of kin selective spite (Hamilton, 1970, 1971).

Why do not mutant mosquitoes which neutralize the activity of the symbiont take over the population? One might argue that such a mutant is not possible or similarly, that the symbionts evolve much faster than their hosts and hence never let the host take control. Such an explanation seems to be possible when one considers the maintenance of male-killing spiroplasms in populations of *Drosophila*. Alternatively group selection arguments can be presented but the power of group selection is doubtful. More probably it is the case that because there is little or no mortality when the cytoplasmic incompatibility factor is at fixation, there is little or no advantage in neutralizing it.

It is proposed that the diverse and complicated effects seen when populations of mosquitoes from geographically distinct areas are interbred, are the consequence of interactions between symbionts and their respective development inhibiting agents. These effects are regarded as side products of a system which evolved for other reasons than to keep species separate. It would be predicted that systems of the type modelled second would produce unidirectional incompatibility when mating with a population with no intrapopulational incompatibility. Unidirectional incompatibility is common. Matings between populations each with their own system of cytoplasmic incompatibility should produce bidirectional incompatibility. This has been confirmed in *Nasonia* hybrids (Breeuwer & Werren, 1990) and *D. similis* (O'Neill & Karr, 1990).

The author wishes to thank Alan Grafen, Bill Hamilton, John Maynard Smith and Andrew Read for commenting on and discussing this paper.

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