Wolbachia as a possible means of driving genes into populations

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SUMMARY

Cytoplasmic incompatibility consists of sterility in cross matings, the crossing type being maternally inherited. It can be explained by the action of *Wolbachia* symbionts which are transmitted through the egg cytoplasm and leave an imprint on the sperm which prevents it fertilizing unless it is 'rescued' by the action of the same type of *Wolbachia* in the egg. Thus matings between infected males and uninfected females are sterile, but the reciprocal matings are fertile. Hence uninfected females are at risk of failing to transmit their uninfected cytoplasm if they cross mate, but infected females are at no such risk. Therefore natural selection favours the infected state and in two wild insect populations the infection has been observed spreading. If a gene for inability to transmit malaria could be introduced into *Wolbachia* and if this could be introduced into *Anopheles* (where these symbionts appear not to occur naturally), release of a limited number of such insects should trigger a process of displacement of malaria vectors, by the non-vector type. A simple model is used to demonstrate the limitations to this process which would be introduced by immigration.

Key words: cytoplasmic inheritance, *Wolbachia*, *Culex*, *Anopheles*, genetic control, immigration.

CYTOPLASMIC INCOMPATIBILITY IN *CULEX* MOSQUITOES

The fact that one or both of the reciprocal crosses between various different geographical strains of the mosquito *Culex pipiens* may be sterile has been known for many years (Marshall, 1938; Ghelelovitch, 1952; Laven, 1959). From the unidirectionally fertile crosses it was possible to make successive backcrosses of female hybrids to the strain which provided the males for the original cross. In this way Laven (1959) showed that the crossing type of both males and females was inherited down the maternal line with no influence from the chromosomal genes introduced by the male parents. He therefore named the phenomenon cytoplasmic incompatibility (which has more recently been abbreviated to CI).

Yen & Barr (1973) showed that if *Culex* larvae are reared in tetracycline, a strain could be produced and bred with new compatibility properties – the males had become universally compatible, whereas the females had lost their compatibility with the untreated males of their original strain. These changes were associated with loss of rickettsia-like bacterial symbionts from the ovarian cytoplasm which had been first observed many years before by Hertig (1936) and named *Wolbachia pipientis*.

Symbiont-free (aposymbiotic) strains of *Cx. pipiens* do not occur naturally but have fairly normal fitness if produced by antibiotic treatment. These uninfected females are consistently rendered sterile or largely sterile by mating with infected males, whereas the infected females make fertile matings with uninfected males. It is assumed that CI between different *Culex* strains, each of which carry symbionts, arises from differences in these symbionts, though the nature of these differences is not yet known.

In the 1960s and 70s attempts were made to use CI for genetic control of *Cx. quinquefasciatus* (vector of urban filariasis), either as a substitute for sterile males produced by irradiation (Laven, 1967) or as a means of replacement of a wild population by a released strain chosen for its bi-directional CI with the wild population. If a sufficient number of the strain with foreign cytoplasm was released to constitute a majority, selection would be expected to favour it because the sterile matings would destroy the reproductive potential of equal numbers of the wild and released strains and this would represent a larger percentage of whichever is rarer. Provided that the sterility is absolute, the cytoplasmic replacement is expected to be accompanied by replacement by the chromosomal genome of the released strain, which might carry a semi-sterilising male linked translocation or a gene for inability to transmit filariasis. The principle of cytoplasmic replacement was proved in outdoor caged populations (Curtis, 1976) but the ability to achieve useful replacement of the chromosomal genome was hampered by incomplete CI in older males (Singh, Curtis & Krishnamurthy, 1976). Further work on genetic control of *Cx. quinquefasciatus* seems unlikely because WHO policy for filariasis control now emphasizes the much improved drug regimes for

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treatment of human populations, and vector control is given at most a supplementary role.

THE DYNAMICS OF *WOLBACHIA* IN INSECT POPULATIONS

CI has also been observed in mosquitoes of the *Aedes scutellaris* complex, in *Drosophila*, cherry flies, flour moths, rice plant hoppers and other insects and has been consistently associated with presence of *Wolbachia*; the rule about infected males sterilising uninfected females, but the reciprocal cross being fertile, holds good in all cases (Werren, Zhang & Guo, 1995). O'Neill *et al.* (1992) developed a PCR assay for 16S rRNA and found remarkable similarity between the *Wolbachia* symbionts in distantly related insects, strongly suggesting that some infections had been acquired by horizontal transfer from other insects.

Drosophila have been freed of their *Wolbachia* and then re-infected by egg injection with extracts from other *Drosophila* or from *Aedes albopictus*, after which normal CI properties are observed in crosses (Braig *et al.* 1994).

In outline, the above described rules of CI, when crossing or selfing infected and uninfected stocks, can be explained by the model shown in Table 1 in which, if there are *Wolbachia* in a male, they are not transmitted through the sperm, but imprint the sperm in such a way that it is unable to complete karyogamy, which prevents it being able to fertilise, unless it is 'rescued' by a complementary substance produced by the same type of *Wolbachia* in the female. The uninfected male has no imprint on its sperms and, with or without the rescue substance in the egg, fertilisation goes on normally.

The plausibility of this model was enhanced because it successfully predicted the outcome when the *Wolbachia* from two bi-directionally incompatible strains of *Drosophila* (O'Neill & Karr, 1990) were both injected into the same eggs, thus creating a doubly infected strain (Sinkins, Braig & O'Neill, 1995). Males of the doubly infected strain were found to be incompatible with females of each of the natural singly infected strains, but doubly infected females were compatible with singly, doubly or uninfected males. All these relationships fit with the sperm imprint/egg rescue model (Table 2). Each type of *Wolbachia* is assumed to imprint the sperm with a different substance and the sperm of doubly infected males is doubly imprinted. Each type of egg can only rescue sperms imprinted by the same type of *Wolbachia* which the egg carries; hence the bi-directional incompatibility between the two singly infected stocks. Doubly imprinted sperms need doubly infected eggs to rescue them and the doubly infected eggs have more than enough capacity to rescue each type of singly imprinted sperm. It must be briefly pointed out that this relatively straightforward picture was complicated by effects of rearing under crowded conditions on density of *Wolbachia* (Sinkins *et al.* 1995).

It was perceived by Caspari & Watson (1959), long before the symbiotic causation of CI was discovered, that maternal inheritance plus unidirectional incompatibility would lead to selection for the type whose females are not sterilized in cross matings but whose males do sterilize females of the other type, i.e. selection would favour those which are now recognized as the *Wolbachia* infected type. This selection arises because the infected female would transmit its cytoplasm no matter which male it mates with, whereas the uninfected female would fail to transmit its cytoplasm if it cross mates.

Fine (1978) viewed the activities of *Wolbachia* as subtle manipulations of the reproduction of its host so as to aid its own propagation, by eliminating some of the competitors with its host. It should be emphasized that this tendency for the frequency of *Wolbachia* to increase is nothing to do with cross-infection (horizontal transmission) which is very rare: it follows from strictly vertical transmission coupled with unidirectional CI.

The spreading process has been observed in mixed populations in the laboratory. Sinkins *et al.* (1995) observed displacement of singly infected *Drosophila* by doubly infected ones as would be expected from the model in Table 2.

In the field, Turelli & Hoffmann (1991) observed spread of *Wolbachia*-infected *D. simulans* along the length of the Central Valley of California at the expense of the uninfected type. A similar process was observed in small brown plant hoppers in Japan (Hoshizaki & Shimada, 1995). In the *Drosophila* case a mitochondrial variant was observed by Turelli, Hoffmann & McKechnie (1992) to 'hitch hike' with the spreading *Wolbachia*, which is to be expected considering that mitochondria are maternally inherited and any two maternally inherited entities will show complete linkage so long as there are no exceptions to the rule of maternal inheritance. Turelli *et al.* (1992) found that there are exceptions to this rule and that the spread in California reached a limit which conformed to their model incorporating incomplete maternal inheritance of *Wolbachia* plus a fitness disadvantage for the infected type.

Another natural limit to the rule, that *Wolbachia* infections will always spread to fixation, would be set by immigration of uninfected females already inseminated with sperm of uninfected males. Table 3 shows a simple model of a mixed infected and uninfected population with immigration from another population, which is so large that emigration from the mixed population could make no appreciable impact on it. Complete maternal inheritance and no differences in fitness are assumed. If, in the mixed population, the initial frequency of

Table 1. Model of how *Wolbachia* acts on fertility in crosses via 'sperm imprint' and 'egg rescue' mechanisms

				Male infection	
				Uninfected	*Wolbachia*
		Egg rescue	Sperm imprint...	0	−w
Female infection	Uninfected	0		0(f)	−w(s)
	Wolbachia	+w		+w(f)	0(f)

f, fertile; s, sterile.

Table 2. Extension of the 'sperm imprint' and 'egg rescue' model to single and double infections by different *Wolbachia* strains (A, B or both) which cause bi-directional incompatibility when the singly infected strains are crossed (Sinkins *et al.* 1995)

				Male infection			
				Uninf.	A	B	A & B
		Egg rescue	Sperm imprint...	0	−a	−b	−a −b
Female infection	Uninf.	0		f	s	s	s
	A	+a		f	f	s	s
	B	+b		f	s	f	s
	A & B	+a +b		f	f	f	f

f, fertile; s, sterile.

Table 3. Simple model of the outcome of matings in a population containing both *Wolbachia* infected and uninfected individuals and with uninfected immigrants (frequencies are shown in parentheses)

		Males			
		Uninfected (q)	*Wolb.* (p)	Uninfected immigrants (m)	Cytotype totals
Females	Uninf. (q)	Uninfected (q^2)	Sterile (pq)	Uninfected (m)	Uninfected = q^2+m
	Wolb. (p)	*Wolbachia* (pq)	*Wolbachia* (p^2)	– –	*Wolbachia* = p^2+pq
		Overall total...			$1-pq+m$

q_1 (next gen.) $= (q^2+m)/(1-pq+m)$.

uninfecteds is q, the frequency of uninfected x uninfected matings would be q^2. To calculate the frequency of uninfected progeny one must allow for the contribution of immigrants, m, and the fact that a proportion pq will be missing from the progeny because of the sterile matings.

Fig. 1 shows the result of successively applying the equation from Table 3 using a programmable calculating machine. If m is zero, the elimination of the uninfected type is inevitable but would be initially slow if one starts at a high uninfected frequency, because there would be relatively few cross matings. Where there is uninfected immigration, elimination of the uninfected type always stops before completion. Furthermore, if the initial proportion of uninfecteds is high, selection against them may be so weak that it is outweighed by immigration and the population may move towards fixation of the *uninfected* type. Therefore, if one wanted to initiate selection against the uninfected

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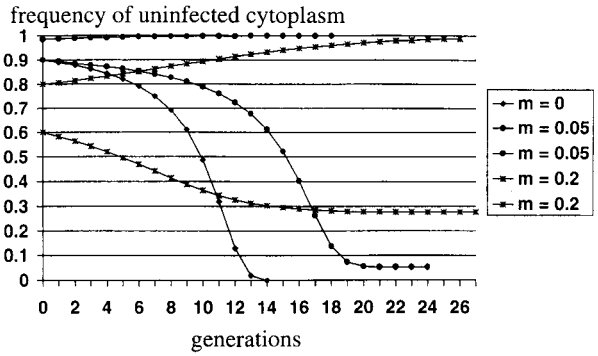

Fig. 1. Predictions from the equation shown in Table 3 about selection for or against the uninfected state in the presence of *Wolbachia* infected individuals and immigration of uninfected individuals.

type one would have to release enough of the infected type to exceed a threshold level, as indicated for the cases of $m = 0{\cdot}05$ or $0{\cdot}2$ in Fig. 1.

POSSIBLE USE OF *WOLBACHIA* TO DRIVE GENES FOR INABILITY TO TRANSMIT MALARIA INTO *ANOPHELES* POPULATIONS

Why might we want to initiate selective elimination of the uninfected type? The answer is that, in parallel with the great popularity of trying to genetically manipulate *Anopheles* mosquitoes so that they are non-susceptible (refractory) to the malaria parasites which they normally carry, a relatively small number of researchers are thinking about ways in which refractoriness genes might be driven into wild populations without the need for mass releases (Curtis, 1968, 1992; Curtis & Graves, 1988; Kidwell & Ribeiro, 1992; Sinkins, Curtis & O'Neill, 1997). CI is one of the promising candidates for this difficult task, but the system using bi-directional CI with the wild population is not an option because PCR tests by the method of O'Neill *et al.* (1992) on several laboratory and wild *Anopheles* populations have shown no existing *Wolbachia* infection in them (Sinkins, 1996).

If unidirectional CI is to be used as a gene driving system, it would be essential that the gene which it was desired to drive was maternally inherited. Otherwise, if it was conventionally bi–parentally inherited, the fertile matings of uninfected males to infected females would rapidly break down the linkage between the driver and the gene which was supposed to be driven, and fixation of the driver would achieve nothing useful.

There is a recent encouraging precedent for engineering a gene for refractoriness to a human pathogen (*Trypanosoma cruzi*, the agent of Chagas' disease) into a bacterial symbiont (*Rhodococcus rhodnii*) of one of the pathogen's insect vectors (*Rhodnius prolixus*) (Duravasula *et al.* 1997). A shuttle plasmid was used to transfect a synthetic gene for the humoral immunity polypeptide cecropin A into *R. rhodnii*. These bacteria are not transovarially transmitted like *Wolbachia*, but are picked up when the young insect feeds on the faeces of older ones. The bacteria play an essential nutritive role for the insects, which fail to develop if they do not pick up the bacteria. It has been demonstrated that plasmid transformed bacteria are propagated in a closed colony of the insects and the cecropin A, which the transfected gene produces, makes the insects refractory to infection by *T. cruzi*. It remains to be seen whether this will continue to work in a bug-infested house where not all the faeces would come from bugs carrying the transformed symbiont.

This example is somewhat encouraging to the idea that refractoriness genes could also be introduced into, and expressed in transovarially-transmitted symbionts. *Anopheles* mosquitoes are not known to be nutritionally dependent on symbionts and, if transfected symbionts are to spread in their wild populations, the force of selection would apparently have to be, not simple starvation, but the process illustrated in Table 3 and Fig. 1. In principle, the gene could be introduced directly into the selecting agent *Wolbachia*, or into another symbiont which could be relied upon to be maternally inherited along with it, as discussed by Sinkins *et al.* (1997). They also modelled the idea of transgenesis of the genes causing CI on to the mosquito chromosomes, but even with an isolated population, the threshold frequency for selection to begin to favour the introduced genes would be higher than shown in Fig. 1, which implies the need for large initial releases, and introduction of the whole symbiont therefore seems preferable.

Since existing wild *Anopheles* populations are not infected with *Wolbachia* (Sinkins, 1996), if an infected type could be produced and released at a frequency sufficient to exceed the threshold (Fig. 1) this type should spread. Introduction of *Wolbachia* into *Drosophila* by egg injection is now routine. Sinkins (1996) attempted to apply similar methods to thousands of *Anopheles* eggs, but unfortunately few survived injection and so far none have acquired stable infections. Sinkins *et al.* (1997) suggest that the chances of success would be greater if the technique for purifying *Wolbachia* from the donor tissue was improved.

More attention is now paid to transposable elements than to *Wolbachia* as means for driving genes into wild populations (Kidwell & Ribeiro, 1992). However, one disadvantage of a transposable element (at least those of the P element type) is that, if it reached fixation in a population, it would no longer cause transposition. Thus if, for example, the refractoriness gene became detached from this type of transposable element during the spreading process, there would be no second chance to use that transposable element. The conditions are not quite so stringent with *Wolbachia*, since as shown by

Sinkins *et al.*(1995) and illustrated in Table 2, a doubly-infected strain could be used for a second sweep through a population through which a singly infected strain had already swept. Thus it is to be hoped that efforts to find a way to infect *Anopheles* with *Wolbachia* will continue.

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