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Models to investigate some issues regarding the feasibility of driving refractoriness genes into mosquito vector populations

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Abstract

A genetic driving system extremely closely genetically linked to a refractoriness gene is needed if such genes are to be of any use in the control of vector-borne disease. *Wolbachia* cytoplasmic symbionts and/or appropriate factors from *Wolbachia* incorporated into the mosquito genome may be usable as driving factors. Maximal fitness of refractoriness factors is needed, otherwise any genetic recombination between the refractoriness factor and the driver can be shown, by simple models, to lead ultimately to fixation of the driver no longer linked to the refractoriness factor. Models can also show the serious impact of non-isolation of the target wild population and incompleteness of the refractoriness.
Keywords: driving systems; refractoriness genes; linkage; *Wolbachia*; fitness; immigration

The need for a driving system

It is likely that *Anopheles* strains that have been genetically engineered to be non-susceptible (refractory) to *Plasmodium falciparum* will soon be available. It is sometimes implied that the production of such strains will instantly provide a new method for controlling malaria. However, in fact the production of the genetic constructs giving refractoriness is only a beginning, and the much larger problems will still have to be solved of how to drive these constructs into very large wild vector populations and establish them there stably in such a way that they actually have a worthwhile impact on prevalence of malaria infection in humans and on incidence of morbidity and mortality.

Theoretically one could introduce a high frequency of refractoriness genes by prolonged mass release of males carrying these genes. However, as with release of sterile males, this method would require mass rearing facilities and would be very vulnerable to reversal by the effects of immigration of wild-type females, already mated to wild-type males outside the release area and unwilling to re-mate on arrival in the release area. Use of a mass rearing facility for sterile males would actually be more efficient than for a strain only carrying a refractoriness factor because – as pointed out by Knipling (1955) – if one begins to succeed with sterile males, the target population would decline and the ratio of released males to the residual wild population would become more and more favourable. It is likely that the problem of

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immigration could be minimized by targeting urban vector populations on geographical islands or ecological 'islands' where the same vector species does not exist in the surrounding area. If there is a serious level of disease transmission in such an urban area this could be a very worthwhile approach, as a release programme covering a given urban area would protect far more people than a similar effort to cover a similar sized rural area.

Africa's malaria problem is mainly a rural one and to hope to deal effectively with this vast problem by introduction of refractoriness genes will require that these genes are tightly linked to driving factors which will lead to increase in gene frequency and spreading from small 'seeding' releases as well as 'resistance' to the effects of immigration. Appropriate types of transposons or *Wolbachia* symbionts are the currently favoured forms of driving factor.

The mechanism of action of *Wolbachia*

Wolbachia is maternally transmitted and has evolved the capacity to inactivate sperm of many host male insects and also to 'rescue' sperm activity after the sperms are introduced into females. Thus, matings within uninfected and within infected stains are fertile and self-reproducing and matings of infected females to uninfected males are fertile and yield infected progeny. However, matings of *Wolbachia*-infected males to uninfected females are sterile because the sperms are inactivated by the females and not 'rescued' by the female. Thus the infected state tends to be selected for and has indeed been observed to spread in *Drosophila* (Turelli and Hoffmann 1995). Figure 1 shows a method of calculating the outcome of random mating between infected and uninfected strains and including the effect of immigration of already mated uninfected females from outside the area (Curtis and Sinkins 1998).

		Male parents		Uninf. migrants	Total
		Uninf. (q)	Inf. (p)		
Female parents	Uninf. (q)	q^2	sterile	m	$q^2 + m$
	Inf. (p)	pq	p^2	--	$p^2 + pq$
				TOTAL	$1 - pq + m$
q at following generation = $(q^2+m) / (1 - pq + m)$					

Figure 1. Method of calculating whether the driving effect of *Wolbachia* infection can overcome the effect of immigration of uninfected mated females (Curtis and Sinkins 1998)

This model was applied to simulate successive generations after a single release. If there is no immigration, the frequency of uninfected insects is driven to zero. If there is an immigration rate per generation of 5% or 20% of the population, selection against the uninfected type can still be achieved by making initial releases of sufficient size. If immigration continues steadily, the driving effect of the *Wolbachia* comes to equilibrium with the immigration. Releases of only 10-40% of the wild population size, made once, were sufficient to start the process of selection for the released type and the 'resistance' to immigration should be contrasted with the

massive and prolonged releases and fatal effects of immigration with sterile-male releases aimed at eradication.

Genetic linkage of refractoriness factors to *Wolbachia*

If driving of *Wolbachia* into a population is to do anything useful it is necessary that a gene or transgenic construct for refractoriness to *Plasmodium* is inherited maternally, as *Wolbachia* is. Possibly the construct could be introduced into *Wolbachia* itself or into some other maternally inherited entity such as mitochondria. However, experience shows that maternal inheritance of such entities is not 100% reliable. If occasionally the construct is not inherited maternally there would be the possibility of production of 'recombinant' individuals with the driving *Wolbachia* but without the construct. It seems probable that a transgenic construct would usually entail some degree of fitness cost and the recombinants, with *Wolbachia* but without the construct, would therefore be the fittest type. Simulations by D.Campbell-Lendrum and P.Coleman (Curtis et al. in press) showed that a limited release could lead to almost 100% replacement by the *Wolbachia*-infected *Plasmodium* refractory type, despite the fact that the refractoriness causes a 20% fitness cost. However, they assumed that once in a million there is a failure of maternal inheritance of the refractoriness construct and, because of the relief from the burden of its fitness cost, the end result is fixation of the *Wolbachia*-infected *Plasmodium*-susceptible type, i.e. no sustained impact on malaria transmission. It would be impossible to carry out laboratory studies on a sufficient scale to exclude the possibility of one in a million failures of maternal inheritance. Some would argue that the assumption of a 20% fitness cost is too pessimistic, especially if it is arranged that the refractoriness construct is only expressed when *Plasmodium* are present. *Plasmodium* cause some damage to mosquitoes and refractoriness might contribute some advantage by preventing this damage (Boëte and Koella 2002). However, infection rates are low in wild populations and it can be supposed that if refractoriness contributed a net selective advantage it would already be the wild type in *Anopheles*, since surely the necessary mutations would have occurred in evolutionary history. The model of Campbell-Lendrum and Coleman is available to test the effects of more common occurrence of failures of maternal inheritance and of lower or zero fitness cost of refractoriness.

If refractoriness was successfully introduced into a vector population there would be intense selection on the *Plasmodium* population to evolve an evasion mechanism (as commonly occurs when new plant varieties are introduced conferring resistance to a plant pathogen). This might be made less likely by incorporating two or more independent refractoriness factors into the release strain. However, it should be recognized that this would multiply the problems of ensuring reliable linkage to the driving factor.

Sinkins and Godfray (2004) have pointed out that the technology for inserting transgenic constructs into *Wolbachia* is not yet available and that it might be more feasible to insert the *Wolbachia* factor which 'rescues' inactivated sperm into a mosquito chromosome (a 'nuclear rescue construct' or NRC). The refractoriness construct would then be closely linked to the NRC. They assume that *Wolbachia* could be injected into, and propagated in, *Anopheles* and that a release would lead to fixation of this strain in a wild population. Then it is assumed that a strain carrying an NRC linked to refractoriness is released resulting in fixation of the NRC (Sinkins and Godfray 2004). With their assumptions *Wolbachia* is selectively eliminated from the

population because the fixation of the NRC cancels the selective advantage of *Wolbachia* and the authors expect some failures of maternal transmission of this symbiont.

Reliability of refractoriness

Boëte and Koella (2002) investigated the conditions under which a transposon-refractoriness construct would go to fixation depending on the transposition frequency (i.e. conversions from heterozygosity to homozygosity for the transposon), the fitness cost of the transposon and of refractoriness and on whether the latter is conditional on the mosquito being *Plasmodium* infected.

Assuming that fixation of the transposon-refractoriness construct occurs, Boëte and Koella (2002) studied the extent to which prevalence of human malaria is, or is not, reduced, depending on intensity of transmission of malaria and efficacy of refractoriness. The conclusion is that there will be little or no benefit in terms of reducing malaria prevalence if transmission is very intense, and unless refractoriness is close to 100% effective in mosquitoes that carry this construct.

Conclusion

In the coming years the engineering of refractory *Anopheles* with driving factors will certainly be a rich source of NPS (*Nature* paper synthetase). However, the sobering conclusion emerges from the models that there are many things that could go wrong and prevent any real benefit for populations suffering from malaria. The main requirements for success are nearly isolated target populations, extremely reliable refractoriness factors, extremely reliable driving factors, and extremely close linkage between them. Calling for 100% reliability for any of these is probably unrealistic but, as data emerge on % reliability for each of these aspects, the above types of models, and other more sophisticated ones, should help judgments to be made about whether a given construct has sufficient promise to be worthwhile to scale it up for field trials.

References

Boëte, C. and Koella, J.C., 2002. A theoretical approach to predicting the success of genetic manipulation of malaria mosquitoes in malaria control. *Malaria Journal,* 1 (1), 1-7. [http://www.malariajournal.com/content/1/1/3]

Curtis, C.F., Coleman, P., Kelly, D., et al., in press. The feasability of use of transgenic sterile male versus that of driving transgenes which prevent disease transmission into vector populations. *In:* Boëte, C. ed. *Malaria control with genetically modified mosquitoes*. Landes Biosience, Georgetown.

Curtis, C.F. and Sinkins, S.P., 1998. *Wolbachia* as a possible means of driving genes into populations. *Parasitology,* 116 (Suppl. S), S111-S115.

Knipling, E.F., 1955. Possibilities of insect control or eradication through the use of sexually sterile males. *Journal of Economic Entomology,* 48, 459-467.

Sinkins, S.P. and Godfray, H.C., 2004. Use of *Wolbachia* to drive nuclear transgenes through insect populations. *Proceedings of the Royal Society of London. Series B. Biological Sciences,* 271 (1546), 1421-1426.

Turelli, M. and Hoffmann, A.A., 1995. Cytoplasmic incompatibility in *Drosophila simulans*: dynamics and parameter estimates from natural populations. *Genetics,* 140 (4), 1319-1338.