

- 17 Mansfield, J.M. and Paulnock, D.M. (2005) Regulation of innate and acquired immunity in African trypanosomiasis. *Parasite Immunol.* 27, 361–371
- 18 Frank, S.A. (1999) A model for the sequential dominance of antigenic variants in African trypanosome infections. *Proc. Biol. Sci.* 266, 1397–1401
- 19 Seed, J.R. (1978) Competition among serologically different clones of *Trypanosoma brucei gambiense* in vivo. *J. Protozool.* 25, 526–529
- 20 Agur, Z. *et al.* (1989) Ordered appearance of antigenic variants of African trypanosomes explained in a mathematical model based on a stochastic switch process and immune-selection against putative switch intermediates. *Proc. Natl. Acad. Sci. U. S. A.* 86, 9626–9630

1471-4922/\$ – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.pt.2007.09.006 Available online 19 November 2007

Research Focus

Can transgenic mosquitoes afford the fitness cost?

Louis Lambrechts^{1,2}, Jacob C. Koella³ and Christophe Boëte⁴

¹ Department of Entomology, University of California, One Shields Avenue, Davis, CA 95616, USA

² Génétique et Evolution des Maladies Infectieuses, UMR CNRS-IRD 2724, Centre de Recherche IRD, 911 Avenue Agropolis, B.P. 64501, Montpellier Cedex 5, France

³ Division of Biology, Imperial College London, Silwood Park Campus, Ascot, SL5 7PY, UK

⁴ Laboratoire de Parasitologie Evolutive, CNRS UMR 7103, CC 237, Université Pierre & Marie Curie, 7 Quai Saint Bernard, 75252 Paris Cedex 5, France

In a recent study, *SM1*-transgenic *Anopheles stephensi*, which are resistant partially to *Plasmodium berghei*, had higher fitness than non-transgenic mosquitoes when they were maintained on *Plasmodium*-infected blood. This result should be interpreted cautiously with respect to malaria control using transgenic mosquitoes because, despite the evolutionary advantage conferred by the transgene, a concomitant cost prevents it from invading the entire population. Indeed, for the spread of a resistance transgene in a natural situation, the transgene's fitness cost and the efficacy of the gene drive will be more crucial than any evolutionary advantage.

Spread of a resistance transgene and mosquito fitness

Marrelli *et al.* [1] reported recently that transgenic *Anopheles stephensi* that carry the *SM1* transgene (see Glossary) and are thus resistant partially to the rodent malaria parasite *Plasmodium berghei* [2] are more fit than non-transgenic individuals when they are maintained on malaria-infected mice. In their cage experiment, initiated with equal proportions of transgenic and non-transgenic mosquitoes, the frequency of the transgene increased rapidly in the population. The fitness advantage was owing to higher fecundity and lower mortality of transgenic mosquitoes than of non-transgenic ones [1].

The publication of this result has generated some optimism for the development and potential use of genetically modified mosquitoes for malaria control, especially in the European media [3]. Indeed, the success of introducing antimalaria genes into a natural vector population by releasing laboratory-produced transgenic mosquitoes will depend strongly on the effect of the transgene on the insect's fitness (i.e. its ability to survive and reproduce) [4]. To date, transgenic mosquitoes have not been associated with a fitness

advantage but, rather, in most studies, with a cost [5–7]. These new data therefore deserve a closer look to understand more completely how harbouring a transgene conferring resistance against malaria influences mosquito fitness.

Being transgenic and paying for it..

Marrelli *et al.*'s study [1] confirms, without much surprise, that, in certain conditions, malaria decreases the mosquito's fitness (see, e.g. Refs [8,9]). Therefore, when mosquitoes were fed on infected blood, the frequency of transgenic mosquitoes was driven from 50% to 70% in less than ten generations. However, the frequency then remained constant, suggesting that the fitness of the transgene is determined by overdominance (i.e. heterozygote superiority) [1]. By combining their empirical data with simple population genetics, Marrelli *et al.* estimated that harbouring at least one copy of the *SM1* transgene (i.e. being homozygous or heterozygous for *SM1*) confers a 50% benefit and that, in homozygotes (but not in heterozygotes), this benefit is reduced by a 35% cost [1], so that the heterozygotes have highest fitness. Therefore, despite a

Glossary

Fitness: In evolutionary biology, the fitness of an individual (or a gene) describes its relative ability to reproduce, as defined by the change of frequency of the individual's genotype (or the gene) from one generation to the next. It is often estimated as the life-time reproductive success (number of offspring of an individual or copy number of a gene).

Fixation: A term used in population genetics to describe the situation in which the frequency of an allele has reached 100% in a population.

Gene-drive efficacy: This is the ability of a gene-drive system to bias the frequency of inheritance of a genetic element, which is measured as the departure from Mendelian inheritance in the gametes of a heterozygous individual and ranges from 0 (when half of the gametes carry the genetic element) to 1 (when all the gametes carry the genetic element).

R_0 : This is the basic reproduction number of an infectious agent, which is the mean number of secondary infections caused by a single infected individual in a susceptible population. An infectious agent can persist only if $R_0 > 1$.

***SM1* transgene:** A synthetic gene driving the blood-inducible, gut-specific secretion of a 12-amino-acid peptide (named Salivary gland- and Midgut-binding peptide 1) that inhibits the development of *Plasmodium berghei* strongly in *Anopheles stephensi* mosquitoes.

Corresponding author: Boëte, C. (cboete@gmail.com).

remarkable fitness advantage conferred by the transgene, the concomitant fitness cost in homozygotes prevents it from reaching fixation. Because nearly complete interruption of transmission is required to reduce malaria prevalence significantly, especially in areas with high prevalence [10], fixation of a highly efficient transgene is a prerequisite for any hope in disease control. Thus, this result reinforces the necessity of coupling antipathogenic transgenes to gene-drive systems that are capable of sweeping them into natural populations despite a fitness load [11].

Need for highly efficient gene-drive mechanisms

Naturally occurring selfish-genetic elements, such as transposable elements, meiotic drive genes, homing endo-

nuclease genes and *Wolbachia*, are considered promising candidates for driving transgenes into insect-vector populations (reviewed in Ref. [12]). The ability of a gene-drive mechanism to move a transgene into a population will depend on the efficacy of the gene-drive system and on the balance between the fitness costs and benefits associated with the transgene and the gene driver [4]. The outcome can also be influenced by the initial frequency of the transgene [13] and the nature of the genetic system. For example, a recent theoretical study suggested that it is unlikely that an antipathogen gene, with a high fitness cost, will be driven to fixation by a Y-linked meiotic drive system [14]. By contrast, a model associating epidemiology and population genetics suggested that a resistance

Box 1. Modelling the dynamics of a transgene

Our model uses the population genetic approach described by Marrelli *et al.* [1]. There are two alleles: R denoting the presence of the resistance transgene and S denoting its absence. The fitness of mosquitoes without any transgene (i.e. SS homozygotes) is defined as $W_{SS}=1$. Mosquitoes with one copy of the transgene (i.e. RS heterozygotes) benefit from being resistant. Their fitness is $W_{RS}=1+b$. Mosquitoes with two copies of the transgene (i.e. RR homozygotes) enjoy the same benefit as the heterozygotes. In addition, they suffer from a cost of resistance. Their fitness is written as:

$$W_{RR} = (1 + b)(1 - c) \quad (\text{Equation 1})$$

In generation t , the frequency of gametes carrying the transgene is p and that of gametes without the transgene is $q=1-p$. The frequencies of the three genotypes before selection are therefore p^2 , $2pq$ and q^2 for the RR, RS and SS genotypes, respectively.

In generation $t+1$ (i.e. after selection), the frequency of the transgene becomes:

$$p' = \frac{p^2 W_{RR} + pq(1 + \delta) W_{RS}}{\bar{W}} \quad (\text{Equation 2})$$

where $\bar{W} = p^2 W_{RR} + 2pq W_{RS} + q^2 W_{SS}$ is the mean fitness in the population of mosquitoes and δ gives the efficacy of the gene drive of the transgene. $\delta = 0$ corresponds to Mendelian inheritance, in which half of a heterozygote's gametes carry the transgene, and $\delta = 1$ means that all the gametes of heterozygotes are transgenic.

Equation 2 was iterated until the frequency of the transgene reached an equilibrium.

Note that these equations, in contrast to, for example, Boëte and Koella [10], assume that the benefit of the transgene: (i) is identical in females and males, although males cannot be infected by malaria (and thus suffer from the cost of resistance without profiting from its benefits) and (ii) does not change as the frequency of the transgene increases and thus the probability of infection (and therefore the benefit of being resistant) decreases. Thus, the equations give an optimistic view of the spread of the transgene.

With the parameters given in Marrelli *et al.*'s study ($b=0.5$; $c=0.35$), the equilibrium frequency of the resistance allele is slightly lower than 50% without any gene drive, that is, approximately 75% of the mosquitoes are resistant (p^2+2pq). As gene drive increases, the frequency of the allele increases linearly; fixation requires that the gene drive enables approximately 67% $[(1+\delta)/2]$ of the gametes of heterozygotes to carry the transgene (Figure I).

The epidemiological effect of this drive depends strongly on the efficacy of resistance [10], here denoted as s . The epidemiology of malaria is governed by a parameter R_0 , which is related to the intensity of transmission [20]. Malaria transmission persists if $R_0 > 1$ and becomes extinct if $R_0 < 1$. Because R_0 is influenced by the number of susceptible mosquitoes in a population, the spread of the transgene to frequency p reduces R_0 to

$$\bar{R}_0 = R_0[(p^2 + 2pq)(1 - s) + q^2] \quad (\text{Equation 3})$$

The first term in the bracket gives the frequency of resistant mosquitoes (if one and two copies of transgene confer the same level of resistance) multiplied by the proportion of these that become infected, whereas the second term gives the frequency of susceptible mosquitoes (all of which are infected).

If resistance is 100% effective (i.e. transmission is completely blocked; $s=1$), R_0 decreases to less than 1 if the transgene is near fixation, so that malaria is eliminated. However, if efficacy of resistance is 90% (i.e. 10% of 'resistant' mosquitoes become infected), prevalence y in the human population, which can be calculated from:

$$y = \frac{R_0 - 1}{R_0 + \frac{a}{\mu}} \quad (\text{Equation 4})$$

where a is the mosquito's biting rate and μ is its mortality rate, is only slightly decreased (Figure II).

Note that the overdominance (heterozygote advantage) suggested by Marrelli *et al.* [1] makes some interesting predictions (Figure III). In particular, if the fitness benefit (expressed in homozygotes and heterozygotes) decreases, the frequency of resistance decreases, as expected. However, this change is strongest at low levels of gene drive. As the allele is driven to close to fixation, it is the gene drive rather than natural selection (the effect of the advantage of resistance) that drives evolution. Therefore, the advantage is irrelevant for the conditions that lead to the fixation of the gene. However, the cost of the transgene (expressed only in homozygotes) does determine the efficacy of the gene drive required to fix the transgene. Reducing the cost of resistance would therefore decrease the level of gene-drive efficacy required to reach fixation (Figure III).

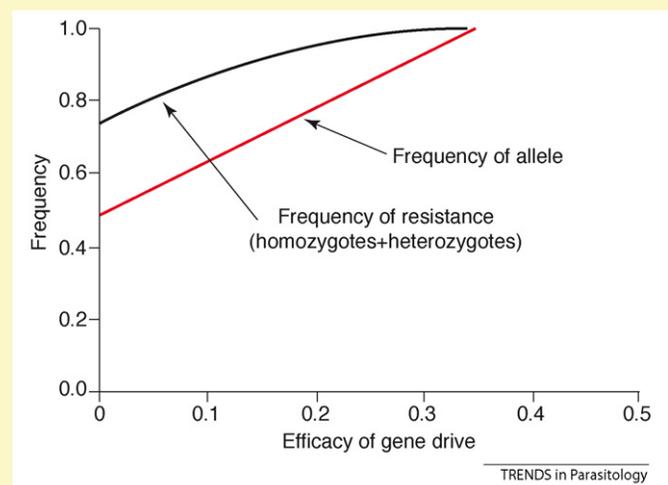


Figure I. Evolutionary equilibrium of resistance gene as a function of the efficacy of the gene drive mechanism. The red line shows the frequency of the allele coding for resistance; the black line shows the frequency of the resistant individuals (i.e. those that carry at least one copy of the resistance allele).

Box 1 cont'd.

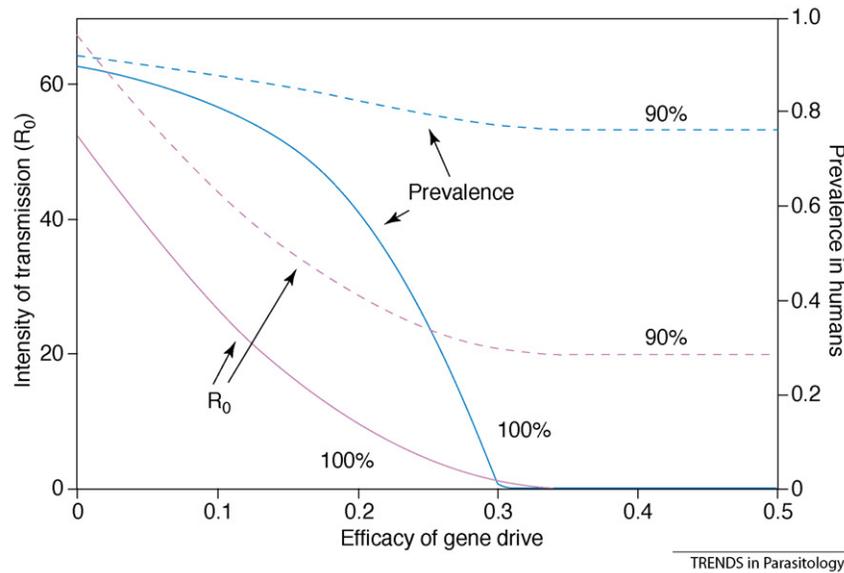


Figure II. Equilibrium of the malaria situation as a function of the efficacy of the gene drive mechanism. The solid lines show the situation where resistance is completely effective; the dotted lines show the situation where resistant mosquitoes can clear only 90% of the infections. The purple lines show the intensity of transmission; the blue lines show the prevalence in the human population.

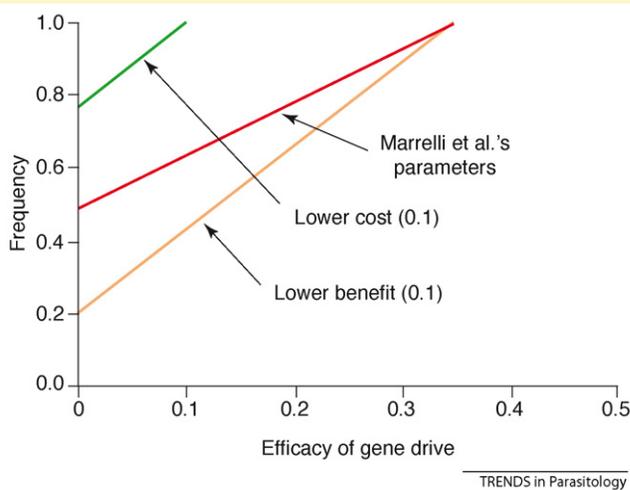


Figure III. Evolutionary equilibrium of the resistance gene as a function of efficacy of the gene drive mechanism. The three lines show the frequency of the resistance allele for different assumptions of the cost and benefit of resistance.

transgene associated with a transposable element might invade a population despite a substantial fitness cost [10].

Here, we use the simpler approach suggested by Marrelli *et al.* [1], which considers population genetics without any epidemiological dynamics and their empirical values of the transgene cost and benefit to assess the gene-drive efficacy that would be required to fix the *SM1* transgene in a mosquito population (Box 1). We observed that, although the transgene is associated with a 50% fitness advantage, the 35% fitness cost paid by homozygotes prevents the transgene's fixation unless it is coupled to a gene driver with an efficacy of at least 35%. Such drivers have been created: in a recent population-replacement experiment in *Drosophila*, a synthetic selfish-genetic element was transmitted successfully at a high frequency (>99%) to the progeny of heterozygous females [15]. However, such

a system remains to be developed and validated in mosquitoes.

It is noteworthy that the overdominance of the transgene measured by Marrelli *et al.* gives qualitatively different influences of the fitness cost and benefit on the gene-drive efficacy required to fix the transgene (Box 1). A closer look at Marrelli *et al.*'s model predicts that the fitness advantage of the transgene influences only the transgene's frequency (if it is not fixed) but that decreasing the cost reduces the level of gene drive required to reach fixation. In other words, if a transgene is overdominant, observing even a strong benefit does not facilitate the fixation of the transgene; it is the cost of the transgene that determines the efficacy of population replacement.

Finally, to make matters worse, our result certainly underestimates the required driver efficacy because

the model is based on the conditions of Marrelli *et al.*'s experiment, which are far more favourable than those that a transgene would encounter in a natural setting.

Extrapolation to a natural situation

As Marrelli *et al.* [1] acknowledge, their conclusions were drawn from a model system in laboratory conditions and therefore remain to be tested with natural mosquito-*Plasmodium* combinations, a prerequisite for any conclusion concerning malaria control [16]. We discuss two of these aspects here. First, the benefit of harbouring the transgene was maximized in the experiment because all mosquitoes were fed on infectious hosts. In any natural situation, in which the probability of feeding on an infectious host is much lower [17], the benefit (but not the cost) of carrying the transgene will be considerably lower. Furthermore, in an epidemiological context, any fitness advantage conferred by the transgene decreases progressively when its frequency rises because increased resistance reduces the rate of disease transmission and thus the probability that a mosquito is infected. Although we argued here that these effects would not be crucial for the fixation of a transgene, they would lead to low penetration of the transgene with inefficient gene drive. Second, although SM1 blocks approximately 80% of parasite transmission [2], transgene fixation in the population is unlikely to affect malaria prevalence in the human population unless its antipathogenic effect is close to 100% (Box 1) (note that, in this context, SM1 does not block *Plasmodium falciparum*, which is the malaria parasite that causes most medical problems). Clearly, this adds some doubts about the potential success of this strategy when thinking about its use in malarious areas, where harbouring a resistance allele will only be a piece of the puzzle [18].

Conclusions

By comparing transgenic malaria-resistant mosquitoes and non-transgenic mosquitoes fed on *Plasmodium*-infected mice, Marrelli *et al.* confirmed that malaria infections are costly to the mosquitoes and that resisting them provides a clear evolutionary advantage. However, despite an optimistic experimental setup compared with the conditions expected in an epidemiologically relevant situation, the benefit of resistance was counterbalanced by a concomitant cost in transgenic homozygotes, so that the transgene could not reach fixation in the population. Because (with the genetic system suggested) it is the cost rather than the benefit, as well as the gene-drive mechanism used to compensate for the cost, that are crucial for the spread of a transgene, their study indicates that the fitness cost of transgenesis still represents a major challenge for the use of transgenic mosquitoes in disease control. Thus, apart from the undeniable social and ethical issues associated with the release of genetically modified organisms [19], a

strong gene-drive mechanism and efficient resistance transgenes are still required desperately before transgenic mosquitoes might be considered as a realistic strategy for malaria control.

Acknowledgements

L.L. is the recipient of a Post-doctoral Marie Curie Outgoing International Fellowship from the European Commission.

References

- Marrelli, M.T. *et al.* (2007) Transgenic malaria-resistant mosquitoes have a fitness advantage when feeding on *Plasmodium*-infected blood. *Proc. Natl. Acad. Sci. U. S. A.* 104, 5580–5583
- Ito, J. *et al.* (2002) Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite. *Nature* 417, 452–455
- Harris, R.F. (2007) Buzz over GM mosquito story. *Curr. Biol.* 17, R259–R260
- Marrelli, M.T. *et al.* (2006) Mosquito transgenesis: what is the fitness cost? *Trends Parasitol.* 22, 197–202
- Catteruccia, F. *et al.* (2003) Impact of genetic manipulation on the fitness of *Anopheles stephensi* mosquitoes. *Science* 299, 1225–1227
- Irvin, N. *et al.* (2004) Assessing fitness costs for transgenic *Aedes aegypti* expressing the GFP marker and transposases genes. *Proc. Natl. Acad. Sci. U. S. A.* 101, 891–896
- Moreira, L.A. *et al.* (2004) Fitness of anopheline mosquitoes expressing transgenes that inhibit *Plasmodium* development. *Genetics* 166, 1337–1341
- Hogg, J.C. and Hurd, H. (1995) *Plasmodium yoelii nigeriensis*: the effect of high and low intensity of infection upon the egg production and bloodmeal size of *Anopheles stephensi* during three gonotrophic cycles. *Parasitology* 111, 555–562
- Lambrechts, L. *et al.* (2006) Environmental influence on the genetic basis of mosquito resistance to malaria parasites. *Proc. Biol. Sci.* 273, 1501–1506
- Boëte, C. and Koella, J.C. (2002) A theoretical approach to predicting the success of genetic manipulation of malaria mosquitoes in malaria control. *Malar. J.* 1, 3
- James, A.A. (2005) Gene drive systems in mosquitoes: rules of the road. *Trends Parasitol.* 21, 64–67
- Sinkins, S.P. and Gould, F. (2006) Gene drive systems for insect disease vectors. *Nat. Rev. Genet.* 7, 427–435
- Cha, S.J. *et al.* (2006) Cage trials using an endogenous meiotic drive gene in the mosquito *Aedes aegypti* to promote population replacement. *Am. J. Trop. Med. Hyg.* 74, 62–68
- Huang, Y. *et al.* (2007) Introducing desirable transgenes into insect populations using Y-linked meiotic drive – a theoretical assessment. *Evolution Int. J. Org. Evolution* 61, 717–726
- Chen, C.H. *et al.* (2007) A synthetic maternal-effect selfish genetic element drives population replacement in *Drosophila*. *Science* 316, 597–600
- Boëte, C. (2005) Malaria parasites in mosquitoes: laboratory models, evolutionary temptation and the real world. *Trends Parasitol.* 21, 445–447
- Bonnet, S. *et al.* (2003) Estimation of malaria transmission from humans to mosquitoes in two neighbouring villages in south Cameroon: evaluation and comparison of several indices. *Trans. R. Soc. Trop. Med. Hyg.* 97, 53–59
- Boëte, C. (2006) Malaria-refractoriness in mosquitoes: just a matter of harbouring genes? In *Genetically Modified Mosquitoes for Malaria Control* (Boëte, C., ed.), pp. 79–88, Eurekah/Landes Biosciences
- Macer, D. (2006) Ethics and community engagement for GM insect vector release. In *Genetically Modified Mosquitoes for Malaria Control* (Boëte, C., ed.), pp. 152–165, Eurekah/Landes Biosciences
- MacDonald, G. (1957) *The Epidemiology of Malaria*. Oxford University Press