Research Focus

Only the good die young: a novel paradigm for mosquito control

Peter F. Billingsley

Sanaria Inc., 9800 Medical Center Drive, Rockville, MD 20850, USA

Andrew Read and colleagues have proposed that insecticides acting late in the vector lifetime are less susceptible to evolutionary pressures, thereby avoiding insecticide resistance. Such late-life acting insecticides would kill the vector before the pathogen's extrinsic cycle is complete, but allow the vector to remain reproductively active. Some examples of late-life acting insecticides are discussed. By targeting older vectors, the dangerous cohorts – those capable of transmitting the fully developed pathogen – are removed.

Old mosquitoes are dangerous mosquitoes

When a mosquito feeds on an infected bloodmeal, two clocks are ticking; their combined timing defines the success or failure of that mosquito as a vector. From the day of emergence as an adult the lifespan clock is counting down the time until the mosquito dies. During her lifetime the female takes bloodmeals every 1–4 days, and lays eggs approximately every three days as each gonotrophic cycle is completed. Any one of the bloodmeals could be infected and, from the moment it is ingested by the vector, the pathogen's own clock is ticking too. In order to pass from the bloodmeal to the salivary glands and back into a host, the pathogen must complete its extrinsic incubation period (EIP) (Glossary) before the vector dies [1] (Figure 1). These clocks help define two important elements of the successful vector. First, old mosquitoes are dangerous; only those that have ingested the pathogen and survived the EIP can possibly transmit the pathogen to another host. Second, as the mosquito ages, with each gonotrophic cycle she has expended an increasing proportion of her lifetime fecundity. For example, *Anopheles stephensi* mosquitoes provided with bloodmeals in the laboratory every 3 days or every day produce 82% or 73%, respectively, of their total eggs in 50% of their lifespan (P.F. Billingsley, unpublished data). Older mosquitoes thus make a decreasing contribution to population replacement [2–4].

In consequence, mosquito survival remains the most crucial component of the transmission process, and models to date demonstrate clearly that transmission is exquisitely sensitive to changes in mosquito survival rate [5,6].

Euthanasia or mass destruction

It is in the interests of both biological players to keep the vector alive for as long as possible. For the vector, lifetime reproductive success will clearly increase as she gets older, even though the relative contribution declines with each gonotrophic cycle (Figure 1). For the pathogen, once it has survived the extrinsic cycle, continued vector survival means that, at each feeding event, there is the potential for transmission to another host. As mosquitoes age they find themselves in a small and declining cohort. Read *et al*. [7] argue that, if older mosquitoes can be targeted in a control program, the overall population size of mosquitoes will not be greatly affected, but the effect on transmission could be profound because mosquitoes would die before becoming capable of transmission, and before the EIP is complete. Targeting lifespan to reduce mosquito longevity clearly satisfies the malaria epidemiological models that, in particular, identify mosquito survival rate as the most vulnerable target for intervention against transmission [1,8]. Such an approach would require the development and successful deployment of what Read *et al*. [7] have termed 'late life acting' insecticides (LLAIs). This is in contrast to conventional insecticide approaches where all vectors are targeted equally, either by killing in spray programs or at the point of feeding using bednets impregnated with insecticide [9–11].

An evolution-proof approach?

The euthanasia approach of LLAIs and the mass destruction approach of conventional insecticides have very different evolutionary outcomes. Conventional insecticides exert a massive selection pressure on the vector population such that onset of resistance to the insecticides can be very rapid; for example, Read *et al*. cite onset of resistance in *An. albimanus* to pyrethroids in just three years [12]. Resistance is a common (even inevitable) outcome wherever mass control programs with conventional insecticides are implemented [10]. Furthermore, resistance to one insecticide leads almost inexorably to cross-resistance against others in the same chemical class and, depending upon the molecular physiological mechanism induced,

Glossary

Extrinsic incubation period (EIP): The time taken by a pathogen to develop in the vector from ingestion in a bloodmeal to eventual transmission at feeding. The EIP is highly dependent upon environmental factors (e.g. temperature) and the pathogen; a mosquito must survive a longer period in order to transmit malaria (EIP = 9–20 days) than to transmit Dengue (EIP = 10–14 days).

Late-life acting insecticide (LLAI): An insecticide that selectively targets older vectors. LLAIs allow the replacement of vector populations by young, reproductively active insects and therefore selection pressure on the insecticides is greatly reduced.

Gonotrophic cycle: This is typically considered to be the time between two oviposition events for a female mosquito. Each gonotrophic cycle is composed of five phases: host seeking, blood feeding (one or more bloodmeals are taken depending upon physiological state and species), a period of egg maturation, searching for an oviposition site, and finally egg-laying.

Corresponding author: Billingsley, P.F. (pbillingsley@sanaria.com).

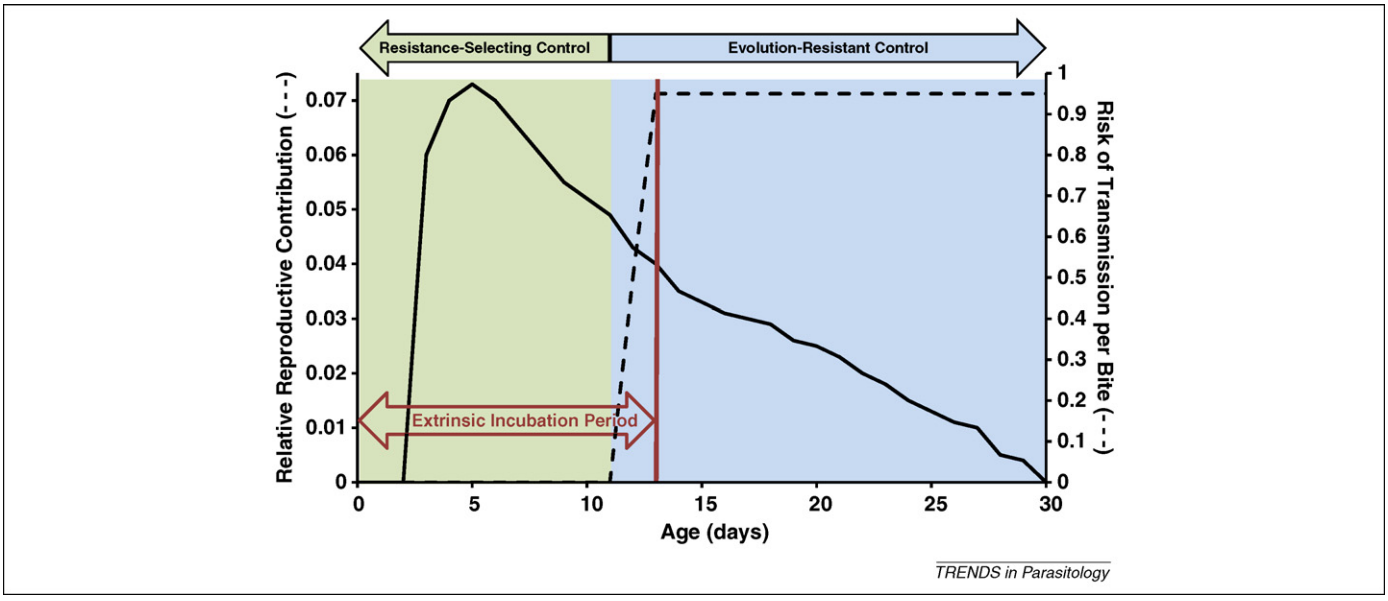

Figure 1. The principle behind late-life acting insecticides (LLAIs). The highest reproductive contribution of a vector occurs early in life. During this period resistance will be subject to strong positive selection because those vectors surviving the control measure will contribute disproportionately to subsequent generations. Such control measures target vectors that are not transmission-competent because the EIP of the pathogen has not been completed. Thus, transmission occurs only late in the vector lifespan, and if LLAIs can be targeted at the older vectors they will reduce transmission and with less likelihood of resistance evolving because most of the reproductive contribution has been made before the LLAIs take effect.

resistance to other classes of insecticides can ensue [9]. One of the reasons for the rapid spread of resistance to conventional insecticides is that, under insecticide pressure, resistant individuals have relatively higher fitness – increased by more than sixfold in the Read *et al*. model [7]. However, with the LLAIs, because a larger proportion of the mosquitoes' lifetime reproductive capacity occurs before the insecticide takes effect (Figure 1), there is little or no selection pressure against the LLAI when the mosquito is producing most of its eggs. This led Read *et al*. to label LLAIs as 'evolution proof' – an ambitious claim, but one would at least expect LLAIs to show a high degree of evolutionary resistance even with long-term deployment. However, although such LLAIs might be particularly effective at controlling *An. gambiae* mosquitoes that have been shown recently to live for several months (T. Lehmann, personal communication), the selection pressure in such bottleneck populations might be sufficiently high to select for resistance. Of course, the selection pressure would also depend very heavily on the mode of action, and one would therefore expect a vector to develop resistance to a LLAI directed against a single vector target (e.g. a monovalent anti vector vaccine) [1,8] more rapidly than an agent with multiple modes of action, such as a live agent [13,14].

Just as the vector is under selection pressure to develop resistance to agents used to control its populations, these same agents can exert selection pressures on the pathogen [15]. If the EIP of the pathogen is not shorter than the lifespan of the vector the pathogen will become extinct. It is therefore possible that, under the pressure of LLAIs, pathogens could evolve that develop more rapidly within the vector in order to reduce EIP. Such phenotypic plasticity in malaria parasites has been known for a long time [16] and, given the remarkable ability of malaria to develop resistance to drugs [17], evolution of resistance to shortened mosquito lifespan might be expected, would be biologically constrained, and could have unpredictable outcomes [18]. When a third element is introduced into the mix, the dynamics of pathogen adaptation to insecticide pressure can become even more complicated. For example, insecticide-resistant *Culex quinquefasciatus* mosquitoes are less likely to transmit filariasis than their non-resistant counterparts [18], and the possible stimulation of innate immune pathways common to bacteria, viruses, fungi and protozoa [19] suggests that LLAIs based on biological agents might have additional benefits.

Can old mosquitoes be targeted selectively?

Altering the age demographic structure of vector populations has been advocated previously [20], but can it be done? Identifying older mosquitoes in order to target control specifically to that population cohort has proved to be difficult, and better characterization of vector population structures based on their comparative physiology and behavior is needed [20,21]. Such work may help to identify age-specific targets and offer tools for monitoring the efficacy of LLAIs. The LLAIs need to do the job of targeting vector longevity, and several promising candidates are on the horizon. These include: (i) fungal pathogens that infect mosquitoes early in their adult life, but manifest their pathological effects only as the mosquito ages [6,22–24]; (ii) the obligate intracellular bacterial symbiont, *Wolbachia*, that, when established successfully in a line of *Aedes aegypti,* halved vector life expectancy [25]; (iii) Densoviruses that can be genetically manipulated to act as paratransgenic control agents [26]; (iv) antivector vaccines, that can decrease the overall life expectancy of mosquitoes and also block transmission of malaria from the vertebrate host to the mosquito [27–29]; or (v) chemical insecticides that accumulate to be effective late in the insect lifespan [7]. Each of these has its own particular advantages and disadvantages, but the pathway to the implementation of a

broad spectrum LLAI looks shortest and most promising for the fungal pathogens. LLAI coverage will need to be considered carefully. Just as transmission is sensitive to reductions in mosquito survival, one expects that poor coverage that misses mosquitoes early in their adult life would negate the effect of the control measure, and especially in zones of high transmission.

New thoughts on old problems

There have been many calls for new additions to the vector control arsenal that have most typically rallied around the perceived need for new chemical insecticides either for direct application or for use on bednets. The LLAI concept offers a new paradigm by adopting long-established principles from old models and by adapting these to reconsider, in a new setting, the consequences of being able to target differentially the most important cohorts of vector populations, perhaps in combination with traditional insecticides. As research progresses on control agents that could be considered LLA insecticides, anophelism without malaria (or in a broader context, mosquitoes without disease transmission) could become the aim of future vector control strategies.

Acknowledgements

Ms M. King is thanked for help with preparation of the manuscript and Prof. J. P. Webster and Dr D. S. Smith for feedback. This paper reflects the views of the author who is solely responsible for its content, and does not reflect the views of Sanaria Inc. or any other organization with which the author may be associated.

References

1 Billingsley, P.F. *et al.* (2008) Mosquitocidal vaccines: a neglected addition to malaria and dengue control strategies. *Trends Parasitol.* 24, 396–400
2 Charlwood, J.D. *et al.* (1997) Survival and infection probabilities of anthropophagic anophelines from an area of high prevalence of *Plasmodium falciparum* in humans. *Bull. Entomol.Res.* 87, 445–453
3 Lines, J.D. *et al.* (1991) Human malaria infectiousness measured by age-specific sporozoite rates in *Anopheles gambiae* in Tanzania. *Parasitology* 102, 167–177
4 Charlwood, J.D. (1995) The rise and fall of *Anopheles arabiensis (Diptera: Culicidae)* in a Tanzanian village. *Bull. Entomol. Res.* 85, 37–44
5 Killeen, G.F. *et al.* (2000) A simplified model for predicting malaria entomologic inoculation rates based on entomologic and parasitologic parameters relevant to control. *Am. J. Trop. Med. Hyg.* 62, 535–544
6 Smith, D.L. and McKenzie, F.E. (2004) Statics and dynamics of malaria infection in *Anopheles* mosquitoes. *Malar. J.* 3, 13
7 Read, A.F. *et al.* (2009) How to make evolution-proof insecticides for malaria control. *PLoS Biol.* 7, e1000058
8 Billingsley, P.F. *et al.* (2006) Immune interactions between mosquitoes and their hosts. *Parasite Immunol.* 28, 143–153
9 Hemingway, J. *et al.* (2004) The molecular basis of insecticide resistance in mosquitoes. *Insect Biochem. Mol. Biol.* 34, 653–665
10 Hemingway, J. *et al.* (2002) An overview of insecticide resistance. *Science* 298, 96–97
11 Lengeler, C. (2004) Insecticide-treated bed nets and curtains for preventing malaria. *Cochrane Database Syst. Rev.* CD000363
12 Penilla, R.P. *et al.* (2007) Cytochrome P450-based resistance mechanism and pyrethroid resistance in the field *Anopheles albimanus* resistance management trial. *Pesticide Biochem. Physiol.* 89, 111–117
13 Thomas, M.B. and Read, A.F. (2007) Can fungal biopesticides control malaria? *Nat. Rev. Microbiol.* 5, 377–383
14 McMeniman, C.J. *et al.* (2009) Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. *Science* 323, 141–144
15 Webster, J.P. *et al.* (2004) Do hosts and parasites coevolve? Empirical support from the *Schistosoma* system. *Am. Nat.* 164 (Suppl 5), S33–S51
16 Boyd, M.F. and Kitchen, S.F. (1937) The duration of the Intrinsic Incubation period in *Falciparum* malaria in relation to certain factors affecting the parasites. *Am. J. Trop. Med.* s1–17, 845–848
17 White, N.J. (2004) Antimalarial drug resistance. *J. Clin. Invest.* 113, 1084–1092
18 Michalakis, Y. and Renaud, F. (2005) Fungal allies enlisted. *Nature* 435, 891–892
19 McCarroll, L. *et al.* (2000) Insecticides and mosquito-borne disease. *Nature* 407, 961–962
20 Baton, L.A. *et al.* (2008) Functional genomics studies on the innate immunity of disease vectors. *Insect Sci.* 15, 15–27
21 Cook, P.E. *et al.* (2007) Predicting the age of mosquitoes using transcriptional profiles. *Nat. Protoc.* 2, 2796–2806
22 Harrington, L.C. *et al.* (2001) Analysis of survival of young and old *Aedes aegypti* (Diptera: Culicidae) from Puerto Rico and Thailand. *J. Med. Entomol.* 38, 537–547
23 Scholte, E.J. *et al.* (2003) Infection of malaria (*Anopheles gambiae* s.s.) and filariasis (*Culex quinquefasciatus*) vectors with the entomopathogenic fungus *Metarhizium anisopliae*. *Malar. J.* 2, 29
24 Blanford, S. *et al.* (2005) Fungal pathogen reduces potential for malaria transmission. *Science* 308, 1638–1641
25 Evans, O. *et al.* (2009) Increased locomotor activity and metabolism of *Aedes aegypti* infected with a life-shortening strain of *Wolbachia pipientis*. *J. Exp. Biol.* 212, 1436–1441
26 Ren, X. *et al.* (2008) Viral paratransgenesis in the malaria vector *Anopheles gambiae*. *PLoS Pathog.* 4, e1000135
27 Foy, B.D. *et al.* (2003) Induction of mosquitocidal activity in mice immunized with *Anopheles gambiae* midgut cDNA. *Infect. Immun.* 71, 2032–2040
28 Dinglasan, R.R. *et al.* (2003) Monoclonal antibody MG96 completely blocks *Plasmodium yoelii* development in *Anopheles stephensi*. *Infect. Immun.* 71, 6995–7001
29 Lal, A.A. *et al.* (2001) Anti-mosquito midgut antibodies block development of *Plasmodium falciparum* and *Plasmodium vivax* in multiple species of *Anopheles* mosquitoes and reduce vector fecundity and survivorship. *Proc. Natl. Acad. Sci. U. S. A.* 98, 5228–5233

1471-4922/$ – see front matter
doi:10.1016/j.pt.2009.11.004 Available online 16 December 2009