Supporting Information

Thailayil et al. 10.1073/pnas.1104738108

SI Text

The impact of remating on release strategies is shown in Fig. S4. We developed a model of the mating dynamics of adult mosquitoes to explore the effects of different frequencies of remating. The model is described in the next paragraphs and readers uninterested in the technical details should skip to the final paragraph.

The adult female mosquito population is described by $\mathbf{N} = \{N_1, N_2, N_3\}$, where the three elements of the vector are the numbers of (1) unmated females, (2) mated and fertile females, and (3) mated but unfertile females due to insemination by a sterile male. We assume that adults recruit to the unmated female class (N_1) at a rate λN_2, where λ is the net fecundity (i.e., including preadult mortality) of a fertile female. Net fecundity will be influenced by density-dependent processes but in the analysis we are interested in population elimination, where λ can be interpreted as net fecundity when rare in the absence of density dependence. We have simplified the system by excluding an explicit time lag. All classes are assumed to suffer mortality at rate μ. Unmated females find mates at rate g and with probability $(1 - x)$ enter the fertile mated class (N_2) and with probability x the unfertile mated class (N_3). If the fitness of sterile and nonsterile males is the same, then x is simply the fraction of sterile males in the population. If the fitness is different, it is the weighted probability of being mated by a sterile male. Finally, females in the mated but unfertile class become receptive to males again (i.e., reenter the N_1 class) at rate h_3 while mated and fertile females exhaust their sperm and start looking for mates at a rate h_2.

With these assumptions the dynamics of the system are

$$\frac{dN}{dt} = \begin{pmatrix} \lambda N_2 + h_2 N_2 + h_3 N_3 - g N_1 - \mu N_1 \\ g(1-x)N_1 - h_2 N_2 - \mu N_2 \\ gx N_1 - h_3 N_3 - \mu N_3 \end{pmatrix},$$

from which the characteristic matrix in v can be constructed,

$$\begin{pmatrix} -g - \mu - v & \lambda + h_2 & h_3 \\ g(1-x) & -\mu - v - h_2 & 0 \\ gx & 0 & -v - \mu - h_3 \end{pmatrix}.$$

Persistence of the population is given by the Routh–Hurwitz criteria on the coefficients of the characteristic equations. Expressed in terms of x, population elimination requires

$$x > \frac{(h_3 + \mu)(-g\lambda + \mu(g + h_2 + \mu))}{g(-h_3\lambda + \mu(h_3 - h_2 - \lambda))}.$$

In *Anopheles* the remating frequency of fertile females is low. Setting $h_2 = 0$ we obtain

$$x > 1 - \frac{\mu}{\lambda}\left[\frac{\mu(g + \mu + h_3)}{g\left(\mu + \left(\frac{h_3}{\lambda}\right)(\lambda - \mu)\right)} \right].$$

Note if mating is very fast ($g \to \infty$), this equation becomes

$$x > 1 - \frac{\mu}{\lambda}\left[\frac{\lambda\mu}{\lambda\mu + h_3(\lambda - \mu)} \right]$$

and further that if there is no remating, then

$$x > 1 - \frac{\mu}{\lambda},$$

which is the classic result that in the absence of remating the fraction of sterile males must be large enough to reduce net recruitment of females to below one (1). The same result can be obtained if both classes of females remate at the same frequency $h_2 = h_3$ and the status of the female is determined purely by the type of male with which she mated last.

[A more sophisticated model might allow the reproductive output of a female as a function of her mating history. Thus, if sterile males transfer sterile sperm at mating—for example if their sterility is due to irradiation—then the females' fecundity may be affected by the dynamics of sperm competition within the spermatheca (2).]

The model described above was used to explore the fraction of sterile males (x) required to eliminate a population that increases when rare at a compound rate of 0.3/d (net fecundity, $\lambda = 0.4 \cdot \mathrm{d}^{-1}$; mortality, $\mu = 0.1 \cdot \mathrm{d}^{-1}$). In the absence of remating by females previously mated to sterile males, the frequency of the latter must exceed 0.75. If nonfertile female mosquitoes are able to remate ($h_3 > 0$), then the sterile male frequency must be larger. The pair of lines marked A in Fig. S4 shows that as the remating rate increases (or, equivalently, its reciprocal, the average time to remating decreases) a greater frequency of sterile males needs to be achieved for population extinction requiring progressively more mosquitoes to be released. When remating is very frequent, extinction is impossible. In calculating A we assumed mosquitoes mate instantaneously ($g \to \infty$); if this is not so, then slightly lower sterile male frequencies are required. This result is illustrated by the lines B in Fig. S4 where it is assumed that relative to an average lifespan of 10 d it takes a mosquito 1 d to find a mate ($g = 1$). A and B are both made up of pairs of lines. In each case the parameter values are the same except for in the lower line we assume 2% of fertile females have remated by the time they have reached half their expected lifespan ($h_2 = 0.004$, a number prompted by *Anopheles* biology). This low remating frequency of fertile females has little effect on the results. Finally, note that if the fitness of sterile males is different from that of wild-type males (either higher or lower), then x can be interpreted as a weighted frequency of the two male types.

1. Knipling EF (1979) *The Basic Principles of Insect Population and Suppression and Management. USDA Handbook* (US Department of Agriculture, Washington, DC).

2. Simmons LW (2001) *Sperm Competition and Its Evolutionary Consequences in the Insects* (Princeton Univ Press, Princeton).
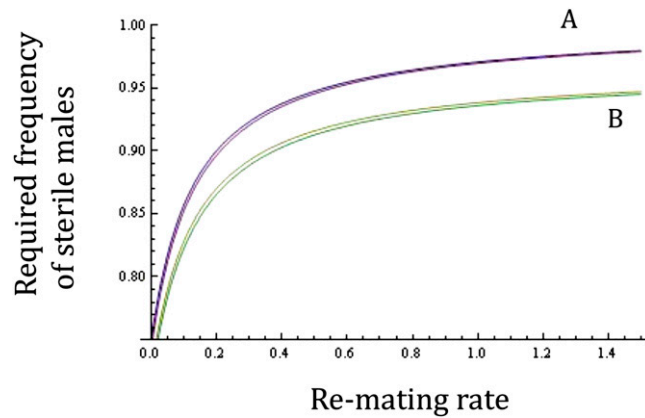
Fig. S1. Reproductive tract of Spm$^+$ and Spm$^-$ males. The image presents a comparison between (*A*) the reproductive tract of an Spm$^+$ male, showing fully developed testes (T) and MAGs, and (*B*) the reproductive tract of an Spm$^-$ male, with normal MAGs but aborted testes development and no sperm. (Scale bar: 0.1 mm.)

Fig. S2. Gene expression in the MAGs is not affected by ds*zpg* injections in Spm$^-$ males. MAGs were dissected from Spm$^+$ and Spm$^-$ male adults. A total of three MAG-specific genes were analyzed in three biological replicates run in duplicate: TGase (AGAP009099), Plugin (AGAP009368), and another MAG-specific gene (AGAP009370). Gene expression was normalized against the ribosomal protein L19 (AGAP004422).

Fig. S3. Transmission image of spermathecae dissected from females mated to a Spm$^-$ (*Left*) and a Spm$^+$ (*Right*) male. The tissue on the *Left* shows the oval shape with transparent appearance characteristic of a virgin spermatheca, whereas the one on the *Right* has the classical round shape of a mated spermatheca filled with spermatozoa that are visible as a dark ring (spermathecal mass, Sm). (Scale bar, 50 μm.)

Fig. S4. Impact of remating on release strategies. Consider a mosquito species that when rare increases at a rate $e^{(\lambda-\mu)t}$, where t is time measured in days, λ is net fecundity per day (the number of eggs laid per day multiplied by the probability of surviving to become adult), and μ is the adult death rate per day. The release of male insects that permanently sterilize females (i.e., remating rate = 0) will drive a population to extinction if the frequency of sterile males in the population is $>(1-\mu/\lambda)$. In the example graphed $\lambda = 0.4$ and $\mu = 0.1$ so the sterile male frequency when there is no remating must exceed 0.75. If mosquitoes are able to remate, then the sterile male frequency must be larger. Lines A show that as the remating rate increases (or, equivalently, its reciprocal, the average time to remating decreases), a greater frequency of sterile males needs to be achieved for population extinction requiring progressively more mosquitoes to be released. When remating is very frequent, extinction is impossible. In calculating A we assumed mosquitoes mate instantaneously ($g \to \infty$); if this is not so, then slightly lower sterile male frequencies are required. This requirement is illustrated by the lines B where it is assumed that relative to an average lifespan of 10 d it takes a mosquito 1 d to find a mate ($g = 1$). A and B are both made up of pairs of lines. In each case the parameter values are the same except for the lower line where we assume 2% of fertile females have remated by the time they have reached half their expected lifespan ($h_2 = 0.004$, a number prompted by *Anopheles* biology). This low remating frequency of fertile females has little effect on the results. Details of the model underlying these calculations are given in *SI Text*.