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Review Article

Playing God: Eradicating Malaria and Mosquitoes in the Developing World with Gene Drives and CRISPR/Cas9

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Abstract

Malaria is one of the leading causes of death in many African nations, and it is one of the top humanitarian problems. There are charities such as Against Malaria Foundation that attempt to reduce malaria infections in humans by distributing bed nets that are protected with insecticides. Each bed net costs about $5 USD to distribute. Their cost-efficiency is estimated to be of around $3k US dollars per life saved. However, they also prevent developmental problems in children that are affected but do not die from the disease. There are also network effects where if most of the population uses bed nets, they make it less likely for other people in the community to get infected as well. One problem with this approach is that the costs remain high and governments are not channeling enough funds to solving this problem. Nevertheless, this is the most effective charity in the world in terms of improving wellbeing, preventing deaths, and decreasing suffering, relative to financial cost. However, mosquitoes could be building up resistance to the insecticides, and therefore other approaches need to be explored. New research has been done on genetically engineering mosquitoes to replace existing populations, making them immune to malaria and other viruses. This could be done with or without gene-drive and CRISPR/Cas9 as tools to modify mosquitoes. Other approaches being researched include exterminating one or all mosquito species. The advantages and disadvantages will be discussed on this paper, along with quantitative estimates for the potential environmental impact, human-life-saving capability and amounts of mosquitoes that would need to be released and how long it would take to achieve the goal, whether it is immunity to malaria or extermination.

Introduction

I initially started researching about applying CRISPR/Cas9 to eliminate lung and breast cancer, but finally ended up realizing that the existing methods can only cure some types and do not totally prevent or eliminate cancer. In living patients, treatments are very limited and involve modifying white cells from a person to make them be able to detect and attack some cancerous cells, and then re-injecting them back into their bloodstream. For newborn babies, one can modify the fertilized egg in vitro to have the person grow up with an enhanced genetic code that would render them immune to some types of cancer. However, we are not yet able to cure all types of cancer, even with total control of the ge-

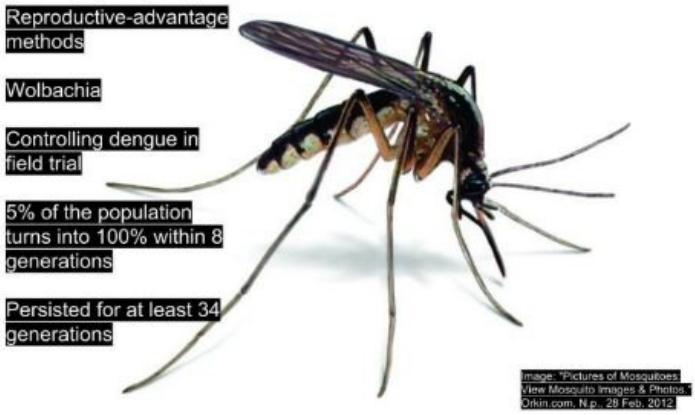
netic code. We can only cure or prevent a few types of cancers. On the other hand, there are diseases that we could possibly prevent or cure almost entirely with much less effort. Some of those cases are diseases carried by mosquitoes, such as dengue, Zika, malaria, Ebola and others.

Malaria, according to GiveWell is "One of the leading killers of children" in Africa [1]. Givewell ranks all charities that exist in the world, and tries to quantitatively estimate which ones can produce the most good if they received additional donations. This is a widely-accepted ranking within the Effective Altruism community, which tries to apply altruism or charitable behavior in the most effective manner [2]. Against Malaria Foundation is the top charity in Givewell's top charities chart. It uses charity funds to distribute "Insecticide-treated nets" that prevent deaths and other cases of malaria [1]. It pays about $5 USD per net. There is a network effect: the more widespread the nets are, the less likely everyone will get infected by malaria. The disease, malaria, is often transmitted by an "Infected female Anopheles mosquito". The mosquito's saliva has parasites that are introduced into a person's blood when the mosquito bites them [3]. Parasites move to the liver to mature and then reproduce. However, huge amounts of funding are currently dedicated to fighting malaria in this way (with bed nets), and the problem has not been fully solved yet. Moreover, Givewell suggests that "Insecticide resistance is a growing concern", although "It Remains difficult to quantify the impact of resistance" [4]. Their cost-effectiveness is of about $3k USD per life saved by using long-lasting insecticide-treated bed nets [4]. The cost is more than justified, but there could be cheaper methods (although riskier ones), such as modifying mosquitoes to make them resistant to malaria or eliminating almost all mosquitoes from the earth [5]. Half of the people in the world have risks of having malaria [6]. In 2015 alone, there were around 212 million cases of malaria and half a million deaths estimated [6]. Since 2010, there was a reduction of 29% in mortality rates from malaria [6]. 90% of the cases are in Sub-Saharan Africa [6].

Reproductive-advantage methods

There exists a naturally-occurring bacterium that can block the development of malaria parasites in mosquitoes [7]. It is called *Wolbachia*, and female insects can be transmitted to her offspring, while females that are not infected cannot produce viable eggs when mating with infected males. This gives infected females a "Reproductive advantage and helps the bacteria spread quickly [7]. *Wolbachia* has already been successfully used for controlling dengue in a field trial, but the bacteria (*Wolbachia*) does not really consistently move from mother to offspring in the malaria-carrying

cells and then, once developed, they break mosquito species: *Anopheles* mosquitoes [7]. However, researchers at Michigan State University have recently found a way to inherit *Wolbachia* infections in *Anophele* mosquitoes stably among multiple generations. This could block the growth of malaria parasites [8]. They were able to persist the infection for at least 34 generations [7]. With only 5% of the population being *Wolbachia*-infected females, all of the mosquitoes became infected within 8 generations [7].



Potential Human Lives Save

Zika

Brazilian authorities have estimated between 497593 and 1482701 cases of Zika virus in the year the Zika outbreaks began. That is 105-6cases only in Brazil. Other countries have not been affected to that extent. There are no reported deaths from Zika. However, the birth defects seem to be dangerous, although rare, and they happen when an infected mother gives birth.

Ebola

During the 2014 Ebola outbreak, there were a total of 11310 deaths [9]. Guinea: 2544 + Sierra Leone: 3956 + Liberia: 4810 = 11310 [9]. That is 104.

Dengue

For dengue, one recent estimate indicates 390 million yearly infections, with 95% credibility: an interval of 284-528 million. That is in the order of 10212-223 infections per year. From those, 96 million (67 to 136 million) are clinically manifested (cause noticeable problems). The total amount of reported deaths from dengue in 2015 was of 1181. That's 103.

Malaria

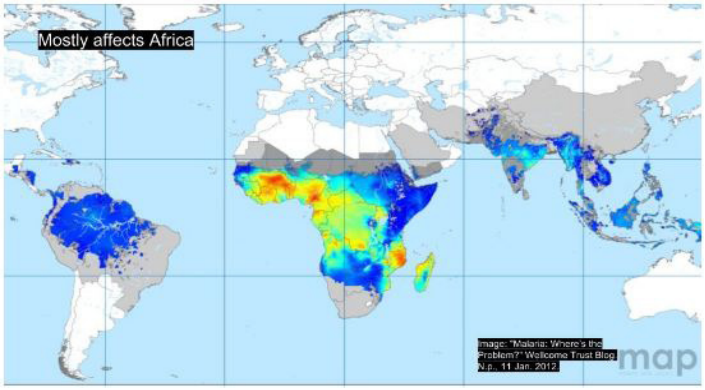
We can attempt to estimate the number of lives that could be saved by eliminating mosquitoes. Not only are mosquitoes the carrier of malaria, but also of dengue, Zika, and Ebola, as well as other diseases. 438,000 people (5×105) lives are lost to malaria every year [10]. The range is: 367,000-755,000. Therefore, in the order of 10512-6. 3 out of 5 of those are children (so they lose more years in their life from malaria compared to other diseases that target the elderly).

Total

In total, there were in the order of 104 deaths from Zika, Ebola and Dengue together. However, there are 5×105 lives lost to malaria every year, consistently. The developmental problems of children affected by Malaria also far outweigh those of the other viruses. For every year that we wait before we release the modified mosquitoes, we are losing half a million lives. Additionally, there are "200 million cases of the disease every year" [10]. This is 2×108 people who are affected but do not die.

Regulatory Hurdles

Because mosquitoes heavily disproportionately affect people in African countries and others that are close to the equator, and those countries are often under-developed, they have little weight in international regulations and decisions. Therefore, despite malaria affecting roughly 212 million people per year and killing 429000, since the affected populations are not very influential, they do not have much weight in the decision to exterminate mosquitoes or making them immune. The risks to the environment are extremely hard to estimate. These risks can affect already-developed areas (such as the US or Europe), while very few people in those nations are affected by the disease. Therefore, researchers, regulators and environmentalists have strong incentives to prevent experimentation and attempts to eradicate the disease. They would be directly impacted by any consequences, while the program would mostly save lives in other nations. As we can see, most viruses that the media portrays as emergencies (Zika, dengue, Ebola) are actually at least an order of magnitude less impactful than Malaria. However, they impact more developed nations, while Malaria does not. Current methods of control for mosquitoes are already controversial because of the unintended impact of pesticides on other insect species, even though they do not use genetic modifications [11]. The chemicals might also be harmful to humans [11].



Largest Field Experiments

Grand Cayman Islands

Oxitec performed a field trial in the Grand Cayman Islands, and reduced a particular species of mosquito by 80%. However, the remaining population could possibly build resistance and mutate to avoid dying before adulthood [12].

Panama

In Panama, Oxitec reported in 2014 that they reduced A. aegypti mosquitoes by 90%, 6 months after they released the genetically modified males [12].

Brazil

In the case of the Zika epidemic, in Brazil, no deaths were reported. However, perhaps because it happened during the Olympic games and many tourists from developed nations were present, an attempt to significantly reduce the mosquito population was carried out. Oxitec, a company from the United Kingdom, released genetically modified mosquitoes in Brazil. It did not use gene-drives, but they used a system that released mosquitoes with

a dominant lethal gene, which turns males sterile [13]. Oxitec designed their mosquitoes to carry the gene and are bred in the presence of an antibiotic, so that they can survive, then mate, and their offspring will not survive the larval phase because they are not in the presence of tetracycline, the antibiotic. Male mosquitoes do not bite people, and thus they do not spread the virus [12]. However, when they mate with wild-type females, their offspring will also die before becoming adults [12]. In three different cities in Brazil, the mosquito populations were reduced by 92%, 94% and 99%.

Oxitec released 800000 (~10^6) insects per week, for half a year. That's a total of 4×6×800000 = 19200000 = ~10^7 insects in total. And they achieved a 90% decrease in the local population of mosquitoes.

Limitations of Dominant-Gene Based Methods

One of the biggest problems of this approach used in Panama, Cayman Islands and Brazil, is that Oxitec's method is only effective in the first generation. After that, more genetically-modified mosquitoes will have to be produced and released periodically. Otherwise, there will likely be rebounds in the number of mosquitoes and those that survived will repopulate the world. Moreover, they could build resistance to this approach.

Timeframes with Reproductive Advantage Methods

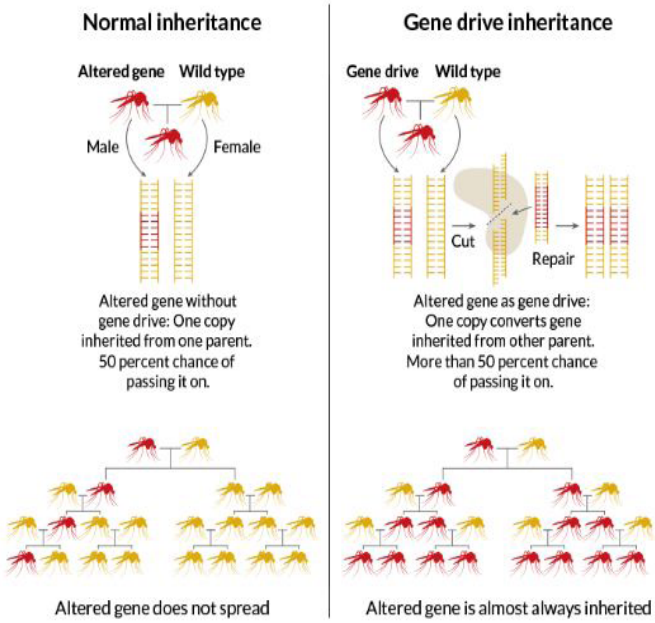
We can attempt to estimate the timeframe after which mosquitoes could become immune to malaria. Mosquitoes have been in the earth for 210 million years, and the consequences of their extinction are unpredictable. Therefore, this approach of making mosquitoes immune to malaria could be less harmful to the environment than the complete elimination of mosquitoes. Some people estimate there are 100 quadrillion mosquitoes [14]. That is 10^{17}. Others estimate 70 quadrillion [15]. That's 7×10^{16}. And that's 10^7 mosquitoes for every human on earth. If we were going to infect mosquitoes with *Wolbachia*, which makes them immune to malaria, it would take 8 generations of female mosquitoes if we release 5% of the existing population in genetically modified mosquitoes. That would be 10^{15} mosquitoes released to exterminate them in about 8×7=56 weeks (1 year). This is based on the trials, in which with only 5% of the population being *Wolbachia*-infected females, all of the mosquitoes became infected within 8 generations [31].

The impact on the environment of making mosquitoes immune to malaria might be minimal, because only one species is targeted, and the edited mosquitoes can be safely eaten by predators [16]. There was another study, at UC: Irvine, where researchers have used CRISPR/Cas9 in order to engineer mosquitoes that were resistant to malaria [17]. This could possibly allow for maintaining the existing population of mosquitoes (thus minimizing environmental impact), while still eliminating the threat of malaria to humans. Other researchers at Imperial College of London have engineered a modified female mosquito that could mostly breed male mosquitoes. This was done by using CRISPR/Cas9, the editing system, in order to cut the female chromosome. This would offset sex ratios and possibly cause the population to collapse [18].

However, the existing trials in Brazil were only performed with 10^7 mosquitoes, which is 8 orders of magnitude below what's needed for infection within 8 generations, using *Wolbachia*. Extrapolating, we could estimate that releasing 10^7 mosquitoes that are immune could result in 10^9 immune mosquitoes after 8 generations, and 10^{11} in 16 generations. 10^{13} in 24 generations, 10^{15} in 32 generations, and 10^{17} in 40 generations, in the best case. Since a generation is 7 weeks on average, that would take 40×7=280 weeks, or 5.4 years. It would take more than 5 years to achieve immunity in all mosquitoes in the world, as a rough estimate, assuming we could apply this to all species. Whether attempting to exterminate mosquitoes or just making them immune to disease, reproductive-advantage-based approaches might be too slow.

Timeframe for Gene-Drive Methods

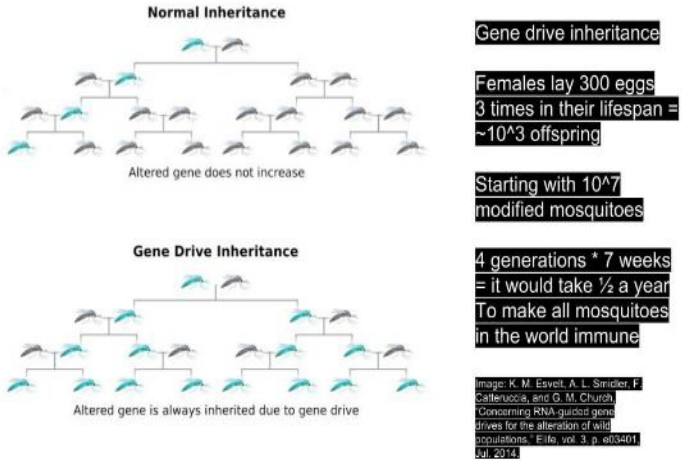
Gene-drive is a method that ensure all offspring by a genetically-modified mosquito will carry the modified gene [19]. This is achieved by cutting the other allele, and repairing it to copy the same gene (the target gene). Therefore, both copies are now the same type. As seen on the image,



the method achieves almost total inheritance, while normally only half of the offspring would inherit the gene. This is quite important when releasing a small percentage of mosquitoes into the wild population, since they need to very quickly spread their genes to the whole population in order to achieve human goals such as mosquito extermination or almost total immunity to malaria and other viruses. Female mosquitoes can lay up to 300 eggs at a time, and they lay eggs up to 3 times before they die [19]. The average mosquito lifespan is of two months [19]. Males usually live up to 10 days, and females live about 6 to 8 weeks [19]. hey lay eggs every three days [19]. Those species that hibernate may live up to six months [19]. Given that females can lay 300 eggs, 3 times in their lifespan, we can estimate they can produce ~1000 offspring, or 10^3. Therefore, if we started with 10^7 mosquitoes, after 1 generation we could have up to 10^{10} mosquitoes that carry the selfish genes, after 2 generations, we would have 10^{13}, 10^{16} after 3 generations, and 10^{19} (more than all existing mosquitoes) could be reached after 4 generations. Since every generation of female mosquitoes is about 7 weeks, it would take 28 weeks, or about half a year. This method is much faster and more effective. It is 10 times faster than infecting mosquitoes with *Wolbachia* to achieve malaria immunity. However, some environmentalists believe that it is impossible to predict the consequences of eliminating an entire species [20].

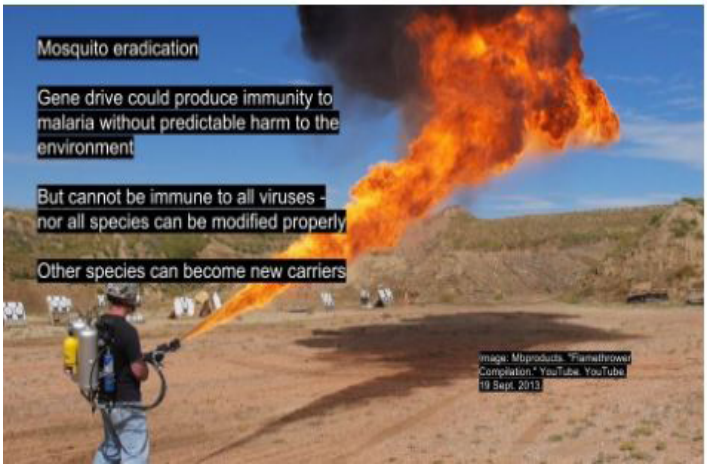
Non-Deadly Impacts of Mosquito-Carried Disease

The Zika virus is one of the most dangerous, as it produces severe birth defects for mothers that are bitten. However, no deaths have been reported. Not all impacts are deaths. This virus has even reached New York (5 people have been diagnosed as positive there) [11]. These mosquitoes can also carry dengue and chikungunya, as well as malaria and zika. The economic impact of dengue fever in a single country (Brazil) was to be of $1.35 billion per year [21]. This is in the order of 10^9 USD. Authorities have been attempting to develop vaccines and attack the virus instead of its host (mosquitoes) [22]. However, a vaccine or cure for Zika could potentially take in the order of 10 years [23]. This time for research not only could affect more humans, but also takes research funds and resources for a problem that could more easily and definitively solved with gene-drive or other methods.



Arguments in Favor of Total Mosquito Eradication

Although malaria-resistant gene drives seem to cause no predictable harm to the environment, and are likely safe, we might not be able to make mosquitoes immune to every disease, and thus they might continue to carry other diseases than malaria, such as new diseases [24]. Moreover, it might be impossible to create gene drives that "Encode antibodies to all of these diseases" [25]. If a cell needs to create non-essential proteins such as antibodies, this will use resources that might otherwise have been used for cell replication and growth, and thus these cells would be selected against other cells with less load, and the organism would eventually develop without those antibodies [26]. Also, other species of mosquitoes could eventually start carrying malaria, so even if one species becomes immune, others might mutate and replace it as carriers [27].



Ecological Impact Estimates of Eradication

Although mosquitoes are believed to pollinate some plants, there are very few studies of mosquitoes acting as pollinators [25]. They function mostly as "Necrophages", that is they consume nectar but are not very helpful in pollination [28]. Another common argument is that mosquitoes might be a food source to birds, fish and amphibians, and those could be impacted by removing mosquitoes [29]. Some estimates by scientists estimate a 50% reduction of migratory bird populations that rest in the tundra, in the Arctic [29]. However, mosquitoes do not show up in birds' stomachs in high amounts, and therefore some believe they are not a primary food source [29]. Also, after an initial short-term transitory phase of reduced fish, bird and amphibians, there could be an increase in them, since mosquitoes could get replaced by other insects as food sources [27]. Environmental impact is not very well understood, and there is a widespread agreement that the consequences of eliminating mosquitoes would be quite unpredictable, although the evidence suggests they are not immediately essential to the survival of any species.

Conclusion

Although the environmental impact of eliminating all malaria-carrying mosquitoes or even all mosquitoes is unpredictable, it might be worth the risk if we can save around 5 million lives in the next decade, as well as preventing suffering from non-lethal cases. However, intermediary approaches could be attempted first, such as spreading immunity to malaria and other diseases via CRISPR/Cas9-engineered mosquitoes and gene-drive. These changes could potentially be reversible with more CRISPR/Cas9 modifications and another gene-drive worldwide experiment. Eventually, if mosquitoes develop resistance after the fact, and become malaria-carriers again, total extermination could be attempted. In any case, gene-drive seems much more promising than approaches based on reproductive-advantage for spreading genes among the population. Gene-drive can achieve a broader effect (~95% vs. 80-90%), ten times faster (half a year vs. more than 5 years).

References

1. Top Charities. GiveWell.
2. Effective Altruism.
3. Malaria Fact Sheet N°94". WHO. March 2014.
4. Against Malaria Foundation. GiveWell.
5. Image: malaria parasites develop inside human red blood c out of it.
6. 10 Facts on Malaria. World Health Organization.
7. Infection Makes Mosquitoes Immune to Malaria Parasites. Department of Health and Human Services, National Institutes of Health, 04 April, 2016 USA.
8. Bian G, Joshi D, Dong Y, Lu P, Zhou G, et al. (2013) *Wolbachia* Invades *Anopheles stephensi* Populations and Induces Refractoriness to Plasmodium Infection. Science 340: 748-751.

9. 2014 Ebola Outbreak in West Africa - Case Counts. Centers for Disease Control and Prevention. 14 April, 2016.

10. Against Malaria Foundation. Giving What We Can.

11. Engber and Daniel (2016) It's Time to Wipe Mosquitoes Off the Face of the Earth. Slate Magazine.

12. Versteeg L, Wang Q, Beaumier CM (2016) Invited Commentary on Genetically Modified Mosquitoes for Population Control of Pathogen-Transmitting Wild-Type Mosquitoes. Curr Trop Med Reports 3: 4-6.

13. Gabrieli P, Smidler A, Catteruccia F (2014) Engineering the control of mosquito-borne infectious diseases. Genome Biol 15: 535.

14. How Many Mosquitoes Are There in the World?

15. How Many Mosquitoes Are There? Is It Even Possible to Estimate Such a Thing?

16. Kim and Meeri (2016) How mosquitoes with 'self-destruct' genes could save us from Zika virus. The Washington Post.

17. Gantz VM, Jasinskiene N, Tatarenkova O, Fazekas A, Macias VM, et al. (2015) Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito *Anopheles stephensi*. Proc. Natl. Acad. Sci 112: E6736-E6743.

18. Hammond A, Galizi R, Kyrou K, Simoni A, Siniscalchi C, et al. (2015) A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. Nat. Biotechnol 34: 78-83.

19. Saey, Hesman T (2016) Gene Drives Spread Their Wings. Science News. Mosquito Facts.

20. Support grows for genetically modified mosquitoes in Zika fight. CBS News. February 16, 2016.

21. Pepin, Kim M, Toledo CM, Scherer L, Maira M, et al. (2013) Cost-effectiveness of Novel System of Mosquito Surveillance and Control, Brazil. Emerging Infectious Diseases. Centers for Disease Control and Prevention.

22. Barnato K (2016) Zika Epidemic on US Doorstep Spurs Vaccine Hunt. CNBC.

23. Inside High-security Laboratory Developing a Zika Vaccine. BBC News. BBC.

24. Lymphatic filariasis, WHO.

25. A Review of the Safety & Efficacy of Genetically Engineered Mosquitoes.

26. Whitlock MC and Bourguet D (2000) Factors Affecting the Genetic Load in *Drosophila*: Synergistic Epistasis and Correlations Among Fitness Components. Evolution 54: 1654-1660.

27. David AS, Kaser JM, Morey AC, Roth AM, Andow DA (2013) Release of genetically engineered insects: a framework to identify potential ecological effects. Ecol Evol 3: 4000-4015.

28. Larson B, Kevan P, Inouye D (2011) Flies and flowers: taxonomic diversity of anthophiles and pollinators F pollinators. Can Entomol 133: 439-465.

29. Fang J (2010) Ecology: A world without mosquitoes. Nature 466: 432-434.