Podcast Interview: Omar Akbari and Kevin Esvelt

PNAS: Welcome to Science Sessions. I'm Paul Gabrielsen.

The *Aedes aegypti* mosquito carries malaria, dengue fever, yellow fever, chikungunya, and Zika. But there may be a way to eliminate this and other harmful invasive species, through an application of gene editing called a gene drive. A gene drive encodes both a gene edit and the ability to copy that edit so that the next generation is guaranteed to inherit it and the edit rapidly spreads through the population.

Kevin Esvelt of the Massachusetts Institute of Technology proposed that the CRISPR/Cas9 gene editing system, first announced in 2013, could be the engine behind a gene drive. Soon after the proposal, however, Esvelt and others expressed concerns about the effect of such a self-propagating gene drive on ecosystems.

In a recent PNAS paper, Omar Akbari of the University of California, San Diego and colleagues presented what could be a safer form of a gene drive. They developed a method of encoding only part of a gene editing system into the *Aedes* mosquito. Akbari's results could lead to a gene drive that achieves the goal of controlling harmful species but carries less risk for unintended and uncontrolled effects on native populations of those same species.

Esvelt begins by telling the story of his gene drive proposal. He was among the first to experiment with the CRISPR/Cas9 system, but almost left the field as CRISPR's popularity skyrocketed. One morning, his outlook changed.

Esvelt: I was walking to work through the Emerald Necklace in Boston, and there was actually a turtle in the water that day, which was a rare spotting. And I was just wondering, are we ever going to edit any of these organisms? In the wild, I mean. And I concluded, well probably not just because whenever we make a change it's for our benefit not that of the organism, and so natural selection tends to wipe the floor with them. So then I wondered, but wait, what would happen if you encoded the CRISPR system that you used to make a genome edit adjacent to the change you're going to make? Then when it mated with a wild organism, the offspring would inherit your change and the instructions for making it, and it would then edit the original version from the other parent to have your new edit. And then, that would ensure inheritance by the next generation and the next and the next and the next.

PNAS: Esvelt's musings led to the gene drive proposal. Others had proposed similar systems, going back even to the 1940s. But now with CRISPR, the idea of a gene drive could become reality.

Esvelt: So the first day was pretty much total excitement and elation at all these possibilities because this could be the key to eradicating malaria, schistosomiasis, all sorts of other diseases. The second day all of my doubts kicked in, thinking about - isn't this going to cause problems if it keeps on spreading, as presumably it would? How are we going to ensure that it's safe? You can't really test it in the field safely because it

would probably just take off, so how do you run a field trial? Is an isolated island enough? What if it gets off?

PNAS: In 2017, Esvelt and colleagues wrote that gene drives could spread remarkably quickly through an invasive animal population, but carry a significant risk of spreading to native populations as well. That risk warrants extreme caution in field trials and more research into safer forms of the technology.

This is where Akbari comes in. In the CRISPR system, the protein Cas9 acts as the scissors, cutting DNA at a location specified by a strand of guide RNA. Akbari and his colleagues encoded the gene for the Cas9 protein into the *Aedes* mosquito genome.

Akbari: Without the presence of the guide RNA, the Cas9 essentially doesn't cut. So, it's off. To turn it on, one would need to either inject the guide RNA into the organism or genetically cross the Cas9-expressing strain to other strains that express guide RNAs.

PNAS: Akbari and colleagues have already employed the Cas9 embedded in the mosquitos' genome to manipulate eye and body color, among other edits. If employed in a gene drive, Akbari's partially-encoded system would be called a split gene drive.

Akbari: So the split drive approach, it's a self-limiting approach. So, it essentially can't spread on its own; you would need to continually supply the Cas9 into the population. So, this type of drive is safe in that it can't spread on its own. The split gene drive approach is a good approach for studying and engineering and designing them in the laboratory and understanding how effective they spread in the presence of Cas9. It could be used in the field as a self-limiting-type approach and I think it would work, but again it would also require significant effort in terms of inundating the population with these gene drive-containing organisms and given that property, it makes it less attractive than a full drive that could actually spread itself.

PNAS: Esvelt says that although Akbari's work can be viewed in the context of a gene drive, it also fulfills one of the basic promises of CRISPR: to accelerate fundamental research.

Esvelt: So the main impact of this paper, which is very well done, is to create tools that will make genome engineering in these mosquitoes fantastically easier.

PNAS: Esvelt's vision of the way forward for invasive and harmful species control is to give communities and regions the tools they need for small-scale ecological engineering.

Esvelt: For almost all potential applications we need to focus on building local drive systems, that is, constructs that will alter a wild population, but only locally. That is, they cannot spread indefinitely. Just because it is hard to see how you're going to get more than 100 countries to agree, even on something like getting rid of these invasive mosquitoes that spread dengue and chikungunya and Zika and yellow fever.

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