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The CRISPR Journal
Volume 1, Number 5, 2018

DOI: 10.1089/crispr.2018.29031.kda

The CRISPR Journal

INTERVIEW

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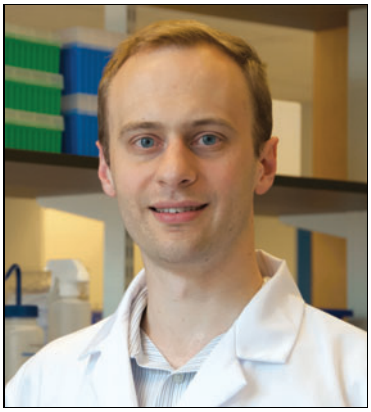
Kevin Davies and Kevin Esvelt



Kevin Esvelt leads the Sculpting Evolution group at the MIT Media Lab—an institution, Esvelt says, that is ''for black sheep who do not fit anywhere else.'' Esvelt has emerged as a leading figure in the potential use of CRISPR-Cas9 to develop strategies, including but not limited to gene drives, to combat tick- and mosquito-borne diseases. He is also leading the discussion of some of the broader societal issues around the real-world deployment of CRISPR in combatting disease, in terms of both engaging affected communities and considerations around potential ecological damage.

After presenting at the recent CRISPRcon meeting in Boston, Esvelt sat down with Executive Editor Kevin Davies to discuss his work on CRISPR and the importance of community engagement.

***Davies*: Kevin, what is your scientific background and how did you end up ''sculpting evolution'' at the MIT Media Lab?**

Esvelt: I have always been fascinated by natural evolution, thanks to a timely visit to the Galapagos when I was in the sixth grade. I wanted to know how is it that so many marvelous creatures are created, and can we learn how that is done and create equally marvelous things ourselves?

Since then, I've learned a bit more, come up with a few ethical objections to the way that natural evolution does things—the apparent total indifference to animal suffering, to any kind of notion of right or wrong. Evolution is *amoral*. I am not saying it is immoral because it is a physical process. But the fact that it does not care about or optimize well-being I view as a fundamental flaw in the universe.

So I do not know if we can ever learn enough about evolution to change that, but you might say that that is our broader goal. Evolution has no moral compass. We do.

***Davies*: Well, this got deep in a hurry!**

Esvelt: Sculpting evolution is an excuse for us to work on anything interesting because evolution applies to any self-replicating informational pattern. That is, it is not just genes. Genes are convenient because they are digital, so they are easy to read.

But it is also culture. Culture evolves in the same sort of way. If culture evolves, then culture is something that is within our purview to try to change because our primary mission, one might say, is to sculpt the evolution of technology development—that is, our ascent up the tree of knowledge, consuming the fruits.

How can we ensure—by changing the fitness landscape governing scientists exploring this tree—that we have the best chance of finding the fruits that we need in order to continue improving human and environmental

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well-being, while avoiding those that could be hazardous to our collective health?

Davies: Before setting up your own lab at the MIT Media Lab, were you always working in evolution?

Esvelt: Yes. I started my PhD at Harvard in the molecular and cellular biology program, but I ended up joining David Liu's lab. He was in chemistry and chemical biology at the time. David is amazing: when I walked in and proposed 10 different project ideas, he said, ''This is fantastic. You'll fit right in. We tried the first two. The third one is really interesting, but I am not sure it will work for these reasons. Fourth…'' and so on. He said, ''But what I am really most interested in is this: you expressed this idea that you wanted to figure out some way to automate directed evolution.''

Directed evolution is where we apply evolutionary principles to optimize the function of typically biomolecules that we do not understand well enough to design new functions rationally. If you don't understand how it works well enough to engineer it, then one alternative is to make a billion variants, test them all, pull out the ones that do what you want the best, and then make a billion variants of those and on and on.

It was really pioneered by Frances Arnold back in the 1980s, and it has gotten better since then. But the problem is that it's a lot of work. And I'm a big fan of laziness: how can we engineer the world such that it solves our problems for us?

The idea was whether there is a natural system that we can harness that evolves on its own that we can tweak to make it evolve the things that we want autonomously. This was inspired by a Gerry Joyce system, where he made a continuous *in vitro* evolution of RNA enzymes, an amazing 1997 paper.[1] The idea was whether we can harness some natural system to make a more general platform for doing this.

This—after six years of effort and many wasted steps—led to phage-assisted continuous evolution. The idea is that you just take a bacteriophage, M13, which requires protein 3, in order to infect a new cell. We remove gene 3, replace it with the gene we want to evolve, and put gene 3 under the control of cellular expression conditions in the host cell such that the host cell will produce protein 3 proportional to the phage doing what we want. That means every phage has an evolutionary incentive to perform the molecular trick that we desire, and in response to its ability to do that, it gets more of this critical protein it needs to continue its life cycle.

Then we sit back and hit start on the pump, and it constantly feeds the lagoon new host cells. The phage evolves a couple of generations an hour in a billion variants in a population, and a couple days later, it spits out what you want. David has used it for a lot of amazing things since then.

Davies: How did that lead into your interest in gene drives?

Esvelt: That touches on how I first got involved in CRISPR. In 2011, I moved to the Wyss Institute, where I was a technology development fellow working primarily with George Church, my fellowship mentor. One of the things I wanted to do was build a PACE phage-assisted continuous evolution system. But the DNA origami folks were growing liter cultures of filamentous wild-type phage because they use the genome as their scaffold, meaning there is phage everywhere, so it kept infecting my cells. I couldn't get a wild culture of *Escherichia coli* with F-plasmids that did not become infected with filamentous phage because it just saturated the air of the entire floor!

I recalled hearing something about a bacterial immune system that could be programmable. So, I initially tried to tinker the *E. coli* CRISPR system to get it to exclude the filamentous phage. It didn't work very well because type 1 systems are not all that great to work with. Then I read Emmanuelle [Charpentier]'s paper on Cas9,[2] and I thought, ''Oh, well, this is great. I'll try it.'' I put it in, added a few guides targeting filamentous phage wild-type gene 3, and recoded the gene in my copy. It worked beautifully.

Then, of course, came Jennifer and Emmanuelle's paper on *in vitro* activity,[3] and I thought, ''Well, this is going to be big.'' But I did not think of using it until Prashant Mali from the Church lab came to me and said, ''So, I have this genome editing platform for analysis completely all worked out for TALENs. You've been working with Cas9 in bacteria. We should collaborate and do this in eukaryotic cells.''

I said, ''Yeah, it's going to be huge, but Jennifer has been doing this for six months. Is this really worth our time? There are probably many other groups that are going to be trying to get it working, too.'' Prashant said, ''This is going to be so big that if we discover one tiny little piece that the other groups miss, it will be worth it.''

As it turned out, our one tiny little piece was—you really do need the whole sgRNA. You cannot truncate it and remove the last hairpin without limiting activity. That led to a year of feverish activity developing new tricks with CRISPR before Prashant and I both realized that there were a lot of other people doing this, and perhaps our services were not required. So, we bowed out.

Around that time, I wondered what happens if you put CRISPR into a eukaryotic cell. What if you teach the cell to do genome editing on its own so that editing would be recursive down generations? This insight just hit me, and then I thought, ''Wait a minute, are there not genes that do this naturally? Is that not what I-SceI does in yeast? Is that not like a homing endonuclease gene drive? Did somebody not try put I-SceI in mosquitoes, like engineer mosquitoes or something like that?''

I found Austin Burt's original gene drive paper[4] and thought, ''Wow, this guy was a genius. He thought of this a decade ago.'' But you cannot really engineer homing endonucleases to cut new targets.

***Davies*: That was in the early 2000s, right, Austin Burt's paper?**

Esvelt: Yes. I had wondered what happens if you encode the CRISPR system for making a change along with the change in the genome. That way, when your organism mates with another organism, the offspring inherit one copy of the edit and the CRISPR system used to make that edit. So, in the offspring's germline, it will cut the wild-type version and replace it with itself. That ensures the next generation inherits, and editing happens again, meaning this system distorts inheritance in its favor. This is a very common phenomenon in nature. This is exactly how this I-SceI homing endonuclease works in the wild. It cuts chromosomes that do not have it and copies itself over using the cell's natural repair mechanisms.

I learned that Austin Burt, who was at Imperial College, London, had first proposed we harness these naturally occurring gene drive systems that work by homing endonucleases to edit wild populations, in especially malarial mosquitoes, to eradicate malaria eventually. In fact, Austin had been working on doing this along with Gates Foundation backing and the help of Andrea Crisanti, a mosquito biologist at Imperial, for the almost 10 years.

But the challenge they faced is that it is fiendishly difficult to work with the homing endonucleases and to get them to target the sequences you want. So, they were anticipating a 20-year project and a couple of billion dollars to get it working in one species. I realized that, ''with CRISPR, you can probably target many different genes trivially. There is no reason why you could not do a CRISPR-based gene drive … this is amazing. We can eradiate malaria.''

The next day, I thought, ''This will work in pretty much any species that reproduces quickly and sexually—what are we going to do about this?'' Fortunately, George [Church] introduced me to a few other people: Jeantine Lunshof, the ethicist he works with, and Ken Oye, who does political science on syn bio stuff at MIT. We thought about how this could go wrong—what the pros and cons were. Ken suggested we bring it up at a meeting he was organizing on the ecological implications of synthetic biology. We talked about it and got feedback from security types, ecologists, evolutionary biologists, representatives, and environmental nongovernmental organizations.

Everyone pretty much agreed that this is not much of a direct physical threat, just because it is slow. It only spreads over generations. It is easily detectable if you look for it by sequencing. You cannot really hide it. And CRISPR is powerful enough that you cannot really build a gene drive that cannot be targeted with CRISPR, meaning whatever one person does, another person can override. And anything slow, obvious, and easily blocked is not much of a threat.

So, we weren't really worried about misuse in that sense, but we were deeply worried about an accident or unauthorized use. We wanted to ensure that this kind of research was done transparently and used appropriate safeguards.

***Davies*: You were grappling with the potential ethical and societal implications of this technology, even before demonstrating that it could work in an experimental fashion?**

Esvelt: We were very fortunate in that this was a new time for biology because pre-CRISPR, most things failed! You couldn't assume that something would work just by knowing which parts you were going to use. But by this time, after a year and a bit of working with CRISPR, we knew that the rules were different. The thing about a CRISPR-based gene drive is that it is exactly CRISPR genome editing, just made heritable.

***Davies*: You mentioned CRISPR and gene drives in the context of malaria. Did you think about working in that area yourself?**

Esvelt: We did talk to some local mosquito biologists: Flaminia Catteruccia and her student Andy Smidler based at Harvard School of Public Health. They joined us in writing the original paper in which we described CRISPR-based gene drive in *eLife*,[5] and with Ken and others, we wrote a piece on regulation and how research should be open in science.[6]

That was how we ended up emphasizing: if you are going to build these things or are just using CRISPR with a vector that encodes both the nuclease and the guides in one piece of DNA, if those guides target the genome, and the whole thing self-inserts, even accidentally, you might make a gene drive without even realizing it.

Or we were concerned people might make a CRISPR-based gene drive for reasons other than editing wild populations, perhaps not even realizing that it might, if it escaped into the wild, affect a wild population. These were our concerns. We also really wanted to make sure that anyone doing this research was doing it in the open and thinking very carefully about these issues and inviting outside criticism.

As it turned out, another group[7] had independently invented a CRISPR-based gene drive just a few months after our publication, and they had never seen our work and proposed safeguards. They did not realize at first that it might spread in the wild, and they performed their initial experiments in fruit flies without that, and therefore did not use the frankly very stringent safeguards that we had proposed.

***Davies*: That was the group in California?**

Esvelt: These are brilliant, well-meaning scientists, and they came from a completely different background. It was their first time working with CRISPR, developing new tools. They had not really been tool developers before either, and they had just never come across the notion of a gene drive or anything like that, and why would they? They were developmental biologists. This is the problem with modern science. You have to be lucky enough to have the right kind of background even to have a chance at predicting the consequences of what you are working on. Any time you move into a different area, you might develop a tool that can be combined with somebody else's tool that you may not even be aware of that could have some pretty powerful implications for society.

To underscore how profound an impact this can be, six years ago, pre-CRISPR in eukaryotes, no human had imagined that we might be able to edit entire wild species. Austin envisioned doing it for one species with a tremendous amount of effort, but no one ever imagined that we might be able to do this routinely someday. The concept was completely absent from science fiction at the time. Literally, no human ever conceived that we might be able to do this. All of a sudden, boom, it looks like we can.

We are lucky that it is not much of a physical or ecological threat, just because it is slow, obvious, and easily overwritten. It didn't have to be that way. Imagine if CRISPR was a little less versatile. Suppose you have a much larger PAM requirement, and they all require that, and it is built into the mechanism, so you cannot engineer them to be better. So, you can hit any sequence typically one every 256 bases, say. Then you could build a gene drive that would work in the wild, but you could engineer yours to lack CRISPR-accessible target sites, meaning it could not be overwritten. Then your security implications are completely different if you remove the easily overwritten, slow, and obvious, sure, but if you cannot do anything about it, that would be a problem.

***Davies*: You have been working off Cape Cod, on Nantucket and Martha's Vineyard. Were you looking for a real-world example where you could potentially explore in a confined way a gene drive scenario?**

Esvelt: The Mice Against Ticks project was born of a desire to find a potential local application where I could talk to a local community about the idea of using CRISPR to engineer the environment, probably without a gene drive, and having a community tell us whether this was a good idea.

We did a direction-finding meeting, gathered people together, including some representatives of these islands off the coast of Massachusetts—Nantucket and Martha's Vineyard. These islands have some of the highest rates of tick-borne disease, particularly Lyme disease, in the country. Lyme disease is the most common vector-borne disease in the United States. The Centers for Disease Control and Prevention estimates well over 300,000 cases a year. Katherine Bui, a pediatrician I am somehow lucky enough to have married, says, ''The West Coast has earthquakes. The South has hurricanes. The middle of the country has tornadoes. The natural disaster of the Northeast is Lyme disease.''

The islands have it worst. It is an ecological problem. Bluntly, we like to engineer the environment to make fragmented forests. We love forests, but we cut them up with roads and houses, so you get maximized perimeters, and we get rid of all the wolves. We maximize the deer because we don't kill enough deer with automobiles, let alone guns.

A ton of deer means a ton of ticks. Fragmented forests mean you get a lot more white-footed mice—the best reservoir of tick-borne disease. The fraction of ticks biting white-footed mice relative to things that cannot infect them with disease goes way up. So, you maximize the reservoir. You maximize the tick vector. You maximize the number of infected ticks.

***Davies*: What is the strategy to combat this problem?**

Esvelt: Tackle the mice. Can we use CRISPR to engineer immunity into the mice? Because these are islands, you have two options—normally you would only have one. If you release enough mice with engineered genetic

cassettes and provide resistance to either Lyme disease or to tick bites directly, then you can disrupt the ecological cycle of transmission. That is, if you make this immunity heritable and release enough mice with protective alleles, on average, descendants will inherit at least one copy of protective alleles and disrupt the cycle.

There are only so many mice there on the islands, and there is not a whole lot of gene flow with the mainland, so you just need to release enough heritably immunized mice. As long as you're not using a gene drive, it is not going to spread on the mainland. So, it should be just an island-by-island thing.

I thought we might be able to do this because we are pretty good at engineering rodents. White-footed mice are actually more distant from lab mice than lab mice are to rats, but they are still rodents. There is a vaccine against Lyme disease. We know it is antibody mediated. It is possible that tick resistance is antibody mediated. If so, in either case, we can isolate antibodies that are highly protective.

If we were to encode those antibodies in the germline such that newborn mice begin expressing them, then you could make mice that would be heritably resistant to disease, take that acquired resistance from some mice, and put it back into mice.

I was well aware of people's wariness of genetically modified organisms (GMOs) but specifically of GMOs created by introducing genes from foreign organisms. People view this as unnatural. When we approached communities, on the advice of the local residents, they just said, ''Contact the Boards of Health, have a town hall meeting.'' This is New England town hall democracy. The Boards of Health will just advertise the meeting. They will get a few dozen local residents come in to hear you talk, and they will yell at you and tell you if they are interested—which is pretty much how it happened.

As I recall, the communities said, ''Yes, we are very interested in this, but we would really rather you not use any foreign DNA. If you are going to engineer mice, we would prefer them to be 100% mouse, as it were. But please immunize them against everything you can.'' This notion of using antibodies is great, but if you are not using any foreign DNA, that means you cannot do any form of gene drive, even a local drive.

***Davies*: Has the local community given you the thumbs-up to go ahead?**

Esvelt: They have established steering committees, one for each island, and the steering committees can end the project for their island at any time, just by calling a vote and saying no, and that is it. Then we walk away. They tend to be packed with extremely well-credentialed people because that is the nature of the islands. The chair of the Nantucket steering committee, Howard Dickler, was in charge of the National Institute of Allergy and Infection Diseases' immunology branch. John Goldman is an editor of the *Journal of Immunology*, and they are two on the Nantucket steering community.

Also on the committee is Danica Connors, who is absolutely wonderful. She is a herbalist, deeply skeptical of anything GMO. She might still vote against it at the end of the day, but she is very interested in seeing if it can be done because if everything turns out perfectly, maybe she could support it.

So, there is no gene drive on the islands at all for Mice Against Ticks. The community says exactly what they want, and then we try to do it, and it is not going to happen for many years because research is slow.

***Davies*: What are some of the most exciting potential applications of CRISPR that might bear fruit in this ''sculpting evolution'' context?**

Esvelt: There are two. The New World screwworm is one of those species that Darwin was not aware of, but he said, in effect, it was hard to conceive of how a benevolent creator could possibly have made the *Ichneumonidae*, the wasps that paralyze caterpillars and lay their eggs, and the caterpillars are eaten alive from the inside.

The New World screwworm, *Cochliomyia hominivorax*, is far worse than a parasitic wasp because it does the same thing to mammals. Each year, millions of wild and domesticated animals are eaten alive in excruciating agony.

We have eradicated this species from all of North America already using the sterile insect technique. But in South America, it is more entrenched, more varied geography. The sterile insect technique cannot do it, but a gene drive could. Can the Mercosur countries come together to agree to do this, or do you use something like a daisy drive to do it country by country to show that it works—a more likely potential option?

Perhaps the most important application of the gene drive is to help explore new ways of doing science, that is, to change the scientific ecosystem. The gene drive is unique in that, first, there are not a lot of near-term commercial applications. You really need public acceptance first, which requires starting in the nonprofit space—certainly, for self-propagating, which is the one we know really works. Not much of a business model in one release solves the problem everywhere in the world.

So, not a whole lot of commercial potential right now, meaning not so much IP pressure, meaning why not do open research? We are ethically obligated to disclose

our research plans publicly and invite people's feedback because if we do not, we are effectively denying people a voice in decisions intended to affect them from which they will not be able to opt out.

Even if you only care about practical benefits of saving lives, and you do not care at all what people think, doing it the ethical way and giving all the communities a voice, I would say, is most likely to result in your desired outcome, even if you do not care about those particular ethical concerns. I think everyone should come together to ensure that gene drive research is open. We have a standard preregistration template we have been working on, explaining what your general project is and why it is worthwhile.

I think we should change science to make it faster because we desperately need new advances to continue the progress in human and also animal well-being that we have gradually been working on. Advances will come faster with more open science. I think that the ability to receive advice from others before you run experiments also makes it less likely that we will, in ascending the tree of knowledge, taste a fruit that proves to be catastrophic.

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