



The usual suspects

From Hercule Poirot to CSI, most detective stories and TV police shows revolve around the investigators narrowing down their list of suspects. At the start of the story, there are often as many suspects as there are characters, but thanks to varying measures of deductive power and luck, the good guys systematically strike suspects off their list until finally they are left with only one – the culprit.

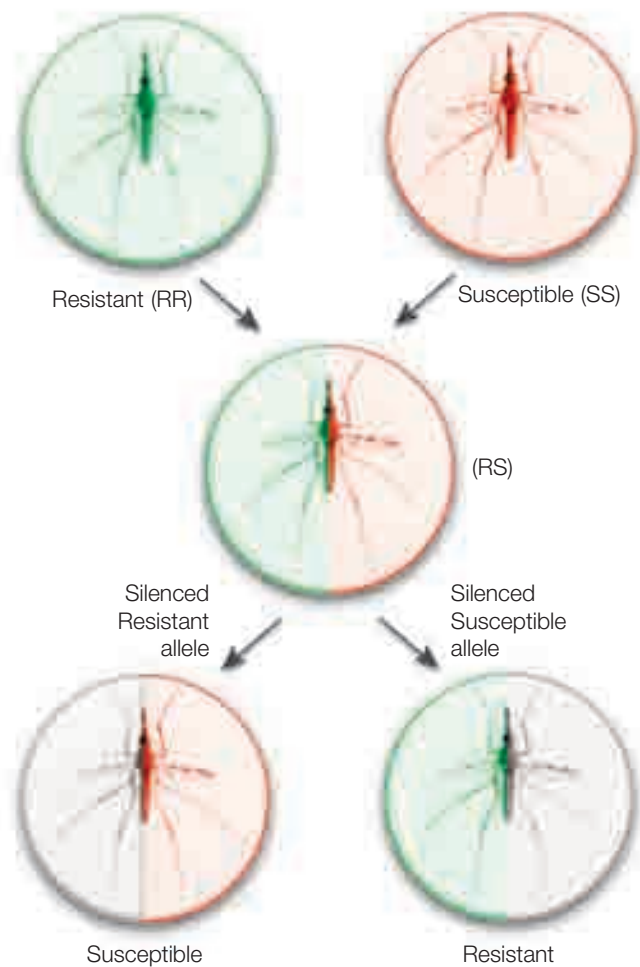
Although they use different interrogation techniques, geneticists often face the same task of whittling down a list of suspects – and in some cases, they too are dealing with killers. In this particular story, the killer’s identity is known from the start: malaria, a disease that claims almost one million human lives a year. The parasites that cause malaria are also well known to scientists. Belonging to the genus *Plasmodium*, they spend part of their life cycle inside humans or another mammalian host – depending on the exact species of parasite – and another part inside mosquitoes. And just as we suffer from malaria, mosquitoes infected with *Plasmodium* parasites do to. However, this is where the mystery begins: not all mosquitoes that are infected with malaria parasites fall ill. Instead, some are resistant to the parasite, meaning that their immune system is able to eliminate the disease-causing agent from their bodies. Why can some individual mosquitoes fight off the parasite better than others? Enter our lead investigators: Lars Steinmetz, joint head of the Genome Biology Unit at EMBL Heidelberg, and EMBL alumni Rui Wang-Sattler, now at the Helmholtz Zentrum in Munich, and Stephanie Blandin, now at the

Institut National de la Santé et de la Recherche Médicale (INSERM) in Strasbourg, France.

To get to the bottom of this enigmatic variation in individual resistance, Lars, Rui and Stephanie turned to *Anopheles gambiae* mosquitoes, the main carriers of the parasite that causes the most severe form of human malaria in Africa, and to *Plasmodium berghei*, which causes malaria in rodents. The scientists compared the complete DNA sequences, or genomes, of mosquitoes that are resistant to malaria with those of mosquitoes that are susceptible to the disease. This comparison suggested that a section of one of the mosquitoes’ chromosomes is linked to the insects’ ability to fend off *Plasmodium*. But as this section contains 975 genes, this still left our investigators plenty of suspects.

“To really understand this matter, we needed to go further,” says Rui. “But the question was how to go from this section of DNA to the single gene that makes mosquitoes resistant to malaria,” Stephanie adds. This is when Lars’ expertise was called upon.

Lars and his group had previously developed a way of doing just this in baker’s yeast. To go from a large DNA section, or genetic interval, to the gene that causes a particular trait, they took two yeast strains with different versions, or alleles, of a particular gene and crossed them, obtaining one strain of yeast with both alleles of that gene. They could then take these yeast cells, divide them into two sets and delete – or knockout – one allele in one set and the other allele in the other. As both sets of cells were genetically identical for that one gene, any differences in

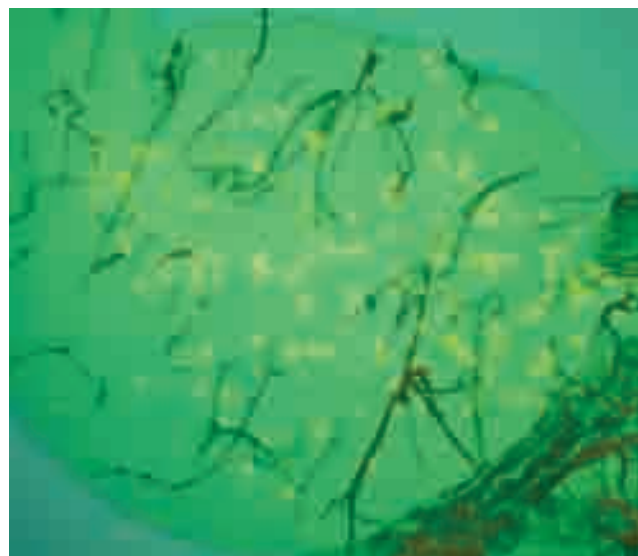


Reciprocal allele-specific RNAi entails obtaining individuals each carrying the 2 different alleles of interest, silencing one or other of those alleles, and comparing the results.

the traits of the two sets of cells must stem from the fact that they had different alleles of that gene. By doing this systematically for all the genes in the interval, Lars and colleagues were able to narrow down their suspects and determine exactly which genes played a role in a particular trait. “Unfortunately, we can delete genes in yeast, but not in mosquitoes,” Stephanie explains, “so we couldn’t just apply that same method to mosquitoes.”

Nevertheless, the fundamental problem was the same, so it made sense to try a similar approach – it just had to be one that would work in mosquitoes. Although you can’t knock genes out in mosquitoes, you can knock them down. Rather than altering an organism’s DNA sequence as they would in a gene knockout, scientists knock a gene down by using specially engineered RNA molecules to interfere with the gene’s expression, i.e. to hinder the production of the protein that the gene encodes. Lars, Stephanie and Rui took advantage of technological advances in this field of RNA interference and created a new method that essentially uses this knockdown approach in the same way that the previous method used gene deletions. This meant devising a way to knock down not just a specific gene, but a specific allele of that gene.

To test their new technique – called reciprocal allele-specific RNA interference – Stephanie and her colleagues at INSERM produced mosquitoes that each carried two different alleles of a gene called TEP1: one allele from the strain of malaria-resistant mosquitoes, and the other from a strain of susceptible mosquitoes. They then individually knocked down each of the alleles, creating mosquitoes whose only functional version of TEP1 was either the ‘resistance’ or the ‘susceptibility’ allele.

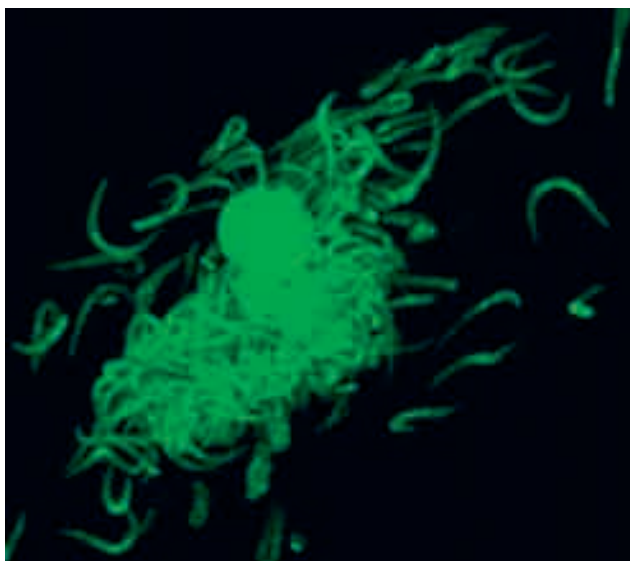


More parasites survived (fluorescent green dots) in the midgut of a mosquito with only the ‘susceptibility’ allele turned on (right) than in a genetically identical mosquito with only the ‘resistance’ allele turned on (left), which contained mainly dead parasites (black dots).

When they compared the ability of these otherwise identical mosquitoes to fight off *Plasmodium*, the scientists found that the immune systems of mosquitoes whose only functional TEPI allele came from the resistant strain were able to kill *Plasmodium* parasites, whereas in mosquitoes with only the 'susceptibility' allele, parasite survival was much higher. In short, silencing different alleles produced mosquitoes with different degrees of resistance to malaria, meaning that an individual mosquito's resistance to the *Plasmodium* parasite depends largely on which form(s) of this one gene it carries.

The team didn't choose TEPI at random. Previous work by Stephanie and others in the lab of former EMBL Director General Fotis Kafatos had shown that this gene encodes a protein that attaches itself to malaria parasites in the mosquito's gut, so that they are then eliminated by the mosquito's immune system. Nevertheless, Lars points out, "If you consider all the complexity that could have been going on, we were lucky – not only did our new technique work, but the biology fell into place too: we'd picked the right gene!"

Our investigators did what all those detectives in mystery stories dream of: they devised a way to efficiently narrow down the suspect list and identify the culprit. Crucially, scientists will now be able to do the same in many other situations, as this method is applicable to many different species and cell types, including human cell lines in culture. "Along with other advances like the ability to induce pluripotent stem cells, this kind of technique really opens up the door to understanding the function of individual alleles," Lars says, "and to do this in a single individual." In this respect, the new technique is complementary to pre-existing approaches, in



Approximately 12 days after infection, a *Plasmodium* oocyst is bursting open, releasing thousands of developing parasites (labeled green) into the mosquito's bloodstream.

which scientists had to scour genetic data from many individuals. "And you can apply it to any organism in which you can do RNA interference, which is a huge expansion of the method we developed for yeast," Lars emphasises.

In the meantime, as far as mosquitoes' resistance is concerned, Stephanie and colleagues in the malaria field have a culprit: TEPI. This culprit may, however, have accomplices, so the scientists would like to investigate the roles of other mosquito genes in increasing – or decreasing – an individual mosquito's ability to defeat the malaria parasite.

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They are also investigating human malaria, for which evidence seems to point to the same suspect. If TEPI enables mosquitoes to fight the parasites that cause human malaria as it does for the rodent malaria parasite, this could prove to be a boon to malaria eradication programmes, a large part of which focus on eliminating the mosquitoes that transmit the disease. "Knowing which allele or alleles confer resistance to malaria could help to make mosquito eradication programmes more effective," Stephanie posits. If scientists can identify the alleles responsible, and distinguish between resistant and susceptible mosquitoes in the wild, such programmes could be restricted to areas where they are most necessary: areas where most mosquitoes are susceptible, and therefore likely to carry the disease. Thus, in a plot twist that's reminiscent of the best detective stories, this winged enemy may, in fact, turn out to be an ally.

Blandin SA, Wang-Sattler R, Lamacchia M, Gagneur J, Lycett G, Ning Y, Levashina EA, Steinmetz LM (2009) Dissecting the genetic basis of resistance to malaria parasites in *Anopheles gambiae*. *Science* 326: 147-150