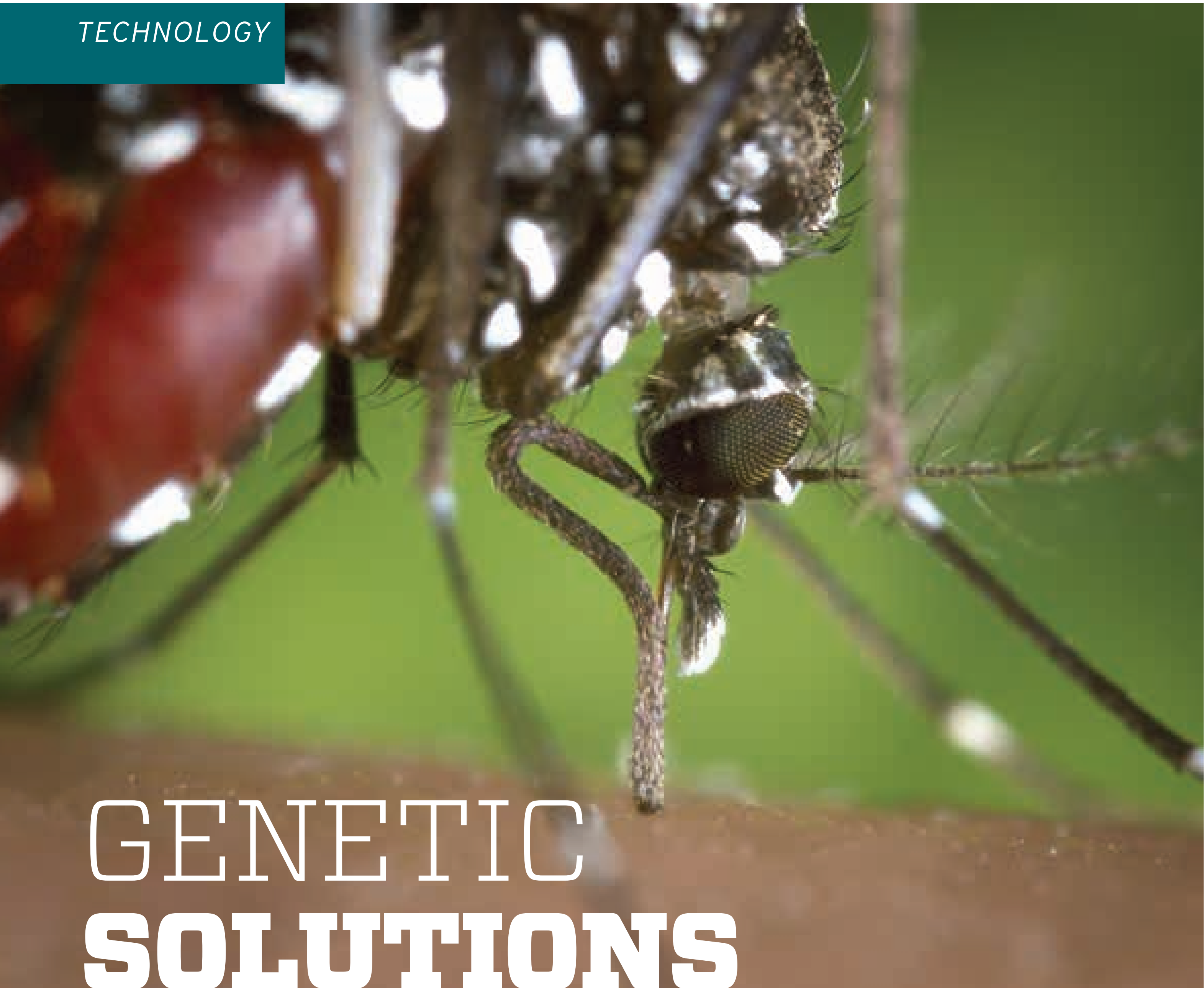

GENETIC SOLUTIONS

Transgenic mosquitoes are set free in Bahia to fight dengue fever

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For animals, mating is a way to perpetuate the species. In the case of *Aedes aegypti*, the mosquito that transmits dengue fever, this basic process is being inverted. By means of genetic manipulation, a population of lab-raised male mosquitoes was given a modified gene. This gene produces a particular protein, killing the offspring that result from mating with normal female mosquitoes. The release of such males could lead to the reduction of a significant number of these insects, reducing the spraying of insecticides necessary to eliminate them and thus reducing the incidence of dengue fever in human beings.

The first genetically modified mosquitoes were set free in December 2010, after Brazil's National Biosafety Technical Committee (CTNBio) granted approval. The transgenic strain of the *Aedes aegypti* mosquito, which was developed by the British company Oxford Insect Technologies (Oxitec), is to be released this month in Brazil's Juazeiro region, in the state of Bahia. This work will be done by biologist Margareth Capurro of the Biomedical Sciences Institute (ICB) of the University of São Paulo (USP) in partnership with Moscamed Brasil, a company headquartered in the town of Juazeiro.

Dengue fever is one of the world's leading public health problems, especially in tropical countries like Brazil. According to the World Health Organization (WHO), 50 million people are infected every year, resulting in 550 thousand hospital stays and 20 thousand deaths. Currently, the only sure way to control this disease is to eliminate the insect that transmits it – namely, the *Aedes aegypti* mosquito. The transgenic mosquitoes developed by Oxitec could become a possible answer for this problem. The males of the OX513A strain – the name given by Oxitec – are set free to mate with wild females. The offspring inherit a lethal protein and die when they are still in the larva, or pupa, stage. They are modified to survive when given tetracycline, an antibiotic, so that they can be reproduced in the lab. Without this antidote, which represses the synthesis of the lethal protein, no survivors would be left to be released into nature. The transgenic strain contains a fluorescent genetic marker that becomes visible when the mosquitoes are exposed to ultraviolet light. This ensures better quality control of the production of these mosquitoes and their dispersion in the field. The continuous release of these genetically modified insects in large quantities is expected, in time, to reduce the wild population of mosquitoes, in hopes of reducing the spread of the disease.

The elimination of female mosquitoes in the larva stage is a strategy that is currently being tested

The story of Capurro's work with these mosquitoes began with a chance encounter during a conference in 2007. At the conference, Capurro met British scientist Luke Alphey, from the University of Oxford, the founder of Oxitec. He proposed a test, in Brazil, of the transgenic mosquitoes he had developed. At the time, the Brazilian researcher did not believe the experiment would be feasible because of legal and bureaucratic obstacles. A short while later, she changed her mind and decided to conduct the experiment.

To this end, she requested permission from CTNBio, the entity responsible for the regulation of transgenic products in Brazil, to import the insects. "The permission to import the insects was granted on September 21, 2009," Capurro recalls. "One week later, we received an envelope with 5,000 eggs from Oxitec, at no cost." The researcher started to raise the modified *Aedes aegypti* in her insect lab at the ICB. However, it became necessary to raise the insects on a large scale to set them free and test them in the wild. In addition, the need arose to find an adequate site to do so; the site must be isolated and have a population of wild mosquitoes. Aldo Malavasi,

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Larvae of transgenic *Aedes* with fluorescent genetic marker

a former professor at USP and founder of Moscamed, proposed producing the transgenic mosquitoes in his biofactory, free of charge, and suggested that the mosquitoes be released in isolated towns in the region of Juazeiro. Capurro accepted the proposal, and the company and USP made an agreement to conduct the trial. "These tests will provide us with exposure, technical training and, at the same time, we will have an alternative to control these insects," Malavasi explained. Through cobalt radiation, the company produces sterile males of the medfly (*Ceratitis capitata*) and of the New World screwworm fly (*Cochliomyia hominivorax*). These flies were set free in the regions of Juazeiro and Petrolina, in the Vale do São Francisco river valley, state of Pernambuco, to compete with wild males for females (*see* Pesquisa FAPESP *issue 133*).

Masses of insects - "As we have been raising a mass of insects for some time already, we are going to conduct the experiment, with the help of the infra structure provided by Moscamed, to multiply the transgenic mosquitoes," Malavasi explains. "To this end, we have built a laboratory to work with the transgenic insects. Our lab has already been approved by the CTNBio."

Moscamed's team has chosen the appropriate places to conduct the field tests, which will take place in the semi-dry climate of the region that surrounds Juazeiro. "The tests will be conducted in five isolated sites that are separated from the town of Juazeiro by plantations, highways or unpopulated areas, and where there is a high incidence of the *Aedes aegypti*," says Capurro. "In the water storage tank of a single house we found 300 larvae of the mosquito." The researcher emphasizes another advantage of working in the chosen places. "Because of Moscamed's work in the region, the local population is already accustomed to insects being set free in the environment," she explains. "This is why the local inhabitants are not afraid of the mosquitoes that we are going to release." Capurro emphasizes that only the male mosquitoes, which do not bite and do not transmit the disease, will be set free. Now that the CTNBio has granted permission, the next step will be to conduct a dispersion study to assess the size of the local *Aedes aegypti* population. This is necessary to calculate how many transgenic mosquitoes will have to be set free. Capurro explains that 5 to 10 transgenic mosquitoes need to be released for each wild male. The researcher does not expect a significant reduction of the wild population after the first lab-produced mosquitoes are released. "We will have to set the transgenic mosquitoes free for at least two summer seasons to significantly reduce the existing population," she says.

There are good reasons to expect that this experiment will be successful in Brazil, especially based on the results of trials in other places where Oxitec's transgenic mosquitoes have been released. Tests conducted last year in the Cayman Islands, which involved the release of 3 million genetically modified mosquitoes, showed an 80% reduction in the wild mosquito population. Similar results were obtained in Malaysia. These successful results have led other countries to use the British company's transgenic mosquitoes in trials as well. Oxitec claims on its web site that France, India, Singapore, Thailand, the United States, and Vietnam have already approved the importation of these insects.

The path chosen by Oxitec to develop genetically modified mosquitoes is just one of many that are being taken around the world. An example of such work was cited in early 2010 in an article in *Proceedings of the National Academy of Sciences* (*PNAS*). The article was written by a team of international scientists, including Brazilian biologist Osvaldo Marinotti, a former researcher of USP and currently a professor at the

THE PROJECT

Promoting mortality in Aedes aegypti *infected by the dengue fever virus – nº 08/10254-1*

TYPE
Ordinary Research Grant

COORDINATOR
Margareth Capurro – USP

INVESTIMENT
R$ 347,263.34 (FAPESP)

University of California, Irvine (UCI). Instead of producing *Aedes aegypti* males that leave a mortal gene for their descendants, they developed a transgenic female that is unable to fly.

The males remain – The muscles that support the mosquitoes' flying ability are stronger in females. Nobody knows exactly why this is so, but it is believed that this occurs because the female mosquitoes draw blood from other animals, including human beings, and carry the eggs. The females carry more weight and therefore need to have stronger wings. On a genetic level, the explanation for this difference is that the muscles that drive the flight of the female depend on a protein, called actin-4, which is coded (produced) by a gene that is much more active in the females than in the males. The males have the same gene, but its expression is milder.

The males carry another kind of acting, which also acts on the flight muscles. Scientists, aware of this difference, created a gene that produces a toxic substance for actin-4, preventing this protein, which is found in the flight muscles, from performing its function. This modification results in females that develop normally until the larva phase, but are unable to fly once they reach the adult phase. Subsequently, they are unable to leave the water, and they die without reproducing or feeding on blood. This way, they can neither have offspring nor transmit dengue fever. The transgenic males are able to fly, but this is not a problem because the males only feed on nectar and plant juices. They do, however, remain sexually active and mate with wild females, thus transmitting the gene that prevents female offspring from flying.

Professor Capurro has also studied other strains of genetically modified mosquitoes. One strain involves the mosquito that transmits malaria, and another strain involves the mosquito that transmits dengue fever. In the first case, Capurro takes a gene from ticks responsible for the production of an antimicrobial peptide – a protein fragment – called microplusin. "This gene is modified so that it can be inserted into a mosquito," she explains. "Once the gene is inside the insect's genome, it produces the microplusin, which eliminates the *Plasmodium protozoa*, a single-sell microorganism that is the agent of malaria, before it is transmitted to a human being."

In the case of the other strain of modified dengue fever mosquito, a project funded by FAPESP, Capurro's genetic manipulation leads to the disease-carrier's death via apoptosis (cell death) after feeding on blood "The existence of the dengue fever virus activates the protein that induces the apoptosis, thus causing the cell death of all the tissues in the infected mosquitoes, and leads to the female's death. As a result, the viral transmission is 100% blocked," Capurro explains. Several gene introduction techniques are currently being tested with the aim of releasing new transgenic mosquitoes into the wild. One of these techniques is referred to as Medeia, because it induces, by means of biotechnological systems, the death of the non-transgenic offspring resulting from the mating of the normal females with the genetically manipulated males. "Only the offspring that carry the transgenic gene survive. The introduction of the transgenic gene into a population of mosquitoes through the Medeia technique takes only eight generations." An additional benefit will be available if time and research studies prove that these strategies of using genetic engineering to create transgenic mosquitoes are efficient. This form of control will reduce the need to use insecticides and larvicides, which, while less expensive in the short term, result in insects becoming resistant. For this reason, the use of transgenic and sterile mosquitoes seems to be a promising option for the future. ■

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Aedes: the male, on the left, is harmless; the female transmits dengue fever

The use of transgenic mosquitoes will reduce the use of insecticides. Consequently, the insects do not acquire resistance to these poisons