Advancing Genetic Control of Destructive Fruit Flies

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Scientia

ADVANCING GENETIC CONTROL OF DESTRUCTIVE FRUIT FLIES

Fruit flies cause significant annual damage to fruit crops globally by laying their eggs into healthy, living fruit tissue. The difficulty in predicting the attacks and controlling the flies before it is too late leads farmers to spray pesticides that can have damaging consequences for surrounding ecosystems. **Dr Nicholas M. Teets** and his team from the University of Kentucky's Department of Entomology aim to eliminate the need for pesticides in the battle against these insect pests, through the development of sterile insects that are easy to rear and release en masse.

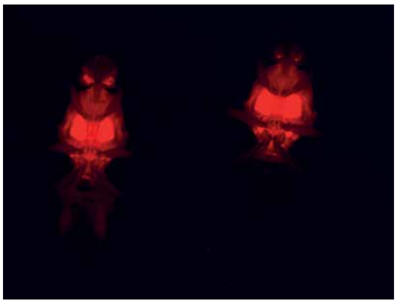
Controlling Destructive Flies

Many of us will be familiar with common fruit flies, belonging to the genus *Drosophila* – those tiny flies that turn up seemingly the moment you leave a piece of fruit uneaten for too long. But some *Drosophila* species, along with their distant cousins, the 'true' fruit flies – belonging to the insect family Tephritidae – are far worse than a simple household nuisance. These flies' fondness for laying eggs in living fruit before it is ready for harvesting mean they cause significant agricultural damage. In extreme infestations the fly larvae can cause millions of dollars' worth of damage, posing a threat to food security.

Controlling fruit flies is notoriously difficult. By the time a fruit fly infestation is detected, the damage is already done, so farmers resort to spraying pesticides in advance. However, pesticides traditionally used to control destructive insects also have a harmful effect on beneficial insects, such as pollinators, and can disrupt fragile ecosystems on land and in waterways.

A solution to this problem is to sterilise lab-reared male fruit flies using high doses of radiation before releasing them – the 'Sterile Insect Technique' (SIT). SIT is also being developed as a method of controlling insects that carry diseases, such as mosquitoes. The technique can effectively reduce pest numbers by preventing the females mated by sterile males from producing fertilised eggs. Unfertilised eggs simply fail to develop. Because the female fruit flies often only mate once, the more females the sterile males can attract, the more effective the population control will be. As it is only the fly larvae that cause damage to fruit, the released male fruit flies do not cause any damage to crops themselves, and simply die naturally or become food sources for other animals.

However, the radiation traditionally used to sterilise male fruit flies also causes unintended damage to other parts of the fly. The males rely on complex courtship displays, fighting with other males and physically demanding movements to attract female fruit flies and secure mating opportunities. Males sterilised with



radiation experience reduced muscle function, weaker courtship displays, and shortened lifespans, making them less effective at competing for mates than their wild counterparts. To offset this, scientists previously mass reared enormous numbers of males to be sterilised and released, effectively saturating the population with sterilised males to compensate for their decreased fitness. This solution

Dead eggs

Hatched eggs and larvae

is not ideal though, due to the cost of mass rearing, radiation sterilisation, transport and release of large numbers of insects.

Dr Nicholas Teets, previously at the University of Florida and now at University of Kentucky, and his collaborators have been investigating methods of improving the fitness of sterilised males by increasing the quantities of protective antioxidants that the flies produce within their cells. They achieved this by adding extra copies of the genes involved in the production of these antioxidants. The result is an improved lineage of fruit flies that resist the damage caused by radiation, and thus outperform their wild counterparts. As this lineage of fruit flies still requires males to be sterilised using high doses of radiation, Dr Teets and his team are continuing their investigations into how insects respond to physiological stressors, including radiation and extreme temperatures. Additionally, Dr Teets is now focusing on a genetically modified lineage of fruit flies that contain a 'genetic switch' for sterility, eliminating the need for radiation entirely.

Physiological Stressors

The radiation used to sterilise male fruit flies is not unlike that used to destroy cancer cells in humans; the target cells are destroyed, but some healthy cells are damaged in the process. In humans, these non-target cells have time to heal and regain full function, but fruit flies have relatively short lives, so the damage persists for the duration of their lives and affects their ability to find and secure mating opportunities. The negative effect of the radiation is similar to effects from other environmental stressors, offering an opportunity to identify and exploit the natural defences insects have to these damaging factors.

Dr Teets previously demonstrated that increasing antioxidant production in fruit flies protected males from the damaging effects of sterilising radiation. Creating lineages of fruit flies that produce extra antioxidants involves inserting extra copies of the genes that produce antioxidants in their cells. These inserted genes are called transgenes.

When compared with sterilised wild counterparts, the improved transgenic lineages – those possessing the additional genes – had reduced radiation damage, better muscle control, and secured more matings with female fruit flies. 'The increased antioxidants protected the flies from oxidative damage to lipids, which is one of the main ways that radiation harms an organism,' explains Dr Teets.

In addition to vastly improving the efficacy of SIT control for the fruit fly, this research provides new strategies for improving classic SIT in a number of insect species, and there are other naturally occurring antioxidants that could be exploited to further protect flies from radiation damage. 'While this work is exciting, new strategies for genetic sterilisation will likely remove the need for radiation treatment in the future,' Dr Teets explains, 'but the process of mass rearing, sterilising, shipping, and releasing insects for Sterile Insect Technique is highly stressful, so we are developing and testing strategies to improve this process.'

A Genetic Switch

Following the successful creation of these transgenic lineages, Dr Teets is now shifting his focus to fruit flies with transgenes that exert control over the fertility of the insects without the need for radiation. These lineages are sterile by default, unless exposed to an antibiotic which is not found in natural environments. This ensures that the eggs produced through

matings with the released transgenic flies never survive in the wild, effectively lowering the population.

Within the controlled environment in the laboratory, the 'genetic switch' provided by the transgene can be turned off by exposing the developing flies to the antibiotic. This temporarily pauses the transgene's effect of impeding survival, allowing the transgenic flies to be mass-reared for release. Subsequently, when these mass-reared transgenic flies mate in the wild, their offspring will not be exposed to the antibiotic, and the genetic switch will turn on again, impeding their development and survival.

Previous large-scale field trials have tested the effectiveness of transgenic sterilisation using other pest insect species, such as the mosquito that transmits yellow-fever virus, Zika virus, and Dengue virus. The results have been exceedingly promising, with some populations having been reduced by up to 98%. Additionally, because the insects no longer need to be exposed to radiation for sterilisation, many of the negative effects on mating ability and fitness are reduced or completely removed.

However, releasing transgenic insects is not routinely allowed in the USA, and obtaining the necessary permits requires extensive testing to demonstrate the safety of the technology and mitigate the potential risks associated with release. 'Thus, we are collecting data to identify potential risks associated with genetic sterilisation,' says Dr Teets. 'Specifically, our two big questions are: Is genetic sterilisation likely to be effective in the field, and thus worth the financial investment and regulatory effort? And if there are conditions that result in failure of genetic sterilisation, what are the relative risks?'

Predicting and Mitigating Risks

One of the key risk factors involved in releasing transgenic insects into the environment is 'incomplete penetrance' of the transgene. In the case of sterile transgenes, this means some of the offspring survive even in the absence of the reversing antibiotic. To identify the potential scenarios under which such an event may occur, Dr Teets and his team have been rigorously testing transgenic sterile fly lineages within their lab.

The researchers are currently investigating whether variation in the genetic make-up of fruit flies could lead to the transgene being less effective or failing entirely in some offspring. Because the transgenic sterile flies will be mating with wild fruit flies, the naturally variable genes of the wild flies may interact with the transgene in unforeseen ways. Through repeated controlled mating events between transgenic and wild fruit flies in the lab, the team has screened thousands of developing fly eggs to assess survival rates. Their initial results are promising, demonstrating an offspring survival rate of less than 2% across all mating events.

The transgenic fruit flies and their offspring will be exposed to natural variations in environmental conditions in the wild. Thus, Dr Teets and his team are replicating ecologically relevant conditions in their experiments, such as temperature, water availability, and food availability and quality, to test whether the flies' physiological responses to these factors affect the activity of the transgene. This work continues from their investigations into the production of protective molecules by fruit flies when exposed to environmental stressors.

Finally, the team is researching whether the fruit flies are able to develop resistance to transgenic male releases. Similar to insecticide resistance, the continual release of sterile males creates a strong selection pressure for flies that are able to survive following sterile releases. This resistance can develop through several routes, including the ability to avoid mating with transgenic males and preferentially mating with wild males, or the ability to withstand the toxic effects of the transgene. By simulating continual releases in large cages in the laboratory, Dr Teets and his team are testing the extent to which natural fly populations can evolve resistance to this technology.

The Future of Transgenic SIT

Dr Teets and his team have demonstrated the viability of transgenic lineages in advancing SIT control of fruit fly populations. Their current research aims to predict and mitigate the risks associated with releasing transgenic flies into the environment, and inform policies surrounding the licensing for these technologies. Their promising research has the potential to prevent significant destruction to fruit crops by these insect pests, without causing additional or persisting environmental damage, and could be extended to other pest species in the future.

Meet the researcher

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Dr Nicholas Teets earned his PhD in Entomology from the Ohio State University, continuing on to postdoctoral research in insect physiology and molecular biology technologies. Dr Teets is currently an Assistant Professor in the Department of Entomology at the University of Kentucky, where his research interests include developing and testing transgenic control technologies for agricultural pest insects, and investigating thermal physiology and environmental stress tolerance in a range of insect species. Dr Teets also devotes his time to teaching future entomologists at the University of Kentucky in subjects such as insect biology and climate science. Dr Teets and his advisees within his laboratory have successfully secured grants to support their past and ongoing research, from a range of organisations including the USDA. He has also achieved many awards for his research, including the Entomological Society of America Early Career Professional Research Award. In addition to his research and teaching activities at University of Kentucky, Dr Teets has also been an instructor for the Living Arts and Sciences Science Explorers Program, an after-school STEM program for underprivileged schools.

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