Some classes of metabolites produce characteristic fragments or neutral losses in their MS/MS spectra that can be used as signatures for unique chemical functional groups. For example, the MS/MS spectra of phosphatidylcholines are characterized by a fragment at *m*/*z* 184.07. For instances in which the MS/MS data uploaded by a user do not match any compound in the database, the new version of the METLIN database will search the MS/MS data for characteristic fragments that can be used for molecular classification. The search can also be performed manually by accessing the 'fragment search' or 'neutral loss search' options. These tools provide a new mechanism by which unknown metabolites can be chemically classified, and they take advantage of the large amount of MS/MS data in the library.

To highlight the new database functionalities, we performed MS/MS on select peaks from the metabolite extracts of *E. coli* and human serum. These data were uploaded to the METLIN database, and fragment matching was performed using the automated feature described above. Representative examples of metabolites identified on the basis of the mass spectrometry and MS/MS data using this method are shown in **Supplementary Figures 28–32**. The compounds identified ranged from lipids to smaller, polar metabolites. Additionally, representative examples of unknown compounds that were classified by characteristic fragments are also shown.

With the combination of the METLIN functionalities described here and the increasing speed of QTOF instrumentation for performing MS/MS, there is the potential to reduce the untargeted metabolomic workflow to just two steps (**Fig. 1**). Using high–scan-speed QTOF instruments, mass spectrometry and MS/MS data can be acquired simultaneously in a single run. Quantitative information can then be extracted from the data using the bioinformatic software XCMS Online, and metabolites can be identified simultaneously by matching the MS/MS data with MS/MS data in the METLIN database in an automated fashion, an approach that is self-directed or autonomous in nature.

With this truncated workflow, the time needed to perform untargeted profiling and the subsequent metabolite identification may be reduced to minutes or hours as compared to the days or weeks needed with the traditional workflow. The results shown here from automated MS/MS matching highlight the applicability of the method for performing high-throughput, untargeted metabolomics using this type of accelerated workflow. Moreover, we have shown that the coverage of the METLIN database enables the characterization and identification of thousands of naturally occurring metabolites in biological samples. Thus, the new METLIN database has the potential to expedite the workflow for untargeted metabolomics as more investigators obtain mass spectrometry instrumentation that can produce high-quality MS/MS data with increasing speed and sensitivity.

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Ralf Tautenhahn[1,2], Kevin Cho[1,2], Winnie Uritboonthai[1,2], Zhengjiang Zhu[1,2], Gary J Patti[3–5] & Gary Siuzdak[1,2]

[1]Department of Chemistry, Center for Metabolomics, The Scripps Research Institute, La Jolla, California, USA. [2]Department of Molecular Biology, The Scripps Research Institute, La Jolla, California, USA. [3]Department of Chemistry, Washington University School of Medicine, St. Louis, Missouri, USA. [4]Department of Genetics, Washington University School of Medicine, St. Louis, Missouri, USA. [5]Department of Medicine, Washington University School of Medicine, St. Louis, Missouri, USA.
e-mail: gjpattij@wustl.edu or siuzdak@scripps.edu

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Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes

To the Editor:

Our paper published last year described the results of preliminary release experiments showing that engineered sterile male mosquitoes could mate with females in a wild population in the Cayman Islands[1]. This trial was supported by simple simulation models indicating that sustained release of sufficient numbers of such males should substantially suppress a target population within a few weeks or months[2–4]. In the following letter, we describe a field release experiment testing this proposition.

The sterile insect technique is an environmentally friendly, species-specific method of pest control that is used to successfully control several agricultural pest insects[5]. Large numbers of sterile insects are released to mate with their wild counterparts and thereby reduce their reproductive potential. However, despite its attractive features, this technique is not in operational use against mosquitoes, in part because of damaging effects of sterilizing doses of radiation on the released mosquitoes[6–8]. Following a similar principle, we have proposed that engineered males carrying a dominant lethal transgene could be released to mate with wild females; the resulting progeny would die as a result of the lethal effect of the transgene. We named this system RIDL (release of insects carrying a

dominant lethal gene)[9,10]. The *Aedes aegypti* RIDL strain, OX513A, has a single transgenic sequence encoding a red fluorescent marker and tetracycline-repressible late-acting dominant lethality[3].

Approximately 3.3 million engineered OX513A males (male mosquitoes do not bite) were released in a 23-week period in 2010 in a field site in Grand Cayman, a British Overseas Territory in the Caribbean. The preliminary release experiments showed that OX513A males could mate with wild females[1] and, together with simulation models[3], indicated a minimum release rate of 3,150 males per hectare (ha) per week to induce population collapse in the absence of immigration. We aimed for twin targets of the release of >4,000 males per ha per week and a 10:1 ratio of sterile to wild males in the field.

Releasing large numbers of engineered sterile males into a wild mosquito population should have several measurable effects on the wild population, including, in temporal order, an increase of the male-to-female ratio, females mating with engineered males and suppression of the target population. The mosquito population—OX513A and wild—was monitored using adult traps and ovitraps, which mimic natural oviposition sites (**Supplementary Notes**). Adult traps were also used to monitor the numbers of OX513A males in the field, as an input of males should change the sex ratio of the population. Larvae hatched from field-collected eggs from ovitraps were screened for fluorescence to determine paternity (fluorescent larvae had an OX513A father, and nonfluorescent larvae had a wild-type father). These monitoring methods were used to evaluate the field trial endpoints: sterile-to-wild male ratio ≥10:1, fluorescence ratio ≥50% and statistically significant population suppression relative to control site(s).

Initial releases of OX513A males across a 55-ha area (areas A, B and C, period 1; **Fig. 1**) and then across a reduced 32-ha area (areas A and B, period 2) were restricted by production difficulties to an average of 1,400 (95% CI 990–1,800) males per ha per week and 3,900 (95% CI 2,600–5,300) males per ha per week, respectively (**Fig. 1c**). These release rates also did not achieve the target OX513A-to-wild male ratios, reaching a 1.9:1 ratio (95% bootstrap CI 1.2:1 to 2.8:1, n = 967) in period 1 and a 4.8:1 OX513A-to-wild ratio (95% bootstrap CI 2.6:1 to 7.9:1, n = 1,994) in period 2 (**Fig. 2a**). We therefore further reduced the release area to 16 ha (area A, period 3). In this final period, we released ~14,000 (95% CI 13,700–14,500) males per ha per week for the first 3 weeks and later reduced this to ~7,700 (95% CI 6,900–8,500) adult males per ha per week, which was supplemented with ~4,900 (95% CI 3,800–6,000) adults eclosing from ~5,600 (95% CI 4,500–6,800) pupae deployed in field. This gave a mean OX513A-to-wild male ratio in the release area of 25.2:1 (95% bootstrap CI 17.8:1 to 34.9:1, n = 3,155) in the first 4 weeks of period 3. We also found an increase in the proportion of the field-collected eggs carrying the fluorescent marker, with a peak of 88% (**Fig. 2b**), implying that the majority of wild females were mating with OX513A males.

We used the ovitrap index—the proportion of ovitraps in each area with one or more eggs after 1 week—as our primary measure of population density (**Fig. 2c**). Until early in period 3, the weekly ovitrap indices for area A were very similar to those in areas C (likelihood ratio (LR) P = 0.63) and B (LR P = 0.13) but were significantly lower (~40%) than those in an external untreated control site (LR P = 0.026). After that time point, the index in area A was highly significantly lower than those in all other areas (P < 0.0001 for each pairwise comparison).

Over the last 7 weeks of the release period, the mean ovitrap index in the untreated areas was 49% (95% CI 43–55%; **Fig. 2c**). In contrast, the mean ovitrap index in area A was 10% (95% CI 7–14%), which is an 80% reduction relative to the untreated areas, indicating strong population suppression in the treated area during this period. The degree of suppression that is possible in such a trial is limited by immigration of wild females from surrounding areas, such as area B, as well as, potentially, from eggs laid at an earlier period.

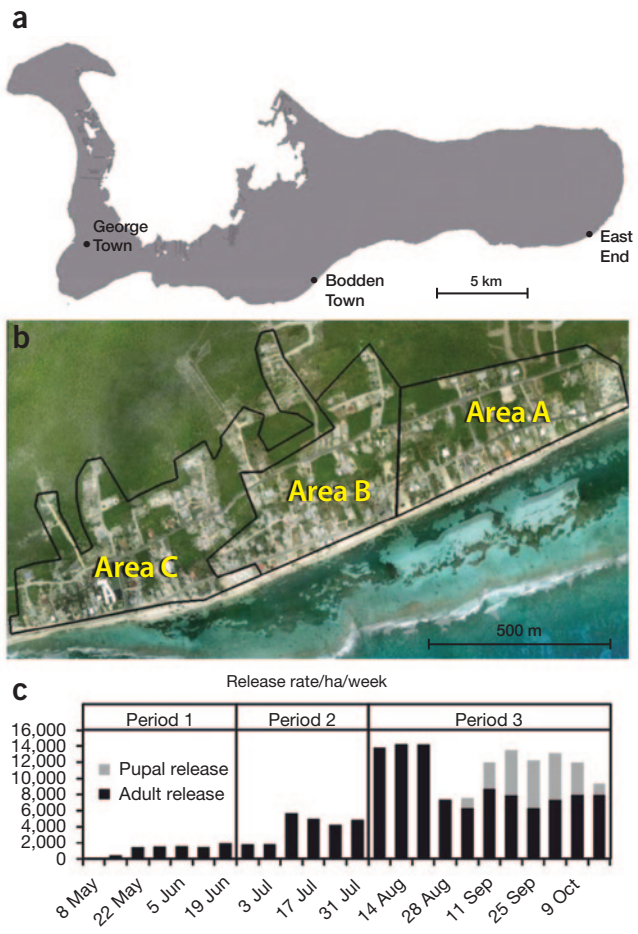

Figure 1 Field site and mosquito releases in 2010. (**a**) Map of Grand Cayman showing the locations of East End and Bodden Town, which were the treatment and external control sites, respectively, and the capital, George Town. (**b**) Aerial photograph of East End showing areas A, B and C. (**c**) Weekly numbers of adult males released per hectare from direct adult release (solid bars) and emerging from pupal deployment (shaded bars). Releases occurred 3 times per week; release numbers shown for each week are the sum of these three releases. During period 1, all treatment areas received treatment, during period 2, areas A and B were treated, and during period 3, only area A was treated.

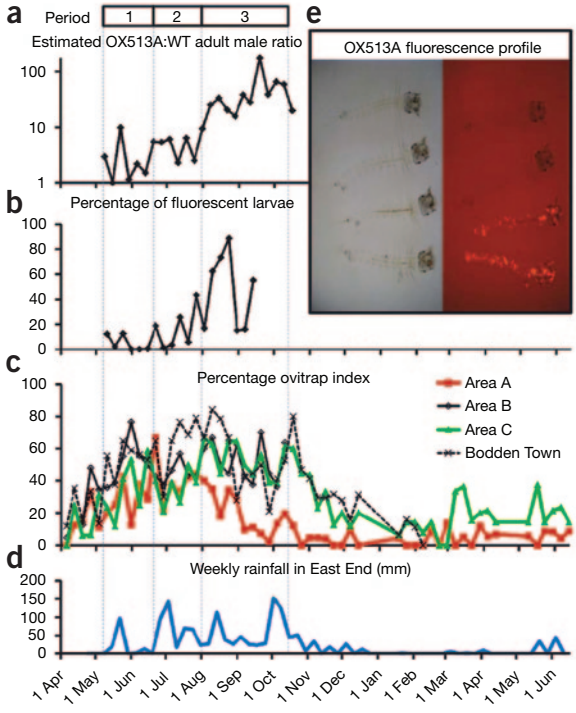

Figure 2 Effect of periodic release of OX513A male mosquitoes on a wild population. Release of engineered males should lead to an increase in the male-to-female ratio of the field population, deposition of hybrid eggs after mating of released males with wild females and, finally, suppression of the target population if sufficient sterility or mortality is induced. (**a**) The ratio of OX513A to wild-type (WT) adult males estimated from the sex ratio of adults caught in BG-Sentinel traps. Treatment periods 1–3 are indicated (see also **Fig. 1**). Increasing the input rate (males per ha per week) and, later, declining wild population numbers resulted in an increase in this ratio over time. (**b**) Percentage of larvae recovered from ovitraps in treated areas with the RIDL transgene as detected by fluorescence. This percentage is plotted only into September 2010, as the number of eggs collected after that time became too low to act as a reliable measure because of suppression of the target population. (**c**) Ovitrap index in East End (areas A–C) and the external untreated control site, Bodden Town. Area C received low-level treatment early in the trial (period 1) for a duration and level that were not considered effective. Because of its close proximity and ecology to area A, area C provided a largely 'untreated' internal control that was highly comparable to area A. From the beginning of August, the ovitrap index in area A declined relative to all control areas. All populations declined later, which is typical of seasonal variation driven by rainfall. However, even after some rainfall from March 2011, by June, the population in area A was still below that in control area C. (**d**) Weekly rainfall in East End in 2010 (mm). (**e**) Red fluorescence of OX513A larvae. Four larvae are shown, of which the upper two are wild-type and the lower two are OX513A. The larvae were imaged under normal illumination (left) and epifluorescence (right). Red fluorescence of the OX513A larvae is caused by expression of DsRed2 (ref. 3).

The time delay inherent in sterile-male–release methods (the population reduction occurs in the progeny of released insects), combined with female monogamy, means that we were releasing a sufficient number of males of sufficient quality to achieve suppression at least 4–6 weeks before the point at which population suppression was detected (5 August 2010; **Fig. 2c**). This indicates that suppression was achieved by releasing approximately 3,500 males per ha per week, which gave a male-to-female ratio in adult traps of 3:1, corresponding to an overflooding ratio of ~5:1.

This trial was conducted in an area with no conventional control targeting *A. aegypti* and a relatively high initial population density; larviciding and removal of breeding sites within an integrated program would greatly reduce the number of sterile mosquitoes required. The positive outcome and successful demonstration of population suppression is encouraging for genetic control strategies in general and, in particular, validates the potential of OX513A RIDL mosquitoes for population suppression.

Note: Supplementary information is available at http://www.nature.com/doifinder/10.1038/nbt.2350.

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Angela F Harris[1,2], Andrew R McKemey[3], Derric Nimmo[3], Zoe Curtis[3], Isaac Black[3,4], Siân A Morgan[3], Marco Neira Oviedo[3], Renaud Lacroix[3], Neil Naish[3], Neil I Morrison[3], Amandine Collado[3,4], Jessica Stevenson[3], Sarah Scaife[3], Tarig Dafa'alla[3], Guoliang Fu[3,4], Caroline Phillips[3], Andrea Miles[3], Norzahira Raduan[3,5], Nick Kelly[1], Camilla Beech[3], Christl A Donnelly[6], William D Petrie[1] & Luke Alphey[3,4]

[1]Mosquito Research and Control Unit, Grand Cayman, Cayman Islands. [2]Vector Group, Liverpool School of Tropical Medicine, Liverpool, UK. [3]Oxitec Limited, Oxford, UK. [4]Department of Zoology, University of Oxford, Oxford, UK. [5]Medical Entomology Unit, Infectious Diseases Research Centre, Institute for Medical Research, Kuala Lumpur, Malaysia. [6]Medical Research Council Centre for Outbreak Analysis and Modelling, Department of Infectious Disease Epidemiology, Faculty of Medicine, Imperial College London, St Mary's Campus, London, UK. e-mail: luke.alphey@oxitec.com

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