Gene Drives: Dynamics and Regulatory Matters-A Report from the Workshop "Evaluation of Spatial and Temporal Control of Gene Drives," April 4–5, 2019, Vienna

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BioEssays

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Gene Drives are regarded as future tools with a high potential for population control. Due to their inherent ability to overcome the rules of Mendelian inheritance, gene drives (GD) may spread genes rapidly through populations of sexually reproducing organisms. A release of organisms carrying a GD would constitute a paradigm shift in the handling of genetically modified organisms because gene drive organisms (GDO) are designed to drive their transgenes into wild populations and thereby increase the number of GDOs. The rapid development in this field and its focus on wild populations demand a prospective risk assessment with a focus on exposure related aspects. Presently, it is unclear how adequate risk management could be guaranteed to limit the spread of GDs in time and space, in order to avoid potential adverse effects in socio-ecological systems.

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The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/bies.201900151

DOI: 10.1002/bies.201900151

The recent workshop on the "Evaluation of Spatial and Temporal Control of Gene Drives" hosted by the Institute of Safety/Security and Risk Sciences (ISR) in Vienna aimed at gaining some insight into the potential population dynamic behavior of GDs and appropriate measures of control. Scientists from France, Germany, England, and the USA discussed both topics in this meeting on April 4–5, 2019. This article summarizes results of the workshop.

1. Population Biology and Control Options

Nick Barton emphasized that while GDs are based on simple technology, developing ways to use them safely is far more complex and uncertain. In nature, complex mechanisms have evolved to suppress viruses and transposons, including elaborate epigenetic mechanisms that suppress selfish genes. Although we have evolved alongside a variety of natural drives, application of artificial drives may be dangerous. Most eukaryote genomes carry a substantial burden from defunct transposons, and devote substantial genetic resources to combating selfish elements; those elements may well be an important cause of extinction. Barton raised the question whether the application of synthetic drives could ever be a local, rather than global, decision, given the high risk of unintended dispersal: GDs may be able to jump to nontarget species, just as insecticide resistance can move between species (e.g., from *Anopheles gambiae* to *A. coluzzii*).[1] If the driver reduces fitness, selection favors resistance and a GD may quickly become attenuated. Thus, a "parliament of genes" may stabilize Mendelian rules, despite the prevalence of selfish genes.[2] Although modeling may give valuable insight into population dynamics—as Barton showed for a Wolbachia release in Australia[3]—the predictions of a model will be correct only under the circumstances and variables used. Models may seriously mislead if applied when key factors are unknown, or too numerous to be taken into account.

Philipp W. Messer took up the tendency of resistance development against GDs. For clustered regularly interspaced short palindromic repeats (CRISPR)-drives in particular, resistance represents a serious obstacle that should be most prominent in suppression-drive applications. He explained that the evolution of resistance is still difficult to predict as a few mismatches can be enough to destroy a target sequence for homing endonuclease-based GDs. Here, the multiplexing-approach may help lower resistance rates, but should not be overestimated, as first results indicate: Champer et al.[4] have shown that multiplexing is not actually multiplicative and resistance could not be suppressed as effectively as initially thought. With regard to the genetic status of the population, the spread of resistance alleles can also represent a genomic alteration induced by the drive. Those individuals are not necessarily equivalent to the wild-type but rather, constitute a fraction of the population that influences spread of the drive. In an individual-based simulation aimed to model the spread of a suppression drive against an invasive rodent population on an island, Messer illustrated the importance of conducting spatially realistic simulations. The dynamics observed in such spatial models can be fundamentally different from the models of "well-mixed" populations currently used in many studies, revealing new phenomena such as "chasing dynamics" where wild-type individuals can quickly recolonize empty areas and the drive never succeeds. These and other complexities that arise in realistic models complicate predictions on the fate of concerned populations.

Florence Débarre brought a deeper investigation of potential control options for GDs into the discussion. She asked whether the release of reversal drives could stop a drive that was either accidentally released or had to be neutralized due to unanticipated effects. Débarre presented mathematical models of the potential population dynamics, first in well-mixed population, and second, considering a population over a spatially continuous environment.[5] The model referred to a reversal drive of the so-called "CATCHA"-type. CATCHA, a "Cas9-Triggered Chain Ablation" of Cas9 is based on a gRNA that cleaves the Cas9 gene and inserts its own gRNA-coding sequence against Cas9.[6] The model focusses on allele frequencies over time. Deterministic versions indicate that the drive can only be stopped if it has an introduction threshold, i.e., if there is a frequency above which the drive has to be introduced in a wild-type population in order to spread. In the absence of such a threshold, the introduction of a reversal drive leads to rock-paper-scissors like dynamics, the frequencies of the original drive and the reversal drive oscillating over time. These oscillations are of large amplitude, however, so that either construct can be lost by chance, often leading to the recovery of the wild-type population in stochastic versions of the nonspatial model.

2. Applications of Gene Drives

Marc Schetelig, in his contribution to agricultural applications for GDs, also referred to options for control as a dependence of a GD on a second product. Sensitizing or optogenetic drives may be used in this regard where optogenetic genes, e.g., induce susceptibility to light beams. According to Schetelig, effective control of pests for a specific time should be the goal of most GD applications. In contrast, suppression drives with the aim of species elimination and systems that are prone to resistance should be avoided. Also, resistance races with chains of GDs against the other drives might not be reasonable in terms of economic and control efficiency. Concerning regulation Schetelig noted that a case-by-case evaluation would represent the most appropriate solution because the term "gene drive" represents a multitude of possible strategies—technically, ethically, and in terms of applicability. For each system, the state-of-the-art tools should be compared for their suitability and compared in a risk assessment to new tools. This is different from the current risk assessment in Germany and many other countries, which evaluates only the novel tool without comparing it to possibly inferior "state-of-the-art" control options.

Nikolai Windbichler elucidated current developments in the development of transmission-blocking GDs for malaria control. From this viewpoint as a potential applicator, four challenges need to be addressed to bring replacement drives to the field. First, how can antimalarial effector molecules, characterized under laboratory conditions and never exposed to heterogeneous and genetically diverse *Plasmodium* parasites, been tested? Second, what is the long-term persistence of genetic constructs in the target population?[7] Third, which issues arise from the molecular complexity of the GD constructs that currently envision incorporation of multiple large transgenes? For a CRISPR-drive in particular, the compound fitness cost of expressing Cas9 and a cargo gene may reduce the efficiency and persistence of the drive. Lastly, regulatory complexity poses an additional challenge for GD releases. For addressing these challenges, a strategy for the design, phased testing and roll-out of transmission-blocking GDs was presented: The construction of minimal, genetically simple GD components out of mosquito genes as well as the molecular separation of the drive and effector functions allows the testing of these in the absence of a full GD.[8] Therefore, a cargo gene could be driven non-autonomously along with the drive component, where the cargo interferes with pathogen transmission. In first releases, a nondriving effector component alone would be tested for its transmission-blocking effect and its expected behavior in the field. In a second phase, a nondriving Cas9-strain would be co-released to enable local spread of the cargo gene. Finally, both effector and drive strains now resulting in full GD could be released in a third phase of this approach.

3. Reasons for Concern

Harald Meimberg shifted the focus to the question how GDs affect the integrity of ecosystems and to what extent they may fit with the concept of protection goals. Protection goals are considering generally a) the protected goods, b) biodiversity, and c) human well-being. Especially problematic is the treatment of biodiversity and ecosystem services as synonym in the context of risk assessment. Hence, if all ecosystem services are protected, so is biodiversity. Functional redundancy might allow the conclusion that a species can be removed from an ecosystem without perturbing the function of the system as a whole. Therefore, with this concept, if reduced to its function the value of a species is lower, if another species shows a similar function. This is especially relevant for GD applications, because here, a removal of single species from the ecosystem is attempted. To rely on potential compensation by other species hides the fact that the initial status of the system cannot be reestablished. In addition, stressors often interact and produce combined effects on biodiversity or ecosystem services.

4. Hype and Concern in the Discourse

Christophe Boëte highlights the fact that GD is highly transformative and shows signs of post-normal science (applications are associated with uncertainty and concerned with high stakes). GD is indeed characterized as a potentially powerful emerging technology that is confronted with multiple ethical dilemmas. There are dual concerns about this technology: the research itself and its potential applications. Concerns over potential application, in the light of the current rudimentary control options, clearly make it a candidate for the precautionary principle. Another burden for this new technology is the as yet open question of whether the potential benefits outweigh the potential costs. As seen for other innovations, communication is often a key aspect in current research but as discussed by B. Wynne, lay people are not so lay, and can have expertise too, making the public one of the stakeholders.[9] Clearly, this calls for inclusion, dialogue, and co-construction, instead of the development of communication strategy. This is a question of how to organize discussion versus how to obtain a consent and in that sense, this task implies great responsibilities. One of them is transparency and it has been already been chipped away as revealed by the publication of the so-called "gene drive files" (cp. http://genedrivefiles.synbiowatch.org/). Regarding communication of the technology, the independence of the actors involved is also key and the implication of the International Life Science Institute (ILSI), financed by Monsanto and CoopLife Int. and banned by the WHO in 2006 is not reassuring,[10] knowing the current interest of the agro-business for GD approaches. Given the potential of GD and the associated risks, the implication of the UN agencies in public health (WHO), in food safety (FAO), and in environment (UNEP) is highly needed to avoid the stranglehold of lobbyists and non-state actors on research and applications.

5. Perspectives

This workshop shed some light on issues that may become relevant for efforts to achieve control and to limit exposure to GD. At the current stage of development, prediction of limits in spread and persistence is severely restricted by insufficient knowledge of the behavior of GD. Orientation on the potential exposure related characteristics of GD can be obtained from models in well-defined configurations, but they remain highly dependent on a realistic choice of the potentially relevant parameters. With regard to the effect of a GD, we have to be aware of the crucial significance of biodiversity for the preservation of the "services" of ecosystems that we rely on. In the light of the insight that initial states cannot be fully reestablished after an intervention, application plans for GD have to consider the precautionary principle. Furthermore, achieving a high level of control as well as abilities to limit the spread of GDs should represent integral parts of responsible research and development.

Acknowledgements

The authors are grateful to Samson Simon and Mathias Otto from the German Federal Agency for Nature Conservation (BfN) for fruitful cooperation during the preparation of this workshop. The workshop was organized as part of the project "Risk Assessment of Synthetic Gene Drive Systems" funded by the German Federal Agency for Nature Conservation (BfN) (support code 3518 84 0500).

Conflict of Interest

The authors declare no conflict of interest.

Keywords

gene drive, modeling, population control, population dynamics

Received: August 28, 2019
Published online:

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