EKAH Newsletter (Feb. 2024)

Genome Editing, Gene Drives, Xenotransplantation

BioEssays
Active genetics comes alive: Exploring the broad applications of CRISPR-based selfish genetic elements (or gene-drives)
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Abstract: Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based “active genetic” elements developed in 2015 bypassed the fundamental rules of traditional genetics. Inherited in a super-Mendelian fashion, such selfish genetic entities offered a variety of potential applications including: gene-drives to disseminate gene cassettes carrying desired traits throughout insect populations to control disease vectors or pest species, allelic drives biasing inheritance of preferred allelic variants, neutralizing genetic elements to delete and replace or to halt the spread of gene-drives, split-drives with the core constituent Cas9 endonuclease and guide RNA (gRNA) components inserted at separate genomic locations to accelerate assembly of complex arrays of genetic traits or to gain genetic entry into novel organisms (vertebrates, plants, bacteria), and interhomolog based copying systems in somatic cells to develop tools for treating inherited or infectious diseases. Here, we summarize the substantial advances that have been made on all of these fronts and look forward to the next phase of this rapidly expanding and impactful field.

Kommentar:
Dieser Übersichtsartikel beschreibt viele grundlegende Aspekte von Gene-Drives, aber auch abgeleitete und zukünftige Anwendungen in verschiedensten Bereichen, inklusive Gene-Drives, die einen anderen Gene-Drive bremsen oder inaktivieren können. Der Artikel ist ziemlich technisch und detailliert, mit diversen konkreten Beispielen.

Trends Genet. 2023 May;39(5):347-357.
doi: 10.1016/j.tig.2023.02.010.Epub 2023 Mar 28.
Synthetic gene drives as an anthropogenic evolutionary force

Asher D Cutter

Abstract: Genetic drive represents a fundamental evolutionary force that can exact profound change to the genetic composition of populations by biasing allele transmission. Herein I propose that the use of synthetic homing gene drives, the human-mediated analog of endogenous genetic drives, warrants the designation of 'genetic welding' as an anthropogenic evolutionary force. Conceptually, this distinction parallels that of artificial and natural selection. Genetic welding is capable of imposing

complex and rapid heritable phenotypic change on entire populations, whether motivated by biodiversity conservation or public health. Unanticipated possible long-term evolutionary outcomes, however, demand further investigation and bioethical consideration. The emerging importance of genetic welding also compels our explicit recognition of genetic drive as an addition to the other four fundamental forces of evolution.

Kommentar:

Dies ist ein interessanter Übersichtsartikel, der den Einsatz von “Gene-Drives” unter dem Aspekt der Evolution betrachtet. Ein Gene-Drive wirkt sich demnach wie ein negativer Selektionsmechanismus aus, der ganze Populationen oder Spezies negativ beeinflussen kann und, im Extremfall, verschwinden lassen kann.

Ecol Lett. 2023 Sep:26 Suppl 1:S62-S80.
doi: 10.1111/ele.14194.

Incorporating ecology into gene drive modelling

Jaehee Kim, Keith D Harris, Isabel K Kim, Shahar Shemesh, Philipp W Messer , Gili Greenbaum

Abstract: Gene drive technology, in which fast-spreading engineered drive alleles are introduced into wild populations, represents a promising new tool in the fight against vector-borne diseases, agricultural pests and invasive species. Due to the risks involved, gene drives have so far only been tested in laboratory settings while their population-level behaviour is mainly studied using mathematical and computational models. The spread of a gene drive is a rapid evolutionary process that occurs over timescales similar to many ecological processes. This can potentially generate strong eco-evolutionary feedback that could profoundly affect the dynamics and outcome of a gene drive release. We, therefore, argue for the importance of incorporating ecological features into gene drive models. We describe the key ecological features that could affect gene drive behaviour, such as population structure, life-history, environmental variation and mode of selection. We review previous gene drive modelling efforts and identify areas where further research is needed. As gene drive technology approaches the level of field experimentation, it is crucial to evaluate gene drive dynamics, potential outcomes, and risks realistically by including ecological processes.

Kommentar:
Gene-Drives wurden bisher nur unter Laborbedingungen oder grösseren Käfig-Experimenten untersucht. Der Einsatz im Feld unter natürlichen Bedingungen wird bisher durch mathematische Modellierungen untersucht. Diese Studie argumentiert, dass solche Modelle vermehrt auch ökologische Kriterien (z.B. Populationsstrukturen, “Life History”) einbeziehen sollten, um aussagekräftige Resultate zu liefern.

Nat Commun. 2021 Jul 28;12(1):4589.
doi: 10.1038/s41467-021-24790-6.

Gene-drive suppression of mosquito populations in large cages as a bridge between lab and field

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Abstract: CRISPR-based gene-drives targeting the gene doublesex in the malaria vector Anopheles gambiae effectively suppressed the reproductive capability of mosquito populations reared in small laboratory cages. To bridge the gap between laboratory and the field, this gene-drive technology must be challenged with vector ecology. Here we report the suppressive activity of the gene-drive in age-structured An. gambiae populations in large indoor cages that permit complex feeding and reproductive behaviours. The gene-drive element spreads rapidly through the populations, fully supresses the population within one year and without selecting for resistance to the gene drive. Approximate Bayesian computation allowed retrospective inference of life-history parameters from the large cages and a more accurate prediction of gene-drive behaviour under more ecologically-relevant settings. Generating data to bridge laboratory and field studies for invasive technologies is challenging. Our study represents a paradigm for the stepwise and sound development of vector control tools based on gene-drive.

Kommentar:

In dieser Studie wurden Mückenpopulationen in Gefangenschaft einem Gene-Drive ausgesetzt, der die Geschlechtsdifferenzierung steuert. Es werden dadurch mehr Männchen gebildet, was die reproduktive Effizient der Population reduziert. Es wird somit die Population verkleinert, ohne dass ein direkter letaler Effekt beteiligt ist. Die Studie wurde an einem Überträger diverser tropischer Krankheiten ausgeführt (*Anopheles gambiae*), unter Bedingungen, die näher an der Realität der Mücken in ihrer natürlichen Umwelt sind als in früheren Studien.

Nat Commun. 2024 Jan 8;15(1):372.
doi: 10.1038/s41467-023-44399-1.

Gene drive and genetic sex conversion in the global agricultural pest Ceratitis capitata

Angela Meccariello, Shibo Hou , Serafima Davydova , James Daniel Fawcett , Alexandra Siddall , Philip T Leftwich , Flavia Krsticevic , Philippos Aris Papathanos , Nikolai Windbichler

Abstract: Homing-based gene drives are recently proposed interventions promising the area-wide, species-specific genetic control of harmful insect populations. Here, we characterise a first set of gene drives in a tephritid agricultural pest species,the Mediterranean fruit fly *Ceratitis capitata* (medfly). Our results show that the medfly is highly amenable to homing-based gene drive strategies. By targeting the medfly transformer gene, we also demonstrate how CRISPR-Cas9 gene drive can be coupled

to sex conversion, whereby genetic females are transformed into fertile and harmless XX males. Given this unique malleability of sex determination, we modelled gene drive interventions that couple sex conversion and female sterility and found that such approaches could be effective and tolerant of resistant allele selection in the target population. Our results open the door for developing gene drive strains for the population suppression of the medfly and related tephritid pests by co-targeting female reproduction and shifting the reproductive sex ratio towards males. They demonstrate the untapped potential for gene drives to tackle agricultural pests in an environmentally friendly and economical way.

Kommentar:
In dieser Studie wird eine Strategie beschrieben, ein invasives Schadinsekt (*Ceratitis capitata*; medfly) mit einem Gene-Drive zu bekämpfen, das Weibchen in Männchen umwandelt. Diese sind weniger schädlich und auf die Länge werden durch den Mangel an Weibchen die Populationen reduziert. Früher wurde *Ceratitis capitata* mit grossem Aufwand durch das massenweise Aussetzen von sterilisierten Männchen erfolgreich bekämpft.

Trends Plant Sci. 2024 Feb;29(2):179-195.
doi: 10.1016/j.tplants.2023.10.016.Epub 2023 Nov 17.
CRISPR enables sustainable cereal production for a greener future
Sunny Ahmar, Babar Usman, Goetz Hensel, Ki-Hong Jung, Damian Gruszka

Abstract: The clustered regularly interspaced short palindromic repeats (CRISPR)/ CRISPR-associated protein 9 (Cas9) system has become the most important tool for targeted genome editing in many plant and animal species over the past decade. The CRISPR/Cas9 technology has also sparked a flood of applications and technical advancements in genome editing in the key cereal crops, including rice, wheat, maize, and barley. Here, we review advanced uses of CRISPR/ Cas9 and derived systems in genome editing of cereal crops to enhance a variety of agronomically important features. We also highlight new techno- logical advances for delivering preassembled Cas9-gRNA ribonucleoprotein (RNP)-editing systems, multiplex editing, gain-of-function strategies, the use of artificial intelligence (AI)-based tools, and combining CRISPR with novel speed breeding (SB) and vernalization strategies.

Diese Übersichtsstudie listet viele GE-Anwendungen in Getreide auf (v. a. Reis, Mais, Weizen, aber auch Gerste). Es werden die angestrebten und erreichten Charakteristika der entsprechenden Linien besprochen und das allgemeine Potential der Technologie in der Getreide-Züchtung diskutiert.

Nat Rev Mol Cell Biol. 2024 Jan;25(1):34-45.
doi: 10.1038/s41580-023-00663-2.Epub 2023 Oct 4.
Base editing of organellar DNA with programmable deaminases
Jin-Soo Kim, Jia Chen
• 10.1038/s41580-023-00663-2

Abstract: Mitochondria and chloroplasts are organelles that include their own genomes, which encode key genes for ATP production and carbon dioxide fixation,

respectively. Mutations in mitochondrial DNA can cause diverse genetic disorders and are also linked to ageing and age-related diseases, including cancer. Targeted editing of organellar DNA should be useful for studying organellar genes and developing novel therapeutics, but it has been hindered by lack of efficient tools in living cells. Recently, CRISPR-free, protein-only base editors, such as double-stranded DNA deaminase toxin A-derived cytosine base editors (DdCBEs) and adenine base editors (ABEs), have been developed, which enable targeted organellar DNA editing in human cell lines, animals and plants. In this Review, we present programmable deaminases developed for base editing of organellar DNA in vitro and discuss mitochondrial DNA editing in animals, and plastid genome (plastome) editing in plants. We also discuss precision and efficiency limitations of these tools and propose improvements for therapeutic, agricultural and environmental applications.

Kommentar:
Genom-Editierung zielt typischerweise auf des Kern-Genom. Interessant wären jedoch auch Applikationen mit DNA-Editierung der kleinen Genome in den Organellen (Mitochondrien, Chloroplasten). Viele menschliche Erbkrankheiten werden verursacht durch genetische Disposition in den Mitochondrien. In der Anwendung in der Landwirtschaft wäre der Einsatz von DNA-Editierung im Chloroplastengenome interessant, da viele relevante Funktionen für die Leistung der Pflanzen im Chloroplastengenom kodiert sind (z.B. Komponenten der photosynthetischen Maschinerie, Herbizid-Resistenzen etc.). In dieser Studie werden CRISPR-unabhängige Mechanismen der Genomeditierung für Organellen-Genome diskutiert.

Biotechnol Adv. 2023 Dec:69:108248.
doi: 10.1016/j.biotechadv.2023.108248. Epub 2023 Sep 2.
CRISPR/Cas9-mediated genome editing techniques and new breeding strategies in cereals - current status, improvements, and perspectives
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Abstract: Cereal crops, including triticeae species (barley, wheat, rye), as well as edible cereals (wheat, corn, rice, oat, rye, sorghum), are significant suppliers for human consumption, livestock feed, and breweries. Over the past half-century, modern varieties of cereal crops with increased yields have contributed to global food security. However, presently cultivated elite crop varieties were developed mainly for optimal environmental conditions. Thus, it has become evident that taking into account the ongoing climate changes, currently a priority should be given to developing new stress-tolerant cereal cultivars. It is necessary to enhance the accuracy of methods and time required to generate new cereal cultivars with the desired features to adapt to climate change and keep up with the world population expansion. The CRISPR/Cas9 system has been developed as a powerful and versatile genome editing tool to achieve desirable traits, such as developing high-yielding, stress-tolerant, and disease- resistant transgene-free lines in major cereals. Despite recent advances, the CRISPR/Cas9 application in cereals faces several challenges, including a significant amount of time required to develop transgene-free lines, laboriousness, and a limited number of genotypes that may be used for the transformation and *in vitro* regeneration. Additionally, developing elite lines through genome editing has been restricted in many countries, especially Europe and New

Zealand, due to a lack of flexibility in GMO regulations. This review provides a comprehensive update to researchers interested in improving cereals using gene-editing technologies, such as CRISPR/Cas9. We will review some critical and recent studies on crop improvements and their contributing factors to superior cereals through gene-editing technologies.

Kommentar:
Diese Übersichtsarbeit widmet sich weniger der GE-Technologie als solcher, sondern deren Anwendung in Getreiden. Sie listet eine grosse Zahl von GE-Projekten in Reis, Mais, Weizen und Gerste auf und gibt damit einen Überblick über den Stand des Forschungsfeldes und der angestrebten Effekten in Getreiden.

Virology
Volume 587, October 2023, 109862
Molecular breeding of livestock for disease resistance
Fei Gao, Pan Li, Ye Yin, Xuguang Du, Gengsheng Cao, Sen Wu, Yaofeng Zhao

Abstract: Animal infectious diseases pose a significant threat to the global agriculture and biomedicine industries, leading to significant economic losses and public health risks. The emergence and spread of viral infections such as African swine fever virus (ASFV), porcine reproductive and respiratory syndrome virus(PRRSV), porcine epidemic diarrhea virus (PEDV), and avian influenza virus(AIV) have highlighted the need for innovative approaches to develop resilient and disease-resistant animal populations. Gene editing technologies, such as CRISPR/Cas9, offer a promising avenue for generating animals with enhanced disease resistance. This review summarizes recent advances in molecular breeding strategies for generating disease-resistant animals, focusing on the development of disease-resistant livestock. We also highlight the potential applications of genome-wide CRISPR/Cas9 library screening and base editors in producing precise gene modified livestock for disease resistance in the future. Overall, gene editing technologies have the potential to revolutionize animal breeding and improve animal health and welfare.

Kommentar:
Diese Übersichtsarbeit beschreibt die verschiedenen Strategien, mit GE-Methoden Nutztiere resistenter zu machen gegen virale Krankheiten. Da virale Erreger den Wirt oft über die Bindung an spezifische Rezeptoren infizieren, besteht eine zentrale Strategie darin, solche Rezeptoren zu eliminieren.