Part One:
Self-Spreading Biotechnology Challenges International Law

Chapter 1:
The Emergence of Self-Spreading Biotechnology

Legal practitioners and scholars alike often tend to break down the facts of a case to only those aspects they deem essential for appreciating that case from the legal perspective. At the interface of science and law, however, this habit runs the risk of oversimplifying the facts, which can result in the legal analysis being incomplete or even incorrect. Therefore, to provide a solid factual basis for the ensuing legal discussion, this first chapter undertakes a concise review of the recent advances in molecular biology in general and the emergence of self-spreading biotechnology in particular.

Since apprehending these developments requires a general understanding of the underlying biological principles, the first section will provide a brief introduction to genetics and molecular biology (A.). For a more detailed account, extensive monographs and treatises are available.[1] Subsequently, techniques for *genome editing* will be discussed, which are methods for precisely modifying the genetic information of any organism (B.). These techniques also enable the development of *engineered gene drives*, which are methods to increase the inheritance of a genetic modification in wild populations (C.). Besides gene drives, *horizontal environmental genetic alteration techniques* are developed to modify large numbers of individual organisms of the same generation simultaneously (D.). The last section will address approaches that also involve self-spreading techniques but are not aimed at genetically modifying their target organisms (E.).

1 For a compelling and non-technical introduction, see *Siddhartha Mukherjee*, The Gene (2016). On plant breeding, see *Noël Kingsbury*, Hybrid: The History and Science of Plant Breeding (2009). For an introduction to genetics, see *Benjamin A. Pierce*, Genetics (7th ed. 2020). For a detailed account of molecular biology, see *Bruce Albers* et al., Molecular Biology of the Cell (6th ed. 2015). For a treatise on modern biotechnology, see *David P. Clark* et al., Molecular Biology (3rd ed. 2019).

A. Principles of Genetics and Molecular Biology

When *Gregor Mendel* described his observations on the heredity of traits in pea breeding in 1866,[2] he was not aware of the underlying biological principles. However, the discovery of these principles later validated Mendel's assumptions. In the last few decades, large progress has been made in the study of the molecular biology of the cell. Today, we have discovered not only how genetic information is stored, but also how this information is processed, passed on to subsequent generations and how genetic variations contribute to evolution. The present section will briefly recall the most important aspects of molecular biology (I.), natural genetic change and inheritance (II.), and human-made or *anthropogenic* genetic change (III.).

I. Basics of Molecular Biology

Genetic information is encoded in sequences of *nucleic acid*. Nucleic acids are made up of chains of *nucleotides*. Nucleotides are molecules comprised of sugar, phosphate, and a *nitrogenous base*. There are four different types of nitrogenous bases in nucleic acid: *adenine* (A), *cytosine* (C), *guanine* (G), and *thymine* (T). Because only the base differs in each of the four types of nucleotides, a polynucleotide chain resembles a necklace made from sugar and phosphate, from which hang the four types of beads (the bases A, C, G, and T).[3] The sequence in which these bases occur in a polynucleotide chain encodes genetic information, similar to a human message written in an alphabetic script.[4] The term *gene* often refers to pieces of nucleic acid encoding a certain genetic characteristic.[5] In contrast, the term *genome* denotes the entirety of an organism's genetic (or heritable) material.[6]

Most organisms carry their genome in *deoxyribonucleic acid* (DNA). DNA consists of two strands of nucleotides that usually take the form of a *double helix*.[7] These strands are complementary to each other, which

2 *Gregor Mendel*, Versuche über Pflanzen-Hybriden (Experiments on Plant Hybrids), 4 (1866) Verhandlungen des Naturforschenden Vereins zu Brünn 3.
3 *Albers* et al. (n. 1), 175.
4 *Ibid.*, 5.
5 *Pierce* (n. 1), 12; cf. 'gene', in: *Eleanor Lawrence* (ed.), Henderson's Dictionary of Biology (16th ed. 2016), 224.
6 *Albers* et al. (n. 1), 7; cf. 'genome', in: Henderson's Dictionary of Biology (n. 5), 228.
7 *Albers* et al. (n. 1), 176.

means that any A base on the first strand always pairs with T on the second strand, and G always pairs with C.[8] This principle is important for how DNA is replicated during cell division (*mitosis* and *meiosis*): in these processes, the two strands of the DNA double helix are pulled apart, and each serves as a template for synthesis of a new complementary strand. These newly formed complementary strands then pair with the original strands forming an additional DNA double helix.[9]

In *eukaryotes* (i.e. organisms whose cells possess a complex structure including a membrane-enclosed nucleus[10]), DNA is organized in sets of *chromosomes*, which are compact packages of long, thread-like DNA strands and associated proteins.[11] Most eukaryotes possess two copies of each chromosome, one of which is inherited from each parent. These pairs of *homologous chromosomes* (or *homologs*) are usually alike in structure and size and carry the genetic information for the same set of hereditary characteristics.[12] Of any given gene, the corresponding variants situated on the maternal and paternal chromosomes are called *alleles*.[13] When both alleles of a certain gene are identical, the organism is called *homozygous* with respect to that gene.[14] On the other hand, when the alleles encode different information, the organism is referred to as *heterozygous*.[15] The only *non-homologous* chromosome pair are the sex chromosomes in males of many species, where a Y chromosome is inherited from the father and an X chromosome from the mother.[16]

Eukaryotic *gametes* (i.e. eggs and sperm[17]) are *haploid*, which means that they only possess one copy of each gene. The formation of gametes, which is called *meiosis*, follows a different procedure than regular (*mitotic*) cell division and is discussed below.[18] When the egg becomes fertilized

8 *Ibid.*
9 *Ibid.*, 4.
10 Cf. 'Eukarya', in: Henderson's Dictionary of Biology (n. 5), 192.
11 *Albers* et al. (n. 1), 180–181.
12 *Pierce* (n. 1), 21.
13 Cf. 'allele', in: Henderson's Dictionary of Biology (n. 5), 20.
14 Cf. 'homozygous', in: *ibid.*, 267.
15 Cf. 'heterozygous', in: *ibid.*, 260.
16 *Albers* et al. (n. 1), 180. Note that some species have different sex determination systems, including such that rely fully or in part on environmental factors. See generally *Pierce* (n. 1), 83–89; on environmental sex determination, see *F. J. Janzen/P. C. Phillips*, Exploring the Evolution of Environmental Sex Determination, Especially in Reptiles, 19 (2006) Journal of Evolutionary Biology 1775.
17 Cf. 'gamete', in: Henderson's Dictionary of Biology (n. 5), 220.
18 See *infra* section A.II.2.

by the sperm in sexual reproduction, the egg and sperm each contribute one set of chromosomes, which are subsequently merged into the new homologous chromosome set of the offspring.[19] This process constitutes the molecular background behind *Mendel's laws* on the inheritance of traits, which will be discussed below.[20]

The cells of each species have a characteristic number of chromosomes.[21] For example, the cells of most mosquito species possess 2n=6 chromosomes (i.e. 3 pairs);[22] human cells possess 2n=46 (i.e. 23 pairs) and pigeon cells possess 2n=80 chromosomes (i.e. 40 pairs).[23] Some eukaryotic organisms, in particular many plants that are bred as crops, are *polyploid* which means that they possess more than two chromosomal copies. For example, ancestral wheat has seven pairs of chromosomes (i.e. 2n=14), whereas contemporary bread wheat is *hexaploid*, meaning that it possesses six sets of seven chromosomes each (i.e. 6n=42).[24]

The process of implementing the information stored in the genome is called *gene expression*. Gene expression commonly appears as a two-step process. First, in *transcription*, segments of the DNA sequence are guiding the synthesis of snippets of *ribonucleic acid* (RNA).[25] RNA is closely related to DNA but appears as a single-stranded chain of nucleotides (as opposed to DNA, which consists of two complementary nucleotide strands).[26]

In the second step, called *translation*, the RNA molecules created in the first step direct the synthesis of *proteins*.[27] Proteins, which are polypeptide chains composed of amino acids,[28] are then responsible for actually implementing the genetic information by performing various functions within the cell. Many proteins are enzymes that catalyse chemical reactions. Other

19 *Pierce* (n. 1), 21.
20 See *infra* section A.II.3.
21 *Clark* et al. (n. 1), 13–15.
22 *Karamjit S. Rai/William C. Black*, Mosquito Genomes, 41 (1999) Advances in Genetics 1, 5–6.
23 *Pierce* (n. 1), 13.
24 *Clark* et al. (n. 1), 45.
25 *Albers* et al. (n. 1), 4.
26 RNA also has a biochemical composition that slightly differs from that of DNA: it uses a different sugar as its backbone (ribose instead of deoxyribose) and the base *thymine* (T) is replaced by *uracil* (U), which however are compatible with each other. See *ibid.*
27 *Ibid.*
28 Cf. 'protein', in: Henderson's Dictionary of Biology (n. 5), 475.

proteins form structural components, help transport substances or perform various regulatory, sensory, communication, or defence functions.[29]

Notably, not all information stored in the genome of an organism (*genotype*) is necessarily expressed in the physical, physiological, biochemical, or behavioural characteristics of that organism (*phenotype*). The genotype merely determines the *boundaries* for development, while the phenotype is determined by the interplay of various genes and by environmental factors.[30]

II. Natural Genetic Change and Inheritance

Evolution denotes the development of new types of living organisms by the accumulation of genetic variations over several generations.[31] The main triggers of genetic variation are *genetic mutation* (1.) and the *recombination and segregation of DNA* from two individuals during sexual reproduction (2.).[32] These mechanisms lead to genetic inheritance in line with the principles discovered by Mendel (3.). The frequency at which alleles occur in the gene pool is influenced by several factors.[33] One of these factors is *natural selection*, which results from the fact that different phenotypes resulting from genetic variation have different rates of physical and reproductive fitness in different environments.[34]

1. Genetic Mutation

The term *mutation* generally denotes a change in the amount of chemical structure of DNA.[35] Mutations can take the form of *point mutations*, which are local changes in the DNA sequence such as the substitution of one base pair with another, but may also appear as large-scale genome rearrangements, including deletions, duplications, insertions and even translo-

29 *Pierce* (n. 1), 439; *Albers* et al. (n. 1), 6.
30 *William S. Klug* et al., Concepts of Genetics (2019), 82–85.
31 Cf. 'evolution', in: Henderson's Dictionary of Biology (n. 5), 195.
32 See 'genetic variation', in *ibid.*, 227.
33 The study of these factors is called *Population Genetics*, see *Pierce* (n. 1), 765–795.
34 *Richard C. Lewontin*, The Units of Selection, 1 (1970) Annual Review of Ecology and Systematics 1, 1.
35 Cf. 'mutation', in: Henderson's Dictionary of Biology (n. 5), 371–372.

cations of DNA from one chromosome to another.[36] Mutations that occur in the body cells are called *somatic mutations* and are only passed on to the immediate descendants of that cell. However, mutations occurring in germline cells can be inherited by the offspring organism.[37]

Mutations are caused by several factors. Most mutations arise from failures in the cell's own mechanisms by which DNA is replicated, recombined, or repaired.[38] However, damage to DNA can also be caused by external influences such as heat, metabolic accidents, radiation of various sorts, or exposure to chemical substances in the environment.[39]

Another source of genetic change is so-called *transposable DNA elements* or *transposons*, which are DNA elements that possess the property of changing their position within the genome.[40] Transposable elements are *selfish genetic elements* that can bias their transmission to subsequent generations in their favour. Transposable elements thus are naturally occurring *gene drive* mechanisms that can spread through populations at a higher rate than it would normally be expected under the laws of *Mendelian inheritance*.[41]

Cells contain multiple systems that can recognize and repair many types of damaged or altered DNA.[42] Since most spontaneous changes are remedied by these mechanisms, only very few of them cause a permanent alteration of the genome. The mutation rate across all living organisms is approximately one nucleotide change per 10^{10} (ten billion) nucleotides each time the DNA is replicated.[43] This rate appears to create an *equilibrium* between genetic stability and genetic variability, which are both required to maintain permanent life.[44]

Due to the double-helical structure of DNA, damage on one DNA strand can easily be repaired by taking the second, complementary strand as a template.[45] *Double-strand breaks*, i.e. complete cuts affecting both DNA

36 *Albers* et al. (n. 1), 217–218.
37 Cf. 'mutation', in: Henderson's Dictionary of Biology (n. 5), 372.
38 *Albers* et al. (n. 1), 217–218.
39 *Ibid.*, 266.
40 Cf. 'transposable genetic elements', in: Henderson's Dictionary of Biology (n. 5), 598; see *Thomas Wicker* et al., A Unified Classification System for Eukaryotic Transposable Elements, 8 (2007) Nature Rev. Genet. 973, 973.
41 See *infra* section A.II.3.
42 See *Albers* et al. (n. 1), 269–276.
43 *Ibid.*, 239.
44 Cf. *ibid.*, 238–239.
45 *Ibid.*, 268–271.

strands, are more dangerous to the cell. If such a cut remains unrepaired, it can cause the chromosome to break down into fragments and lead to the loss of genes when the cell divides.[46] Cells possess two different mechanisms to repair double-strand breaks. *Homology-directed repair* (HDR, also called *homologous recombination*) fully restores the damage by using the homologous chromosome as a template. Therefore, HDR can only be applied in those phases of the cell cycle in which a sister chromosome is present.[47] In other cases, the damage is repaired by *non-homologous end joining* (NHEJ), in which the broken ends are brought together and rejoined. This generally involves losing a number of nucleotides at the site of joining, which results in a point mutation. For this reason, some genome editing techniques make use of NHEJ by introducing double-strand breaks at specific locations in the DNA in order to induce mutations there.[48]

2. Sexual Reproduction

Sexual reproduction and the associated recombination of DNA is a second important source of genetic variation. Sexual reproduction consists of two processes. The first is *meiosis*, which denotes the formation of *haploid* gametes in which the number of chromosomes is reduced by half. The second process is *fertilization*, in which the egg cell and the sperm cell fuse to form a *zygote* in which the maternal and paternal chromosome sets are joined to form the new diploid genome of the offspring.[49]

The process of meiosis begins with a stem cell that is *diploid*, which means that the cell possesses two complete sets of chromosomes, of which one set is of maternal and one is of paternal origin. During meiosis, each set of chromosomes is first replicated, resulting in four complete sets. These are then distributed to a total of four *haploid* gametes in two successive cell divisions. During this process, genetic variation is generated by two different mechanisms. Firstly, *inter-chromosomal recombination* causes a 'reshuffling' of genetic information between the corresponding maternal and paternal chromosomes after they have been replicated.[50] Secondly, during cell division, the resulting chromosomes are randomly distributed

46 *Ibid.*, 274.
47 *Ibid.*, 278–279.
48 See *infra* section B.I.
49 *Pierce* (n. 1), 28.
50 *Albers* et al. (n. 1), 1004–1010.

onto the gametes, so that each gamete receives either the maternal or the paternal copy of each chromosome (*chromosomal segregation*).[51] Consequently, each of the four resulting gametes carries a different combination of alleles. Despite certain differences, these processes are essentially the same in plants and animals.[52]

3. Mendel's Laws of Inheritance

The molecular biology of sexual reproduction I described above forms the background for Mendel's observations on the principles (or 'laws', as they are often called) of inheritance.[53] The first, called *principle of segregation*, describes the observation that diploid organisms possess two *alleles* (i.e. variants of a given gene) for any particular trait and that these alleles segregate during meiosis. Consequently, for any given gene, half the gametes will carry one allele and half the other.[54] The second observation, termed *independent assortment*, is that alleles for separate traits are passed on independently from each other (which occurs as a result of the chromosomal segregation during meiosis).[55] Mendel's third rule, called the *principle of dominance*, describes the consequences of segregation and independent assortment: whenever an organism possesses two different genes for a particular trait, only one of them (the *dominant* allele) is expressed in the phenotype.[56] The other allele, which is called *recessive*, remains part of the genotype and will be passed on to half of the organism's gametes.

III. Anthropogenic Genetic Change

Humankind has been a source of genetic change for a long time. On the one hand, human activity such as land development, exploitation of resources, and pollution is the main cause of the decline and extinction

51 *Pierce* (n. 1), 33–34.
52 *Ibid.*, 38.
53 See *Mendel* (n. 2).
54 *Pierce* (n. 1), 53–54; 'segregation of alleles', in Henderson's Dictionary of Biology (n. 5), 532.
55 *Pierce* (n. 1), 62–63; see 'independent assortment', in Henderson's Dictionary of Biology (n. 5), 285.
56 *Pierce* (n. 1), 53; see 'dominance', in Henderson's Dictionary of Biology (n. 5), 164.

of species.[57] On the other hand, humans have domesticated and improved animal and plant species for thousands of years.[58] For most of the time, the only method to improve cultivated species was *selective breeding*, which refers to selectively mating strains that possess desired traits such as increased productivity or resistance.[59]

After the principles of genetics were discovered in the early twentieth century, novel breeding techniques such as *hybridization* were developed, which however still relied on utilizing naturally occurring genetic mutations.[60] In the late 1920s, it was discovered that certain mutagenic agents such as radiation and chemicals increase the rate of genetic mutations in an organism, and that these agents can be used to accelerate breeding by creating large amounts of mutants and then selecting individuals with desired characteristics.[61] This technique is today known as *mutation breeding* or *mutagenesis*.[62]

In the following decades, genetic science advanced quickly. Major milestones include the decryption of the chemical structure and molecular functioning of DNA in 1966, the first creation of a transgenic organism in 1973, and the development of the first methods for sequencing DNA in 1977 and multiplying DNA segments in 1983.[63] The first genetically modified crop, an antibiotic-resistant tobacco plant, was produced in 1982.[64] The modification of endogenous genes became possible with the development of *gene targeting* methods based on homologous recombination.[65] More recently, the development of *genome editing* techniques substantially extended the possibilities to modify genetic information.

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57 See *Russell Lande*, Anthropogenic, Ecological and Genetic Factors in Extinction and Conservation, 40 (1998) Researches on Population Ecology 259.

58 On the history of plant breeding, see the extensive monograph by *Kingsbury* (n. 1).

59 *Rolf H. J. Schlegel*, Concise Encyclopedia of Crop Improvement (2007), 5–52; *Kingsbury* (n. 1), 155–186.

60 *Schlegel* (n. 59), 53–135.

61 *Hermann J. Muller*, Artificial Transmutation of the Gene, 66 (1927) Science 84; see *B. S. Ahloowalia* et al., Global Impact of Mutation-Derived Varieties, 135 (2004) Euphytica 187; *Schlegel* (n. 59), 96–101; *Kingsbury* (n. 1), 266–272.

62 Cf. 'mutagenesis' and 'mutation breeding', in Henderson's Dictionary of Biology (n. 5), 371–372.

63 *Pierce* (n. 1), 9–11.

64 *Robert T. Fraley* et al., Expression of Bacterial Genes in Plant Cells, 80 (1983) PNAS 4803.

65 *Wenfang Tan* et al., Gene Targeting, Genome Editing, 25 (2016) Transgenic Research 273, 274–275; *Almudena Fernández* et al., A History of Genome Editing in Mammals, 28 (2017) Mammalian Genome 237, 237.

B. Genome Editing

A central challenge in biotechnology is to induce genetic changes at *specific locations* in the genome, i.e. at a particular site of the DNA sequence within a certain chromosome. If existing genes are to be modified or knocked out, the genetic modification must necessarily take place at the location of the targeted gene. In conventional *mutation breeding* (where random mutations are induced by exposing the organisms to certain chemicals or radiation), the search for an individual bearing a mutation at the desired genomic location or showing the desired traits in its phenotype is a laborious and time-consuming step.

In addition, it is now known that not only the existence of a certain gene but also its position in the genome can be decisive for its phenotypic expression.[66] Hence, when transgenes are to be inserted into an organism, it is not always sufficient to achieve integration of these transgenes at a random location in the genome of the plant, as is the case with earlier genetic engineering methods.[67]

For many years, strategies of efficiently inducing precise, targeted genome alterations were laborious and limited to certain organisms.[68] Furthermore, these techniques often required drug-selectable markers or left behind unwanted DNA sequences associated with the modification method.[69] However, in the last decade, a number of techniques have been developed that allow for the introduction of double-strand breaks at specific locations of an organism's DNA, which can be used to site-specifically insert, delete or replace genetic information. These techniques are commonly denoted as *genome editing* techniques.

The following section outlines the general principles underlying genome editing (I.). Subsequently, the most relevant techniques are described (II.). This is followed by an overview of potential and already existing applications of genome editing techniques (III.) before the technical

66 See, in particular, *Matthew V. Rockman* et al., Selection at Linked Sites Shapes Heritable Phenotypic Variation in C. Elegans, 330 (2010) Science 372.

67 See *Schlegel* (n. 59), 157–174; *Götz Laible* et al., Improving Livestock for Agriculture, 10 (2015) Biotechnology Journal 109, 112–113; *Katia Pauwels* et al., Engineering Nucleases for Gene Targeting: Safety and Regulatory Considerations, 31 (2014) New Biotechnology 18.

68 *Jeffry D. Sander/J. K. Joung*, CRISPR-Cas Systems for Editing, Regulating and Targeting Genomes, 32 (2014) Nature Biotech. 347, 347; see *Tan* et al. (n. 65), 273–275.

69 *Sander/Joung* (n. 68), 347.

challenges and limitations of these methods (IV.) as well as environmental risks and ethical concerns (V.) are addressed.

I. Functioning of Genome Editing

Most current approaches to genome editing follow the same principles. Firstly, a double-strand break is induced at the location in the genome where the modification is intended. This can be achieved by using *site-directed nucleases*. *Nucleases* are naturally occurring enzymes that can cleave the nucleotide chain of nucleic acid.[70] A *site-directed* nuclease (SDN) can bind to a specific DNA sequence and then cleave the DNA at this location. After such a break has been induced, intra-cellular DNA repair mechanisms will attempt to repair the break. These mechanisms can be harnessed to introduce the intended modification. Generally, genome editing techniques based on SDNs are categorized as follows:[71]

SDN-1: In its most basic form, only the SDN is delivered to the organism without a repair template. The cell will repair the DNA break by *non-homologous end joining* (NHEJ), which means that the two loose ends of DNA are simply joined together.[72] This process tends to add or leave out a small number of nucleotides. Hence, NHEJ often induces small mutations (so-called *indels*) at the cleavage site, which can be used to knock out a specific gene.[73] Alternatively, larger DNA sequences can be 'deleted'

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70 Cf. 'nuclease', in: Henderson's Dictionary of Biology (n. 5), 392; *Albers* et al. (n. 1), 464–465.

71 See *Maria Lusser/Howard V. Davies*, Comparative Regulatory Approaches for Groups of New Plant Breeding Techniques, 30 (2013) New Biotechnology 437; *Thorben Sprink* et al., Regulatory Hurdles for Genome Editing: Process- vs. Product-Based Approaches in Different Regulatory Contexts, 35 (2016) Plant Cell Reports 1493, 1504 and Figure 2 on p. 1498. Note that some publications (e.g. *Motoko Araki* et al., Caution Required for Handling Genome Editing Technology, 32 (2014) Trends in Biotechnology 234, 235; European Commission, New Techniques Working Group (NTWG): Final Report, not officially published (2012), 14–16; *Maria Lusser* et al., New Plant Breeding Techniques: State-of-the-Art and Prospects for Commercial Development (2011), 19) also refer to these methods as ZFN-1, ZFN-2 and ZFN-3, implying the use of ZFN as site-directed nuclease to induce a double-strand break (see *infra* section B.II.1). The editing pathways, however, are identical to those of other SDNs.

72 *Pierce* (n. 1), 575–576; see *supra* section A.II.1.

73 *Thomas Gaj* et al., ZFN, TALEN, and CRISPR/Cas-Based Methods for Genome Engineering, 31 (2013) Trends in Biotechnology 397, 400; *Lusser/Davies* (n. 71), 440; *Sander/Joung* (n. 68), 347; *Pauwels* et al. (n. 67), 19 and Figure 2 on p. 20.

by inducing breaks at either end of the targeted sequence.[74] It is also possible to induce mutations at several locations in one step.[75]

SDN-2: The second option is to direct the DNA repair by providing a 'repair template'. This template consists of a small DNA snippet which is identical (*homologous*) to the target site except for one or a few differing base pairs.[76] When the cell employs *homology-directed repair* (HDR),[77] it relies on the given template to repair the break, which leads to the inclusion of the pre-determined mutation at the target site.[78]

SDN-3: A double-strand break can also be used to introduce larger pieces of new DNA. This can be achieved by supplying a piece of 'donor' DNA which has ends corresponding to the DNA sequence at the intended cleavage site.[79] In between these homologous ends, the donor DNA may contain new genetic information.[80] Similar to SDN-2, the cell will rely on the donor DNA as a template for homology-directed repair, which results in the incorporation of the new sequence at the intended location.[81]

As mentioned above, most cell types and organisms have two pathways to repair DNA double-strand breaks, namely NHEJ and HDR.[82] If the cell relies on NHEJ, a given repair template is ignored and the resulting mutations will be random, as in SDN-1.[83] Hence, for SDN-2 and SDN-3 it is necessary that the damage is repaired by HDR.[84] Since both repair mechanisms operate in different phases of the cell cycle, timed delivery of the SDN and the repair template can influence which repair mechanism

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74 Cf. *Huanbin Zhou* et al., Large Chromosomal Deletions and Heritable Small Genetic Changes Induced by CRISPR/Cas9 in Rice, 42 (2014) Nucleic Acids Res. 10903.

75 See *Le Cong* et al., Multiplex Genome Engineering Using CRISPR/Cas Systems, 339 (2013) Science 819; *An Xiao* et al., Chromosomal Deletions and Inversions Mediated by TALENs and CRISPR/Cas in Zebrafish, 41 (2013) Nucleic Acids Res. e141.

76 *Sprink* et al. (n. 71), 1504.

77 See *supra* section A.II.1.

78 *Sander/Joung* (n. 68), 347; *Lusser/Davies* (n. 71), 440.

79 *Gaj* et al. (n. 73), 400.

80 *Sprink* et al. (n. 71), 1504.

81 *Gaj* et al. (n. 73), 400; *Lusser/Davies* (n. 71), 440; *Sprink* et al. (n. 71), 1504.

82 See *supra* section A.II.1. There are a number of other DNA repair mechanisms, including *single-strand annealing*, *alternative end joining*, and *microhomology-mediated joining*. See *Rodolphe Barrangou/Jennifer A. Doudna*, Applications of CRISPR Technologies in Research and Beyond, 34 (2016) Nature Biotech. 933, 933 for further references.

83 *Sprink* et al. (n. 71), 1504.

84 Cf. *Gaj* et al. (n. 73), 400.

is used.[85] Depending on the particular circumstances, the likelihood of achieving the desired mutation varies between 1 % and, in some cases, over 50 %.[86] Usually, a larger number of individuals need to be treated to identify a small number who carry the desired mutation using screening techniques.[87]

II. Engineered Nuclease Techniques for Site-Specific DNA Cleavage

The mechanisms described above require a DNA double-strand break to be induced at the target site. Hence, the main challenge of genome editing lies not in stimulating the repair, but in cleaving DNA at specific locations. This became first possible in the 1990s with the discovery of so-called *meganucleases*, whose 18 base pair long recognition site could be manipulated to target desired chromosomal sites. With meganucleases, it became possible for the first time to introduce DNA double-strand breaks at *predictable* locations. However, the recognition sites of meganucleases are randomly scattered in the genome and redesigning these recognition sites to target specific genes was very laborious.[88]

More recently, a number of techniques have been developed to engineer site-directed nucleases that can target virtually any DNA sequence. These techniques include engineered *zinc finger nucleases* (1.), synthetic *transcription-activator-like effector nucleases* (2.), and the *CRISPR-Cas* technique (3.).

1. Zinc Finger Nucleases

The first genome editing method that could be virtually universally applied was the *zinc-finger nuclease* (ZFN) technique.[89] Zinc finger nucleases are artificial constructs generated by fusing a non-specific nuclease domain (responsible for cleaving the DNA) to an engineered zinc finger DNA-

85 *Steven Lin* et al., Enhanced Homology-Directed Human Genome Engineering by Controlled Timing of CRISPR/Cas9 Delivery, 3 (2014) eLife e04766.

86 *Sander/Joung* (n. 68), 347; see *Christopher D. Richardson* et al., Enhancing Homology-Directed Genome Editing by Catalytically Active and Inactive CRISPR-Cas9 Using Asymmetric Donor DNA, 34 (2016) Nature Biotech. 339.

87 *Sander/Joung* (n. 68), 347.

88 *Christian Jung* et al., Recent Developments in Genome Editing and Applications in Plant Breeding, 137 (2018) Plant Breeding 1, 2.

89 Cf. *Gaj* et al. (n. 73), 399; *Fernández* et al. (n. 65), 238–239.

binding domain.[90] Zinc fingers, which are a structural component shared by various DNA-binding proteins, can recognize certain three base pair long DNA sequences.[91] Scientists were able to engineer zinc fingers to recognize almost any of the 64 possible three-base pair combinations that can occur in DNA.[92] Additionally, arrays of multiple zinc fingers can be constructed to increase their specificity; these arrays can recognize DNA sequences of up to 18 base pairs in length.[93] These engineered zinc finger domains are then fused to a nuclease domain in order to create a *zinc finger nuclease* that will cleave DNA at the recognition site programmed in the zinc finger array.

The first ZFN was created and applied *in vitro* in 1996,[94] while the first successful application for targeted mutagenesis was reported in 2002.[95] After that, the ZFN technique has been applied to edit the genome of many plants and animals,[96] including mammals.[97] ZFNs were also applied in clinical trials to cure HIV.[98] For many years, ZFNs were the only available approach for inducing site-specific cuts in nucleic acid. However, the development of custom-made ZFN complexes remained laborious and expensive.[99]

2. Transcription Activator-Like Effector Nucleases

Transcription activator-like effector nucleases (TALENs) are structurally very similar to ZFNs since they also consist of a nuclease domain (responsible for cleaving DNA) and a DNA-binding domain (responsible for at-

90 *Pierce* (n. 1), 574.
91 *Ibid.*, 472–473; *Gaj* et al. (n. 73), 398.
92 See *C. O. Pabo* et al., Design and Selection of Novel Cys2His2 Zinc Finger Proteins, 70 (2001) Annual Review of Biochemistry 313.
93 *Gaj* et al. (n. 73), 398.
94 *Y. G. Kim* et al., Hybrid Restriction Enzymes, 93 (1996) PNAS 1156.
95 *Marina Bibikova* et al., Targeted Chromosomal Cleavage and Mutagenesis in Drosophila Using Zinc-Finger Nucleases, 161 (2002) Genetics 1169.
96 See *Dana Carroll*, Genome Engineering with Zinc-Finger Nucleases, 188 (2011) Genetics 773, 776.
97 *Fernández* et al. (n. 65), 239.
98 *Pablo Tebas* et al., Gene Editing of CCR5 in Autologous CD4 t Cells of Persons Infected with HIV, 370 (2014) N. Engl. J. Med. 901.
99 *Sander/Joung* (n. 68), 348.

taching to specific DNA sequences).[100] Here, the DNA-binding domain is derived from naturally occurring TALE proteins that are secreted by the *Xanthomonas* bacteria. These proteins possess DNA-binding domains composed of series of *amino-acid repeats* that each recognize a single base pair.[101] Like zinc fingers, several TALE repeats can be linked together to recognize continuous DNA sequences. TALENs are equally efficient as ZFNs but relatively easier to design.[102] Therefore, the new technique was quickly adopted by a broad range of scientists after it had been developed in 2011.[103] Since then, TALENs were applied to edit the genome of numerous organisms.[104]

3. CRISPR-Cas

CRISPR/Cas9 was discovered as a novel technique for genome editing in 2012. CRISPR denotes adaptive immune systems used by prokaryotes (i.e. bacteria and archaea) to defend themselves against viruses and other foreign DNA elements.[105] These mechanisms memorize the genetic characteristics of past invaders and, when they intrude again, recognize and

100 Cf. *Jens Boch* et al., Breaking the Code of DNA Binding Specificity of TAL-Type III Effectors, 326 (2009) Science 1509; *Matthew J. Moscou/Adam J. Bogdanove*, A Simple Cipher Governs DNA Recognition by TAL Effectors, 326 (2009) Science 1501; see *J. K. Joung/Jeffry D. Sander*, TALENs, 14 (2013) Nature Reviews Molecular Cell Biology 49, 49.

101 *Gaj* et al. (n. 73), 399.

102 *Joung/Sander* (n. 100), 49; *Sander/Joung* (n. 68), 348.

103 *Joung/Sander* (n. 100), 49.

104 See e.g. *Sanyuan Ma* et al., Highly Efficient and Specific Genome Editing in Silkworm Using Custom TALENs, 7 (2012) PLOS ONE e45035; *Xiao* et al. (n. 75); *Kulbhushan Chaudhary* et al., Transcription Activator-like Effector Nucleases (TALENs), 16 (2016) Engineering in Life Sciences 330, 334–335.

105 Approximately 46 % of bacteria and 90 % of archaea carry CRISPR loci in their genomes. Despite their similarity in role and function, there are many different CRISPR systems that are extremely variable in characteristics such as genetic locus, protein composition, RNA processing, and effector complex structure. The variety of natural CRISPR systems can be harnessed for various genome editing purposes. See *Philippe Horvath* et al., Applications of the Versatile CRISPR-Cas Systems, in: Rodolphe Barrangou/John van der Oost (eds.), CRISPR-Cas Systems (2013) 267; *Eugene V. Koonin* et al., Diversity, Classification and Evolution of CRISPR-Cas Systems, 37 (2017) Current Opinion in Microbiology 67.

destroy them.[106] When a prokaryote is first infected by a virus, it integrates short fragments of the viral DNA into special regions of its own genome. These regions are called CRISPR (from *clustered regularly interspaced short palindromic repeats*).[107] The CRISPR array is then continuously transcribed into RNA snippets called crRNA (from *CRISPR RNAs*).[108] These crRNAs combine with CRISPR-associated (*Cas*) proteins that can cleave DNA to form an *effector complex*. If at some point the same virus enters the cell again, the crRNA will immediately bind to its corresponding sequence in the viral DNA. Subsequently, the associated Cas protein will cleave and thereby destroy the viral DNA.[109]

The discovery of CRISPR-Cas as a genome editing tool was preceded by two decades of research into natural CRISPR systems.[110] When the occurrence of CRISPR sequences in prokaryotes was first discovered in the genome of the bacterium *Escherichia coli* in 1987,[111] the function of these sequences was still unclear.[112] In 2005, a systematic analysis of CRISPR arrays revealed that they are derived from foreign genetic elements[113] and that viruses are unable to infect prokaryotes carrying DNA sequences cor-

106 *Emmanuelle Charpentier* et al., CrRNA Biogenesis, in: Rodolphe Barrangou/John van der Oost (eds.), CRISPR-Cas Systems (2013) 115, 137.

107 *Albers* et al. (n. 1), 434.

108 *Ibid.*

109 *Pierce* (n. 1), 574–575.

110 See *Eric S. Lander*, The Heroes of CRISPR, 164 (2016) Cell 18. Note that this article has been criticized for not adequately representing the share of some researchers in the discovery of CRISPR/Cas9 as a genome editing technique, cf. *Heidi Ledford*, The Unsung Heroes of CRISPR, 535 (2016) Nature News 342; *Tracy Vence*, "Heroes of CRISPR" Disputed, The Scientist, 19 January 2016, available at: https://www.the-scientist.com/?articles.view/articleNo/45119/title/-Heroes-of-CRISPR--Disputed/ (last accessed 28 May 2022).

111 *Yoshizumi Ishino* et al., Nucleotide Sequence of the {i}ap Gene, Responsible for Alkaline Phosphatase Isozyme Conversion in Escherichia Coli, and Identification of the Gene Product, 169 (1987) Journal of Bacteriology 5429.

112 *Patrick D. Hsu* et al., Development and Applications of CRISPR-Cas9 for Genome Engineering, 157 (2014) Cell 1262.

113 *Francisco J. Mojica* et al., Intervening Sequences of Regularly Spaced Prokaryotic Repeats Derive from Foreign Genetic Elements, 60 (2005) Journal of Molecular Evolution 174; *C. Pourcel* et al., CRISPR Elements in Yersinia Pestis Acquire New Repeats by Preferential Uptake of Bacteriophage DNA, and Provide Additional Tools for Evolutionary Studies, 151 (2005) Microbiology 653; *Alexander Bolotin* et al., Clustered Regularly Interspaced Short Palindrome Repeats (CRISPRs) Have Spacers of Extrachromosomal Origin, 151 (2005) Microbiology 2551.

responding to their own genomes.[114] Two years later, experiments showed that CRISPR acts as an adaptive immunity system in which Cas enzymes control both the acquisition of spacers (i.e. the insertion of non-coding viral DNA into the prokaryote's own genome) and the defence against intruding foreign DNA.[115] In the following years, many further details were revealed[116] and the first steps to rebuilding the CRISPR-Cas9 nuclease system were taken.[117] In the course of these efforts, a third essential component of the CRISPR-Cas9 system was discovered: so-called *trans-activating crRNA* (*tracRNA*) facilitates the generation of crRNAs,[118] but it also has an auxiliary role in nuclease activity by keeping the Cas protein active.[119]

In 2012, two research groups made substantial discoveries that led to the use of CRISPR as a genome editing tool. Both groups demonstrated that Cas9 protein derived from bacteria of the *Streptococcus* genus is able to cleave purified DNA *in vitro*. They also showed the Cas9 protein can be 'programmed' to cleave DNA at specific sites by providing an engineered crRNA that contains the target sequence.[120] In addition, one of the groups constructed a single *guide RNA (sgRNA)* by fusing the engineered crRNA with tracrRNA (which, as mentioned above, supports the cleavage of DNA

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114 *Mojica* et al. (n. 113), 180.

115 *Rodolphe Barrangou* et al., CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes, 315 (2007) Science 1709.

116 See *Hsu* et al. (n. 112), 1266; *Luciano A. Marraffini*, CRISPR-Cas Immunity in Prokaryotes, 526 (2015) Nature 55. Important publications include: *Hélène Deveau* et al., Phage Response to CRISPR-Encoded Resistance in Streptococcus Thermophilus, 190 (2008) Journal of Bacteriology 1390; *Philippe Horvath* et al., Comparative Analysis of CRISPR Loci in Lactic Acid Bacteria Genomes, 131 (2009) International Journal of Food Microbiology 62; *Andrea Quiberoni* et al., Streptococcus Thermophilus Bacteriophages, 20 (2010) International Dairy Journal 657; *Josiane E. Garneau* et al., The CRISPR/Cas Bacterial Immune System Cleaves Bacteriophage and Plasmid DNA, 468 (2010) Nature 67; *Rimantas Sapranauskas* et al., The Streptococcus Thermophilus CRISPR/Cas System Provides Immunity in Escherichia Coli, 39 (2011) Nucleic Acids Res. 9275.

117 See, *inter alia*, *Garneau* et al. (n. 116); *Elitza Deltcheva* et al., CRISPR RNA Maturation by Trans-Encoded Small RNA and Host Factor RNase III, 471 (2011) Nature 602, 602–603.

118 *Deltcheva* et al. (n. 117), 602–603.

119 *Martin Jinek* et al., A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity, 337 (2012) Science 816, 816.

120 *Giedrius Gasiunas* et al., Cas9–crRNA Ribonucleoprotein Complex Mediates Specific DNA Cleavage for Adaptive Immunity in Bacteria, 109 (2012) PNAS E2579–86, E2583; *Jinek* et al. (n. 119), 817.

by keeping the Cas protein active).[121] With the development of sgRNAs, only two components were required for genome editing, namely a Cas protein and a customized sgRNA in which the target sequence is 'programmed'. This meant a major breakthrough in harnessing CRISPR-Cas9 for genome editing.[122] Shortly after, two simultaneous studies demonstrated that CRISPR need not be limited to bacteria, but can also be applied to eukaryotes, in particular to mammals such as mice and humans.[123] Furthermore, it was shown that multiple guide RNAs can be used to induce multiple double-strand breaks in one single step.[124]

Since its discovery, the CRISPR/Cas9 genome editing technique was rapidly adopted by many commercial and non-commercial researchers. It widely replaced other genome editing techniques such as TALENs, since CRISPR is said to be more precise, easier to apply and cheaper to prepare.[125] Further refinements of the technique are published constantly at the time of writing. For instance, different Cas proteins can be used to achieve different cleavage characteristics.[126] Another example is the so-called *base editing* approaches, which aim at exchanging single bases in RNA[127] or base pairs in DNA[128] without cleaving the nucleotide chain.[129] In 2020, *Emmanuelle Charpentier* and *Jennifer Doudna* were awarded the Nobel Prize in Chemistry 'for the development of a method for genome editing'.[130]

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121 *Jinek* et al. (n. 119), 819–820.
122 *Heidi Ledford*, CRISPR, the Disruptor, 522 (2015) Nature 20, 23.
123 *Cong* et al. (n. 75); *Prashant Mali* et al., RNA-Guided Human Genome Engineering via Cas9, 339 (2013) Science 823.
124 *Cong* et al. (n. 75); *Mali* et al. (n. 123).
125 *Ledford* (n. 122), 21–22.
126 *Bernd Zetsche* et al., Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System, 163 (2015) Cell 759; *Guocai Zhong* et al., Cpf1 Proteins Excise CRISPR RNAs from MRNA Transcripts in Mammalian Cells, 13 (2017) Nature Chemical Biology 839; see also *Heidi Ledford*, Alternative CRISPR System Could Improve Genome Editing, 526 (2015) Nature News 17.
127 *David B. T. Cox* et al., RNA Editing with CRISPR-Cas13, 358 (2017) Science 1019.
128 *Nicole M. Gaudelli* et al., Programmable Base Editing of A•T to G•C in Genomic DNA Without DNA Cleavage, 551 (2017) Nature 464.
129 See *Emily Mullin*, CRISPR 2.0 Is Here, and It's Way More Precise, MIT Technology Review, 25 October 2017, available at: https://www.technologyreview.com/s/609203/crispr-20-is-here-and-its-way-more-precise/ (last accessed 28 May 2022).
130 Royal Swedish Academy of Sciences, The Nobel Prize in Chemistry 2020 (07 October 2020), available at: https://www.kva.se/en/pressrum/pressmeddelanden/nobelpriset-i-kemi-2020 (last accessed 28 May 2022).

III. Applications of Genome Editing Techniques

Genome editing techniques, especially those using the CRISPR-Cas method, are extremely versatile and can be applied in all areas of molecular biology. Prospective and already-existing applications can be found, *inter alia*, in agriculture (1.), basic research and medicine (2.), approaches to modify the human genome (3.), and industrial biotechnology (4.).

1. Agriculture

Like conventional genetic engineering techniques, genome editing is widely used in agriculture, where it can be applied to either directly incorporate heritable mutations or to accelerate conventional breeding. In livestock breeding, for example, genome editing is applied to improve traits relevant to the quality and quantity of animal products such as milk, meat, and wool.[131] It can also be used to increase animal health and welfare, for instance by breeding variants that are resistant to certain diseases.[132] The development of hornless dairy cattle variants could spare calves the pain- and stressful dehorning commonly practised in industrial livestock farming.[133] Moreover, the organs of pigs are modified through genome editing with the aim of making their organs usable for pig-to-human transplantations.[134]

Besides livestock, genome editing is extensively used to improve crop plants.[135] For instance, the genes encoding for *polyphenol oxidase* (PPO), an enzyme that causes browning to fruit and vegetables when cut or bruised,

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131 *Iuri V. Perisse* et al., Improvements in Gene Editing Technology Boost Its Applications in Livestock, 11 (2020) Frontiers in Genetics 614688, 8–11; *Abdul Jabbar* et al., Advances and Perspectives in the Application of CRISPR-Cas9 in Livestock, 63 (2021) Molecular Biotechnology 757, 760–762.

132 *Perisse* et al. (n. 131), 11; cf. *Kristin M. Whitworth* et al., Gene-Edited Pigs Are Protected from Porcine Reproductive and Respiratory Syndrome Virus, 34 (2016) Nature Biotech. 20.

133 Cf. *Daniel F. Carlson* et al., Production of Hornless Dairy Cattle from Genome-Edited Cell Lines, 34 (2016) Nature Biotech. 479; *Felix Schuster* et al., CRISPR/Cas12a Mediated Knock-in of the Polled Celtic Variant to Produce a Polled Genotype in Dairy Cattle, 10 (2020) Sci. Rep. 13570.

134 *Peter J. Cowan* et al., Xenogeneic Transplantation and Tolerance in the Era of CRISPR-Cas9, 24 (2019) Current Opinion in Organ Transplantation 5.

135 *Reema Rani* et al., CRISPR/Cas9, 38 (2016) Biotechnology Letters 1991; *Ming Luo* et al., Applications of CRISPR/Cas9 Technology for Targeted Mutagenesis,

were successfully knocked out in various species.[136] Genome editing also allows to confer or improve the resistance of plants to diseases,[137] insect pests,[138] or drought stress.[139] Another important field of application lies in conferring herbicide resistance to various crop plants.[140] Furthermore, genome editing can serve to improve the nutritious characteristics of food crops.[141] One approach aims to produce bread wheat with lower levels of gluten immunogenicity that can be consumed by people suffering from celiac disease.[142]

Like in conventional genetic engineering, the CRISPR-Cas components can be introduced into the target organism by using vectors such as the plant pest bacterium *Agrobacterium tumefaciens* or viral plasmids that encode them.[143] The use of vectors involves the introduction of foreign genetic elements into the target organism, which either are not incorporated into the plant's genome or can later be removed.[144] In many jurisdictions,

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Gene Replacement and Stacking of Genes in Higher Plants, 35 (2016) Plant Cell Reports 1439.

136 *Norfadilah Hamdan* et al., Prevention of Enzymatic Browning by Natural Extracts and Genome-Editing: A Review on Recent Progress, 27 (2022) Molecules 1101.

137 *Giuseppe Andolfo* et al., Genome-Editing Technologies for Enhancing Plant Disease Resistance, 7 (2016) Front. Plant Sci. 1813; *Naghmeh Nejat* et al., Plant-Pathogen Interactions, 37 (2017) Critical Reviews in Biotechnology 229.

138 *Shaily Tyagi* et al., Genome Editing for Resistance to Insect Pests: An Emerging Tool for Crop Improvement, 5 (2020) ACS Omega 20674.

139 *Damiano Martignago* et al., Drought Resistance by Engineering Plant Tissue-Specific Responses, 10 (2019) Frontiers in Plant Science 1676; *Abdul Sami* et al., CRISPR-Cas9-Based Genetic Engineering for Crop Improvement Under Drought Stress, 12 (2021) Bioengineered 5814.

140 *Huirong Dong* et al., The Development of Herbicide Resistance Crop Plants Using CRISPR/Cas9-Mediated Gene Editing, 12 (2021) Genes 912; *Amjad Hussain* et al., Herbicide Resistance: Another Hot Agronomic Trait for Plant Genome Editing, 10 (2021) Plants 621.

141 *Kathleen L. Hefferon*, Nutritionally Enhanced Food Crops; Progress and Perspectives, 16 (2015) International Journal of Molecular Sciences 3895; *Yongwei Sun* et al., Generation of High-Amylose Rice Through CRISPR/Cas9-Mediated Targeted Mutagenesis of Starch Branching Enzymes, 8 (2017) Front. Plant Sci. 298.

142 *Aurelie Jouanin* et al., CRISPR/Cas9 Gene Editing of Gluten in Wheat to Reduce Gluten Content and Exposure–Reviewing Methods to Screen for Coeliac Safety, 7 (2020) Frontiers in Nutrition 51.

143 See *Zheng Gong* et al., Non-GM Genome Editing Approaches in Crops, 3 (2021) Frontiers in Genome Editing 817279, 2.

144 *Je W. Woo* et al., DNA-Free Genome Editing in Plants with Preassembled CRISPR-Cas9 Ribonucleoproteins, 33 (2015) Nature Biotech. 1162, 1162; *San-*

however, the regulatory regime for GMOs is already triggered by the transient presence of transgenic elements in the organism.[145] Researchers are therefore developing methods for editing plant genomes without introducing foreign DNA.[146] For instance, preassembled gRNA-Cas9 protein complexes (so-called *ribonucleoproteins*) can be delivered to the plant cell by vector-less methods such as direct injection or by transfection.[147] These complexes cleave their chromosomal target sites immediately after entering the cell and rapidly degrade afterwards.[148]

2. Basic Research and Medicine

The CRISPR-Cas technique may also serve as an important tool in basic research and medicine.[149] For instance, CRISPR-Cas can serve as a tool for genome-wide screens, including for genes involved in tumour growth and metastasis.[150] In medical research, genome editing can be used to generate disease models, such as for human lung cancer in mice,[151] which might accelerate the identification of suitable therapies.[152] Researchers were also able to recreate a naturally occurring mutation that provides innate resistance to HIV.[153] Another study successfully corrected an inherited mutation in mice and thus cured the metabolic disease *tyrosinemia*.[154] Moreover, the original function of CRISPR as an immune system could

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wen Huang et al., A Proposed Regulatory Framework for Genome-Edited Crops, 48 (2016) Nature Genetics 109, 109.

145 See chapter 3, sections A.I.1 and A.IV.2.

146 *Chidananda N. Kanchiswamy* et al., Non-GMO Genetically Edited Crop Plants, 33 (2015) Trends in Biotechnology 489; *Gong* et al. (n. 143), 2–8.

147 *Woo* et al. (n. 144); *Gong* et al. (n. 143), 4–6.

148 *Woo* et al. (n. 144), 1162; see *Sojung Kim* et al., Highly Efficient RNA-Guided Genome Editing in Human Cells via Delivery of Purified Cas9 Ribonucleoproteins, 24 (2014) Genome Research 1012.

149 See *Barrangou/Doudna* (n. 82).

150 *Ophir Shalem* et al., High-Throughput Functional Genomics Using CRISPR–Cas9, 16 (2015) Nature Rev. Genet. 299.

151 *Andrea Ventura* et al., In Vivo Engineering of Oncogenic Chromosomal Rearrangements with the CRISPR/Cas9 System, 516 (2014) Nature 423.

152 *Barrangou/Doudna* (n. 82), 936.

153 *Pankaj K. Mandal* et al., Efficient Ablation of Genes in Human Hematopoietic Stem and Effector Cells Using CRISPR/Cas9, 15 (2014) Cell Stem Cell 643.

154 *Hao Yin* et al., Genome Editing with Cas9 in Adult Mice Corrects a Disease Mutation and Phenotype, 32 (2014) Nature Biotech. 551.

also be harnessed to develop new antimicrobial and antiviral applications that might be able to replace conventional drugs such as antibiotics.[155]

Various approaches using genome editing for therapeutic purposes in humans have already advanced to clinical trials.[156] Some studies seek to treat cancer by editing the immune cells of patients *in vitro*, selecting and expanding cells which contain the desired modification, and infusing these cells back into the patient.[157] Another promising application is *gene therapy*, in which genetic disorders are corrected to treat diseases that cannot be cured with conventional therapies.[158] In 2019, CRISPR was successfully used to treat humans suffering from the genetic disorder *sickle-cell anaemia*.[159] In 2020, CRISPR was used for the first time to edit genetic information in a human *in vivo* in an attempt to treat the heritable eye disease *Leber congenital amaurosis*.[160]

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155 *Chase L. Beisel* et al., A CRISPR Design for Next-Generation Antimicrobials, 15 (2014) Genome Biology 516; *Robert J. Citorik* et al., Sequence-Specific Antimicrobials Using Efficiently Delivered RNA-Guided Nucleases, 32 (2014) Nature Biotech. 1141; *David Bikard* et al., Exploiting CRISPR-Cas Nucleases to Produce Sequence-Specific Antimicrobials, 32 (2014) Nature Biotech. 1146; *Ahmed A. Gomaa* et al., Programmable Removal of Bacterial Strains by Use of Genome-Targeting CRISPR-Cas Systems, 5 (2014) mBio e00928–13; see generally *Barrangou/Doudna* (n. 82), 937–938.

156 *Barrangou/Doudna* (n. 82), 937; *Filipe V. Jacinto* et al., CRISPR/Cas9-Mediated Genome Editing: From Basic Research to Translational Medicine, 24 (2020) Journal of Cellular and Molecular Medicine 3766; *Matthew P. Hirakawa* et al., Gene Editing and CRISPR in the Clinic: Current and Future Perspectives, 40 (2020) Bioscience Reports.

157 *Hirakawa* et al. (n. 156), 4–11; *Jacinto* et al. (n. 156), 3768–3769.

158 *Jacinto* et al. (n. 156), 3771–3774.

159 *Rob Stein*, In a 1st, Doctors in U.S. Use CRISPR Tool to Treat Patient with Genetic Disorder, NPR, 29 July 2019, available at: https://www.npr.org/sections/health-shots/2019/07/29/744826505/sickle-cell-patient-reveals-why-she-is-volunteering-for-landmark-gene-editing-st?t=1617188222805 (last accessed 28 May 2022); *Heidi Ledford*, CRISPR Gene Therapy Shows Promise Against Blood Diseases, 588 (2020) Nature 383; see *Haydar Frangoul* et al., CRISPR-Cas9 Gene Editing for Sickle Cell Disease and B-Thalassemia, 384 (2021) N. Engl. J. Med. 252; *Erica B. Esrick* et al., Post-Transcriptional Genetic Silencing of BCL11A to Treat Sickle Cell Disease, 384 (2021) N. Engl. J. Med. 205.

160 *Rob Stein*, In a 1st, Scientists Use Revolutionary Gene-Editing Tool to Edit Inside a Patient, NPR, 04 March 2020, available at: https://www.npr.org/sections/health-shots/2020/03/04/811461486/in-a-1st-scientists-use-revolutionary-gene-editing-tool-to-edit-inside-a-patient (last accessed 28 May 2022); *Heidi Ledford*, CRISPR Treatment Inserted Directly into the Body for First Time, 579 (2020) Nature 185.

3. Human Germline Editing

The therapeutic applications mentioned above aim at editing somatic cells, i.e. body cells whose genetic information is not heritable.[161] Basic research studies usually work with embryonic or post-embryonic stem cells that cannot develop into viable organisms.[162] However, genome editing can also be applied to modify the genes of reproductive germline cells or fertilized egg cells (*zygotes*), including early human embryos.[163] Researchers have already demonstrated the use of CRISPR-Cas in human embryos in a number of studies.[164]

In November 2018, it was revealed that a Chinese biophysicist had used CRISPR to edit the genomes of embryos in an attempt to confer genetic resistance to HIV.[165] While the researcher claimed that the babies were born healthy,[166] some contended that the genetic modification could have life-shortening effects.[167] The undertaking was widely condemned[168] and

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161 Cf. *Mali* et al. (n. 123); see *supra* section B.III.2.

162 *Zhao Zhang* et al., CRISPR/Cas9 Genome-Editing System in Human Stem Cells, 9 (2017) Molecular Therapy – Nucleic Acids 230; *Jacinto* et al. (n. 156), 3769–3770.

163 Cf. *R. Vassena* et al., Genome Engineering Through CRISPR/Cas9 Technology in the Human Germline and Pluripotent Stem Cells, 22 (2016) Human Reproduction Update 411.

164 *Puping Liang* et al., CRISPR/Cas9-Mediated Gene Editing in Human Tripronuclear Zygotes, 6 (2015) Protein & Cell 363; *Xiangjin Kang* et al., Introducing Precise Genetic Modifications into Human 3PN Embryos by CRISPR/Cas-Mediated Genome Editing, 33 (2016) Journal of Assisted Reproduction and Genetics 581; *Lichun Tang* et al., CRISPR/Cas9-Mediated Gene Editing in Human Zygotes Using Cas9 Protein, 292 (2017) Molecular Genetics and Genomics 525; *Hong Ma* et al., Correction of a Pathogenic Gene Mutation in Human Embryos, 548 (2017) Nature 413.

165 *Antonio Regalado*, Exclusive: Chinese Scientists Are Creating CRISPR Babies, MIT Technology Review, 25 November 2018, available at: https://www.technologyreview.com/2018/11/25/138962/exclusive-chinese-scientists-are-creating-crispr-babies/ (last accessed 28 May 2022).

166 *He Jiankui*, About Lulu and Nana: Twin Girls Born Healthy After Gene Surgery as Single-Cell Embryos (31 March 2021), available at: https://www.youtube.com/watch?v=th0vnOmFltc (last accessed 28 May 2022).

167 *Jon Cohen*, Did CRISPR Help – Or Harm – The First-Ever Gene-Edited Babies?, Science News, 01 August 2019, available at: https://www.sciencemag.org/news/2019/08/did-crispr-help-or-harm-first-ever-gene-edited-babies (last accessed 28 May 2022).

168 See *Natalie Kofler*, Why Were Scientists Silent over Gene-Edited Babies?, 566 (2019) Nature 427; *Jon Cohen*, Inside the Circle of Trust, 365 (2019) Science 430;

the responsible scientist was later sentenced to prison for 'illegal medical practice'.[169]

4. Industrial Biotechnology

It is assumed that genome editing based on the CRISPR-Cas technique will have a large impact on all industries related to bacteria, fungi, and yeast.[170] For instance, engineered versions of these organisms may help to produce biofuels[171] or chemicals required for antibiotics.[172] In the food industry, genome editing may be used to improve fermentation-based manufacturing, e.g. by vaccinating useful bacteria against phages or by depleting certain microbial populations while preserving others.[173]

IV. Technical Challenges of CRISPR-Cas Based Genome Editing

Although the CRISPR-Cas technique quickly became the prevalent technique for genome editing, it still involves a number of technical challenges affecting both the efficacy and the safety of the techniques. These challenges include the potential for off-target effects (1.), genetic mosaicism (2.), and the delivery of the CRISPR components into the target organism (3.).

1. Off-Target Effects

A major challenge in the application of CRISPR for genome editing is potential *off-target effects*, i.e. the introduction of double-strand breaks

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Karen M. Meagher et al., Reexamining the Ethics of Human Germline Editing in the Wake of Scandal, 95 (2020) Mayo Clinic Proceedings 330.

169 *David Cyranoski*, What CRISPR-Baby Prison Sentences Mean for Research, 577 (2020) Nature 154.

170 *Barrangou/Doudna* (n. 82), 938.

171 Cf. *Owen W. Ryan* et al., Selection of Chromosomal DNA Libraries Using a Multiplex CRISPR System, 3 (2014) eLife e03703; *Ching-Sung Tsai* et al., Rapid and Marker-free Refactoring of Xylose-fermenting Yeast Strains with Cas9/CRISPR, 112 (2015) Biotechnology and Bioengineering 2406.

172 *He Huang* et al., One-Step High-Efficiency CRISPR/Cas9-Mediated Genome Editing in Streptomyces, 47 (2015) Acta Biochimica et Biophysica Sinica 231.

173 Cf. *Kurt Selle/Rodolphe Barrangou*, CRISPR-Based Technologies and the Future of Food Science, 80 (2015) Journal of Food Science R2367, R2370-R2371.

at other than the desired location, which might lead to unwanted mutations. One reason for this is that Cas proteins have a certain tolerance for mismatches between the introduced guide RNA and the target DNA sequence.[174] Furthermore, complex genomes often contain multiple copies of sequences that are identical or highly similar to the intended DNA target.[175]

The frequency of off-target effects depends on many factors and varies among cell types.[176] Algorithms can help anticipate the locations of off-target mutations.[177] However, there appears to be no scientific consensus about the general likelihood and extent of off-target effects. A publication reporting unexpected mutations in mice after CRISPR-Cas9 was applied to edit their genome *in vivo*[178] was criticized for using an insufficient experimental design and wrongly interpreting data.[179] As noted above, there have also been concerns about the safety of human germline editing using CRISPR-Cas9.[180] In any case, many researchers are seeking to increase the precision of CRISPR,[181] including by identifying alternative

174 *Seung W. Cho* et al., Analysis of Off-Target Effects of CRISPR/Cas-Derived RNA-Guided Endonucleases and Nickases, 24 (2014) Genome Research 132, 134; *Xiao-Hui Zhang* et al., Off-Target Effects in CRISPR/Cas9-Mediated Genome Engineering, 4 (2015) Molecular Therapy – Nucleic Acids e264, 1.

175 *Gaj* et al. (n. 73), 400.

176 *Sander/Joung* (n. 68), 349–350; *Zhang* et al. (n. 174), 3.

177 See *Maximilian Haeussler* et al., Evaluation of Off-Target and On-Target Scoring Algorithms and Integration into the Guide RNA Selection Tool CRISPOR, 17 (2016) Genome Biology 148; *Hong Zhou* et al., Whole Genome Analysis of CRISPR Cas9 SgRNA Off-Target Homologies via an Efficient Computational Algorithm, 18 (2017) BMC Genomics 826.

178 *Kellie A. Schaefer* et al., Unexpected Mutations After CRISPR-Cas9 Editing in Vivo, 14 (2017) Nature Methods 547.

179 *Christopher J. Wilson* et al., Response to "Unexpected Mutations After CRISPR-Cas9 Editing in Vivo", 15 (2018) Nature Methods 236; *Caleb A. Lareau* et al., Response to "Unexpected Mutations After CRISPR-Cas9 Editing in Vivo", 15 (2018) Nature Methods 238.

180 *Michael V. Zuccaro* et al., Allele-Specific Chromosome Removal After Cas9 Cleavage in Human Embryos, 183 (2020) Cell 1650–1664.e15.

181 *Muhammad Naeem* et al., Latest Developed Strategies to Minimize the Off-Target Effects in CRISPR-Cas-Mediated Genome Editing, 9 (2020) Cells, 3–9; *Manuel M. Vicente* et al., The Off-Targets of Clustered Regularly Interspaced Short Palindromic Repeats Gene Editing, 9 (2021) Frontiers in Cell and Developmental Biology 718466; cf. *William T. Garrood* et al., Analysis of Off-Target Effects in CRISPR-Based Gene Drives in the Human Malaria Mosquito, 118 (2021) PNAS.

Cas proteins.[182] Furthermore, researchers work on developing methods to identify off-target mutations more efficiently.[183]

2. Genetic Mosaicism

Another challenge of applying the CRISPR-Cas technique in multicellular embryos or whole organisms lies in the potential creation of *genetic mosaics*, which denotes the simultaneous presence of wild-type cells and modified cells in the resulting organism.[184] The reason for this phenomenon is that CRISPR-Cas is not 100 % efficient, which means that the desired mutation may not occur equally in all cells of the organism.[185] Genetic mosaics may lead to major phenotypic changes or cause the expression of lethal genetic mutations.[186]

3. In Vivo Delivery of CRISPR-Cas Components

There are many different methods for delivering the CRISPR components into the cell, depending on the organism and other particular circumstances.[187] Most methods do not insert the CRISPR components themselves into the organism, but rather genetic elements encoding for sgRNA and a Cas protein.[188] While delivery of these elements into cell cultures *in*

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182 *Naeem* et al. (n. 181), 9–12; see *Zetsche* et al. (n. 126); *Sergey Shmakov* et al., Discovery and Functional Characterization of Diverse Class 2 CRISPR-Cas Systems, 60 (2015) Molecular Cell 385; *Zhong* et al. (n. 126); see also *Barrangou/Doudna* (n. 82), 934.

183 *Zhang* et al. (n. 174), 4–5; *Naeem* et al. (n. 181), 3–6.

184 *Araki* et al. (n. 71), 234; *Maryam Mehravar* et al., Mosaicism in CRISPR/Cas9-Mediated Genome Editing, 445 (2019) Developmental Biology 156, 156–159; see *Shuo-Ting Yen* et al., Somatic Mosaicism and Allele Complexity Induced by CRISPR/Cas9 RNA Injections in Mouse Zygotes, 393 (2014) Developmental Biology 3; *Uros Midic* et al., Quantitative Assessment of Timing, Efficiency, Specificity and Genetic Mosaicism of CRISPR/Cas9-Mediated Gene Editing of Hemoglobin Beta Gene in Rhesus Monkey Embryos, 26 (2017) Human Molecular Genetics 2678.

185 *Pierce* (n. 1), 577.

186 *Hagop Youssoufian/Reed E. Pyeritz*, Human Genetics and Disease, 3 (2002) Nature Rev. Genet. 748; see *Ma* et al. (n. 104), 2–4.

187 *Sander/Joung* (n. 68), 352–353.

188 Cf. *Alexis C. Komor* et al., CRISPR-Based Technologies for the Manipulation of Eukaryotic Genomes, 168 (2017) Cell 20, 27.

vitro is comparatively easy to accomplish, delivery *in vivo*, i.e. the insertion into cells of living host organisms, remains a challenging task.[189] These challenges include the limited carrying capacity of vectors, their immunogenicity (i.e. CRISPR components engaging a response by the host organism's immune system) and the limited efficiency of delivery and editing, which is significantly lower compared to *in vitro* editing.[190] Therefore, other approaches like non-viral vectors[191] and protein-based delivery, in which a preassembled Cas9-sgRNA complex is directly inserted into the organism by various methods,[192] are being developed.

V. Environmental Risks and Ethical Concerns Connected to the Use of Genome Editing

Aside from the aforementioned technical challenges, the use of genome editing faces several other criticism and concerns, particularly in the context of agricultural uses (1.) and when applied to humans (2.).

1. Alleged Environmental Risks of Genome Editing in Agriculture

Regarding commercial applications including in agriculture, critics primarily point to the general limitations of genome editing techniques pointed out above.[193] In particular, it is argued that genome editing techniques are prone to inducing off-target mutations that, similar to conventional genetic engineering methods, might lead to unintended effects including the accumulation of toxins and residues, and an increase in allergens.[194]

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189 *Pierce* (n. 1), 577.

190 *Rubul Mout* et al., In Vivo Delivery of CRISPR/Cas9 for Therapeutic Gene Editing, 28 (2017) Bioconjugate Chemistry 880, 882–883.

191 *Ling Li* et al., Challenges in CRISPR/CAS9 Delivery, 26 (2015) Human Gene Therapy 452; *Sander/Joung* (n. 68), 352.

192 *Mout* et al. (n. 190), 880–882; cf. *Ming Wang* et al., Efficient Delivery of Genome-Editing Proteins Using Bioreducible Lipid Nanoparticles, 113 (2016) PNAS 2868.

193 See *supra* section B.IV.

194 *Sarah Z. Agapito-Tenfen/Odd-Gunnar Wikmark*, Current Status of Emerging Technologies for Plant Breeding: Biosafety and Knowledge Gaps of Site Directed Nucleases and Oligonucleotide-Directed Mutagenesis, Biosafety report 02/2015 (2015), 32; *Ricarda A. Steinbrecher*, Genetic Engineering in Plants and

It is furthermore contended that the primary aim of many efforts was to avoid the existing regulatory processes for GMOs,[195] even though the risks and uncertainties related to genome-edited organisms were similar to those relating to products of conventional genetic engineering, such as increased toxicity of the resulting organism or horizontal gene transfer to native species, which may have unintended consequences for ecosystems and biological diversity.[196] Others claim that organisms containing no transgenic elements did not give rise to any new type of risks that would require governance beyond the existing regulation of new traits.[197]

Notably, many of the potential environmental impacts of conventional GMOs recognized in scientific literature appear to be related to the presence of transgenes in these organisms[198] and would thus not be caused by

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the "New Breeding Techniques (NBTs)" (2015), 3, but see *Miguel A. Sánchez/Wayne A. Parrott*, Characterization of Scientific Studies Usually Cited as Evidence of Adverse Effects of GM Food/Feed, 15 (2017) Plant Biotechnology Journal 1227.

195 *Steinbrecher* (n. 194), 1; see *Woo* et al. (n. 144), 1162 who assume that: 'Editing plant genomes without introducing foreign DNA into cells may alleviate regulatory concerns related to genetically modified plants.' Also see *Emily Waltz*, Gene-Edited CRISPR Mushroom Escapes US Regulation, 532 (2016) Nature News 293; *Emily Waltz*, CRISPR-Edited Crops Free to Enter Market, Skip Regulation, 34 (2016) Nature Biotech. 582.

196 *Araki* et al. (n. 71), 236; *Christoph Then/Andreas Bauer-Panskus*, Playing Russian Roulette with Biodiversity: Uncontrolled Applications of Gene Editing Threaten Biodiversity, the Rights of Consumers and Farmers, as Well as the Future of Animal and Plant Breeding (2017), 14–21; *Steinbrecher* (n. 194); also see Conseil d'État, Confédération paysanne et autres, 03 October 2016, N° 388649, para. 28; CJEU, Confédération paysanne et al. v. Premier ministre et al., Judgment of 25 July 2018, C-528/16, para. 48; on the questionable statements of the Court regarding the risk of organisms modified through targeted mutagenesis, see *Felix Beck*, All About that Risk? A (Re-)Assessment of the CJEU's Reasoning in the "Genome Editing" Case, 17 (2019) EurUP 246, 250–251.

197 *Robin Fears*, Assessing the Security Implications of Genome Editing Technology: Report of an International Workshop, Herrenhausen, Germany, 11–13 October 2017 (2018), 13 and 19; *Fyodor D. Urnov* et al., A Call for Science-Based Review of the European Court's Decision on Gene-Edited Crops, 36 (2018) Nature Biotech. 800–802; *Rodolphe Barrangou*, CRISPR Craziness: A Response to the EU Court Ruling, 1 (2018) The CRISPR Journal 251; *Martin Wasmer*, Roads Forward for European GMO Policy, 7 (2019) Front. Bioeng. & Biotechnol. 367, 7.

198 Cf. *Aristidis M. Tsatsakis* et al., Environmental Impacts of Genetically Modified Plants: A Review, 156 (2017) Environmental Research 818; also see *José L. Domingo*, Safety Assessment of GM Plants: An Updated Review of the Scientific Literature, 95 (2016) Food and Chemical Toxicology 12. Furthermore, see

transgene-free edited organisms. However, while it is comparatively easy to identify GMOs containing transgenes, it is often difficult or even impossible to conclusively determine whether an organism that only contains one or several point mutations has occurred naturally or has been modified using genome editing techniques.[199]

2. Risks and Ethical Concerns Relating to Human Genome Editing

The advent of genome editing in humans, particularly in the human germline,[200] has re-fuelled pre-existing ethical debates.[201] It has been warned that therapeutic applications of genome editing in the human germline, such as correcting mutations that give rise to hereditary diseases, could have unpredictable consequences on future generations,[202] in particular in light of the still limited knowledge of human genetics, gene-environment interactions and the interplay of various traits and conditions in

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NASEM, Genetically Engineered Crops (2016), 15, which concluded that there was 'no conclusive evidence of cause-and-effect relationships between GE crops and environmental problems', even though it was admitted that 'the complex nature of assessing long-term environmental changes often made it difficult to reach definitive conclusions'.

199 *Lutz Grohmann* et al., Detection and Identification of Genome Editing in Plants: Challenges and Opportunities, 10 (2019) Frontiers in Plant Science 236.

200 See *supra* section B.III.3.

201 Cf. *David Baltimore* et al., Biotechnology. A Prudent Path Forward for Genomic Engineering and Germline Gene Modification, 348 (2015) Science 36; *Edward Lanphier* et al., Don't Edit the Human Germ Line, 519 (2015) Nature News 410; Leopoldina Nationale Akademie der Wissenschaften et al., The Opportunities and Limits of Genome Editing (2015); *Kelly E. Ormond* et al., Human Germline Genome Editing, 101 (2017) American Journal of Human Genetics 167; German Ethics Council, Intervening in the Human Germline: Opinion (2019); *Sean C. McConnell/Alessandro Blasimme*, Ethics, Values, and Responsibility in Human Genome Editing, 21 (2019) AMA Journal of Ethics E1017–1020; *Seppe Segers/Heidi Mertes*, Does Human Genome Editing Reinforce or Violate Human Dignity?, 34 (2020) Bioethics 33; *Sebastian Schleidgen* et al., Human Germline Editing in the Era of CRISPR-Cas: Risk and Uncertainty, Inter-Generational Responsibility, Therapeutic Legitimacy, 21 (2020) BMC Medical Ethics 87; also see UNESCO General Conference, Universal Declaration on the Human Genome and Human Rights (11 November 1997), Records of the General Conference, 29th session, Vol. 1: Resolutions, p. 41; *Federico Lenzerini*, Biotechnology, Human Dignity and the Human Genome, in: Francesco Francioni/Tullio Scovazzi (eds.), Biotechnology and International Law (2006) 285.

202 *Lanphier* et al. (n. 201), 410.

the human body.[203] Moreover, it was warned that human germline gene editing could pose a substantial risk for aneuploidy.[204]

Beyond that, there are strong concerns of both the scientific community and the general public about non-therapeutic applications, i.e. the theoretical possibility of applying genome editing for human enhancement or eugenic purposes.[205] Therefore, many researchers have called for a global moratorium on human germline editing to discuss the connected scientific, ethical and legal issues.[206] However, there will likely be more instances of genome-edited humans in the future, as shown by a Russian scientist seeking to create a germline modification to prevent a type of hereditary deafness.[207]

In response, the *World Health Organization* (WHO) established a multidisciplinary expert panel which concluded that 'it would be irresponsible at this time for anyone to proceed with clinical applications of human germline genome editing'.[208] The WHO's Director-General stated that 'regulatory authorities in all countries should not allow any further work

203 *Baltimore* et al. (n. 201), 37; *Ormond* et al. (n. 201), 169–171; Leopoldina Nationale Akademie der Wissenschaften et al. (n. 201), 25–26.

204 *Zuccaro* et al. (n. 180).

205 *Lanphier* et al. (n. 201), 410; *Baltimore* et al. (n. 201), 37; *Ormond* et al. (n. 201), 171–172.

206 Cf. *Baltimore* et al. (n. 201); *Lanphier* et al. (n. 201); Leopoldina Nationale Akademie der Wissenschaften et al. (n. 201), 27; *Francis S. Collins*, Statement on NIH Funding of Research Using Gene-Editing Technologies in Human Embryos (28 April 2015), available at: https://www.nih.gov/about-nih/who-we-are/nih-director/statements/statement-nih-funding-research-using-gene-editing-technologies-human-embryos (last accessed 28 May 2022); European Group on Ethics in Science and New Technologies, Statement on Gene Editing (2016), available at: https://ec.europa.eu/info/sites/default/files/research_and_innovation/ege/gene_editing_ege_statement.pdf (last accessed 28 May 2022).

207 *David Cyranoski*, Russian 'CRISPR-Baby' Scientist Has Started Editing Genes in Human Eggs with Goal of Altering Deaf Gene, 574 (2019) Nature 465; see WHO Advisory Committee on Developing Global Standards for Governance and Oversight of Human Genome Editing, Human Genome Editing: As We Explore Options for Global Governance, Caution Must Be Our Watchword (08 November 2019), available at: https://www.who.int/news/item/08-11-2019-human-genome-editing-as-we-explore-options-for-global-governance-caution-must-be-our-watchword (last accessed 28 May 2022).

208 WHO Advisory Committee on Developing Global Standards for Governance and Oversight of Human Genome Editing, Report of the First Meeting (2019), 3.

in this area until its implications have been properly considered'.[209] The WHO also established a *Human Genome Editing Registry* to collect information on clinical trials using human genome editing technologies.[210] The Registry, which covers both somatic and germline clinical trials, lists 133 research projects as of May 2022.[211] In the future, the Registry is planned to also cover research using genome editing technologies on human embryos and germline cells even when there is no attempt to initiate a pregnancy.[212]

C. Engineered Gene Drives

According to the Mendelian *principle of segregation* in sexually reproducing organisms, each of the two parents normally contributes a random half of its genetic information to the genome of their offspring. Consequently, a genetic mutation occurring in only one of the parents is statistically inherited by only half of its offspring. A newly emerged mutation thus spreads rather slowly through a natural population. Whether it can prevail depends on evolutionary factors, particularly on whether it confers a physical or reproductive advantage to the organisms carrying it.[213]

These rules of inheritance and evolution can be circumvented by *gene drives*, which refers to genetic elements that bias inheritance in their favour, resulting in the gene becoming more prevalent in the population over successive generations.[214] In this way, a gene drive can spread through a wild population even if it bears no advantage in evolutionary

209 WHO, Statement on Governance and Oversight of Human Genome Editing (26 July 2019), available at: https://www.who.int/news/item/26-07-2019-statement-on-governance-and-oversight-of-human-genome-editing (last accessed 28 May 2022).

210 WHO, Human Genome Editing Registry, available at: https://www.who.int/groups/expert-advisory-committee-on-developing-global-standards-for-governance-and-oversight-of-human-genome-editing/registry (last accessed 28 May 2022).

211 *Ibid.*

212 *Ibid.*

213 See *supra* section A.II.2.

214 *Luke S. Alphey* et al., Opinion: Standardizing the Definition of Gene Drive, 117 (2020) PNAS 30864.

fitness compared to the wild type allele, hence circumventing the rules of Mendelian inheritance.[215]

While gene drive is a naturally occurring phenomenon (I.), genome editing techniques such as CRISPR-Cas now allow for the development of *engineered* (or *synthetic*) gene drives to genetically modify wild populations of species (II.). Engineered gene drives have a range of prospective applications (III.), although the technique is not without technical limitations and environmental risks (IV.).

I. Natural Gene Drive Mechanisms

Selfish genetic elements are naturally occurring gene drive phenomena that use various molecular mechanisms to bias inheritance in their favour.[216] They typically make use of either of two strategies, namely increasing their own replication (1.) or eliminating competing wild-type gametes or progeny (2.).[217]

1. Over-Replication Mechanisms

Selfish genetic elements relying on *over-replication* bias their transmission to subsequent generations by becoming replicated more often than other genes in the same organism.[218] The most prominent type of over-replication is *transposable elements*, which are DNA elements that are able to change their position within the genome.[219] Their changing presence at random locations in the genome tends to create multiple copies of the

215 *Jackson Champer* et al., Cheating Evolution, 17 (2016) Nature Rev. Genet. 146, 146–147; *Kevin M. Esvelt* et al., Concerning RNA-Guided Gene Drives for the Alteration of Wild Populations, 3 (2014) eLife e03401, 1.

216 For a detailed account of various natural gene drive systems, see the extensive monograph by *Austin Burt/Robert Trivers*, Genes in Conflict (2006); also see (with reference to research on engineered drive systems) NASEM, Gene Drives on the Horizon (2016), 26–30.

217 *Champer* et al. (n. 215), 147. Note that *Burt/Trivers* (n. 216), 5–7 introduce a third strategy called *gonotaxis*, by which they refer to drive systems that bias inheritance by moving preferentially towards the germline, and away from somatic cells, e.g. by distorting meiosis in females (*ibid*., p. 301–324).

218 *Ibid.*, 4.

219 *Ibid.*, 228–300; see *supra* section A.II.1.

same transposable element in the genome,[220] which results in an increased inheritance compared to Mendelian inheritance patterns.[221] Transposable elements are currently not seen to be a feasible vector for engineered gene drives, mainly because they integrate at random, unpredictable loci when moving across the genome.[222]

Another class of gene drive based on over-replication are *homing endonuclease genes*. These genes encode sequence-specific endonucleases that cleave the corresponding DNA sequence in chromosomes lacking them.[223] This triggers the activity of the intra-cellular DNA repair mechanisms that are also utilised for genome editing.[224] If the cut is repaired by *homology-direct repair*, the intact chromosome inhibiting the drive will be used as a template, and the drive components will be copied onto the damaged chromosome along with any genes.[225] However, the application of other repair mechanisms such as *non-homologous end joining* can lead to the formation of resistances against the drive mechanism.[226]

2. Interference Mechanisms

Natural gene drives relying on *interference* increase the frequency in which they are inherited by disrupting the transmission of the alternative, 'wild type' allele.[227] There are various molecular pathways to achieve this. Many systems gain a fitness advantage over the wild type allele by either impeding the viability of the wild-type gametes or by killing progeny that carries the wild type allele.[228] Other mechanisms, called *Meiotic Drive*, bias the

220 *Ibid.*, 231–232.
221 *B. Charlesworth/C. H. Langley*, The Population Genetics of Drosophila Transposable Elements, 23 (1989) Annual Review of Genetics 251.
222 *Malcolm J. Fraser*, Insect Transgenesis, 57 (2012) Annual Review of Entomology 267, 272–273; NASEM, Gene Drives on the Horizon (n. 216), 27.
223 *Austin Burt/Vassiliki Koufopanou*, Homing Endonuclease Genes, 14 (2004) Current Opinion in Genetics & Development 609, 609; NASEM, Gene Drives on the Horizon (n. 216), 27.
224 See *supra* section A.II.1.
225 *Champer* et al. (n. 215), 151.
226 *Ibid.*
227 *Burt/Trivers* (n. 216), 4.
228 *Ibid.; Champer* et al. (n. 215), 147.

transmission of alleles during the segregation of chromosomes in meiosis (i.e. the formation of gametes).[229]

Interference mechanisms can be classified by their goal. So-called *selfish sex chromosomes* distort the sex ratios of the progeny in favour of one of the sexes. For instance, the so-called *X-shredder* mechanism is composed of endonucleases that cleave the female-determining X chromosome during spermatogenesis, leading to a bias towards male progeny.[230] It has been proposed that the X-shredder mechanism could be used to employ gene drives for population suppression, for instance in disease vector and pest control.[231] Other drive elements reverse the sex of their host by converting XY males into females.[232]

The second class of interference mechanisms is *autosomal killers*, which propagate genetic elements located on non-sex-determining (i.e. *autosomal*) chromosomes and have no direct influence on sex ratios. This includes the *Maternal-Effect Dominant Embryonic Arrest* (*Medea*) mechanism found in flour beetles, which is a combination of a maternally-expressed toxin and an antidote expressed by those zygotes that carry the Medea element, leading to the survival of only those zygotes.[233] Other examples are the

229 Note that the term *Meiotic Drive* appears to be used inconsistently. Some authors refer to it as *any* drive mechanism that distorts the rules of Mendelian inheritance (e.g. NASEM, Gene Drives on the Horizon (n. 216), 28; *Shannon R. McDermott/Mohamed A. F. Noor*, The Role of Meiotic Drive in Hybrid Male Sterility, 365 (2010) Philos. Trans. R. Soc. B 1265), while others use the term more narrowly as referring only to those mechanisms that interfere with the process of *meiosis*, i.e. the formation of gametes (*Terence W. Lyttle*, Cheaters Sometimes Prosper, 9 (1993) Trends in Genetics 205; *Champer* et al. (n. 215), 152; 'meiotic drive', in: Henderson's Dictionary of Biology (n. 5), 340).

230 *Burt/Trivers* (n. 216), 60–73; *Austin Burt*, Site-Specific Selfish Genes as Tools for the Control and Genetic Engineering of Natural Populations, 270 (2003) Proc. R. Soc. B 921, 926.

231 *Anne Deredec* et al., The Population Genetics of Using Homing Endonuclease Genes in Vector and Pest Management, 179 (2008) Genetics 2013; *Roberto Galizi* et al., A Synthetic Sex Ratio Distortion System for the Control of the Human Malaria Mosquito, 5 (2014) Nature Comms. 3977.

232 *Burt/Trivers* (n. 216), 78–91.

233 Cf. *R. W. Beeman* et al., Maternal-Effect Selfish Genes in Flour Beetles, 256 (1992) Science 89; *Chun-Hong Chen* et al., A Synthetic Maternal-Effect Selfish Genetic Element Drives Population Replacement in Drosophila, 316 (2007) Science 597; see *Champer* et al. (n. 215), 152–154; *Austin Burt/Andrea Crisanti*, Gene Drive, 13 (2018) ACS Chemical Biology 343, 344.

t-haplotype in mice[234] and the *Segregation Distorter* system in the fruit fly species *Drosophila melanogaster*,[235] which both drive through populations by disabling sperm cells not containing their elements. Similar mechanisms also exist in plants, fungi, and nematodes.[236]

Maternal-effect toxin-antidote systems might be applied as a method to create *underdominance* gene drives.[237] Underdominance, or *heterozygous disadvantage*, denotes a genetic condition in which heterozygotes (or their progeny) have a lower relative fitness compared to (parental) homozygotes.[238] Drive systems based on underdominance have the potential to be both spatially self-limiting and reversible to the original genetic state, and might therefore be used in developing safe methods for propagating desired genetic changes in natural populations.[239]

II. Development of Engineered Gene Drives

The idea of using naturally occurring gene drives to suppress species that are vectors of human diseases like yellow fever and malaria has been discussed since 1960.[240] *Austin Burt* first proposed the idea of using homing endonuclease genes to propagate genetic modifications to natural

234 *Lee M. Silver*, The Peculiar Journey of a Selfish Chromosome, 9 (1993) Trends in Genetics 250; *K. G. Ardlie*, Putting the Brake on Drive, 14 (1998) Trends in Genetics 189; see *Burt/Trivers* (n. 216), 21–37.

235 Cf. *Yuichiro. Hiraizumi/James F. Crow*, Heterozygous Effects on Viability, Fertility, Rate of Development, and Longevity of Drosophila Chromosomes that Are Lethal When Homozygous, 45 (1960) Genetics 1071; *Amanda M. Larracuente/Daven C. Presgraves*, The Selfish Segregation Distorter Gene Complex of Drosophila Melanogaster, 192 (2012) Genetics 33; see *Burt/Trivers* (n. 216), 38–45.

236 Cf. NASEM, Gene Drives on the Horizon (n. 216), 28; *Burt/Trivers* (n. 216), 20–21.

237 The use of underdominance as a method to achieve gene drives was suggested by *Omar S. Akbari* et al., Novel Synthetic Medea Selfish Genetic Elements Drive Population Replacement in Drosophila; a Theoretical Exploration of Medea-Dependent Population Suppression, 3 (2014) ACS Synthetic Biology 915.

238 *Pierce* (n. 1), 786.

239 *R. Guy Reeves* et al., First Steps Towards Underdominant Genetic Transformation of Insect Populations, 9 (2014) PLOS ONE e97557; *Omar S. Akbari* et al., A Synthetic Gene Drive System for Local, Reversible Modification and Suppression of Insect Populations, 23 (2013) Current Biology 671.

240 *G. B. Craig* et al., An Inherited Male-Producing Factor in Aedes Aegypti, 132 (1960) Science 1887.

populations in 2003.[241] The first successful creation of a gene drive in mosquitoes with HEGs was reported in 2011.[242] However, the difficulty in engineering HEGs to cut new target sequences posed a major obstacle to the development of universal gene drive techniques.[243] Therefore, attempts were made to build 'synthetic' HEGs using engineered nucleases like ZFN and TALENs.[244] Besides the disadvantages of these nucleases known from genome editing, namely their laborious and expensive construction and limited specificity,[245] they also suffered from evolutionary instability due to off-target cleavage.[246]

The discovery of the CRISPR-Cas technique for genome editing has enhanced the capabilities of gene drive research.[247] In principle, gene drives can be engineered by introducing DNA encoding for the CRISPR-Cas component into the host organism along with any desired payload gene. The cell expresses the components which then cleave the host's DNA at the target sequence in the wild-type chromosome. After that, the mechanism relies on the intra-cellular homology-directed repair mechanism, which remedies the break and copies the gene drive elements from the mutant chromosome.[248]

In 2015, researchers reported the first successful developments of gene drives based on the CRISPR-Cas technique in fruit and vinegar flies,[249]

241 See *Burt* (n. 230).

242 *Nikolai Windbichler* et al., A Synthetic Homing Endonuclease-Based Gene Drive System in the Human Malaria Mosquito, 473 (2011) Nature 212.

243 Cf. *Ryo Takeuchi* et al., Redesign of Extensive Protein–DNA Interfaces of Meganucleases Using Iterative Cycles of in Vitro Compartmentalization, 111 (2014) PNAS 4061; *Summer B. Thyme* et al., Reprogramming Homing Endonuclease Specificity Through Computational Design and Directed Evolution, 42 (2014) Nucleic Acids Res. 2564, 2574.

244 *Alekos Simoni* et al., Development of Synthetic Selfish Elements Based on Modular Nucleases in Drosophila Melanogaster, 42 (2014) Nucleic Acids Res. 7461.

245 See *supra* sections B.II.1 and B.II.2.

246 *Simoni* et al. (n. 244), 7471; *Esvelt* et al. (n. 215), 2; *John M. Marshall/Omar S. Akbari*, Gene Drive Strategies for Population Replacement, in: Zach N. Adelman (ed.), Genetic Control of Malaria and Dengue (2015) 169, 179.

247 *Robyn R. Raban* et al., Progress Towards Engineering Gene Drives for Population Control, 223 (2020) Journal of Experimental Biology, 1.

248 See *Esvelt* et al. (n. 215), 4–8; NASEM, Gene Drives on the Horizon (n. 216), 32; *Raban* et al. (n. 247), 4.

249 *Valentino M. Gantz/Ethan Bier*, The Mutagenic Chain Reaction: A Method for Converting Heterozygous to Homozygous Mutations, 348 (2015) Science 442; *Fang Li/Maxwell J. Scott*, CRISPR/Cas9-Mediated Mutagenesis of the White and

yeast,[250] and two mosquito species.[251] Since then, CRISPR-based gene drives have been developed in a number of other species, demonstrating its potential to drive genetic changes at virtually any genomic location through natural populations.[252]

III. Potential Applications of Engineered Gene Drives

The application of engineered gene drives is currently being discussed in several different areas and for various purposes, including the management of infectious diseases (1.), the protection of biological diversity (2.), and agriculture (3.).

Generally, gene drives can either be employed to propagate desirable genetic changes to a target population (*modification drive*), reduce the abundance of a target species, or exterminate it locally or globally (*suppression drive*).[253] Depending on the desired outcome, different drive strategies might be preferable. They are generally classified by several attributes relating to their efficiency, specificity and stability.[254] Some systems, called *low-threshold* or *invasive drives*, are fast-spreading and require a comparatively low number of initial releases. In contrast, *high-threshold* or *local drives* spread more slowly and need higher numbers of initial releases relative to the size of the target population, which could allow for locally confined releases.[255] In any case, the pace at which a gene drive spreads also depends

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Sex Lethal Loci in the Invasive Pest, Drosophila Suzukii, 469 (2016) Biochemical and Biophysical Research Communications 911.

250 *James E. DiCarlo* et al., Safeguarding CRISPR-Cas9 Gene Drives in Yeast, 33 (2015) Nature Biotech. 1250.

251 *Valentino M. Gantz* et al., Highly Efficient Cas9-Mediated Gene Drive for Population Modification of the Malaria Vector Mosquito Anopheles Stephensi, 112 (2015) PNAS E6736–43; *Roberto Galizi* et al., A CRISPR-Cas9 Sex-Ratio Distortion System for Genetic Control, 6 (2016) Sci. Rep. 31139.

252 *Champer* et al. (n. 215), 151; *Marshall/Akbari* (n. 246), 180; *John Min* et al., Harnessing Gene Drive, 5 (2018) Journal of Responsible Innovation S40, S43–S45; *Ethan Bier*, Gene Drives Gaining Speed, 23 (2022) Nature Rev. Genet. 5, 5.

253 *Esvelt* et al. (n. 215).

254 *Champer* et al. (n. 215), 147.

255 *Ibid.*, 148; *Min* et al. (n. 252), S41; cf. *Sumit Dhole* et al., Invasion and Migration of Spatially Self-limiting Gene Drives, 11 (2018) Evolutionary Applications 794, 800–802. Other authors distinguish between *localized* and *non-localized drives*, depending on the potential of drive systems to spread beyond their initial release site, cf. *Raban* et al. (n. 247).

on characteristics of the target population such as mating dynamics and generation time.[256]

1. Control of Vector-Borne Diseases

The use of gene drives in the fight against vector-borne diseases has been discussed since 1960[257] and is their most prominent application.[258] In particular, the fight against human *malaria* has attracted much attention. Malaria is an infection caused by parasitic microorganisms of the *Plasmodium* genus, which are transmitted by mosquitoes of the *Anopheles* genus.[259] Malaria occurs in tropical and subtropical regions and caused approximately 627,000 deaths in 2020, predominantly in Africa.[260] Besides malaria, several other severe diseases are transmitted by insects, including *Dengue* and *Yellow Fever*.[261]

a) Modification Drives

Gene drives could be used to genetically modify populations of disease vector species in order to reduce their ability to transmit a given pathogen.[262] For instance, it was shown that CRISPR-Cas9 can be used to engineer a gene drive that spreads a resistance gene against the malaria pathogen *Plasmodium* to populations of the malaria vector mosquito *Anopheles stephensi*.[263]

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256 *Esvelt* et al. (n. 215), 3.
257 See *Craig* et al. (n. 240).
258 See *Stephanie James/Karen Tountas*, Using Gene Drive Technologies to Control Vector-Borne Infectious Diseases, 10 (2018) Sustainability 4789.
259 Cf. *Austin Burt* et al., Gene Drive to Reduce Malaria Transmission in Sub-Saharan Africa, 5 (2018) Journal of Responsible Innovation S80, S66–S67.
260 WHO, World Malaria Report 2021 (2021), 24; also see *Burt* et al. (n. 259), S66–S67.
261 See, *inter alia*, *Galizi* et al. (n. 231).
262 See generally *John M. Marshall/Charles E. Taylor*, Malaria Control with Transgenic Mosquitoes, 6 (2009) PLOS Medicine e1000020; NASEM, Gene Drives on the Horizon (n. 216), 50–54; *H. C. J. Godfray* et al., How Driving Endonuclease Genes Can Be Used to Combat Pests and Disease Vectors, 15 (2017) BMC Biology 81, 4–6; *Burt* et al. (n. 259), S70–S72; *Bier* (n. 252), 9–12.
263 Cf. *Gantz* et al. (n. 251); *Astrid Hoermann* et al., Converting Endogenous Genes of the Malaria Mosquito into Simple Non-Autonomous Gene Drives for Popula-

b) Suppression Drives

Alternatively, a gene drive could be employed as a 'genetic equivalent of insecticides', i.e. to suppress or even eradicate the vector species.[264] This could be achieved by either biasing the sex ratio of the progeny[265] or by propagating a mutation that confers sterility.[266] Researchers have already used CRISPR-Cas9 to develop a gene drive system that causes sterility in female *Anopheles gambiae* mosquitoes.[267] A major challenge is genetic mutations which arise after a number of generations and confer resistance to the drive.[268] However, in 2018 a drive system targeting the *doublesex* gene in *Anopheles gambiae* reportedly reached a 100 % prevalence among mosquitos after 7–11 generations, which caused the population to collapse in a small-scale cage trial.[269] In 2020, a male-biased sex-distorter gene drive was developed as an additional, complementary approach.[270]

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tion Replacement, 10 (2021) eLife e58791; also see *Junitsu Ito* et al., Transgenic Anopheline Mosquitoes Impaired in Transmission of a Malaria Parasite, 417 (2002) Nature 452; *Esvelt* et al. (n. 215), 12; for an overview of other approaches, see *John M. Marshall*, The Cartagena Protocol and Releases of Transgenic Mosquitoes, in: Brij K. Tyagi (ed.), Training Manual: Biosafety for Human Health and the Environment in the Context of the Potential Use of Genetically Modified Mosquitoes (GMMs) (2015) 163, 165.

264 *Bier* (n. 252), 7; see *Anne Deredec* et al., Requirements for Effective Malaria Control with Homing Endonuclease Genes, 108 (2011) PNAS E874–80; *Burt* et al. (n. 259), 570–571.

265 Cf. *Nikolai Windbichler* et al., Targeting the X Chromosome During Spermatogenesis Induces Y Chromosome Transmission Ratio Distortion and Early Dominant Embryo Lethality in Anopheles Gambiae, 4 (2008) PLOS Genetics e1000291.

266 Cf. *T. A. Klein* et al., Infertility Resulting from Transgenic I-PpoI Male Anopheles Gambiae in Large Cage Trials, 106 (2012) Pathogens and Global Health 20; see *Marshall* (n. 263), 164; *Bier* (n. 252), 8–9.

267 *Andrew Hammond* et al., A CRISPR-Cas9 Gene Drive System Targeting Female Reproduction in the Malaria Mosquito Vector *Anopheles Gambiae*, 34 (2016) Nature Biotech. 78.

268 *Andrew M. Hammond* et al., The Creation and Selection of Mutations Resistant to a Gene Drive over Multiple Generations in the Malaria Mosquito, 13 (2017) PLOS Genetics e1007039; *Bier* (n. 252), 7.

269 *Kyros Kyrou* et al., A CRISPR–Cas9 Gene Drive Targeting *Doublesex* Causes Complete Population Suppression in Caged *Anopheles Gambiae* Mosquitoes, 36 (2018) Nature Biotech. 1062.

270 *Alekos Simoni* et al., A Male-Biased Sex-Distorter Gene Drive for the Human Malaria Vector Anopheles Gambiae, 38 (2020) Nature Biotech. 1054.

c) Current State of Development

While genetically modified insects were already released into the environment in a number of instances,[271] there have so far been no reported environmental releases of organisms carrying a synthetic gene drive.[272] Instead, experiments are confined to cage trials,[273] and computational models are used to evaluate various gene drive methods and release strategies by simulating simplified field settings including circumstances such as seasonal weather.[274] Altering, reducing or eliminating a mosquito species may have various ecological effects on other species that they interact with as prey, predator, competitor or disease vector, and may also open ecological niches that may be colonized by other species.[275] It has also been suggested that species could be reintroduced from sheltered laboratories or island populations once disease eradication is complete.[276]

A research consortium named *Target Malaria* is currently exploring the use of engineered gene drives to bias the sex ratio or reduce the female fertility in the mosquitoe species *Anopheles gambiae*.[277] In September 2018, regulators in Burkina Faso granted permission to Target Malaria for the

271 *R. Guy Reeves* et al., Scientific Standards and the Regulation of Genetically Modified Insects, 6 (2012) PLOS Neglected Tropical Diseases e1502; see *infra* section E.III.

272 Cf. *Burt* et al. (n. 259), S75–S76.

273 *Andrew Hammond* et al., Gene-Drive Suppression of Mosquito Populations in Large Cages as a Bridge Between Lab and Field, 12 (2021) Nature Comms. 4589.

274 Cf. *Philip A. Eckhoff* et al., Impact of Mosquito Gene Drive on Malaria Elimination in a Computational Model with Explicit Spatial and Temporal Dynamics, 114 (2017) PNAS E255–E264; *Ace R. North* et al., Modelling the Suppression of a Malaria Vector Using a CRISPR-Cas9 Gene Drive to Reduce Female Fertility, 18 (2020) BMC Biology 98; *Paola Pollegioni* et al., Detecting the Population Dynamics of an Autosomal Sex Ratio Distorter Transgene in Malaria Vector Mosquitoes, 57 (2020) The Journal of Applied Ecology 2086.

275 *Godfray* et al. (n. 262), 6 and additional file 1, note 11; see *Aaron S. David* et al., Release of Genetically Engineered Insects: A Framework to Identify Potential Ecological Effects, 3 (2013) Ecology and Evolution 4000; *Andrew Roberts* et al., Results from the Workshop "Problem Formulation for the Use of Gene Drive in Mosquitoes", 96 (2017) Am. J. Trop. Med. Hyg. 530. Also see *infra* section C.IV.3.

276 *Esvelt* et al. (n. 215), 14.

277 See Target Malaria, Male Bias and Female Fertility, available at: https://targetmalaria.org/what-we-do/our-approach/male-bias-and-female-fertility/ (last accessed 28 May 2022).

experimental release of up to 10,000 genetically modified mosquitoes.[278] The mosquitoes did not contain a gene drive, but were modified to be sterile (i.e. incapable of sexual reproduction) and to carry fluorescent markers, which allows the identification of modified individuals.[279] The mosquitoes were generated in the United Kingdom and tested in containment in Italy before they were imported to Burkina Faso in the form of eggs in November 2016.[280] Following cage trials in Burkina Faso, approximately 6,400 genetically modified male (i.e. non-biting) mosquitoes were experimentally released in a village in Burkina Faso in July 2019.[281] The release was followed by a 20-day 'recapture period' and a monitoring period to verify the disappearance of the transgene from the environment.[282] In the next project phase, Target Malaria plans to release non-drive mosquitoes with a male bias.[283]

278 Cf. Target Malaria, Target Malaria Welcomes the Decision of the National Biosafety Agency of Burkina Faso to Approve a Small-Scale Release of Genetically Modified Sterile Male Mosquitoes (n.d.), available at: https://targetmalaria.org/wp-content/uploads/2021/07/statement_authorisation_nba_bf-1.pdf (last accessed 28 May 2022); *Keith R. Hayes* et al., Risk Assessment for Controlling Mosquito Vectors with Engineered Nucleases: Controlled Field Release for Sterile Male Construct: Risk Assessment Final Report (2018); *Ike Swetlitz*, Researchers to Release First-Ever Genetically Engineered Mosquitoes in Africa, STAT, 05 September 2018, available at: https://www.statnews.com/2018/09/05/release-genetically-engineered-mosquitoes-africa/ (last accessed 28 May 2022). The decision appears to be unpublished, nor was it notified to the Biosafety Clearing-House, see chapter 3, section A.II.3. On non-drive applications of genetically modified insects generally, see *infra* section E.II.

279 Target Malaria, Results of the Small-Scale Release of Non Gene Drive Genetically Modified Sterile Male Mosquitoes in Burkina Faso (2021), 1–2; *Franck A. Yao* et al., Mark-Release-Recapture Experiment in Burkina Faso Demonstrates Reduced Fitness and Dispersal of Genetically-Modified Sterile Malaria Mosquitoes, 13 (2022) Nature Comms. 796, 2; cf. *Hayes* et al. (n. 278), 14; *Windbichler* et al. (n. 265), 2.

280 Target Malaria was criticized for not having notified the import of the mosquitoes into Burkina Faso in line the pertinent international regulations, but claimed that these rules did not apply because the mosquitoes were first tested in containment before being released; see chapter 3, section A.II.1.g).

281 Target Malaria (n. 279), 2–3.

282 *Ibid.*, 3; *Yao* et al. (n. 279), 6–7.

283 Target Malaria (n. 279), 3.

2. Control of Invasive Species

Invasive species often cause severe damage to the local environment up to the extinction of local species, as well as substantial economic losses, particularly on islands.[284] It has been suggested that suppression drives could be employed to control or eradicate these species from islands or continents[285] or even to cause their global extinction.[286]

The application of suppression drives has been proposed to eradicate non-indigenous rodents such as rats and mice species.[287] Gene drives could constitute a more efficient, more species-specific and non-toxic alternative to conventional methods to suppress invasive species.[288] In theory, gene drives might also be used to aid threatened species by genetically enhancing them or by increasing their ecological niches.[289]

The application of gene drives to control invasive species is currently investigated by several universities, government and not-for-profit organizations that have established a joint program on *Genetic Biocontrol of Invasive Rodents*.[290] Furthermore, New Zealand's *Predator Free 2050* program, which aims at eliminating all rats, possums and stoats by 2050, is sometimes associated with suppression drives,[291] but there appear to exist no concrete plans to actually employ gene drive techniques as part of the program.[292]

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284 *S. L. Goldson* et al., New Zealand Pest Management, 45 (2015) Journal of the Royal Society of New Zealand 31, 32–35; *Min* et al. (n. 252), S47.

285 *Esvelt* et al. (n. 215), 15; NASEM, Gene Drives on the Horizon (n. 216), 54–56.

286 *Bruce L. Webber* et al., Opinion, 112 (2015) PNAS 10565, 10565.

287 *Karl J. Campbell* et al., The Next Generation of Rodent Eradications, 185 (2015) Biological Conservation 47, 51–52.

288 *Ibid.*

289 *Esvelt* et al. (n. 215), 15; see *Kent H. Redford* et al., Genetic Frontiers for Conservation (2019); *Jesse L. Reynolds*, Engineering Biological Diversity: The International Governance of Synthetic Biology, Gene Drives, and De-Extinction for Conservation, 49 (2021) Current Opinion in Environmental Sustainability 1, 2.

290 Island Conservation, The Genetic Biocontrol of Invasive Rodents (GBIRd) Program, available at: http://www.geneticbiocontrol.org/ (last accessed 28 May 2022).

291 See *Kevin M. Esvelt/Neil J. Gemmell*, Conservation Demands Safe Gene Drive, 15 (2017) PLOS Biology e2003850, 1–2; *Brian Owens*, Behind New Zealand's Wild Plan to Purge All Pests, 541 (2017) Nature News 148.

292 Cf. Predator Free 2050 Limited, Current Research Projects, available at: https://pf2050.co.nz/current-research-projects/ (last accessed 28 May 2022).

3. Agriculture

In agriculture, gene drives might be applied to fight plant pests in various ways. One study suggested that a suppression drive might be applied in the fruit crop pest *Drosophila suzukii*, which poses an economic threat to soft summer fruits such as blueberries and strawberries.[293] Another approach is to use *sensitizing drives* to remove herbicide or pesticide resistances that pest species have developed over time,[294] such as the western corn rootworm's resistance to *Bacillus thuringiensis* toxins[295] or the mutations allowing horseweed and pigweed to resist the herbicide glyphosate.[296] Alternatively, *sensitizing drives* might be used to render pest populations vulnerable to substances that have not affected them before; this would potentially allow for the development of less toxic and more species-specific pest control agents.[297] Finally, gene drives could be applied to render pest species less harmful without impeding their viability, for instance by reprogramming insects to avoid human crops or by disabling the desert locust's capacity to form large, damaging swarms.[298]

IV. Limitations and Risks of Applying Engineered Gene Drives

Engineered gene drive techniques are still subject to several limitations (1.) and risks (2.). In addition, concerns arise from the potential ecological effects of suppressing target species (3.) as well as from the potential transboundary effects of gene drives (4.).

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293 Cf. *Li/Scott* (n. 249), 916; *Anna Buchman* et al., Synthetically Engineered Medea Gene Drive System in the Worldwide Crop Pest Drosophila Suzukii (2018) PNAS 201713139; see also NASEM, Gene Drives on the Horizon (n. 216), 58.

294 *Ibid.*, 57–58.

295 *Aaron J. Gassmann* et al., Field-Evolved Resistance by Western Corn Rootworm to Multiple Bacillus Thuringiensis Toxins in Transgenic Maize, 111 (2014) PNAS 5141.

296 *Todd A. Gaines* et al., Gene Amplification Confers Glyphosate Resistance in Amaranthus Palmeri, 107 (2010) PNAS 1029; *Xia Ge* et al., Rapid Vacuolar Sequestration, 66 (2010) Pest Management Science 345; NASEM, Gene Drives on the Horizon (n. 216), 57–58.

297 *Esvelt* et al. (n. 215), 15; *Min* et al. (n. 252), S46–S47.

298 *Min* et al. (n. 252); see *Ryohei Sugahara* et al., Knockdown of the Corazonin Gene Reveals Its Critical Role in the Control of Gregarious Characteristics in the Desert Locust, 79 (2015) Journal of Insect Physiology 80.

1. Limitations of Current Gene Drive Techniques

Current gene drive techniques are subject to four major challenges. First of all, gene drives only work in organisms that reproduce sexually, since they rely on biasing the inheritance of genetic information from both parents.[299] Therefore, gene drive systems will not function in organisms that reproduce asexually, including viruses and bacteria.[300] Organisms that employ a mix of sexual and asexual reproduction, including many plants,[301] are expected to be highly resistant to gene drives.[302]

Secondly, depending on the number of initial releases, gene drives require many generations to spread through a population. Hence, they are an unsuitable means to address species that have long generation times compared to human-relevant time frames.[303]

The third group of challenges concerns the potential formation of resistances.[304] When the cell repairs the drive-induced DNA break not by homology-directed repair but by joining together the 'loose ends' of DNA (non-homologous end joining), small mutations will alter the target sequence and hence inactivate the drive components.[305] One approach to solve this is to address only genes that are important for fitness so that any resistant organism will not reproduce.[306] However, there is no scientific certainty yet about the degree to which evolving resistances inhibit gene drives.[307] While some studies reported that mutations inevitably arise,[308]

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299 *Esvelt* et al. (n. 215), 9; NASEM, Gene Drives on the Horizon (n. 216), 49; *Min* et al. (n. 252), S48.

300 *Esvelt* et al. (n. 215), 9.

301 See NASEM, Gene Drives on the Horizon (n. 216), 50.

302 *Min* et al. (n. 252), S48; *Esvelt* et al. (n. 215), 9; see *Douglas W. Drury* et al., CRISPR/Cas9 Gene Drives in Genetically Variable and Nonrandomly Mating Wild Populations, 3 (2017) Science Advances e1601910.

303 *Esvelt* et al. (n. 215), 9; NASEM, Gene Drives on the Horizon (n. 216), 49; *Min* et al. (n. 252), S48.

304 See *J. J. Bull*, Evolutionary Decay and the Prospects for Long-Term Disease Intervention Using Engineered Insect Vectors, 2015 (2015) Evolution, Medicine, and Public Health 152.

305 *Champer* et al. (n. 215), 151; *John M. Marshall* et al., Overcoming Evolved Resistance to Population-Suppressing Homing-Based Gene Drives, 7 (2017) Sci. Rep. 3776, 2; *Charleston Noble* et al., Evolutionary Dynamics of CRISPR Gene Drives, 3 (2017) Science Advances e1601964.

306 *Esvelt* et al. (n. 215), 14.

307 *Raban* et al. (n. 247), 5.

308 Cf. *Robert L. Unckless* et al., Evolution of Resistance Against CRISPR/Cas9 Gene Drive, 205 (2017) Genetics 827; *Jackson Champer* et al., Novel CRISPR/Cas9

another study demonstrated with mathematical models that CRISPR-Cas drive systems are likely to be highly invasive.[309]

The fourth limitation of engineered gene drives is that their evolutionary stability can be limited.[310] This depends on the particular circumstances and whether the drive decreases the organism's fitness. Especially when the drive imposes a fitness cost on the organism, drive-bearing individuals might be outcompeted by wild-types that have higher evolutionary fitness.[311] This might require repeated releases of altered organisms, which can be included in containment strategies.[312]

2. Risks Related to Gene Drive Applications

The application of gene drives also imposes a number of (potential) risks. Some of these risks are shared with other genetic engineering techniques, such as that payload genes delivered with a gene drive may have unanticipated detrimental effects.[313] Furthermore, the drive might evolve into a harmful construct after being released.[314] For instance, the drive construct might produce off-target mutations in the target genome which continue to spread as long as the mutation does not render the drive construct itself inoperative.[315] The use of gene drives could also pose risks to human health, for example by increasing the organism's capacity to transmit pathogens.[316] In addition, several risks originate from the functioning of gene drives and their potential effects.

Gene Drive Constructs Reveal Insights into Mechanisms of Resistance Allele Formation and Drive Efficiency in Genetically Diverse Populations, 13 (2017) PLOS Genetics e1006796.

309 Cf. *Charleston Noble* et al., Current CRISPR Gene Drive Systems Are Likely to Be Highly Invasive in Wild Populations, 7 (2018) eLife e33423.

310 See NASEM, Gene Drives on the Horizon (n. 216), 34–36.

311 *Esvelt* et al. (n. 215), 9; *Min* et al. (n. 252), S49.

312 NASEM, Gene Drives on the Horizon (n. 216), 36.

313 *Champer* et al. (n. 215), 156; see *supra* section B.V.

314 *Fears* (n. 197), 14.

315 *Webber* et al. (n. 286), 10566.

316 Cf. *Fears* (n. 197), 14; *Roberts* et al. (n. 275), 531; *John L. Teem* et al., Problem Formulation for Gene Drive Mosquitoes Designed to Reduce Malaria Transmission in Africa: Results from Four Regional Consultations 2016–2018, 18 (2019) Malaria Journal 347, 7–8.

a) Unintended Geographic Spread

Gene drives might spread beyond their intended target population. Even if not intended to alter or eradicate a species globally, gene flow enabled by human activity or disruptive events, or simply movement of individuals from one population to another,[317] may enable a gene drive to spread beyond its intended geographical range.[318] Thus, invasive gene drives in principle have the potential to spread transgenes globally throughout an entire species.[319] Furthermore, there is a potential risk that invasive gene drives might accidentally escape from laboratories, which requires the adoption of adequate safeguards.[320]

b) Intended but Unauthorized Spread

A gene drive might also be spread through deliberate unauthorized transport and release. When a gene drive system offers substantial economic benefits, such as suppressing an agricultural pest species, previous exam-

317 In this context, one study showed that the t-haplotype, a selfish genetic element in house mice which might also be used for synthetic gene drives (see the references in n. 234), manipulates host behaviour and increases the propensity of mice carrying it to migrate into foreign populations, cf. *Jan-Niklas Runge/Anna K. Lindholm*, Carrying a Selfish Genetic Element Predicts Increased Migration Propensity in Free-Living Wild House Mice, 285 (2018) Proc. R. Soc. B 1333.

318 NASEM, Gene Drives on the Horizon (n. 216), 37–38; *Kenneth A. Oye* et al., Regulating Gene Drives, 345 (2014) Science 626, 627; *Webber* et al. (n. 286), 10556. This problem has been acknowledged before the arrival of synthetic gene drive techniques, in particular with regard to the release of genetically modified viruses for pest control, cf. *Elena Angulo/B. Cooke*, First Synthesize New Viruses Then Regulate Their Release? The Case of the Wild Rabbit, 11 (2002) Molecular Ecology 2703, 2706.

319 *John M. Marshall*, The Cartagena Protocol and Genetically Modified Mosquitoes, 28 (2010) Nature Biotech. 896, 897; *Marshall* (n. 263), 167; also see *Yehonatan Alcalay* et al., The Potential for a Released Autosomal X-Shredder Becoming a Driving-Y Chromosome and Invasively Suppressing Wild Populations of Malaria Mosquitoes, 9 (2021) Front. Bioeng. & Biotechnol. 752253, proposing that it was 'unlikely' that a self-limiting autosomal X-shredder gene drive would become invasive after being released into the environment.

320 Cf. *Burt* (n. 230), 927, noting that 'the ease and rapidity with which these selfish genes can invade a population applies not just to planned releases, but also to unintentional releases of laboratory escapees'. Also see *Omar S. Akbari* et al., Safeguarding Gene Drive Experiments in the Laboratory, 349 (2015) Science 927 and chapter 5, section C.III.

ples from conventional biocontrol[321] suggest that individuals will likely seek advantage by moving drive-equipped organisms to other locations, even when such movement is illegal.[322] Whether a gene drive can persist and continue to spread in other locations depends on the characteristics of both the target organism and the drive and includes factors such as fitness, conversion rate, population structure and ecological interactions with other species.[323] In some cases, gene drives might be confined to certain (sub-)populations by employing highly specific 'precision drives'.[324]

c) Undesired Spread to Non-Target Species

Gene drives, or parts of it, could spread into non-target species through *horizontal gene transfer*, which denotes the movement of genes between distinct species.[325] There are mechanisms that allow for horizontal gene transfer between unrelated bacterial species,[326] between bacteria and plants (e.g., through *Agrobacterium tumefaciens*[327]), between bacteria and animals,[328] and between plants through hybridization.[329] The potential for horizontal gene transfer must therefore be evaluated for any species targeted by a gene drive in order to avoid an undesired spread into non-target species.[330]

321 Cf. *Angulo/Cooke* (n. 318), 2704–2705; *Peter O'Hara*, The Illegal Introduction of Rabbit Haemorrhagic Disease Virus in New Zealand, 25 (2006) Revue scientifique et technique (International Office of Epizootics) 119.
322 *Esvelt/Gemmell* (n. 291), 2; *Min* et al. (n. 252), S48.
323 NASEM, Gene Drives on the Horizon (n. 216), 39.
324 Cf. *Esvelt* et al. (n. 215), 10–11; *Oye* et al. (n. 318), 627.
325 Horizontal gene transfer is also referred to as 'lateral gene transfer', cf. Henderson's Dictionary of Biology (n. 5), 268.
326 *Pierce* (n. 1), 271.
327 Cf. *Pavel Krenek* et al., Transient Plant Transformation Mediated by Agrobacterium Tumefaciens, 33 (2015) Biotechnology Advances 1024.
328 *Julie C. Dunning Hotopp*, Horizontal Gene Transfer Between Bacteria and Animals, 27 (2011) Trends in Genetics 157.
329 *Pierce* (n. 1), 818; NASEM, Gene Drives on the Horizon (n. 216), 39–40.
330 NASEM, Gene Drives on the Horizon (n. 216), 39; *Webber* et al. (n. 286), 10566; *Virginie Courtier-Orgogozo* et al., Agricultural Pest Control with CRISPR-based Gene Drive, 18 (2017) EMBO Reports 878; *Fears* (n. 197), 14.

d) Dual Use of Gene Drive Techniques

The advent of gene drive techniques also raised concerns over biosecurity and potential dual-use applications.[331] In theory, mosquitoes might be engineered to transmit a pathogen that is normally not vector-borne or even to deliver a toxin.[332] Other scenarios involve the use of gene drives for targeted attacks on crop plants.[333] Currently, the malicious use of gene drive techniques appears unlikely due to its high engineering complexity compared to other potential biohazards.[334] Nevertheless, the potential of gene drives for dual-use applications cannot be discounted[335] and resembles previous instances of so-called *Dual Use Research of Concern*, e.g. studies that increased the transmissibility of the highly pathogenic avian influenza virus H5N1.[336]

3. Potential Ecological Effects of Suppressing a Target Species

The potential removal of a target species or its substantial reduction in abundance in its native habitat range raises ethical[337] as well as ecological

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331 Cf. *Min* et al. (n. 252), S57–S58.

332 *David Gurwitz*, Gene Drives Raise Dual-Use Concerns, 345 (2014) Science 1010; NASEM, Gene Drives on the Horizon (n. 216), 160–161; see *Jeffrey A. Lockwood*, Insects as Weapons of War, Terror, and Torture, 57 (2012) Annual Review of Entomology 205, 221–222.

333 *Gurwitz* (n. 332); *Oye* et al. (n. 318), 627.

334 NASEM, Gene Drives on the Horizon (n. 216), 160; on the low feasibility of using gene drives to modify the he human genome, see Committee on Strategies for Identifying and Addressing Potential Biodefense Vulnerabilities Posed by Synthetic Biology et al., Biodefense in the Age of Synthetic Biology (2018), 79.

335 See *Jim Thomas*, The National Academies' Gene Drive Study Has Ignored Important and Obvious Issues, The Guardian, 09 June 2016, available at: https://www.theguardian.com/science/political-science/2016/jun/09/the-national-academies-gene-drive-study-has-ignored-important-and-obvious-issues (last accessed 28 May 2022).

336 NASEM, Gene Drives on the Horizon (n. 216), 159; cf. *Sander Herfst* et al., Airborne Transmission of Influenza A/H5N1 Virus Between Ferrets, 336 (2012) Science 1534.

337 Cf. *Jonathan Pugh*, Driven to Extinction? The Ethics of Eradicating Mosquitoes with Gene-Drive Technologies, 42 (2016) Journal of Medical Ethics 578; *Axel Hochkirch* et al., License to Kill?, 11 (2018) Conservation Letters e12370; *Tina Rulli*, CRISPR and the Ethics of Gene Drive in Mosquitoes, in: David Boonin (ed.), The Palgrave Handbook of Philosophy and Public Policy (2018) 509;

concerns.[338] Since most species are embedded in complex ecosystems, in which they are connected to other species through food webs or as competitors for ecological niches, removing a certain species might lead to unintended environmental effects.[339] This could include the disruption of food webs as well as the facilitation of other, possibly invasive species or undesired negative effects for non-target species.[340] Hence, targeting one species can potentially produce cascade effects on several other species or destabilize entire ecosystems.[341] Since the ecological trophic networks are highly complex, these effects can be difficult to predict.[342]

Until now, research on ecological consequences of gene drives has mostly focused on mosquito species that transmit malaria, in particular *Anopheles gambiae*.[343] Some argue that the removal of this species was unlikely to cause ecological harm since it did not represent a keystone species and sufficient alternatives, especially from within the *Anopheles* genus were available.[344] Others warn that the removal of *Anopheles gambiae* could cause cascading community effects, disrupt food webs and potentially lead to a loss of diversity in the affected community.[345] Besides natural ecosystems, gene drive applications may also pose risks to agriculture, e.g. when the dominance of a pest species is enhanced, which may cause damage to crops or livestock.[346]

Swiss Federal Ethics Committee on Non-Human Biotechnology, Gene Drives: Ethical Considerations on the Use of Gene Drives in the Environment (2019), 5; see chapter 3, section B.VIII.

338 See *Teem* et al. (n. 316), 8–9; *John B. Connolly* et al., Systematic Identification of Plausible Pathways to Potential Harm via Problem Formulation for Investigational Releases of a Population Suppression Gene Drive to Control the Human Malaria Vector Anopheles Gambiae in West Africa, 20 (2021) Malaria Journal 170.

339 *Oye* et al. (n. 318), 627; NASEM, Gene Drives on the Horizon (n. 216), 40–41; *Webber* et al. (n. 286), 10556; *Bier* (n. 252), 7.

340 *Webber* et al. (n. 286), 10566; NASEM, Gene Drives on the Horizon (n. 216), 40.

341 *David* et al. (n. 275), 4010.

342 NASEM, Gene Drives on the Horizon (n. 216), 40.

343 *Esvelt* et al. (n. 215), 10; see *David* et al. (n. 275); *C. M. Collins* et al., Effects of the Removal or Reduction in Density of the Malaria Mosquito, Anopheles Gambiae S.L., on Interacting Predators and Competitors in Local Ecosystems, 33 (2019) Medical and Veterinary Entomology 1.

344 NASEM, Gene Drives on the Horizon (n. 216), 41; *Roberts* et al. (n. 275), 531–532; *Min* et al. (n. 252), S47–S48.

345 *David* et al. (n. 275), 4010.

346 Cf. *Fears* (n. 197), 14.

In general, it can be concluded that the risks and ecological effects of employing gene drives in wild populations have not yet been sufficiently scrutinized,[347] and it is generally acknowledged that further studies examining the ecological consequences of applying gene drives in specific species and environments are needed.[348] In October 2018, Target Malaria launched a four-year project to study the ecology of *Anopheles gambiae* and to analyse their position in local ecological foods webs.[349] Reportedly, this involves the use of *DNA barcoding*, where excretions of predators are analysed for traces of DNA originating from *Anopheles gambiae*.[350] Scientists also seek to develop drive-neutralizing systems such as 'reversal drives' to halt or undo the spread of a gene drive if it is found to cause unintended effects.[351]

4. Potential Transboundary Effects of Gene Drives

It appears to be undisputed that engineered gene drives, especially invasive drive systems, have the potential to cause transboundary effects. Most importantly, a gene drive might move into foreign territories – either by natural gene flow or intentionally or unintentionally aided by human action – and continue to spread to local populations there.[352] This also means that the risks associated with an unintentional release of a gene

347 Cf. NASEM, Gene Drives on the Horizon (n. 216), 113; also see *David* et al. (n. 275); *Esvelt* et al. (n. 215), 9–10.

348 Cf. *T. Kuiken* et al., Shaping Ecological Risk Research for Synthetic Biology, 4 (2014) Journal of Environmental Studies and Sciences 191; *Esvelt* et al. (n. 215), 10; *Oye* et al. (n. 318), 627; *Webber* et al. (n. 286), 10556; NASEM, Gene Drives on the Horizon (n. 216), 40–41.

349 University of Oxford, Department of Zoology, New Project Led by Oxford University's Zoology Department to Study the Community Ecology of the African Mosquito Vectors of Malaria (15 June 2017), available at: https://www.zoo.ox.ac.uk/article/new-project-led-oxford-universitys-zoology-department-study-community-ecology-african (last accessed 28 May 2022); cf. *Sarah Zhang*, No One Knows Exactly What Would Happen If Mosquitoes Were to Disappear, The Atlantic, 24 September 2018, available at: https://www.theatlantic.com/science/archive/2018/09/mosquito-target-malaria/570937/ (last accessed 28 May 2022).

350 Cf. *ibid.*

351 *Esvelt* et al. (n. 215), 10; *Bier* (n. 252), 15–17.

352 NASEM, Gene Drives on the Horizon (n. 216), 157; *Marshall* (n. 319), 896; *Oye* et al. (n. 318), 628; *Redford* et al. (n. 289), 41; *Connolly* et al. (n. 338), 61; *Raban* et al. (n. 247), 1–4.

drive are higher than with other genetically modified organisms.[353] In theory, a gene drive could also have transboundary effects without actually crossing a boundary. For instance, the gene drive-based removal of a certain predator species could facilitate the dominance of a non-altered invasive species and subsequently its spread into a neighbouring state's territory.

With regard to proposed gene drive applications in the mosquito species *Anopheles gambiae*, it has been argued that their removal from a particular environment was unlikely to cause ecological harm, particularly because the species is not known to be the sole or primary food source for any other species.[354] Others have warned that 'ecosystems are connected in myriad ways and that a handful of organisms introduced in 1 [sic] country may have ramifications well beyond its own borders'.[355] Previous releases of genetically modified insects have also raised concerns about their compliance with the Cartagena Protocol,[356] scientific standards on risk assessments,[357] and impacts on organic farmers.[358]

If a gene drive has transboundary effects, the environment of the foreign state, in particular its biological diversity, will be primarily affected.[359] However, it also appears possible that individual goods might be impaired, e.g. by the loss of ecosystem services or due to contamination of farmland with drive-equipped organisms. Depending on the circumstances, individual damage could take the form of personal injury, property damage, or economic loss.

D. Horizontal Environmental Genetic Alteration Agents (HEGAAs)

As shown in the previous section, engineered gene drives can be used to increase the probability that a certain genetic modification is passed on

353 NASEM, Gene Drives on the Horizon (n. 216), 149.
354 *Roberts* et al. (n. 275), 531–532; *Collins* et al. (n. 343), 10–11.
355 *Esvelt/Gemmell* (n. 291), 5.
356 Cf. *Marshall* (n. 319); *Marshall* (n. 263), 165–167.
357 Cf. *Reeves* et al. (n. 271).
358 Cf. *R. Guy Reeves/Martin Phillipson*, Mass Releases of Genetically Modified Insects in Area-Wide Pest Control Programs and Their Impact on Organic Farmers, 9 (2017) Sustainability 59.
359 *René Lefeber*, The Legal Significance of the Supplementary Protocol: The Result of a Paradigm Evolution, in: Akiho Shibata (ed.), International Liability Regime for Biodiversity Damage (2014) 73, 75–76.

to subsequent generations. Hence, gene drives aim at achieving a *vertical* propagation of genetic modifications. A different approach is so-called *horizontal environmental genetic alteration agents* (HEGAAs), which perform the same genetic modification in a multitude of individuals of the same generation.[360] HEGAAs are biological agents that can spread through horizontal transmission, such as pathogens or symbionts, and have been engineered to alter the genome of their target organism by using sequence-specific genome editing techniques.[361] In contrast to gene drives, HEGAAs are not necessarily aimed at increasing the rate of transmission of a genetic modification to subsequent generations but rather at modifying large amounts of already-living organisms. However, by targeting germline cells, HEGAAs can also be used to confer heritable alterations.[362]

In 2016, the United States' *Defense Advanced Research Projects Agency* (DARPA) launched a research program funding the development of HEGAAs to genetically modify already-growing crop plants in the field.[363] The program, called *Insect Allies*, proposed to use insects to transmit viral HEGAAs to mature crop plants in order to genetically modify these plants within the same growing season.[364] The most prospective approach is to integrate a CRISPR system into a benign virus that would modify the genetic material of the crop plant in cells infected by the virus.[365] According to DARPA's call for proposals, at least three transgenes should be expressed by the virus to result in a *gain of function* phenotype (i.e. a phenotype that possesses new functions compared to the wild type[366]) in the crop plants.[367] The call required a 'large greenhouse demo' to be performed at the end of the four-year project term.[368]

According to recipients of grants from the Insect Allies program, traits of interest predominantly include resistance to disease, drought or insects,

360 *R. Guy Reeves* et al., Agricultural Research, or a New Bioweapon System?, 362 (2018) Science 35.
361 See *supra* section B.II.
362 *Evan E. Ellison* et al., Multiplexed Heritable Gene Editing Using RNA Viruses and Mobile Single Guide RNAs, 6 (2020) Nature Plants 620, 620.
363 DARPA, Broad Agency Announcement: Insect Allies: HR001117S000 (2016), 4–6.
364 *Ibid.*, 6.
365 *Reeves* et al. (n. 360); see *Ellison* et al. (n. 362).
366 Cf. 'gain-of-function', in: Henderson's Dictionary of Biology (n. 5), 219.
367 Cf. DARPA (n. 363), 8.
368 *Ibid.*, 6.

all of which are of value to farmers.[369] However, the technique might equally be used to confer *detrimental* traits to crops, and thus result in the generation of a new class of biological weapons.[370] Furthermore, the approach faces multiple technical challenges,[371] such as that the envisaged application will almost invariably generate a mixture of the intended edit, along with random mutations at the target chromosomal site (where each individual plant has the potential to gain a unique set of mutations), unintended off-target mutations and individual plants that remain unaltered.[372] Infected insects could also disperse beyond their intended geographical scope and lead to the infection of untargeted plants. It has therefore been argued that the approach was 'beyond any risk assessment ever performed in the field of biotechnology'.[373]

E. *Self-Spreading Biotechnology Not Involving Genetic Alteration of the Target Organism*

While synthetic gene drives and HEGAAs are aimed at conferring permanent genetic modifications to their target organisms, other instances of

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369 Cf. Boyce Thompson Institute, BTI Receives DARPA "Insect Allies" Award to Develop Viruses and Insects for Maize Improvement (27 July 2017), available at: https://btiscience.org/explore-bti/news/post/bti-receives-darpa-insect-allies-award-to-develop-viruses-and-insects-for-maize-improvement/ (last accessed 28 May 2022); Ohio State University, College of Food, Agricultural, and Environmental Sciences, Insect Allies: How the Enemies of Corn May Someday Save It (16 October 2017), available at: https://cfaes.osu.edu/news/articles/insect-allies-how-the-enemies-corn-may-someday-save-it (last accessed 28 May 2022); Pennsylvania State University, Penn State Team Receives $7M Award to Enlist Insects as Allies for Food Security (20 November 2017), available at: http://news.psu.edu/story/495037/2017/11/20/research/penn-state-team-receives-7m-award-enlist-insects-allies-food (last accessed 28 May 2022), 36.

370 *Todd Kuiken*, DARPA's Synthetic Biology Initiatives Could Militarize the Environment: Is that Something We're Comfortable with? (28 March 2018), available at: http://www.slate.com/articles/technology/future_tense/2017/05/what_happens_if_darpa_uses_synthetic_biology_to_manipulate_mother_nature.html (last accessed 28 May 2022); *Reeves* et al. (n. 360).

371 See *Kevin Pfeifer* et al., Insect Allies – Assessment of a Viral Approach to Plant Genome Editing, 18 (2022) Integrated Environmental Assessment and Management.

372 *Reeves* et al. (n. 360), 36.

373 *Samson Simon* et al., Scan the Horizon for Unprecedented Risks, 362 (2018) Science 1007.

self-spreading biotechnology pursue different goals. For instance, genetically modified viruses can be used to control agricultural pests (I.) or as self-disseminating vaccines (II.). Another example is the suppression of insect populations by releasing large numbers of individuals genetically modified to be sterile (III.). Moreover, the heritable *Wolbachia* bacterium is used to suppress infectious diseases transmitted by mosquitoes (IV.).

I. Use of Genetically Modified Viruses in Plant Pest Control

Genetically modified viruses can be used to control plant pests such as insects or bacteria.[374] For instance, a commercial enterprise located in the United States has developed a genome-edited virus to control the so-called *citrus greening disease* (also known as *Huanglongbing*), which is a bacterial disease that infects citrus fruit trees.[375] In the United States alone, this bacterial disease has caused billions of US dollars in losses since it was first detected in 2005.[376]

To render citrus trees resistant to this disease, genes derived from spinach that encode for antibacterial proteins were added to a harmless strain of the *citrus tristeza* virus.[377] The trees are then artificially infected with the virus, where it triggers the production of *defensin* proteins that kill the bacterium responsible for the disease.[378] The genetic material encoding defensins is not inserted into the citrus chromosome, but only transiently expressed as long as the virus is present in the plant.[379] According to an environmental impact statement produced during the authorization procedure, no adverse impacts on the environment or human health are expected by the use of the modified virus.[380] However, the virus may be

374 Cf. *Jennifer S. Cory* et al., Field Trial of a Genetically Improved Baculovirus Insecticide, 370 (1994) Nature 138.
375 Cf. *Heidi Ledford*, Geneticists Enlist Engineered Virus and CRISPR to Battle Citrus Disease, 545 (2017) Nature News 277; APHIS, Southern Gardens Citrus Nursery, LLC Permit to Release Genetically Engineered Citrus Tristeza Virus: Draft Environmental Impact Statement (2018).
376 *Ledford* (n. 375), 277.
377 APHIS, Draft Environmental Impact Statement on Release of Engineered Citrus tristeza virus (n. 375), 33–34.
378 *Ibid.*
379 *Ibid.*, 33.
380 *Ibid.*, 33–39.

present in products derived from crops that are susceptible to the virus.[381] Moreover, the virus might be delivered to untargeted plants by insects.[382]

II. Self-Disseminating Vaccines

Scientists have proposed to harness the self-propagating capabilities of viruses to develop self-disseminating vaccines.[383] This could be achieved either by modifying a pathogenic wild-type virus not to cause illness or by inserting gene sequences from the target pathogen into a benign but quickly-dispersing virus.[384] Once released, this modified virus would move through its target populations but confer immunity rather than causing disease.[385] According to scientists, self-disseminating vaccines could be designed to be either indefinitely 'transmissible' or merely 'transferable', meaning that only individuals to which the vaccine is administered would be able to pass it on to other individuals.[386] A different study suggested using transgenic mosquitoes as 'flying vaccinators' to deliver vaccines via blood-feeding.[387]

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381 It was concluded that this posed no health risk because the citrus tristeza virus was not pathogenic to humans and, since virtually all citrus produced in Florida was infected with the virus, the virus likely was already 'consumed on a regular basis', cf. *ibid.*, 10–11.

382 Cf. *Michelle Heck*, Insect Transmission of Plant Pathogens: A Systems Biology Perspective, 3 (2018) mSystems e00168–17; but note that the modified virus strains reportedly are either not transmissible or have extremely low transmissibility by insects, see APHIS, Draft Environmental Impact Statement on Release of Engineered Citrus tristeza virus (n. 375), 8. There appear to be no independent or peer-reviewed studies available on the questions of transmissibility and hazardousness to human health.

383 See *Crystal Watson* et al., Technologies to Address Global Catastrophic Biological Risks (2018), 45–47.

384 *James J. Bull* et al., Transmissible Viral Vaccines, 26 (2018) Trends in Microbiology 6.

385 *Filippa Lentzos/R. Guy Reeves*, Scientists Are Working on Vaccines that Spread Like a Disease. What Could Possibly Go Wrong?, Bulletin of the Atomic Scientists, 18 September 2020, available at: https://thebulletin.org/2020/09/scientists-are-working-on-vaccines-that-spread-like-a-disease-what-could-possibly-go-wrong/ (last accessed 28 May 2022); *Filippa Lentzos* et al., Eroding Norms over Release of Self-Spreading Viruses, 375 (2022) Science 31, 32.

386 *Scott L. Nuismer/James J. Bull*, Self-Disseminating Vaccines to Suppress Zoonoses, 4 (2020) Nature Ecology & Evolution 1168, 1169.

387 *D. S. Yamamoto* et al., Flying Vaccinator; a Transgenic Mosquito Delivers a Leishmania Vaccine via Blood Feeding, 19 (2010) Insect Molecular Biology 391.

The first known field trial of a transmissible vaccine was carried out by Spanish researchers in 2001, targeting two infectious diseases threatening the European rabbit population.[388] In light of the COVID-19 pandemic, efforts to develop self-disseminating vaccines have received renewed attention.[389] To date, they are primarily discussed as a means to control the spread of *zoonoses*, i.e. pathogens of animal origin that can be transmitted to humans,[390] such as Ebola,[391] MERS, and SARS-CoV-2.[392] Currently, about 10 institutions worldwide are known to do significant work on self-disseminating vaccines.[393] A research project funded by DARPA aims at 'creating the world's first prototype of a self-disseminating vaccine designed to induce a high level of herd immunity (wildlife population level protection) against Lassa virus […] and Ebola'.[394]

Outside of experiments, the deployment of self-disseminating vaccines will likely face considerable technical challenges, such as identifying appropriate targets for intervention and ensuring that the immunity is maintained in the long term.[395] The approach also raises dual-use concerns, because the research could be repurposed to develop self-spreading,

388 *Juan M. Torres* et al., First Field Trial of a Transmissible Recombinant Vaccine Against Myxomatosis and Rabbit Hemorrhagic Disease, 19 (2001) Vaccine 4536.

389 Cf. *Michael Cogley*, Could Self-Spreading Vaccines Stop a Coronavirus Pandemic?, The Telegraph, 31 January 2020, available at: https://www.telegraph.co.uk/technology/2020/01/28/could-self-spreading-vaccines-stop-global-coronavirus-pandemic/ (last accessed 28 May 2022); *Nuismer/Bull* (n. 386); *Rodrigo Pérez Ortega*, Can Vaccines for Wildlife Prevent Human Pandemics?, Quanta Magazine, 24 August 2020, available at: https://www.quantamagazine.org/can-vaccines-for-wildlife-prevent-human-pandemics-20200824/ (last accessed 28 May 2022); *Lentzos/Reeves* (n. 385).

390 Cf. 'zoonosis', in: Henderson's Dictionary of Biology (n. 5), 638.

391 *Yoshimi Tsuda* et al., A Replicating Cytomegalovirus-Based Vaccine Encoding a Single Ebola Virus Nucleoprotein CTL Epitope Confers Protection Against Ebola Virus, 5 (2011) PLoS Neglected Tropical Diseases e1275.

392 *Nuismer/Bull* (n. 386); *Scott L. Nuismer* et al., Eradicating Infectious Disease Using Weakly Transmissible Vaccines, 283 (2016) Proc. R. Soc. B; *Aisling A. Murphy* et al., Self-Disseminating Vaccines for Emerging Infectious Diseases, 15 (2016) Expert Review of Vaccines 31.

393 *Lentzos/Reeves* (n. 385).

394 UC Davis, Big Win: New Countermeasures to Eliminate Pandemic Risk, available at: https://www.preemptproject.org/s/BIG-WIN-New-Countermeasures.pdf (last accessed 28 May 2022); see DARPA, PREventing EMerging Pathogenic Threats (PREEMPT) (17 November 2020), available at: https://www.darpa.mil/program/preventing-emerging-pathogenic-threats (last accessed 28 May 2022).

395 *Lentzos/Reeves* (n. 385); *Lentzos* et al. (n. 385), 31–32; see *Bull* et al. (n. 384), 9–14.

potentially irreversible biological weapons.[396] Theoretically, transmissible vaccines could even be applied to humans,[397] although this would raise serious ethical and human rights-related concerns.[398]

III. Mass Releases of Sterile Genetically Modified Insects

Another strategy to suppress populations of insect species that are plant pests or disease vectors is to release masses of individuals genetically modified to be sterile.[399] This builds upon the conventional *sterile insect technique*, in which male insects are sterilized by irradiation.[400] The use of genetically modified insects seeks to increase the efficiency and flexibility of these programs, as conventional approaches offer limited ways to separate the sterilized males wanted for release from females, which are undesired for release because they still can lay eggs and transmit diseases through biting.[401]

In contrast to gene drive applications, the use of non-drive sterile insects for population suppression requires continuous releases of large numbers of modified individuals. The use of genetically modified sterile insects can thus be seen as a 'precursor' to gene drive applications, where additional genetic components are used to disseminate the genetic modification conferring sterility within the target population.[402] In the past, genetically modified insects have already been released in a number of cases in various countries.[403]

396 *Lentzos/Reeves* (n. 385); *Lentzos* et al. (n. 385), 33.
397 *Murphy* et al. (n. 392); *Bull* et al. (n. 384), 14; see *Lentzos/Reeves* (n. 385), noting that 'there is no clear evidence that anybody is actively working' on self-spreading vaccines for humans.
398 *Watson* et al. (n. 383), 46–47.
399 *Marshall* (n. 263), 164.
400 Cf. *W. Klassen/C. F. Curtis*, History of the Sterile Insect Technique, in: Victor A. Dyck/J. Hendrichs/A. S. Robinson (eds.), Sterile Insect Technique (2005) 3.
401 Cf. *Reeves/Phillipson* (n. 358), 4–5.
402 See *supra* section C.III.1.b).
403 Cf. *Reeves* et al. (n. 271), 1.

IV. Use of Wolbachia to Suppress Mosquito-Vectored Infectious Diseases

An alternative approach to suppress certain infectious diseases not necessarily involving genetic modification is to introduce strains of *Wolbachia* into the *Aedes aegypti* mosquito.[404] Wolbachia is a heritable, intra-cellular bacterium that naturally occurs in many insect species.[405] Its presence within *Aedes aegypti* shortens the lifespan of these mosquitoes[406] and reduces their ability to spread viruses such as *Dengue fever*[407] and *Zika*.[408]

An initiative called *World Mosquito Program* has announced plans to release *Wolbachia*-carrying mosquitoes in a number of countries, claiming that their approach neither suppressed mosquito populations nor involved genetic modification.[409] Deployments of Wolbachia-infected mosquitoes in *Townsville* in Australia[410] as well as in *Yogyakarta* in Indonesia[411] were reported to effectively reduce the local transmission of Dengue to humans.

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404 See *Marshall* (n. 263), 163–164.

405 Cf. *Laura R. Serbus* et al., The Genetics and Cell Biology of Wolbachia-Host Interactions, 42 (2008) Annual Review of Genetics 683.

406 *Conor J. McMeniman* et al., Stable Introduction of a Life-Shortening Wolbachia Infection into the Mosquito Aedes Aegypti, 323 (2009) Science 141; *Luciano A. Moreira* et al., Human Probing Behavior of Aedes Aegypti When Infected with a Life-Shortening Strain of Wolbachia, 3 (2009) PLoS Neglected Tropical Diseases e568.

407 *T. Walker* et al., The *W*Mel *Wolbachia* Strain Blocks Dengue and Invades Caged *Aedes Aegypti* Populations, 476 (2011) Nature 450.

408 *Luciano A. Moreira* et al., A Wolbachia Symbiont in Aedes Aegypti Limits Infection with Dengue, Chikungunya, and Plasmodium, 139 (2009) Cell 1268; *Heverton L. Carneiro Dutra* et al., Wolbachia Blocks Currently Circulating Zika Virus Isolates in Brazilian Aedes Aegypti Mosquitoes, 19 (2016) Cell Host & Microbe 771; see *Champer* et al. (n. 215), 156.

409 See World Mosquito Program, Our Wolbachia Method, available at: https://www.worldmosquitoprogram.org/en/work/wolbachia-method (last accessed 28 May 2022).

410 *Scott L. O'Neill* et al., Scaled Deployment of *Wolbachia* to Protect the Community from Dengue and Other *Aedes* Transmitted Arboviruses, 2 (2018) Gates Open Research 36.

411 *Ewen Callaway*, The Mosquito Strategy that Could Eliminate Dengue, Nature News, 20 August 2020, available at: https://www.nature.com/articles/d41586-020-02492-1 (last accessed 28 May 2022).

F. Summary

Genetic change is a natural phenomenon that has been influenced by humankind for a long time. However, modern biotechnology has made significant advancements in the last decade. Especially the discovery of the CRISPR system and its development as a versatile tool for genome editing has vastly enlarged the 'molecular toolbox'. Applications of these new possibilities already exist and can be expected to arise in many areas including agriculture, basic and medical research (including gene therapy and genome editing in the human germline) and industrial biotechnology.

However, the probably most significant advancement is the development of engineered gene drives and other self-spreading techniques, which can either bias the Mendelian rules of inheritance or even spread horizontally within the same generation of organisms. This potentially allows one to confer new traits to natural populations of species or crop plants within a single generation. But it also makes it possible to inhibit the reproductivity of organisms and thereby suppress populations of species, potentially to the point of extinction.

The technological leap made with self-spreading biotechnology cannot be overestimated: while conventional GMOs are developed in the laboratory and can be thoroughly tested before being released into the environment, self-spreading techniques inherit the 'molecular toolbox' itself and the genetic modification is carried out in the target organism and without direct human intervention. Thus, the advent of self-spreading biotechnology means that 'the laboratory moves into the environment'.[412] However, the ecological effects of these techniques have not yet been sufficiently scrutinized, and there is a substantial likelihood that they are released into the environment before their risks are fully understood. This poses considerable challenges to existing scientific conventions but, as will be discussed in the subsequent chapters, also to international law.

412 *Samson Simon* et al., Synthetic Gene Drive: Between Continuity and Novelty (2018) EMBO Reports e45760, 2.