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Statement on the Handling of Gene Drives

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Gene Drive is a technology for facilitating the spread of a particular genetic trait, such as a mutation, which has the potential to alter the genetics of a whole population of interest in a certain geographical area. The technical difficulty in employing Gene Drive has been overcome by combining CRISPR/Cas9, a known genome editing technique, which has expanded the horizon of Gene Drive's possible applications. Gene Drive technology is expected to be widely used by researchers in various fields, particularly in studies on deliberate extinction of organisms that act as vectors for pathogens and alien species.

On the other hand, organisms modified by Gene Drive technology (Gene Drive Organisms) have the potential to rapidly spread certain heritable characteristics in the population of interest, in a non-Mendelian fashion. For that reason, users of these organisms must be very careful when handling them.

Given this background, for extensive discussions about the safety and ethically appropriate handling of Gene Drive, a working group on Gene Drive has been established in the AAPGS. Members of the working group have been exchanging views on Gene Drive with experts at home and abroad, including procedures for appropriate handling of Gene Drive.

In view of the potentially significant impact of Gene Drive on the environment, the AAPGS would like to call your attention to the following recommendations about Gene Drive:

1. Share information on Gene Drive with all researchers at the institution:
   Be aware of the debates on Gene Drive at home and abroad. The academic community is discussing the potential benefits and risks in using this technology. The essential first step is to share information on and relating to Gene Drive with researchers inside the institution. Please see the Appendix for reference information.

2. Protocols or planned experiments involving Gene Drive technology
   Gene Drive is likely to be used with CRISPR/Cas9; thus, if a protocol or planned recombinant DNA experiment describing CRISPR/Cas9 has been submitted to the institutional review board and use of Gene Drive is suspected from the description, the institution is recommended to contact the applicant to determine whether it involves Gene Drive.

3. Be certain that appropriate containment measures are taken
   As indicated above, Gene Drive Organisms have the potential to rapidly spread certain characteristics in the population of interest. Thus, it is extremely important to take appropriate containment measures in compliance with the Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms. If a protocol or planned experiment involving Gene Drive has been submitted, the institutional review board at the research institution is recommended to fully confirm that appropriate containment measures have been taken. For specific examples of containment measures, please see the Appendix.

We recommend that all research institutions act immediately in response to this warning. Share information on Gene Drive with all researchers at the institution and gather internal information about it. Then institutional reviews of protocols or planned experiments involving Gene Drive can be carried out appropriately.

Should you have any comments or information on Gene Drive, please contact the Secretariat of the AAPGS.

Thank you in advance for your understanding and cooperation in this matter.

If you have any questions regarding this communication, please contact:
Secretariat for the Academic Association for Promotion of Genetic Studies in Japan (AAPGS)
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Appendix

1 Gene Drive

Gene Drive is a general term for a phenomenon in which inheritance of a particular genetic trait is spread preferentially in biological populations, or the technology to accomplish this. Gene Drive is capable of spreading a particular gene trait in non-Mendelian fashion, and thus involves the risk of replacing a genetic trait throughout an entire population (Figure 1).

In nature, homing nuclease, meiotic drive, etc. have been observed as Gene Drive, but in recent years, development of artificial Gene Drive with CRISPR/Cas9 has become increasingly popular. With artificial Gene Drive, experiments using yeast, *Drosophila*, and mosquitos demonstrated that targeted genetic types were modified at very high rates in laboratory populations (in the case of *Drosophila*, average 97% in F2 flies). Gene Drive with CRISPR/Cas9 can be applied by preparing a simple construct in which the host genome sequence is placed between DNAs of Cas9 and the guide RNA (Figure 2) and then introducing it into a germ cell. The elimination of technical barriers to the application of Gene Drive has led many researchers to explore possible applications of Gene Drive in search for measures against infectious diseases, solutions to agricultural problems, means of preserving species, etc. Thus far, Gene Drive studies on malaria-transmitting mosquito populations have been ongoing, and there are successful reports on mosquitos that have lost the capacity to retain the malarial protozoan, and on mosquitos with artificially modified reproductive capacity.

(This page was prepared based on the texts of *Gene Drive on the Horizon*, Summary, Pp1-3, 2016; Committee on Gene Drive Research in Non-Human Organisms, The National Academic Press)

Mendelian Inheritance　　Biased Inheritance with Gene Drive

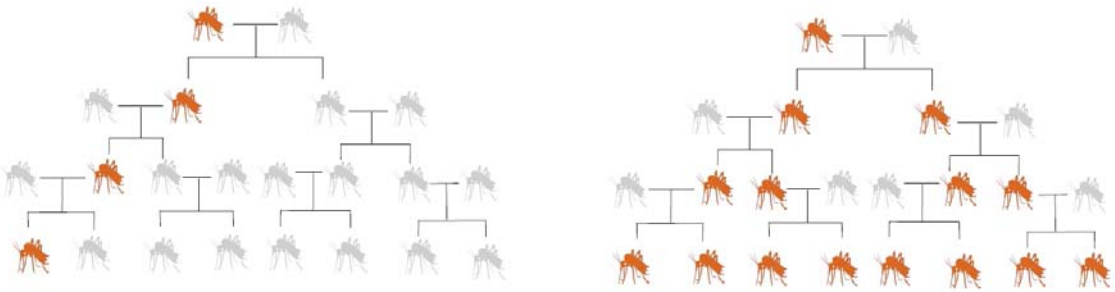

Figure 1. Schematic diagrams of inheritance: Mendel's law and Gene Drive

In non-biased heredity (Mendelian inheritance), since offspring inherit one allele from each parent (50%), preferential spread of a particular trait rarely occurs. In biased heredity with Gene Drive, theoretically, all of the offspring in a population become homozygous for the factor introduced with Gene Drive. As a result, such a trait rapidly spreads in the population, and the whole species can acquire that genetic trait after several generations.

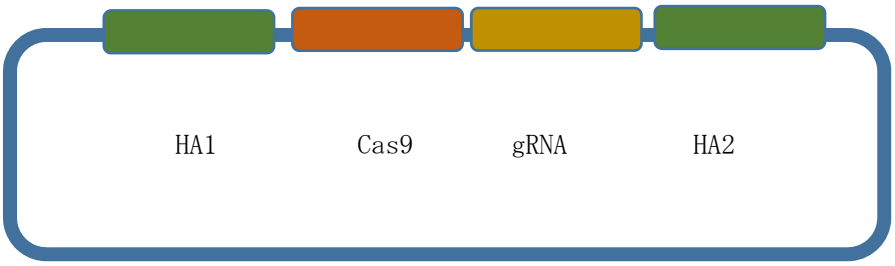

Figure 2. A construct for Gene Drive using CRISPR/Cas9

HA1 & HA2: "Homology Arm 1" and "Homology Arm 2". Sequences homologous with genome sequences of the species of interest.

Cas9: A base sequence encoding the Cas9 protein. In use, it would be combined with a promoter expressed in a germ cell or other specific cell.

gRNA: The base sequence of the guide RNA. The guide RNA is produced by transcription using a promoter suitable for expressing a small RNA.

2 Containment measures

If Gene Drive Organisms escape into the environment, it would have significant impact compared to those that general living modified organisms (LMOs) could have. Thus, when carrying out an experiment involving Gene Drive, enhanced containment measures must be taken, compared to standard measures required for LMOs. Also, researchers have been discussing the need of additional safeguards so as not to release such traits into the environment, even in the event of an accidental escape. From this perspective, researchers should take containment measures conforming to the Cartagena Act and the R&D Type 2 Ministerial Ordinance*, and then provide high-level containment measures and additional safeguards suitable for the experiment or experimental organisms involving Gene Drive.

*Cartagena Act: Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms
R&D Type 2 Ministerial Ordinance: Ministerial Ordinance Providing Containment Measures to Be Taken in Type 2 Use of Living Modified Organisms for Research and Development

[Examples of enhanced containment measures]

<u>Facility, installation and equipment</u>

- Installing multiple doors, air curtains, and/or an airlock.
- Using containment by air flow devices such as blast air fans or air showers.
- Filters for air conditioning equipment and air vents.
- Multiplexed containers or devices for holding organisms.
- Place containers for holding organisms in a low-temperature chamber.
- Surrounding containers for holding organisms with mineral oil or adhesive sheets.
- Keeping pesticides in the lab.
- Lab coats, shoes, etc. dedicated to the Gene Drive laboratory.
- Containers holding Gene Drive Organisms should be so labeled.
- Posting a sign saying "Gene Drive Experiment in Progress" outside a room while implementing a Gene Drive experiment.

<u>Considerations for implementing Gene Drive experiments</u>

- Prohibit the entry of all personnel other than authorized personnel during Gene Drive experiments.
- Wear lab coats, shoes, etc. dedicated to the Gene Drive laboratory, and remove them at the end of the experiment (to prevent inadvertent transfers of seeds or insects out of the laboratory).

- Never conduct a Gene Drive experiment concurrently with other experiments.
- Permit only skilled, experienced persons perform Gene Drive experiment.
- Keep Gene Drive Organisms separated from other organisms while holding.
- Inactivate or anesthetize organisms before taking them out of holding containers.
- Manage the number of Gene Drive Organisms depending on their characteristics (e.g., the number of mice kept in the cage, the number of vials if yeasts or *Drosophila*, etc.).
- Keep detailed records of the experiment.

[Examples of additional safeguards]

The following measures may be taken to enhance containment:

Category	What to consider	Examples
Genetic engineering technique	◆ Place genetic factors needed in Gene Drive in different loci. ◆ Use a sequence that does not exist in wild types as a target of RNA-dependent nuclease. ◆ Use a promoter to regulate expression of RNA-dependent nuclease ◆ Make use of a factor inhibiting Gene Drive. ◆ Design organisms incapable of exchanging genes with wild organisms.	⬧ Arrange the Cas9 and gRNA sequences in different loci. ⬧ Use a GFP gene sequence as the target of Cas9. ⬧ Regulate expression of Cas9 by the "inducible promoter + Cas9" cassette. ⬧ Regulate activity of Cas9 with anti-Cas9 antibodies or Cas9 inhibitory proteins. ⬧ Use *Drosophila* with abnormal chromosomes.
Geography	◆ Carry out experiment in a facility located in an environment (including climate, season) where the organisms cannot live. ◆ Carry out experiments in an area where wild species capable of crossing with the organisms do not exist. ◆ Carry out experiments in places that can limit possible spread, even if the organisms escape into the environment.	⬧ Carry out experiments on *Aedes aegypti* (dengue mosquito) in Sapporo during the winter. ⬧ Carry out experiments with marine fish in an inland area. ⬧ Carry out experiments in an isolated place (such as an isolated island)

3 References

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