To Catch a Clone

A new use of laser technology identifies clones among flowering plants Tim Dickinson & Nadia Talent

Conducting field research around the world and bench work in the ROM’s own laboratories, ROM curators study the life sciences, art, archaeology, and world cultures. This new column offers a glimpse into their techniques.

In their studies, botanists must understand the patterns of variation they observe in groups of plants in order to categorize them. If they see a local population of plants that differs from other populations nearby, they might think they are seeing a new species. But if the “different-looking” plants are all genetically identical—in other words are members of a clone—they aren’t necessarily considered a species, since individual plants in a species are expected to be genetically variable.

Most plants reproduce sexually just like humans and other animals, and therefore the DNA varies from one individual to another. But long before humans created Dolly the cloned sheep, some plants have been able to reproduce asexually, by cloning—the offspring are exact replicas of the parent, and have exactly the same DNA.

Some plants clone themselves by sprouting new shoots from their roots or by sending out runners, and the new clones are located near the parent plant. Others, such as hawthorn trees, are able to clone themselves through their seeds, and these clones can be dispersed far from the parent plant. Recently, a technique used for more than 30 years for cancer diagnosis and other purposes has been adapted to reveal how a plant reproduces. Our lab is one of a small number that has been able to use this technique to tell whether seeds, in our case from the hawthorns we study, were produced sexually or asexually.

Briefly, here’s how reproduction works in flowering plants such as hawthorns. Sexual reproduction involves two fertilizations. First, a cell divides by a process called meiosis, halving the number of chromosomes, and thus the amount of DNA. This is similar to the way animals reproduce. But unlike animals, the plant parents make pairs of gametes (sex cells) instead of just one each—in the ovule there is one egg cell and one “central cell,” while in the pollen grains, there are two sperm. The egg cell is fertilized by one sperm; this produces an embryo from which a new plant can grow. The central cell is fertilized by the...
SEED FORMATION IN HAWTHORNS

Fluorescence data from cytometer analysis reflects how seed contents differ for sexual and asexual reproduction.

SEXUAL (NON-CLONAL) REPRODUCTION

Prior to fertilization
- central cell nuclei (each 1m)
- egg (1m)
- sperm nuclei (each 1p)
- pollen tube

After fertilization
- endosperm nucleus (2m + 1p)
- zygote (1m + 1p)

SEXUAL SEED CYTOMETER FLUORESCENCE DATA

CLONAL (ASEXUAL) REPRODUCTION

Prior to fertilization
- central cell nuclei (each 2m)
- egg (2m)
- sperm nuclei (each 1p)
- pollen tube

After fertilization
- endosperm nucleus (4m + 1p)
- clonal proembryo (2m)

ASEXUAL SEED CYTOMETER FLUORESCENCE DATA

*egg that develops into an embryo without fertilization

Other sperm; this forms the endosperm, a nutritive tissue that helps the seed grow. Plants that reproduce asexually make clones of themselves by producing egg cells without meiosis, and embryos without fertilization.

Telling the two types of embryos apart—those of clonal seed and those of sexual seed—is difficult. But the two kinds of endosperm have an exciting difference—because meiosis does not occur, endosperm nuclei from clonal seeds contain more DNA.

We find out how much DNA there is in the endosperm by measuring its fluorescence. First we release the nuclei from cells of the endosperm and embryo—which contain the DNA that we are interested in—by chopping individual seeds with a razor blade. We add a stain that binds to the DNA and then activate it by shining the laser beam of an instrument called a flow cytometer on it. The cytometer measures fluorescence of the individual nuclei as they flow past the laser. The amount of fluorescence is proportional to the amount of DNA.

A graph of the fluorescence reveals that endosperm signals differ significantly for the two reproduction methods. In clonal seeds, the endosperm peak is further to the right because there is more DNA in each nucleus.

That’s how we tell a clonal seed from a sexual one, and learn which hawthorn trees are cloning themselves—which informs our decisions about whether to recognize a new species.

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Above left, top to bottom: Hawthorn flowers; closeup of a flower; hawthorn fruits; hawthorn fruit in section, showing the woody stone that encloses the seed (at the left, the mature seed in section shows the tissues analyzed by the cytometer); seeds are chopped in stain in order to release the nuclei.

Above right, top diagram: Hawthorn flower in section, and the pathway of the pollen tube (blue dashed line) from the stigma to an ovule. Middle: Events within the ovule at the time of double fertilization (two arrows). Bottom: Events within the ovule when the egg is produced without meiosis, and develops into an embryo without fertilization; only the central cell is fertilized (arrow). In these diagrams m and p stand for maternal and paternal sets of chromosomes. As more sets of chromosomes are present in the endosperm nuclei, the endosperm’s cytometry signal (purple) shifts to the right.