Biology	Name:	Date:

Mendel in a Box – Genetics at your fingertips

In the 19th century, the monk Gregor Mendel carried out various crossing experiments with peas and then formulated three central rules of inheritance. You will recreate some of the experiments, but not with peas, but with an herb called *Arabidopsis thaliana*. It is the most important model plant in science, it is the botanist's mouse, so to speak. Since it does not grow as large as the pea, you can easily grow it on your windowsill.

The seeds for Mendel in a Box are based on different varieties of Arabidopsis, which differ slightly in their genetic blueprint. Columbia (Col) is the wild mother plant with hairy leaves and normal flower structure (four white petals, all sex organs). From Col, two purebred mutant lines were created by mutation and selection: *wuschelig* (*wus, engl.: fuzzy*) and *kahl* (*ka, engl.: bald*)[1] . *wuschelig* is named after its flowers, which have no sex organs and an abundance of white petals. *kahl,* on the other hand, has no leaf hairs at all, the leaves are completely smooth (see page 2).

For this experiment, you will sow the offspring of different crosses between these lines and observe how they develop. A graphical overview of the mutants and which crosses you are looking at can be seen on page 2. The following seeds are included in this box (the number of pots to be filled is in brackets):

- purebred parentals for Col and *ka*[2] (1 pot each)
- *ka* F1: the offspring of a cross between Col and *ka* (2 pots)
- *ka* F2: the offspring of a *ka* F1 plant[3] (5 pots)
- *wus* F2: the progeny of a plant ("*wus F1"*) containing the mutant and the wild
- *wus* gene (5 pots)
- *ka x wus* F2: the offspring of a plant (*ka x wus* F1) created by crossing a *ka* F1
- and "*wus* F1" plant (16 pots)

Divide into groups of 2-3 students. One group is responsible for preparing the pots for the other groups, who sow the seeds.

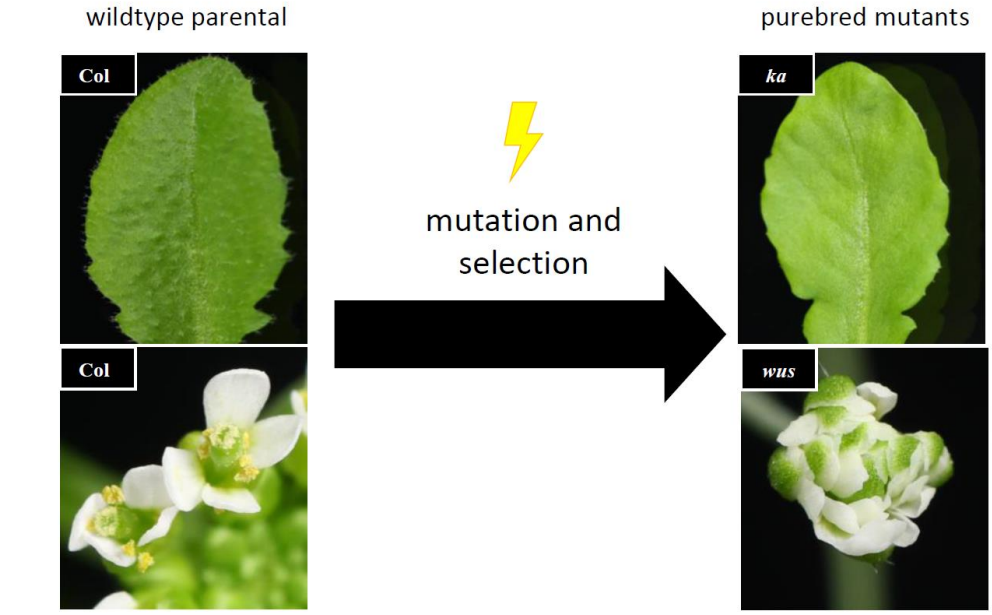
Tasks:

1. Proceed in your groups according to the instructions (preparing pots, sowing seeds → see page 3)
2. In your groups, think about the following tasks and write down your suggested solutions in your notebooks
    a. Name properties that make *Arabidopsis thaliana* a suitable model plant.
    b. Hypothesise about the characteristics that the different plant lines in the pots will have.

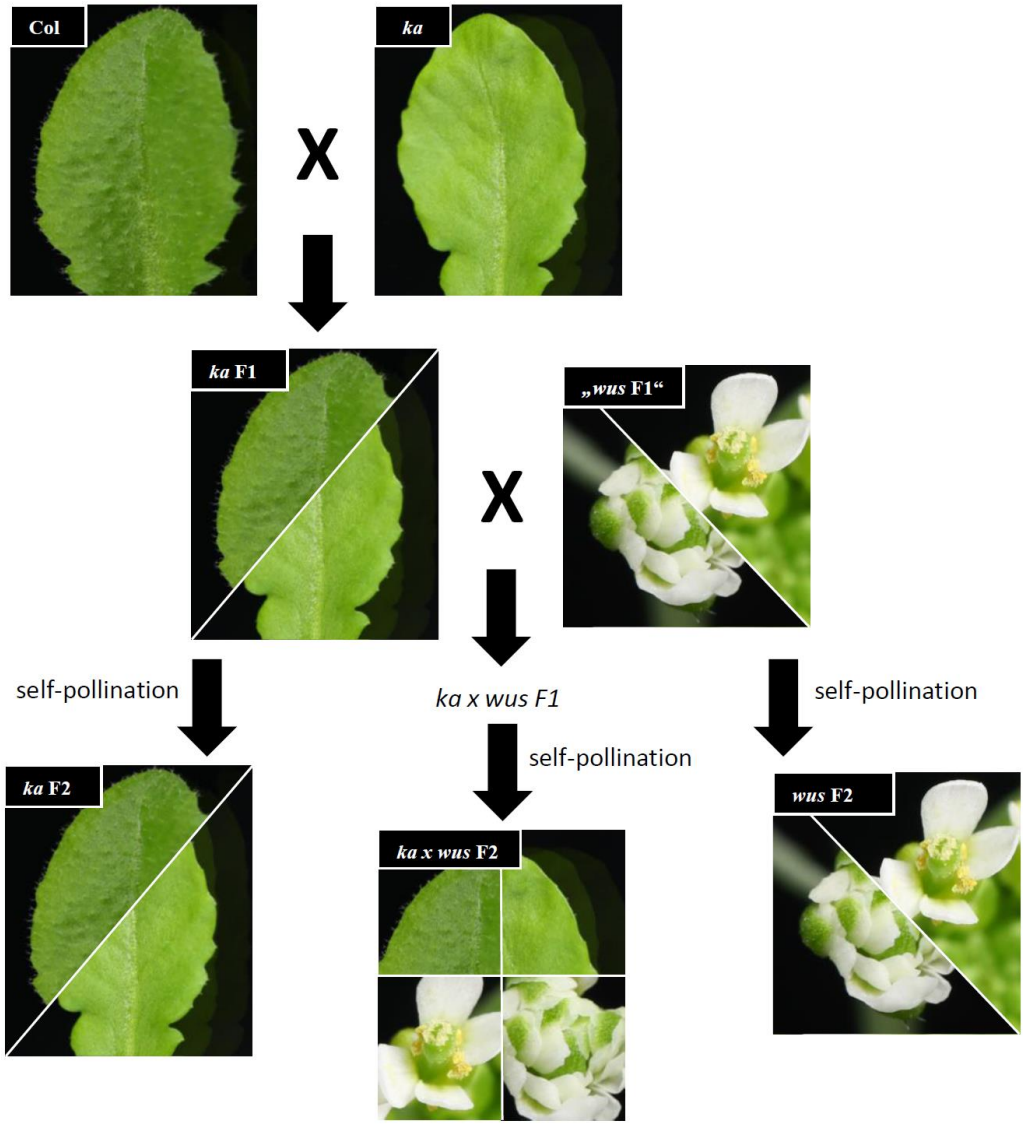
[1] In biology, new mutants are usually named after their appearance and scientists can get very creative. Other mutants that have been officially published are: *superman*, *yoda* and *schnarchzapfen (sleepyhead in German)*.

[2] *wus* mutants do not possess sexual organs and therefore do not produce offspring. Purebred *wus* plants can therefore not be crossed or propagated.

[3] Arabidopsis is facultatively self-pollinating. This means that the plant normally self-pollinates but can also be artificially crossed.



crossing scheme

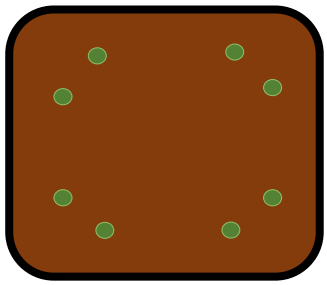


Group 1: Preparing pots

The other groups need the pots to sow their seeds, so work quickly! Fill a bucket with soil and gradually add water. Mix the soil well so that no large clumps remain. **Do not add too much water**, there should be no standing water in the bucket and the soil should only be damp, not soaking wet! If you see standing water in the bucket, pour it off. Remove any large pieces of wood. Fill all the pots **to the brim, do not** press the soil as the seeds need **airy soil**. Get the filled pots ready for sowing and clean everything up when you are done with the pots.

Remaining groups: Sowing seeds

Each group is provided with seeds in small plastic pots. You can find out which seeds you have and how many pots to fill on page 1. In addition, each group needs a label for each pot, toothpicks, and a small piece of cellulose for each pot with seeds (handkerchief, paper towel or similar, approx. 10x10 cm).
Label the tags for your pots and place the paper in front of you on the table or a plate. Label the paper with the name of the seeds you have been given and open the corresponding tube. Tilt the contents of the tube in one sweep onto the paper so that the seeds are well distributed on the wet paper. Now get the pots you need from group 1.



Sow eight seeds in the corners of the pot but leave some space around the edge!

Moisten a toothpick with your mouth or some water and carefully pick up a seed with the tip. **Attention! Be careful not to crush the seeds and only pick up one seed.** Lay the tip of the toothpick onto the soil and roll it slightly in your fingers. This should loosen the seed. **Do not submerge the seeds in soil** (Arabidopsis is light-germinating). Make notes of which corners of the pot you have already filled. If some soil or the seed does not come off the toothpick, use some paper to clean the toothpick and take a new seed. When you have distributed eight seeds into all the pots, place them in the tray and put the cover on top with the ventilation slides open.

Place the tray on the windowsill and switch on the plant lamp (if available). Now it's time to wait and observe!

While the plants are growing

Leave the cover on the tray for the first two to three weeks while the plants are still small (less than six leaves). Regularly check the wetness of the soil as the seeds and seedlings are highly drought-sensitive.

After 1-2 weeks: If you have pots with reduced germination, transfer **young** seedlings between pots of the same genotype. **Discard** seedlings in all pots until there are only **four per pot** to leave sufficient room for the plants.

1. Do you see a difference between the first two leaves (cotyledons) and the following ones? What could be the reason for this?

Once the cover is off, you need to make sure that you are regularly watering the plants, so watch for the soil dryness or the leaves collapsing and looking weak. Arabidopsis does not like standing water, so only add about 0.75 litres of water to the tray. If the water is still standing in the tray after a day, you have added too much. On average, young plants need to be watered much less often than older ones (approx. 1-2x per week at the beginning, 2-3x per week towards the end).

1. Why do older plants need more water than young ones?

2. Do you see differences in the growth rate between the different pots? What factors could play a role here?

Worksheet for analysis

As soon as the *wus plants* start flowering, you can start analysing the data. Compare the appearance of the different lines and note the phenotypes observed.

1. Look at the populations. Do the phenotype distributions match your expectations? Make a Punnett square and compare the expected distributions with the hypotheses you made earlier.

2. Based on your results, what phenotype should the plants "*wus* F1" and *ka x wus* F1 have? Why?

3. Why is it not possible to obtain seed with exclusively $wus^{-/-}$ genotype? Can you think of a way to obtain these lines anyway?

4. Gregor Mendel crossed the different pea lines with themselves several times before starting his experiments in order to obtain purebred lines. Hypothesise why this was important for the success of his experiments!

5. If you cross *wus* and *ka* plants with each other, the phenotype distributions correspond perfectly to Mendel's 3rd law. What does this say about the position of the two genes on the *Arabidopsis thaliana* genome?

6. Compare the general appearance of the *wus* F2 and *ka* F2. Do you notice any differences? What about the *ka x wus* F2 cross? What could be the reason for the differences?