Mendel in a Box – Teacher's guide

"Mendel in a Box" is a teaching kit that explains Mendel's laws of inheritance. The pupils grow *Arabidopsis thaliana* plants in class and observe the phenotypes over several weeks. I hope you will have a lot of fun with this box!

The experiment is divided into two lessons: Sowing and analysis, additional tasks or topics can be addressed while the plants are growing. The analysis can start about 8-14 weeks after sowing, depending on light conditions and temperature.

Components of "Mendel in a Box with plant lamp" (MB-01):

- 2 trays and covers
- 30 pots
- 30 labels
- Toothpicks
- 1 plant lamp (not included in MB-02)
- Teaching material
- Seeds in plastic tubes

Components which are additionally required:

- Bucket and water
- Cellulose paper (handkerchiefs, paper towels or similar)
- approx. 8-10 litres of potting soil

Essential information beforehand!

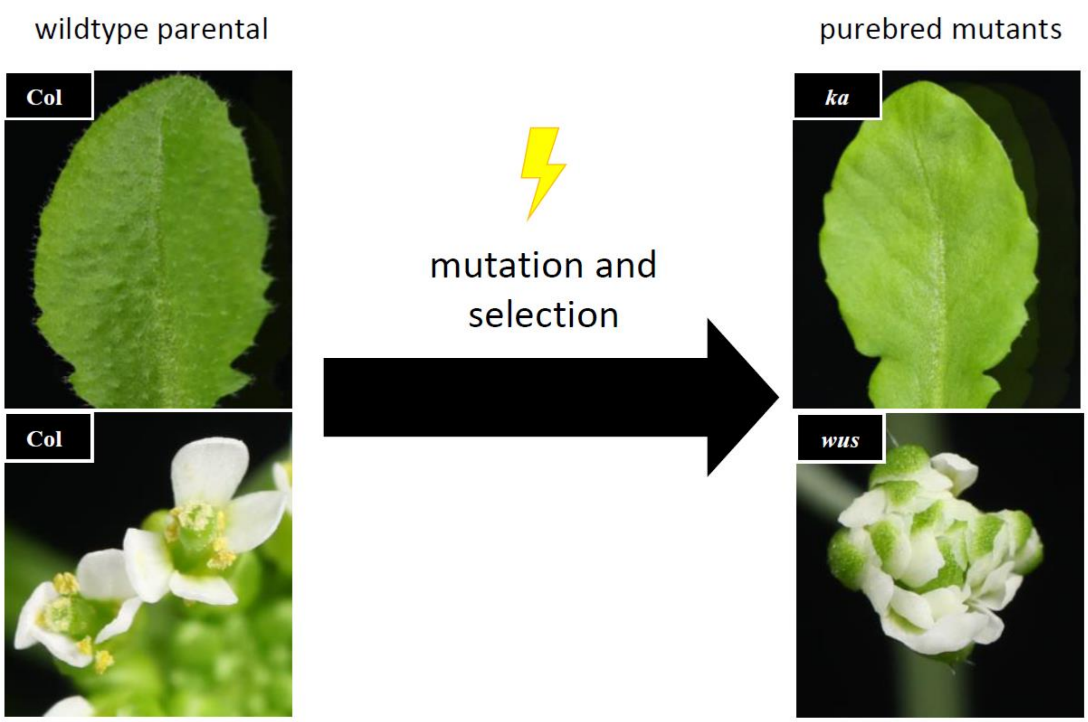
1. With "Mendel in a Box", the pupils work with real living beings. This does not guarantee that everything will always go according to plan. Be prepared to be flexible!
2. The seeds must be stratified before the first lesson. This means you have to fill the plastic tubes with water and put them in a refrigerator. The cold will ensure more uniform and faster germination. It is recommended to store the seeds in the refrigerator for three to five days before the experiment.

3. If you are pressed for time, the first evaluation of results can already be done after approx. 2-3 weeks, as *ka/ka* plants do not have plant hairs on the true leaves, i.e. on the first leaves after the two cotyledons. For *wus* plants, however, you have to wait until the first flowers open.

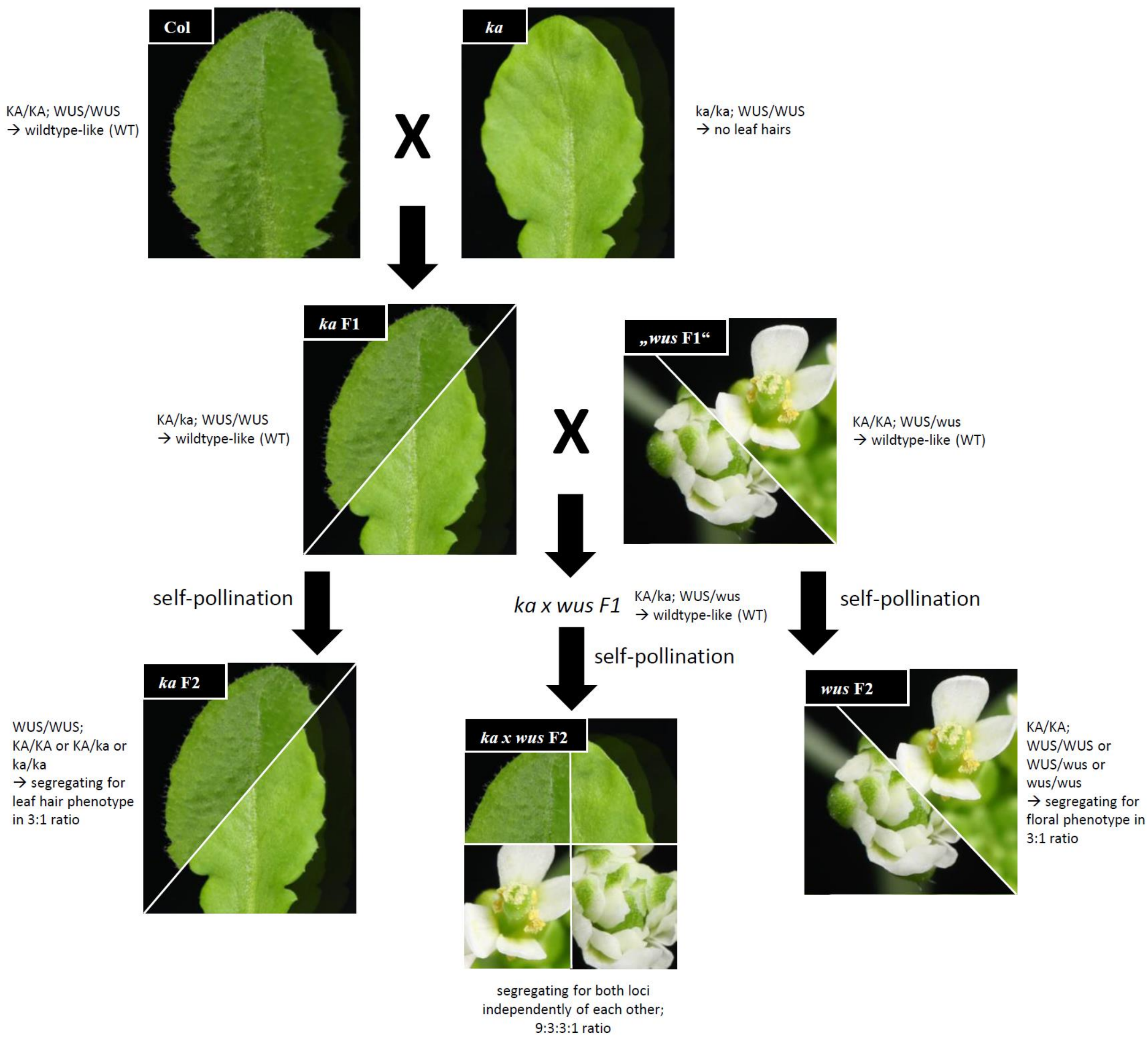
The following seeds are included in „Mendel in a Box“:

- Col (wildtype parental)
- *ka/ka* (mutant parental)
- *KA/ka* (F1)
- *ka* segregating (F2)
- *wus* segregating („F2“, homozygotes not fertile, can’t be propagated)
- *KA/ka WUS/wus* segregating (third Mendelian law)

➔ you can find and overview of the geno- and phenotypes on the next page



crossing scheme



Lesson overview

1st lesson (45-60 min.): Sowing

- Introduction to Mendel and classical genetics, if applicable
- Reading through the worksheet
- divide into groups, Group 1: Preparation of the pots

While preparing the pots for the other groups:

- Allocation of the seeds (note the number of pots!), prepare labels and paper
- Solving the other questions if students have to wait longer for pots

Once the pots are distributed:

- Group 1: tidy up, answer questions
- other groups: Sowing and answering questions
- evaluation of the tasks and questions

General advice

- Group 1 should be assigned first so that the pots are available quickly
- Group size max. 2-3 pupils
- labels should be written legibly to facilitate evaluation
- When sowing, make sure that the seeds are not crushed, placed **on top of** the soil and pupils write down which pot corners are already filled

After sowing:

- the cover cannot be placed in line with the tray, it will sit on the pots, keep ventilation slider open!
- Turn on the plant lamp (**heavily recommended! Arabidopsis is light-germinating**), do not forget the timer function.
- Avoid draughty windows and fluctuating temperatures, especially during germination. Arabidopsis ideally grows under constant warm temperatures (seal windows, keep heating running, maybe germinate in some distance to windows)

While the plants are growing

- Check regularly for wetness of soil. **Especially in the beginning, seeds and seedlings are highly drought-sensitive**
- If pots contain more than four plants, remove them with tweezers or fingers and, if necessary, place them in pots with too few plants (after 1-2 weeks)
- Regularly observe plant growth together with the pupils (see worksheet for sample questions).

Experiments that can be carried out in parallel

With "Mendel in a Box", you cannot only explain the laws of inheritance in a more tangible way, but also obtain fresh material for other teaching units in other classes. The following series of experiments are also conceivable with "Mendel in a Box" (feel free to write to us if you have additional ideas!):

- Leaf morphology with the binocular microscope; differences between upper and lower leaf surfaces, leaf hairs
- Leaf anatomy with the microscope; leaf cross-section, tissue layers in leaves of dicotyledonous land plants
- Flower structure of angiosperms; comparison of *wus* and wild-type flowers

2nd lesson (45-90 min.): analysis

- Review of the premises from the 1st lesson, hypotheses and, application of previously learnt knowledge
- Counting the phenotypes
- Answering the questions from the worksheet together or in small groups

Excursion: Statistical analysis

It can be exciting to make an excursion into the statistical analysis of such data in order to be able to make statements about whether the null hypothesis can be considered falsified or not. Here a chi-square test is used, which can be calculated very easily and comprehensibly on the blackboard (see appendix for a calculation example).

Additional background information

The seeds of "Mendel in a Box" consist of different generations and crosses of three lines (one parent, two mutants) of *Arabidopsis thaliana*, also known as thale cress. *A. thaliana* is the most important dicotyledonous model plant in plant research and its genome was therefore completely sequenced in 2000.

The mutant lines in this box are *kahl (ka)* and *wuschelig (wus)*, the parent Columbia (Col). The mutants were each found by mutagenesis using ethyl methane sulfonate (EMS). EMS reacts with nucleotides to cause G/C -> A/T mutations, a method used extensively in agricultural breeding and research.

Plants that are homozygous mutants for *ka* (*ka/ka*) do not develop leaf hairs (trichomes) on their true leaves. *wus/wus* plants show a defective flower structure. This is a homeotic mutation in which the identity for the stamina and carpel is lost, the flower is "filled" and has only petals and sepals. In ornamental breeding this is a very popular phenotype, seen in various begonias or chrysanthemums, but often only the stamina are mutated. As *wus/wus* plants do not produce gametes, the line cannot be maintained homozygously. The mutants were found by mutagenesis of different ecotypes of *Arabidopsis thaliana.* Strictly speaking, the parent of *wus* is not Col, but Landsberg erecta (Ler). Therefore, the mutants also differ to some extent in their general appearance. However, to make it easier for the students, only Col was given as the parent.

Appendix

Example Chi-square test

The chi-square test checks whether an observed distribution of classes (in this case phenotypes) corresponds to the expected distribution based on the previously established hypothesis. Possible hypotheses in this case would be, for example:

Null hypothesis H_0: The *wus* and ka *alleles* are inherited recessively/non-Mendelian.

Alternative hypothesis H_1: The *wus* and ka *alleles* are not inherited recessively/mendelian.

One calculates X^2, which is then compared with values in a table to arrive at the p-value for our statistical test.

$$\chi_c^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

X^2 is calculated from the sum of the squared difference between the observed (O) and expected (E) number of phenotypes, divided by the expected number for each phenotype class. Thus, for each phenotype in the population, one compares how much the number of plants deviates from the expected distribution based on Mendel (null hypothesis). If the deviation is large, X^2 will also be large, and thus the p-value (see below).

Let us take as an example a segregating F2 population for *wus* and *ka* with 64 individuals observed. For the calculation, the following table can be filled in together with the students from left to right:

phenotype	O (observed distribution in pots)	E (expected distribution based on Mendel, 9:3:3:1)	O - E	(O - E)²	(O - E)²/E
Hairy leaves, normal flowers	38	36	2	4	0,11
Bald leaves, normal flowers	14	12	2	4	0,33
Hairy leaves, fuzzy flowers	9	12	3	9	0,75
Bald leaves, fuzzy flowers	3	4	1	1	0,25
				Sum = X²	1,44

Now consult a table to determine the p-value:

n	p						n
	0,5 %	1 %	2,5 %	5 %	10 %	50 %	
1	0,0000	0,0002	0,0010	0,0039	0,0158	0,4549	1
2	0,0100	0,0201	0,0506	0,1026	0,2107	1,3863	2
3	0,0717	0,1148	0,2158	0,3518	0,5844	2,3660	3
4	0,2070	0,2971	0,4844	0,7107	1,0636	3,3567	4
5	0,4117	0,5543	0,8312	1,1455	1,6103	4,3515	5

n is the degrees of freedom, which is always the number of classes minus 1 (i.e. 3 for the third Mendelian law, 1 for the second law). You then look for the calculated X^2 value in the corresponding row (in the example: 1.44) and see in which column you end up in, which gives you the p-value. In this case, the p-value is between 10% and 50% and thus above the classical significance level of 5%.

The p-value indicates the probability of obtaining such an observed distribution again if the null hypothesis is correct, i.e. if there is a Mendelian inheritance. If $p < 5\%$, H_0 can be considered falsified. So there would be a reason to assume that there is no Mendelian inheritance because the distribution is far enough away from Mendelian expectations.

It should be very difficult to obtain a significant result with $p < 5\%$ because the population tested here is comparatively small and genetically there is always an underlying Mendelian inheritance. Nevertheless, it can be the basis for an exciting excursion to talk about scientific studies, reproducibility, and statistical tests.

Answer sheets

Sowing – answers

1. 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

      - small
      - grows quickly
      - small genome
      - robust/undemanding
      - facultative self-pollinator, allows crossing and easy propagation
      - easily transformable (genetically modifiable)

   b. Hypothesise about the characteristics that the different plant lines in the pots will have.

      - purebred parentals for Col and *ka* → **as on page 2**
      - *ka* F1: the descendants of a cross between Col and *ka* → **like Col**
      - *ka* F2: the offspring of a *ka* F1 plant → **3:1; hairy : without leaf hairs**
      - *wus* F2: the descendants of a plant ("*wus F1*"), which is the mutant and wild *wus* gene contains → **3:1; normal flowers : fuzzy flowers**
      - *ka x wus* F2: the offspring of a cross between a *ka* F1 and "*wus* F1" plant → **9:3:3:1; like Col : without hairs, normal flowers : hairy, fuzzy: without hairs, fuzzy**

While the plants are growing - answers

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

   - the first two leaves are the cotyledons and have a much simpler morphology (small, circular leaves and little vascularisation).
   - The plants for the cotyledons are already laid out in the embryo in the seed, they serve the rapid absorption of energy by the sun
   - a complex leaf structure would be more energy-intensive and take longer, which would put it at a competitive disadvantage

2. Why do older plants need more water than young ones?
   - older plants are larger and therefore need more water for their growth
   - they also have more roots and can therefore absorb water more quickly

3. Do you see differences in the growth rate between the different pots? What factors could play a role here?
   - plants often grow faster on the edges of the bowls, as well as on the side facing the window, because they get more light
   - in addition, wus *mutants* are usually smaller, but often flower earlier (see also analysis - answers, Task 5)

analysis - answers

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.
   - purebred parentals for Col and *ka* → **as on page 2**
   - *ka* F1: the descendants of a cross between Col and *ka* → **like Col**
   - *ka* F2: the offspring of a *ka* F1 plant → **3:1; hairy : without hair**

	KA	ka
KA	KA/KA	KA/ka
ka	KA/ka	ka/ka

   - *wus* F2: the progeny of a plant ("*wus F1*") containing the mutant and the wild *wus* gene → **3:1;** normal flowers : fuzzy flowers

	WUS	wus
WUS	WUS/WUS	WUS/wus
wus	WUS/wus	wus/wus

- *ka x wus* F2: the offspring of a cross between a *ka* F1 and "*wus* F1" plant → **9**:**3**:**3**:**1**; **Col** : **without hair, normal flowers** : **hairy, fuzzy** : **without hair,fuzzy**

	KA WUS	ka WUS	KA wus	ka wus
KA WUS	KA/KA; WUS/WUS	KA/ka; WUS/WUS	KA/KA; WUS/wus	KA/ka; WUS/wus
ka WUS	KA/ka; WUS/WUS	ka/ka; WUS/WUS	KA/ka; WUS/wus	ka/ka; WUS/wus
KA wus	KA/KA; WUS/wus	KA/ka; WUS/wus	KA/KA; wus/wus	KA/ka; wus/wus
ka wus	KA/ka; WUS/wus	ka/ka; WUS/wus	KA/ka; wus/wus	ka/ka; wus/wus

2. Based on your results, what phenotype should the plants "*wus* F1" and *ka x wus* F1 have? Why?
   - both wild type, as they are heterozygous for the respective loci

3. Why is it not possible to obtain seed with exclusively *wus*$^{-/-}$ genotype? Can you think of a way to obtain these lines anyway?
   - *wus* $^{-/-}$ is sterile
   - propagation by asexual reproduction/cloning (e.g., by grafting or hormonal induction of new plants from vegetative material).

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!
   - purebred lines are homozygous for all loci (although of course he did not know this explicitly)
   - this was the only way he could ensure that his crossings would work, because he could thus safely know how the "genotype" of his plants was composed

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?
   - there is no linkage, the genes are located on different chromosomes

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?

wus - plants tend to have the following phenotypes:

- smaller plants
- blunt, shorter and broader fruits
- denser, apically clustered inflorescences with shorter pedicels
- fewer side shoots
- tends to grow more upright
- rounder leaves

The *ka x wus* F2 plants will also segregate for the general phenotypes. This is because the Col mother plants used to generate the mutants were not genetically identical. Thus, these additional traits segregate in parallel with those described. The mutants are homozygous for their respective mutations, but not in the whole genome. For this, one would have to self-pollinate the plants for at least 5 generations.