Abstract

Sexual reproduction and its profound effect on the evolution of anatomical, physiological and behavioural traits are of major interest in evolutionary biology. Especially, reproduction via outcrossing requires numerous adaptations and incurs multiple costs, which can be avoided when reproducing via self-fertilization. Therefore, species reproducing mostly or solely via self-fertilization often share the phenomenon called 'selfing syndrome' which involves the loss or diminution of traits involved in cross-fertilization. A convenient model species for studying this phenomenon is the nematode *Caenorhabditis elegans*, which in nature reproduces mostly via self-fertilization of hermaphrodites, occasionally crossing with males which are very rare in populations. In this species, selfing syndrome traits are observed on morphological (e.g., decreased sperm size in comparison to closely related outcrossing species), physiological (e.g., rare production of copulatory plugs) and behavioural (e.g., hermaphrodites being resistant to mating with males) levels.

The aim of this thesis was to study whether the transition of the reproductive system from mostly selfing (androdioecy) to obligatory outcrossing (dioecy) leads to the evolution of reproductive traits towards reduction of selfing syndrome in *Caenorhabditis elegans*. The studies constituting the chapters of this thesis were based on populations from a larger evolutionary experiment which involved obligatorily outcrossing *C. elegans* populations, established by introducing a mutation changing their reproductive system onto the genetic background of isogenic populations. I expected that change in the reproductive system would create selective pressure towards more efficient outcrossing, which would result in the evolution of higher fitness and improved reproductive behaviour after 100 generations in comparison to the ancestral populations.

The first study was designed to test the hypothesis that *C. elegans* populations transformed from androdioecy to obligatory outcrossing will after nearly 100 generations of evolution have higher fitness than their ancestors (just after changing the mating system), whereas a similar increase should not be observed in simultaneously evolving wild-type (control) populations. Similarly, in the second study, I expected that males after nearly 100 generations of obligatory outcrossing will evolve higher fertilization efficiency than observed in their ancestors. Specifically, the traits tested were: the time required for a male to find a female, duration of their physical contact, efficiency in fertilizing females

and time spent on tail-chasing behaviour, as well as relationships between the studied traits. The last study aimed at assessing to what extent the maintenance of obligatorily outcrossing populations created via the introduction of *fog-2(q71)* mutation can be affected by spontaneous gene conversion events restoring wild-type (selfing) phenotype.

In Study 1, I observed fitness increase in populations after nearly 100 generations in comparison to their ancestors. However, this effect was present both in the obligatorily outcrossing and in wild-type populations, suggesting overall further adaptation to the laboratory conditions rather than to the novel reproductive system. Nevertheless, taking into consideration the lack of genetic variation at the onset of the evolutionary experiment, this can be considered a rapid evolutionary change.

In Study 2, none of the male behavioural traits measured changed in the populations after nearly 100 generations of obligatory outcrossing in comparison to their ancestors. Their efficiency in fertilizing females also did not increase. The reasons can be, similarly as in Study 1, too weak selective pressure imposed by outcrossing or too low mutational input in the relevant genes that selection could operate on, within the timespan covered in the experiment.

Study 3 revealed gene conversion between *fog-2* and *ftr-1*, restoring the function of *fog-2*, in five out of 35 tested obligatorily outcrossing populations. This indicates that the spontaneous loss of the desired mutation is a non-negligible factor that can affect the maintenance of obligatorily outcrossing populations in a long-term study and points to the necessity of applying several methods to control their genotype, including the regular performance of visual screenings and random sampling of experimental females for their ability to produce self-progeny.

A general conclusion from the studies included in this thesis is that nearly 100 generations of obligatory outcrossing did not lead to detectable evolution of traits specific to outcrossing, either in reproductive behaviour (Study 2) or on fitness level (Study 1, where fitness increase was significant in comparison to ancestors but this change was not higher in obligatorily outcrossing populations than in the wild-type) under the experimental setup applied. This lack of response to the altered reproductive system could result from several factors combined: strength of the selective pressure, the genetic variance available in the relevant traits, because of low genetic diversity at the onset of the study and a limited input of beneficial mutations under given population size and the experiment timespan.