Abstract

Among the factors shaping biodiversity, sexual reproduction plays a significant role. It has a profound impact on the world around us. Sexual reproduction can influence how populations adapt to new environments and the evolution of traits related to outcrossing. These traits can be both cooperative and conflicting. Sexual reproduction also comes with costs, such as the cost of males. Self-fertilization is a form of sexual reproduction that, in many ways, resembles asexuality because it occurs without outcrossing. Consequently, populations that reproduce through self-fertilization avoid most of the costs associated with sexual reproduction. However, self-fertilization also has consequences. In populations where self-fertilization is dominant, a "selfing syndrome" is often observed, which leads to the loss or degeneration of traits associated with outcrossing. Moreover, self-fertilization can limit the adaptive potential of a population because, similar to asexual reproduction, there is no recombination of genetic information between genomes from different individuals.

Thanks to modern genetic methods, it is possible to alter the reproductive system of certain organisms. One such organism is the nematode *Caenorhabditis elegans*, a widely used model organism. It is famous for its short life cycle, small size, and ease of laboratory cultivation and genetic modification. The dominant form of reproduction in this species is hermaphroditism, where individuals are capable of self-fertilization but unable to cross-fertilize each other. There are also male individuals capable of fertilizing hermaphrodites, however, in the majority of populations they make up less than 1% of the population. In *C. elegans*, the "selfing syndrome" is evident both at the physiological level, such as the absence of inducing paralysis in hermaphrodites, which facilitates mating with males, and at the behavioural level, such as hermaphrodites rejecting male sperm or shorter copulation time.

In this study, an experimental evolution approach was used to investigate whether changing the reproductive system of *C. elegans* from nearly exclusive self-fertilization to obligate outcrossing would affect adaptation to new environmental conditions and the re-evolution of traits related to outcrossing. This study was part of a larger evolutionary experiment in which the initial populations had an isogenic genetic background. To create obligately outcrossing populations, the production of sperm in hermaphrodites was blocked by introgressing the *fog-2(q71)* allele, effectively transforming them into functional females. Both reproductive systems (wild-type mainly using self-fertilization and the *fog-2* obligate outcrossing) evolved for over 100 generations at two temperatures: 20°C (control) and 24°C (elevated temperature).

The first hypothesis formulated at the beginning of the experiments assumed that changing the reproductive system would impose selective pressure on traits associated with outcrossing, leading to the re-evolution of traits degenerated by the "selfing syndrome." The second hypothesis was related to adaptation to the new environment depending on the reproductive system. The rate of adaptation in the new environment was expected to be higher in obligatorily outcrossing populations, relative to populations mainly reproducing through self-fertilization.

In the first study, predictions derived from the first hypothesis were tested. The introduction of obligate outcrossing in populations exhibiting the "selfing syndrome" should result in strong selective pressure on traits associated with outcrossing, leading to the evolution of more "efficient" reproductive behaviours related to copulation and fertilization. The first chapter examined the rate of female fertilization in *fog-2* populations that evolved for 100 generations under the changed reproductive system and their ancestors in which the system was changed but the populations did not undergo evolution. To ensure the reproducibility of the results, the studies for each population were repeated in 2 or 3 independent blocks. The study identified 5 populations in which a higher rate of fertilization likely evolved compared to their ancestors. However, there was substantial variation between blocks, which suggests that these results should be treated with caution.

The second study aimed to test the second hypothesis mentioned above. The fitness of both wild-type (self-fertilizing) populations and obligately outcrossing populations, along with their ancestors, was measured after 100 generations of evolution at two temperatures: 20°C and 24°C. In this experiment, it was predicted that populations evolving at 24°C would adapt to this environment, resulting in higher fitness compared to their ancestors. Additionally, it was expected that adaptation would be more pronounced in populations reproducing through obligate outcrossing. This study was also repeated in 2 or 4 independent blocks. The predictions of the tested hypothesis were not confirmed in this study. Similar to the first study, substantial variation between blocks was detected. Additionally, genetic background was a significant factor influencing the differences between evolutionary populations and their ancestors.

The third chapter of the study describes the methodology developed to estimate fitness, as described in the second study. This method allows for high-throughput estimation of fitness in populations of nematodes, measured through competition with a strain of *C. elegans* labelled with the green fluorescent protein (GFP). By using a model trained with convolutional neural networks (CNN), the model "learned" to classify nematodes visible in images as either fluorescent or non-fluorescent animals. The performance of the model was evaluated using precision and recall parameters. Additionally, its performance was compared to manual counting from images. In both measures, the model achieved good results and reduced the time required for image analysis by a factor of 20, while avoiding human errors.