Sordaria fimicola: meiotic divisions experiment

Abstract

The purpose of this investigation is to determine the frequency of meiotic divisions analyzed from hybrid crossings collected from different strains of the fungus Sordaria fimicola. The experiment was conducted to demonstrate hybrid crossings with MI and MII patterns of ascospores within the asci. Over the course of seven days, the sample of Sordaria was incubated and fused under laboratory conditions. In the outer areas of the blocks of agar, hyphae growth from the mutant tan strain (t-g+) and wild-type black strain (t+g+) was visible through the " X-shaped" and outer rims of the Petri dish.

By identifying the amounts of non-hybrid and hybrid MI and MII asci, the observation of ascospores within the asci displayed the one possible pattern of MI, and the four possible patterns of MII. The first part of the laboratory experiment formed a hypothesis predicting that 8 ascospores would result from two stages of Meiosis and one stage of Mitosis. After calculating the frequency of crossing over, the map distance of the gene to the centromere in the tan colored gene observed was 32 map units, significantly different from the projected null hypothesis and expected 26 map units.

Introduction

Many research investigations utilize the common fungus Sordaria fimicola as a primary and reliable model organism for displaying genetics due to its firm structure and life cycle. Mapping the distance between the tan gene (t-g+) and the centromere requires careful preparation of a fused sample of Sordaria already containing hybrid and non hybrid arrangements in the ascus. By measuring the amounts of hybrid MI (non-crossover) asci and MII (crossover) asci, and calculating the frequency of crossover, the percentage

of Asci may also be calculated from the rate of crossovers throughout the experiment. With an understanding of frequency of crossover, biological ideologies such as adaptation, mutation, and recombination are expressed fully within the experiment. The null hypothesis states that there will not be a considerable difference between the expected 26 map units and the observed map distance from the collected class data (Helms, Kosinski, Cummings, 350). Collective effort from each bench to calculate the correct amount of asci assigned will certainly affect the frequency of calculation and rejection or acceptance of the null hypothesis.

Biological evolution closely relates to the process of Sordaria crossovers. Mendel’s Law of Independent Assortment is directly validated through the life cycle of the fungus. As a member of Ascomycota, Sordaria fimicola practices “ strict sexual reproduction”, and provides the easiest visualization of meiosis I, II, and mitotic division found in the ascus (Volk). Some characteristics that display the easiness of observation lie in the Sordaria fimicola structure. Lengthened nature of the ascus prevents the overlapping of ascospores. Therefore, carefully ruptured perithecia are rightly lined up according to the production of meiosis of tan and black spores: making it relatively easier to perform with more efficiency in counting MI and MII patterns. With its phenotype almost equivalent to its genotype, due to the absence of another dominant allele, the accurate physical traits are examined directly from the genetic makeup of Sordaria (Helms, Kosinski, Cummings, 334).

During hybrid crossovers in Prophase I, a tetrad forms four haploid nuclei, each of which then form two haploid nuclei, leading to a total of eight

ascospores in a single ascus. Generally, Sordaria is a common fungus for genetics research because of various reasons centered on the easiness in the demonstration of Meiosis, observation of structure, and/or behavior of its life cycle. Growth of the Sordaria fungus is a significant factor and dependent variable carried out throughout the study. The Ascomycota fungus only grows under the conditions of decomposing vegetation, making it available for nutrients to be absorbed and increase hyphae growth and extension (“ Meiosis and Recombination in Sordaria Fimicola”). The results of this study could contribute to a broader knowledge of mutation, biodiversity, and segregation. Further applications towards investigating meiotic and mitotic crossovers and map distances may soon propose new interpretations of Mendel’s laws.

Materials and Methods

During week one of the experiment, wild -type black (+) and mutant tan (t) cultures of Sordaria fimicola were obtained and while using aseptic technique, placed in a sterile Petri dish divided into four subsections labeled for the two gene colors. After a metal spatula was disinfected into 95% ethanol, it was heated using a Bunsen burner and cooled for 10 to 15 seconds.

While carefully lifting the lid of the Petri dish slightly to prevent contamination, a block of agar was removed and transferred faced down for mycelium linkage and crossing agar. After re-flaming the spatula and repeating proper aseptic technique, the process was repeated with wild type (+) black strain and two mutant (t) tan strains positioned on the marks of the Petri dish indicating the labeled plus(+) sign. After all necessary blocks of

agar have been placed in the proper sections of the Petri dish, the plates were incubated in 22 to 24°C temperature in the dark for 7 days.

During week two, a plate of Sordaria fimicola containing the fusion of black and tan strains were obtained for the analysis of hybrids and non hybrids within the 8 produced ascospores. Using a toothpick, the surface of the plate along the “ X-shaped area” was scraped gently to collect a sample of perithecia. A slide of perithecia was prepared by dropping water on a slide the collected perithecia, and then secured with a coverslip. Before placing the slide under a 10x Objective microscope, the slide was first gently pressured with a pencil eraser or equivalent pressure pointer rupturing the perithecia without destroying the structure of the ascus. Using the microscope, slides were examined to locate hybrid and non hybrid asci. Class data on numbers of MI, MII, Total Asci, percentage of crossover, and frequency were calculated. A Chi -Square Test was performed since necessary. (Helms, Kosinski, Cummings 336 -350).

Discussion

Based on the individual bench results, the number of total MI and MII asci counted depended on the number of asci assigned per person. For example, since there were only two bench members in Bench B and each bench member in the class were assigned to find and count 5 hybrid crossovers each, consequently, there was a total of 10 MI and MII asci for Bench B, shown on the table. According to the Biology Lab manual, 26 map units was the published map distance of the tan spore gene from the centromere (Helms 350).

The level of frequency is closely related to how “ loosely” or “ tightly” linked genes are on the chromosome. For this experiment, the deviations between the frequencies of the benches individually does not seem drastic, although the results from Bench F shows a slight over calculation of total asci counted, therefore resulting with the highest frequency level of 34. 6, way over the expected 26 map units. Analyzing the class data as a whole, with 276 total MI and MII Asci counted, the percent (%) of Asci showing crossover was 64%, giving a frequency of 32 map units.

In order to justify if there is a significant difference between the 32 map units observed and the 26 map units expected, we perform a Chi -Square calculation. With Ï‡² equaling 16. 291, my conclusion is that the class data demonstrates a much higher frequency than expected. The degree of freedom (dÆ’) for the experiment was 1, from n-1, with 2 attributes MI and MII. Since the probability value (p) was greater than (>)0. 05, we rejected the null hypothesis and accepted the alternative hypothesis asserting that our observed frequency of 32 map units is significantly different from the expected 26 map units provided by published results. Possible Sources of error can be closely examined from the bench data results. Besides an over calculation of MI and MII asci, mentioned earlier that produced inconsistent figures, another source of miscalculation may have come from counting/including hybrid crossovers that had a 3-1-2 or 2-3-1 abnormal arrangement. Many times students were obligated to restructure a new slide of perithecia because their slide either did not have enough hybrids, or they ruptured the vulnerable perithecia incorrectly, proving very time consuming. Overall, the conducted lab was precise in calculating the frequency.

Sordaria fimicola investigations have multiple purposes and applications. If conducted correctly, the fungus demonstrates an accurate arrangement of spores resulting from the meiotic and mitotic divisions. In a very similar laboratory experiment, Meiosis and Recombination in Sordaria Fimicola, the same approaches of the two labs shared common procedures including: crossing a wild type and mutant type gene, growing the hyphae in rotting vegetation, and calculating the genetic map distances. Calculating the number of map units will be consistent throughout most Sordaria fimicola studies because the frequency of crossing over is always divided by 2(because frequency of recombination is exactly . 5 of frequency crossed over) proved in most investigations. The easiness of growing agar on Petri dishes and crossing a wild type and mutant gene increases recombination of genetic material, leading to increases in the range of genotypes, paving a way towards future increases in biological development.

References

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