Letters in
General Microbiology

The Adaptiveness of Temperature in Different Strains of *Neurospora discreta*

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Abstract

The genus *Neurospora* is known as orange bread mold found in tropical areas. It has a haploid life cycle, and the species are used as a model organism because they are easy to grow and are easily recognizable when changes of fitness and adaptiveness occur (Jacobson 2006). Strains of *Neurospora discreta* from Alaska, New Mexico, Switzerland, Gabon, and the Ivory Coast were studied to see how different environment affect their adaptiveness and reproduction cycle. Crossing of these strains helped showed whether the progeny was able to adapt to the high or low temperature.

Introduction

Neurospora species are well adapted and mostly found on vegetation. A particular study noticed that *Neurospora* completely occupied a different niche regardless of the temperature within the habitat. They range from southern North America to Alaska, and were even found to be under bark that was damaged by wild fire. The change in temperature within these areas did not affect the growth rate of *Neurospora*, in fact it continued to grow at a normal rate (Jacobson 2006). More specifically, *N. discreta* can be found throughout western North America, Europe, and Central Africa whereas *Neurospora crassa* is only found tropical areas (Greenwald 2010). Also, it is believed that there is a greater genetic diversity in *N. discreta* than most other species of *Neurospora* (Dettman 2006). The causes of this diversity are unknown and something that may be able to explain the evolution and dispersal of *Neurospora* species, including *N. discreta*. This would lead to a better understanding of how these fungal organisms are able to adapt to their environment, which is important due to the fact that fungal species around the world play a very important ecological role.

Likewise, *Neurospora discreta* also demonstrates fitness to sustain. Fitness can be measured by counting the number of conidia which are asexual spores produced by the fungus, as well as the growth rate measured each day. The ability of any organism to grow in its environment is important because it must grow at a certain rate to remain competitive with other species in its survival. While *N. discreta* is able to reproduce sexually, it is also important that asexual reproduction, or conidial production, remains high since fungal species require another organism with a different mating type, something that is not always found in nature (Greenwald 2010). This ability of the fungus to adapt to a lack of suitable mates shows a strong fitness in its environment. On the other hand, sexual reproduction and the ability to perform such, is a necessary asset that the organism must have in nature. The ability of the progeny to survive in its surroundings is also very important so that the species does not become extinct. If the various strains are able to mate and produce progeny that are able to withstand a variety of environments, they can then be considered a "more fit" species.

Materials & Methods

Obtaining of Fungal Strains

Eight strains of *Neurospora discreta* were provided by the lab of Dr. Kwangwon Lee, Rutgers University—Camden. Two strains were previously acquired from Alaska (9981 and 9978), two from New Mexico (9982 and 8579), two from Western Africa (9973 and 9969), and two from Switzerland (9993 and 9992).

Low-Glucose Race Tube Media Production

LGRT media was produced in order to allow the strains to grow in long, glass race tubes (six tubes to one set) to determine growth rate of the strains in different temperatures. Ingredients of this media include Vogel's salt, water, L-arginine, HCl, and D-glucose. The pH was then adjusted to exactly 5.8. The media was poured into the race tubes and allowed to dry before the strains were inoculated.

Race Tube Inoculation and Growth Rate Measurement

Three trials for each strain, to be grown in each temperature, were inoculated and placed into chambers that had been set at specific temperatures. The higher temperature was set at approximately 22˚C and the lower

temperature was set at approximately 13°C. The growth of each strain was measured daily for fourteen days. Growth rate was determined by averaging the amount grown by each strain over any given day. The growth rate of each trial was then averaged for the growth rate of that strain in that temperature.

Crossing Media Production

Crossing media was produced in order to provide a suitable environment that the various strains could be crossed in. Crossing media contains Westergaad's salts, water, sucrose, and biotin. The pH of the solution was adjusted to 6.5 before it was poured into large test tubes on a slant.

Crossing of Strains

In order to successfully cross strains of *Neurospora discreta*, it was necessary that different strains were of different mating types. Strains were paired up with other strains that had the opposite mating type, and were chosen based upon differences in latitudes. The strains that were crossed are as follows:

9981 (Alaska) with 8579 (New Mexico)
9978 (Alaska) with 8579 (New Mexico)
8579 (New Mexico) with 9969 (Ivory Coast)
9982 (New Mexico) with 9993 (Switzerland)
9982 (New Mexico) with 9992 (Switzerland)
9982 (New Mexico) with 9973 (Gabon)
9981 (Alaska) with 9992 (Switzerland)
9978 (Alaska) with 9992 (Switzerland)
9981 (Alaska) with 9993 (Switzerland)
9978 (Alaska) with 9993 (Switzerland)

The crosses performed were able to give a well-rounded idea of the ability of different strains to mate with one another, regardless of latitudinal or ecological differences. The crosses are currently waiting to be put through any type of experimentation.

Minimal Media Production

Minimal media was produced in order to promote conidial growth of the various strains in the two different temperatures. It contains Vogel salts, water, and agar, with the pH being adjusted to 5.8. The solution was poured into test tubes, but kept at an even level as to not have slanting within the test tube.

Inoculation onto Minimal Media

Each strain was inoculated twice onto the minimal media in order to account for conidial production in the two different temperatures. One trial per sample was placed into the chamber with the lower temperature, and the other into the chamber with the higher temperature (these chambers are the same ones in which the race tubes were stored). The strains were allowed to produce conidia for fourteen days.

Conidial Count

After fourteen days, the test tubes were removed from the chambers and small amounts were extracted into a series of dilutions made with water. Two of the solutions, 10^{-2} and 10^{-3} were used to count the amount of conidia produced by each strain, rather than using one dilution and not having comprehensive data. These dilutions were then applied drop-wise onto a hematocytometer and counted manually through a compound light microscope at 400X.

Results

This study looks to see how various strains of *Neurospora discreta* from different countries responds to the temperature change. The adaptiveness of the strains was measured using growth rate in race tubes and conidial count. Summaries of the measure of growth rate in race tubes are shown in Figure I. This table compares all the strains from various regions to show how they vary.

After watching the growth of these strains, 9978-Alaska had the highest growth rate in the lowest temperature with an average of 5.96 cm by day. 9982-New Mexico had the highest growth rate in high temperature with an average of 10.6 cm by day. Other than these two maximums there seems to be no real difference among the growth rates between the strains from the different latitudes in each temperature.

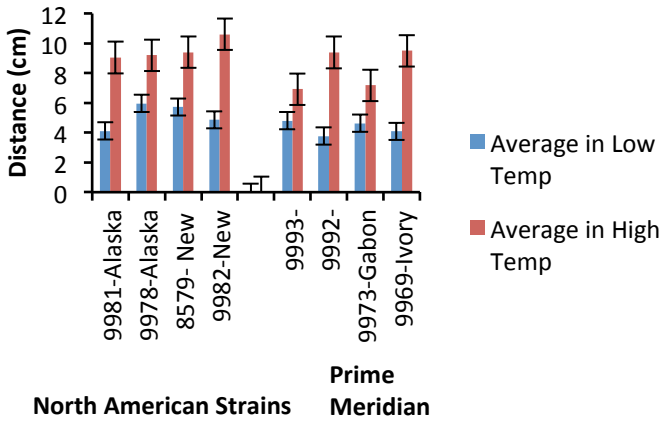

Figure I: Summary of all the strains to show a comparison. Error bars were made using standard deviation.

North American Strains	Average in Low Temp	Average in High Temp
9981	4.1	9.04
9978	5.96	9.2
8579	5.73	9.4
9982	4.87	10.6
European/African Strains	Average in Low Temp	Average in High Temp
9993	4.79	6.92
9992	3.76	9.38
9973	4.63	7.18
9969	4.09	9.5

Table I: Summary of average growth rate in race tubes.

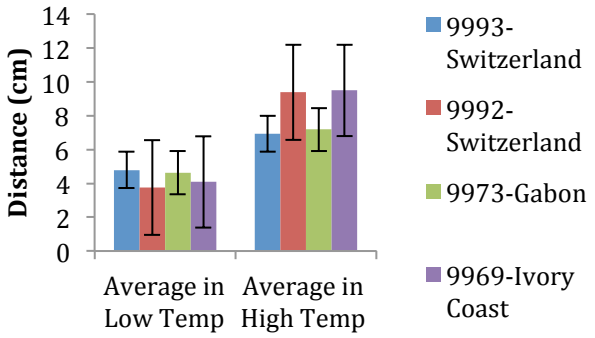

Figure 2. Summary of European and African strains' growth rate. Error bars were made using standard deviation.

Summary of the growth of just the European/African strains are shown in Figure II. This figure shows the overall difference between the various strains from Europe and Africa. It shows the growth rate which can be used to compare how well the strains grow in both the high and low temperatures.

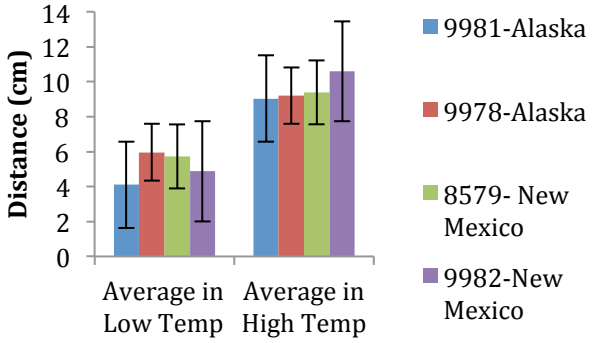

Figure 3. Summary of North American strains' growth rate. Error bars were made using standard deviation.

The overall difference of growth rate was also measured in the various North American strains. This gave the option of comparing growth rate of various strains in high and low temperatures.

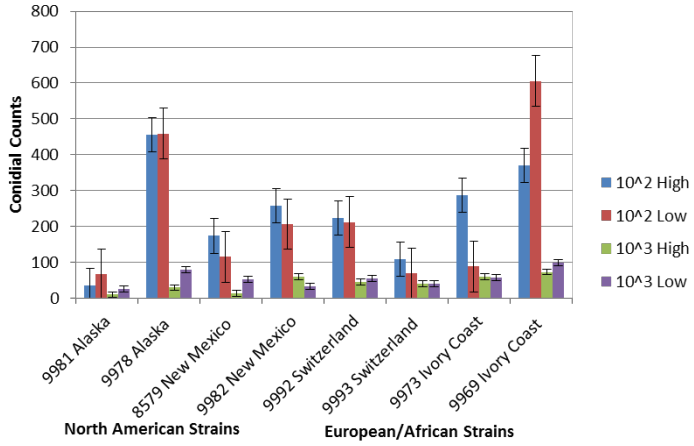

Figure 4. Summary of asexual conidial production by all strains. Error bars were made using standard deviation.

Other than measuring growth rates, conidial count was another way to determine a strains' adaptiveness. In figure 4, conidial counts are compared to show how it differed in the North American strains and European/African strains. The strain with the highest conidial production in high temperature was 9978-Alsaka with 455 in the 10^{-2} dilution The strain with the highest conidial production in low temperature 9969 from Ivory Coast with 605 in the 10^{-2} dilution.

Discussion

A difference in lower temperature growth rates was looked for in order to see if northern strains have precedence over the more southern strains. This could mean genetic adaptation and variation for these strains, seeing as *Neurospora* is typically found in more tropical climates. Within the first week, all strains seemed to be growing at quicker pace in warmer temperature, and some seemed to have a quicker rate than others in the lower temperature. As the data from the growth rate was analyzed, it was determined that there was not a significant difference in growth among the strains in either the cold and hot temperatures. It was anticipated that the northern strains would have a more prevalent growth rate in the lower temperatures than the southern strains, but this was seemingly incorrect. To ensure that less human error or data error could exist, it has further been hypothesized that adding one more strain from each location, as well as better partnering the strains so that the various latitudes line up, could have the potential to give a stronger set of data, through which a larger statistical difference may appear.

There was also no statistical difference between the different strains in terms of conidial production. It was hypothesized that the northern strains would produce a larger amount of conidia in the lower temperature and the southern would produce more conidia in the higher temperature. However, it was determined that the exact opposite occurred. An African strain produced the most conidia in the lower temperature and an Alaskan strain produced the most in the higher temperature. Similar changes could be made to this part of the experiment to further separate the averages and provide a statistical difference in the data.

The crosses of the various strains have not yet been tested and/or analyzed for statistical differences. It is hypothesized that the progeny of these strains will be able to adapt to a variety of environments with a higher level of fitness than its parent strains. This is important because having successful progeny is one of the most important factors in continuing a species so that it cannot become extinct. These crosses will be photographed using a Scanning Electron Microscope to see if there are any physical differences between them and their parent

strains. The growth rate and conidial production of these crosses will also be researched further to determine if there is an affinity for any specific environment that can be recreated in a laboratory setting. This may further give insight into the evolution and potential speciation of fungal species.

Acknowledgements:

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References:

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