How heritable is phenotypic plasticity in Funaria hygrometrica (Funariaceae)? A study involving haploid sib families grown on ash environments.

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ABSTRACT

*Funaria hygrometrica* is a moss that is a successful colonizer of recently burnt soils, substrates which typically revert back to pre-burn conditions within 2 years. *F. hygrometrica* lacks much genetic variation within populations and yet is able to prosper in rapidly changing environments. I hypothesized that its phenotypic plasticity would be very heritable to allow populations of *F. hygrometrica* to adopt different phenotypes and hence different life strategies that would allow survival without needing a large pool of variation. I grew haploid sib families on control and ash agar, which mimicked a recently burnt environment, and estimated spore germination proportions and measured length and branching of sporelings within populations. Results were discussed as evidence that *F. hygrometrica* adopts a colonizer growth plan on non-burnt environments, but a fugitive growth plan on recently burnt environments. In addition, high levels of heritability of plasticity were found for protonema length and branching.

INTRODUCTION

Wildfires have great effects on not only the biota of a region, but also on the soil (Dietert 1979; Hoffman 1966; Table 1). The changed environment only lasts for about 2 years (Dietert 1979; Hoffman 1966), but it sets the stage for new life strategies used by new actors. Fugitive moss species are typically the first to grow on the recently burnt environment. They are characterized by focusing mostly on sexually reproduction, making many sporophytes and
making them often, while not growing much horizontally. Fugitive species are followed by colonizers, which grow more by asexual propagation. Populations of colonizers typically live for years longer than fugitives, but do not do as much sexual reproduction (During 1979).

*Funaria hygrometrica* is a common moss that can successfully grow in many different environments (Shaw 1991), including recently burnt substrates (Hoffman 1966; Thomas et al. 1994). During (1979) categorized it as a fugitive species because of its preference for burnt sites and adeptness at sexual propagation upon such substrates. Because *F. hygrometrica* has the ability to survive and reproduce in varying environments with relatively low levels of genetic variation within populations (Shaw 1991), it is a good candidate species in plasticity studies. Furthermore, families of haploid sibs can easily be obtained because one mature sporophyte contains the parent and likely clonal offspring (Parihar 1961).

Heritability is the proportion of a population’s phenotypic variation that can be accounted for by its genetic variation, and heritability changes based on the environment (Falconer 1960). *Funaria hygrometrica*, like all mosses, has a haploid-dominant lifestyle, large family sizes, and very small spores (Parihar 1961). Broad-sense heritability can easily be calculated from common garden studies, which remove environmental variation, and it contains no dominance term because of the gametophyte’s ploidy (Shaw 1991). Scheiner and Lyman (1989) calculated the heritability of phenotypic plasticity for some traits in *Drosophila melanogaster*, but this study will attempt to expand their study to plant biological systems. Plasticity may be very important to plants because they cannot manipulate their environments in the ways that animals can (Buryova and Shaw 2005).

My question is: how heritable is phenotypic plasticity in *F. hygrometrica*. My hypothesis is that plasticity of growth and branching will be highly heritable in *F. hygrometrica*. 
A population of *F. hygrometrica* was obtained, growing as a weed in the greenhouse. It grew on top of a cloth, which wicked from a reservoir of water between two plastic trays of same length and width, but different depth. The wick was on top of the shallow plastic tray. It was watered as needed to maintain the reservoir with distilled H$_2$O, kept 10 cm under 4 × 40°C fluorescent light bulbs and allowed to grow without any other temperature modification. Plants were allowed to continue to grow after mature sporophytes appeared. Test samples were harvested as needed from the population.

To establish haploid sib families, mature sporophytes were harvested (n = 10), and dipped into undiluted bleach for 20 s. Next, a flame-sterilized forceps was used to transfer each sporophyte into separate 1.5 ml Eppendorf tubes, filled with 1.2 ml distilled water. Each sporophyte was then crushed with forceps until spores had been released. Tubes were vortexed for 5 s, and then, 0.4 ml of the mixture were applied to agar on a 20 ml petri plate. Plates were set 10 cm under 4 × 40 V fluorescent light bulbs with a 12:12 (light:dark) photoperiod. Plates contained one of two types of 1% agar to create two different environments for germination. Petri plates with BactoAgar were prepared with either 1:10 Hoaglund’ solution or 1:10 Hoaglund’s solution and 5% ash. The ash medium was made by mixing wood ash with distilled water, stirring the mixture for 2 hr., and incubating the mixture at room temperature for 22 hr. This technique was modified from Thomas et al. (1994). The supernatant was then filtered through filter paper. Concentrated Hoaglund’s solution and BactoAgar were then added, and the resulting mixture was autoclaved. Plates were haphazardly rearranged daily to equalize any aberrations that might have resulted from spatial differences. In each family plate, 2 transects were drawn on the bottom of each plate as chords of its circumference. Observations were made...
after 8 days to assess germination proportions. Spore germination proportions were estimated as
the number of germinated spores counted out of the first 50 spores encountered along transects.

A new treatment was devised because no germination was observed on the 5% ash
treatment. The new treatment differed only from the 5% ash treatment in being 0.5% ash (Table
2). This trial, like the previous one, had haploid sibs being grown on the control as well as the
ash agar. Observations were made along transects for this trial after only 5 days. Germination
proportions were assessed in the same manner as in the previous trial. In addition, 10 individuals
were selected from each plate along transects, and length and branching data were collected on
those individuals. For family S, only 4 individuals were selected because those were the only
sporelings that were visible from transects. The purpose of this was to assess variation within
each family and among families. Length data were collected as the distance in pixels determined
from a microscope camera at 100X magnification of the longest part of the protonema.

Branching data were collected as the number of branches per sporeling and were log transformed
to normalize them.

Two-way analyses of variance (ANOVA) were used to test if an interaction existed
between family and environment for a trait. One-way ANOVA were used to examine
differences among families on each environment. Spore germination proportions were arcsin
square-root transformed in order to normalize the data, and a Paired t-Test was used to test
differences within haploid sib families across treatments. Broad-sense heritability of each trait
was estimated as the ratio of mean square among families in one environment to the sum of
mean square error and mean square family (Lynch and Walsh 1998). Mean square values were
obtained from ANOVA. Heritability of plasticity was estimated as the ratio of mean square of
family × environment interaction for a trait to the sum of mean square error and mean square of family × environment interaction (Scheiner and Lyman 1989).

RESULTS

Higher concentrations of ash equated to higher pH, calcium, potassium, phosphorus, magnesium, sulfur, and iron levels in the agar (Fig. 1, Table 2). For the 5% ash trial after 8 days, 3 of the 10 families germinated on the control substrate, while no family germinated on the 5% ash substrate (Fig. 2). For the 0.5% ash trial after 5 days, 3 of the 10 families had germinated on control agar, and 9 families had germinated on 0.5% ash agar (Fig. 3). Families had significantly higher germination rates on 0.5% ash agar than on control agar ($t = 4.819$, $P < 0.001$). Low germination rates on control agar were consistent across both trials. Two out of 3 families grew longer and branched more on control agar than they did on ash agar. Heritabilities of plasticity of branching and sporeling length were estimated as being very high (Fig. 4, Fig. 5, Table 3).

DISCUSSION

My results matched the predictions of my hypothesis. My methodology of using ash agar is justified because its composition correlated well with observations made on real fires (Southorn 1979).

Spores tended to germinate the most on 0.5% ash agar. One of the most prominent differences among the agar was the pH. The 0.5% ash agar had a pH within the optimal range, whereas the control was barely within the acidity range that permitted any germination (Dietert 1979). Acidity was not reported to be an important factor by Hoffman (1966), but Dietert (1979) and Thomas et al. (1994) concluded it to be one of the most important factor in growth and germination. Besides pH, the ash agar differed from the control in having different concentrations of minerals and nutrients. *Funaria hygrometrica* is reported to be generally
insensitive to mineral and nutrient levels (Dietert 1979; Hoffman 1966), but mineral and nutrient levels on recently burnt substrates are likely to be great enough to inhibit growth (Dietert 1979). The control agar would have been at more optimal levels, which might explain that the families that were able to germinate on the more acidic control agar grew more horizontally than their clones on 0.5% ash agar two-thirds of the time. Furthermore, the proximate mechanism of the result of more germination on 0.5% ash agar can be explained in light of the relative unimportance of mineral and nutrient levels by its being at the optimal pH.

During (1979) referred to *F. hygrometrica* as a fugitive species, which would mean that it would invest most of its energy in sporophytes instead of horizontal growth. The fugitive class of mosses are classified as being gone from an area within 2 years, but *F. hygrometrica* has been reported to persist for around 4 years on mine sites (Shaw 1991), which is a characteristic length of time that colonizer type species survive (During 1979). The ultimate cause of high rate of germination on 0.5% ash agar, but lower horizontal growth relative to the control, might be able to be explained by the fugitive and colonizer life strategies. *F. hygrometrica* might have the strategy of fugitive on recently burnt environments, but it has the strategy of colonizer on other environments. The ability to have different life strategies would be mediated by the species’ high plasticity.

Plasticities of branching and length were found to be very highly heritable. This matches with Shaw and Bartow, (1992) who found high levels of phenotypic plasticity (between a metal-rich environment and a control one) for several traits including leaf and cell dimensions in *F. hygrometrica*. They did not find very much genetic variation, however, which might imply that the plasticity is highly heritable. Lavergne and Molofsky (2007) suggested that reed canary grass (*Phalaris arundinacea*) had been responding to a selection pressure toward higher
plasticity of growth traits. *P. arundinacea* is an invasive species that grows in a wide variety of environments and is highly plastic (Lavergne and Molofsky 2007). Because *F. hygrometrica* grows in rapidly changing environments (Dietert 1979) and lacks much variation within populations (Shaw 1991; Shaw and Bartow 1992), it may be responding to selection pressures toward higher plasticity of growth traits.

This study could be strengthened by including more populations of *F. hygrometrica* as well as some populations of other ubiquitous, pyrophilous mosses such as *Ceratodon* (Shaw and Beer, 1999). With more populations, more accurate estimates of heritability could be calculated. Studies that incorporate gametophore formation in addition to protonemata growth would also be enlightening. Evidently, heritability of plasticity in *F. hygrometrica* is something that should prove to be useful and interesting to continue studying.

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LITERATURE CITED


FIGURE LEGENDS

FIGURE 1. Acidity in pH of control agar, 0.5% ash agar, and 5% ash agar.

FIGURE 2. Spore germination proportion for 10 families, each on control and 5% ash substrates, after 8 days. No germination was observed on the 5% ash substrate, and 3 out of 10 families
germinated on the control substrate. Proportions were estimated by counting the number of
sporelings out of the first 50 spores observed along transects of plates.

FIGURE 3. Spore germination proportion for 10 families, each on control and 0.5% ash
substrates, after 5 days. Nine out of 10 families germinated on the 0.5% ash substrate, and 3 out
of 10 families germinated on the control substrate. Proportions were estimated by counting the
number of sporelings out of the first 50 spores observed along transects of plates.

FIGURE 4. Mean (± 1 S.E.) sporeling length of 10 families, each on control and 0.5% ash
substrates, after 5 days. Means were calculated from 10 haphazardly selected (4 for family S on
control) individuals on each plate. Lengths were determined in pixels from pictures taken from a
photographic microscope at 100X magnification.

FIGURE 5. Mean (± 1 S.E.) sporeling branching of 10 families, each on control and 0.5% ash
substrates, after 5 days. Means were calculated from 10 haphazardly selected (4 for family S on
control) individuals on each plate.
Figure 1

Figure 2
Figure 3.

Figure 4.
Figure 5.