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Mushroom’s spore size and time of fruiting are strongly related: is moisture important?

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Most basidiomycete fungi produce annual short-lived sexual fruit bodies from which billions of microscopic spores are spread into the air during a short time period. However, little is known about the selective forces that have resulted in some species fruiting early and others later in the fruiting season. This study of relationships between morphological and ecological characteristics, climate factors and time of fruiting are based upon thorough statistical analyses of 66,520 mapped records from Norway, representing 271 species of autumnal fruiting mushroom species. We found a strong relationship between spore size and time of fruiting; on average, a doubling of spore size (volume) corresponded to 3 days earlier fruiting. Small-spored species dominate in the oceanic parts of Norway, whereas large-spored species are typical of more continental parts. In separate analyses, significant relationships were observed between spore size and climate factors. We hypothesize that these relationships are owing to water balance optimization, driven by water storage in spores as a critical factor for successful germination of primary mycelia in the drier micro-environments found earlier in the fruiting season and/or in continental climates.

Keywords: fungi; fruiting; fruiting body; phenology; spore; spore size

1. INTRODUCTION

Many basidiomycete fungi produce annual ephemeral sexual fruit bodies from which billions of spores are spread into the air within a time span that often lasts for only a few days. In temperate and boreal ecosystems, most mushrooms (Agaricomycetidae) fruit during the autumn. To our knowledge, nothing is known about which selective forces and evolutionary adaptations make some mushroom species fruit early while others fruit late in autumn. The fruit bodies and spores of fungi fruiting early and late in the season experience different climatic conditions. Early in the season, higher average air temperatures will cause more rapid desiccation of fruit bodies and more rapid loss of vigour than later in the season. Most probably, spores released early in the season lose water more rapidly and, perhaps most important, the germinating spores and highly exposed primary mycelia will be more prone to desiccation. Towards the end of the season, the risk of desiccation decreases, but at the same time the metabolic activity will decrease as a result of lower temperatures. In addition, the risk of frost and snow that may damage fruit bodies and suppress spore release increases, at least in boreal and alpine ecosystems.

Based on these considerations we hypothesize that early fruiting fungi have evolved characteristics that favour water conservation. Larger spores contain more water, as well as more nutrients, which is an essential resource during the germination process and the phase of initial growth of the primary (homokaryotic) mycelia. We also hypothesize that the crucial role of water availability for establishment is reflected in the spatial distribution of species with small and large spores, respectively: species with smaller spores being more prevalent in areas with a moister climate than species with larger spores. The nutritional mode (e.g. saprotrophic or mycorrhizal symbiont) may also be related to time of fruiting [1]; mycorrhizal species being linked to the phenological cycle of the host plant.

In this study, we analyse relationships between different species characteristics and the fruiting time (day of fruiting) of autumn-fruiting species of mushroom-forming fungi (Agaricomycetidae) in Norway. Statistical analyses of digitized herbarium and field records of mushrooms were used to evaluate the relationship between fruit body size and shape, spore size, shape and colour, nutritional strategy (saprotrophy versus mycorrhizal symbiosis), geographical distribution and time of fruiting as response.

2. MATERIAL AND METHODS

The study is based upon recorded day of fruiting for 66,520 records of mushroom-forming fungi in Norway, made in the period 1940–2008, and representing 271 species in 43 genera (see electronic supplementary material, table S1). The data were obtained from the Norwegian Mycological Database, University of Oslo. Although the analysed records were sampled in a non-systematic manner throughout Norway by many collectors, the sampling shares properties with random sampling processes. All records are scaled to municipalities, which give a rough estimate of geographical location in Norway. For each species, data on fruit body size, fruit body shape, spore size, spore colour and nutritional mode were obtained from Knudsen & Vesterholt [2] (see table 1 for further explanation of the variables). Average spore length and breadth measurements were used to calculate spore volume (using the equation of a revolution ellipsoid), which was used as a measurement of spore size. All records in the same genus are assumed to have the same nutritional mode. Based on present day knowledge, species in the investigated genera are thought to have similar nutritional modes, but we recognize that this is a crude approximation. The variables fruit body size, shape and spore size and colour are species-specific constants. In addition, geographical coarse scaled climatic data were available for each record. Norway is divided into five main climatic regions (electronic supplementary material, figure S1), for which monthly temperature and precipitation means were estimated.

For the statistical analyses, we used the R-software [3]. The effects of fruit body shape, fruit body size, fruit body shape, spore size, spore colour and nutritional mode on the day of fruiting (Day) were analysed by linear mixed modelling [4]. We applied a backward Q1 elimination of the explanatory variables starting with interaction...

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Table 1. Predictor variables related to day of fruiting (response) in the statistical analyses. Species characteristics were obtained from Knudsen & Vesterholt [2].

<table>
<thead>
<tr>
<th>predictor</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>species</td>
<td></td>
</tr>
<tr>
<td>genus</td>
<td></td>
</tr>
<tr>
<td>geography</td>
<td>longitude/latitude&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>year</td>
<td>year of fruiting</td>
</tr>
<tr>
<td>nutritional mode</td>
<td>Ectomycorrhizal versus saprotroph log (base = 2)</td>
</tr>
<tr>
<td>spore size</td>
<td>spore length/breadth</td>
</tr>
<tr>
<td>spore shape</td>
<td>0 = white, 1 = pink, grey and brown, 2 = black</td>
</tr>
<tr>
<td>spore colour</td>
<td>cap diameter</td>
</tr>
<tr>
<td>fruit body size</td>
<td>cap diameter/stem diameter</td>
</tr>
<tr>
<td>fruit body shape</td>
<td>(stoutness)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Adjusted to the centre of gravity of municipalities in Norway.

3. RESULTS

A significant relationship was found between the spore size and the day of fruiting (table 2 and electronic supplementary material, table S2). The estimated effect, $\beta_1 = -3.06 + 0.74$, suggests a 3 day earlier mean fruiting time associated with each doubling of spore size (figure 1a). The random contribution of genera (s.d. = 11.31) was larger than the contribution from species in genera (s.d. = 7.63), and the residuals were dominant (s.d. = 21.37).

The spatial distribution of spore size, conditional on genera, year and day of fruiting, was statistically significant (electronic supplementary material, table S3); records of small-spored species were more frequent than expected towards western and oceanic parts of Norway, whereas records of large-spored species were more frequent than expected in the more continental parts of Norway (figure 1b). The spatial component of variation in spore size explained approximately 30.3 per cent of the variation after accounting for genera and time.

A separate analysis, where the relationship between spore size and climate (precipitation and temperature) was investigated, revealed that the spore size is influenced by both precipitation and temperature (electronic supplementary material, table S4). Large-spored species were related to higher temperatures, but the effect was less pronounced when precipitation was higher (figure 2). At higher temperatures, spore size decreased with increasing precipitation. However, when conditioned on both year and geography, the spore size was influenced only by temperature ($\beta_1 = 0.014 \pm 0.001$), and not by precipitation ($p$-value = 0.14). This indicates that at least some of the geographical distribution detected is linked to the climatic influence of spore size. Scatter plots of spore size against monthly mean temperature and monthly precipitation are provided in the electronic supplementary material, figure S2.

We found no significant ($p > 0.05$) effects of fruit body size, fruit body shape, spore colour and nutritional mode on day of fruiting when correcting for geographical and species effects (electronic supplementary material, table S2).

4. DISCUSSION

Our analyses demonstrated that spore size is significantly related to time of fruiting of autumn-fruiting mushrooms: species fruiting early in the season in general producing larger spores than late-fruiting species. Fungal spores that spread through air may easily lose water by evaporation. Since temperatures on average are higher and precipitation lower early in the season than later (see electronic supplementary material, table S5), spores that are shed early are at higher risk of desiccation. Furthermore, if a spore lands on a suitable substrate and starts to germinate and form a primary mycelium, the hyphae are likely to desiccate more rapidly when temperatures are high and precipitation low. This puts stronger demands on water supply from the spore. Hence, we argue that a selection pressure for more voluminous spores in...

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Table 2. Analysis of day of fruiting by a linear mixed model [4], where the effects of fruit-body size, fruit-body shape, spore size, spore shape, spore colour and nutritional mode on the day of fruiting (dependent) were analysed. The effects of geography and year were always included in the models to account for confounding effects of these elements. Additional effects of the biological characteristics were subjected to a backward elimination. Then only the spore size showed significant effect on the day of fruiting. The effects of the interaction term between spore size and nutritional mode and the main effect of nutritional mode was eliminated using a sequential test. (numDF, numerator degree of freedom; denDF, denominator degree of freedom in F-test with $p$-values indicating that the effect of spore size is statistically significant.)

<table>
<thead>
<tr>
<th>predictor</th>
<th>numDF</th>
<th>denDF</th>
<th>F-value</th>
<th>p-value</th>
</tr>
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<tbody>
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<td>intercept</td>
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<td>65711</td>
<td>17022.81</td>
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</tr>
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<td>65711</td>
<td>25.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>year</td>
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<td>65711</td>
<td>18.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>spore size</td>
<td>1</td>
<td>227</td>
<td>17.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>nutritional mode</td>
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<td>41</td>
<td>1.47</td>
<td>0.232</td>
</tr>
<tr>
<td>spore size : nutritional mode</td>
<td>1</td>
<td>226</td>
<td>0.15</td>
<td>0.697</td>
</tr>
</tbody>
</table>

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Figure 1. (a) The normalized time of fruiting plotted against the logarithm of spore size. The logarithm (base 2) provides a unit change corresponding to a doubling of spore size. The fruiting time was adjusted for collection year and geography, and we allowed for a stochastic contribution by genera and species in genera. The statistics were resolved by a linear mixed effect model [4]. The dashed lines are 95% pointwise confidence intervals. (b) The estimated geographical distribution of spore size in Norway constrained by genera, year and day in year. Isopleths indicate the anomaly in spore size and the 0 isopleth (blue) is the average constrained spore size with scale of log(spore size) base 2. There appears a clear tendency for species with smaller spores (purple) to occupy coastal areas with higher precipitation while species with larger spores (red–orange) are associated with more continental and drier inland areas (electronic supplementary material, table S5). The statistics here employ a thin-plate spline with offsets [5]. The dashed lines (lower) and dots (upper) are 95% pointwise confidence interval bands, respectively. More information about the climatic conditions is given in the electronic supplementary material, table S5 and figure S1.

The significant relationship between spore size and geography, where records of small-spored species were more frequent than expected towards western and oceanic parts of Norway, supports the idea that spore size is related to water requirements of the germinating primary mycelia. Hence, the present-day distributional patterns suggest selection for larger spore sizes in more continental climates. This hypothesis is supported by the significant relationships observed between spore size and climate conditions. Large-spored species are expected when temperature increases but a less pronounced effect when the precipitation increases. Furthermore, at higher temperatures, decrease in spore size is expected with increased levels of precipitation.

In general, little is known about which selective forces drive the evolution of the very divergent life-history characteristics of fungi. This study exemplifies the value of combining data from a variety of different sources, in this case digitized herbaria and field records, climate data and known species characteristics, to address questions on the evolution and role of fungal life-history characterization.

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3 R Development Core Team. 2008 R: A language and environment for statistical computing. Vienna, Austria:


