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Sexual Fecundity is Correlated to Size in the Lichenized Fungus *Xanthoparmelia cumberlandia*

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Abstract. *There are no standard measures of fungal fitness, and yet descriptions of natural selection in fungi require an understanding of how to compare the success of two individuals. Success, or fitness, is normally understood to be a combination of survival and reproduction.* Xanthoparmelia cumberlandia *is a sexual, lichenized fungus. By recording the size of, and number of sexual structures on, individual lichens we demonstrate a significant correlation between size and reproductive effort in this species, showing that size is an easily measured surrogate of fitness. Published data of other lichen species, for example* Umbilicaria spodochroa *or* Xanthoria parietina, *also show a correlation between size and sexual fecundity, indicating that the correlation may be a general feature of sexual lichens. However, patterns of resource allocation differ between lichen species. Published data collected from* U. spodochroa *are linear, demonstrating that larger lichens allocate equivalent resources to growth and reproduction. In contrast, the data of* X. cumberlandia *are curved, indicating that in this species larger lichens allocate a disproportionate share of resources to reproduction.*

Organisms are variable, and the variability causes one individual to be more successful than another; because the variation is heritable a lineage can evolve. To understand the evolution of fungi we first must understand how to measure differences in the success of two individuals. Success is normally understood as fitness, but there are no standard measures of fitness in fungi (Pringle & Taylor 2002). Fitness may be quantified as the combination of survival and reproduction (Day & Otto 2000). If we are to understand basic hypotheses related to the adapative significance of fungal phenotypes, for example why a species is sexual or asexual, we first must find useful ways to measure and understand which fungal individuals are more or less fit within a habitat.

Lichens are symbiotic associations of fungi and either algae or cyanobacteria; individual lichens are easily counted or measured. The fitness of an individual, sexual lichen might be measured as female fecundity, by counting the number of female sexual structures (ascogonia), or as male fecundity, by counting the number of male sexual structures (pycnidia). But these structures are hardly visible and so counting ascogonia or pycnidia can be difficult or impossible. Sexual spore-bearing structures, the apothecia, are formed by the fusion of female and male sexual propagules. Apothecia are more easily counted than ascogonia or pycnidia, and although no data relate apothecial numbers to spore numbers for any species of lichen, it is reasonable to assume that there is some correlation between the two parameters. But counting apothecia can be time consuming, especially when an individual lichen possesses hundreds or even a thousand apothecia. In contrast, measuring the size of an individual lichen is straightforward, but useful only if apothecial number is correlated to size.

In this study we demonstrate than an aspect of fungal fitness, apothecial number, is correlated to the size of a fungal individual. *Xanthoparmelia cumberlandia* is a sexual, lichenized fungus. Apothecia are retained on the thallus of the lichen for the lifetime of an individual; an apothecial count is a direct measure of the reproductive effort expended by a lichen in its lifetime. By counting apothecia and measuring the size of *X. cumberlandia* individuals, we demonstrate that this aspect of fungal fitness is correlated to the size of an individual. Sizes are easily measured and for this reason may serve as a useful estimate of fungal fitness. In fact the technique is analogous to measuring the biomass of a plant when biomass is a predictor of fruit and seed number—this is a methodology that is commonly employed by plant population biologists.

MATERIALS AND METHODS

Species.—*Xanthoparmelia cumberlandia* is a foliose lichen commonly distributed throughout California and western (excluding Nevada) and eastern North America (Fig. 1). Apothecia are common, and appear as cup-shaped structures at the center of the thallus. Apothecial discs are darkly pigmented, although the margins are typ-

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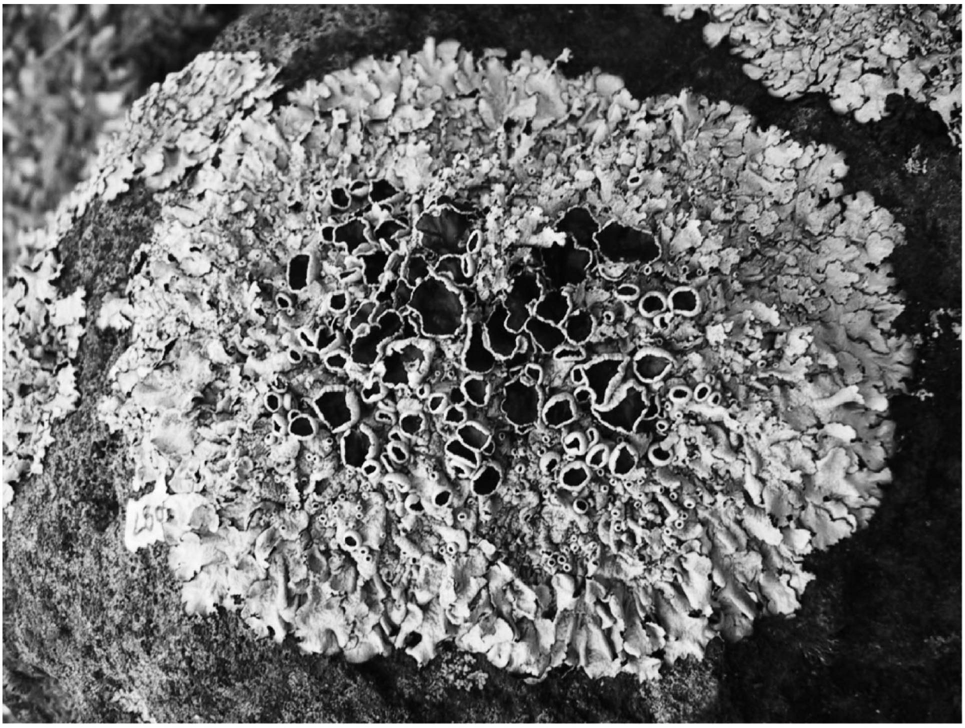

FIGURE 1. *Xanthoparmelia cumberlandia* growing in the University of California Botanical Garden.

ically toothed, rolled, and the same color as the thallus. Pycnidia are also common, and appear as black dots on the surface of the thallus. Ascogonia are not visible to the unaided eye. The lichen *X. cumberlandia* does not create specialized asexual propagules e.g., isidia or soredia. The species is usually found on exposed or somewhat shaded siliceous and other noncalcareous outcrops or boulders in open and dry habitats. Descriptions of the species can be found in Brodo et al. (2001) and McCune and Geiser (1997).

Study site and sampling.—Lichens measured in this study were found within the University of California at Berkeley's Botanical Gardens during June and July of 2002. A total of 101 lichens were sampled from the ''Southern Africa'', ''New World Desert'', and ''California'' displays on rocks that are densely populated with individuals of *X. cumberlandia*. We chose to measure lichens of a variety of sizes, and included only those individuals whose boundaries were easily defined.

Measures of area.—Transparent plastic sheets were taped over an individual lichen and the perimeter of the lichen traced using an ultra fine point permanent marker. Each of the lichens was traced by only one of the authors (D. Chen) so that a consistent technique was employed over the course of the study. Tracings were scanned and files opened in Adobe Photoshop 6.0.1 (Adobe Systems Inc. 2001). The interior of each tracing was painted black using the ''paintbucket'' command. Filled images were saved as ''Tagged Image File'' or ''tif'' files. Images were subsequently measured with NIH Image 1.62 (a program available from the NIH website, rsb.info.nih.gov/nih-image/download.html). Measures were taken by counting the number of black pixels in each image and converting the count to a standard measure of area, in this case, cm^2. For details on using NIH Image 1.62 to gather morphometric data see Ruzin (2002; available from microscopy.berkeley.edu).

After tracing each lichen, a spray-bottle of water was used to wet the lichen and open apothecia. A digital photo was taken and used to count apothecia.

Apothecial counts.—One of two methods was used to count apothecia. Initially, the number of apothecia on each *X. cumberlandia* was determined visually by taping write-on transparency film over the photo of an individual lichen. Apothecia were simultaneously marked with a fine point permanent marker and counted with a hand-tally counter. Marking each apothecium as it was counted ensured that a single apothecium was not counted twice. Very large lichens, and those that grew around the curves of rocks, were difficult to fit into a single picture. In these cases thread was used to halve or otherwise divide the lichen and each piece photographed and counted separately.

Our technique was refined when we discovered that instead of using a permanent marker to mark apothecia we could open the photos in Adobe Photoshop version 6.0.1 (Adobe Systems Inc. 2001) and use the mouse and ''airbrush'' tool to mark each apothecium. By this technique, the number of apothecia was still recorded with a hand-tally counter. The advantage of using Adobe Photoshop to mark apothecia was the ability to enlarge photos as we counted so that apothecia growing closely together were more easily distinguished.

Analyses.—Data were first explored with a simple correlation statistic using JMP version 3 (SAS Institute Inc. 1999).

We used maximum likelihood methods to determine the relationship between fecundity (the number of apothecia, A_i) and size (S_i). We compared two models: a linear model (where the predicted fecundity $\hat{A}_i$ of individual i increases

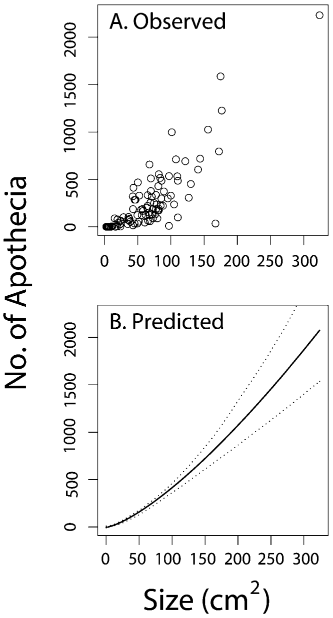

FIGURE 2. Fitting a curved model to data of individual *X. cumberlandia*. — A. Data. B. The model $\hat{A}_i = -2.8 + 0.78(S_i^{1.4})$. Dashed lines are 95% confidence intervals.

linearly with size S_i): $\hat{A}_i = b + mS_i$ and a non-linear model (in which larger individuals allocate proportionately more or less to fecundity): $\hat{A}_i = b + m(S_i)^e$. Fitted parameters include an intercept (b), the slope of the relationship between fecundity and size (m) and exponent e. Values of e greater than one would indicate that larger individuals allocate proportionately more to reproduction, values less than one would indicate that fecundity saturates with size. As the number of apothecia is a positive integer value we used a Poisson likelihood to estimate the parameter vector p (b, m, and if the non-linear model, e). The negative log-likelihood is thus:

$$\mathrm{L}(\boldsymbol{A}|p) = \sum_{i=1}^{j} -\hat{A}_i + A_i \times \log(\hat{A}_i) - \log[\Gamma(A_i + 1)]$$

where $\boldsymbol{A}$ is the j length vector of observed apothecial counts (A_i's).

We used a likelihood ratio test to determine which model best fit the data. A likelihood ratio test compares nested models by determining whether the extra parameter (in our case, exponent e) significantly increases the model fit. Significance is assessed by the difference in likelihoods of the two models, which is distributed as a chi-square distribution with degrees of freedom determined by the difference in parameters (in this case, one; Hilborn & Mangel 1997). Maximum likelihood analyses were completed using the R statistical package (available from www.r-project.org).

RESULTS

Area and sexual fecundity.—Surface area and sexual fecundity were strongly correlated (Pearson's correlation coefficient = 0.82, $p < 0.00005$; Fig. 2A). The smallest lichen measured 2.57 cm^2 and had no apothecia, and the largest lichen measured 323.81 cm^2 and possessed 2,230 apothecia. The median size of a lichen was 64.68 cm^2; the median number of apothecia per individual was 172.

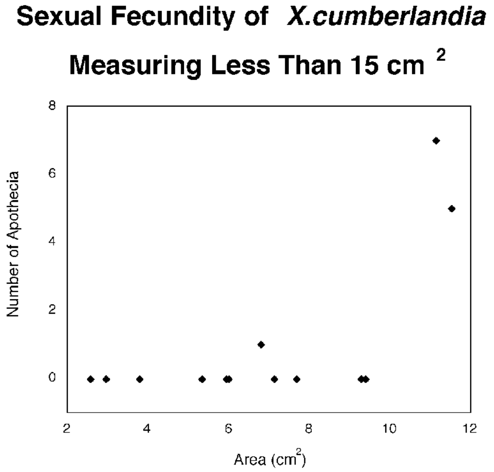

FIGURE 3. Individuals that measure less than 10 cm^2 possess few, if any, apothecia.

Size at sexual maturity.—With one exception, *X. cumberlandia* measuring less than 10 cm^2 were not sexual (Fig. 3). Only one lichen larger than 10 cm^2 was not sexual, and it measured 16.47 cm^2.

Fitting a predictive model to the data.—Both the linear model and non-linear model gave significant fits to the data, however, a maximum likelihood ratio test indicated that the curved model was a significantly better fit than the linear model (log likelihood of curved model = 5,133.69; probability that the linear model is a better fit <0.0001). Parameters of the saturating model were estimated to be:

$$\hat{A}_i = -2.8 + 0.78(S_i^{1.4}) \text{ (Fig. 2B)}$$

DISCUSSION

Reproductive effort, as measured by the number of apothecia, is correlated to the size of an individual *X. cumberlandia*. The apothecia of a lichen are permanently bound to the lichen thallus and an apothecial count is a measure of the fecundity of an individual over its lifetime; therefore size may be an accurate surrogate of fitness. The inference is analogous to measuring biomass for a plant species when biomass is correlated to fruit or seed number. Furthermore, in these data lichens measuring less than 10 cm^2 possessed no apothecia, but lichens larger than 10 cm^2 were sexual, indicating a developmental threshold before which *X. cumberlandia* are sexually immature. Finally, a predictive model of apothecial number and thallus size was non-linear, demonstrating that larger lichens allocate proportionately more resources to reproduction versus growth.

The age of an animal or plant often is used to estimate reproductive maturity and capacity, as is commonly demonstrated in life tables (Begon et al. 1996), but the age of a fungus may not be a simple correlate of size. For example Golm et al. (1993) examined *X. cumberlandia* growing in a Tulsa cemetary and found that size was normally distributed within each of three age classes e.g., a 29.2 year old lichen might be 60 to 140 mm large. Similarly, Hestmark (1997) found that the age and size of individuals of the lichenized fungus *Lasallia pustulata* were not correlated. As illustrated by the data of this study, the fecundity of a lichen is correlated to the size of the lichen, and so for lichens the size and not the age of an individual should be used to gauge reproductive maturity and capacity. This observation also is true for clonal plants, where correlations between age and size also are weak (Watkinson & White 1985).

The correlation of sexual fecundity and size, and a developmental threshold before which lichens are sexually immature, may be a general feature of sexual lichens. For example, Ramstad and Hestmark (2001) grouped apothecial numbers into classes, and found that reproductive effort was predicted by the mass and diameter (and not age) of thalli of the saxicolous and umbilicate lichen *Umbilicaria spodochroa*. These data also provided evidence of a minimum diameter and mass at which *U. spodochroa* was sexually mature. Pentecost and Rose (1985) recorded a significant correlation between thallus dry weight and apothecial dry weight in *Xanthoria parietina*. Hestmark (1992) collected data from a largely asexual lichen, *Lasallia pustulata*, and found that sexuality was correlated to size (as measured by diameter) and that there was a minimim diameter below which *L. pustulata* was not sexual.

The curved relationship between size and sexual fecundity in these data indicate that as an individual of *X. cumberlandia* grows very large, a thallus allocates a greater fraction of resources to apothecial production, but other species may behave differently. For example Ramstad and Hestmark (2001) found that the relationship between thallus mass and apothecial number (measured as the minimum number of apothecia within a given apothecial class) was linear; thus providing evidence for a constant allocation of resources to growth and reproduction. In contrast, individuals of *Cladonia furcata*, *Cetraria islandica*, and *Peltigera canina* end growth as apothecia develop (Jahns et al. 1978; Jahns & Schuster 1981; Jahns & Frey 1982), and individuals of *Baeomyces rufus* die shortly after apothecia are made (Jahns 1982).

The methodology employed in this study might be adjusted to include alternative measures of size or reproductive effort. For example, the technique used to measure size is straightforward but, in fact, other and quicker estimates of size may also be viable. For example, size might be measured by recording the diameter of circular lichens (Hestmark 1992; Pringle, unpubl. data). We do not consider male reproductive effort, which might be measured by counting numbers of pycnidia, nor do we consider female reproductive effort, as measured by numbers of ascogonia. However, it is likely that numbers of pycnidia and ascogonia also are correlated to lichen size. Nor do we consider asexual lichens, although it would be possible to measure or estimate the number of isidia or soredia of an asexual individual and correlate that number to lichen size.

Although an individual lichen may be asexual and sexual, apparently many species are either sexual or asexual and not both (Bowler & Rundel 1975; Hestmark 1991). The progeny of a sexual species may be at a disadvantage in competition with asexual species because sexual spores must recapture symbionts shortly after germination. For this and other reasons, Hestmark (1992) hypothesized that individuals presented with an option to be asexual will evolve to be asexual. However, recapturing symbionts may not be so difficult. Clayden (1998) and Ott (1987) found that in quarry spoils or a dune environment free-living algae are common and in fact Ott (1987) demonstrated that the sexual *Xanthoria parietina* can capture substrate more quickly than two sympatric soredious lichens. Ott (1987) hypothesized that *X. parietina* may use the soredia of sympatric lichens to capture a photobiont, and so at least in this case the sexual lichen is not at a disadvantage. Furthermore, individual lichens of a population are genetically distinct, implying that within a species, sexual propagules are more successful than asexual propagules and that sexual propagules can successfully locate photobionts (DePriest & Been 1992; Kroken & Taylor 2001).

We cannot judge, in this study, which aspects of the biotic or abiotic environment are shaping the evolution of lichens. But we can offer a tool with which to explore this subject; relating size to environmental variables (e.g., light, precipitation, substrate; see Hestmark 2000) may help us to understand how a lichen is adapted to a habitat, for example if larger lichens (with greater fitness) are correlated to habitats that also maintain greater numbers of free-living symbionts. Data on single species may eventually help us to understand the differences between species, as for example why a lichen is sexual or asexual.

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