Lichen Speciation in the Section Cocciferae: *Cladonia incrassata* and *Cladonia cristatella*

SERENA A. KORTEPETER

Edward Tuckerman named two lichen species, *Cladonia cristatella* and *C. cristatella* var *paludicola*, in the late 1800s. Modern lichenologists have synonymized *C. cristatella* var *paludicola* with *C. incrassata* Florke. This usage of nomenclature was investigated through observation and through the use of chemotaxonomy. A morphological and chemical intermediate was found between *C. cristatella* and *C. incrassata*. This intermediate is perhaps Tuckerman's *C. cristatella* var *paludicola*, or, alternatively, a distinct species related to both.

Introduction

Taxonomists seek to impose a logical system upon the diversity of nature. Using specific nomenclature, they categorize organisms according to a variety of consistent, reliable characteristics. Controversies tend to arise as descriptions become more and more exclusive, defining fine lines between similar species. Important from an evolutionary standpoint, taxonomy gives evolutionists a means of tracking convergent and divergent species. It also allows conservationists to know exactly what organisms they must attempt to preserve, and how to go about doing so both inside and outside protected land.

The research on lichens featured in this paper took place in Myles Standish State Forest, a nature reserve approximately one hour's drive south of Boston. The forest features a series of unique microhabitats within a very small area, established by frost pits. These are depressions into which cold air sinks, simulating certain climates of a more northerly latitude. Each subclimate contains a unique vegetative zone.[1] The pits have sandy, acidic soil, which, while hostile to vascular plants, is an ideal environment for lichens.

Lichens arise from mutually beneficial symbioses or controlled parasitisms between a mycobiont and a photobiont. The mycobiont is a fungal thallus (a group of fungal filaments) which hosts either an alga or a cyanobacterium (the photobiont) attached by fungal hyphae or stands of fungal cells.

Lichens play important roles in the ecosystems they inhabit and are useful for ecological research. Lichens colonize and decompose substrate surfaces such as rock and wood while utilizing their residual nutrients. Because of their ability to withstand harsh conditions, lichens usually are the first colonizers of various surfaces, and can thus be used for dating such substrates through lichenometry.[2] They act as soil binders, especially in sandy areas. Some are also able to incorporate nitrogen into organic compounds, or "fix" it; nitrogen is useless in its naturally occurring, gaseous form. Scientists also use lichens as indicators of pollution because of their sensitivity to sulfur dioxide (SO_2), nitrogen dioxide (NO_2), and ozone (O_3). Lichens absorb these compounds and accumulate heavy metals and radionuclides. Lichens are also sensitive to anthropogenic disturbances along pathways where soil is frequently compressed.[2]

Cladonia Browne is a common lichen genus conspicuous for its large size and unique anatomy. The thallus is made up of layered fungal tissue and forms two basic structures, the horizontal primary squamules (primary thallus) and the vertical podetium (secondary thallus). Squamules are small leaf-like structures, irregularly arranged and densely packed, adhering to the substrate. They lie flat in order to maximize photosynthetic production and may form soredia, or propagative buds, on their lower margins. Soredia appear under a microscope as flaky, white dust. They are aggregations of algal and fungal cells that clonally reproduce. The podetium is an erect, hollow structure originating from the upper side of the primary squamules. Podetia branching patterns vary and generally terminate in cup-like structures or in apothecia, which contain developing spores. Asci and ascospores are present in many species and are considered to be sexual propagules.[3]

The more conspicuous of the *Cladonia* lichen flora fall under the Section *Cocciferae* Delise. Such lichens appear red due to the presence of rhodocladonic acid, a naphthoquinone pigment.[4] The members of this Section that are found in Massachusetts include *C. incrassata* Florke), *C. cristatella* Tuck., *C. digitata* Hoffm, *C. bacillaris* Genth, *C. macilenta* Hoffm, *C. deformis* Hoffm, *C. pleurota* Florke, *C. floekeana* Florke, and *C. didyma* Fee.[5]

Edward Tuckerman, an American lichenologist, inventoried and named species from around the world near the end of the last century. He named the species *C. cristatella* and the related *C. cristatella* var *paludicola* ("swamp inhabiting"), now widely identified with *C. incrassata*. Many lichenologists, however, have questioned whether *C. cristatella* var *paludicola* and *C. incrassata* are two distinct species. The goal of the research described in this paper was to compare and contrast *C. incrassata* and *C. cristatella,* the objects of this taxonomic dispute, and to look for intermediates between the two as evidence for an evolutionary relationship.

Methods

This study began as a comparison of *C. cristatella* and *C. incrassata*. The two species were studied with regard to their microhabitat preferences, including substrate preference, surrounding conditions, and cohabitants (i.e. organisms living in close proximity). Such a study required field collecting as well as laboratory, herbarium and library research.

Collections of lichens were made during several field trips to the study site. Specimens were air-dried and examined under a dissecting microscope. The first task was to be able to differentiate between the two species in the field, since, at first glance, they appear quite similar. This identification problem, however, demanded investigation into a number of associated species. In the Farlow Reference Library and Herbarium at Harvard University, sample

SERENA A. KORTEPETER is a recently graduated concentrator in biology at Harvard College. This article grew out of her research on lichens at Myles Standish State Forest during the summer of 1994. Ms. Kortepeter is involved in drama and social service, and is currently deciding between careers in medicine and marine biological research. She wishes to thank the Ford Foundation for the support that made this work possible.

Table 1. Summary of TLC results on collected specimens.

Sample	*Barbatic Acid*	*Usnic Acid*	*Squamatic Acid*
C. cristatella	+	+	-
C. incrassata	+	+	+
Unknown form	+	-	-

specimens of *C. cristatella, C. incrassata, C. cristatella* var *paludicola, C. cristatella* f. *incrassata* and *C. cornucopioides* var *incrassata* were carefully studied with respect to their morphology.

Both morphological and chemotaxonomic characters are used in describing and distinguishing species. Portions of every collected thallus were analyzed for secondary chemical compounds using thin layer chromatography (TLC). This technique is used to detect trace chemicals using diagnostic solvent systems.[6] TLC is a routine process that supplements data gathered in traditional reagent "spot-tests," and is an excellent method for screening lichen substances. This assay is commonly used in instances when morphological characters are not easily interpreted. The podetia of the lichens in question were placed in acetone, which solvates lichen acids. This solution was micropipetted onto a silica plate, which was then run through a solvent system so that the chemicals diffused up the plate. Acids were then identified by color and by their position on the plate.[6] This study used solvent system "A," made from 180 ml toluene, 45 ml 1,4-dioxane, and 5 ml acetic acid. The acids were fixed with sulfuric acid (H_2SO_4) and the plate dried in an oven. The plate was then analyzed under long- (500 nm) and short-wave (350 nm) ultraviolet light. The presence of usnic acid and barbatic acid characterize *C. cristatella*. The same acids, in addition to squamatic acid, are found in *C. incrassata*. *C. incrassata* samples can be identified under ultraviolet light because squamatic acid fluoresces an ice blue color under these conditions.

Results

Within the context of the Northeastern climate of Myles Standish, lichens are affected by the diverse microhabitats and vegetative zones created by the different elevations of the frost pits they inhabit.[1] The soil at Myles Standish is sandy and acidic and is often disturbed, yet lichens of all species thrive there. From field observation it is clear that *C. incrassata* is much more selective in its habitat choice than *C. cristatella*. *C. cristatella* can live in a wide range of conditions. It grows in conditions of illumination ranging from low, attenuated light to about 90% ambient light on dry or wet substrates, usually in open areas. Its preferred substrate choices are decaying wood and leafy debris, but it will grow on damp peat, such as is found on the shores of Grassy Pond in Myles Standish. *Cladonia cristatella* proliferates in 50% ambient light and damp substrate. *Cladonia cristatella* is usually accompanied by other *Cladonia*, such as *C. macilenta, C. atlantica* (Evans), and *C. grayi* (Merr), as well as by the genus *Cladina* (Ahti), various members of the family Iridaceae, and in swampy areas, by swamp sedges and sundew.

Cladonia incrassata, on the other hand, thrives in damp, shaded areas. *C. incrassata* grows most frequently on the vertical surfaces of rotting trees. Its squamules are closely associated with the substrate and its podetia arise from these primary structures in maturity. This same formation is tipped on its side when the rotting tree breaks and falls to the ground. *C. incrassata* is also found in swampy areas on peat in conditions ranging from 3% to 90% ambient light.

C. cristatella and *C. incrassata* can be distinguished by sight on the basis of several morphological characters. *Cladonia cristatella* has small, flat, serrated, sorediate squamules, which are evanescent. They are light green on top and white on the underside, and cover their substrate sparsely. Their podetia are approximately 1-2 cm tall, and are cylindrical, broadening toward the apothecium. The podetia branch multiple times and are usually free of squamules, at times presenting a wrinkled appearance due to slight striation, revealing the inner thallus. The bulbous scarlet apothecia are typical of the Section *Cocciferae* and seem to fall over the edges of the supporting podetium.[5,7,8]

C. incrassata can be identified by their contrastingly large, sorediate squamules, which grow thickly. Their club-shaped podetia are a greenish to grayish-yellow color and range from 0.5 to 2 cm tall. The podetia are unbranched or simply branched, with a scaly appearance due to their vertical striations. At the top of the podetia are the red-orange apothecia, which stay within the bounds of the head.

Morphologically speaking, the relationship of Tuckerman's *Cladonia cristatella* var *paludicola* to other members of the species *C. cristatella* is problematic. Tuckerman describes *C. cristatella* var *paludicola* as having "conspicuously powdery squamules and the short, simple apothecia."[8] His samples were sorediate and contained squamatic acid. Such a description identifies *C. incrassata* more readily than *C. cristatella*; it therefore seems that *C. incrassata* and *C. cristatella* var *paludicola* are similar, if not indistinguishable.

It was to substantiate these observations that this study was taken to a chemical level. If Tuckerman is correct in stating that *C. cristatella* var *paludicola* is only a variety of *C. cristatella*, the two should share similar basic chemistry. However, a study by Stenroos et al. concerning aromatic substances in *Cladonia* found very little chemical similarity between these two supposedly related taxa. The study concluded that *C. cristatella* contains (in percentage of dry weight) 1% usnic acid and 0.7% barbatic acid, and that *C. incrassata* contains 6.3% squamatic acid, 2.0% usnic acid, 0.9% didymic acid, 0.2% condidymic acid and traces of subdidymic acid and barbatic acid.[4] TLC assays were used to test the hypothesis that *C. cristatella* would contain usnic and barbatic acid and that *C. incrassata* would contain squamatic acid, in addition to these two. This hypothesis proved to be valid, except that some samples contained only barbatic acid (Figure 1, previous page). The author wondered if these samples could be some previously unknown form chemically and morphologically intermediate between *C. cristatella* and *C. incrassata*. As these samples were all found growing in swampy areas, it seemed possible that they were Tuckerman's *C. cristatella* var *paludicola.* This possibility was ruled out, however, when the samples in Tuckerman's herbarium tested positive for squamatic acid under UV light. The identity of these particular samples remains unknown.

Discussion

Could *Cladonia cristatella* and *C. incrassata* be part of a growth and reproduction continuum? *C. incrassata* reproduces asexually by soredia, later gaining the ability to reproduce sexually through asci and ascospores as it forms podetia in maturity. *C. cristatella*, on the other hand, reproduces solely through sexual processes, possibly having secondarily lost its ability for asexual reproduction in the course of evolution. While the possibility of a continuum remains an interesting theory, it seemed less and less viable as this research progressed and the two species appeared increasingly distinct. It could be asserted, however, that the two are distantly related and may have diverged from a common ancestor in response to reproductive isolation and natural selection.

Knowledge of speciation patterns is necessary for accurate taxonomic records, which inform broader issues related to conservation. Solving the controversy that has been discussed here is important for the preservation of the species involved. If there is indeed an intermediate organism between *C. cristatella* and *C. incrassata,* it needs to be investigated. If this species is not identified, described and studied, there is no assurance that it will survive anthropogenic ecological damage.

Acknowledgments

I wish to thank Professor Samuel Hammer for a year of good teaching, inspiration and guidance. Thanks also to the Harvard Herbarium staff: Prof. Donald H. Pfister, Carolyn Hesterberg, Dr. Benito Tan, and Jerry Caccavio. I am also very grateful to the Ford Foundation for the funding that made this research possible.

References

(1) Hammer, S. 1994. *A Preliminary Report on the Lichens of Myles Standish State Forest with a Focus on the Potential Impact of Non-Designated Off-Road Vehicle Trails on the Lichen Flora at Sensitive Sites* (unpublished manuscript copy).

(2) Amadjian, V. 1993. *The Lichen Symbiosis* (New York: John Wiley and Sons, Inc.).

(3) Hammer, S. 1993. *A Revision of the Lichen Genus* Cladonia *in the Western United States* (Ann Arbor, MI: Dissertation Services).

(4) Stenroos, S., et al. 1989. The Composition and Contents of Aromatic Substances in *Cladonia*, section Cocciferae. *Annalles Botanical Fennici*. 26: 133-48.

(5) Thomson, J. W. 1967. *The Lichen Genus* Cladonia *in North America* (Toronto: University of Toronto Press).

(6) White, F. J., and P. W. James. 1985. A New Guide to Microchemical Techniques for the Identification of Lichen Substances. *British Lichenology Society Bulletin.* 57: 1-41.

(7) Hale, M. E. 1969. *How to Know the Lichens* (Dubuque, IA: William C. Brown).

(8) Tuckerman, E. 1882. *Synopsis of North American Lichens Vol 1* (Boston: Cassino).