Journal of Invertebrate Pathology 139 (2016) 34–41

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

# Fine-scale spatial genetic structure of a fungal parasite of coffee scale insects

Doug Jackson *, Ash T. Zemenick, Brian Malloure, C. Alisha Quandt, Timothy Y. James

*Department of Ecology and Evolutionary Biology, University of Michigan, 2019 Kraus Nat. Sci. Bldg., 830 North University, Ann Arbor, MI 48109, USA*

ARTICLE INFO

*Article history:*
Received 6 March 2016
Revised 12 July 2016
Accepted 18 July 2016
Available online 19 July 2016

*Keywords:*
*Lecanicillium lecanii*
Entomopathogen
Population genetics
Spatial structure
Metapopulation

ABSTRACT

The entomopathogenic fungus *Lecanicillium lecanii* persists in a highly dynamic network of habitat patches (i.e., a metapopulation) formed by its primary host, the green coffee scale *Coccus viridis*. *Lecanicillium lecanii* is an important biological control of both *C. viridis* and the coffee rust, *Hemileia vastatrix*. Successfully managing this biocontrol agent will depend on an increased understanding of the characteristics of its dispersal, as migration between occupied and unoccupied patches is essential for the persistence of this metapopulation. In the present study, we employ a population genetics approach, and show that in our study system, a coffee farm in the Soconusco region of southern Mexico, *L. lecanii* is characterized by clear spatial genetic structure among plots within the farm but a lack of apparent structure at smaller scales. This is consistent with dispersal dominated by highly localized transport, such as by insects or rain splash, and less dependence on longer distance dispersal such as wind transport. The study site was dominated by a few multi-locus microsatellite genotypes, and their identities and large-scale locations persist across both study years, suggesting that local epizootics (outbreaks) are initiated each wet season by residual propagules from the previous wet season, and not by long-distance transport of propagules from other sites. The index of association, a measure of linkage disequilibrium, indicates that epizootics are primarily driven by asexual, clonal reproduction, which is consistent with the apparent lack of a teleomorph in the study site and the presence of only a single mating type across the site (MAT-1-2-1). Although the same predominant clonal genotypes were found across years, a drastic difference in genotypic diversity was witnessed across two sites between the two years, suggesting that interclonal selection was occurring. In light of the dispersal limitation of *L. lecanii*, spatial structure may be an essential axis of management to ensure the persistence of *L. lecanii* and preserve the ecosystem services provided by this versatile biocontrol agent in this and similar coffee farms.



## 1. Introduction

Due to the ubiquity of populations that are distributed across networks of habitat patches, and the difficulty of empirically assessing such metapopulations, the persistence of metapopulations has proven to be a topic of great theoretical interest (Hanski, 1999; Levins, 1968). Persistence of metapopulations is ultimately a matter of two factors: the local extinction rate and the rate of migration between patches. Therefore, translating the insights gained by this theory to ecosystems of interest requires knowledge of extinction and migration rates under field conditions. This can be difficult to obtain for more cryptic organisms with short life cycles, such as fungi and bacteria, which, despite their microscopic propagules, also exhibit dispersal limitation (Peay et al., 2010).

Molecular population genetics provides powerful tools to infer dispersal patterns (Broquet and Petit, 2009) and obtain evidence of migration between patches. The distribution of alleles at multiple spatial scales, and the extent to which these distributions adhere to or depart from panmixia, can provide information about the connectedness of metapopulation habitat patches and the persistence of populations within these patches. These methods are particularly powerful for microorganisms, which are not as amenable to other techniques for ascertaining migration characteristics (e.g., tagging, visual observations, etc.).

In the present study, we apply a population genetics approach to gain insight into the dispersal characteristics of *Lecanicillium lecanii*, an important biological control of two potential pests of coffee in southern Mexico: the green coffee scale, *Coccus viridis*,

* Corresponding author.
*E-mail address:* dougjack@dougjackson.net (D. Jackson).

http://dx.doi.org/10.1016/j.jip.2016.07.007
0022-2011/

and coffee rust, *Hemileia vastatrix* (Jackson et al., 2012a; Vandermeer et al., 2009).

The primary host of *L. lecanii* in the study site is *C. viridis*. Nearly all of the biomass of *L. lecanii* in the farm is generated by infections of *C. viridis*, while infections of *H. vastatrix* account for a minuscule fraction of *L. lecanii* biomass, and are therefore likely to play a negligible role in the population dynamics of *L. lecanii* in this system. Populations of *C. viridis* can reach in the thousands on a single coffee plant (Perfecto and Vandermeer, 2006); these large concentrations provide the abundance and density of hosts necessary for *L. lecanii* to proliferate in local epizootics, which can infect and ultimately kill nearly 100% of the *C. viridis* populations on a coffee plant (Jackson et al., 2009; MacDonald et al., 2013).

The distribution of *C. viridis* itself is a consequence of its interaction with a second organism: its mutualistic partner *Azteca sericeasur*, an arboreal-nesting ant that provides protection from predators to *C. viridis* while feeding on the carbohydrate-rich honeydew that *C. viridis* excrete. *Azteca sericeasur* creates carton nests in shade trees, which form a canopy over the coffee plants, and occasionally nests in the coffee plants themselves. In our study system, a coffee farm in the Soconusco region of southern Mexico, *A. sericeasur* nests are distributed in a spatially clustered pattern, despite the shade trees being distributed in a pattern that is statistically indistinguishable from a uniform distribution (Vandermeer et al., 2008). These clusters of nests, and the associated clusters of large *C. viridis* populations in the coffee plants surrounding these nests, form a spatially-extended landscape of resource-rich habitat patches for *L. lecanii* to exploit.

This landscape of habitat patches in which the *L. lecanii* metapopulation exists is temporally variable due to the pronounced wet-dry seasonality of the region. In the wet season, conditions are favorable for the scale insects (Jackson et al., 2014a) and for *L. lecanii* (Reddy and Bhat, 1989), and this is the season during which ongoing epizootics are most prevalent. In the dry season, the scale populations contract (Jackson et al., 2014a) and conditions are unfavorable for the fungus, which is almost exclusively found as dry, residual infections on *C. viridis* cadavers (unpublished data).

Thus, for *L. lecanii* to persist in this system, it must function as a metapopulation in a landscape of habitat patches that are separated spatially and which expand and contract in a highly dynamic fashion throughout the course of a year. Persistence of *L. lecanii* is a function of two factors: first, its ability to avoid extinction in a given site (defined as a cluster of coffee plants populated by *C. viridis* surrounding an *A. sericeasur* nest or tight cluster of nests), either via infected cadavers that persist through the dry season or by a pool of propagules in the soil (Jackson et al., 2012b) or other environmental reservoir; and second, the dispersal ability of the fungus, which determines its migration rate and thus its ability to infect newly formed populations of *C. viridis* or rescue patches from which the fungus has been locally extirpated.

Understanding the influence of dispersal on the persistence of the *L. lecanii* metapopulation, and by extension the robustness of this metapopulation to management actions that may alter the spatial structure of the system, such as the pruning or felling of shade trees, is important for the management of *C. viridis* and *H. vastatrix*, which can become significant pests if they escape control (Vandermeer et al., 2010). Despite this, relatively little is known about the dispersal of *L. lecanii* under field conditions. Outstanding questions include: Is there apparent spatial genetic structure within a landscape, and if so, is it consistent with wind dispersal or smaller-scale processes such as rain splash or insect dispersal? Is dispersal a consequence of clonal reproduction, as is suggested by the fact that a teleomorph (the sexual reproductive stage) of *L. lecanii* has yet to be observed in this system? To what extent are local epizootics initiated by long-distance dispersal from external sources versus residual propagules from the previous year's epizootics? Is the system dominated by a few successful genotypes or by a diverse and variable suite of genotypes?

To begin to answer these questions, and to help illuminate the extent to which migration between patches is promoting persistence of this metapopulation, we employed a population genetics approach based on a nested, hierarchical sampling across two years. From these data, we were able to identify unique genotypes; determine their spatial distribution within and across years; calculate the amount of genetic variation that was partitioned at a variety of spatial scales; test for clonality and evidence of sexual recombination; and test for spatial autocorrelation.

## 2. Material and methods

The study was performed at Finca Irlanda, a coffee farm in the Soconusco region of southwest Chiapas, Mexico (15°11′ N, 92°20′ W), the site of a 45-hectare plot that has been under continuous and intensive study for over a decade (Perfecto et al., 2014, 2003; Perfecto and Vandermeer, 2008; Vandermeer et al., 2010, 2008, 2002; Vandermeer and Perfecto, 2006). Annual rainfall is ca. 4500 mm, and the elevation ranges from ca. 900–1150 m. The farm encompasses a total area of approximately 300 ha, and is managed as a commercial polyculture (Moguel and Toledo, 1999), with 30–50% shade cover and almost 100 tree species in total, primarily *Inga* spp. The total number of trees with dbh > 10 cm in the 45 ha study plot was 7294, or approximately 162/ha, in 2010; and 6145 (ca. 137/ha) in 2011. The total number of *A. sericeasur* nests in the study plot was 624 and 581 in 2010 and 2011, respectively. Large populations of *C. viridis*, on the order of 1000–2000 individuals per coffee plant (Perfecto and Vandermeer, 2006), are typically found only directly adjacent to an *A. sericeasur* nest on a few coffee plants (generally less than 5).

Samples of infected *C. viridis* individuals were collected in 2010 and 2011. To determine how genotypic variation was partitioned at multiple spatial scales, we employed a nested design: samples were collected from five sites (sites A-E), from four coffee plants at each site, and from 10 branches chosen haphazardly at different heights within each coffee plant. Our sites were defined as the coffee plants surrounding a single *A. sericeasur* nest or group of nests in adjacent shade trees. Nested sampling is an effective method for obtaining information about the effect of spatial scale, particularly for clustered populations such as this (Storfer et al., 2007).

Our sampling scheme was designed to balance coverage with sampling effort, allowing us to obtain a representative sample of the unit of interest (infected *C. viridis* individuals) across nested spatial scales while accounting for the sparse distribution of *C. viridis* populations in the plot and the substantial time and effort involved in hiking between sites across difficult terrain. The number of branches sampled within each plant (10) was also influenced by the tendency of *C. viridis* individuals to cluster on a small number of branches within a given plant and the average number of branches per plant found previously in a representative sample of coffee plants (n = 430, mean = 31.3, SD = 17.4).

To obtain five sites with four plants hosting sufficient scales infected by *L. lecanii*, it was necessary to select two sites from outside the perimeter of the 45 ha plot (Fig. 1). It was not possible to sample the same plants in both years because individual plants are periodically pruned, cut back to the stump, or replaced between seasons. Also, epizootics of *L. lecanii* may not occur on the same plants in subsequent years. Due to these constraints, only two plants were available in one site (Site B) in 2011. Because an additional plant with sufficient infected scales was available, we also opportunistically sampled an additional plant at Site A in 2011.

We removed infected scales from leaves and berries of the coffee plants using forceps that were surface-sterilized with 95%