SUPPLEMENTARY MATERIAL

Local adaptation of bacterial symbionts within a geographic mosaic of antibiotic coevolution

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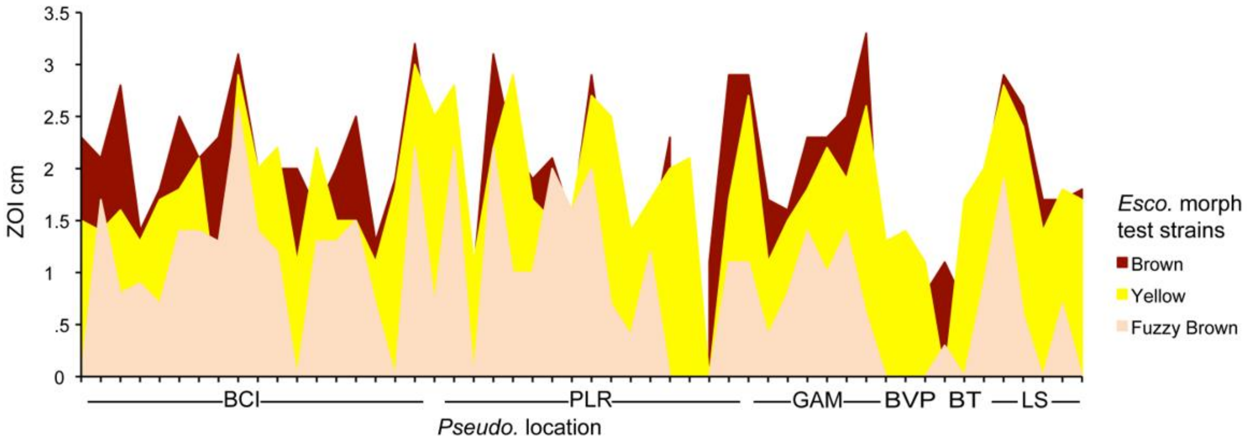
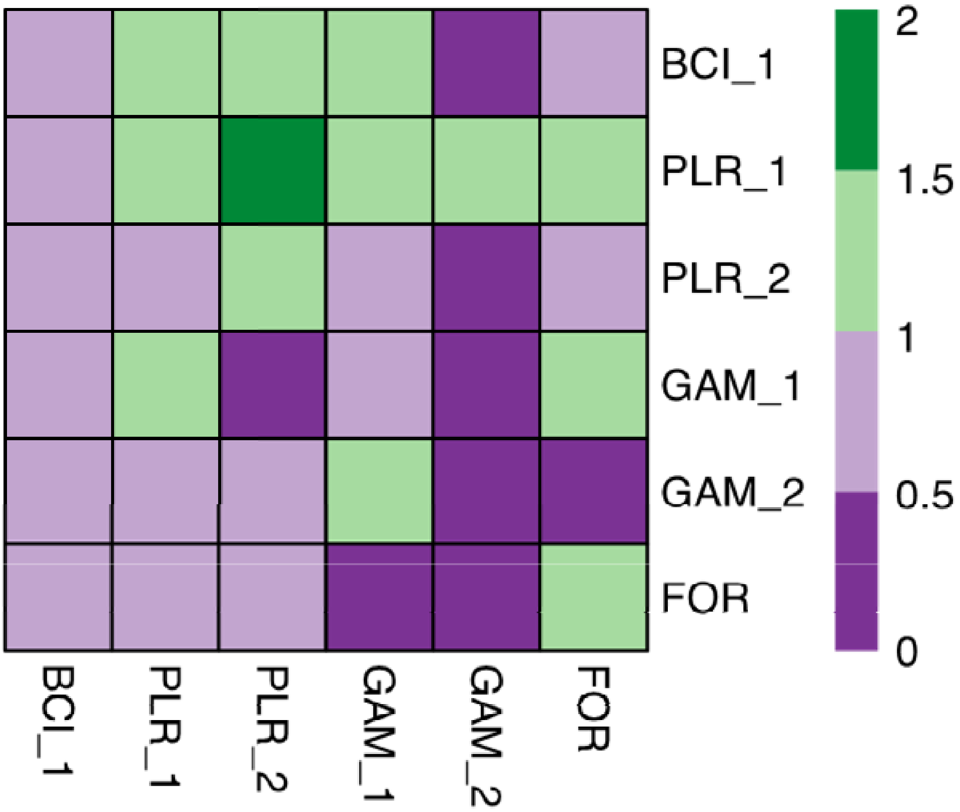
Mutualist	**Parasite Target**	**Parasite Interaction**
Fungal Cultivar	Primary	Labile
Ant Host	Secondary	General
Pseudonocardia	Tertiary	Specific

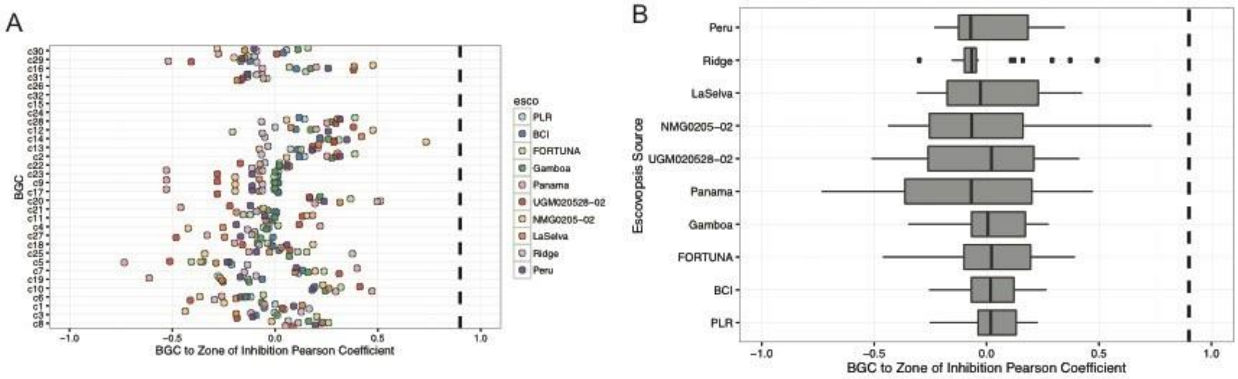
Supplementary Table 1: Summary of microbial symbiont interactions associated with the fungus-farming ant *Apterostigma dentigerum*.



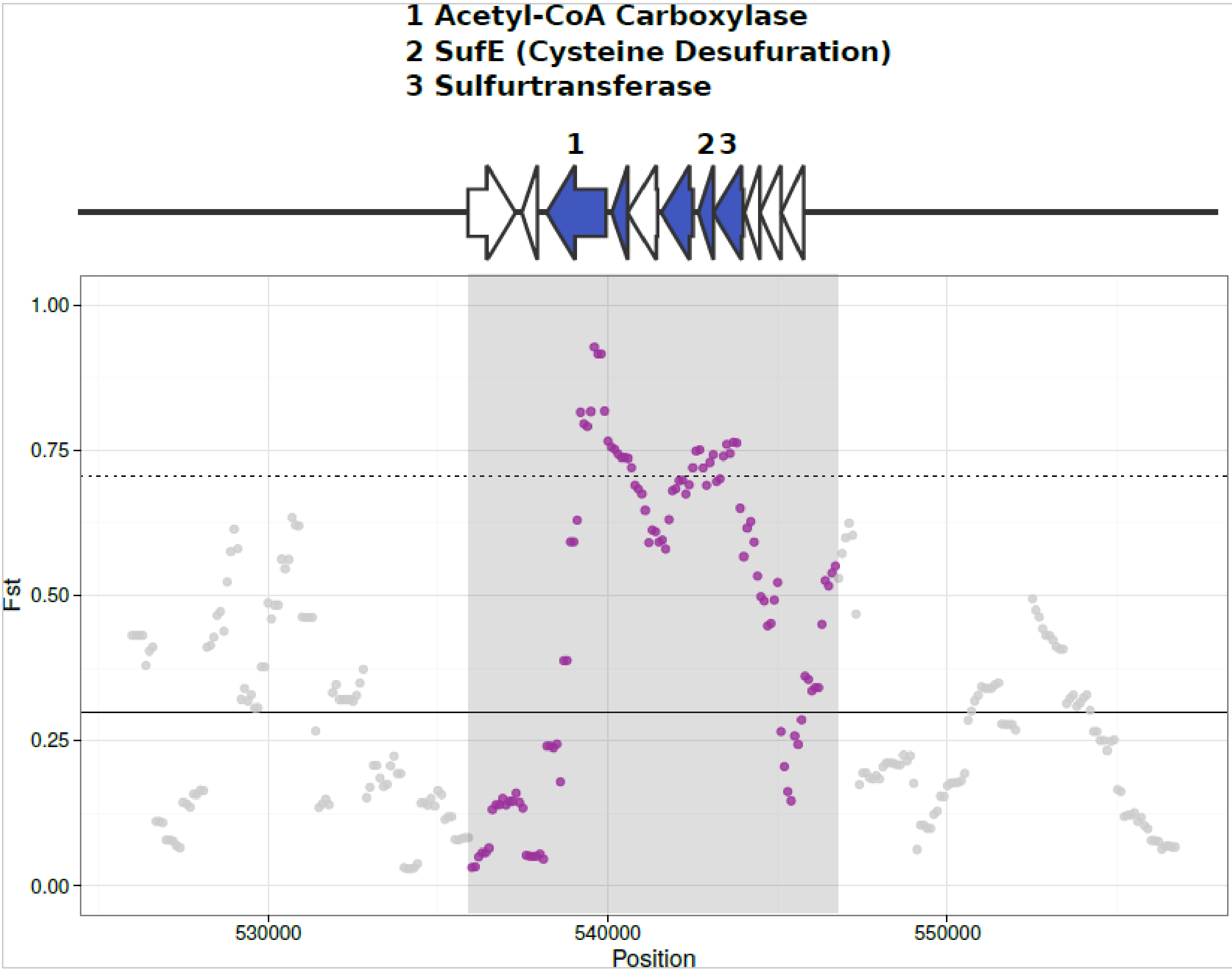
Supplementary Figure 1: Central America *Pseudonocardia* hierarchical inhibition of three parasite lineages (brown, yellow and fuzzy brown). Brown *Escovopsis* inhibition was greater than yellow, which was greater than fuzzy brown in paired t-tests. ZOI corresponds to the length of a zone of inhibition in Petri-plate bioassay experiments.



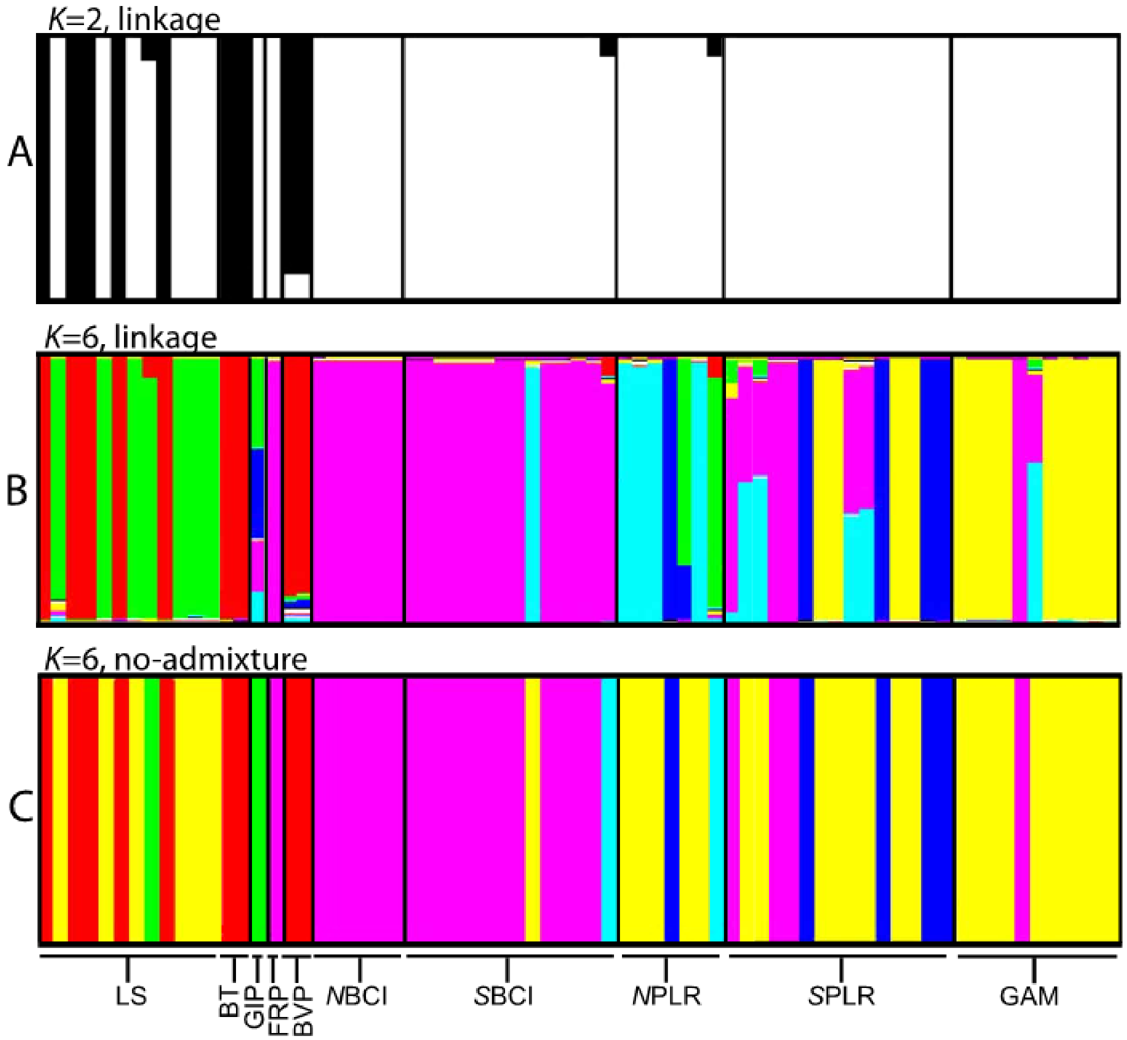
Supplementary Figure 2: Trait mismatches in *Pseudonocardia-Escovopsis* inhibition across Panama. A lack of differentiation is seen between within-colony (i.e., along the diagonal) and among-colony interactions refutes colony-level symbiont selection. In contrast, cultivars have little antibiotic inhibition towards brown *Escovopsis* (see Gerardo, N.M. & E.J. Caldera. 2007. Labile associations between fungus-growing ant cultivars and their garden pathogens. International Society for Microbial Ecology Journal. 1: 373-384). Darker shades indicate a greater zone of inhibition (ZOI) against Escovopsis in Petri plate bioassays.



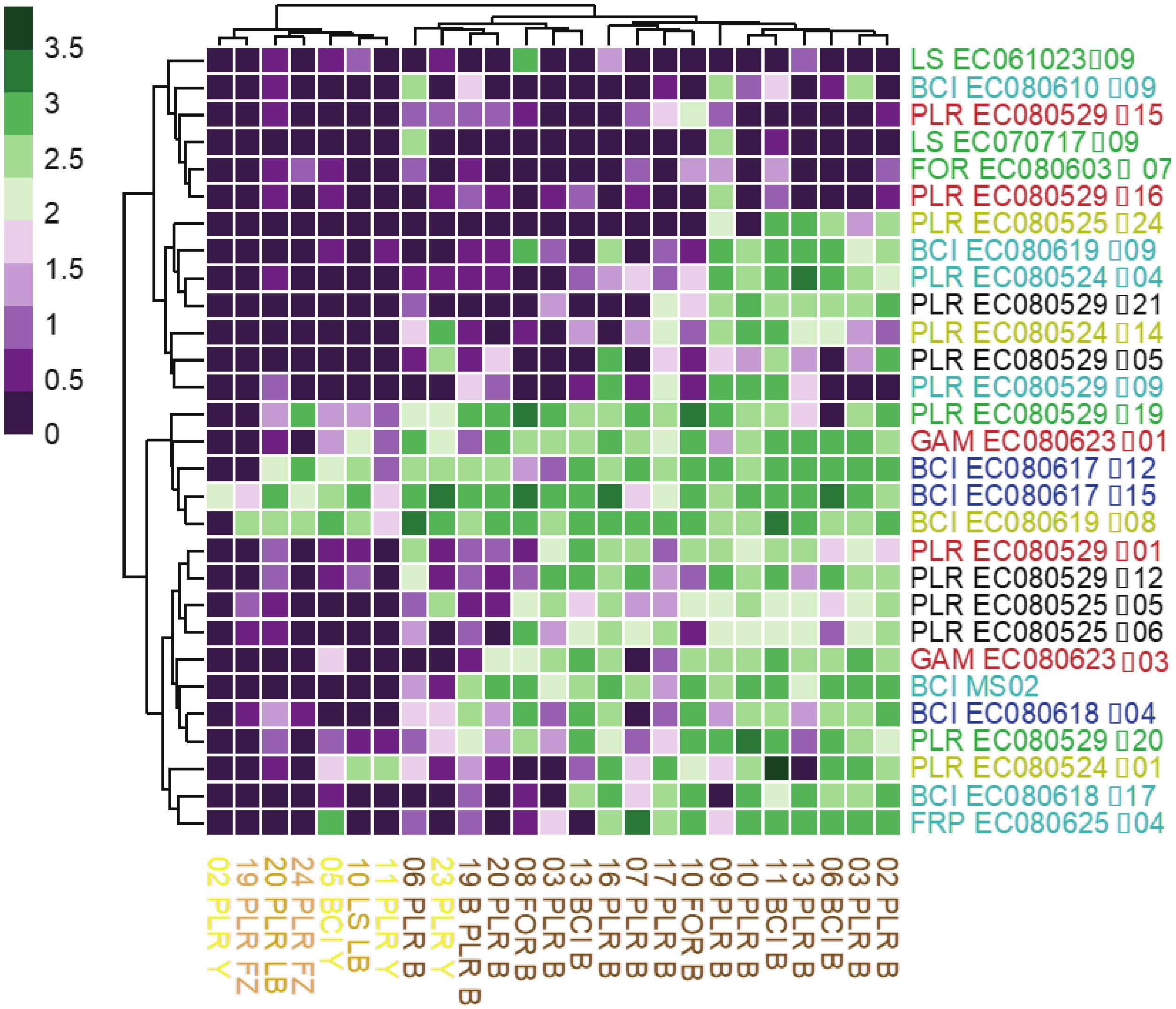
Supplementary Figure 3: Pearson correlations between the presence/absence of a biosynthetic gene cluster and the observed *Escovopsis* zone of inhibition by *Pseudonocardia*. Groupings based on either BGC (a) or *Escovopsis* source (b) do not reach significance.



Supplementary Figure 4: High F_{ST} within three enzymatic bacteriocin regions in a population of *Psudonocardia* symbionts on Barro Colorado Island, Panama.



Supplementary Figure 5: Assignment of *Pseudonocardia* isolates to genetic clusters (K) determined using Bayesian simulations in STRUCTURE. Each vertical line corresponds to the proportion of an individual's genome that corresponds to a particular cluster, indicated by color differences. Panel A presents results of the linkage/admixture model (K=2), as determined by calculating ΔK (see see Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–20). Admixed isolates in A correspond to recombinant sequences identified through tests for recombination and phylogenetic incongruence (see Caldera EJ, Currie CR. 2012. The Population Structure of Antibiotic-Producing Bacterial Symbionts of *Apterostigma dentigerum* Ants: Impacts of Coevolution and Multipartite Symbiosis. Am Nat 180:604–617). B and C show K=6 for the linkage/admixture and no-admixture models, respectively, where the no-admixture model assumes that each isolate derives from a single population and the linkage/admixture model allows individuals to have admixed ancestry. K=2 yielded the highest ΔK values (see Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–20) for both the linkage (A) and no-admixture (not shown) models. Output for both of these models was virtually identical with the exception of admixture in five isolates. These two populations corresponded to the two major clades observed by phylogenetic analysis. As stated in the STRUCTURE documentation, selecting the appropriate K can be difficult when populations conform to isolation by distance (see Caldera EJ, Currie CR. 2012. The Population Structure of Antibiotic-Producing Bacterial Symbionts of *Apterostigma dentigerum* Ants: Impacts of Coevolution and Multipartite Symbiosis. Am Nat 180:604–617). The highest likelihood scores for the linkage model were observed for K=6 (B). For both the linkage and no-admixture models, Ks larger than six did not find additional structure or assign individuals to different populations, but rather only added minimal probability of association to multiple populations for a few isolates. Thus, we also present K=6 for the no-admixture model (C), as it is most consistent with the Evano et al. 2005 method (see Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–20) of selecting K. Similar population clusters were identified for both models; however, the linkage model captured a greater distinction between LS, Costa Rica and the Panamanian isolates, and identified a genetic cluster within NPLR that appears admixed with SPLR and GAM. The linkage model identified the sole isolate from GIP as being highly admixed.



Supplementary Figure 6: Zones of inhibition in various *Escovopsis*-*Pseudonocardia* challenges clustered based on Euclidean distance. Large zones shown in dark green and no inhibition shown in dark purple. *Pseudonocardia* (y-axis) are color coded by fineSTRUCTURE groupings from whole genome sequencing, and *Escovopsis* (x-axis) are color coded by morphotype.