Emerging Perspectives on the Ecological Roles of Endophytic Fungi in Tropical Plants

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9.1 INTRODUCTION

Fungal endophytes live inside of plant tissues (e.g., roots, stems, leaves) without causing apparent harm to their host. Although the definition of the term endophyte is a matter of debate (Wilson, 1995a), throughout this chapter we refer to endophytes as those fungi that live inside of foliar plant tissue, excluding a discussion of stem- (see Evans et al., 2003) and root-associated (i.e., mycorrhizal) fungi. Foliar endophytes are highly diverse and have been documented in nearly all plants sampled (e.g., mosses, liverworts, ferns, conifers, and angiosperms; see Carroll, 1988; Clay, 1988; Petrini, 1991; Arnold et al., 2000, 2003; Arnold, 2002; Davis et al., 2003). Despite the growing recognition of their occurrence among species representing many plant lineages, their ecological roles are still poorly understood.

The best-studied endophytes are Ascomycetes, belonging to the family Clavicipitaceae. These fungi are found growing systemically in the aboveground tissues of some temperate grass species (e.g., Festuca spp.; see Saikkonen et al., 1998; Clay and Schardl,
Infected plants often harbor a single fungal genotype, and asexual endophytes are typically transmitted vertically from maternal plants to their offspring via seeds. Endophytes associated with some domesticated grasses are generally thought to act as mutualistic symbionts (see Clay, 1991; Clay and Scharl, 2002; see also Faeth, 2002). These endophytes, which are intimately associated with their hosts, can confer an array of benefits upon their hosts, including tolerance to heavy metals, increased drought resistance, reduced herbivory, defense against pathogens, and enhanced growth and competitive ability (reviewed by Saikkonen et al., 1998). However, vertical transmission, high specificity, and low within-host fungal diversity appear to represent a special case that does not provide a general model for the majority of host–endophyte associations (Saikkonen et al., 1998; Stone et al., 2000; Faeth and Fagan, 2002).

Whether endophytes of woody angiosperms also confer benefits to their hosts is a subject of current debate. While studies with temperate-zone trees show that in some cases endophyte densities are negatively correlated with herbivores and galling insects (Wilson and Carroll, 1994, 1997; Wilson, 1995b; Gange, 1996; Preszler et al., 1996; Wilson and Faeth, 2001), some authors have argued that defensive mutualisms between endophytes and woody plants are likely to be rare (see Carroll, 1986, 1991; Faeth, 2002). In particular, it has been suggested that herbivorous insects may actually promote endophyte infection via folivory, especially in the case of leaf-mining insects (Faeth and Hammon, 1997; Faeth, 2002). However, considering that endophytes are symbionts that obtain resources from and grow within their hosts, it is highly plausible that endophytes of woody plants have evolved ways to defend their hosts, and thus themselves, from being eaten by herbivores or damaged by pathogens (see Frank, 1996; Herre et al., 1999; Arnold, 2002).

Despite this intriguing possibility for mutualistic interactions between endophytes and their hosts, endophyte research in tropical areas has generally been limited to describing the endophyte species found on particular host plants, e.g., Lodge et al., 1996, Bayman et al., 1998; Rajagopal and Suryanarayanan, 2000). Recent studies in tropical areas have demonstrated that endophytes can be extremely diverse within host plants, even within a single leaf. For example, tropical endophytes represent at least five classes of Ascomycota, with 3 to 20 species often coexisting as highly localized infections within individual leaves (Lodge et al., 1996; Arnold et al., 2000). However, compared with endophyte–grass systems, the ecological roles of endophytic fungi associated with leaves of tropical woody plants are poorly known. Only a few recent studies have focused on the basic ecology of these fungi and their interactions with hosts (Fröhlich and Hyde, 1999; Arnold et al., 2001, 2003; Arnold and Herre, 2003; Suryanarayanan et al., 2003).

In contrast to vertical transmission of endophytes in grasses, endophytes associated with foliage of tropical woody plants appear to be predominantly transmitted horizontally via sporefall (Bayman et al., 1998; Lebrón et al., 2001; Arnold and Herre, 2003; Mejia et al., 2003). Leaves are flushed endophyte-free, and then shortly after emergence, they become densely infected with endophytes. There is some evidence to suggest that insect folivory may influence the abundance and diversity of endophytes (A.E. Arnold, unpublished), but the majority of endophyte infections occur without leaf damage as a precursor. Recent studies indicate that young leaves accumulate endophytes shortly after emergence via epiphytic germination of fungal propagules, which then infect leaves via cuticular penetration or growth through stomates (Arnold and Herre, 2003, Mejia et al., 2003).

We have conducted multiyear surveys of endophytes that are associated with Theobroma cacao (Malvaceae) and several other plant hosts in Panama. We outline our major findings on diversity, host affinity, transmission, interactions, and pathogen resistance in Table 9.1. Additionally, we discuss the following unanswered questions: (1) Which fungal species occupy which hosts? (2) What is the mechanism for differential host affinity? (3)
Table 9.1 Summary of Selected Findings from Field Surveys and Experimental Work on Endophytic Fungi

Major Findings

1. Endophytic fungal (EF) diversity is extremely high within a single host species. In a sample of 126 *T. cacao* leaves (32 mm² of tissue sampled per leaf), 1172 isolates representing 344 morphotaxa were recovered. Within that sample, 20 morphotaxa accounted for roughly 60% of all isolates, with most morphotaxa found only rarely (Arnold et al., 2003). This result is consistent with surveys of endophyte diversity in other families of tropical woody plants (Fröhlich and Hyde, 1999; Arnold et al., 2000; Arnold, 2002), and the prevalence of rare morphotaxa reflects a general pattern among tropical plant-associated fungi (see Gilbert, 2002, Gilbert and Sousa, 2002, Gilbert et al., 2002).

2. EF communities exhibit considerable heterogeneity at small and large spatial scales (Bayman et al., 1998; Arnold et al., 2000, 2003). Although the aggregate fungal communities found on conspecific trees growing within 50 km of each other show relatively high Morsita–Horn similarity (>0.65), that similarity drops off sharply with larger distances (see also Fröhlich and Hyde, 1999).

3. EF transmission is horizontal (among hosts) rather than vertical. Leaves are flushed endophyte-free, and EF are acquired from the habitat over time (see Arnold and Herre, 2003). Leaves appear to saturate in EF density after roughly 2 to 4 weeks.

4. The species diversity of EF communities within leaves increases up to the point of saturation of EF density, generally at 4 to 8 weeks after leaf flush (Rojas et al., unpublished data).

5. EF exhibit differential host affinity. EF communities associated with different host species show striking differences, even when those species are growing in close proximity (Arnold et al., 2000). Specifically, the EF species that tend to dominate the communities in a given host tend to be rare, if they are found at all, in other hosts (Arnold et al., 2003; Herre et al., unpublished).

6. EF growth *in vitro* is strongly affected by the medium. Generally, EF that are commonly found in a given host usually grow best in media that contain extracts of that host species (Arnold and Herre, 2003; Arnold et al., 2003).

7. EF species show a range of dominance interactions *in vitro*, ranging from indifference to active inhibition (Herre et al., unpublished data). The outcome of interactions between any two EF species depends on the medium (Arnold et al., 2003). EF species that commonly occur on a given host generally tend to dominate interactions with more rarely occurring species when tested on medium containing extracts of that host.

8. Hosts with EF-free leaves can be produced by preventing freshly flushed leaves from surface wetting, which is conducive to spore germination and subsequent hyphal infection (Arnold and Herre, 2003). Selected EF can be introduced into leaves in order to conduct experimental tests of the effects of the EF (Arnold et al., 2003, Mejia et al., 2003).

9. Greenhouse trials demonstrate that EF-inoculated leaves resist *Phytophthora* sp. (pathogen) damage, compared with EF-free leaves (Arnold et al., 2003). EF can enhance host antipathogen defenses.

10. Field trials show that EF inoculations can help protect *T. cacao* fruits from loss to pathogen damage (*Phytophthora* sp.) (Mejia et al., 2003, Mejia et al., unpublished).
What is the complete life cycle of the fungi? (4) What is the mechanism of endophyte-mediated host defense?

9.2 QUESTIONS

9.2.1 Which Fungal Species Are in Which Hosts?

Given the diversity of tropical fungi and their hosts, we have not yet begun to scratch the surface of describing how fungi are distributed across hosts. To date, we have isolated endophytic fungi from leaves of eight plant hosts (three vines and five woody plants) in Panama using standard methods (outlined in Arnold et al., 2003). Fungi were grouped to morphotaxa using vegetative features that appeared to conservatively uphold species boundaries as defined by molecular markers (Arnold et al., 2000; Arnold, 2002; Lacap et al., 2003). For the most common and several rare endophytic morphotaxa associated with each host plant species, we used analyses of nrDNA sequence divergence and conducted interaction trials among different isolates to confirm the species boundaries suggested by morphology (see Arnold et al., 2003; Herre et al., unpublished). Further, we used a basic local alignment search tool (BLAST) in order to assign tentative names to the morphospecies (Table 9.2). We emphasize that caution must be used in interpreting the species names given by sequence matches from the BLAST search, primarily due to the incomplete and uneven sampling of taxa in the GenBank database. Therefore, we include the names of our top matches to provide a general idea of the genera and possible species that are commonly found as endophytes in these plants. We note that there is often genetic divergence between isolates that yield the same name as top matches. Given that even small genetic differences can translate to large functional differences (Freeman and Rodriguez, 1993), these observations are consistent with the inference that functional diversity of endophytes is likely to be much greater than the diversity reflected in species names.

To compare differences in host affinity among endophytes, we surveyed and compared the endophytic fungi within two host plant groups. One group consisted of three woody trees on Barro Colorado Island, while the second group consisted of three vines and one woody shrub, all growing in nearby Parque Soberania. Among the endophyte morphotaxa recovered from the trees in the first group (T. cacao [Malvaceae], n = 9 leaves; Heisteria cocinna [Olacaceae], Ouratea lucens [Ochnaceae], n = 3 leaves; Table 9.2), 65.5% were recovered from only one host species (Arnold et al., 2003). Moreover, the most common morphotaxa from one woody host species was usually absent or rare in the other host species. Among the morphotaxa recovered from the second group (Ipomoea philomela, Ipomoea squamata, Merremia umbellata [Convolvulaceae], n = 16 leaves/host species; Witheringia solanacea [Solanaceae], n = 8 leaves; Table 9.2), 75.6% were recovered from only one host species (Van Bael et al., unpublished data). In contrast to the first group, however, several of the most common endophyte–host species were very closely related to the common endophytes in the other host plant species (Table 9.2). This observation of high overlap or similarity among common endophytes in the second group may reflect the relatively higher phylogenetic affinities of these hosts (three Convolvulaceae and one Solanaceae). This raises the question: Do closely related hosts share similar endophytes? A further possibility is that the most common endophytes are more likely to be host generalists, as has been demonstrated for polypores (Gilbert et al., 2002). Further work, in which structured sampling of hosts with different degrees of phylogenetic affinity is done, is needed.
Table 9.2 Species of Endophytic Fungi That Were Frequently Isolated from Leaves of Several Host Plants in Panama

<table>
<thead>
<tr>
<th>Host Plant Family, Species</th>
<th>Top GenBank Matches^a</th>
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</thead>
<tbody>
<tr>
<td><strong>Olacaceae</strong></td>
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<tr>
<td><em>Heisteria coccinna</em></td>
<td><em>Guignardia magniferae</em></td>
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<td></td>
<td><em>Xylaria hypoxylon</em></td>
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<tr>
<td></td>
<td><em>Xylaria arbuscula</em> A</td>
</tr>
<tr>
<td><strong>Malvaceae</strong></td>
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<tr>
<td><em>Theobroma cacao</em></td>
<td><em>Botryosphaeria lutea</em></td>
</tr>
<tr>
<td></td>
<td><em>Colletotrichum gloeosporoides</em> A</td>
</tr>
<tr>
<td></td>
<td><em>Botryosphaeria dothidea</em> A</td>
</tr>
<tr>
<td></td>
<td><em>Botryosphaeria dothidea</em> B</td>
</tr>
<tr>
<td></td>
<td><em>Colletotrichum gloeosporoides</em> B</td>
</tr>
<tr>
<td></td>
<td><em>Phomopsis</em> sp.</td>
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<tr>
<td></td>
<td><em>Colletotrichum gloeosporoides</em> C</td>
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<td></td>
<td><em>Xylaria longipes</em> A</td>
</tr>
<tr>
<td><strong>Ochnaceae</strong></td>
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<tr>
<td><em>Ouratea lucens</em></td>
<td><em>Guignardia endophyllicola</em></td>
</tr>
<tr>
<td></td>
<td><em>Phyllosticta</em> sp.</td>
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<tr>
<td><strong>Convolvulaceae</strong>^f</td>
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<tr>
<td><em>Ipomoea philomega</em></td>
<td><em>Glomerella cingulata</em> A</td>
</tr>
<tr>
<td></td>
<td><em>Xylaria arbuscula</em> B</td>
</tr>
<tr>
<td><em>Ipomoea squamata</em></td>
<td><em>Glomerella cingulata</em> B</td>
</tr>
<tr>
<td></td>
<td><em>Curvularia affinis</em></td>
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<tr>
<td></td>
<td><em>Colletotrichum truncatum</em> A</td>
</tr>
<tr>
<td><em>Merremia umbellata</em></td>
<td><em>Xylaria longipes</em> B</td>
</tr>
<tr>
<td></td>
<td><em>Colletotrichum gloeosporoides</em> D</td>
</tr>
<tr>
<td></td>
<td><em>Colletotrichum truncatum</em> B</td>
</tr>
<tr>
<td><strong>Solanaceae</strong>^f</td>
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<tr>
<td><em>Witheringia solanacea</em></td>
<td><em>Glomerella cingulata</em> C</td>
</tr>
<tr>
<td></td>
<td><em>Colletotrichum truncatum</em> C</td>
</tr>
<tr>
<td><strong>Rubiaceae</strong></td>
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<tr>
<td><em>Faramea occidentalis</em></td>
<td><em>Xylaria</em> sp.</td>
</tr>
<tr>
<td></td>
<td><em>Glomerella cingulata</em> D</td>
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*Note:* Identities are based on BLAST searches of the National Center for Biotechnology Information GenBank database using internal transcribed spacer (ITS) sequences (Altschul et al., 1990).

^a Listed are the fungal species present in GenBank with which endophytes showed the highest affinity. Letters signify samples that were genetically distinct, despite receiving the same name.

^b-c Ranking for the most frequently encountered endophyte species in one T. cacao collection of 10 leaves (Rojas et al., in preparation).

^f Identifications represent the two or three most common fungi per plant species in these families.

^* Note that *C. gloeosporoides* is an anamorph of *G. cingulata.*
9.2.2 What Is the Mechanism for Differential Host Affinity?

In addition to carefully designed surveys of leaves from different species, experiments are important for distinguishing true host affinity from spatial artifacts (i.e., localized dispersal within host crowns) and for examining the mechanisms behind host affinity when it is observed (Arnold et al., 2003). Recent experimental work has demonstrated growth differences among endophyte morphotaxa frequently collected from *T. cacao*, *H. cocinna*, and *O. lucens* when they were plated on separate media containing leaf extracts from each host species. In >75% of the cases, growth rates were higher on media containing extracts of the host species from which they were most frequently isolated in the field surveys (Arnold et al., 2003). Moreover, the growth rates of endophytes *in vitro* (with host plant extracts) corresponded to their relative abundance *in planta*, with common taxa from a given host growing better than rare taxa. In sum, host-specific leaf chemistry appears to favor the growth of some endophytes over others, and highest growth rates are observed when endophytes were cultivated on extracts of the host species for which they displayed highest affinity in the field. By mediating the growth of particular endophyte species, host-specific leaf chemistry may also influence the outcomes of competitive interactions among endophytes or among endophytes, herbivores, and pathogens.

9.2.3 What Is the Life Cycle for Tropical Endophytic Fungi?

Very little work has been done to establish the complete life cycles of the fungal endophytes identified from woody angiosperms. Reproductive structures of some of the fungal associates are readily observed in nature. Fungi typically identified as the most prevalent dicotyledonous taxa (e.g., *Xylaria* spp. and *Colletotrichum* spp.) are also often encountered on the tropical forest floor developing from leaf and wood litter (Bischoff, personal observations). The current dogma is that the fungi contained within the plant reproduce after the plant tissue (e.g., leaves and stems) senesces or abscises (Wilson, 2000). These fruiting structures then provide inocula that lead to new infections of developing leaf and branch tissue (Malloch and Blackwell, 1992).

Although horizontal transmission via spores after leaf senescence is a likely method of dispersal, it is doubtful that it is the only form in which horizontal transmission occurs among the endophytes of woody angiosperms. Species of the grass endophytic genera *Epichloë* and *Balansia* are known to vertically transmit by systemic infection of the host embryo (Freeman, 1902; Clay, 1986). In contrast to these clavicipitaceous endophytes, there has been little evidence of vertical transmission among endophytes of woody dicots. As in previous studies (Bayman et al., 1998; Lebrón et al., 2001), we have observed that seedlings at germination and leaves at emergence lack cultivable endophytes. However, endophytic species have been found associated with host seeds while attached to the parent plant (Petrini et al., 1992; Wilson and Carroll, 1994). These fungi may then disperse with the angiosperm seed, sporulate, and thus provide the inoculum for the newly established seedling. This would help maintain a host–symbiont relationship even in founder events of dispersal. Grass endophytes living asymptotically in plant tissue were discovered over 100 years ago (Vogl, 1898). Despite extensive work focused on this plant–host interaction over the ensuing years, it was not until 1996 that *Neotyphodium* sp. (the anamorph of *Epichloë*) was found to develop a mycelial net and conidiogenous cells along the leaf surface of *Agrostis hiemalis* and *Poa rigidifolia* (White et al., 1996). The authors determined that the epiphyllous conidia are likely responsible for some of the horizontal transmission occurring in the grass–*Epichloë* interaction. It is possible that this inconspicuous mode of dispersal is also occurring in some of the woody endophytic species.

When discussing the spore dispersal and life cycles of endophytes associated with woody plants, we find that there are more questions than answers. This is especially true
of the tropical woody angiosperms. For example, why fungi wait until senescence to reproduce, what cues their reproduction, and how within-leaf competition influences endophyte fitness require further research. Further, due to the high diversity of these endophytes (Arnold et al., 2000), it is likely that many different types of life cycles will be found among these fungi. For example, many of these endophytes are also regarded as pathogens of particular hosts. It may be that these organisms are able to live in an asymptomatic manner in one host but cause disease in another. Detailed studies of these organisms and their dispersal methods may provide clues to host shifting and the origins of symptomatic pathogens in susceptible hosts.

9.2.4 What Is the Mechanism of Host Defense?

Two recent studies have demonstrated that in at least some cases, endophytes can enhance host defenses against pathogens (Arnold et al., 2003; Mejia et al., 2003). Two key methodological discoveries allowed this work to occur. First, we found that by keeping leaves dry as they grew, the leaves remained endophyte-free (E−) (Arnold, 2002; Arnold and Herre, 2003; Mejia et al., 2003; see also Wilson and Carroll, 1994; Wilson et al., 1997). Second, we were able to introduce endophytes into E− leaves, in combinations and concentrations of our choosing, and thereby create endophyte positive (E+) leaves (Mejia et al., 2003; Arnold et al., 2003). Leaves that were E− and E+ could be generated within individual seedlings of T. cacao (Figure 9.1).

In a greenhouse experiment (Arnold et al., 2003), we generated seedlings (n = 70) in which half of the focal leaves were inoculated with a group of seven endophyte species (from the genera Colletotranchum, Xylaria, and Nectria/Fusarium) that had shown previous in vitro activity against a foliar pathogen, Phytophthora sp. Thus, each seedling contained endophyte treated (E+) and untreated (E−) leaves. Eighteen days after endophyte treatments, we applied a strain of Phytophthora sp., isolated previously from symptomatic T. cacao in Panama, to a subset of E+ and E− leaves. The final experiment included all factorial combinations of endophyte (E) and pathogen (P) presence and absence. After 15 additional days, we assessed pathogen damage by determining leaf mortality and the area of damage on surviving leaves.

Leaves without endophytes and with Phytophthora (E−P+) experienced leaf death and abscission 2.8 times more frequently than did leaves inoculated with endophytes (E+P+). Moreover, on P+ leaves that did survive, necrotic lesions were significantly larger on leaves without endophytes (E−P+) than on leaves with endophytes (E+P+). Although the protection by endophytes was apparently localized to individual leaves, entire host plants were affected by the presence or absence of endophytes. For example, when we
considered both leaf loss and leaf damage on retained leaves, surface area available for photosynthesis decreased by 32.3% for E–P+ treatments relative to E–P–, but only by 14.1% for E+P+ treatments relative to E+P– (Arnold et al., 2003).

While this experiment demonstrated that endophytes limit pathogen damage in *T. cacao*, the mechanism for this defense remains unclear. One clue, however, was the apparent localization of defense to endophyte-infected tissues. This observation, combined with observations of interactions among endophytes in *vitro* (Herre et al., unpublished data), suggested that interspecific interactions among endophytes and pathogens may play an important role in mediating host defense. To explore this hypothesis, we assessed *in vitro* interactions between 50 endophyte morphotaxa isolated from *T. cacao* and three major cacao pathogens (*Phytophthora* sp., *Moniliophthora roreri*, and *Crinipellis perniciosa*; Mejia et al., 2003, unpublished data). In interactions on standard media (2% malt extract agar), 40% of the endophyte morphotaxa appeared to antagonize at least one of the pathogen species, while the remaining endophytes had no effect or were themselves antagonized. Interestingly, when we repeated the interaction trials on media containing leaf extracts of *T. cacao*, the outcomes differed qualitatively and quantitatively. Together, these observations suggest that direct interactions among endophytes and pathogens are complex, diverse, and sensitive to host-specific leaf chemistry. The diversity of endophytes and their interactions may contribute to effective antipathogen defense in woody plants. Because host plants must deal with ever-changing and diverse pathogens in tropical forests, this form of defense is likely to be enhanced when endophytes are highly diverse within and among leaves, plants, and host species.

### 9.3 CONCLUSIONS

We are only beginning to understand the ecological role of endophytes in natural tropical communities and to realize their applied potential. It is clear that horizontally transmitted endophytes can enhance and supplement host defense against pathogens. The mechanism of defense appears to be in part affected by the outcome of interspecific competition among endophytes and pathogenic fungi, which in turn appears to be influenced by plant chemistry. There are still many outstanding questions about mechanisms of defense and about the potential mutualism between endophytes and their hosts. For example, what are the costs of harboring endophytes to hosts? What is the relative importance of abundance, diversity, and species composition of endophytes in determining whether antipathogen defense occurs? Do endophytes in woody plants provide other types of defense to their hosts, such as against herbivores? An additional obvious need is to expand the work into other host species, in order to assess the generality and frequency of such endophyte-mediated effects.

In addition, the extent to which the interactions among endophytes and their hosts represent true mutualisms deserves further study. In general, mutualistic interactions between hosts and vertically transmitted symbionts can be easily reconciled with existing theory (reviews by Herre et al., 1999; Leigh, 1999). In contrast, horizontally transmitted symbionts are expected to behave less mutualistically and may tend toward antagonism. Nonetheless, several recent examples of horizontally transmitted mutualists, such as pollinators (Herre, 1999), mycorrhizal fungi (Husband et al., 2002), and endophytic fungi, may challenge the existing theory.
ACKNOWLEDGMENTS

The authors thank Greg Gilbert, Tom Gianfagna, and Prakash Hebbar for essential technical advice and training. They also thank the Smithsonian Institution, the Smithsonian Migratory Bird Center, the Andrew W. Mellon Foundation, the National Science Foundation (DEB 9902346 to Lucinda McDade and A.E.A.), the American Cacao Research Institute, and the World Cacao Foundation for financial support.

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