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History, genetics and pathology of a leaf-cutting ant introduction: a case study of the Guadeloupe invasion

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Abstract As dominant herbivores and notorious pests in their native Neotropics, introduced leaf-cutting ants have the potential for ecological and economic harm. Although a large-scale invasion of leaf-cutting ants has not occurred, an isolated introduction in the Caribbean islands of Guadeloupe provides useful insight into the progress of such an invasion. Since being first detected in 1954, *Acromyrmex octospinosus* has colonized virtually all available land area, defying an aggressive control campaign and damaging agriculture. I attempted to reconstruct the origins and spread of the invasion, as well as screen for the presence of garden pathogens, which could be used for biological control. Mitochondrial sequencing of the *A. octospinosus* complex throughout the Caribbean showed that the probable source of the invasion lies on Trinidad and Tobago or northeast South America. Using historical records and field surveys, the invasion's rate of spread was estimated at 0.51 km/year. Microsatellite genotyping further confirmed the limited dispersal abilities of *A. octospinosus*, showing the presence of isolation by distance (even in a relatively small geographic area) and suggested ubiquitous local inbreeding. Although the invasion likely resulted from the introduction of a single colony, microsatellites showed a high level of genetic variation in the introduced population, likely as a consequence of multiple mating by the queen. A survey showed that the specialized fungus garden pathogen *Escovopsis* exists on the islands, suggesting that the successful spread of the ants was not due to escape from this parasite. Given that chemical control has failed in the past and that biological control using specialized garden pathogens seems improbable, only vigorous quarantine and inspection programs may prevent wide-scale leaf-cutting ant invasions in the future.

Keywords Dispersal · Ecological release · Fungus-gardening ants · Phylogeography · *Pseudonocardia* · Symbiosis

Ants are present in large numbers in many ecosystems and drive numerous ecological processes (Hölldobler and Wilson 1990). Consequently, introduced ant species, especially a few high-impact ant invaders, may cause fundamental changes in their new habitat to the detriment of native organisms. For example, by competitively excluding native seed-dispersing ants, invasive Argentine ants have caused major shifts in plant compositions in the South African shurblands (Christian 2001). Likewise, by eliminating the native ants, which serve as food for horned lizards, Argentine ants have likely led to declines in lizard populations in California (Suarez and Case 2002). Unfortunately, little is known about

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these high-impact invaders before they become established and initiate major ecological changes. Although efforts to elucidate criteria by which potentially invasive ants could be determined are underway (e.g., McGlynn 1999b and Suarez et al. 2005), no high risk taxa have yet been identified prior to major invasions.

Leaf-cutting ant genera *Acromyrmex* and *Atta* are dominant Neotropical herbivores. *Atta* nests may contain millions of workers and consume hundreds of kilograms of leaves per year (see a review by Wirth et al. 2003). Their ability to consume a diverse array plants results from an ancient obligate mutualistic interaction with a fungus cultivar (Weber 1972; Wheeler 1907). Because of their voraciousness and impressive dietary breadth, the leaf-cutters are major agricultural pests of the New World (Cherrett 1968). Hölldobler and Wilson (1990) wrote: "If any leaf-cutter ants, especially *Atta*, were to be established in sub-Saharan Africa or some other part of the Old World tropics, the result might be an ecological catastrophe. The terrestrial ecosystems of these continents are unprepared for these highly organized insects." Although not commonly considered invasive, leaf-cutters can travel with commercial and military cargo and at least two *Acromyrmex* species have been intercepted by US Customs (Suarez et al. 2005). Although the Old World was spared a leaf-cutter invasion, a population of the leaf-cutter ant *Acromyrmex octospinosus* became established in the Caribbean, on Guadeloupe. These islands, previously free of native leaf-cutter ants (Cherrett 1968), provide a unique opportunity to study the dynamics of a leaf-cutter invasion in advance of a possible ecological catastrophe. This invasion may also provide useful insights into basic leaf-cutting ant biology.

A. octospinosus (locally known as *la fourmi-manioc*, or the manioc ant) occupied a 600-hectare area in the vicinity of the Morne-à-l'eau community on the island of Grande Terre at the time of its discovery in 1954 (Blanche 1954). Two years later the local government began an eradication campaign, which focused largely on control through a variety of pesticides, although the use of psychotropic drugs to "impair the vigilance" of *Acromyrmex* was also considered (Kermarrec and Mauleon 1990). Attempts to prevent the westward spread of the ants to the rest of Guadeloupe failed and they also colonized the nearby island of Basse Terre. Subsequently,

large-scale efforts to combat the spread of *Acromyrmex* were abandoned, leaving the ants to freely spread throughout the rest of the islands (Fig. 1).

I attempted to answer a number of general questions aimed at understanding (a) how a leaf-cutter ant invasion originates, (b) how it progresses, and (c) whether biological control may be feasible using the specialized fungus garden pathogen *Escovopsis* (Currie 2001; Currie et al. 2003b). To determine the origin the introduced ants, I conducted a biogeographic study of the *A. octospinosus* complex throughout the Caribbean. The rate of spread of *Acromyrmex* in a novel habitat was estimated from field surveys and historical accounts of the invasion's extent at various points in time. Dispersal patterns were further investigated using a microsatellite analysis of population genetic structure and diversity. Finally, in order to ascertain whether the success of the *Acromyrmex* invasion may have been aided by escape from virulent fungal pathogens, which may eventually be used for biological control, I surveyed the leaf-cutter colonies for *Escovopsis*, as well as for *Pseudonocardia* actinomycete bacteria used to combat the parasitic fungi (Currie et al. 2003a; Currie et al. 1999b).
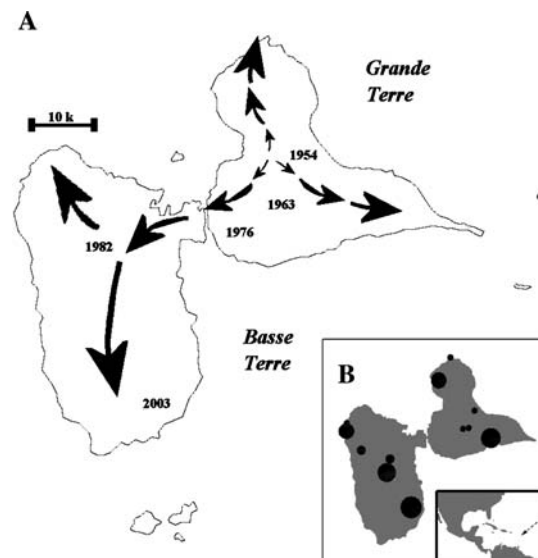

Fig. 1 History of the invasion of Guadeloupe by *Acromyrmex*. After their initial discovery at the community of Morne-à-l'eau on Grande Terre, the ants slowly colonized the both major islands

Materials and methods

Collection

Several dozen nest workers of all sizes were sampled from 60 colonies throughout the Guadeloupe islands of Basse Terre and Grande Terre in December of 2003 (Fig. 1B). Ants and fungi were preserved in 95% ethanol and stored at $-80°C$ when brought to the lab several weeks later.

Origin of the invasion

I used the primers COI and COII, anchored in cytochrome oxidase sub-units I and II, respectively (see Sumner et al. 2004, for primer sequences and PCR conditions) for reconstructing ant phylogenetic relationships. The amplified region also included an intergenic spacer, which was too variable for unambiguous alignment across the species complex and was excluded from the analysis. A neighbor-joining tree was calculated by CLUSTAL with support provided by 1,000 bootstrap replicates. The sequences have been deposited in GenBank (accession numbers EF485021–EF485028).

Rate of spread

From the point of initial detection, the invasion expanded relatively unhindered from central Grande Terre to the north, west, and east (Fig. 1). Using maps of the *A. octospinosus* distribution from Malato et al. (1977) and Therrien (1986), I conducted a regression analysis of the ants' spread in each of these three directions as a function of time. A lack-of-fit *F*-test was confirmed that expansion of the invasion fronts was linear with respect to time. Minitab (v. 11) was used for regression analyses. All regression estimates are presented ±95% confidence interval.

Gene flow

One individual from each colony was selected haphazardly for DNA extraction, which was accomplished using a guanidinium isothiocyanate-based extraction protocol (Mikheyev et al. 2006). DNA was amplified using three pairs of primers re-designed from loci originally isolated by Ortius-Lechner et al. (2002) from a Panamanian population of *A. octospinosus* (the original primers did not amplify DNA from the Guadeloupe population). 10 μl PCR reactions contained 1 μl of genomic DNA, 1× reaction buffer, 1 mM dNTPs, 0.01 μM M13-tailed primer, 0.15 μM other primer (Table 1), 0.18 μM of fluorescently labeled M13 tail, 5 mM $MgCl_2$ and 0.1 U Taq polymerase. PCR reaction conditions involved an initial denaturing step of 94°C for 2 min, followed by 30 cycles of 94°C for 10 s, 60°C for 20 s, and 72°C for 10 s. The reaction was incubated at 72°C for a 1 h final extension step. The length of the amplified microsatellite fragments was read on an ABI PRISM® 3100 genetic analyzer. Four samples did not amplify at one or more loci and were excluded from the analysis. Allelic diversity, Hardi-Weinberg equilibrium and isolation by distance computations were carried out in GenAlEx (v. 6) (Peakall and Smouse 2006). Arlequin (v. 3) was used for the F_{st} calculations (Excoffier et al. 2005).

Fungus garden pathology

Escovopsis screening was conducted by placing 1–2 cm^2 ant-free samples of the fungus garden into a sterile 1.5 ml tubes. Garden contaminants were allowed to colonize the fungus over the course of two weeks at room temperature. I searched for ants covered with a characteristic white bloom during colony excavation in order to screen for the presence of mutualistic *Pseudonocardia* bacteria (Currie et al. 2006; Currie et al. 2003b).

Table 1 Primers used in the microsatellite survey, based on loci originally developed by Ortius-Lechner et al. (2002)

Locus	Primer sequence (5′→3′)
Ech4225	TCTGTTAGTCATAAGTATGTCACTGTCG
	AACCGATATGTGTCGATATGTATTTGTTAG
Ech3385	GAATGAATTAAATAACGCTAGTGAAAGTAG
	GTGATTATCCACTGATTCAGATTTAAAGAG
Ech3197	GATCATTTATCCTCAATTCAATAGTTTG
	GTTTATGGAACCAGTAGACCCTAATGAAG

Note that the first primer in each pair was synthesized with a fluorescently labeled M13 tail for microsatellite analyses

Results

Origins of the invasion

Phylogenetic analyses demonstrated that *A. octospinosus* from Guadeloupe belong to a clade distinct from most Central American and Cuban *A. octospinosus*, while being most closely related to ants from Trinidad and Tobago (Fig. 3). The affinity of Guadeloupe ants with Trinidad and Tobago is further supported by near-identity of the highly variable mitochondrial spacer region, which was excluded from phylogenetic analysis.

Rate of spread

A linear regression model provided a significant fit to the spread estimates, explaining 82% of the variance (regression $F_{1,10} = 46.6$, $P = 4.6 \times 10^{-5}$; Lack-of-fit $F_{2,8} = 2.7$, $P = 0.13$). The average rate of spread for *A. octospinosus* was 0.51 ± 0.20 km/year and an initial introduction was extrapolated to $1,950 \pm 1.8$. According to the linear model, given this rate of spread, the ants would not have colonized the extreme south of Basse Terre, which lay some 30 km south of the invasion front in the mid-1980s, at the time of my surveys. Indeed, I was unable to find ants south of Capesterre-Belle-Eau during my survey. Additional evidence came from interviews with local residents who claimed that no *fourmi-manioc* were present in the extreme south of Basse Terre. According to temperature and precipitation data published by Therrien (1986), these habitats should be well within the range of environments tolerated by *A. octospinosus* on Guadeloupe, making it likely that the ants still have not reached the extreme south of Guadeloupe.

According to the microsatellite analysis, although there was no significant population structure between the two islands ($F_{st} = 0.022$, $P = 0.10$), there was evidence of evidence of weak isolation by distance ($R_{xy} = 0.06$, $P = 0.029$), probably as a result of the ants' poor dispersal abilities. In addition, populations on both islands exhibited substantial heterozygote deficiency at all microsatellite loci, suggesting common local inbreeding (Table 2).

Fungus garden pathology

A variety of fungi colonized the cultivars when the ants were removed, including the specialized garden

Table 2 Alleleic diversity on Guadeloupe

Locus	Alleles	H_o–H_e	P
Ech4225	6	−0.38	4×10^{-13}
Ech3385	3	−0.036	0.10
Ech3197	7	−0.31	4×10^{-20}

The table shows the name of the microsatellite locus according to Ortius-Lechner et al. (2002), the number of alleles, the difference between expected and observed heterozygocity values and their χ^2 probability

pathogen *Escovopsis*, which was observed in seven out of the 60 garden samples ($\sim 12\%$). The identity of *Escovopsis* was further confirmed using spore morphology and through sequencing a section of the *EF1-α* gene (Gerardo and Taerum, unpublished data). Although no forager bore visible amounts of *Pseudonocardia* symbionts, workers inside the garden were often covered with an easily visible film of these bacteria (Fig. 2; Currie et al. 2006).

Discussion

Origins of the invasion

Without exhaustively sampling the Caribbean using microsatellite markers, the exact origin of *A. octospinosus* will remain unknown. However, an introduction from Central America or Cuba can be ruled out. Panamanian *A. octospinosus* may even be a different species (Sumner et al. 2004). Without providing any further justification for their claim, Malato et al. (1977) suggested that the ants were introduced via unregulated commerce from an

Fig. 2 *Acromyrmex* workers with and without actinomycete symbionts. Both workers came from the same colony; the one the left is a forager and has no visible actinomycetes, while the one on the right is a garden worker, and has a white actinomycete bloom

unspecified English-speaking country in the Caribbean. Phylogenetic data support this hypothesis, since the ants are closely related to those on Trinidad and Tobago, although northeastern South America is also a possibility (Fig. 3).

It is most likely that the ants were introduced either as newly-mated queen(s) or an incipient colony associated with potted vegetation, since mature nests are too large and too aggressive to be transported inadvertently. Recent work has shown that probably only one cultivar genotype was introduced on Guadeloupe (Mikheyev et al. 2006). As genetically identical cultivars genotypes are rare on the mainland (Mikheyev et al. 2007), a single-queen introduction seems most likely. Often invaders suffer considerable loss of genetic diversity, relative to source populations, which, paradoxically, may provide a competitive edge to some ants, such as the Argentine ants, by allowing the formation of wide-ranging super-colonies (Holway et al. 1998; Tsutsui et al. 2000). However, for other ants, like the imported red fire ant, secondary introductions may have been important for successful

establishment (Ross and Fletcher 1985; Shoemaker et al. 2006). Ant queens acquire all the sperm they will use during a single mating flight and the rates of polyandry vary between species (Hölldobler and Wilson 1990). In contrast to the singly-mating Argentine and fire ants (Krieger and Keller 2000; Ross et al. 1988), every *Acromyrmex* queen has the potential to bring greater genetic diversity through mating with numerous males (Murakami et al. 2000). Consequently, the levels of diversity on Guadeloupe, are comparable to those seen on the mainland (Ortius-Lechner et al. 2002).

Rate of spread

Historical accounts of *Acromyrmex* spread on Guadeloupe provided a unique insight into the dispersal rate of these ants, which spread at the surprisingly low rate of 0.51 ± 0.20 km/year. The slow and even expansion of the invasion suggests mating flights as a dominant dispersal mechanism, unlike the usual jump dispersal and colony fission associated with other invasive ants (Suarez et al. 2001). The factors that limited within-Guadeloupe spread of *A. octospinosus* by humans are likely the same that limit international transport—that the ants cannot nest opportunistically, requiring large excavated cavities for their fungus garden (Wetterer et al. 1998), and are substantially larger and more visible than most invasive ants (McGlynn 1999a; Mikheyev and Mueller, 2007). Low dispersal ability can also be inferred from molecular data, which show isolation by distance (even over a few tens of kilometers on Guadeloupe) and reduced heterozygocity, which suggests inbreeding (Table 2).

Fig. 3 Phylogeny of the *Acromyrmex octospinosus* species complex based on cytochrome oxidase subunits I and II. While distinct from populations inhabiting Central America, the Guadeloupe population shows a marked affinity with populations from Trinidad and Tobago and eastern South America. These genetic affinities were further supported by nearly identical intergenic spacer sequences. Genbank accession numbers accompany reference sequences

Fungus garden pathology

Considerable evidence exists indicating that invading species gain an ecological advantage by escaping their natural enemies (Keane and Crawley 2002; Mitchell and Power 2003). *A. octospinosus* provides a test of this hypothesis, since garden pathogens of attine cultivars are relatively well-known (Currie et al. 1999a). However, the success of *A. octospinosus* on Guadeloupe is unlikely due to escape from garden pathogens, as evidenced by both presence of *Escovopsis* and
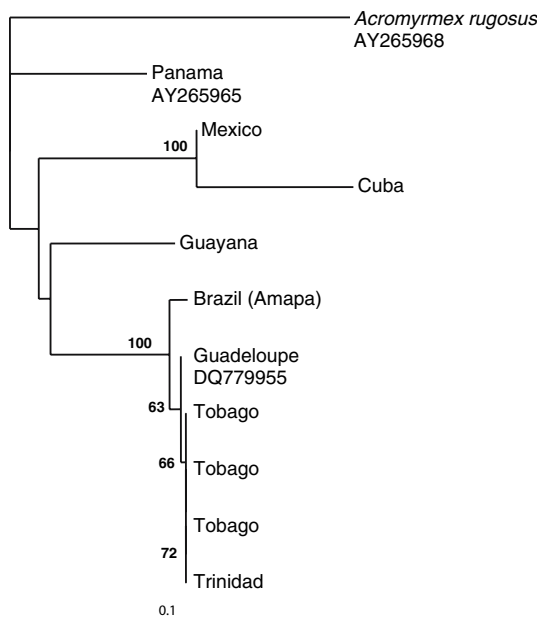
abundance of metabolically costly actinomycetes on the bodies of garden workers, suggesting an ongoing need to protect the garden from pathogens (Poulsen et al. 2003; Fig. 2). The presence of *Escovopsis* in the attine gardens is notable, since dispersing virgin queens appear not to carry the pathogen along with the parental cultivar they take on their mating flight, but instead acquire it only after the onset of foraging (Currie et al. 1999a). Thus, *Escovopsis* must either be sufficiently common in the environment to have been introduced independently, or, less likely, A. *octospinosus* was introduced as a foraging colony, rather than a newly-mated queen.

Implications for further invasions

Although A. *octospinosus* damaged agriculture in Guadeloupe (Blanche 1954), whether they affect natural ecosystems is unknown. The South of Basse Terre, where the ants still appear to be absent, may be a location where the progressive ecological effects of a leaf cutting ant invasion can be still observed.

While most modern-day invasive ants are cryptic and easily transported hidden within goods, A. *octospinosus* appears transport-limited, making introductions a rare event (McGlynn 1999b). On the other hand, even a single queen may carry sufficient genetic diversity to initiate a successful invasion. On Guadeloupe, history has shown that chemical control against such an invasion would be ineffective once it has become fully established (Febvay et al. 1988; Gomel et al. 1989). Given the presence of *Escovopsis* on Guadeloupe, biological control using specialized garden pathogens does not appear promising. However, the limited dispersal ability of A. *octospinosus* may allow the elimination of an incipient population. Such an effort should be rapid and thorough, possibly involving the destruction of all available plant food sources in the vicinity of the introduced A. *octospinosus* population. However, undoubtedly the best defense involves an awareness of the invasive potential of leaf-cutting ants and in addition to an investment into thorough agricultural inspection and quarantine services (Mikheyev and Mueller 2006; Suarez et al. 2005). Although a large number of countries already have such services, their track record is not perfect (e.g., major invasives, such as the red imported fire ant *Solenopsis invicta*,

established despite the activity of the US Customs service). The situation can no doubt be improved by comprehensive studies of the relationship between different inspection and quarantine practices and their relative success at keeping out invaders, and, if necessary, changes in quarantine law.

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