Current Biology

Six decades of museum collections reveal disruption of native ant assemblages by introduced species

Highlights

- Florida's leaf-litter ant communities' changes over a 54-year period are documented

- Nine of ten species that increased most in relative abundance were introduced

- Decreases in abundance occurred in native seed dispersers and specialist predators

- Introduced species differed from native species in key social attributes

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In brief

Booher et al. document changes to leaf-litter ant communities over a 54-year period across the state of Florida, USA. These changes include increases in the relative abundance of non-native species, which now account for 30% of occurrence records. Native species whose abundance decreased included both seed dispersers and specialist predators.

CellPress

Report

Six decades of museum collections reveal disruption of native ant assemblages by introduced species

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SUMMARY

There is a looming environmental crisis characterized by widespread declines in global biodiversity,[1–6] coupled with the establishment of introduced species at accelerated rates.[7–14] We quantified how multi-species invasions affect litter ant communities in natural ecosystems by leveraging museum records and contemporary collections to assemble a large (18,990 occurrences, 6,483 sampled local communities, and 177 species) 54-year (1965–2019) dataset for the entire state of Florida, USA. Nine of ten species that decreased most strongly in relative abundance ("losers") were native, while nine of the top ten "winners" were introduced species. These changes led to shifts in the composition of rare and common species: in 1965, only two of the ten most common ants were introduced, whereas by 2019, six of ten were introduced species. Native losers included seed dispersers and specialist predators, suggesting a potential loss of ecosystem function through time, despite no obvious loss of phylogenetic diversity. We also examined the role of species-level traits as predictors of invasion success. Introduced species were more likely to be polygynous than native species. The tendency to form supercolonies, where workers from separate nests integrate, also differed between native and introduced species and was correlated with the degree to which species increased in their rank abundances over 50 years. In Florida, introduced ants now account for 30% of occurrence records, and up to 70% in southern Florida. If current trends continue, introduced species will account for over half of occurrence records in all Florida's litter ant communities within the next 50 years.

RESULTS AND DISCUSSION

A biodiversity crisis is underway, characterized by the widespread decline in diversity and abundance of many taxonomic groups. Although such reductions are well documented in vertebrate populations,[1–3] trends observed for invertebrates are similarly alarming.[4–6] The consequences of these declines may be particularly severe when diverse native taxa are replaced by introduced species.[7–10] Although invasive species can have acute local impacts and cause extinctions in insular or aquatic ecosystems, their effects in continental systems remain

debated.[11,12] Yet the pace at which species are becoming established in many environments is accelerating,[13,14] and we lack a detailed understanding of the community-wide consequences of multi-species introductions over large areas. Determining the fate of native communities, as entire assemblages of non-native species establish and spread, requires (1) long-term data on the identity and relative abundance of native and non-native taxa over large areas, and (2) decades of recurrent monitoring and species-level identifications to relate patterns to possible changes in the functional and phylogenetic diversity of communities.
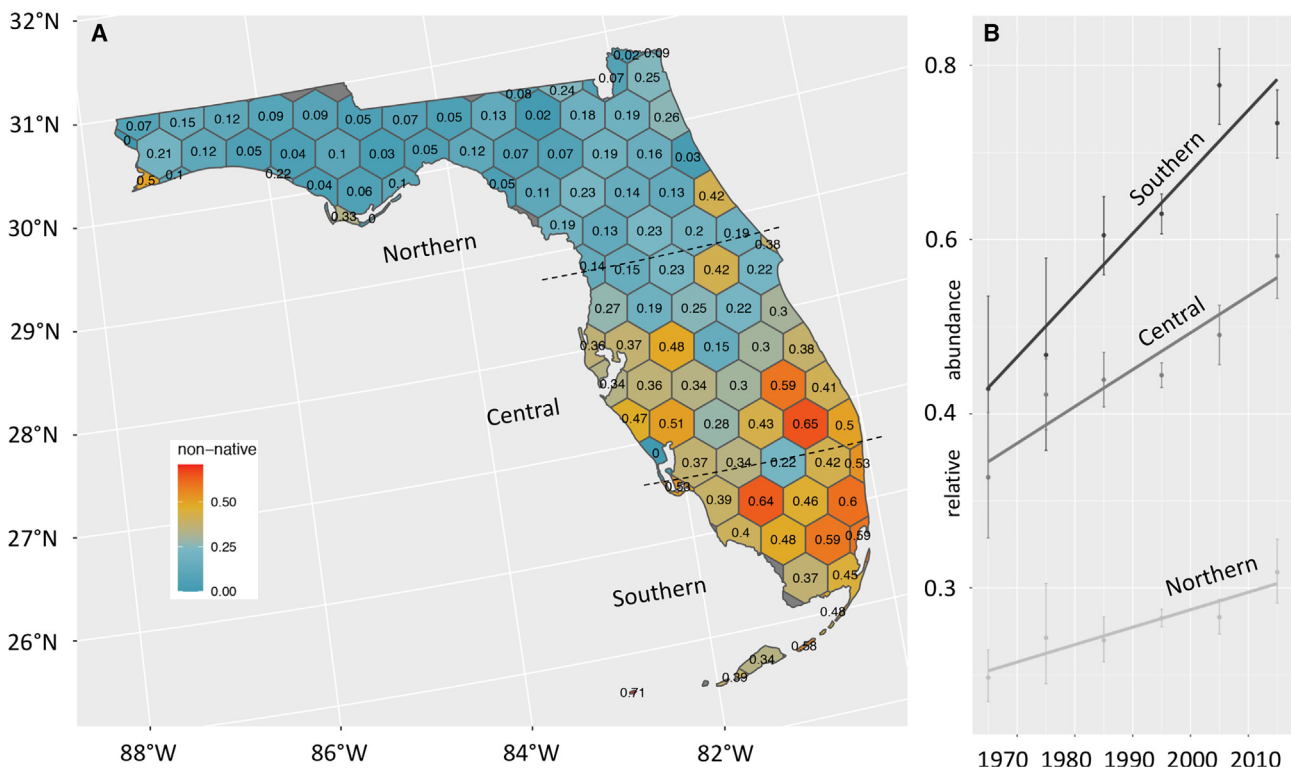
Figure 1. Map of study area showing relative proportion of non-native species spatially and through time

(A) Our sample area covering the state of Florida, USA, separated into 2,000 km² hexagonal bins and divided into three equal latitudinal regions (north, central, and south). Numbers represent the proportion of non-native species in litter ant communities for each bin for all collections from 1965 to 2019.

(B) Beta regressions show increase in proportion of non-native ants collected in samples through time for the three equally divided latitudinal regions of FL. See also Figure S1 and Data S1.

We examined temporal and spatial patterns of native and introduced ant species in Florida (USA) litter communities over a 54-year period by assembling a dataset of 18,990 individual occurrence records from 6,483 collection events (Figures 1A and S1; Data S1). These data include 177 primarily ground- and litter-dwelling ant species and were collated from museum collections as well as historical and contemporary leaf-litter surveys from 1965 to 2019. Collection events were assigned to 2,000 km² hexagonal grid cells and binned by decade (Figure 1A). More than half the species have a limited range within the state; therefore, we divided the study area into three equal latitudinal regions to group species with ranges that correlated with climatic zones. Within each latitudinal band, we estimated the relative abundance of each species, including in years of no detection. This was accomplished by applying a novel statistical approach to deal with biases in the representation of material in museum collections.[15] In ants, traits such as polygyny (multiple queens in a single colony), extreme polydomy (e.g., the tendency to form supercolonies), omnivory, nest opportunism, and worker polymorphism have all been implicated in invasion success.[16–19] We therefore also tested whether these biological traits varied between introduced and native species, or whether functional or phylogenetic similarity predicted winners or losers in this system.

Non-native ants have been common in Florida's ecosystems since the 1960s, and their prevalence has varied with latitude and through time (Figure 1). Out of 63 introduced ant species known to be established in Florida, 52 are ground/litter dwelling (e.g., non-arboreal), and we recorded 51 in our samples.[16] Non-native ants represented 30% of the 177 ground-dwelling species detected in our surveys across the entire state (Figures S2 and S3A). The proportion of non-native species in collection events increased over several decades, from 1965 to 2019. However, the rates of increase varied geographically (Figure 1B), with non-natives increasing from 43% to 73% in southern Florida (beta regression, $p < 0.0001$, pseudo $R^2 = 0.90$), 33% to 58% in central Florida (beta regression, $p < 0.0001$, pseudo $R^2 = 0.90$), and 9% to 22% in northern Florida (beta regression, $p < 0.0001$, pseudo $R^2 = 0.88$) (Figure 1B). Although non-native ants are most prevalent in the most populated counties of Florida (based on the 2020 census; pseudo $R^2 = 0.2047$, $p < 0.0001$), rates of increases in non-natives did not correlate with increases in county populations through time ($R^2 = 0.014$, $p = 0.35$). This suggests that anthropogenic land use and habitat disturbance are not the sole reasons for the increase of non-native ants in natural areas of Florida.

A pattern of non-native winners and native losers in Florida's litter-dwelling ant communities emerges when comparing relative abundances of all species present in collections over the 54-year period (Figures 2 and 3A). Notably, nine of the ten species with the greatest increase in relative abundance, and the top four biggest winners, were all non-native. In contrast,
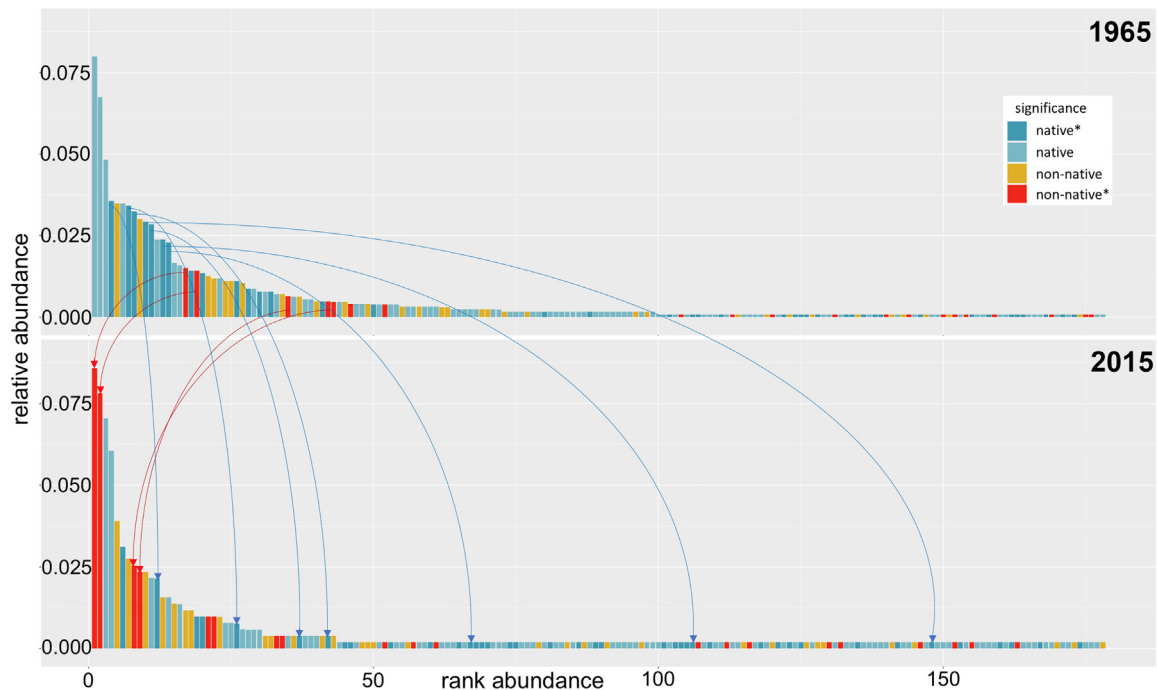
Figure 2. Change in the rank abundance of native and non-native ant species over a 50-year period
Relative abundance ranks calculated using Dirichlet distribution estimates for non-native (red and orange) and native (teal and blue) species that significantly increased (red) or decreased (blue) are denoted with a star. Lines between graphs show species abundance changes between 1965 and 2015 decades.
See also Figures S1–S3 and Data S2 and S5.

nine of the ten biggest losers, including the seven species with the greatest decrease in relative abundance, were all native (Figure 3). Throughout the 54-year period, the relative abundance of individual introduced species could be described with one of four general patterns: increasing through time (15 spp.), decreasing (4 spp.), a short period of increase followed by stability or decline (5 spp.), or remaining at low levels without obvious spread (28 spp.) (Figure S2). We also found a shift in the species abundance distribution of ant species, resulting from the turnover of the 20 most abundant species (Figure 2). This change was driven by increases in abundance of introduced ant species: in 1965 only two of the top ten and four of the top 20 most abundant ants were non-native, while by 2015, seven of the top ten and eleven of the top 20 most abundant ants were introduced (Figure S2). Relative to the taxa that decreased in relative abundance, which were primarily native, the taxa that increased were disproportionately non-native (χ^2, p = 0.01, R^2 = 0.09). Overall, the relative abundance of non-natives increased from 27% to 42% between 1965 and 2019. If this rate of change continues, the relative abundance of non-native species will be greater than that of native species in litter ant communities across the entire state of Florida within 50 years. This pattern supports the finding that widespread species introductions lead to biotic homogenization, even if local species pools are increased (e.g., birds on islands[20]).

The observed temporal changes in relative abundance resulted in increased community dissimilarity in each region (Jaccard dissimilarity; Figure S3A). That is, sampled ant communities are becoming more different relative to the early baseline

collections (p < 0.005, R^2 > 0.93). The rate of change in composition is similar among the three regions and between native and introduced species, when examined separately at near 1% turnover every 20 years (Figure S3A). Despite these changes in relative abundance over this 54-year period, shifts in species composition due to the addition of introduced and loss of native species rarely reduced phylogenetic or functional diversity (Figures S3B and S3C). When controlling for species richness, morphological trait (functional) diversity (Figure S3B) and phylogenetic diversity (Figure S3C) did not increase or decrease through time and had no discernable pattern of change. This maintenance of phylogenetic and functional diversity is likely a consequence of many introduced ants replacing native species of the same genus as the native species, whose abundance is declining. For example, within the three most diverse ant genera with introduced congeners (*Nylanderia*, *Pheidole*, and *Strumigenys*), relative abundance of non-native species increased as native species concurrently decreased (beta regressions for all three genera with R^2 > 0.58 and p < 0.005; Figure S3D). In addition, the retention of phylogenetic diversity may also result from the establishment of genera previously absent from Florida, including *Cardiocondyla* and *Tetramorium*. However, functional redundancy does not necessarily negate the ecological consequences of invasion.[21,22] Notably, three of the seven native species showing the largest decline, *Aphaenogaster carolinensis*, *Pheidole dentata*, and *Solenopsis geminata*, are important seed predators and dispersers. Three others, *Strumigenys louisianae*, *Odontomachus brunneus*, and *Myrmecina americana*, are predatory specialists. For example, *Myrmecina americana*
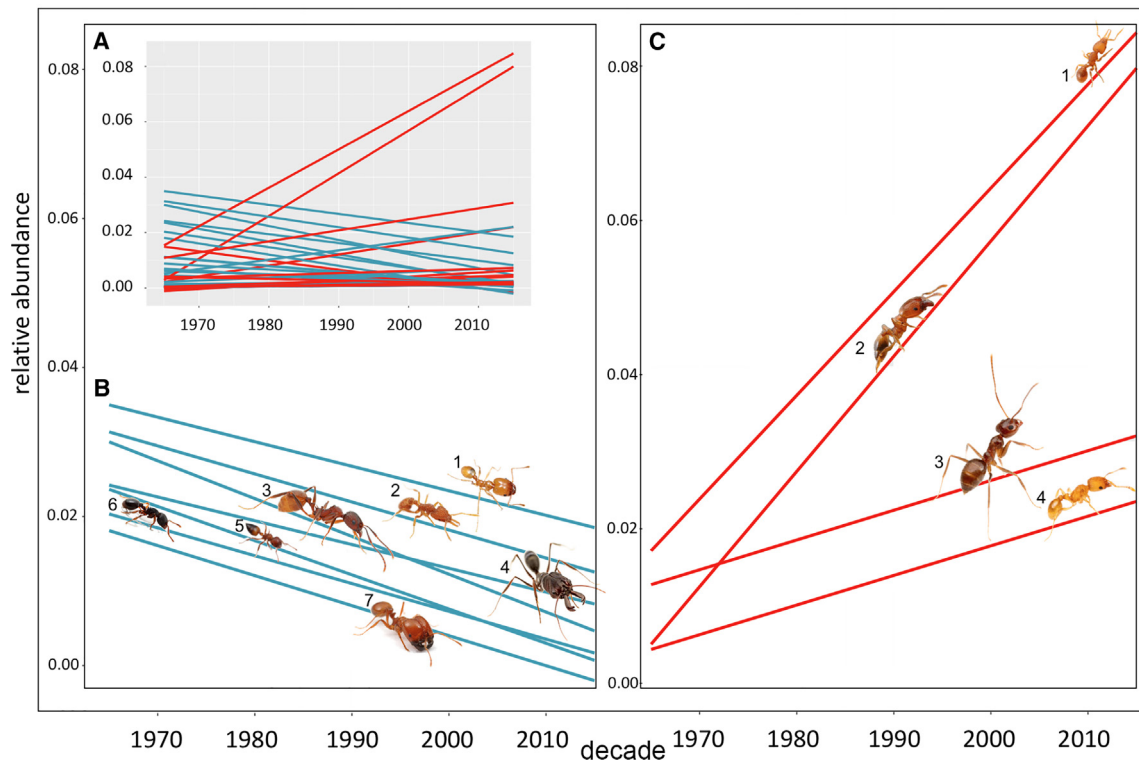
Figure 3. Species with the largest changes in abundance

(A) All 60 species with significant beta-regressions slopes of Dirichlet abundance changes over time for and native (teal) species and non-native (red).

(B) The seven native species with the greatest decreases in relative abundance from 1965 to 2019: (1) *Pheidole dentata*, (2) *Strumigenys louisianae*, (3) *Aphaenogaster carolinensis* (*A. fulva* pictured), (4) *Odontomachus brunneus* (*O. cephalotes* pictured), (5) *Crematogaster minutissima* (*C. missouriensis* pictured), (6) *Myrmecina americana*, and (7) *Solenopsis geminata*.

(C) The four non-native species with the greatest increase in relative abundance from 1965 to 2019: (1) *Strumigenys eggersi*, (2) *Pheidole navigans*, (3) *Nylanderia steinheili* (*N. sp.* pictured), and (4) *Wasmannia auropunctata* (photographs by Alex Wild).

See also Figures S1 and S2 and Data S1.

is a litter dwelling specialist that selectively forages on oribatid mites.[16] In comparison, the non-native winners are all habitat generalists, dietary generalists, or both. One of the biggest winners, the introduced ant *Pheidole navigans*, is also a seed predator; however, it is unknown whether and, if so, how the replacement of multiple species with diverse sizes, bite forces, and seed choices with a single generalist seed predator will impact communities and ant-plant mutualisms.

Habitat loss and disturbance due to urban and agricultural development are the leading proximate factors associated with the decline of native species and the success of many introduced taxa. Although urbanization has increased dramatically in Florida over the past 50 years, quantitative data on the geographic and temporal changes in land use and land cover do not exist at the spatial resolution required for analyses across the time frame of this study. However, by focusing on litter ant communities from primarily natural habitats, we can gain insight into traits that confer competitive advantages. Interestingly, three of five traits previously identified as important predictors of dispersal patterns of invasive ants at global scales[19] were associated with introduced status (polygyny, omnivory, and supercoloniality). Polygyny is often associated with increased colony growth and local population density. We found that

polygyny weakly predicted status (native versus introduced: χ^2, $R^2 = 0.1$, $p = 0.03$) but was not related to changes in relative abundance, from 1965 to 2015 or by 2015 rank (χ^2, $R^2 = 0.01$, $p = 0.48$). We also predicted that dietary generalists would increase in abundance relative to dietary specialists. However, there was no significant relationship between omnivory and either introduced status or changes in relative abundance (Data S5A). Worker polymorphism may increase niche breadth and promote ecological success; however, we found no relationship between polymorphism and native status and/or changes in relative abundance ($p > 0.1$ for both comparisons). As we limited our species to those nesting mainly in litter/ground, it was also no surprise that we did not see differences between introduction status and nesting strata ($p > 0.1$ for both comparisons).

The trait we examined that varied the most between native and non-native species was the degree to which workers from separate nests integrated into other nests, both within and among sites (χ^2, $R^2 = 0.53$, $p = 0.02$) (Data S5; Figure 4A). This difference is stronger when workers that were introduced from nests within 5 m apart were excluded (Figure 4B). Additionally, non-native species always had higher integration scores when compared with their congeneric natives, and the biggest winners also had higher integration scores than losers
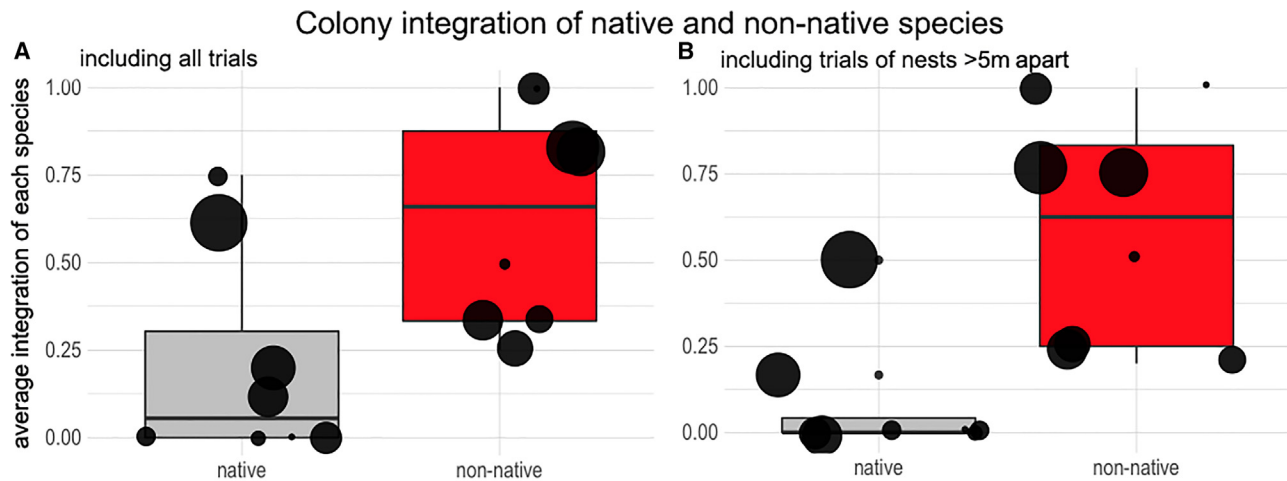
Colony integration of native and non-native species

Figure. 4. Introduced species were more likely to have workers integrate across spatially separate nests without aggression

(A) Integration of conspecific worker introductions from colonies ranging from one to more than 100-km apart are more likely in non-native species (ANOVA, n = 107 trials, R^2 = 0.28, p < 0.035).

(B) The difference between native and non-native species is greater when only including colonies >5 m apart as expected if nearby colonies are multiple nests of the same colony (ANOVA, n = 71 trials, R^2 = 0.36, p < 0.015). Species' point sizes are weighted by the number of integration trials observed for each species and include eight native and eight non-native species (species listed in Data S5B).

(χ^2, R^2= 0.44, p < 0.02), for species increasing or decreasing by 10 or more rank positions between 1965 and 2019. The stronger difference in native versus non-native species integration at large spatial distances arises because there are some native species that locally occupy multiple nests (polydomous) but do not form larger supercolonies made up of nests greater than a few meters apart. For example, the native species *Crematogaster minutissima* has multiple-queen colonies that disperse by budding, so a single colony essentially occupies a larger local area with multiple nests. In contrast, the native ant *Aphaenogaster carolinensis* has a single founding queen that occupies a single nest. Subsequently, as with most native species, *A. carolinensis* workers always are aggressive toward other nests of the same species, even over small spatial scales. *Nylanderia steinheili* was the only non-native species we tested whose workers did not integrate between nests in most trials; however, this still occurred more often than with any native species. Having extreme polydomy, or supercoloniality, is thought to contribute to invasion success by allowing species to obtain higher densities and subsequently monopolize resources and displace native species through competition and predation.[17,20] Our study was limited in terms of the number of species attracted to artificial nests, allowing us to collect enough live colonies to perform integration trials within and between sites. Future efforts to increase the diversity of species, including such trials, will reveal the generality of the result in this and other ecosystems.

The establishment and spread of non-native species in Florida may be an exception due to the state's hot and humid climate. More temperate states have not experienced the same levels of invasion and harbor much fewer non-native species. Many of Florida's non-native fauna originate from tropical and subtropical regions globally, with the most successful non-natives being Neotropical species. Although 42% of Florida's introduced species have an Indomalayan, Australasian, or Afrotropical origin,

these species (n = 24) represented only 18% of our non-native species records (Data S4). The introduced species with the highest relative abundance were from the Neotropics (70% of species occurrence records). Many of these Neotropical species have native ranges that include the flood plains of northern Argentina and southern Brazil, suggesting that natural disturbances in their native range may select for traits (e.g., high competitive ability, frequent nest relocation, polygyny, habitat generality) that promote their success in new environments that they invade.[17–19,23] Consequently, several South American ant species that have evolved defenses against one another in their native range are now interacting with each other in the novel environment of Florida.[23,24] Although native ants do not appear to provide resistance to these invaders, interactions among introduced species may help reduce their abundance or rate of spread. Investigating why some regions appear to be net exporters of introduced species, and how these species interact in areas they are introduced, is a priority for future research.

There are many examples of single introduced species causing reductions in native species in highly disturbed areas or in vulnerable systems like islands. However, our long-term data reveal that even in relatively intact mainland habitats, introduced species can accumulate with community-wide consequences over large spatial scales. Surprisingly, almost none of the introduced ant species we detected are traditionally considered "invasive," based on known ecological or economic impacts.[17] Additional research is needed to understand how relatively inconspicuous introduced species may increase in relative abundance and modify the communities they invade over long periods. In addition, litter-dwelling arthropods are typically surveyed in relatively undisturbed forest and scrub habitats, as these areas are the primary source of "quality" leaf litter where ant and other arthropod diversity is highest. As a result, invasive species that are most common in highly disturbed habitats had extremely sparse records of occurrence in our

collections. For example, the red imported fire ant, *Solenopsis invicta*, is rarely collected using litter extraction methods because they do not nest in litter and are uncommon in relatively undisturbed forested areas where litter accumulates.[25]

Despite the widespread re-organization of Florida litter ants that we documented, and the dominance of fire ants and other invasive ant species in disturbed habitats,[26] 22 native species increased in relative abundance over the last 50 years. Moreover, we did not detect a regional or state-wide extinction of any native ant species of Florida. However, extinctions are 5–10 times more likely to be caused by interactions with non-native species than with other native species.[27] A lag in response of native communities to invasion may therefore be analogous to the "extinction debt" following habitat loss—a delay in population responses due to changes in species interactions and demographic or stochastic processes.[28] The prominent 54-year shift in the numerically dominant ant species from native to introduced (Figure 1) may precede the eventual extinction of some native species that have started to become relatively rare. Areas with unique floras and faunas, such as Florida, may be particularly prone to the establishment of communities of introduced species. Moreover, food web and mutualistic network breakdown may be even more severe as ant communities and the functional roles of their members homogenize/disassemble over large areas.[29]

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2023.03.044.

AUTHOR CONTRIBUTIONS

D.B.B., C.S.M., and A.V.S. conceived of the study; D.B.B. and M.D. collected the data; N.J.G., D.B.B., and M.P.N. performed statistical analyses; and all authors contributed to study design and manuscript preparation.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Florida ant occurrence dataset	This paper	https://doi.org/10.5061/dryad.z8w9ghxgw
Climate data	Worldclim	https://www.worldclim.org/data/bioclim.html
Population growth data of Florida counties	US Census Data	https://www.census.gov/data.html
Phylogenetic trees and alignment	GenBank, this paper, Booher et al.[30]	https://github.com/mpnelsen/Booher_et_al_2023_FL_Ants/tree/main/data
GenBank accession numbers for new sequences	This paper	Myrmica pinetorum (OQ325314); Cremagtogaster ashmeadi (OQ325315); Pheidole dentigula (OQ325316)
Phylogenetic partitions	This paper	https://github.com/mpnelsen/Booher_et_al_2023_FL_Ants/blob/main/data/spreadsheets_text_files/Booher_et_al_2023_Partitions_for_Phylogeny.pdf
Software and algorithms		
Code for relative abundance analyses	Gotelli et al.,[15] this paper	https://github.com/antmuseum/Six-decades-of-museum-collections-reveal-disruption-of-native-ant-assemblages-by-introduced-species
Code for dirichlet statistics	Gotelli et al.[15]	https://github.com/GotelliLab/FAMA
Code for additional statistics	R statistical program, Gotelli et al.,[15] this paper	https://github.com/antmuseum/Six-decades-of-museum-collections-reveal-disruption-of-native-ant-assemblages-by-introduced-species/tree/main
Code for phylogenetic inference	This paper	https://github.com/mpnelsen/Booher_et_al_2023_FL_Ants/tree/main/scripts
Other		
Habitant, artificial ant nests	Doug Booher	manufactured by Lou Kregel and Doug Booher and can be obtained upon request dbooher@antmusuem.com

RESOURCE AVAILABILITY

Lead Contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Doug Booher (dbooher@antmuseum.com).

Materials availability
Specimens collected by or loaned to the authors for this study were deposited in the following natural history museums, UGAMNH, ABS, FMNH.

Data and code availability
Data generated (ant occurrence records) and code used for statistical analyses is available through DOI links on the key resources table or as supplemental information. Gene sequence data generated for the Florida ant phylogeny have been deposited at GenBank and accession numbers are listed on the key resources table. All original code is available on github with links on the key resources table. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study Area
The state of Florida, USA, comprises an area of 170,300 km^2 with a primarily subtropical climate (see supplemental information for more information). Dominant habitat types include cypress, freshwater marl prairie, freshwater slough, coastal lowlands, mangrove, pinelands, and hardwood hammock.[31] Prior to the 1900's only four cities had populations greater than 10,000 (the coastal cities of Jacksonville, Pensacola, Key West, and Tampa.[31] However, agricultural and extraction industries (timber/mining) led to large

land-cover changes in the early 20th century. The population of Florida increased from fewer than three million in 1950 to more than to 21 million residents in 2019,[32] and more than 18% of land in Florida was converted to urban land use by 2010.[33]

Florida has a diverse and well-described ant community.[16] Its mild climate also makes it suitable for many non-native species; 25% (64 species) of Florida's 254 documented ant species are introduced.[19,34,35] Most research on introduced ants in Florida has focused on a few species, particularly the conspicuous red imported fire ant and its impacts on resident arthropods and wildlife.[17,36] With the colonization and spread of more than 50 non-native species over the last 100 years, Florida is an ideal arena to examine both the persistence of introduced species and the resilience of native ant communities as non-native species arrive and spread. Although significant land use changes (timber/mining/agriculture) had already taken place over the first half of the 20th century prior to standard collection methods,[31] and many introduction species were already established by 1950,[34] most had either not yet become established or are continuing to spread (Data S4).

METHOD DETAILS

Detailed methods are provided in the supplemental information and include the following:

Data Collection

We examined changes to Florida's leaf litter ant communities by collating data from museum collections, and from historical and contemporary leaf litter surveys. We focused on collections since the 1960s when standardized sampling of litter-dwelling ant communities became common practice. A total of 19,060 ant specimens representing 6483 collection events were collected over a 54-year period spanning from 1965 to 2019 (Data S1). Collection events were defined in one of two ways, either by a collection code given by a collector, or artificially constructed as a set of species collected by the same collector in the same location in a single day. We focused on collection events that used Berlese or other litter extraction sampling methods (e.g. Winkler sifters) as these methods focus on a particular community of ants – those that live in at least partially canopied areas where litter from trees, shrubs, or herbaceous plants accumulates. We also chose to focus on litter extraction collections as they are comparable in terms of collection area —they are limited by litter volume, typically representing a community sample of ants at the scale of approximately one square meter. However, we also included collection events that did not report method of collection but were likely collected by litter extraction methods as they contained mostly ground- and litter-dwelling species.

Data were gathered from four sources representing historic to modern collections spanning the years 1965-2019: 1. The Archbold Biological Station where Mark Deyrup's collections and hand-written collection notes were deposited, 2. Doug Booher contemporary collections for this study that focused on previously sampled sites decades earlier, 3. AntWeb.org, and 4. The Field Museum of Natural History bulk collections which included Walter Suter's collections (key resources table). Many digitized geographic locations often associated with the presence of a collection in a county and correlated to county centers. For historic collections, to correct GPS locations, we visually validated or changed collection coordinates to represent collection data and not county centers when collection notes provided more accurate location descriptions. Many geographic locations from the same sample were assigned different GPS coordinates by different individuals that had independently estimated and reported coordinate locations. These coordinates were cross-referenced with specimens databased in AntWeb, and individually corrected with descriptive locations from Deyrup's handwritten collection notes. These different data sources often had incongruent associated habitats, locations, or determinations pertaining to the same collection event and if data were unclearly associated with a particular collection date those data were excluded. Ants taken together by collector and day were disassociated from habitat data if only some ants collected that day have that data and were not clearly linked to an exact collection event such as the collector's collection code. For 2019 collections, sites were chosen to cover the cross section of latitudes of Florida in large undisturbed preserved lands having diverse natural habitat types such as tropical hammocks, riparian scrub, and flatwoods. Five litter-samples were taken in four sites at ten areas identified as having large collection efforts informed from historic collections, and were specifically chosen to overlap with 1965-1974 collections. Doug Booher used identical collection methods as Deyrup, sampling litter for arthropods in the same size areas (1m² litter samples), using Berlese funnel extraction methods, and sampling in the least human-disturbed habitats. All ants were collected, sorted, and identified by Doug Booher.

Species were occasionally reported from the same collection event in two or more databases, and in some cases, species belonging to a collection event were reported in one database but not another. All species collected on a single day were cross-referenced by their associated data to either remove duplicates from a specific collection event or combined with other species known from the same collection event. Primarily arboreal species were removed from the dataset, as were those that could not be identified to species (1520 records removed; Data S4). Some ant species of Florida have multiple valid names associated with the same species; this was mainly a problem for non-native ants consistently identified as the same species by different taxonomists. In these cases, ants were combined under a single name *e.g. Pheidole moerens = Pheidole navigans.* Species of the *Solenopsis* diplorhoptrum clade were removed as confirmations of identifications were highly variable, species concepts are not well established, and because we could not assign native or non-native status to many species (3069 records removed). Ant identification was confirmed by Doug Booher and Mark Deyrup. Records for specimens that could not be confirmed or were not reported to inhabit Florida[16] were discarded.

Spatial and temporal bins

Collection events were assigned to 2,000 km^2 hexagonal grid cells and binned by decade (Figure 1A). More than half the species have a limited range within the state; therefore, we divided the study area into three equal latitudinal regions to group species with ranges that correlated with climatic zones.[37] Within each latitudinal band, we estimated the relative abundance of each species, including in years of no detection. This was accomplished by applying a novel statistical approach to deal with biases in the representation of material in museum collections[15] (see *calibrating museum records with field surveys*). Before analyzing the entire data set, we analyzed a curated subset to confirm that estimates of species relative abundance from historical museum records and from contemporaneous field observations collected from the same place and time were highly correlated with one another. This correlation holds for many other taxa including invertebrates, vertebrates, and plants, which validates the use of historical museum records for places and times when no ecological field data collections are available.[15]

Of the 177 litter dwelling species occurring in our dataset of Florida collections, 47% occupy all three regions (84 species), while about 28% inhabit two regions (48 species), and 26% inhabit only one (46 species). Southern Florida is mainly tropical to subtropical and incorporates USDA plant hardiness zones 10-11 (latitudinal band 24.55°- 26.69°); central Florida is subtropical incorporating most of zone 9 (latitudinal band 26.70°- 28.83°); and northern Florida is subtropical/temperate incorporating zone 8 (latitudinal band 28.84° - 30.98°). Unless noted for a specific analysis, we used these three regional divisions and decade time bins divisions for statistical analyses. We chose to examine decades over smaller time period bins as smaller time bins had problems with highly variable collection efforts, often with empty time bins. The major collector over the decades spanning from 1980-2005 (Mark Deyrup) collected samples in more disturbed habitats from 1990-2000 than he did from 1980, but this relationship was not significant when decade bins started mid-decade. For this reason, we chose decade bins starting at mid-decades *e.g.* 1985-1995. Because the start of collections with reliable co-occurrences of litter collections began in 1965, and we used mid-decade binning, the 2015 decade consists of collections only for the years between 2015-2019 and were almost entirely collected by author Doug Booher in 2019.

QUANTIFICATION AND STATISTICAL ANALYSIS

Proportions of non-native ants across Florida

We investigated overall proportions of native and non-native ants at the geographic scale of 2000 km^2. This geographic scale was the finest scale having full coverage over all land areas of Florida and was used to visualize patterns of mean proportions of non-native ants over all collection events within each 2000 km^2 geographic bin (Figure 1A).

Calibrating museum records with field surveys

Before using museum records to document spatial and temporal trends in species relative abundance, we needed to first verify that relative abundances from museum records quantitatively reflected relative abundance of species estimated from standardized field surveys. To do this, we assembled an extensive set of 15,281 specimen field records including all counties in FL, collected from 1955-2019, and then curated a comparable, independent set of 11,185 museum collection records covering the same time and space. These two data sets contained 157 shared species, with 5 unique species recorded only from the field survey and 28 unique species recorded only from the museum records. For all 189 species, there was a strong correlation between the number of field and museum records for each species ($r^2 = 0.62$, $p<10^{-4}$). After transformation with the Dirichlet distribution, the correlation was even stronger for the estimates of relative abundance for each species from field and museum records ($r^2 = 0.70$, $p <10^{-4}$, with $\log_{10}$ transformation of relative abundances). Despite that 28 species known from museum records were never detected in the field surveys, species that are common (or rare) in the field also are common (or rare) in museum collections. These patterns are not unique to Florida ants and were detected for over a dozen other comparisons of museum records and field abundances for mammals, herps, fish, butterflies, bees, trees, and understory plants.[15] These correlations make it reasonable to estimate relative abundance of assemblages from museum records of previous decades for which standardized field survey data are not available.

Estimating relative abundance

To estimate the relative abundance of native versus non-native ants in different times and places, we used the beta distribution, rather than the standard frequentist estimator $p = n/N$ (where n is the number of invasive ants and N is the total number of ants in the collection). The beta distribution deals sensibly with zeroes and generates asymmetric confidence intervals that reflect uncertainty in the sampling. For estimating the relative abundance of each species in the collection, we used the Dirichlet distribution, which is the multinomial analog of the beta distribution.[18] For both the beta and the Dirichlet distributions, we assume that any species present in the Florida ant collections from 1965 to the present, could occur in any sample. Technically, this method assumes there is no colonization or extinction, and the relative abundance of every species is greater than zero at all times. However, if a species is not present in a sample, the estimated relative abundance is small at that time. The larger the total sample size of the collection, the closer the relative abundance estimate is to zero, and the smaller its confidence bound. This makes it ideal for realistically estimating long-term trends of species and detecting "winners" and "losers" for data sets in which absences may reflect detection errors or true absences.

To estimate these trends, we used beta regression models, which assume the response variable (relative abundance) is bounded between 0 and 1. All analyses, were conducted in R version 3.6.2[38] with the packages gtools[39] and betareg[40] for fitting of the Dirichlet distributions and beta regression models, respectively.

Population growth and temporal changes in non-native ant collections

Population data and county shapefiles were downloaded from the United States Census Bureau (USCB, accessed Oct. 25, 2022; key resources table). Coordinates from collection events were intersected with the USCB county shapefile and the proportion of non-native ants in collection events were binned and averaged by decade. Slopes from linear regressions were obtained in R for population growth for each county for the years 1950-2020. Slopes from beta-regressions were obtained using the betareg package in R for the change in non-native proportions for each county for the years 1954-2019.[40] A beta regression analysis was performed using 2020 populations of each county to predict average proportions on non-native ants from collection events from 1954-2019. A linear regression analysis was performed on slope values from population growth predicting rates of changes (slopes) in non-native ants by county.

Functional diversity

We obtained measures for 177 species from images available at AntWeb.org[35] (Data S2). We chose four morphological characteristics correlating with functional traits: eye size, mandible length, body size, and head width. Eye size predicts dietary role and foraging strata. Species with larger eyes tend to be omnivorous vs. fungus farmers and predators. Litter dwelling ants have intermediate eye sizes, subterranean smallest, and surface foragers had largest.[41] Eye size correlates with food particle size with omnivores and predators often smaller than fungivores and granivores, though there is a phylogenetic signal among ant subfamilies.[41] Mandible length relates to diet with ants having longer mandibles tending to be predators.[42,43] Weber's length, a measurement of body size[33] correlates to the size of area an ant may pass through within the leaf litter or soil.[44] Head width also correlates to environmental structural space use and to musculature and diet.[45]

We calculated Dirichlet relative abundance estimates by decade for each of the three divisions of Florida (northern, central, and southern) using decade time bins that represented geographic coverage of collection events. Using Dirichlet abundance estimates as weights, we sampled five species 1000 time with replacement. We chose five species as that number represents a realistic number of species found in litter samples and to control for species richness as functional diversity (FD) is highly correlated with species richness. For each draw we calculated functional diversity and report the mean value of the total number of draws per decade. We used a distance matrix of traits to create a tree to calculate FD of each sampled community. We calculated a gower distance matrix of all species using the 'vegdist' function in the R vegan package R[46] and used this matrix to create an 'average' tree using the hierarchical cluster function 'hclust.'[38] Since all traits were morphometric continuous measures, we calculated FD using Faith's phylogenetic diversity metric in the R package 'picante.'[47] We performed linear regression analyses of the mean function diversity per decade over all decades for each of the three Florida regions (Figure S3B).

Phylogenetic diversity

Matrix assembly

We inferred a phylogeny of the ants of Florida by first selecting NCBI accessions used in a previous study[48] for COI and 11 other nuclear / mitochondrial loci from species known to occur within the broader study area (Florida and Georgia; Data S3). Two COI sequences were generated for common taxa lacking sequence data in NCBI (*Crematogaster ashmeadi*, *Pheidole dentigula*) using the HCO/LCO primers.[49] Sequences were downloaded, parsed and compared with BLAST against a local database[48] to verify the identity of the locus and ensure sequences were not from contaminants or distantly related taxa. Sequences were then aligned using MAFFT[48,50,51] for non-protein-coding loci, or MACSE[52] for protein-coding loci. Profile aligning in MAFFT was then used to align tribe-level alignments for individual loci within subfamilies to one another, and subfamily alignments were then similarly aligned to one another. Alignments were subsequently trimmed using GBLOCKS,[53] concatenated, and assigned to substitution model partitions using the BIC in PartitionFinder 1.1.1.[54] A greedy analysis was run and the substitution models available in RAxML[55,56] were evaluated.

Phylogenetic inference

A maximum likelihood analysis was then run in RAxML using the favored partitioning scheme and GTR+G substitution model for all partitions (key resources table), and 1000 rapid bootstrap replicates[57] performed in the CIPRES Science Gateway.[58] Preliminary analyses revealed a non-monophyletic *Crematogaster*, but alternate relationships lacked strong support. Similarly, two *Tetramorium* species that do not occur in Florida grouped together (*immigrans* and *tsushimae*), but separate from other *Tetramorium* species. As both findings were inconsistent with published phylogenies,[59] we removed the two *Tetramorium* species and constrained monophyly of *Crematogaster*.

Timescaling and grafting

Nodes shared with the phylogeny of Nelson et al.[48] were then identified, and their ages fixed, while ages of all nodes were estimated using the penalized likelihood algorithm in treePL.[60] We used the prime function to identify optimal conditions, and then used a cross-validation procedure to evaluate smoothing values between 1,000 and 1e-6, with a final analysis performed under the optimal smoothing estimate. We then grafted a recent timescaled RADSeq phylogeny of all *Strumigenys* species in the study area[30] onto our broader ant phylogeny. This was achieved by removing the limited *Strumigenys* representatives the family-wide tree and grafting the timescaled RADSeq *Strumigenys* phylogeny onto our ant phylogeny while preserving *Strumigenys* node heights from the RADSeq phylogeny. Associated scripts and files are included on GitHub (key resources table).

Phylogenetic diversity

We calculated Dirichlet relative abundance estimates for each of the three divisions of FL (northern, central, and southern) by decade and drew 1000 random draws of five species with replacement weighted by Dirichlet relative abundance estimates. We chose five species as it represents a realistic number of species found in litter samples and because phylogenetic diversity is highly correlated with species richness, sampling the same number in each draw controlled for species richness. After sampling, species without available phylogenetic data were replaced in each sample with congeneric close relatives (Data S4). Our results were robust to variations in substituted congeneric species as is expected given that phylogenetic diversity metrics have been shown in other ant studies to be insensitive to within species relationships for calculating PD across genera belonging to many subfamilies.[61] For each draw we calculated phylogenetic diversity using the 'pd' function in the R package picante[47] and report the mean value of the total number of draws per decade. The 'pd' function calculates Faith's phylogenetic diversity, the sum of the total phylogenetic branch length of each sampled draw.[62] We performed linear regression analyses of the mean phylogenetic diversity per decade over all decades for each of the three Florida regions (Figure S3C).

Species-level traits

Available species-level natural history data in the form of dietary preference and nesting niches were obtained from Deyrup.[16] Dietary preference was categorized into three possible designations: omnivores, predators, and herbivores while nesting niches were categorized as either ground nesting, arboreal nesting, leaf litter nesting, or a combination of the three. Traits that represent social complexity of ants such as the presence or absence of worker caste polymorphism and multiple queens within a colony (polygyny) were also extracted from Deyrup.[16] However, polygyny data was relatively sparse, as such other literature sources or expert assessments were used to supplement data collection. All data and sources are made available in the supplemental information.

Supercoloniality

The tendency for species to form supercolonies was determined experimentally by introducing conspecific workers from separate nests at different distances from each other. To obtain enough workers for these experiments, we designed and produced 1000 artificial ant nests made of high-fire clay pottery (key resources table). Nest designs mimicked natural cavities found in sticks and nuts, which are common nesting sites for many ant species. Artificial nests were placed in 5x10m arrays with each nest placed one meter apart and held in place with metal staked flags. Nests were placed in June 2018 and retrieved between May 27 and June 25, 2019. Each nest was collected into individual ziplock bags labelled with the site and array position. Collected nests were processed within 24hrs. Each was examined for colony occupancy and colonies were removed from occupied nests and placed into a sealed, breathable container with 1cm of moistened vermiculite with a piece of bark or leaf to provide a new nesting site. Ant colonies were provided water and fed a mix of sugar water, crickets, or spring tails twice a week. Colonies were kept at temperatures between 25-28°C.

We assessed intraspecific behaviors of nest integration, avoidance, or aggression between pairs of colonies by introducing workers from one colony to the other (species listed in Data S5B). We aimed to introduce workers from one colony to another across a range of distances from a few meters apart to more than 50km apart or more to avoid polydomous nests of the same colony. Ant-interaction behaviors were recorded at the time of introduction and again after a 24-hr period. Ants being introduced into another colony were marked with enamel paint and (Testor, 2011 the Testor Corporation, Rockford, IL 61104) diluted in a one-part paint thinner to two parts paint. To paint ants, we used a single hair from a brush to apply a dot to the gaster of the ant. Ants being marked were cooled to 5°C to slow them and allow marking. We first confirmed that handled and marked ants would be accepted back into their parent colony to determine behaviors recorded were not due to the paint itself. All ant species examined allowed painted individuals from their own colony back into their natal nest. However, marked ants were often groomed heavily by their nestmates and often little to no paint was left after 24 hrs. The number of ants introduced into another colony varied from 1-10. Never more than 50% of workers from a colony are marked for trial and number of introduced workers will be equal to or less than the number of workers in foreign colony. The number and paint color were recorded for each trial. At the time of introduction of workers to another colony, a second piece of bark or a small leaf part was placed at the opposite end of the nesting container to provide a secondary unused nesting site for introduced ants so integration was not unduly forced. Many trial introductions were reciprocal, with workers from each colony being introduced to the other. The summary results of these trials were treated as a single trial as they were not independent.

Nest integration was scored if no aggression was noted and the majority of introduced workers could be found within the nesting area of their host colony within the 24hr period. Avoidance was scored when introduced ants and their host workers avoided contact or after non-attacking contact, introduced workers moved away from host workers and nest. Avoidance was also scored if introduced ants created their own nesting site (either in the vermiculite substrate or under the secondary leaf/bark nesting site). Attacks were scored by observed behavior or by examining dead introduced workers for damage such as missing body parts or tears in the exoskeleton. After 24 hrs some scores were in conflict such as some introduced workers might have been killed while others integrate into the nest without obvious aggression. If conflict arose at any point that was scored as avoidance or attack, the summarized score was always the more extreme aggressive behavior.

We used chi-square tests to evaluate traits as predictors of native and non-native status or of winners and losers. Winners and losers were categorized as those having significant positive or negative beta-regressions of Dirichlet abundance estimates (binned collections by decade; Data S5A). Integration data was limited to those taxa that colonized our artificial nests in large enough number to perform trials within and among sites, and only 7 of these species (3 native and 4 non-native) has significant beta-regressions. We therefore also evaluated the effect of supercoloniality for species that increased or decreased by at least 10 rank positions (5 native and 5 non-native species).