

STUDIES ON THE INVASION BIOLOGY OF SOCIAL INSECTS

A Dissertation

by

ALEXANDER JOSEPH BLUMENFELD

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Chair of Committee,	Edward L. Vargo
Committee Members,	Robert T. Puckett
	Micky D. Eubanks
	Thomas M. Chappell
Head of Department,	Phillip E. Kaufman

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## ABSTRACT

Social insects (*e.g.*, ants, termites) are among the most prolific group of invasive organisms worldwide. The rapid expansion of both their ecological (*i.e.*, habitats) and global (*i.e.*, countries/continents) distributions has likely been facilitated by the world's most successful invader – humankind. Therefore, for my PhD research, I performed several investigations into the invasions of social insects to gain further insight into how their invasions have been and are currently being shaped in the Anthropocene.

For my first study, I compiled a comprehensive dataset of termite interceptions at US ports of entry spanning the years 1923 to 2017 to elucidate broad patterns in the spread of invasive termites to the US. My main findings included a strong regional bias in both the origin (*i.e.*, country/continent) and destination (*i.e.*, port of entry/US region) of interceptions and convincing evidence that invasive termites utilize bridgeheads (*i.e.*, previously invaded locations) to expand their global range. In my next two studies, I reconstructed the invasion histories of two prominent invasive termites – *Coptotermes formosanus* (native to East Asia) and *Reticulitermes flavipes* (native to North America). By leveraging existing sample sets previously collected from a large geographic range (*i.e.*, both native and invasive ranges), robust genetic datasets, and approximate Bayesian computation, I inferred a complex invasion history for both species, with multiple invasions from their respective native ranges occurring in conjunction with bridgehead invasions (*i.e.*, invasions originating from a non-native locality). For my final study, I examined *Tapinoma sessile*'s (odorous house ant) invasion of the urban environment (*i.e.*,

cities) from its native natural environments (*e.g.*, forests) across the US. By integrating genetic, chemical, and behavioral data, I discovered strong differentiation between urban and natural populations of the ant in each locality, suggesting cities may be restricting gene flow between habitats and exerting intense selection pressure. Overall, the findings from each of my studies highlight humankind's powerful and ever-growing influence on the ecological and global distribution of species.

## DEDICATION

To my wife, Yee, and my boy, Tiger.

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### **Contributors**

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## TABLE OF CONTENTS

	Page
ABSTRACT .....	ii
DEDICATION .....	iv
ACKNOWLEDGEMENTS .....	v
CONTRIBUTORS AND FUNDING SOURCES.....	vi
TABLE OF CONTENTS .....	viii
LIST OF FIGURES.....	xi
LIST OF TABLES .....	xv
1 INTRODUCTION.....	1
1.1 General invasion biology .....	1
1.2 Social insect invaders .....	8
1.3 Studies on the invasion biology of termites & ants.....	13
2 GEOGRAPHY, OPPORTUNITY AND BRIDGEHEADS FACILITATE TERMITE INVASIONS TO THE UNITED STATES .....	17
2.1 Introduction .....	17
2.2 Methods.....	20
2.2.1 Data acquisition, standardization and filtration.....	20
2.2.2 Sources of termites .....	21
2.2.3 Propagule pressure .....	22
2.2.4 Bridgehead interceptions.....	23
2.3 Results .....	24
2.3.1 Sources & destinations of termites.....	26
2.3.2 Propagule pressure & bridgehead interceptions.....	29
2.4 Discussion .....	32
2.5 Conclusion.....	36
3 BRIDGEHEAD EFFECT AND MULTIPLE INTRODUCTIONS SHAPE THE GLOBAL INVASION HISTORY OF <i>COPTOTERMES FORMOSANUS</i> .....	38



3.1	Introduction .....	38
3.2	Methods .....	42
3.2.1	Sample collection and molecular methods .....	42
3.2.2	Raw read quality filtering and processing .....	43
3.2.3	Genetic diversity and population structure .....	44
3.2.4	Phylogenetic analysis .....	46
3.2.5	Invasion history .....	46
3.2.6	Exploring changes in population sizes .....	48
3.3	Results .....	49
3.3.1	Population structure .....	49
3.3.2	Phylogenetic analysis .....	54
3.3.3	Invasion history .....	55
3.3.4	Demographic history .....	56
3.4	Discussion .....	57
4	EXTENSIVE HUMAN-MEDIATED JUMP DISPERSAL WITHIN AND ACROSS THE NATIVE AND INTRODUCED RANGES OF THE INVASIVE TERMITE <i>RETICULITERMES FLAVIPES</i> .....	66
4.1	Introduction .....	66
4.2	Methods .....	72
4.2.1	Population sampling and sequencing .....	72
4.2.2	Population structure and phylogenetic relationship .....	74
4.2.3	Assessing invasion history .....	75
4.3	Results .....	77
4.3.1	Population structure .....	77
4.3.2	Phylogenetic relationship .....	82
4.3.3	Invasion history .....	84
4.4	Discussion .....	87
4.5	Conclusion .....	96
5	CONSISTENT SIGNATURES OF URBAN ADAPTATION IN A NATIVE, URBAN INVADER ANT <i>TAPINOMA SESSILE</i> .....	98
5.1	Introduction .....	98
5.2	Methods .....	103
5.2.1	Study sites and sampling .....	103
5.2.2	Genetic analyses .....	104
5.2.3	Population and colony structure analyses .....	105
5.2.4	Breeding structure analyses .....	107
5.2.5	Chemical analyses .....	108
5.2.6	Behavioral assays .....	110
5.3	Results .....	111
5.3.1	Population and colony structure analyses .....	111

5.3.2 Breeding structure analyses .....	117
5.3.3 Chemical analyses .....	119
5.3.4 Behavioral analyses .....	121
5.4 Discussion .....	122
5.5 Conclusion.....	130
6 SUMMARY .....	132
6.1 Overview .....	132
6.2 Future research directions .....	133
REFERENCES .....	137
APPENDIX A. COLLECTION LOCATIONS FOR <i>COPTOTERMES</i> <i>FORMOSANUS</i> .....	181
APPENDIX B. COLLECTION LOCATIONS FOR <i>RETICULITERMES FLAVIPES</i> .....	182

## LIST OF FIGURES

Figure	Page
Figure 1: (a) Time series of non-native termite interceptions from 1925 to 2017 and (b) time series of interceptions for each world region from 1925 to 2017 (interceptions of unknown origin not pictured) .....	24
Figure 2: (a) Origin of non-native termites intercepted at US ports of entry, with the country supplying the most termites in each of the eight world regions highlighted and (b) US interceptions of non-native termites grouped by state, with the five states intercepting the greatest number highlighted .....	27
Figure 3: The proportion of interceptions in each state from the eight world regions defined in the study (shown in the bottom right); the size of each pie chart is proportional to the number of interceptions received.....	28
Figure 4: (a) The negative significant relationship between the distance from the US to the originating country and the overall number of interceptions from that country; (b) positive significant relationship between the number of interceptions from a country with the number of unique termite species it exports; and (c) positive significant relationship between the average value of vegetable imports and the number of interceptions since 1991 .....	29
Figure 5: The relationship between (a) the number of interceptions of a species with its establishment probability, modeled using a binomial GLM; (b) the number of world regions a species inhabits with its interceptions, modeled using a Poisson GLM; and (c) the number of world regions a species inhabits with its secondary interceptions, modeled using a Poisson GLM. For (a), the black dots at 0 represent species which have not become established within the US, while the black dots at 1 represent species established within the US .....	30
Figure 6: The primary and secondary interception percentages of alien termites from this study, with the primary percentage bar to the left of the species name and the secondary percentage bar to the right. The maps to the left of the primary interception bars represent the native world region of each termite, with the countries the termite is native to colored. The pie charts to the right of the secondary interception bars represent the interception makeup of species secondarily intercepted at least 25% of the time. The color code indicates the world region of origin for the secondarily intercepted species. The bolded names represent species already established within the US. The percentage of unknown interceptions ( <i>i.e.</i> , port of origin unknown) is not pictured, so bars may not add up to 100% .....	31

Figure 7: (a) Pie charts of fastSTRUCTURE assignments (for  $K = 5$ ) for each sampling location of *C. formosanus* in its native and introduced range. Pie chart size is proportional to the number of samples. (b) fastSTRUCTURE assignment for each individual sampled for  $K = 5$  and 15. Each color represents a distinct genetic cluster and each vertical bar represents an individual.....51

Figure 8: (a) Principal component analysis (PCA) of all *C. formosanus* individuals. The axes represent the first two principal components (PC). Only the first 20 PC's (out of 324) are shown in the eigenvalue inset graph, with the black bars representing the two plotted PCs. (b) Discriminant analysis of principal components (DAPC) with best support for  $K = 15$  genetic clusters. The axes represent the first two linear discriminants (LD). The first inset graph shows the cumulative variation explained by the PCs, with only the PCs in the black shaded area utilized for the DAPC. The second inset graph depicts the eigenvalues for all linear discriminants, with the black bars representing the two plotted LDs .....52

Figure 9: Co-ancestry matrix between each pair of individuals inferred using fineRADstructure. Each pixel represents the individual co-ancestry coefficient between two individuals. Low co-ancestry coefficient values are depicted by yellow colors, whereas high values are indicated by darker colors.....53

Figure 10: The sampling locations are colored according to their fastSTRUCTURE assignments ( $K = 15$ ). For clarity, bootstrap values are only indicated for major branching events. Samples from Hawaii are highlighted with a round tip.....54

Figure 11: (a) Graphical representation of the most likely invasion history scenario for *C. formosanus* out of Asia tested through ABC RF. Thin dotted lines represent bottleneck events. Time is not to scale, with S indicating sampling time. (b) Estimation of the variation of effective population size through time for three invasive localities using Stairway Plot 2. The solid red line is the estimate of the median effective population size, and the light and dark red shaded areas represent the 95% and 75% confidence intervals, respectively. (c) Nucleotide diversity in the introduced and native range. All native and introduced localities were first analyzed while grouped together, then independently.....57

Figure 12: Sampling map and fastSTRUCTURE assignment for each individual of *R. flavipes* for  $K = 4$ . Each vertical bar represents an individual and each color represents a distinct genetic cluster. Individual fastSTRUCTURE assignments are geographically located in the native and introduced ranges of *R. flavipes* .....78

- Figure 13: Principal component analysis (PCA) of *Reticulitermes flavipes* individuals. Each circle represents an individual. Each individual is colored according to its population of origin; introduced populations are depicted in reddish colors, native populations are colored in grey. Individuals are grouped according the discriminant analysis of principal components (DAPC) with best support for  $K = 4$  genetic clusters.....80
- Figure 14: Coancestry matrix between each pair of individuals inferred using fineRADstructure. Each pixel represents a pair of individuals. Coancestry coefficients between two individuals are designated on a color spectrum. Low values are shown in yellow; higher values are shown in darker colors.....82
- Figure 15: Maximum likelihood phylogenetic tree of *R. flavipes* individuals from RAxML. Individuals are colored according to their fastSTRUCTURE assignments ( $K = 4$ ). Samples from the introduced ranges are highlighted with an emphasized tip. The phylogenetic tree is rooted with 16 *R. virginicus* samples .....83
- Figure 16: Graphical representation of the invasion pathway of *R. flavipes* out of the eastern USA inferred through ABC RF in France, Canada and Chile. The estimated time of introduction and rate of admixture is provided for each introduction event. The large 95% CI, however, calls for caution in interpreting those values .....86
- Figure 17: (a) Sampling locations of *Tapinoma sessile* across the United States. For each locality, nests were sampled in both natural and urban environments, depicted as light-colored (natural) and dark-colored (urban) numbers in the figure. The thickened black lines connecting some nests represent nests that were found to belong to the same colony. Additionally, the stars next to each number denote the social structure of the colony - white and black stars represent monogyne and polygyne colonies, respectively. Note that for each locality, the counting always begins with the first natural nest; also, note that no urban nests were found in Colorado. (b) Bayesian inference tree based on 145 COI sequences of *T. sessile* across the four localities, with one *T. melanocephalum* sequence as an outgroup. (c) PCA based on the microsatellite data of each individual from each nest sampled in the overall data set (dots represent individuals). (d) STRUCTURE analysis based on the microsatellite data across four values of  $K$ , which correspond to the levels of hierarchy present within the overall data set ( $2 = \text{habitat}$ ;  $4 = \text{locality}$ ;  $7 = \text{habitat} \times \text{locality}$ ) ..... 112
- Figure 18: Population structure of *Tapinoma sessile* across the United States. Clustering of nests in (a) Indiana, (b) Arkansas, (c) California and (d) Colorado using a PCA and STRUCTURE on the microsatellite markers. For

each locality, the light-shaded and dark-shaded ellipses in the PCA represent natural nests and urban nests, respectively. Additionally, two runs of STRUCTURE are shown for each locality, which correspond to best  $K$  (*i.e.*, genetic clusters) as inferred by two different methods (Evanno above and Puechmaille below) ..... 114

Figure 19: (a) Isolation-by-distance plots for each locality. (b) Comparisons of genetic differentiation ( $F_{ST}$ ) between each pair of nests, both between and within habitats. Each gold dot on a boxplot represents the mean of the group, and only significant pairwise comparisons are shown (as determined by Dunn's test with  $p$ -values adjusted according to the Holm method;  $*p < .05$ ,  $**p < .01$ ,  $***p < .001$ ). Note that Colorado contains only intrahabitat comparisons for both (a) and (b), as nests were only found in natural habitats ..... 116

Figure 20: Breeding structure of *Tapinoma sessile* overall and across the four localities. (a) The percentage of monogyne and polygyne nests in natural and urban habitats, as well as the number of queens collected in nests where at least one queen was found. (b) The average relatedness among workers within a nest in urban and natural habitats. For both (a) and (b), each smaller dot represents a nest, and each gold dot denotes the mean of the habitat ..... 118

Figure 21: Clustering of nests in (a) Indiana, (b) Arkansas, (c) California and (d) Colorado using a PCA on each nest's CHC profile. For each locality, the light-shaded and dark-shaded ellipses in the PCA represent natural nests and urban nests, respectively. Additionally, the boxplots illustrate the CHC differentiation of nests among and between habitats. Each smaller dot on the boxplots represents the difference between a pair of nests, while each gold dot denotes the mean of the group. Only significant pairwise comparisons are shown (as determined by Dunn's test with  $p$ -values adjusted according to the Holm method;  $*p < .05$ ,  $**p < .01$ ,  $***p < .001$ ). ..... 120

Figure 22: Violin plots of aggression between nests, with aggression compared within and among habitats for each locality (except Colorado). The gold dot on each violin represents the mean, and only significant pairwise comparisons are shown (as determined by Dunn's test with  $p$ -values adjusted according to the Holm method) ..... 122

## LIST OF TABLES

Table	Page
Table 1: Interceptions of non-native termites broken down by world region and country. For each world region, the three countries contributing the most termites to interceptions are listed, with the two most commonly intercepted non-native termite species also noted. ....	25
Table 2: The number and average number of alleles, observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity, inbreeding coefficient ( $F_{IS}$ ) and fixation index ( $F_{ST}$ ) for each locality and habitat across the 12 microsatellite loci. ....	113

# 1 INTRODUCTION

## 1.1 General invasion biology

Charles Elton's *The Ecology of Invasions by Animals and Plants* (Elton, 1958) laid the foundation for the field of invasion biology, being the first major scientific work to collate and examine the various impacts invasive species have within their new environment(s). Although it took about 30 years for research (*i.e.*, published studies) focused on biological invasions to become more commonplace (E. Lowry et al., 2012), the field is now flourishing. For instance, an exact search of the term “biological invasion” in Google Scholar yields only 174 results between 1958 and 1990; for the subsequent 30-year period (1991 – 2021), this search produces 20,900 results (~11,000% increase).

While the field of invasion biology may be relatively young, biological invasions are not a recent phenomenon. Since humans first began domesticating plants (*i.e.*, crops) and animals (*i.e.*, livestock, pets), these ‘goods’ have been regularly transported by humans during the colonization of new territory (Crosby, 2004). Although domesticated goods are not typically labeled as invasive species (*i.e.*, harmful non-native species), they can have a profound influence within their new environment. For example, the domestic cat *Felis catus* could be considered an invasive species given the devastating impact(s) it has on wildlife throughout the world [*e.g.*, Legge et al. (2020); Y. Li et al. (2021); López-Jara et al. (2021)]. In the US alone, *F. catus* has been estimated to kill up to 4 billion birds and 22.3 billion mammals annually (Loss, Will, & Marra, 2013). More recently, the trade of exotic pets (*i.e.*, non-native/non-domesticated species, but not necessarily invasive) has



emerged as an additional ‘intentional’ pathway for non-native species to become established and invasive within novel territory (Gippet & Bertelsmeier, 2021). Although it has not been definitively proven to be the direct result of the exotic pet trade, the invasion of the Burmese python *Python molurus* in Florida is thought to be one such example of an exotic pet that escaped containment and subsequently became established (Willson, Dorcas, & Snow, 2011). In addition to these intentional introductions, the non-intentional introduction of species has often occurred during human colonization events. Early and famous examples of such non-intentional introductions include the Norway rat *Rattus norvegicus* and the black rat *Rattus rattus*, two now cosmopolitan rodent species that are thought to originate from northern China (Nowak & Walker, 1999) and Southern Asia (Aplin et al., 2011), respectively. In more modern times, the advent of the global human transportation network has provided near limitless opportunity for non-intentional introductions to occur (Hulme, 2021), with both airline and ocean transport/shipping having been implicated as drivers of invasion(s) (Liebhold, Work, McCullough, & Cavey, 2006; McCullough, Work, Cavey, Liebhold, & Marshall, 2006). Consequently, rates of invasion have now risen to unprecedented levels, and no end is in sight (Seebens et al., 2018; Seebens et al., 2017).

The fact that invasive species exist at all has been seen as a paradox, *i.e.*, how are non-native species able to establish within new environments where they are not locally adapted and proliferate to the extent that they sometimes displace native (*i.e.*, locally adapted) species (Sax & Brown, 2000)? Most introductions of species to novel territory fail to establish, and only a small percentage of those introduced species that do establish

go on to become invasive (Mack et al., 2000; Tobin, 2018). Even species that go on to become invasive may require multiple opportunities to successfully colonize novel territory. For example, the European Starling *Sturnus vulgaris* is now among the most abundant and widespread North American bird species, yet it needed (at a minimum) nine introductions before establishing (Lever, 1985). The main reason most introductions do not result in establishment is likely a combination of demographic and environmental stochasticity (Sax & Brown, 2000). Even if species are able to survive the journey to a new location, the colonizing population will likely be small and therefore more sensitive to demographic stochasticity (Engen, Bakke, & Islam, 1998). When combined with extrinsic factors of the environment, such as temporal (*e.g.*, seasons, severe weather events) and/or spatial (*e.g.*, climate, suitable habitat) variability, demographic stochasticity may intensify, potentially resulting in the rapid decline and local extinction of colonizing species (Lande, Engen, & Saether, 2003; Schreiber & Lloyd-Smith, 2009). Furthermore, the small size of colonizing populations may induce a strong Allee effect [*i.e.*, negative population growth rate resulting from low population size/density (Allee, 1938)], which could also inhibit establishment or further dispersal (Dennis, Assas, Elaydi, Kwessi, & Livadiotis, 2016; Drake, 2004). Therefore, to beat the odds and succeed in establishing, organisms need to be genetically suited to the new environment (*i.e.*, genotypic match) and propagules need to be introduced into sufficiently habitable locales during opportune times of the year (*i.e.*, environmental match), with more optimal environmental matches likely necessary for a species to subsequently become invasive. Indeed, recent meta-analytic work has yielded two exciting results that support this

hypothesis – 1) invasive species largely conserve their climatic niche between their native and introduced ranges (C. Liu, Wolter, Xian, & Jeschke, 2020) and 2) smaller climatic niche shifts are observed in invasive species when compared against non-invasive alien species (Bates, Ollier, & Bertelsmeier, 2020).

While the prominent determinants of a successful invasion are likely the environmental/climatic match (both temporally and spatially) between the donor and recipient regions and the general invasibility of the environment [*e.g.*, Lovell, Blackburn, Dyer, and Pigot (2021)], characteristics of a species also influence invasion success (Sakai et al., 2001). Not dissimilar to species adept at colonizing previously disturbed habitat (*i.e.*, pioneer species), the success of invading species has been hypothesized to be promoted if they possess broad ecological requirements and tolerances (*i.e.*, generalist species), r-selected life histories, and/or a proclivity to inhabit disturbed/anthropogenic habitat (Baker & Stebbins, 1965; Sakai et al., 2001; Sax & Brown, 2000; Stohlgren & Schnase, 2006). Broad quantitative studies (*e.g.*, meta-analyses) have therefore been performed to gauge the validity of such hypotheses, with much of the research focused on plant [*e.g.*, Davidson, Jennions, and Nicotra (2011); Lloret et al. (2005); Mathakutha et al. (2019); Pysek, Prach, and Smilauer (1995); van Kleunen, Schlaepfer, Glaettli, and Fischer (2011); van Kleunen, Weber, and Fischer (2010)] and bird [*e.g.*, Blackburn and Duncan (2001); Cardador and Blackburn (2019); Cassey, Blackburn, Sol, Duncan, and Lockwood (2004); Sol and Lefebvre (2000); Sol, Timmermans, and Lefebvre (2002)] invaders. While several traits have been found to correlate with establishment success and/or invasiveness, many exceptions exist both within and across taxonomic groups (Kolar & Lodge, 2001).

However, a few characteristics do appear more broadly applicable; notably, that greater propagule pressures (*i.e.*, the frequency of introduction events and number of individuals per event) and larger native ranges of alien species positively correlate with invasion success (Novoa et al., 2020). Although neither characteristic is an inherent trait of a species, both are likely influenced by inherent traits (*e.g.*, choice of habitat, dietary/climatic restrictions). Furthermore, both characteristics can also be tied to the distinctive traits of pioneering species, which, as stated above, have been thought to confer greater invasiveness. For instance, greater propagule pressures may arise more frequently in species with r-selected life histories and/or anthropogenic distributions (Hufbauer et al., 2012), and larger native ranges likely result from broader ecological tolerances [*e.g.*, Devictor, Julliard, and Jiguet (2008)].

Interestingly, invasive populations themselves can serve as source populations for future invasions, a phenomenon recently termed the ‘bridgehead effect’ (Lombaert et al., 2010). Since the Lombaert et al. (2010) study on the worldwide invasion of the Harelquin ladybird *Harmonia axyridis*, strong evidence for a bridgehead effect has been found in a number of organisms [*e.g.*, plants (Vallejo-Marín et al., 2021; van Boheemen et al., 2017), insects (Ascunce et al., 2011; Ryan et al., 2019), and pathogens (Fontaine et al., 2021; Leduc et al., 2015)]. One line of reasoning regarding the apparent prevalence of bridgehead introductions posits that bridgehead populations evolve higher ‘invasiveness’ compared to their native conspecifics (*i.e.*, acquire new traits that facilitate future invasions); however, empirical evidence of this kind of adaptive evolution in bridgehead populations is lacking (Bertelsmeier & Keller, 2018). Moreover, significant adaptations

may not even be necessary for invasive species to establish in new territory given climatic niches are mostly conserved between their natal and novel territories (Bates et al., 2020; C. Liu et al., 2020) and traits facilitating their successful invasions already exist in their native range (Sakai et al., 2001). Therefore, the adaptive evolution of invasiveness within bridgehead populations may be unlikely. Instead, the prevalence of bridgehead introductions likely has a more straightforward explanation. For one, invasive populations may simply become more integrated within the human trade and transportation network (*e.g.*, establishing within ‘hub’ nodes), thus increasing the number of potential novel locations they can reach/colonize (Banks, Paini, Bayliss, & Hodda, 2015). Two, the great densities some invasive populations achieve [*e.g.*, due to increased resource availability, enemy release/reduction, novel weapons, etc. (Catford, Jansson, & Nilsson, 2009)] may increase their propagule pressure in comparison to their native relatives (Simberloff, 2009), thereby enhancing the success rate of their subsequent invasions. Regardless of the primary reason, invasions stemming from bridgeheads figure to play a larger role in the coming decades given the increasing interconnectedness of the world.

Finally, discourse surrounding the field of invasion biology generally frames non-native species as being the sole perpetrators of invasions, despite numerous examples of ‘native invaders’ with impacts that rival those of non-native invasive species (Carey, Sanderson, Barnas, & Olden, 2012; Valéry, Fritz, & Lefeuvre, 2013). Like the invasions of non-native species, native invaders typically arise via human activities, such as human-mediated environmental change (*e.g.*, global warming) (Valéry, Fritz, Lefeuvre, & Simberloff, 2008) and/or habitat modification (*e.g.*, urbanization) (Didham, Tylianakis,

Gemmell, Rand, & Ewers, 2007). In these cases, human activities facilitate population expansion through either the creation of new habitat(s) or resources or the decline/extirpation of other native species (*i.e.*, increased availability of already present habitat/resources) (Carey et al., 2012). Examples of native invaders now exist across a wide variety of taxa. For instance, a decrease in fire frequency due to human-mediated activities (*e.g.*, livestock grazing, road construction) lead to the rapid range expansion of the western juniper *Juniperus occidentalis* across the western US (Burkhardt & Tisdale, 1976; R. F. Miller, 2005; Wall, Miller, & Svejcar, 2001). In Oregon specifically, western juniper's range exploded from only 456,000 hectares in 1936 to 2.2 million hectares in 1988 (33,500 hectares per year), with ensuing declines observed among its competition (*e.g.*, big sagebrush *Artemisia tridentata*, aspen *Populus tremuloides*) (R. F. Miller, 2005). Perhaps a more iconic example lies in the recent outbreak of the mountain pine beetle *Dendroctonus ponderosae* across western North American pine forests (Audley et al., 2020). A combination of human-exacerbated climate change (*e.g.*, mild winters, hot/dry summers) and poor management strategies (*e.g.*, fire suppression) provided the beetle with idyllic breeding conditions (*i.e.*, a high density of mature, weakened trees), resulting in an outbreak an order of magnitude greater than any previous outbreak (435 million m<sup>3</sup> in timber losses) (Taylor, Carroll, Alfaro, & Safranyik, 2006; Walton, 2007). Clearly, the ability of a species to expand its range and inflict detrimental impact upon the environment does not rely on its 'nationality'; therefore, the biological invasion/invasive species label need not be wholly applied to non-native species.

## 1.2 Social insect invaders

One group of organisms particularly adept at exploiting humankind's global transportation network is social insects (Bertelsmeier, 2021). The term social insect applies to any insect that displays a specific range of sociality (*i.e.*, eusociality), with species in the orders of Blattodea (*i.e.*, termites) and Hymenoptera (*i.e.*, ants, bees, and wasps) being the most well-known social insects. However, eusociality has evolved in several different insect orders [*e.g.*, Coleoptera (S. M. Smith, Kent, Boomsma, & Stow, 2018), Hemiptera (Shibao, 1998), and Thysanoptera (Crespi, 1992)]. The defining characteristics of eusociality are 1) a reproductive division of labor, 2) cooperative brood care, and 3) overlapping generations of adults within a colony (Wilson, 1971, 1975). Social insects represent just 2% of all insect species; yet, the majority of the world's worst invasive insects, as determined by the IUCN, are social insect invaders (IUCN, 2020). Termites (Blattodea: Isoptera) and ants (Hymenoptera: Formicidae) are the only groups entirely composed of eusocial species, and, interestingly, are also the most prolific of the social insect invaders. Approximately 240 ant species (Bertelsmeier, Ollier, Liebhold, & Keller, 2017) and 28 termite species (Evans, Forschler, & Grace, 2013) are now established outside of their native range, including dominant invasive pest species such as the Argentine ant *Linepithema humile* (Tsutsui, Suarez, Holway, & Case, 2000), the red imported fire ant *Solenopsis invicta* (Caldera, Ross, DeHeer, & Shoemaker, 2008), and the Formosan subterranean termite *Coptotermes formosanus* (Vargo, Husseneder, Woodson, Waldvogel, & Grace, 2006). The invasions of the latter two species may be the

most costly of any invasive insect, with some annual estimates approaching \$8 billion for *S. invicta* and \$30 billion for *C. formosanus* (Bradshaw et al., 2016). So, why are social insects – and specifically, termites and ants – such prolific invaders?

Specific, yet distinct, life history traits appear to enhance the invasion success of these two social insect groups (Eyer & Vargo, 2021). For termites, a recent review identified three important features that all invasive termites share – they all 1) consume wood, 2) nest in their food (*i.e.*, wood-nesters), and 3) readily generate secondary reproductives [*i.e.*, reproductives descended from the founding primary reproductives (Vargo, 2019)] (Evans et al., 2013). The first two characteristics speak to the importance of global trade (Banks et al., 2015) and propagule pressure (Simberloff, 2009) in driving invasion rates, as wood is a heavily traded commodity. The third ensures that virtually any piece of wood containing such a termite species is a reproductively viable propagule (Evans et al., 2013), thus increasing the probability of establishment once introduced to a new locality. Two prominent invasive termites (*i.e.*, globally invasive) are the foci of two of my dissertation chapters – the aforementioned Formosan subterranean termite *C. formosanus* and the eastern subterranean termite *Reticulitermes flavipes*. *Coptotermes formosanus* is currently recognized by the IUCN as one of the 100 worst invasive species in the world (Lowe, Browne, Boudjelas, & De Poorter, 2000), establishing notable invasive populations in Hawaii and the southeastern US (Jiasi Wang & Grace, 1999). Previous studies using microsatellite markers (Husseneder et al., 2012) or mitochondrial DNA (Austin et al., 2006; Fang, Huang, & Zhong, 2008; T. R. C. Lee, Cameron, Evans, Ho, & Lo, 2015; H.-F. Li, Ye, Su, & Kanzaki, 2009; Yeap, Othman, & Lee, 2009) have



been unable to clearly resolve the invasion history; therefore, the native source(s) of these invasive US populations are yet to be determined. *Reticulitermes flavipes* is a species of termite native to the eastern US that has become invasive throughout the world, most notably in South America (Austin, Szalanski, Scheffrahn, & Messenger, 2005; Clément et al., 2001; Su, Ye, Ripa, Scheffrahn, & Giblin-Davis, 2006) and Europe (Austin et al., 2012; Clément et al., 2001; Ghesini, Messenger, Pilon, & Marini, 2010; Hernández-Teixidor, Suárez, García, & Mora, 2019; Kollar, 1837; Weidner, 1937). Unlike *C. formosanus*, the native range of *R. flavipes* is well established. Additionally, the native source of the European and South American populations is thought to have been uncovered, as colonies in a *R. flavipes* population from Louisiana share the same colony breeding structure exhibited by their invasive relatives (Perdereau, Bagnères, Dupont, & Dedeine, 2010; Perdereau et al., 2015; Perdereau, Dedeine, Christidès, Dupont, & Bagnères, 2010); however, this invasion pathway has not yet undergone rigorous quantitative testing.

Like termites, most invasive ants possess a suite of traits which facilitate their invasion success. The prominent characteristics include a polygyne (*i.e.*, multi-queen) colony structure, colony foundation through budding, and reduced internest aggression (Bertelsmeier et al., 2017; Eyer & Vargo, 2021; McGlynn, 1999; Tsutsui & Suarez, 2003). The multiple reproductive queens that head polygyne colonies enable colonies to achieve a high rate of growth (Boulay, Arnan, Cerdá, & Retana, 2014) and subsequently monopolize environmental resources (Tsutsui & Suarez, 2003), which may explain why invasive ants predominantly display polygyny. Oftentimes, polygyne colony structure is

associated with colony foundation through budding (Cronin, Molet, Doums, Monnin, & Peeters, 2013), whereby daughter queens disperse from their natal nests on foot to nearby nesting sites with the assistance of nestmate workers (Hölldobler & Wilson, 1977; Peeters & Ito, 2001). Budding may also favor invasion success as the assistance of the worker force during the early stages of colony foundation promotes secondary colony establishment success through increased survival and reproduction rates (Cronin et al., 2013). The dispersal range of colonies that spread via budding is more limited than colonies that disperse via nuptial flights (Peeters & Ito, 2001), thus the establishment of many genetically similar colonies across a landscape may also occur (Schultner, Saramäki, & Helanterä, 2016), potentially giving rise to supercolonies. Supercolonies are populations of ants comprised of many distinct, genetically similar nests that lack aggression toward one another (Helanterä, 2022; Tsutsui & Suarez, 2003), which circumvents the issue of intraspecific competition and facilitates an increased interspecific competitive ability (Eyer & Vargo, 2021). Overall, the combination of polygyne colony structure and dispersal via budding may greatly enhance the likelihood of initial establishment, as transported propagules will have an elevated reproductive capacity and budding will promote secondary colony formation (Tsutsui & Suarez, 2003; Yang et al., 2012). The subsequent reduction in internest aggression resulting from this limited dispersal may then enable the spread and dominance of the species throughout the newly invaded environment (Eyer & Vargo, 2021; Tsutsui & Suarez, 2003). Indeed, the presence of supercolonies has been observed in many species of invasive ants, such as the Argentine ant *Linepithema humile* (Holway, Suarez, & Case, 1998), the yellow crazy ant *Anoplolepis*

*gracilipes* (Abbott, 2005), the bigheaded ant *Pheidole megacephala* (Fournier, De Biseau, & Aron, 2009), and the tawny crazy ant *Nylanderia fulva* (Eyer, McDowell, et al., 2018).

As mentioned previously, in human-altered environments native species possess the ability to significantly expand their range and subsequently generate impacts that rival those of more traditional invasive species; likewise, native ant populations expanding into new areas within their native range may exhibit traits more traditionally associated with invasive ant populations. The odorous house ant *Tapinoma sessile* is one such ‘native invasive’ organism appearing to display the traits of its invasive counterparts. First described by Thomas Say in 1836 (Say, 1836), the ant soon garnered a reputation as a serious ‘house infesting species’ across much of the continental US (M. R. Smith, 1928). Likely the most widely distributed native North American ant species, *T. sessile* originally inhabited a variety of natural habitats across the continent (*e.g.*, forests, grasslands, bogs) prior to becoming highly abundant in urban environments (Buczkowski & Bennett, 2008; Menke et al., 2010). Although the ant is still prevalent throughout these various natural environments, a strong dichotomy now appears to exist between natural- and urban-dwelling colonies. Natural-dwelling colonies are typically small (<200 workers) and consist of a single nest headed by a single queen (*i.e.*, monogyne), whereas urban colonies tend to be larger (>100,000 workers) and composed of several interconnected nests that each house numerous reproductive queens (*i.e.*, polygyne), with low internest aggression over large landscapes (Buczkowski, 2010; Buczkowski & Bennett, 2008; Menke et al., 2010). Interestingly, this transition of small, monogyne natural colonies to large, polygyne urban colonies resembles the invasions of many true invasive ants (Eyer & Vargo, 2021),

a phenomenon not previously elucidated for any other native North American ant. Despite this fascinating juxtaposition, most previous work on *T. sessile* has been based on behavioral studies (Buczkowski, 2010; Buczkowski & Bennett, 2008; Buczkowski & Krushelnycky, 2011), while the genetic underpinnings of the colonies have largely not been analyzed. However, the one previous genetic study on the ant did identify some interesting patterns. Notably, that the natural-urban colony transition appears to have occurred consistently and repeatedly throughout its distribution, rather than the urban phenotype being derived and then spread across the continent from a single natural source (Menke et al., 2010). This result therefore suggests that the invasive qualities of *T. sessile* are inherent within the species, thus creating a unique opportunity to compare their transition with that of more traditional invasive ants.

### **1.3 Studies on the invasion biology of termites & ants**

My dissertation concerns invasions performed by both native and non-native social insect invaders. For my first chapter, I explored the path(s) non-native termites take to invade the US using US port of entry data dating back to the early 1900s. Port of entry data refer to the interceptions of pest species at a country's various ports of entry (*e.g.*, airports, seaports, and land borders), and thus has the potential to illuminate invasion pathways. Recently, this kind of data has been used to analyze patterns of spread in another group of social insects – ants [*e.g.*, Bertelsmeier et al. (2018); C.-C. Lee et al. (2020); Suarez, Holway, and Ward (2005); Suhr et al. (2019); Ward, Beggs, Clout, Harris, and O'Connor (2006)]. Therefore, using these port of entry data, I evaluated the prominent origin and destination points of introductions, how interceptions have fluctuated through time, the

correlation between introduction pressure and establishment success, and the effect of trade on interceptions. Additionally, I investigated the prevalence of the bridgehead effect among non-native termites, as non-native ants have been recently found (also using port of entry data) to be spread via bridgeheads (Bertelsmeier et al., 2018). Overall, regional biases in interceptions and convincing evidence that termites utilize bridgeheads to expand their global range were found. This work was published in *Biological Invasions* (Blumenfeld & Vargo, 2020).

For my next two chapters, I reconstructed the invasion histories of two invasive termites – *C. formosanus* and *R. flavipes*. While traditional population genetic approaches can describe the genetic structure and relationships between native and invasive populations, elucidating clear invasion routes from such methods is more challenging. However, the recent incorporation(s) of approximate Bayesian computation (ABC) into population genetics (Beaumont, Zhang, & Balding, 2002; Cornuet et al., 2014; Pudlo et al., 2016) has enhanced the field's ability to decipher the origin(s) of invasive populations and led to an explosion in invasion reconstruction research [*e.g.*, Fontaine et al. (2021); Fraimout et al. (2017); Sherpa et al. (2019); Vallejo-Marín et al. (2021)]. With ABC, different invasion scenarios are able to be compared quantitatively, removing much of the ambiguity from earlier, more traditional methods (Beaumont, 2010). Importantly, ABC can handle the evaluation of complex invasion scenarios (*e.g.*, multiple introductions, bridgehead introductions, admixture events). Therefore, for both projects/species, the following methods were carried out. First, extensive sampling was performed across the native and introduced ranges, followed by double digest restriction-site associated DNA

sequencing to obtain markers of high resolution (*i.e.*, single-nucleotide polymorphisms, or SNPs). These two initial steps were performed prior to the beginning of my PhD career and therefore not conducted by me. Second, population structure and phylogenetic analyses were performed using the SNPs to assess the relationships between populations (both within and between native and invasive populations), thereby facilitating the formation of potential invasion history models. Last, the SNPs were subject to ABC analysis in order to compare invasion models and ultimately select the most likely invasion history. For both species, complex invasion histories were inferred (*e.g.*, multiple invasions from the native range and bridgehead invasions from the invasive range). The *C. formosanus* study was published in *Communications Biology* (Blumenfeld et al., 2021) and the *R. flavipes* study was published in *Molecular Ecology* (Eyer et al., 2021).

For my final chapter, I examined the urban invasion of *T. sessile* from its native natural habitats throughout the US. Specifically, I investigated the population genetic and breeding structure of *T. sessile* across four broad geographic regions in order to compare colonies of the ant within and between its regions and habitats of residence. Colonies were sampled and analyzed in both natural and urban environments to not only test for the existence of supercolonies, but also to test for a transition of monogyne colonies in more natural settings to polygyne colonies in urban settings. The social structure of a colony was found to be a plastic trait in both habitats, although extreme polygyny (*i.e.*, multi-queen colonies) was confined to urban habitats. Additionally, strong differentiation between urban and natural populations was identified in each geographic region (where both natural and urban colonies could be found – see Chapter 4), indicating cities may

restrict gene flow and exert intense selection pressure. This work was published in *Molecular Ecology* (Blumenfeld, Eyer, Helms, Buczkowski, & Vargo, 2021).

Overall, the findings from each of my chapters highlight the substantial and increasing influence humankind has in shaping both the local and global distribution of species.

## 2 GEOGRAPHY, OPPORTUNITY AND BRIDGEHEADS FACILITATE TERMITE INVASIONS TO THE UNITED STATES<sup>1</sup>

### 2.1 Introduction

The worldwide trend towards globalization has promoted the accidental transfer of animal and plant species throughout the world (Banks et al., 2015; Westphal, Browne, MacKinnon, & Noble, 2008). Introduction rates of alien species have been shown to match up remarkably well with modern, human-mediated events (Bertelsmeier et al., 2017); therefore, alien species range expansion appears to be a trademark of the Anthropocene (Capinha, Essl, Seebens, Moser, & Pereira, 2015; Lewis & Maslin, 2015). The rate at which these alien species are spreading to novel countries is still increasing (Seebens et al., 2018; Seebens et al., 2017) and may continue to rise for the foreseeable future (Seebens et al., 2015), despite focused efforts to mitigate invasions over the last half-century (Hulme, 2009). Even though many species do not succeed in establishing, those that persist and then spread from their initial introduction point can become invasive species (*i.e.*, harmful alien species) (Tobin, 2018). The detrimental impacts of invasive species are well documented (Bradshaw et al., 2016; Simberloff et al., 2013), and pose a tremendous threat to biodiversity, agriculture and general human health. As prevention of their introduction or rapid response treatment programs remain the most cost-effective

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<sup>1</sup>Reprinted with permission from “Geography, opportunity and bridgeheads facilitate termite invasions to the United States” by Blumenfeld, A. J., & Vargo, E. L., 2020. *Biological Invasions*, 22(11), 3269–3282, Copyright [2021] by Springer Nature.



approach of reducing their impacts (Finnoff, Shogren, Leung, & Lodge, 2007; R. P. Keller, Lodge, & Finnoff, 2007; Reaser et al., 2020), it is important to fully understand the pathways by which they are spreading in order to prevent invasions or generate early detections.

Recently, port of entry data have been utilized to elucidate patterns in the spread of alien species in a wide variety of organisms, including beetles (Haack 2001), mosquitoes (Derraik, 2004), ants (Bertelsmeier et al., 2018; Suarez et al., 2005; Suhr et al., 2019), lizards (Chapple, Whitaker, Chapple, Miller, & Thompson, 2013) and general plant pests (McCullough et al., 2006). The data specifically refer to interceptions of pest species at a country's various ports of entry (*e.g.*, airports, seaports or land borders), and has the potential to reveal novel insights into invasion pathways. Records of port of entry interceptions in the US date back to the early 1900s, when the USDA Animal and Plant Health Inspection Service (APHIS) first began publishing annual lists of the intercepted pest species. The primary data compiled for each interception include the name of the pest species, date of the interception, country of origin, US port of entry and item on which the pest was found (*e.g.*, trade commodity, packaging). Therefore, this historical port of entry dataset offers a robust opportunity to identify patterns in species invasion pathways. For example, Bertelsmeier et al. (2018) utilized 100 years' worth of port of entry records to find that invasive ants more frequently originated from countries where the ant had previously invaded and established, a phenomenon known as the 'bridgehead effect' (Lombaert et al., 2010). This phenomenon has been found to play a role in the invasions of several different organisms (Javal et al., 2019; Lombaert et al., 2010; van Boheemen et

al., 2017), with Ascunce et al. (2011) the first to describe this phenomenon in a eusocial organism, in the spread of the red imported fire ant *Solenopsis invicta* out of South America. Given the recent finding that the bridgehead effect appears prevalent in many ant invasions, bridgeheads may also play a vital role in the invasions of other eusocial organisms.

Termites are a group of eusocial insects that consume the cellulose and lignocellulose found in dead wood, grass, microepiphytes, leaf litter, and cultivated fungi (Hartke & Baer, 2011). They perform beneficial ecosystem services in their natural environments, primarily improving soil quality (Black & Okwakol, 1997; Dawes, 2010), and thereby productivity (Nash & Whitford, 1995), of an ecosystem, making them critical members of their community (Holt & Coventry, 1990; Whitford, 1991). However, the services they perform that make them key members of their natural environments render them destructive in urban environments, as they can heavily infest man-made structures (Rust & Su, 2012). Of the approximately 3000 described termite species, 80 are currently designated as serious urban pests (Rust & Su, 2012) and 28 species have become established in countries outside their native range (*i.e.*, alien species) (Evans et al., 2013). Worldwide, urban pest termites necessitate expensive repairs, prevention and control efforts by humans (Ghaly & Edwards, 2011; Scharf, 2015), with recent damage estimates approaching \$40 billion annually (Rust & Su, 2012). Invasive termites threaten to exacerbate these costs within the US, as two of the most destructive urban termite pests in the world are now established there (Chouvenc, Scheffrahn, & Su, 2016; Evans et al., 2013)—the Formosan subterranean termite *Coptotermes formosanus* and the Asian

subterranean termite *C. gestroi*. Moreover, invasive termites could become the cause of more traditional negative invasive effects, as their expansion out of urban environments is occurring in the southeastern US (Evans, Forschler, & Trettin, 2019). To mitigate and possibly prevent costs associated with future invasive termite establishments, a more thorough knowledge of their pathways into the US is necessary. We used almost 100 years of port of entry data of non-native termites to the US to analyze and elucidate their invasion pathways.

## **2.2 Methods**

### *2.2.1 Data acquisition, standardization and filtration*

Termite interceptions were acquired from USDA APHIS through their (1) published annual reports and (2) current computerized database, resulting in a catalog of interceptions spanning almost 100 years, from 1924 to 2017. Country and termite species names have fluctuated greatly over the past century, so the data were standardized to facilitate downstream analysis. Country names were changed to align with recognized countries as of 2019, and termite species names were changed to align with the current taxonomy, reflecting both updates of genus/species names (Krishna, Grimaldi, Krishna, & Engel, 2013) and synonymies of two or more species (Austin, Szalanski, Scheffrahn, Messenger, et al., 2005; Scheffrahn et al., 2015; Scheffrahn, Krecek, Szalanski, & Austin, 2005). Additionally, Hawaii was listed in the USDA records as a port of origin for some interceptions. Given its statehood, we chose to treat Hawaii solely as a member of the US and therefore excluded interceptions originating from Hawaii from all analyses except for the bridgehead analysis, as Hawaii could potentially act as an important bridgehead for

alien termites (see Bridgehead section below). However, US territories such as American Samoa, Guam, Puerto Rico and the US Virgin Islands were treated as foreign countries given their closer associations with their geographic neighbors—Oceania for American Samoa and Guam and the Caribbean for Puerto Rico and the US Virgin Islands.

Non-native termites were the focus of this study, so only non-native termite interceptions were analyzed. Non-native termites were assigned to one of two groups, when applicable: 1) alien—non-native and established somewhere outside of their native range or 2) invasive—harmful alien species. The port of entry records did not designate whether the pests intercepted were native or non-native to the US, so designations were performed primarily based on the distributions given in Krishna et al. (2013). Additionally, data from Evans et al. (2013) were used to determine whether the non-native termite species intercepted had established a population outside of its native range (*i.e.*, alien). Finally, species recognized as pests of significant economic importance by Rust and Su (2012) were designated as invasive for this study, as the detrimental impacts of alien termites primarily occur in the urban environment. All of the following analyses were conducted in R (R Core Team, 2019).

### 2.2.2 Sources of termites

To identify countries and regions acting as major source of interceptions, we analyzed the total number of non-native termite interceptions originating from each country. Countries were also assigned to one of eight world regions to identify trends among larger land masses – 1) Africa, 2) Asia, 3) Caribbean, 4) Central America, 5) Europe, 6) North America, 7) Oceania or 8) South America. Trade and distance data were also analyzed in

conjunction with the region and country data in order to ascertain the most important factors influencing interception rates. Trade data (*i.e.*, value of imports) was obtained from the World Bank and dated back to 1991 (<https://wits.worldbank.org/>). Three trade categories were tested for a link with interceptions – 1) overall (*i.e.*, all imports), 2) wood and 3) vegetables (the latter two reflect the goods most commonly associated with the termite interceptions). The monetary value for all three import categories was adjusted for inflation to reflect the value as of 2017, the last date for which data were collected. The geographical distance between a country and the US was measured as the distance between the capital of the country and the capital of the US state to which the country had introduced termites most frequently. When two or more US states were tied as a country's most frequent destination, the state which had intercepted more total termites was chosen. Additionally, a Poisson GLM was created to elucidate the individual and interactive effects of trade and distance on interceptions. As the trade data only went back to 1991, interception and distance data prior to this date were also excluded.

### 2.2.3 Propagule pressure

Propagule pressure refers to the number of individuals colonizing a new locality and the rate at which this colonizing force arrives to the locality (Lockwood, Cassey, & Blackburn, 2005; Simberloff, 2009), with increases in propagule pressure found to enhance colonization success in many organisms (Petri, Alatalo, Anne, Kumpulainen, & Suhonen, 2003; Suarez et al., 2005; Veltman, Nee, & Crawley, 1996; Woodford, Hui, Richardson, & Weyl, 2013). There are currently five alien termites present in the United States—*Coptotermes formosanus*, *C. gestroi*, *Cryptotermes brevis*, *Nasutitermes corniger* (Evans

et al., 2013) and a yet unidentified species of *Heterotermes* (Scheffrahn & Su, 1995; Szalanski, Scheffrahn, Austin, Krecek, & Su, 2004)—all with multiple documented interceptions over the last 90 years. This highlights the role of opportunity (*i.e.*, increased propagule pressure) for a non-native termite in becoming established in a novel country. To identify the influence propagule pressure has on the spread of non-native termites to the US, we first modeled the establishment probability at the species level using a binomial generalized linear model (GLM). We then constructed two Poisson GLMs to identify the relationship between the number of world regions a species inhabits with its number of both overall interceptions and secondary interceptions. All three models were tested for significance against their null equivalents (*i.e.*,  $y \sim 1$ ) using a chi-squared test.

#### 2.2.4 Bridgehead interceptions

Given that the bridgehead effect appears widespread among invasive species, including invasive ants, a similar investigation is warranted for invasive termites. For all invasive termites intercepted, we calculated the proportion of primary interceptions (interceptions from countries within their native range) and secondary interceptions (interceptions from countries outside their native range). Additionally, interceptions originating from Hawaii were included as foreign introduction events in order to evaluate the potential bridgehead status of the state, as there are no termites native to the islands (Tong et al., 2017). Therefore, each interception originating from Hawaii was considered a secondary interception. Six alien termites are present in Hawaii, including three of the four already established on the US mainland (*C. formosanus*, *C. gestroi* and *Cr. brevis*) (Tong et al., 2017).

### 2.3 Results

We identified 906 non-native termite interceptions originating from outside the US from the last 100 years of USDA records. On an annual basis, the number of non-native termite interceptions increased until reaching a peak in the late 1970s, at which point a steady decline has occurred until the present day (Fig. 1a). Also, in 1984 the USDA began including the month associated with the interceptions, enabling the possible identification of seasonal effects upon interceptions. However, seasonal effects appear negligible for termites, as only two months had significantly different means (June – December;  $p =$

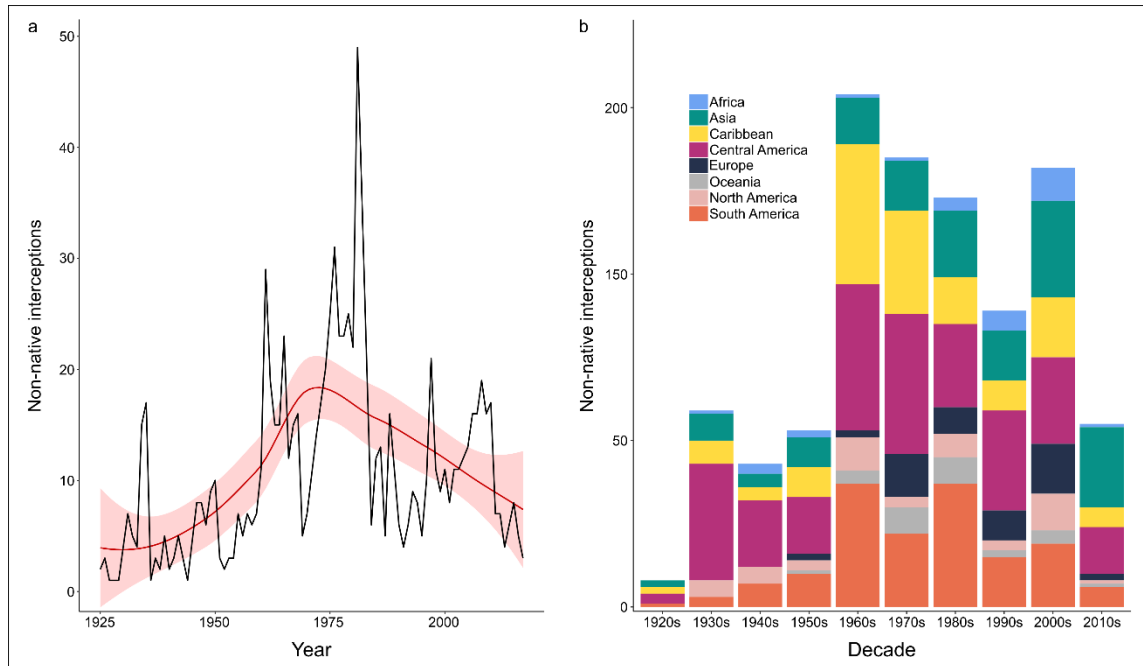


Figure 1: (a) Time series of non-native termite interceptions from 1925 to 2017 and (b) time series of interceptions for each world region from 1925 to 2017 (interceptions of unknown origin not pictured)

0.0232).

All interceptions were identified to at least the family level, with 904 and 620 further down to genera and species, respectively. In total, four families, 32 genera and 75

species were represented in the data. *Nasutitermes corniger* (119 interceptions), *Cryptotermes brevis* (61), *N. ephratae* (52), *Coptotermes formosanus* (45) and *Kaloterme flavicollis* (38) were the five most commonly intercepted termites. *Nasutitermes* was the most abundant (384) and rich (11 species) genus intercepted, followed by *Coptotermes* (173) and *Cryptotermes* (70) in abundance and *Neotermes* (seven species) and *Incisitermes* (six species) in richness. At the family level, Termitidae was the most abundant (447) and rich (38 species) family intercepted, due in large part to the high number of *Nasutitermes* spp. interceptions, followed by Rhinotermitidae in abundance (273 interceptions from 14 species) and Kalotermitidae in richness (181 interceptions from 22 species). Stolotermitidae ranked last in both respects (five interceptions represented by one species).

Table 1: Interceptions of non-native termites broken down by world region and country. For each world region, the three countries contributing the most termites to interceptions are listed, with the two most commonly intercepted non-native termite species also noted.

<b>World Region</b>	<b>Unique species</b>	<b>Interceptions to species/total<sup>1</sup></b>	<b>Locality (interceptions to species/total interceptions)</b>	<b>Species (interceptions)</b>
Central America	19	172/256 (67.2%)	Honduras (46/71)	<i>Nasutitermes corniger</i> (20) <i>N. ephratae</i> (12)
			Costa Rica (30/49)	<i>N. corniger</i> (10) <i>N. ephratae</i> (8)
			Panama (35/43)	<i>N. corniger</i> (12) <i>N. ephratae</i> (9)
South America	30	107/157 (68.2%)	Brazil (30/45)	<i>Coptotermes testaceus</i> (6) <i>N. corniger</i> (6)
			Colombia (26/31)	<i>Heterotermes tenuis</i> (7) <i>N. corniger</i> (4)



<b>World Region</b>	<b>Unique species</b>	<b>Interceptions to species/total<sup>1</sup></b>	<b>Locality (interceptions to species/total interceptions)</b>	<b>Species (interceptions)</b>
			Chile (17/19)	<i>Neotermes chilensis</i> (12) <i>Porotermes quadricollis</i> (5)
Caribbean	18	124/142 (87.3%)	Bahamas (22/24)	<i>N. rippertii</i> (11) <i>Cryptotermes brevis</i> (3)
			Jamaica (16/17)	<i>H. convexinotatus</i> (5) <i>N. corniger</i> & <i>N. nigriceps</i> (3)
			Puerto Rico (13/16)	<i>N. corniger</i> (7) <i>Cr. brevis</i> (3)
Asia	22	72/140 (51.4%)	China (13/27)	<i>C. formosanus</i> (8) <i>Cr. brevis</i> (2)
			Philippines (11/21)	<i>N. luzonicus</i> (3) 8 others (1)
			Japan (15/18)	<i>C. formosanus</i> (8) <i>Reticulitermes speratus</i> (5)

<sup>1</sup>Interceptions to species refers to interceptions identified down to the species level, while the total reflects all interceptions originating from a country, including interceptions identified to the species level, or down to only the genus or family levels (see Results)

### 2.3.1 Sources & destinations of termites

Of the eight world regions defined in the study, Central America (256), South America (157), the Caribbean (142) and Asia (140) were the greatest contributors of non-native termites to the US (Fig. 1b; Table 1). Interceptions originated from 88 countries across the world (Fig. 2a), arriving at ports of entry in 29 different states and Washington DC (Fig. 2b). By country, the top five exporters of termites to the US were Honduras (71), Costa Rica (49), Mexico (48), Brazil (45) and Panama (43), and the five states intercepting the most termites were Florida (232), New York (104), California (95), Louisiana (82) and

Texas (70). Additionally, a geographical bias in interceptions appears to be present within the US. In the southern and eastern portions of the US, interceptions primarily originated from the Caribbean, Central America and South America, while interceptions in the west primarily originated from Asia, North America (Mexico) and Oceania (Fig. 3). As the results above allude to, a significant negative relationship was found between the distance from the US to the originating country and the overall number of interceptions from that country ( $r = -0.36$ ;  $p = 0.0014$ ; Fig. 4a). Also, a significant positive correlation was found between the overall number of interceptions from a country and the number of unique non-native termite species ( $r = 0.86$ ;  $p < 2.2 \times 10^{-16}$ ; Fig. 4b), suggesting increased

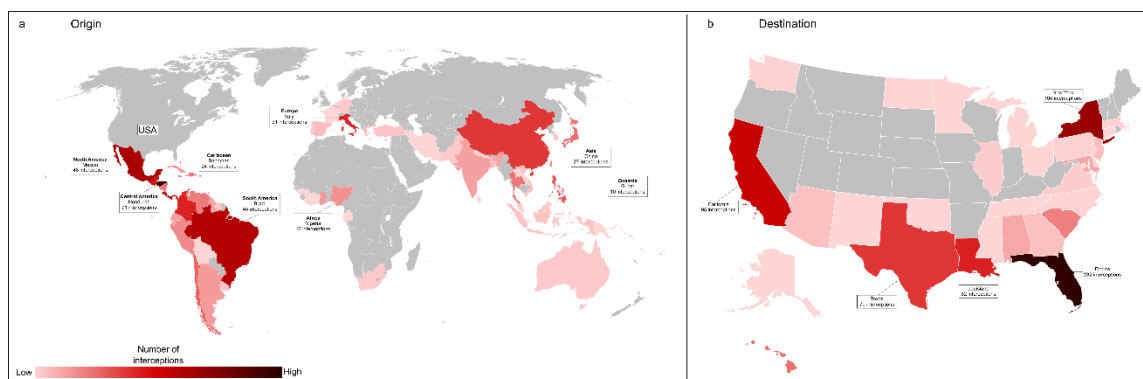


Figure 2: (a) Origin of non-native termites intercepted at US ports of entry, with the country supplying the most termites in each of the eight world regions highlighted and (b) US interceptions of non-native termites grouped by state, with the five states intercepting the greatest number highlighted

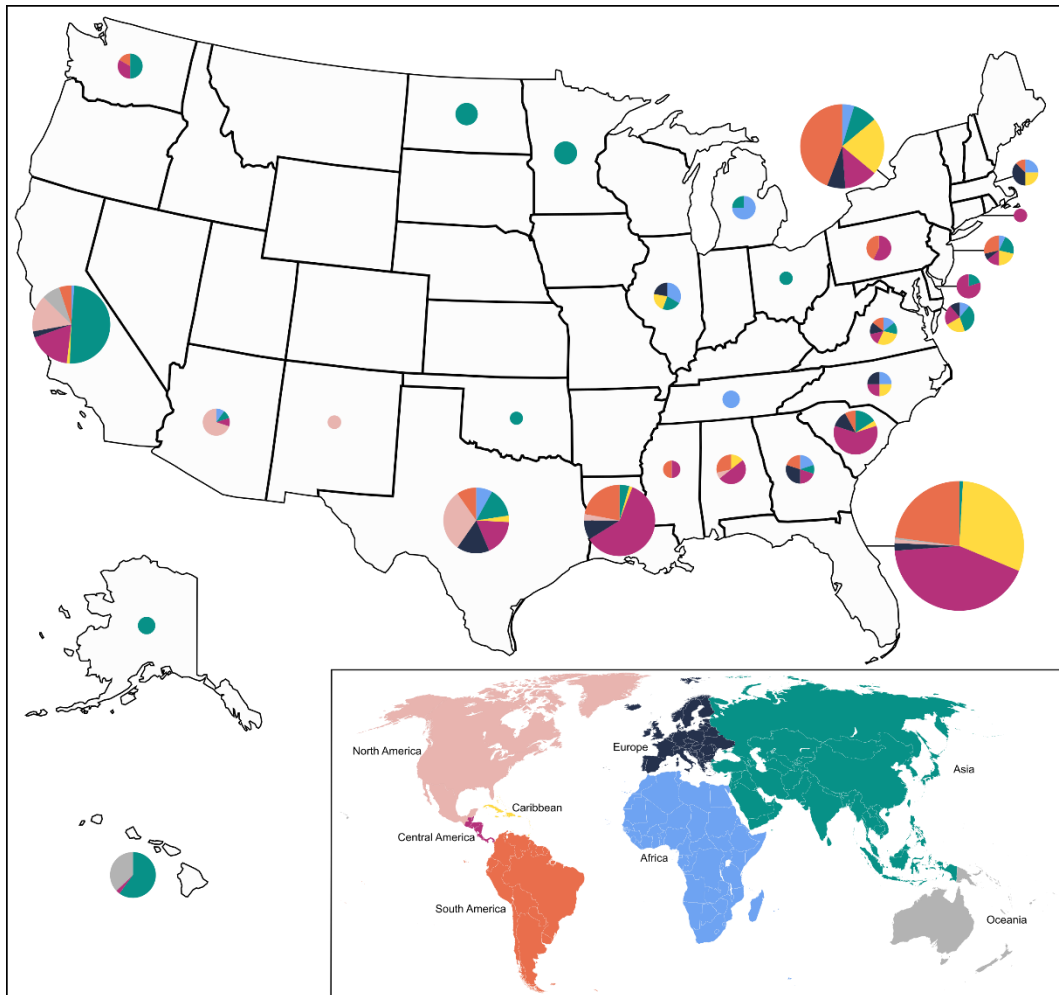


Figure 3: The proportion of interceptions in each state from the eight world regions defined in the study (shown in the bottom right); the size of each pie chart is proportional to the number of interceptions received

diversity from a country is most likely a result of increased interceptions rather than a reflection of the diversity of termite fauna existing within a country.

Trade was both individually and interactively evaluated to elucidate its possible connection with interceptions. First, the three trade categories (overall, wood and vegetable) were plotted against interceptions, and after the removal of any outliers, only vegetable trade remained significant ( $r = 0.46$ ;  $p = 3.3 \times 10^{-5}$ ; Fig. 4c). A GLM was then constructed to test for an interaction effect between trade and distance, and as the two

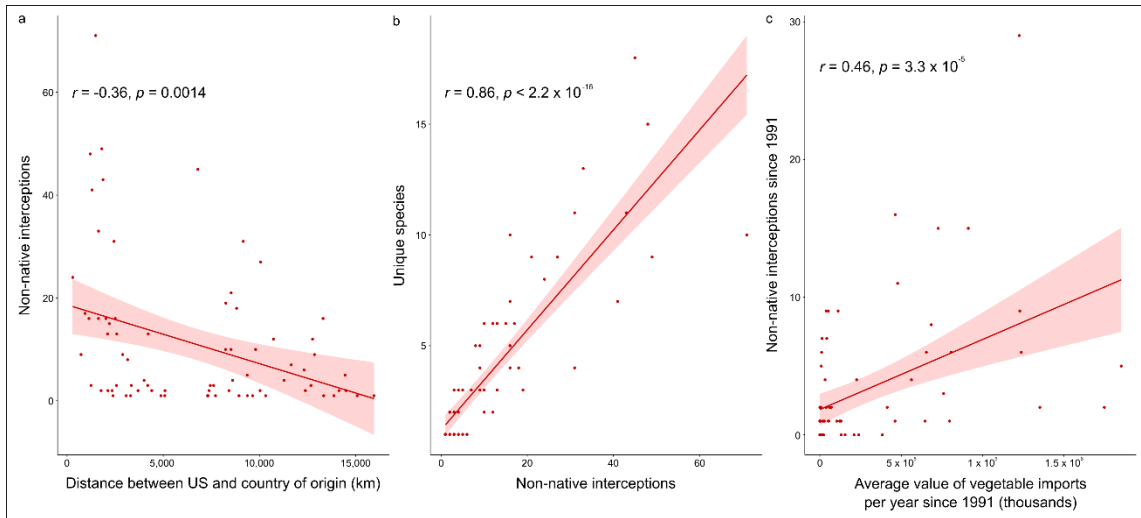


Figure 4: (a) The negative significant relationship between the distance from the US to the originating country and the overall number of interceptions from that country; (b) positive significant relationship between the number of interceptions from a country with the number of unique termite species it exports; and (c) positive significant relationship between the average value of vegetable imports and the number of interceptions since 1991

individual trade categories better correlated with interceptions than overall trade, overall trade was excluded from the model. However, distance and vegetable trade remained the only significant factors, with no interactions significant.

### 2.3.2 Propagule pressure & bridgehead interceptions

The binomial GLM identified a significant association between the number of times a non-native termite was intercepted and the likelihood of its establishment, with increased interceptions resulting in a higher probability of establishment ( $\chi^2 = 16$ ;  $df = 73$ ;  $p = 7.8 \times 10^{-5}$ ; Fig. 5a). Additionally, the Poisson GLM's revealed that species inhabiting more world regions were more likely to be intercepted at US ports of entry ( $\chi^2 = 631$ ;  $df = 73$ ;  $p < 2.2 \times 10^{-16}$ ; Fig. 5b), and also more likely to be secondarily intercepted ( $\chi^2 = 407$ ;  $df$

= 73;  $p < 2.2 \times 10^{-16}$ ; Fig. 5c). Overall, these results suggest that greater propagule pressure increases the chance of a successful invasion.

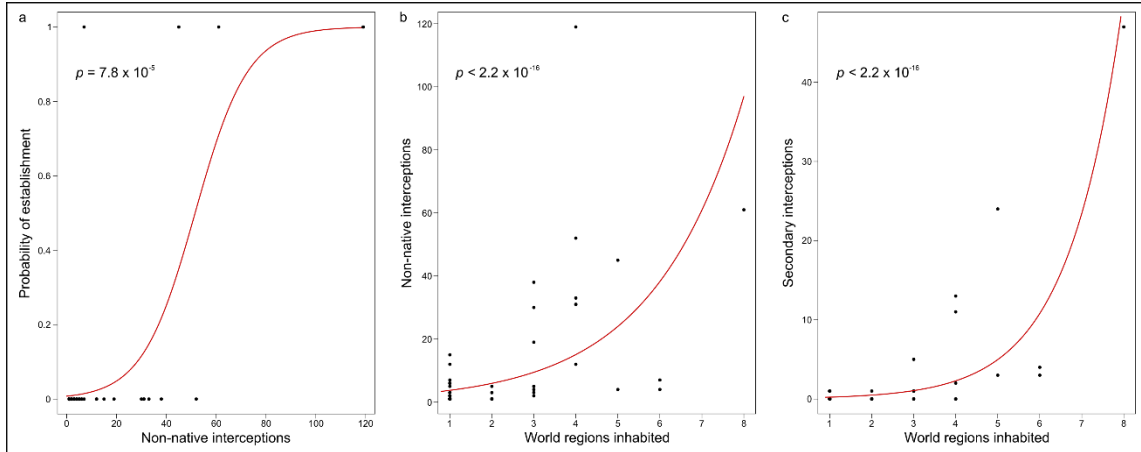


Figure 5: The relationship between (a) the number of interceptions of a species with its establishment probability, modeled using a binomial GLM; (b) the number of world regions a species inhabits with its interceptions, modeled using a Poisson GLM; and (c) the number of world regions a species inhabits with its secondary interceptions, modeled using a Poisson GLM. For (a), the black dots at 0 represent species which have not become established within the US, while the black dots at 1 represent species established within the US

We found 67 interceptions that originated from Hawaii to add to the above interceptions for our bridgehead analysis, with 64 identified down to species—the majority belonging to *Cr. brevis* (36) and *C. formosanus* (21). Evans et al. (2013) reported that 25 termites are both non-native to the US and established outside of their native range across the world, of which 12 were intercepted at US ports of entry. Of these 12, 11 were deemed pests of significant economic importance by Rust and Su (2012) and therefore could be classified as invasive for this study as potentially harmful to their invaded environments. Ideally, we would have compared invasive termites to non-invasive species, but as there was only a single non-invasive species, such a comparison would be inappropriate. Instead, we compared the number of interceptions that were primary versus

those that were secondary among the alien species to determine the prevalence of bridgehead invasions across the alien termites. Overall, 48% of the interceptions of alien species were primary and 46% were secondary (Fig. 6a), with secondary interceptions largely originating from two regions: Hawaii (37%) and the Caribbean (27%) (Fig. 6b). There were also significant differences among these 12 species in their proportion of

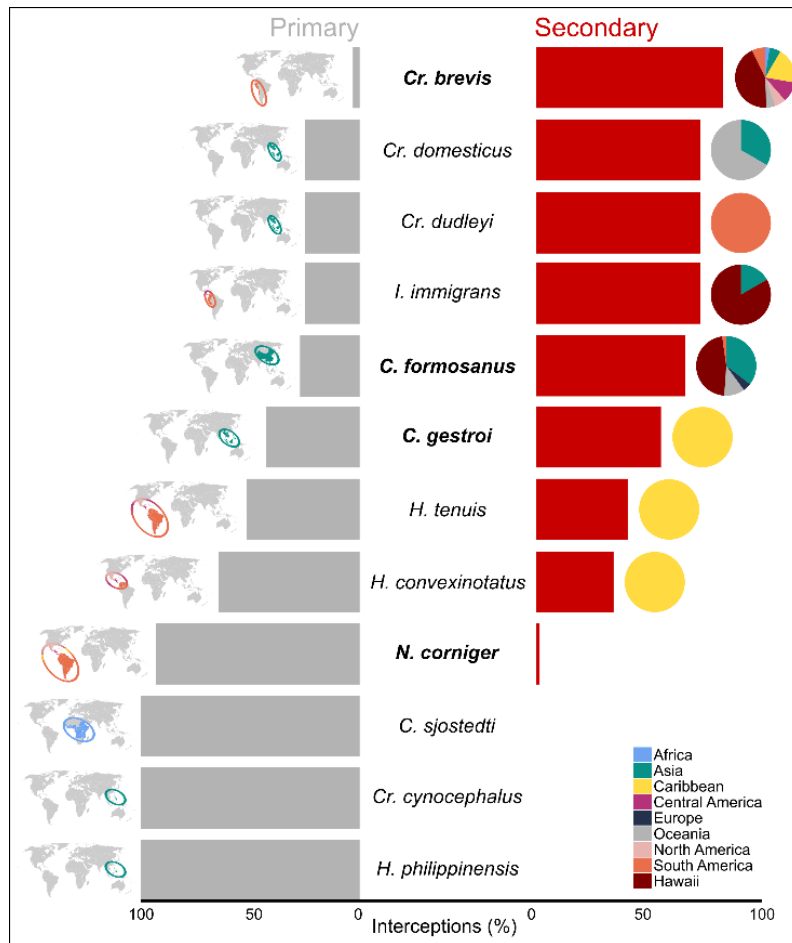


Figure 6: The primary and secondary interception percentages of alien termites from this study, with the primary percentage bar to the left of the species name and the secondary percentage bar to the right. The maps to the left of the primary interception bars represent the native world region of each termite, with the countries the termite is native to colored. The pie charts to the right of the secondary interception bars represent the interception makeup of species secondarily intercepted at least 25% of the time. The color code indicates the world region of origin for the secondarily intercepted species. The bolded names represent species already established within the US. The percentage of unknown interceptions (*i.e.*, port of origin unknown) is not pictured, so bars may not add up to 100%

primary versus secondary interceptions ( $\chi^2 = 201$ ;  $df = 11$ ;  $p < 0.001$ ), indicating certain species more frequently spread through bridgeheads than others. These differences appear linked with the geographical distribution of the alien species, with those inhabiting more world regions more likely to be secondarily intercepted ( $\chi^2 = 110$ ;  $df = 10$ ;  $p < 2.2 \times 10^{-16}$ ).

## **2.4 Discussion**

Given their prominent pest status within the urban environment, termites are associated with substantial negative economic consequences. Continued urbanization (Seto, Fragkias, Güneralp, & Reilly, 2011) and globalization (Hulme, 2009) of the planet threaten to exacerbate these consequences across the world through expansion of their primary pest habitat and increased ability to disperse between these habitats, respectively. Additionally, the cryptic nesting habits of termites create difficulty in both eradication and assessing successful eradication once they become established (Evans et al., 2013; Thorne, Vargo, Adams, & Johnson, 2019); in fact, only two known successful eradications of introduced termites have ever occurred (Bain & Jenkin, 1983; Mitchell, 2002). Furthermore, as re-introduction and re-establishment may be likely without sufficient knowledge of invasion pathways, identifying these pathways and important source locations of invaders are necessary to increase likelihoods of both invasion prevention and early detection (Finnoff et al., 2007; R. P. Keller et al., 2007; Reaser et al., 2020). Our results indicate a geographical bias is present in the number of interceptions, as the three closest geographic regions to the US were also the three regions from where non-native termites originated the most. Also, as evidenced in Figure 2, different regions of the US

do not receive the same proportions of non-native termites from the rest of the world, suggesting each state has a unique intake rate based on their individual trading profile. Therefore, shipments from our geographic neighbors require heightened vigilance, and each state (or region) should develop its own risk management plan for potential termite invaders, rather than a one-size-fits-all approach blanketing the entire US.

The 28 alien termite species collectively share three traits: 1) wood-eaters, 2) wood-nesters and 3) readily generate secondary reproductive (Evans et al., 2013). The first two shared characteristics strongly suggest the importance of trade as a dispersal mechanism, given wood is a commonly traded commodity and a heavily utilized packing material (*e.g.*, crating, pallets). Indeed, we found overall trade to be a significantly positive factor in explaining the number of non-native termite interceptions coming from a country; however, this correlation was only slightly positive, perhaps due to regional biases in trading profiles (see above). The third characteristic refers to the eusocial nature of termites, in that a reproductive division of labor exists within colonies so that only certain members of the colony reproduce (Vargo, 2019). Secondary reproductives are members of the colony which develop and become reproductively active within a pre-existing colony. In lower termites (*e.g.*, Kalotermitidae, Rhinotermitidae), secondary reproductives develop from nymphs or workers (Myles, 1999), and in rarer cases soldiers (Thorne, Breisch, & Muscedere, 2003). In higher termites (*e.g.*, Termitidae), they can develop from nymphs or alates (adultoids) (Noirot, 1985). These secondary reproductives are able to supplement the reproductive output of the primary reproductives (*i.e.*, the founding queen and king), or replace their output in the event of their death (Vargo, 2019). Taken together,



termite species that live and nest in wood, as well as readily generate secondary reproductives have great invasive potential, as any piece of wood serving as a nest or foraging site can potentially be a viable propagule (Lockwood et al., 2005; Simberloff, 2009), provided individuals are present who have the potential to develop into secondary reproductives. Worryingly, groups of termites that share these three traits make up the bulk of the interceptions, including Kalotermitidae, *Coptotermes* spp., *Heterotermes* spp., *Nasutitermes* spp. and *Reticulitermes* spp. (Myles, 1999). Given the significant trends for species inhabiting more world regions to be introduced more often leading to increased propagule pressure, more non-native termites threaten to become established within the US in the near future. Termites not yet established within the US that share the three invader traits and had a high number of interceptions include *N. ephratae* (52 interceptions/4 world regions), *K. flavicollis* (38 interceptions/3 world regions) and *N. nigriceps* (33 interceptions/4 world regions).

The recently termed ‘bridgehead effect’ also appears to be playing a significant role in the dispersal of some invasive termites to the US. For instance, *Cr. brevis* appears to disperse primarily via bridgeheads; it was intercepted from seven of the eight world regions and 96.5% (n = 83) of its known interceptions (*i.e.*, when originating port could be determined) were secondary. *C. formosanus* was also commonly intercepted from bridgeheads, with interceptions originating from four of the eight world regions and 71% (n = 45) of its known interceptions were secondary. However, other species mainly originated from their native countries, chiefly *N. corniger*, with 98.2% (n = 111) of its known interception coming from its native range. To a large extent, this was influenced

by its vast native range which encompasses four world regions, stretching from Brazil to southern Mexico and including the Caribbean (Evans et al., 2013). Therefore, bridgeheads could be expected to play a larger role for species with narrower native ranges, as they gain increased access to human transport networks through establishment in other countries (Banks et al., 2015; Hulme, 2009; Westphal et al., 2008).

The status of the bridgehead effect as an important feature of future studies on invasion processes and dynamics was recently noted (Ricciardi et al., 2017), and several mechanisms have been suggested as the main driving force behind the effect (Bertelsmeier & Keller, 2018). One popular explanation put forward in many studies (De Kort, Mergeay, Jacquemyn, & Honnay, 2016; Grapputo, Boman, Lindstrom, Lyytinen, & Mappes, 2005; Lombaert et al., 2010; N. Miller et al., 2005; Zepeda-Paulo et al., 2010) suggests that introduced populations acquire traits that confer greater invasiveness, making them more likely than their native counterparts to further disperse throughout the world. However, non-evolutionary mechanisms could also explain the propensity of introduced populations to become sources of future invasions. For one, many species attain far greater densities in their invaded ranges than in their native range (Elton, 1958; Parker et al., 2013; Torchin, Lafferty, & Kuris, 2001), for several possible reasons (*e.g.*, enemy release, increased resource availability (Catford et al., 2009)). Increased densities will subsequently lead to increased opportunities for the species to spread again. Two, as the spread of alien species is often linked with human transport and trade networks, introduced populations will likely be located in prime locations to further spread (*i.e.*, transport hubs) (Banks et al., 2015; Westphal et al., 2008). Increased globalization has facilitated an increased connectedness

of hubs around the world (Banks et al., 2015), possibly enabling bridgehead populations to piggyback off this network. This explanation seems most likely for termites, since a positive association between trade and interceptions was identified, as well the status of wood as a global trade commodity and packing material. Given the lack of direct empirical evidence for adaptive evolution of introduced populations (*i.e.*, evolution of invasiveness), the most influential mechanism driving the bridgehead effect cannot be precisely known (Bertelsmeier & Keller, 2018). Regardless of the mechanism, US port of entry interceptions indicate that as many as eight alien termites may have utilized bridgeheads to successfully invade the US.

## **2.5 Conclusion**

Here, we performed a quantitative assessment of US port of entry interceptions for non-native termites, unlocking insights about their global dispersal patterns. With almost 100 years' worth of data, we identified factors which also play crucial roles in the general invasion dynamics of other species, including geographical distance, propagule pressure and bridgeheads. Specifically, interceptions were found to originate more often from world regions closest to a state, increased propagule pressure results in a higher likelihood of becoming intercepted and thereby established, and bridgeheads likely play a role in termite invasions to the US. By shedding light on the influential factors affecting termite spread to the US, this study provides further evidence towards the importance of bridgeheads and increased propagule pressure as significant drivers of global invasion rates in general. Moreover, direct empirical work is recommended in the field of bridgehead biology to determine if true adaptive evolution is the main driving force behind

invasions generated from bridgehead populations. As introductions arising from these populations are more probable than introductions originating from native populations (Bertelsmeier & Keller, 2018), careful surveillance of bridgeheads is warranted.

### 3 BRIDGEHEAD EFFECT AND MULTIPLE INTRODUCTIONS SHAPE THE GLOBAL INVASION HISTORY OF *COPTOTERMES FORMOSANUS*<sup>2</sup>

#### 3.1 Introduction

Biological invasions are a defining feature of the Anthropocene (Capinha et al., 2015; Lewis & Maslin, 2015), a byproduct of globalization where human transport and trade have facilitated the transfer of organisms throughout the world (Banks et al., 2015; Hulme, 2009; Meyerson & Mooney, 2007). Remarkably, the accumulation of introduced species worldwide has yet to reach saturation (Seebens et al., 2017), and the harmful effects these invasive species have on the communities and ecosystems they invade cannot be overstated (Bellard, Cassey, & Blackburn, 2016; Simberloff et al., 2013). The success of invasive species in their new environments has often been considered paradoxical, as they are able to persist and outcompete native, locally adapted species despite experiencing bottlenecks that reduce their genetic diversity, and thereby possibly their fitness (Allendorf & Lundquist, 2003; Schrieber & Lachmuth, 2017). However, there is growing evidence that genuinely paradoxical invasions are not so common (Estoup et al., 2016), as the loss of genetic diversity in invasive populations is less frequent and less intense than previously expected (Bossdorf et al., 2005; Roman & Darling, 2007; Uller & Leimu, 2011). In addition, low genetic diversity in introduced populations measured at neutral markers

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<sup>2</sup>Reprinted with permission from “Bridgehead effect and multiple introductions shape the global invasion history of a termite” by Blumenfeld, A. J., Eyer, P.-A., Husseneder, C., Mo, J., Johnson, L. N. L., Wang, C., Grace, J. K., Chouvenec, T., Wang, S., & Vargo, E. L., 2020. *Communications Biology*, 4(1), 196, Copyright [2021] by Springer Nature.

(*e.g.*, microsatellites) does not necessarily correlate with low variation in ecologically relevant traits (Estoup et al., 2016). Indeed, quantitative variation is usually lost at a reduced rate during invasions compared to diversity at molecular markers (Dlugosch & Parker, 2008), and pre-adaptive traits that confer success in the invaded range may render reduced genetic diversity inconsequential (Facon et al., 2006; Hufbauer et al., 2012). Furthermore, the degree of genetic loss may differ under distinct invasion histories. The amount of genetic diversity brought to the introduced population increases with the size of the propagule and additional re-introductions during multiple introduction events from the same or genetically distinct source populations (Facon et al., 2006). In rare cases, genetic diversity might be higher within an introduced population than its native, source populations (Facon, Pointier, Jarne, Sarda, & David, 2008). Sometimes, introductions originate from an already invasive population rather than a native population—a phenomenon known as the ‘bridgehead effect’ (Ascunce et al., 2011; Bertelsmeier et al., 2018; Lombaert et al., 2010). This may lead to an extreme loss of diversity, as subsequent introductions arise from an already depauperate introduced population (Bertelsmeier & Keller, 2018). Investigating patterns of genetic diversity within the native and introduced populations of a species may provide insights into past demographic events and allow for reconstructing its invasion history (Cristescu, 2015; Estoup & Guillemaud, 2010).

The Formosan subterranean termite *Coptotermes formosanus* (Shiraki) is currently recognized by the IUCN as one of the 100 worst invasive species in the world (Lowe et al., 2000), establishing invasive populations in Japan, Hawaii, and the southeastern United States (Jiasi Wang & Grace, 1999). Like all invasive termites, this species nests in and

feeds on wood, thereby increasing its chance of being transported through merchandise trade (Evans et al., 2013). *Coptotermes formosanus* is thought to be native to eastern Asia, though its exact origin remains unclear. It has long been suspected to originate from the vicinity of Formosa (*i.e.*, Taiwan), where the type specimen was described (Shiraki, 1909). A southern China origin was also suggested due to the presence of termitophilous beetles associated with *C. formosanus* colonies (Kistner, 1985); however, these beetles were later found to also occur within colonies in Japan (Maruyama & Iwata, 2002; Maruyama, Kanao, & Iwata, 2014). This southern China origin was previously supported by the high diversity of *Coptotermes* species present (24 species (G. Li, 2000)), but the recent identification of at least nine synonymized species of *C. formosanus* in the region undermines this hypothesis (Chouvenc, Li, et al., 2016). Recent phylogeographic studies using mitochondrial DNA (mtDNA) have also struggled to determine the origin of this species, as the variation of this marker is extremely low. These studies found either no variation between samples from Taiwan, China, and Japan (T. R. C. Lee et al., 2015; Yeap et al., 2009), or extremely low levels (Austin et al., 2006; Fang et al., 2008; H.-F. Li et al., 2009). Even the complete mitochondrial genome reveals more than 99.9% similarity, with only a six nucleotide difference between three Japanese islands (Tokuda, Isagawa, & Sugio, 2012). Overall, these studies have failed to conclusively identify the origin of the species within East Asia; however, they all suggest that the Chinese, Taiwanese, and Japanese populations are closely related, hinting at an early human-mediated movement of the termite throughout this region (Fang et al., 2008; T. R. C. Lee et al., 2015; H.-F. Li et al., 2009; Vargo, Husseneder, & Grace, 2003; Yeap et al., 2009).

Several studies have also attempted to reconstruct the invasion history of *C. formosanus*. However, these studies have similarly suffered from the lack of genetic variation in the mtDNA present within native populations (Austin et al., 2006; Broughton & Grace, 1994; Korman & Pashley, 1991; Jiasi Wang & Grace, 2000). As a result, no mtDNA variation was found in Hawaii (Broughton & Grace, 1994), and only 0–0.3% of variation was found on three mtDNA genes despite global sampling, with clades separated by a maximum of 3 bp differences (H.-F. Li et al., 2009). Although the lack of mtDNA variation hampers the reconstruction of the invasion history of this species, several studies have found that introduced populations do belong to the same clade, suggesting that US populations of *C. formosanus* arise from at least two introduction events out of eastern Asia (Austin et al., 2006; Gentz, Rubinoff, & Grace, 2008; Korman & Pashley, 1991; Vargo et al., 2006; Jiasi Wang & Grace, 2000). Based on microsatellite markers, at least five different sources of introduction have been suggested (Husseneder et al., 2012), with high similarity between the populations of Hawaii, Louisiana, and North Carolina (Vargo et al., 2006). This finding suggests that these introduced populations either stem from a common native source population or that the mainland US population originated from a Hawaiian bridgehead. Conversely, strong differences in cuticular hydrocarbon signatures between Hawaiian and continental US samples of *C. formosanus* suggest that Hawaiian populations may not be the source of the continental US populations (Haverty, Nelson, & Page, 1990). Overall, despite many studies attempting to elucidate this termite's path out of eastern Asia, its exact invasion history remains unresolved.



In this study, we aimed to determine the origin(s) and the number of introduction events of *C. formosanus* out of eastern Asia and into the US. We sampled this species in both its native and introduced ranges and used double digest restriction-site associated DNA sequencing (ddRADseq (Peterson, Weber, Kay, Fisher, & Hoekstra, 2012)) to obtain markers of high resolution (*i.e.*, single-nucleotide polymorphisms, or SNPs). We first conducted population structure and phylogenetic analyses of the global *C. formosanus* population to assess genetic structure within its native range and determine the genetic relationship between native and introduced populations. Second, we used approximate Bayesian computation (ABC) to decipher its worldwide routes of invasion. Finally, we investigated introduction-induced effects on population demography, such as population bottlenecks, expansions, migration, and admixture, to assess the consequences of the invasion on the global genetic patterns of this species.

## **3.2 Methods**

### *3.2.1 Sample collection and molecular methods*

*Coptotermes formosanus* colonies were sampled in both their native and introduced ranges, with workers stored in 100% ethanol for subsequent sequencing. In the native range, colonies were sampled across thirteen localities in mainland China (southcentral China—Beihai, Changsha, Guilin, Hainan, Hengyang, Nanning, and Xinyu; eastern China—Fuzhou, Hangzhou, Jieyang, Lufeng, Wenzhou, and Xiamen), Hong Kong, and Taiwan. In the introduced range, colonies were sampled in mainland Japan and Okinawa, as well as in Hawaii, Texas, Louisiana, Mississippi, and Florida (Appendix A). Total genomic DNA of 359 workers was extracted following a modified Genra Puregene

extraction method (Gentra Systems, Inc., Minneapolis, MN, USA), then libraries were prepared and sequenced at the Texas A&M AgriLife Genomics and Bioinformatics Service facility following the protocol of Peterson et al.<sup>48</sup>. Briefly, genomic DNA was first digested with the restriction enzymes SphI and EcoRI. Following restriction digestion, each sample was ligated with unique indexed adapters. Then, samples were PCR amplified with iProof™ High-Fidelity DNA Polymerase (Bio-Rad), and purified using AMPure XP beads (Beckman Coulter Inc.) to make the ddRADseq library. Each library pool was size selected to a range of 300–500 bp using the BluePippin system (Sage Science Inc.). Quantity and size distribution were assessed using the Qubit® 2.0 Fluorimeter (Life Technologies Corp.) and Bioanalyzer 2100 System (Agilent Technologies). Amplified fragment libraries were then pooled in equimolar amounts and sequenced on six lanes of an Illumina HiSeq 2500 machine to generate 150 bp pair-end reads. The sampling and library preparation/sequencing were performed prior to the beginning of my PhD career and therefore not conducted by me.

### *3.2.2 Raw read quality filtering and processing*

Raw sequences for each lane were examined separately to check for read quality and adapter contamination using FastQC v0.11.8 (Andrews, 2010), with reads of the two lanes then concatenated after ensuring no lane discrepancies (*i.e.*, R1's & R2's combined separately). Forward and reverse reads were assembled and SNPs were generated using the de novo pipeline of Stacks v.2.41 (Rochette, Rivera-Colón, & Catchen, 2019). The main parameters for the analysis were optimized following the r80 loci method (Paris, Stevens, & Catchen, 2017). Briefly, a representative subset of samples was taken from the

main dataset to run through the de novo pipeline under varying values of its most influential parameter (-M, the number of mismatches allowed between putative alleles), in order to identify the value that produced the greatest number of polymorphic loci found in 80% of the population. After parameter optimization, filtered reads were run through the de novo pipeline of Stacks, which built and genotyped the paired-end data, as well as called SNPs using the population-wide data per locus. Only SNPs present in at least half of the individuals in all populations were kept for downstream analyses. In addition, alleles at low frequency ( $<0.05$ ) and loci with high heterozygosity ( $>0.7$ ) were filtered out as these are likely byproducts of sequencing errors and paralogs (Benestan et al., 2016). Furthermore, SNPs with  $<5\times$  mean coverage and exceeding  $200\times$  mean coverage were filtered out using VCFtools v.0.1.15 (Danecek et al., 2011), to buffer against unlikely SNPs and avoid highly repetitive regions of the genome. To prevent linkage disequilibrium (LD) between SNPs from affecting the population structure and phylogenetic analyses, only one random SNP per locus was kept. All subsequent file format conversions were accomplished through PGDSpider v.2.1.1.5 (Lischer & Excoffier, 2011).

### 3.2.3 Genetic diversity and population structure

Genetic diversity (expected heterozygosity ( $H_E$ ), observed heterozygosity ( $H_O$ ), inbreeding coefficients ( $F_{IS}$ )), and population differentiation ( $F_{ST}$ ) indices for each locality were calculated in Stacks. Population structure among the 22 sampled locations was analyzed using three complementary approaches. First, population structure was assessed by estimating the most likely number of genetic clusters (*i.e.*,  $K$ ) in the dataset using

fastSTRUCTURE v1.040 (Raj, Stephens, & Pritchard, 2014). fastSTRUCTURE runs were parallelized and automated using Structure\_threader (Pina-Martins, Silva, Fino, & Paulo, 2017). Different values of  $K$  ranging from 1 to 22 were analyzed, and the best value was selected using the chooseK.py function from the fastSTRUCTURE package. Plots were created by Distruct v2.3 (Chhatre, 2019) (available at <http://distruct2.popgen.org>). Second, we used both a principal component analysis (PCA) and discriminant analysis of principal components (DAPC) to estimate clustering in the data. DAPC describes clusters in genetic data by creating synthetic variables (discriminant functions) that maximize variance among groups while minimizing variance within groups (Jombart, Devillard, & Balloux, 2010). We first performed the PCA, then ran the find.clusters clustering algorithm using the PCA results to infer the most likely number of genetic groups, as DAPC requires prior groups to be defined. The Bayesian information criterion was used to select the most likely number of genetic clusters. Finally, the function optim.a.score to identify the optimal number of principal components to inform the DAPC, as too few components could hinder discriminatory power between groups, while too many could lead to overfitting. Both the PCA and DAPC were run in R (R Core Team, 2020) through the adegenet package (Jombart, 2008). Third, we used the program fineRADstructure v0.3.2 (Malinsky, Trucchi, Lawson, & Falush, 2018) to infer population structure via shared ancestry among *C. formosanus* individuals. Modified from fineSTRUCTURE (Lawson, Hellenthal, Myers, & Falush, 2012), fineRADstructure is specifically designed for RADseq data, and does not require information about location of loci on chromosomes or phased haplotypes. Loci were first reordered according to LD, as strong LD combined

with unsorted loci could result in an overconfident clustering of individuals. A coancestry matrix was then constructed from the sorted loci and individuals were assigned to populations with a burn-in period of 100,000 and 100,000 Markov chain Monte Carlo iterations. Finally, a tree was constructed from the default parameters, and results were visualized in R through scripts provided with the program (available at [http://cichlid.gurdon.cam.ac.uk/fineRAD\\_structure.html](http://cichlid.gurdon.cam.ac.uk/fineRAD_structure.html)).

#### *3.2.4 Phylogenetic analysis*

Maximum likelihood (ML) phylogeny among *C. formosanus* individuals was inferred using RAxML v8.2.12 (Stamatakis, 2014). We applied an acquisition bias correction to the likelihood calculations as the alignments were composed exclusively of SNPs (Leaché & Oaks, 2017), removing all invariant sites in the alignments with the Phrynomics R script (available at <https://github.com/bbanbury/phrynomics/>). We then conducted a rapid bootstrap analysis and search for the best-scoring maximum likelihood tree using the extended majority rule (MRE)-based bootstopping criterion (Pattengale, Alipour, Bininda-Emonds, Moret, & Stamatkis, 2010) to determine an appropriate amount of bootstrap replicates. All searches were performed using the GTR+G nucleotide substitution model.

#### *3.2.5 Invasion history*

We inferred the invasion routes and colonization history of *C. formosanus* by selecting the most likely evolutionary scenario using ABC (Beaumont et al., 2002). The number of competing scenarios exponentially increases with the number of potential source populations and demographic events compared in the analysis (Estoup & Guillemaud,

2010; Lombaert et al., 2010), which requires considerable computational effort. Therefore, to more efficiently allocate this effort, we utilized a recently developed random forests (RF) machine learning tool to conduct model selection and parameter estimation (ABC RF (Pudlo et al., 2016)). ABC RF requires a considerably reduced number of simulated datasets compared with alternative methods, while also providing a more reliable estimate of the posterior probability for the best model. We also decreased the required computational effort by inferring the invasion history of *C. formosanus* through a step-by-step analysis (six different steps), which is commonly performed in ABC studies (Fraimout et al., 2017; Javal et al., 2019; Ryan et al., 2019). The mainland Japan and Okinawa populations were included in the ABC analysis as a member of the eastern Asia region given their strong clustering within the region in the population genetic and phylogenetic analyses (see “Results”). In addition, two localities (Xinyu and Mississippi) were excluded from all ABC steps as only one sample was available for each location. Briefly, the first step aimed at identifying which region(s) of the native range (*i.e.*, eastern Asia, southcentral China, or an admixture of both) have contributed to the introduction of *C. formosanus*, with the introduced US range pooled as a single population. The second and third steps aimed at determining which region(s) of eastern Asia (*i.e.*, only the Hong Kong region, only the other localities within eastern Asia, or an admixture of both) played a role. The fourth and fifth steps tested for the origin of the Hawaiian population and the possibility of a bridgehead effect in Hawaii; thus, Hawaii was analyzed separately from the mainland US. Finally, the sixth step considered the occurrence of a distinct introduction event to Florida.

Model simulations were first run in DIYABC v2.0 (Cornuet et al., 2014), with at least 10,000 simulations per model performed on 2000 randomly sampled SNPs for each of the steps above. Priors were set uniform for all model parameters and selected based on historical records. The timing of introduction events to the US was set to between 50 and 300 years ago, with the condition that the introduction in Hawaii (for steps 4–6) occurred prior to the introduction to the southeastern US, consistent with historical records (Husseneder et al., 2012). In addition, for all scenarios tested, the decrease in effective size of an introduced population was allowed to vary between 1 and 100 migrants, and the duration of the bottleneck set to vary between 0 and 50 years for each introduction event. The range of all other priors was adjusted by evaluating the posterior distributions of the preliminary simulated datasets, then setting the prior distribution as wide as possible while retaining biological meaning. All summary statistics included in the DIYABC software were used for each analysis, and both model selection and parameter estimation were performed through ABC RF (Pudlo et al., 2016; Raynal et al., 2018), available in the `abcrf` R package.

### 3.2.6 *Exploring changes in population sizes*

We inferred the demographic history of each locality by using Stairway Plot 2 (X. Liu & Fu, 2020) to investigate recent changes in population size (*e.g.*, bottleneck, expansion, admixture, etc.). Unlike traditional skyline plot methods for demographic inference which compute a likelihood for a whole sequence (X. Liu, Fu, Maxwell, & Boerwinkle, 2010), Stairway Plot 2 instead calculates the composite likelihood of a given SNP frequency spectrum (SFS) (X. Liu et al., 2010; Nielsen, 2000). This method uses the expected

number of mutation(s) per base pair to measure time and  $\theta$  per base pair to measure population size ( $\theta = 4N_e\mu$ , where  $N_e$  is the effective population size and  $\mu$  is the mutation rate per generation). The full catalog of SNPs was retained for this analysis; however, only SNPs with no missing data (by population) were used in the SFS calculations due to the difficulty of integrating missing data when modeling the SFS under coalescent approaches. Folded SFSs for each locality were generated by the `vcf2sfs` R script (S. Liu, Ferchaud, GrønkJær, Nygaard, & Hansen, 2018).

### **3.3 Results**

The 359 samples yielded 0.16–43.7 million paired reads per individual, with an average of 12.9 million reads. Thirty-four individuals had a high amount of missing data (*i.e.*,  $\geq 30\%$ ), and were thus removed from the dataset. The final dataset contained 22,229 polymorphic loci and 33,601 SNPs for 325 individuals from the 22 populations, with an average coverage of 44 $\times$  and 6.8% of missing data. To prevent linkage from affecting the population structure and phylogenetic results, only one random SNP per locus was kept.

#### *3.3.1 Population structure*

Substantial structure was observed among the *C. formosanus* populations from fastSTRUCTURE, with  $K = 15$  best explaining the structure in the data (Fig. 1a, b). At this value of  $K$ , 10 out of the 15 native populations represent distinct genetic clusters, with the five remaining localities mostly grouping with their geographic neighbors. Conversely, the five US states segregate into two genetic clusters, with one of the clusters comprised primarily of individuals from Florida. In addition, the populations of mainland Japan and Okinawa do not cluster together. Overall, when  $K = 15$ , the native and the invasive US



populations share no strong ties with one another, and  $K$  must be decreased to five before clustering between the two becomes apparent (Fig. 1b). At  $K=5$ , the entire US range clusters as one genetic entity, with its strongest tie to the native range being the Hong Kong region.

The PCA and DAPC revealed similar results to that of fastSTRUCTURE. For the PCA, samples from a given native locality mostly cluster together, suggesting that different native localities are genetically distinct from each other (Fig. 2a). Three main clusters are apparent: (1) southcentral China populations, (2) eastern China/Japan populations, and (3) introduced US populations (Fig. 2a). Again, Hong Kong and adjacent regions were most similar to the invasive US populations. The `find.clusters` function found strong support for 15 genetic clusters, with southcentral China populations again distancing themselves from eastern China/Japan and invasive US populations; however, the DAPC could not effectively distinguish between eastern China/Japan and US populations (Fig. 2b). Notably, the US invasive samples were grouped into two separate clusters: (A) one cluster including all US invasive populations (including some Florida samples, and excluding Mississippi), and (B) a second cluster including the other samples from Florida were split between the genetic clusters.

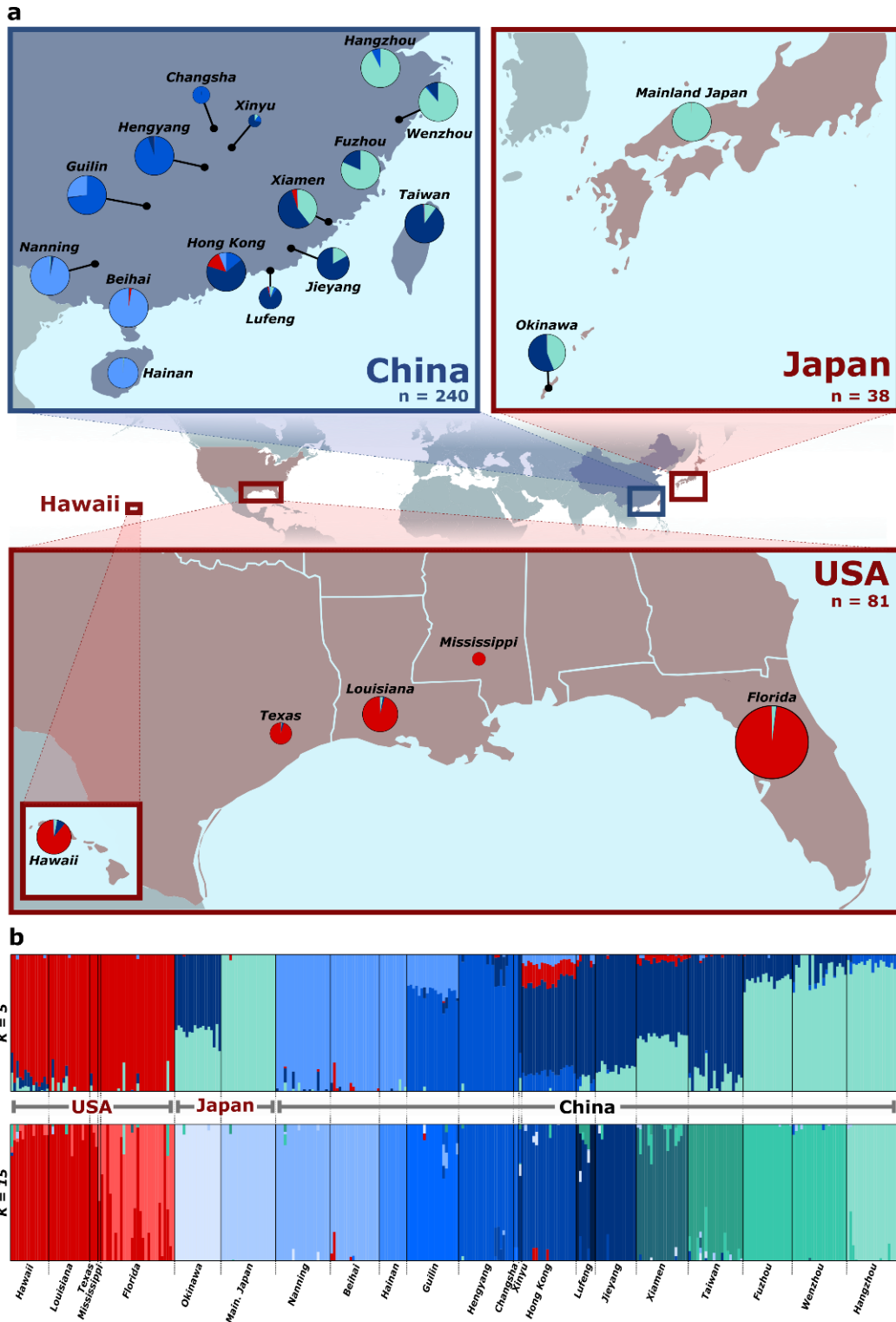


Figure 7: (a) Pie charts of fastSTRUCTURE assignments (for  $K=5$ ) for each sampling location of *C. formosanus* in its native and introduced range. Pie chart size is proportional to the number of samples. (b) fastSTRUCTURE assignment for each individual sampled for  $K=5$  and 15. Each color represents a distinct genetic cluster and each vertical bar represents an individual

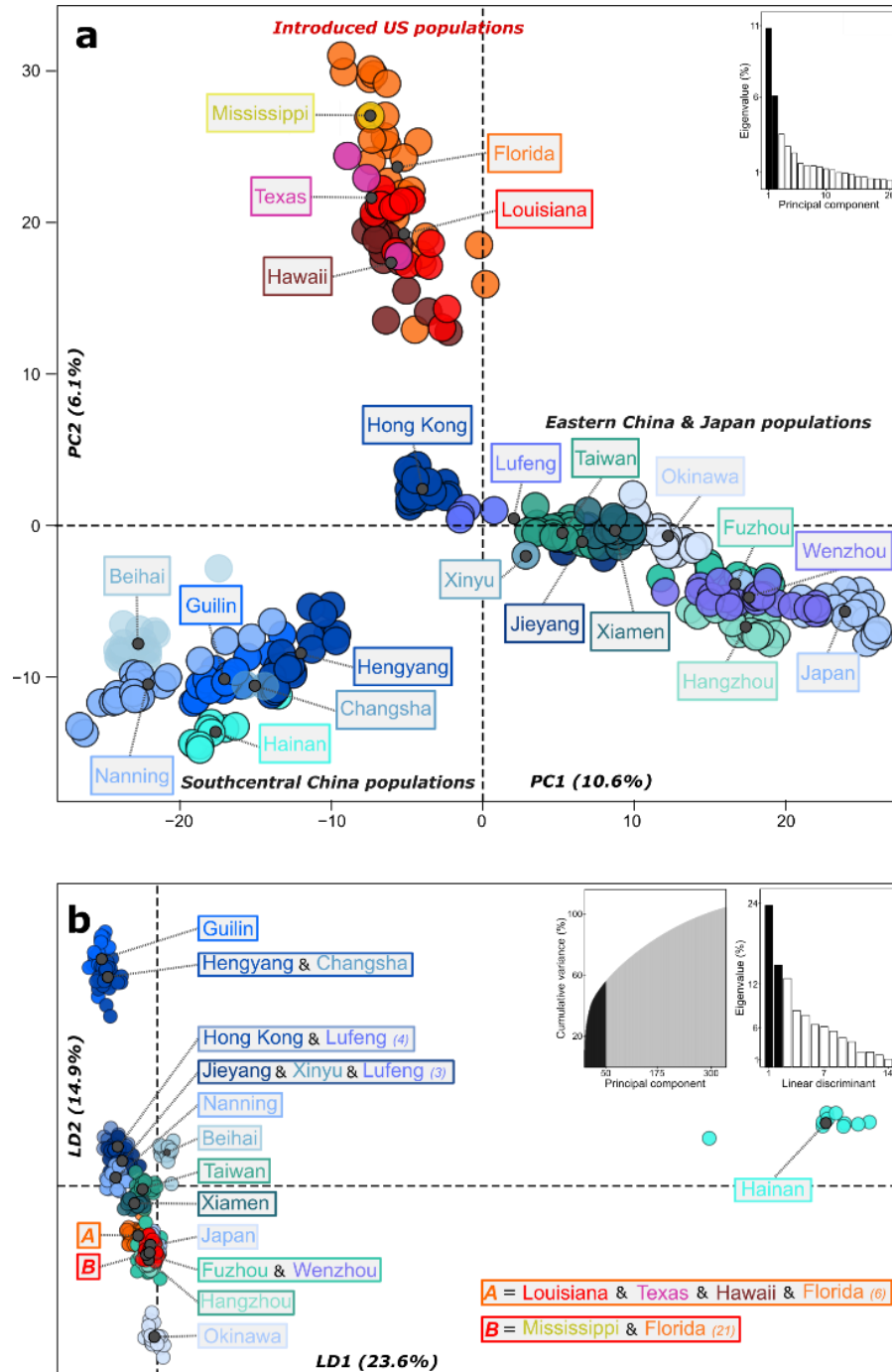


Figure 8: (a) Principal component analysis (PCA) of all *C. formosanus* individuals. The axes represent the first two principal components (PC). Only the first 20 PC's (out of 324) are shown in the eigenvalue inset graph, with the black bars representing the two plotted PCs. (b) Discriminant analysis of principal components (DAPC) with best support for  $K=15$  genetic clusters. The axes represent the first two linear discriminants (LD). The first inset graph shows the cumulative variation explained by the PCs, with only the PCs in the black shaded area utilized for the DAPC. The second inset graph depicts the eigenvalues for all linear discriminants, with the black bars representing the two plotted LDs

Similar patterns were identified using fineRADstructure with samples belonging to a given locality highly related to one another, indicative of the high population structure in the native range (Fig. 3). Notably, the entire US introduced population, including Hawaii, clusters together. This analysis also uncovered the three distinct clusters identified by the PCA analysis—two solely comprising geographically adjacent native regions

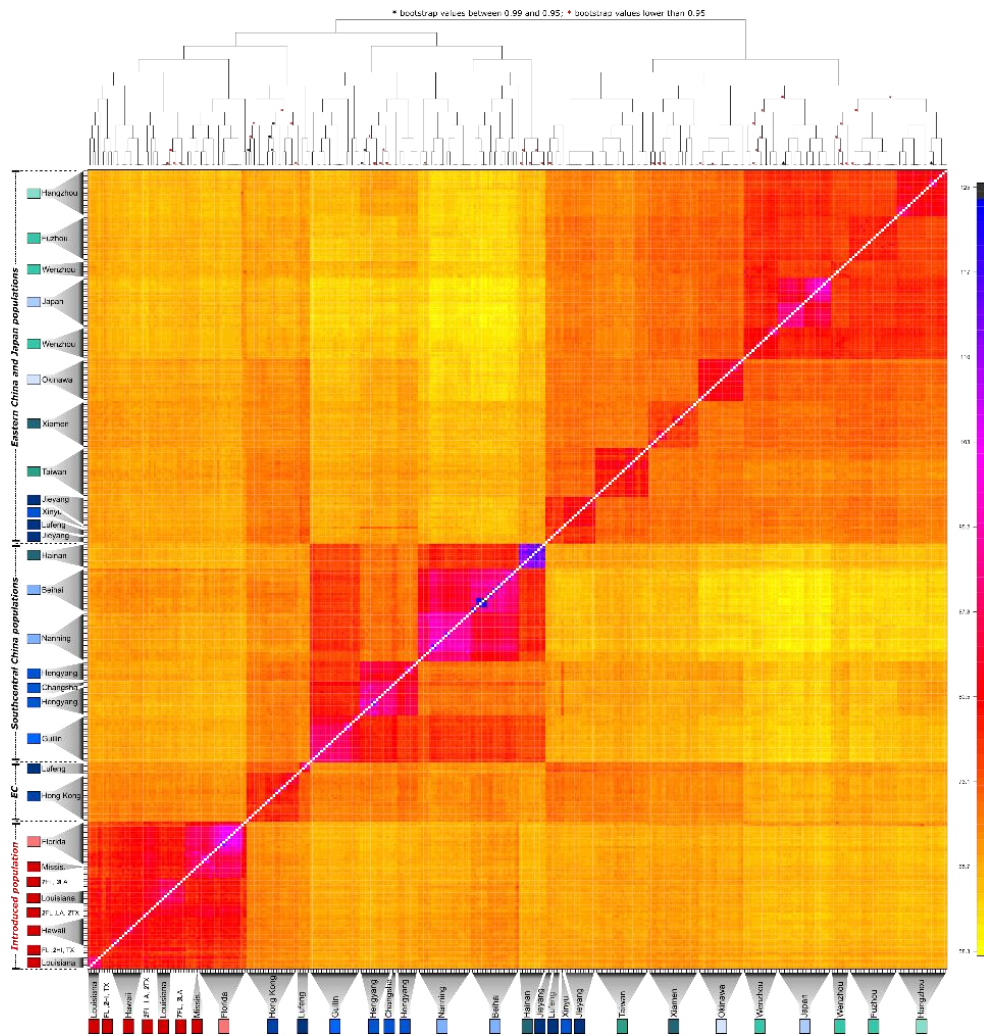


Figure 9: Co-ancestry matrix between each pair of individuals inferred using fineRADstructure. Each pixel represents the individual co-ancestry coefficient between two individuals. Low co-ancestry coefficient values are depicted by yellow colors, whereas high values are indicated by darker colors.

(southcentral China populations in one cluster and eastern China/Japan populations in the other) and one grouping the entire US invasive region with Hong Kong (Fig. 3).

### 3.3.2 Phylogenetic analysis

We constructed ML phylogenies for the full set of individuals using a further refined dataset of SNPs to determine if there were any strongly supported phylogenetic lineages. The 22,229 unlinked SNPs were stripped of invariant sites, leaving 21,542 SNPs to construct the tree. The MRE-based bootstrapping criterion was satisfied by 400 bootstrap replicates, with the best-scoring likelihood and majority rule extended consensus trees for the SNP dataset having a middling amount of support throughout the topology; however, the tree was consistent with results from the clustering analyses. First, the strong

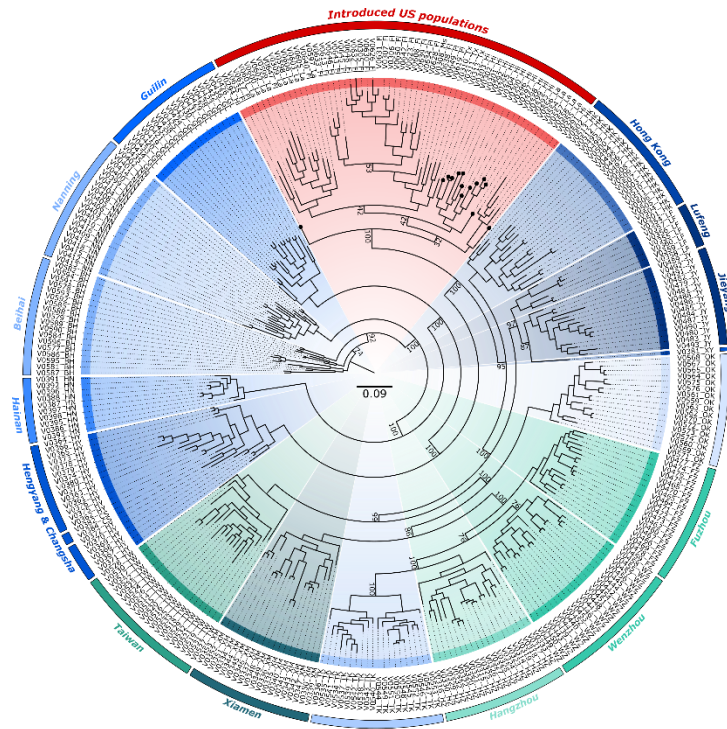


Figure 10: The sampling locations are colored according to their fastSTRUCTURE assignments ( $K = 15$ ). For clarity, bootstrap values are only indicated for major branching events. Samples from Hawaii are highlighted with a round tip

population structure in the native range is again apparent as almost every native population represents its own branch of the tree (Fig. 4). In addition, the invasive US populations fall out as a single clade and appear most closely related to Hong Kong (Fig. 4). Interestingly, samples from Hawaii cluster at the base of this “introduced” branch, despite the presence of five Louisianan samples segregated within the Hawaiian samples.

### 3.3.3 *Invasion history*

The first step of the ABC analysis found the most support for the introduced US population originating from admixture between southcentral and eastern Asia scenario (S1c; 375 out of 500 RF votes), rather than from southcentral China (46 RF votes) or eastern Asia (79 RF votes) exclusively. For the second step, the Hong Kong region (*i.e.*, Hong Kong, Jieyang, Lufeng, Okinawa, Taiwan, and Xiamen), was separated from eastern Asia (*i.e.*, sub-eastern Asia—Fuzhou, Hangzhou, mainland, and Wenzhou), with the introduced US population best explained by admixture between eastern China and Hong Kong (S2c) when considering only two-population admixture. However, this scenario was outvoted (only 31 RF votes) in the third step when the possibility of admixture between all three populations was considered, regardless of the first admixture event (S3b, c and d; 469 cumulative RF votes). In addition, sub-steps 2A and 2B confirmed the inclusion of the Japanese populations into the two eastern Asian sub-regions.

When the Hong Kong region was reintegrated within eastern Asia, the fourth step (analyzing Hawaii separately from the mainland US) was not conclusive, as two scenarios gained a similar number of RF votes. The first one suggested that Hawaii and the US mainland originated independently from eastern Asia and southcentral China, respectively

(S4c; 155 RF votes), while the other proposed that Hawaii results from admixture between eastern Asia and southcentral China, with the US mainland arising from a Hawaiian bridgehead (S4g; 136 RF votes). This discrepancy seemed to be driven by a split between most Florida samples and the rest of the mainland, which is also depicted in fastSTRUCTURE. Indeed, sub-step 4 subsequently confirmed a Hawaiian bridgehead to the US mainland (to Louisiana/Texas; Sub4c; 194 RF votes), when the US mainland was split between Florida and Louisiana/Texas. The fifth step confirmed that both eastern Asian regions were involved in the invasion of Hawaii and Louisiana/Texas (S5c; 291 RF votes), when the Hong Kong region was separated from eastern Asia (*i.e.*, sub-eastern Asia).

Finally, the sixth and final step revealed that the population in Florida most likely resulted from admixture between Louisiana/Texas (49%) and southcentral China (51%) 87 years ago, and Louisiana/Texas to originate solely from the admixed Hawaiian bridgehead population 98 years ago (Fig. 5a; S6c; 220 RF votes). Also, the first introduction to Hawaii was estimated to have occurred 138 years ago from admixture between the Hong Kong region (48%) and sub-eastern Asia (52%).

#### 3.3.4 Demographic history

The demographic history of each population was inferred through Stairway Plot 2, using an average of 14,138 SNPs per population after SNPs with missing data were filtered out. Distinct demographic histories were present in both the native and the introduced US ranges (Fig. 5b). Most native populations have experienced a gradual decline in their effective population size, while Fuzhou, Hangzhou, Jieyang, and the two Japanese



populations experienced a bottleneck followed by a period of rapid growth. In the US populations, Hawaii and Louisiana both underwent a gradual decline in their effective population size, while Florida experienced a bottleneck followed by a period of rapid growth (Fig. 5b), corroborating the ABC results of an additional and distinct introduction event within Florida.

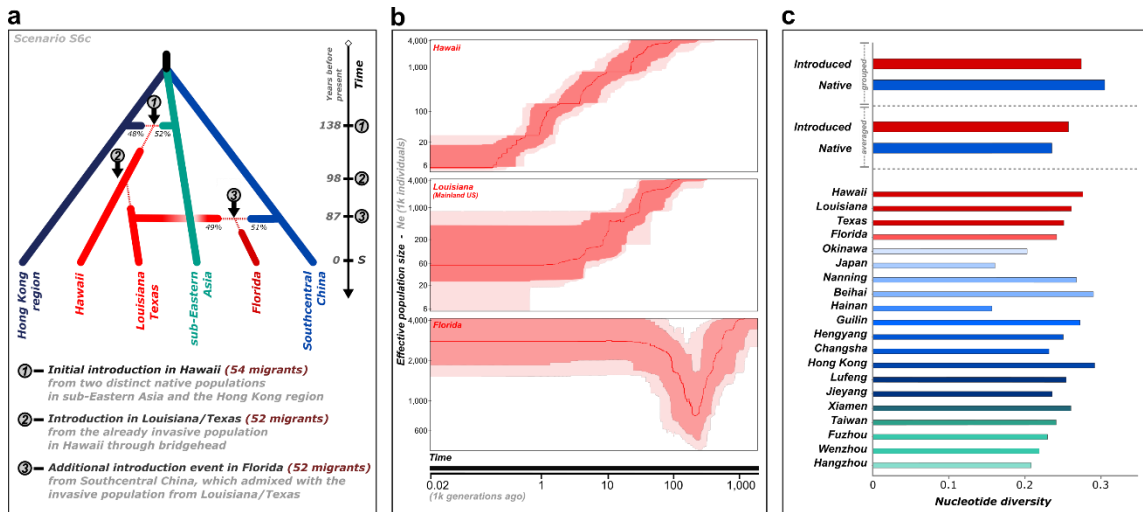


Figure 11: (a) Graphical representation of the most likely invasion history scenario for *C. formosanus* out of Asia tested through ABC RF. Thin dotted lines represent bottleneck events. Time is not to scale, with S indicating sampling time. (b) Estimation of the variation of effective population size through time for three invasive localities using Stairway Plot 2. The solid red line is the estimate of the median effective population size, and the light and dark red shaded areas represent the 95% and 75% confidence intervals, respectively. (c) Nucleotide diversity in the introduced and native range. All native and introduced localities were first analyzed while grouped together, then independently

### 3.4 Discussion

Our study unravels the global invasion history of *C. formosanus*, retracing its invasion pathway out of eastern Asia and assessing introduction-induced effects on its population demography and genetic diversity. Our findings reveal that the global distribution of *C. formosanus* has been shaped by multiple introductions out of eastern Asia, coupled with a bridgehead event. The complex invasion history of *C. formosanus* began with an initial



introduction in Hawaii (~1870) that originated from at least two distinct events, and their admixture, out of sub-eastern Asia and the Hong Kong region. This introduced Hawaiian population later served as the source for the invasion of the US mainland (~1930), where an additional introduction event from southcentral China occurred in Florida (~1940). These dates match up well with the generally accepted timeline of its invasion to the US. The first recorded observation of this species in the US dates back to the early 1900s in Hawaii (Swezey, 1914, 1915), although there is evidence that it had been established as early as 1869 (Su & Tamashiro, 1987). Within the mainland US, *C. formosanus* was first recorded in South Carolina (1957 (Chambers, Zungoli, & Hill, 1988)), Texas (1965 (Beal, 1967)), and Louisiana (1966 (Spink, 1967)). However, its strong association with military ports receiving and storing equipment and supplies from the Pacific theater after World War II (Spink, 1967) led to the widely held theory that it was introduced to the US mainland around this time period, aligning closely with our time estimates.

Much of the diversity present in the native range is highly structured among the native populations, with almost every native population representing a unique genetic cluster. The PCA and low values of  $K$  revealed two primary groups within the sampled Chinese range (eastern and southcentral China). Such a split is found in other eastern Chinese organisms (Shi, Michalski, Welk, Chen, & Durka, 2014; Ye et al., 2016), and has been proposed to be a relic of the Last Glacial Maximum that would have restricted available habitat for subtropical species to the extreme southern edge of China (Qiu, Fu, & Comes, 2011).

There was a slight reduction of genetic diversity within the introduced US range compared to the entire native range. Yet, the genetic diversity within each US population was equivalent to the diversity present in each of the native populations, indicating this termite's invasion has not been accompanied by a drastic loss of diversity at the population level (Fig. 5c). This finding differs from a previous study comparing the diversity of *C. formosanus* between its native and introduced range using microsatellite markers, which found substantially reduced diversity in each introduced population sampled relative to the native range (Husseneder et al., 2012). However, RADseq derived SNPs have been found to more accurately estimate genome-wide diversity than microsatellites (Fischer et al., 2017; Lemopoulos et al., 2019), which may explain the contrasting results obtained in the present study. This outcome is perhaps surprising because the founding event following an introduction usually reduces diversity within invasive populations, suggesting that multiple introduction events from distinct source populations may have prevented and even reversed the loss of diversity within the invasive range of *C. formosanus*.

Our clustering, phylogeographic and ABC results consistently show that eastern China is the prominent source of the invasive populations in Hawaii and the mainland US, congruent with the hypothesis of Husseneder et al. (2012). Interestingly, Husseneder et al. (2012) also identified two genetic clusters within the US mainland, with South Carolina being distinct from Louisiana and North Carolina. Unfortunately, samples from South Carolina were not included in our analyses, preventing the identification of a possible link between South Carolina and the second cluster we found in Florida. In addition, we are

unable to rule out a Japanese influence in the *C. formosanus* invasion of the US, and whether the Japanese populations (Mori, 1987) are invasive. While the demographic histories for both Okinawa and mainland Japan did display evidence of a bottleneck, genetic diversity statistics of the two populations were not drastically different from Chinese populations and clustering between the two is not present unless  $K$  is substantially reduced.

Human and merchandise transportation hubs have been shown to be an important factor in the spread of invasive species throughout the world (Banks et al., 2015; Floerl, Inglis, Dey, & Smith, 2009; Hulme, 2009; Westphal et al., 2008), and eastern Asia includes some of the largest and busiest ports in the world, such as Hong Kong, Shanghai, and Tokyo. The long history of both trade and immigration between China and the Kingdom of Hawaii dates back to the late 1700s, and centers around the southeastern region of China (*i.e.*, Hong Kong and neighboring areas) and Honolulu (main port of Hawaii) (Gay, 1967; Nordyke & Lee, 1989). Notably, the population of Chinese in Hawaii drastically increased from 364 individuals in 1852 to 18,254 individuals in 1884 (Nordyke & Lee, 1989), with most immigrants originating from this southeastern portion of China. Japan has also had a long history of immigration to Hawaii, from both the mainland (Boyd, 1971) and Okinawa (Matsumoto, 1982), as almost 200,000 Japanese moved to Hawaii between 1886 and 1924 (Boyd, 1971). These large-scale immigration events from eastern Asia coincide with the first suspected evidence of a subterranean termite in Hawaii (Su & Tamashiro, 1987).

Our ABC analysis suggests that the US mainland populations of *C. formosanus* likely arose from an already established invasive population in Hawaii through bridgehead rather than from an independent introduction directly from the native range. Indeed, cases of introduced populations themselves becoming the source of further introductions are being recognized more commonly (Correa et al., 2019; Javal et al., 2019; Lesieur et al., 2019; Sherpa et al., 2019; van Boheemen et al., 2017), including in other eusocial insects like invasive ants. For example, global phylogeographic analysis of the red imported fire ant *Solenopsis invicta* revealed that after its primary introduction event into the southeastern US from South America, this southeastern US population served as the source for its further spread to the rest of the world (Ascunce et al., 2011; Yang et al., 2012). Furthermore, ants as a whole display striking secondary introduction rates, with over 75% of ants intercepted at US and New Zealand ports of entry originating from locations where they had already been introduced (Bertelsmeier et al., 2018). While termite interceptions at US ports of entry have hinted at their potential to spread via bridgeheads (Blumenfeld & Vargo, 2020), our study empirically elucidates a bridgehead invasion in a non-ant social organism, with *C. formosanus* utilizing Hawaii as a stepping-stone for its subsequent invasion of the US mainland. This suggests that bridgeheads may play a crucial role for social insects in achieving multi-continental distributions, warranting further research into the invasion histories of other globally distributed social insects. For example, the West Indian drywood termite *Cryptotermes brevis* (Walker) likely represents a bridgehead invader, as it is native to the coastal deserts of Peru and Chile and is now invasive on five continents (Evans et al., 2013).

Bridgehead introductions have drastic effects on genetic diversity as introduced populations often experience bottleneck events. While some invasive species merely tolerate this genetic depletion, some benefit from the periodic purge of deleterious alleles through founder effects (Barrett & Charlesworth, 1991; Crnokrak & Barrett, 2002; Eyer, Matsuura, et al., 2018; Roman & Darling, 2007). In this context, bridgehead populations may reduce inbreeding depression in subsequent invasive populations through the purge of deleterious alleles during recurrent founder effects (Facon et al., 2011). On the other hand, reduced genetic diversity in bottlenecked bridgehead populations may promote the rapid evolution of invasive traits, as rates of adaptive evolution substantially increase with reductions in population size (Charlesworth & Eyre-Walker, 2007; Lanfear, Calcott, Kainer, Mayer, & Stamatakis, 2014). For this reason, bridgeheads have been hypothesized to be a stepping-stone for invasion by selecting for invasive traits (Ascunce et al., 2011; Lombaert et al., 2010; N. Miller et al., 2005; Zepeda-Paulo et al., 2010). These traits may increase the ability of an invader to be further spread to novel locations, confer greater ecological advantage that enables them to outcompete native species, and aid in circumventing the low genetic diversity in bottlenecked populations (Eyer, Blumenfeld, & Vargo, 2019; Kolbe et al., 2007; Pearcy, Goodisman, & Keller, 2011; Tsutsui et al., 2000; Whitney & Gabler, 2008). Despite this hypothesis of adaptive spread as a driver of the bridgehead effect, empirical evidence for this evolution of invasiveness is still lacking (Bertelsmeier & Keller, 2018), and the evolution of specific invasive traits within the Hawaiian population of *C. formosanus* remains undetected.

The presence of two genetic clusters in the mainland US signaled that the invasion pattern was more complex than just a single introduction from the Hawaiian bridgehead, and subsequent analysis confirmed a separate introduction event from southcentral China had indeed occurred. It seems most likely this additional event occurred within Florida, given it clustered separately from the other US populations and that the scenario describing Florida as a result of admixture between Louisiana/Texas and southcentral China was found most probable, as well as its unique demographic history. We also considered the possibility of interspecies admixture being the cause of Florida clustering separately, as a sister species of *C. formosanus*, *C. gestroi* (Wasmann), is also established in Florida (Su, Scheffrahn, & Weissling, 1997). These species have overlapping nuptial flights (Chouvenc, Scheffrahn, Mullins, & Su, 2017) and form tandem pairs of reproductive individuals (Chouvenc, Helmick, & Su, 2015; Chouvenc, Sillam-Dussès, & Robert, 2020); however, this hypothesis was ultimately found to be unlikely, as hybridization should be identifiable at low values of  $K$ . Furthermore, these two species are also sympatric in Hawaii and Taiwan, and none of the three regions display a highly negative  $F_{IS}$  commonly observed due to hybridization. Instead, Florida as a separate genetic cluster appears to stem from a distinct introduction event out of southcentral China.

Multiple events out of the native range from different source populations differ from the invasion pattern observed in another invasive subterranean termite, *Reticulitermes flavipes*. This species is native to the eastern US and has been introduced to France, Canada, the Bahamas, Uruguay, and Chile. Interestingly, most introduced

populations of this species seem to originate specifically from New Orleans, Louisiana (Perdereau et al., 2013; Perdereau et al., 2015). While New Orleans is an important hub for global trade, this species is also present in major trading cities along the eastern seaboard that have seemingly played no role in their spread. This suggests that certain traits of *R. flavipes* colonies within the New Orleans region may have pre-adapted this population to invasion, such as their distinct breeding structure and reduced antagonism between non-nestmates (Perdereau et al., 2015; Vargo, 2019), which they share with introduced populations in France (Clement & Bagnères, 1998; Perdereau, Bagnères, et al., 2010; Perdereau et al., 2015; Perdereau, Dedeine, Christidès, & Bagnères, 2010; Perdereau, Dedeine, Christidès, Dupont, et al., 2010) and Chile (Perdereau et al., 2015). Therefore, this finding is similar to the hypothesis suggested for bridgehead populations, whereby the evolution of specific traits conferring higher invasiveness primes a population for further invasion. This similar scenario has been coined the ‘*Anthropogenically Induced Adaptation to Invade*’ and suggests the evolution of adaptations to human-modified habitats in specific native populations favor their subsequent spread (Hufbauer et al., 2012). Such local pre-adaptation to invasion has been observed in the native range of the little fire ant *Wasmania auropunctata*, with natural populations mostly displaying small non-dominant colonies headed by sexually produced reproductives, while anthropogenic populations shift to large and dominant supercolonies headed by clonal reproductive (Fournier et al., 2005). The similar life-histories of native anthropogenic populations and invasive populations suggest that these traits, which evolved within its native range, may act as pre-adaptations to human-altered habitats and favor its worldwide invasion

(Hufbauer et al., 2012). Yet, despite being one of the most widespread invasive termites worldwide, introduced colonies of *C. formosanus* do not appear to have experienced a major shift in their breeding system or colony structure when compared to native colonies (Husseneder et al., 2012; Thoms et al., 2009; Vargo & Husseneder, 2011). Therefore, the worldwide invasion of this termite seems unrelated to these life-history traits. However, as native samples in this study were collected solely from human-disturbed habitats, we cannot be certain *C. formosanus* has not already undergone selection toward anthropogenic landscapes in their native range, like *W. auropunctata*. Whether there are other physiological factors enhancing their ability to thrive in human-modified habitats or whether no specific pre-adaptation is required, meaning each native population has the capacity to produce an invasion viable propagule, remains to be seen. Overall, these findings stress the need for comparative research between the introduced and native range, where key evolutionary processes promoting invasions may be occurring.



## 4 EXTENSIVE HUMAN-MEDIATED JUMP DISPERSAL WITHIN AND ACROSS THE NATIVE AND INTRODUCED RANGES OF THE INVASIVE TERMITE

### *RETICULITERMES FLAVIPES*<sup>3</sup>

#### **4.1 Introduction**

The transport of species beyond their native ranges by human activity is breaking down biogeographical barriers and causing global reorganization of biota (Capinha et al., 2015; van Kleunen et al., 2015), with the ensuing invasions posing a serious threat to biodiversity, agriculture and human health (Simberloff et al., 2013). Successful invaders must disperse into a geographically distant area, establish a viable and fertile population, and spread throughout this new environment, where the biotic and abiotic pressures may differ from those they faced in their native range (Kolar & Lodge, 2001). Biological invasions have long been seen as paradoxical, as the invasion process was thought to occur in spite of the reduction of genetic diversity that typically follows introductions of invasive species (Sax & Brown, 2000). However, data from a growing number of studies suggest that biological invasions are not always associated with a loss of genetic diversity, and that a loss of genetic diversity is not always accompanied with inbreeding costs and a loss of adaptive potential (Blumenfeld et al., 2021; Estoup et al., 2016; Eyer, Matsuura, et al., 2018; Facon et al., 2006; Roman & Darling, 2007). In addition, the ecological dominance

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<sup>3</sup>Reprinted with permission from “Extensive human-mediated jump dispersal within and across the native and introduced ranges of the invasive termite *Reticulitermes flavipes*” by Eyer, P.-A., Blumenfeld, A. J., Johnson, L. N. L., Perdereau, E., Shults, P., Wang, S., Dedeine, F., Dupont, S., Bagnères, A.-G., & Vargo, E. L., 2021. *Molecular Ecology*, 30(16), 3948-3964, Copyright [2021] by John Wiley and Sons.

of invaders in their novel environments is not necessarily the result of superior competitive ability compared to native species, but may simply involve the filling of vacant niches (Bates et al., 2020; Dlugosch, Anderson, Braasch, Cang, & Gillette, 2015; Dlugosch & Parker, 2008).

Several life-history traits may enhance the invasive success of some species (Eyer & Vargo, 2021). Specific breeding systems, modes of dispersal or physiological characteristics may influence the ability of species to spread and to become established. Investigating the mechanisms underlying the invasion process requires determining whether these traits differ between introduced and native populations. Such differences may arise after the introduction due to new ecological pressures occurring in the invaded area (S. R. Keller & Taylor, 2008; Wares, Hughes, & Grosberg, 2005), or they may already be present within native populations, thereby preadapting the source population for invasion success. Therefore, determining the source population of invasive species is critical to conduct comparative studies of life-history traits between introduced and native ranges to understand how they evolved under distinct biotic and abiotic pressures (Barker, Andonian, Swope, Luster, & Dlugosch, 2017).

Investigating invasion mechanisms also requires knowledge of the invasion history, in which a series of demographic events may influence the invasion process and patterns of genetic diversity. The introduced range may consist of a single invasive population. This introduced population may have originated from a single introduction out of the native range, or from multiple introductions out of the native range, either from the same or different source populations. In contrast, the introduced range may comprise

multiple invasive populations, which may originate from separate introduction events from one source population, or from different source populations out of the native range (Acevedo-Limón, Oficialdegui, Sánchez, & Clavero, 2020; Oficialdegui et al., 2019). Finally, an established invasive population itself may become a source for subsequent invasions, a phenomenon coined the “bridgehead effect” (Bertelsmeier & Keller, 2018; Lombaert et al., 2010). Therefore, reconstructing invasion histories is important for explaining the global distribution of genetic diversity and understanding adaptive evolution in new environments (Cristescu, 2015; van Boheemen & Hodgins, 2020).

A bottleneck event following an introduction usually results in a loss of genetic diversity in the introduced population (Dlugosch & Parker, 2008), but the amount of genetic diversity lost may vary under different invasion scenarios. The degree to which genetic diversity is reduced may be limited when the initial colonizing force is large, when the introduced population is subsequently reinvaded by additional individuals during multiple introduction events, and/ or when the introduced population is invaded by individuals from several genetically distinct source populations (Facon et al., 2006). Sometimes, when there are several introductions from different source populations and these interbreed within an invasive population, genetic diversity may even be higher within this population than its native source population(s) (Facon et al., 2008). In contrast, the bridgehead effect may result in a severe loss of diversity, as subsequent introductions arise from an already depauperate introduced population. The bridgehead effect has been suggested to promote the spread of phenotypic traits enhancing invasion success in secondary invasive populations, as these traits are already selected for and widespread in

the initial introduced population, although there is limited support for such a phenomenon (Bertelsmeier & Keller, 2018). Investigating patterns of genetic diversity in native and introduced populations can therefore provide insights into the introduction history of invasive species (*e.g.*, (Geburzi, Ewers-Saucedo, Brandis, & Hartl, 2020; Hirsch, Richardson, Pauchard, & Le Roux, 2021; Resh et al., 2021; Wesse, Welk, Hurka, & Neuffer, 2021; Winkler et al., 2019)).

*Reticulitermes flavipes* is a subterranean termite species native in the eastern USA, where it ranges from Texas to Massachusetts. The termite has become invasive in localities both near to and distant from the eastern USA. In both its native and introduced ranges, this termite species is responsible for large amounts of damage to human structures (Evans et al., 2013; Shults et al., 2021). This includes the western USA (Austin, Szalanski, Scheffrahn, & Messenger, 2005; McKern, Szalanski, & Austin, 2006), the Province of Ontario in Canada (Kirby, 1965), the Bahamas (Scheffrahn, Chase, Mangold, Krčec̆k, & Su, 1999), Chile (Clément et al., 2001) and Uruguay in South America (Austin, Szalanski, Scheffrahn, & Messenger, 2005; Su et al., 2006) and France, Germany, Austria and Italy in Western Europe, where it was first reported in 1837 (Clément et al., 2001; Ghesini et al., 2010; Kollar, 1837; Weidner, 1937). This species has also been reported (GBIF) from Mexico and the outermost regions of Spain (Canary Island; (Hernández-Teixidor et al., 2019)) and Portugal (Azores; (Austin et al., 2012)).

The native and invasive populations of *R. flavipes* have been the focus of numerous studies investigating its breeding system. In the French invasive range, colonies are large, readily fuse together and contain several hundred neotenic (worker or nymph- derived

reproductives that replace the primary or alate-derived reproductives who found new colonies) (Dronnet, Chapuisat, Vargo, Lohou, & Bagnères, 2005; Perdereau, Bagnères, et al., 2010; Vargo & Husseneder, 2009). Although substantial variability in breeding structure is present among the native USA populations of *R. flavipes*, colonies from most native populations are spatially less expansive, fuse only occasionally and are headed by a monogamous pair of primary reproductives or a few neotenic (Aguero, Eyer, Martin, Bulmer, & Vargo, 2021; Aguero, Eyer, & Vargo, 2020; Vargo, 2019; Vargo & Husseneder, 2009; Vargo et al., 2013). Interestingly, colonies in a population from Louisiana share some of the same traits as those in France (Perdereau, Bagnères, et al., 2010; Perdereau et al., 2015; Perdereau, Dedeine, Christidès, Dupont, et al., 2010).

Previous genetic analyses based on microsatellite markers and mtDNA haplotypes have shown that the introduced French population of *R. flavipes* exhibits an average decrease in genetic diversity of 60%–80% compared to native USA populations (Perdereau et al., 2013). The analysis also revealed the occurrence of three main genetic clusters within the native USA range – the “Eastern cluster” (West Virginia, Virginia, Delaware, North and South Carolina), the “Gulf Coast cluster” (Florida and Eastern Mississippi– Louisiana) and the “Southern Louisiana cluster” (the New Orleans and Baton Rouge regions in Louisiana) (Perdereau et al., 2013). Notably, some microsatellite and mtDNA haplotypes found in France were unique to the Southern Louisiana cluster (Perdereau et al., 2013). This finding, together with similarities in chemical profiles and breeding structures found between France and Louisiana (Perdereau et al., 2015; Perdereau, Dedeine, Christidès, & Bagnères, 2010), suggested that the French population

of *R. flavipes* was introduced from Louisiana, most likely during the 17th and 18th centuries via wood and plant trade between New Orleans and the major French ports on the Atlantic coast (Dronnet et al., 2005; Perdereau et al., 2013; Perdereau, Bagnères, et al., 2010).

Although the Louisiana origin of the invasive French population appears well supported, several points remain unclear. First, Perdereau et al. (2019) recently identified a French haplotype more closely related to the “Eastern cluster” than the “Southern Louisiana cluster,” suggesting multiple native populations from the USA may have invaded France. Additionally, the source(s) of the Canadian and Chilean invasions remain unidentified. Although several populations of *R. flavipes* occur in the Northeastern and Midwestern USA (*i.e.*, adjacent to Ontario), the only haplotype found in Canada was shared with populations in Louisiana and France (Perdereau et al., 2013). Therefore, it is unclear whether the Canadian population arose from a primary introduction from Louisiana or from a secondary introduction through France (*i.e.*, bridgehead introduction), as eastern Canada and France share a close historical bond. Similarly, the unique haplotype of Chile was closest to one shared between Louisiana and France (Perdereau et al., 2013), raising the same question regarding primary versus secondary introduction. Overall, these findings suggest a complex invasion history for *R. flavipes* and raise the question of how many native populations may have served as sources for the introduced populations and what the role of bridgeheads might be in the global distribution of this species.

Here, we used population genetic analyses and approximate Bayesian computation random forest (ABC-RF) to investigate the invasion history of *R. flavipes*. Using ddRADseq, we first generated a SNP data set sequencing 23 native populations in the USA and six introduced populations in France, Germany, Chile, Uruguay, the Bahamas and Canada. We then assessed patterns of genetic structure within the entire native range of the species, and within each of the introduced populations. Finally, in order to elucidate the invasion history of *R. flavipes*, we compared support for different invasion scenarios modeling the number, size and origin of each introduction event using ABC-RF.

## **4.2 Methods**

### *4.2.1 Population sampling and sequencing*

A total of 257 individuals of *R. flavipes* were collected from 29 populations spanning both native (USA) and different introduced populations in Europe (*i.e.*, France, Germany), North America (Canada and Bahamas) and South America (Chile and Uruguay) (Figure 1; Appendix B). In addition, 19 individuals of the sister species *R. virginicus* were collected to serve as an outgroup for the phylogenetic analysis. Samples were stored in 96% ethanol at 4°C until DNA extraction. Total genomic DNA was extracted from each individual using a modified Gentra Puregene extraction method (Gentra Systems, Inc.). DNA quality was assessed by agarose gel electrophoresis and DNA concentration was measured with Qubit 2.0 Fluorometer (Invitrogen). Nondegraded genomic DNA (100–300 ng) was used to construct ddRAD libraries. Libraries were prepared and sequenced at the Texas A&M AgriLife Genomics and Bioinformatics Service facility using SphI and EcoRI restriction enzymes following the protocol of Peterson et al. (2012). Each sample

was identified using unique combinatorial barcodes of six and eight base pairs. Samples were amplified through PCR with iProof High-Fidelity DNA Polymerase (Bio-Rad). PCR products were purified using AMPure XP beads (Beckman Coulter Inc.). Libraries were size-selected to a range of 300–500 bp using the BluePippin system (Sage Science Inc.). Libraries were sequenced on six flowcell lanes using an Illumina HiSeq 2500 (Illumina Inc.) to generate 150 bp paired-end reads. The sampling and library preparation/sequencing were performed prior to the beginning of my PhD career and therefore not conducted by me.

The paired-end reads were checked for quality control using Fastqc v0.11.8 (Andrews, 2010). Forward and reverse reads were demultiplexed from their barcodes, assigned to each sample and assembled using Stacks v.2.41 (Rochette et al., 2019). Reads were first aligned to the *R. flavipes* reference genome (Zhou et al. unpublished data) using the Burrows-Wheeler Aligner (H. Li & Durbin, 2009). Aligned reads were then run through the reference-based pipeline of Stacks, which built and genotyped the paired-end data, as well as called SNPs using the population-wide data per locus. Only SNPs present in at least 70% of individuals in half of the populations were kept for downstream analyses. Furthermore, SNPs with mean coverage lower than 5× and higher than 200× were removed using VCFtools v.0.1.15 (Danecek et al., 2011), to prevent unlikely SNPs and highly repetitive regions. Low frequency alleles (<0.05) and highly heterozygous loci (>0.7) were sorted out, as they probably represent sequencing errors and paralogs (Benestan et al., 2016). A single random SNP was kept for each locus, to prevent linkage disequilibrium that may potentially affect population structure and phylogenetic analyses.



The data set was formatted for downstream software programs using PGDSpider v.2.1.1.5 (Lischer & Excoffier, 2011).

#### 4.2.2 Population structure and phylogenetic relationship

Expected ( $H_E$ ) and observed ( $H_O$ ) heterozygosity, inbreeding coefficient ( $F_{IS}$ ), and population differentiation values ( $F_{ST}$ ) were calculated using Stacks (Rochette et al., 2019). Population structure among the 23 native and six introduced populations was analyzed using three complementary approaches.

First, the most likely number of genetic clusters (*i.e.*,  $K$ ) in the data set was estimated, and individuals were assigned into each of them using fastSTRUCTURE v1.040 (Raj et al., 2014). The algorithm ran following an admixture model with allele frequencies correlated and did not use a priori information on localities. The algorithm was parallelized and automated using Structure\_threader (Pina-Martins et al., 2017), and ran for  $K$  ranging from 1 to 29. The chooseK.py function was used to select the most likely number of genetic clusters. Plots were created by Distruct v2.3 (Chhatre, 2019) (available at <http://distruct2.popgen.org>).

Second, genetic clustering was estimated using a principal component analysis (PCA) and a discriminant analysis of principal components (DAPC). DAPC uses discriminant functions that maximize variance among groups while minimizing variance within groups (Jombart et al., 2010). The most likely number of genetic groups was first inferred by the find.clusters algorithm on the principal component analysis (PCA) outputs, with the Bayesian information criterion utilized to select the number of genetic groups. The optimal number of principal components to inform the DAPC (*i.e.*, maximizing

discriminatory power between groups, while preventing overfitting) was then defined using the function `optim.a.score`. Both the PCA and DAPC were performed in R (R Core Team, 2020) using the `adegenet` package (Jombart, 2008).

Third, population structure was visualized using the relatedness matrix produced by the `RADpainter` and `fineRADstructure` software (Malinsky et al., 2018). This method calculates coancestry between samples as an independent assessment of population structure. Analyses ran using default parameters of 100,000 burnin and 100,000 MCMC iterations, and results were visualized in R through scripts provided with the program (available at <http://cichlid.gurdon.cam.ac.uk/fineRADstructure.html>).

Phylogenetic relationships among *R. flavipes* individuals were inferred using maximum likelihood (ML) analysis implemented in `RAxML v8.2.12` (Stamatakis, 2014). Phylogenetic relationships were also estimated using a Bayesian analysis. After filtering, only 16 out of the 19 individuals of *R. virginicus* were used as an outgroup; these *R. virginicus* samples were not used in any other analyses. An acquisition bias correction was applied to the likelihood calculations, removing invariant sites from the alignment through the `Phrynomics` R script (available at <https://github.com/bbanbury/phrynomics/>). The rapid bootstrap analysis and search for the best scoring maximum likelihood tree was performed using the extended majority rule (MRE)-based bootstopping criterion (Pattengale et al., 2010) under the GTR+G nucleotide substitution model.

#### 4.2.3 Assessing invasion history

The global invasion history of *R. flavipes* was inferred through ABC analyses by comparing support for different invasion scenarios. The scenarios varied according to the

origin(s) of introduced populations, the founding population size, the bottleneck duration and the admixture rate if multiple sources were detected. To reduce computational effort, model selection and parameter estimation were performed using the recently developed random forests (RF) machine learning method (ABC-RF) available in the `abcrf` R package (Pudlo et al., 2016; Raynal et al., 2018). This method requires a reduced number of simulated data sets while still providing robust posterior estimates. To reduce computational effort, we also only tested scenarios relevant to biological and historical data; for example, we did not consider that the Chilean and Canadian introduced populations could be the source of the French population. A step-by-step approach (nine different steps divided into four parts) was used to infer the different episodes of the invasion history of *R. flavipes*, as this type of approach is commonly performed in ABC studies to distribute the computational effort (Fraimout et al., 2017; Javal et al., 2019; Ryan et al., 2019). The introduced populations in Germany, Uruguay and the Bahamas were not used in ABC computations as they were represented by too few individuals. Briefly, the first part estimated whether each introduced population (*i.e.*, France, Canada and Chile) arose from independent or bridgehead introduction events (Part A). As this first part indicated that the French population may have played a role in the introductions to Canada and Chile, we first sought to decipher the source(s) of the introductions to France alone (Part B). Next, we attempted to identify the sources of the Canadian (Part C) and Chilean (Part D) populations using France as a potential source. For all scenarios tested, introduction events were followed by a decrease in effective population sizes that varied from one to 100 migrants for a duration of zero to 50 years. Divergence time is given in

generations, with a generation length of 1 year. Posterior distributions of preliminary simulated data sets were used to adjust the range of other priors as wide as possible while retaining biological meaning. For each step, 10,000 simulated data sets, including all of the summary statistics implemented in diyabc v.2.1.0 (Cornuet et al., 2014), were generated per scenario from 2000 randomly sampled SNPs. Priors were set uniform for all model parameters and selected based on historical records. Simulated data sets were first generated by DIYABC, and later exported for model selection and parameter estimation in ABC-RF.

### 4.3 Results

The 257 *R. flavipes* samples yielded an average of 7.0 million paired reads per individual (range: 0.03–23.5). A total of 28 individuals were removed due to a significant amount of missing data ( $\geq 60\%$ ) or low coverage ( $\leq 9.5\times$ ). After filtering, the final data set contained 229 individuals of *R. flavipes* from 29 populations and included 51,116 SNPs, with an average coverage of  $27\times$  and 32% missing data. Weak inbreeding was found within *R. flavipes* populations ( $F_{IS} \pm SE = -0.053 \pm 0.031$ ). Consequently, values of observed heterozygosity ( $H_O \pm SE = 0.196 \pm 0.031$ ) were higher than values of expected heterozygosity ( $H_E \pm SE = 0.135 \pm 0.020$ ).

#### 4.3.1 Population structure

Strong genetic structure was uncovered among the *R. flavipes* individuals from fastSTRUCTURE, with  $K = 4$  best explaining the structure in the data (Figure 1). At this value of  $K$ , more than half of the individuals in the data set (57.2%) were clearly assigned to one of the four clusters (assignment probability higher than 99%; 73.3% of individuals

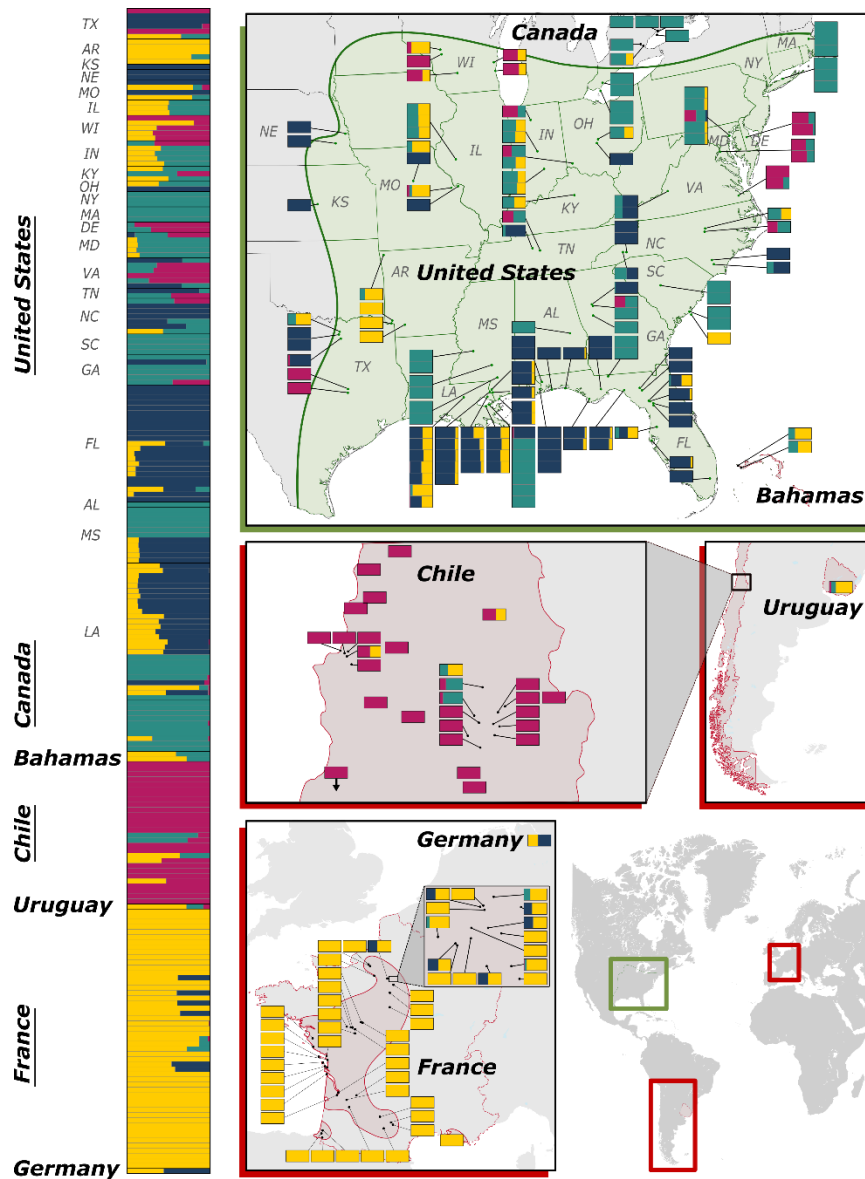


Figure 12: Sampling map and fastSTRUCTURE assignment for each individual of *R. flavipes* for  $K = 4$ . Each vertical bar represents an individual and each color represents a distinct genetic cluster. Individual fastSTRUCTURE assignments are geographically located in the native and introduced ranges of *R. flavipes*

were assigned to a unique cluster probability higher than 80%). However, the strong genetic structure uncovered among individuals in the native range was inconsistent with their geographic origin, as neighboring samples often exhibited completely different

assignment profiles (Figure 1). This pattern was also found when populations from the native range were analyzed separately. In the French introduced range, most samples could be assigned to the same cluster, although some samples from the Paris region had a mixed assignment; a similar mixed assignment was found for the lone German sample. A comparable pattern was observed in the Chilean introduced range, with most samples displaying fixed assignments and only a few with mixed assignments. Although a single genetic group was mostly found within each introduced population (France, Chile and Canada), the three introduced populations were separately assigned to three different genetic groups and did not segregate into a single “introduced” cluster; a finding also uncovered at lower values of  $K$ . Because the genetic clustering of the native range did not consistently align with geographic origin, inferring a source population for each introduced population becomes difficult. For example, most samples from Chile were assigned to the same cluster as samples from New York, Wisconsin and Texas. Similarly, although the introduced population in France shared its strongest tie to the native range with Arkansas, France also had ties with Louisiana, Missouri and even one sample in South Carolina. The origin of the samples in Canada was even more complicated, as the genetic cluster present in this population was spread across most native localities. Similar findings were uncovered for different values of  $K$ .

Similar results to that of fastSTRUCTURE were uncovered using the PCA and DAPC approach (Figure 2). The PCA indicated strong differentiation across the *R. flavipes* samples, as they broadly segregated along the two axes. For most localities, genetic clustering was not associated with geography, as samples from a given locality did not

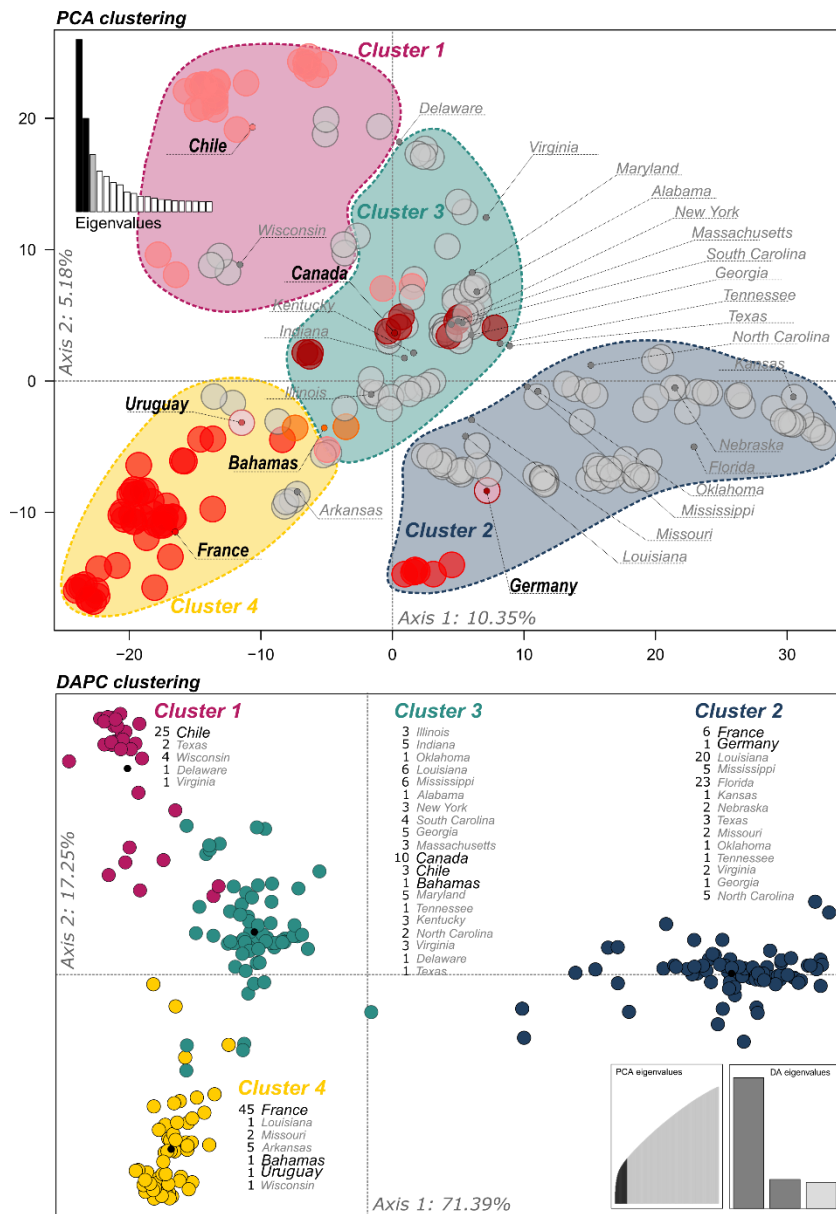


Figure 13: Principal component analysis (PCA) of *Reticulitermes flavipes* individuals. Each circle represents an individual. Each individual is colored according to its population of origin; introduced populations are depicted in reddish colors, native populations are colored in grey. Individuals are grouped according the discriminant analysis of principal components (DAPC) with best support for  $K = 4$  genetic clusters

always cluster together. Likewise, low genetic similarity was observed between geographically neighboring localities. Interestingly, such a pattern was also found to a

lesser extent in the introduced populations of France and Chile (only a single sample was available from Germany and Uruguay, and just two from the Bahamas). In France, most of the samples segregated together, except for six individuals from the Paris region, which clustered separately from the rest of the main population and had mixed assignments. A similar pattern was observed for the samples from Chile, with three samples clustering apart from the main Chilean population. Interestingly, fastSTRUCTURE found the occurrence of two and three genetic clusters in the Chilean and French populations respectively, when those populations were analyzed separately. The find.clusters algorithm found the best support for four genetic clusters in the data set (Figure 2). Notably, the introduced localities of *R. flavipes* did not cluster together; instead, the different introduced populations were spread across the four different DAPC clusters, with some even split between two clusters (Chile and France). Remarkably, a similar pattern was observed from localities within the native range, with samples from a given locality clustering into two (*e.g.*, Texas, Mississippi, Wisconsin) or even three (Louisiana) distinct DAPC clusters.

The coancestry matrix highlighted similar patterns when clustering individuals based on their level of relatedness (Figure 3). Using fineRADstructure, all samples from a given locality were no more related to one another than they were to samples from another locality (Figure 3). This result is indicative of a weak geographic structure in the native range, as most localities were disjunct in the coancestry matrix. Notably, the same pattern was observed for the introduced populations, with clustering observed in two (Canada) or three (France and Chile) distinct coancestry groups. Accordingly, although



significant, the mean genetic differentiation between populations was rather low (mean  $F_{ST} \pm SE = 0.091 \pm 0.054$ ).

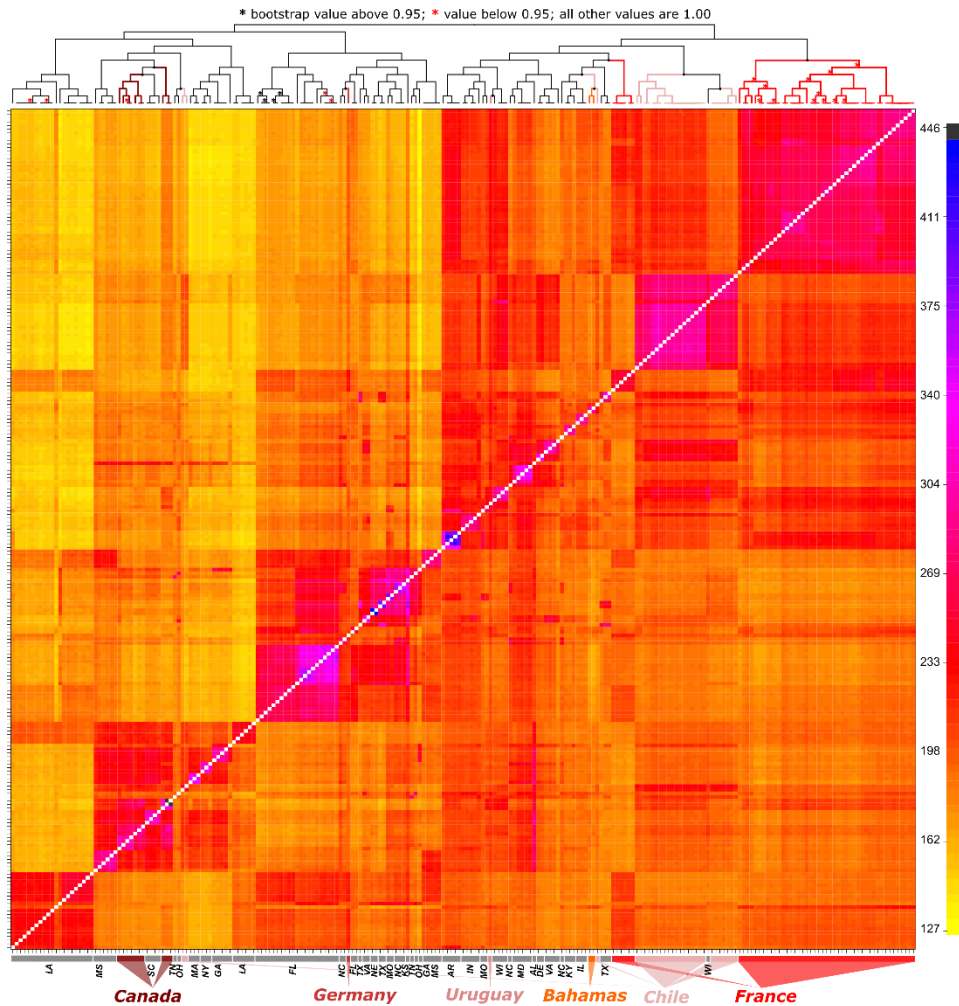


Figure 14: Coancestry matrix between each pair of individuals inferred using fineRADstructure. Each pixel represents a pair of individuals. Coancestry coefficients between two individuals are designated on a color spectrum. Low values are shown in yellow; higher values are shown in darker colors

#### 4.3.2 Phylogenetic relationship

The ML phylogeny was constructed on 29,875 SNPs after filtering out invariant sites, using 650 bootstrap replicates, as suggested by the MRE-based bootstopping-criterion.

Overall, the tree was consistent with results from the clustering analyses, despite weak bootstrap support throughout the topology (Figure 4). Interestingly, the entire introduced range did not fall out as a single clade; instead, introduced populations arose throughout different branches of the tree. Furthermore, all invasive populations fall out as at least two (Canada and Bahamas) or more different clades (France and Chile). This result also suggests that different introduced populations arose from separate introduction events out of the native range, and that there were several introduction events in most invasive populations (similar findings were found when Bayesian inferences were used to build the tree).

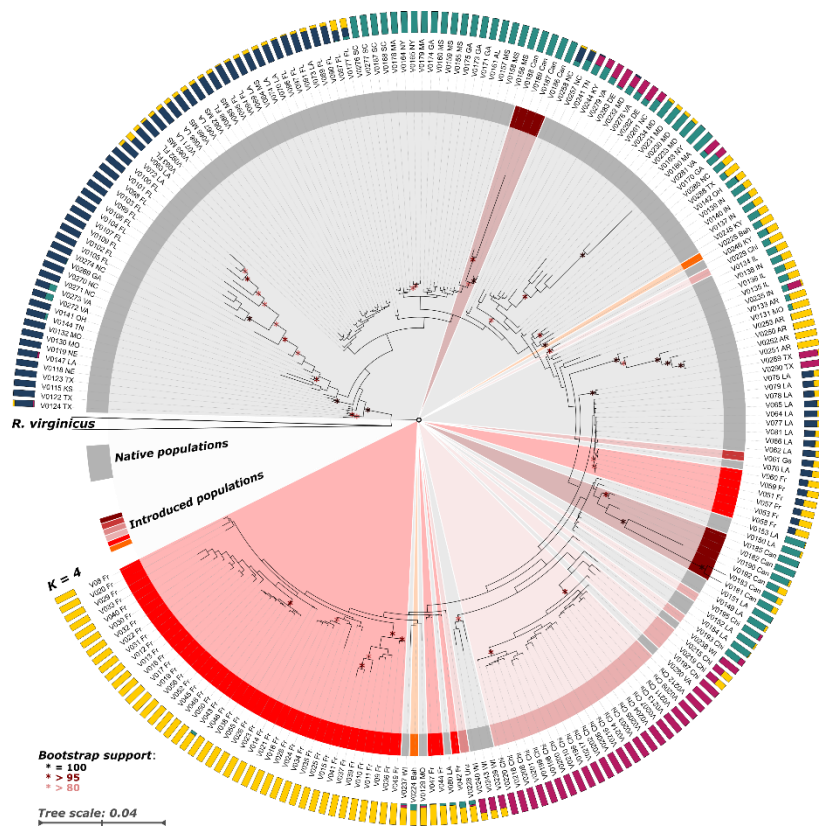


Figure 15: Maximum likelihood phylogenetic tree of *R. flavipes* individuals from RAxML. Individuals are colored according to their fastSTRUCTURE assignments ( $K = 4$ ). Samples from the introduced ranges are highlighted with an emphasized tip. The phylogenetic tree is rooted with 16 *R. virginicus* samples

### *4.3.3 Invasion history*

Part A of the ABC analysis found that introduced populations in Canada and Chile most likely originated, at least partially, from bridgehead introductions from the previously introduced population in France (Figure 5), rather than directly from the native range. The RF votes were mostly split between three scenarios describing a bridgehead introduction from France to either Canada (220 RF votes), Chile then Canada (221 RF votes) or both countries (215 RF votes).

When analyzing the introduced French population alone in part B, the first step found that this introduced population could not be unambiguously assigned to a single origin, as all three regions of the native range received a substantial amount of support (Louisiana/Mississippi: 417 RF votes, East: 414 RF votes and Central: 169 RF votes). The “least bad” single introduction event scenario (151 RF votes) was outvoted when compared against a two- population admixture scenario (319 RF votes, second step); and this two population admixture scenario (271 RF votes) was itself outvoted by scenarios simulating the contemporary French population arising through admixture of all three native regions (394 RF votes, third step). When groups of scenarios were compared, the group of scenarios with admixture outvoted the group without admixture in the second step (660 against 340 RF votes); and the group of scenarios with three-population admixture outvoted the group with two- population admixture in the third step (612 against 388 RF votes). The fourth step of part B (further dividing the native range) found that Massachusetts, Maryland and New York (222 RF votes) obtained the highest support as the origin for the French population. However, several other source populations obtained

a significant number of RF votes, casting doubt on the ability to undeniably assign the introduced population of France to a unique source. This ambiguity is further emphasized when the scenarios were divided into groups, as both the Southeastern region (504 RF votes) and the rest of the native range (496 RF votes) obtained an almost identical number of RF votes. Overall, these findings suggest the occurrence of multiple introduction events out of the native range. However, at both large (step 1) and finer scales (step 4), no scenario received a majority vote, preventing a definitive determination of the source for the introduced population in France and calling for caution in the appraisal of the estimated parameters.

Part C aimed at analyzing the origins of the Canadian introduced population, using the French introduced population as a potential source. ABC-RF analyses revealed that the most probable scenario for the origin of the Canadian population was an introduction from a French bridgehead and its admixture with a separate introduction event from the native range (405 RF votes), rather than originating entirely from the native range (227 RF votes) or a French bridgehead (368 RF votes). The presence of a French bridgehead is also supported, as the group of scenarios including a bridgehead event (623 RF votes) outvoted the group without a bridgehead event (377 RF votes). When the native range was further divided, ABC-RF analyses also failed to confidently link the origin of the Canadian introduced population to a unique geographic region, as several source populations obtained a significant number of RF votes. This doubt is also emphasized when groups of scenarios were compared, as both the Southeastern region (520 RF votes) and the rest of the native range (480 RF votes) obtained a similar number of RF votes.

A similar invasion history was identified for Chile in part D, as a bridgehead from France combined with an additional introduction event from the native range was found most likely (506 RF votes), rather than entirely from the native range (339 RF votes) or a French bridgehead (155 RF votes). Similar to the origin of the introduced populations of France and Canada, ABC-RF did not confidently infer the source of the Chilean population when the native range was divided. Several source populations obtained a similar number of RF votes when each scenario was analyzed separately, and the Southeastern group of scenarios (539 RF votes) obtained a similar number of RF votes to the group that included the rest of the native range (461 RF votes). Overall, the ABC results cast doubt on the ability to connect each introduced population to one or a few specific source populations, as most simulated scenarios poorly fit the observed data set

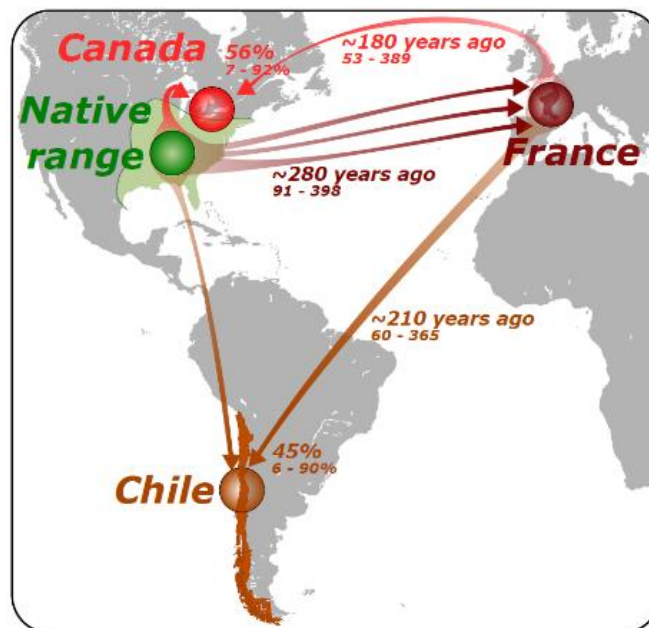


Figure 16: Graphical representation of the invasion pathway of *R. flavipes* out of the eastern USA inferred through ABC RF in France, Canada and Chile. The estimated time of introduction and rate of admixture is provided for each introduction event. The large 95% CI, however, calls for caution in interpreting those values

with no scenario receiving a clear majority of the votes. This finding is also suggested by the divergence between the simulated and observed data sets present in the LDA graphs, potentially highlighting that more sophisticated scenarios are needed to better explain the data. Although all of the posterior probabilities and posterior parameter estimates were generated for the “least bad” scenario in each step, we call for caution in interpreting those values given the ambiguous results obtained in most steps.

#### **4.4 Discussion**

Our study provides insights into the invasion history of the termite *Reticulitermes flavipes*, highlighting frequent and recent human-mediated jump dispersal in both the native and introduced range of this species. We first revealed strong genetic structure among individuals within the native range of this species with individuals grouping into four distinct clusters. Yet, these clusters were not strictly associated with geography, as highly different individuals were found in the same locality and highly similar ones in localities separated by several thousand kilometers. This finding indicates extensive movement of colonies throughout the native range, probably through human transportation of wood. We also highlight a complex invasion history with multiple introduction events out of the native range and bridgehead spread from the introduced population in France. The apparent genetic shuffling within the native range limits our ability to assign an exact source population(s) for the different introduced ranges. However, similar to the effect of multiple introductions into the invasive range, admixture in the native range prior to invasion can potentially favor invasion success by increasing the genetic diversity later conveyed to the introduced ranges.

Our findings revealed the occurrence of multiple introductions from different native localities serving as sources for the invasive ranges of France, Chile and Canada. Additionally, Canada and Chile received secondary invasions from the introduced population in France, which acted as a bridgehead. Some previous results indicated that there may have been several introductions into France (Perdereau et al., 2019). *Reticulitermes flavipes* was first reported in Europe (Austria) in 1837 and was first reported in France as *R. santonensis* in 1924 (Feytaud, 1924), where it was widespread and therefore probably introduced much earlier (Bagnères et al., 1990). Despite being unable to definitively link its source population(s) to the New Orleans region as previously suggested (Perdereau et al., 2013; Perdereau et al., 2015), our data, based on a larger sample size and more informative markers, do not rule out this possibility. Our data instead suggest that individuals genetically similar to this invasive population were found across the entire native range, from Louisiana to Maryland. However, it is possible that the French population originated from colonies originally from the New Orleans region that had been transported elsewhere within the native range, such as South Carolina or Arkansas. Such long-distance jump dispersal within the native range can therefore hamper the clear identification of the source population(s). Likewise, although our results suggest that the Canadian and Chilean introduced populations originated from admixture between the introduced population of France and native localities in the northern range of *R. flavipes*, these results suffer from low confidence, potentially due to genetic mixing between native localities. Although the French connections with Louisiana and eastern Canada are well-established, France also has historical ties with Chile. Notably, most of

the human immigrants to Chile between the 18th and 20th centuries come from the Basque region of Southern France (Domingo, 2006), where *R. flavipes* occurs. During the 18th century, Chile experienced massive immigration from this region, reaching 27% of the total Chilean colonial population. Overall, these findings indicate that jump dispersal may not be restricted to a single region within the native range of this species. Instead, such dispersal appears common in *R. flavipes* in both its native and invasive ranges, suggesting that this species possesses traits that promote its spread and have contributed to its global distribution.

The genetic patterns observed in the native range of *R. flavipes* may be explained by numerous and recent jump dispersal events across the native range, probably mediated via human trade and transportation. This finding exemplifies species spread by stratified dispersal, whereby individuals disperse at different spatial scales, from local to long-distance movement (Shigesada, Kawasaki, & Takeda, 1995). Local scale dispersal relies on the biological dispersal ability of the species, ranging from short-range (*i.e.*, budding) to moderate dispersal (*i.e.*, nuptial flights). In contrast, long-distance dispersal is often human-mediated and therefore considered stochastic and difficult to identify. Notably, our study revealed both genetically distinct individuals inhabiting the same locality and genetically similar individuals separated by several thousand kilometers. The geographic distance separating highly similar individuals far exceeds the biological dispersal ability of this species, which suggests that these individuals were artificially transported to a different locality. Additionally, the finding of genetically distinct individuals within the same or adjacent localities indicates a low level of mixing between those individuals. This



may stem from reduced local dispersal, whereby transported individuals inbreed and do not disperse far from their landing point. A high proportion of new reproductives of *R. flavipes* do in fact couple with their nestmates during mating flights (25%); however, the proportion of inbred founders is significantly reduced among established colonies (DeHeer & Vargo, 2006). Therefore, this inbreeding depression may select against the interbreeding of artificially transported colonies. Also, *R. flavipes* usually disperses through nuptial flights, which should enhance gene flow over large scales (Vargo, 2003). Consequently, a scenario where transported individuals interbreed and do not disperse far from their landing point may not alone explain the pattern observed in this study. The finding of highly genetically different individuals within the same locality therefore suggests that some of the long-distance jump dispersal events are probably too recent to allow transported individuals to admix with local colonies and homogenize the gene pool within populations.

The global spread of invasive species is strongly influenced by long-distance jump dispersal events, even once established within an introduced range (Suarez, Holway, & Case, 2001). Jump dispersal events are more effective, and often required, for species to rapidly reach a widespread distribution (Bertelsmeier, 2021; Gippet, Liebhold, Fenn-Moltu, & Bertelsmeier, 2019). For example, the worldwide distribution of the Argentine ant has been shown to primarily stem from human-mediated jump dispersal, rather than from its classical spread through colony budding, as the latter would have to be three orders of magnitude higher to explain its actual distribution (Suarez et al., 2001). This finding is also typified in the global distribution of the red imported fire ant *Solenopsis*

*invicta*, which utilized long-range jump dispersal to first invade the southeastern USA, and subsequently Asia and Australia from this USA bridgehead (Ascunce et al., 2011). In general, human-mediated jump dispersal appears common in eusocial invaders with a global distribution, like ants (Bertelsmeier et al., 2017; Bertelsmeier et al., 2018) and termites (Blumenfeld & Vargo, 2020; Buczkowski & Bertelsmeier, 2017). These multiple long-distance movements are also observed among regions within invasive ranges, across a wide variety of taxa, such as the aforementioned *S. invicta* throughout the southern USA (Lofgren, 2019) and China (Ascunce et al., 2011), the western mosquitofish *Gambusia affinis* in New Zealand (Purcell & Stockwell, 2015), and plants in China (Horvitz, Wang, Wan, & Nathan, 2017). Many studies have demonstrated the role of human-mediated jump dispersal in shaping invasive distributions and genetic diversity. However, it often remains unclear whether long-distance dispersal pre-exists in the native range of invasive species, and whether it plays a role in determining the pattern of genetic diversity observed at the global scale of these species.

Native ranges of many invasive species often remain geographically structured (Beck, Schmuths, & Schaal, 2008; Leinonen, O'Hara, Cano, & Merila, 2008; Verhoeven, Macel, Wolfe, & Biere, 2011; Voisin, Engel, & Viard, 2005). For example, native populations of *S. invicta* are strongly geographically differentiated (Ross, Krieger, Keller, & Shoemaker, 2007). Though rare long-distance dispersal of *S. invicta* has been reported (Ahrens, Ross, & Shoemaker, 2005), these events occurred far in the past and have been attributed to strong winds during nuptial flights or the rafting of entire colonies during flooding events (Hölldobler & Wilson, 1990), rather than from human-mediated transport

(Ahrens et al., 2005). Native populations of another termite invader *Coptotermes formosanus* in China are highly structured, with distinct native populations representing different genetic clusters (Blumenfeld et al., 2021). This structuring suggests reduced gene flow across populations, and therefore a limited number of human-mediated dispersal events within the native range of this species. Our results stand in sharp contrast with the strong population structure commonly uncovered within the native ranges of invasive species, as frequent jump dispersal appears to have occurred in the native range of *R. flavipes*. Understanding the factors driving the differences between *C. formosanus* and *R. flavipes* may shed light on key evolutionary mechanisms underlying their invasion success. Furthermore, while most studies focus on unraveling invasion pathways out of a native range, our results stress the need to consider evolutionary processes and human-mediated dispersal that may already be present within the native range of an invasive species, as these can affect the level and distribution of genetic diversity in both the native and invasive ranges.

Extensive human-mediated jump dispersal has been reported in the native range of a few species. For example, in the invasive tree *Acacia pycnantha*, extensive transport and replanting throughout its native Australian range prior to its introduction to South Africa resulted in highly admixed genotypes already present in the native range. This feature has consequently prevented an accurate identification of the native source population(s), as highly admixed genotypes and comparable genetic diversity were present in both ranges of the species (Le Roux, Richardson, Wilson, & Ndlovu, 2013). A similar pattern has been found in the North American rangeland weed, *Centaurea diffusa*, where an extremely low

level of population structure in the native range hindered the assignment of its introduced population to its likely native source location (Marrs, Sforza, & Hufbauer, 2008). However, the genetic patterns observed in the *Acacia* and *Centaurea* plants are slightly different than the one observed in *R. flavipes*, as the inability to pinpoint the origins of invasive populations of these plants stems from the near panmixia found across the native range (Le Roux et al., 2013; Marrs et al., 2008). Therefore, the patterns in these other species most likely stem from an ancient and continuous genetic shuffling throughout the native range. In contrast, the lack of geographic structure despite highly genetically different individuals indicates recent and stochastic long-distance dispersal in *R. flavipes*. Consequently, the genetic structure of *R. flavipes* may have been different (with less admixture) in both the native and invasive range(s) a few centuries ago, at the beginning of the French, Canadian and Chilean invasions. The complex genetic structure currently observed, together with multiple introduction events, makes it difficult to accurately reconstruct the invasion history of this species.

The invasion success of termites is tightly linked with their ability to eat wood, nest in wood and cultivated plants, and readily generate secondary reproductives, as all 28 species of invasive termites share these three traits (Evans et al., 2013; Eyer & Vargo, 2021). These traits may enhance the frequency of human-mediated dispersal because any piece of wood serving as a nest or foraging site has the potential to become a viable propagule (Evans et al., 2013; Evans, Inta, & Lai, 2010). However, these traits are common in lower termites like *R. flavipes* and *C. formosanus*, therefore their occurrence in both species cannot explain why *R. flavipes* has experienced a greater frequency of

long-distance dispersal than *C. formosanus*. In *R. flavipes*, repeated human-mediated dispersal could reflect a higher degree of propagule pressure from different USA regions, representing multiple hubs of intense human activity and timber production. Forests and timber production are unequally distributed across the eastern USA (Brown, Schroeder, & Kern, 1999; Howard & Liang, 2019), and may therefore require significant wood transportation throughout this part of the country from high to low timber-producing regions. Similarly, the frequency of human-mediated dispersal may reflect the connectivity between native regions. In the introduced population of *R. flavipes* in France, the distribution of genetic diversity is associated with the construction of the railway network and stations, highlighting its possible role in spreading termites over long distances (Andrieu et al., 2017; Perdereau et al., 2019; Suppo, Robinet, Perdereau, Andrieu, & Bagnères, 2018). In the USA, about 14,000 km of track were active by 1850, mainly in the eastern USA (141,000 km in 1880 and over 400,000 km in 1916) (Adams, 1895; Chandler, 1965). In contrast, the first 10 km railway was built in China in 1881, but less than 13,000 km were in use by 1948 for the whole country. This difference in connectivity may explain the numerous long-distance dispersal events in the native range of *R. flavipes* and their absence in the Chinese native range of *C. formosanus*. Interestingly, the USA railroad network has been suggested to represent a major dispersal mode for the invasive population of *C. formosanus* (Austin, Glenn, & Gold, 2008). Overall, many invasive social insect species originate from South America or East Asia (Eyer, Espinoza, Blumenfeld, & Vargo, 2020; Eyer, Matsuura, et al., 2018; Eyer, McDowell, et al., 2018; Heller, 2004; Ross et al., 2007; Tsutsui et al., 2000). The

population structure observed in most native populations of invasive termites may simply reflect the reduced connectivity between native regions in these areas, potentially resulting from a lack of internal trade among regions or difficulty in reaching isolated geographic areas. Our findings in *R. flavipes* indicate that frequent long-distance dispersal may already be present within the native ranges of some invasive species, especially those originating from regions with a long history of dense transport networks.

Native populations of many invasive species often remain geographically isolated and locally adapted (Verhoeven et al., 2011). In the introduced range, a temporary loss of local adaptation through admixture has been suggested to alter the fitness consequences of admixture in recent invaders (Verhoeven et al., 2011). In our study, the levels of admixture observed in the introduced populations of France and Chile may be explained by numerous introductions from distinct source populations and their interbreeding within the invasive range. However, we cannot rule out the possibility that populations were already admixed before propagules were transported worldwide. Similarly, it is possible that admixed introduced populations re-invaded the native range of *R. flavipes*. In the native range of species, long-distance dispersal enhances gene flow between distant populations that are otherwise isolated. Similar to post- introduction increases of genetic diversity through multiple introduction events (García, Melero, Palazón, Gosálbez, & Castresana, 2017; Kolbe et al., 2004; Stenoien, Fenster, Tonteri, & Savolainen, 2005), admixture between native populations prior to an introduction event may enhance the amount of genetic diversity brought to the invasive range. Admixture may improve invasion success through recombination of distinct genotypes, potentially creating novel

combinations of traits, and/or increasing the level of genetic diversity upon which natural selection can act. Pre- or post-introduction admixture may also relax the inbreeding load by reducing the expression of recessive deleterious alleles or lead to heterosis effects, potentially improving the establishment and early success of invasive species (Drake, 2006; Ellstrand & Schierenbeck, 2000; Hahn & Rieseberg, 2016; S. R. Keller & Taylor, 2008). Overall, increased genetic diversity via admixture may favor subsequent introductions given the novel selection pressures invasive species face in their new environments (Verhoeven et al., 2011).

#### **4.5 Conclusion**

In this study, we infer the occurrence of long-distance jump dispersal in the native range of the termite *R. flavipes*. This long-distance dispersal may facilitate admixture between populations that are otherwise isolated. Admixture in native populations prior to introduction may favor invasion success by increasing the amount of genetic diversity brought to the introduced range, achieving an effect similar to that produced by multiple introductions from the native range. However, pre-introduction admixture may not be as common as multiple introduction scenarios (*i.e.*, post-introduction admixture), because the benefits of admixture in the novel environment of the invasive range are probably higher, and the costs smaller (Rius & Darling, 2014). As native populations are locally adapted, long-distance dispersal and admixture may disturb this local adaptation, thereby reducing population fitness (Palacio-Lopez, Keller, & Molofsky, 2017; Verhoeven et al., 2011). In contrast, populations in invaded ranges are generally too recent to be locally adapted (but see (Batz et al., 2020)). This lack of local adaptation may release introduced

populations from maintaining specific locale-selected allelic combinations, and thereby fully benefit from admixture in early stages of the invasion. The relative roles of pre- and post-introduction admixture in biological invasions should be fertile ground for future studies.



## 5 CONSISTENT SIGNATURES OF URBAN ADAPTATION IN A NATIVE, URBAN INVADER ANT *TAPINOMA SESSILE*<sup>4</sup>

### 5.1 Introduction

Despite urbanization being a relatively recent byproduct of the Anthropocene (Lewis & Maslin, 2015), already about 3% of Earth's land surface is urban (Z. Liu, He, Zhou, & Wu, 2014), with further increases projected throughout the remainder of the 21st century (Gao & O'Neill, 2020; Seto, Güneralp, & Hutyra, 2012). Natural environments undergo marked transformations as a result of urbanization (Grimm et al., 2008), including modifications to landscape composition (*e.g.*, loss of suitable patches, homogenization and connectivity (Groffman et al., 2014; McKinney, 2006)), natural processes (*e.g.*, soil pollution and nutrient cycling (Isaksson, 2015)), and ecological interactions (*e.g.*, competition, predation and pathogens (Rivkin et al., 2019)). Subsequently, local biotic and abiotic interactions are altered and novel selection pressures thereby introduced, suggesting urban environments may be hotspots for microevolution (Alberti, 2015; Johnson & Munshi-South, 2017).

Another hallmark of the Anthropocene appears to be biological invasions, as the worldwide dispersal of plants and animals is heavily influenced by international trade and humankind's transportation network (Banks et al., 2015; Hulme, 2021). Like urbanization,

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these invasions often promote ecological disturbance, whereby invasive species outcompete native species for resources or fill an empty ecological niche and alter the complexion of an ecosystem (Ehrenfeld, 2010). Similarly, ecological disturbance itself may encourage biological invasions by increasing the availability of resources (Lembrechts et al., 2016; Tilman, 1994) and/or altering the composition of communities (Buckling, Kassen, Bell, & Rainey, 2000), potentially implicating urbanization as another human-induced facilitator of invasion. Ants are among the most prolific of these invaders (Jeschke & Wittenborn, 2011), with around 240 invasive species globally that display a significant association with disturbed environments (Bertelsmeier et al., 2017). It is generally assumed that individual and colony-level plasticity in physiology, morphology and behavior may enhance their invasion success. This plasticity may allow for rapid acclimation to novel ecological pressures they encounter within their new environment to quickly rise to ecological dominance.

Many invasive ants possess a suite of shared characteristics that facilitate their success within new/disturbed environments, such as a polygyne social structure (*i.e.*, multiple reproductive queens), dependent colony foundation (*i.e.*, budding) and lack of aggression among non-nestmate workers (Eyer & Vargo, 2021; Lester & Gruber, 2016; McGlynn, 1999; Tsutsui & Suarez, 2003). A polygyne social structure may enhance the survival rate of a colony, as the colony can withstand the death of a single queen or multiple queens. The reproduction of multiple queens also allows for greater colony growth because it relaxes the constraint on the upper limit of their egg-laying capacity (Boomsma, Huszár, & Pedersen, 2014; Boulay et al., 2014). Oftentimes, polygyny is

associated with colony foundation through budding (Cronin et al., 2013). This dispersal strategy entails the founding of new colonies by queens assisted by workers, dispersing from their natal nests on foot to establish new nests nearby (Hölldobler & Wilson, 1977; L. Keller, 1991). Colony foundation through budding is associated with high foundation success, as the help of workers increase survival and reproduction of new nests during the early establishment stage (Cronin et al., 2013). However, this mode of foundation restricts dispersal of the species, often leading to the establishment of many genetically similar colonies across a landscape and thereby a pattern of isolation-by-distance (Schultner et al., 2016). Interestingly, this reduction in dispersal may promote polygyny and polydomy, and may ultimately lead to the formation of supercolonies – extensive colonies comprised of many nests exchanging workers, queens, brood and resources (Tsutsui & Suarez, 2003). This colony structure eliminates intraspecific competition, leading to dense networks of interconnected nests, genetically indistinguishable from each other. The development of highly polygynous supercolonies enables invasive populations to reach tremendous densities and rapidly outcompete native species by allocating a high number of workers to monopolize resources (Tsutsui & Suarez, 2003). For example, populations of the yellow crazy ant *Anoplolepis gracilipes* and the little fire ant *Wasmannia auropunctata* can reach densities of up to 20 million and 240 million ants per hectare, respectively (Abbott, 2005; Souza, Follett, Price, & Stacy, 2008). To date, the association of polygyny, dependent colony foundation and development of a dense polydomous nest structure have been observed in many invasive ants, such as *Linepithema humile*, *Pheidole megacephala*,

*Monomorium pharaonis* and *Nylanderia fulva* (Buczkowski & Bennett, 2009; Eyer, McDowell, et al., 2018; Tsutsui et al., 2000).

Although invasions are generally associated with establishments in new countries or continents, they can also occur along a habitat continuum. The odorous house ant *Tapinoma sessile* is one such invader – native to a variety of natural habitats across North America (e.g., forests, grasslands, bogs, etc.), this ant has become highly abundant in urban environments throughout the United States (Buczkowski, 2010; Buczkowski & Bennett, 2008; Menke et al., 2010). Interestingly, like more traditional invasive ants, *T. sessile* exhibits a transition in its breeding system and social structure between its native and invasive populations. Colonies occurring in natural habitats are typically small (<200 workers) and consist of a single nest headed by a single queen (Buczkowski, 2010). On the other hand, urban colonies tend to be large (>100,000 workers) and made of several interconnected nests, each comprising numerous reproductive queens, with low internest aggression over large landscapes. This suggests the existence of supercolonies in this species within urban environments (Buczkowski, 2010; Buczkowski & Bennett, 2008; Menke et al., 2010). However, these assessments have only been based on behavioral studies (Buczkowski, 2010; Buczkowski & Bennett, 2008; Buczkowski & Krushelnycky, 2011), while the genetic underpinnings of the colonies have not been analyzed. In *T. sessile*, four major mitochondrial clades have been described across the United States (Menke et al., 2010). Remarkably, this shift in life history traits has occurred consistently across its distribution, rather than all urban colonies originating from a single natural population (Menke et al., 2010). Therefore, plasticity in colony structure appears to be

inherent within the species, and the repeated transition of small, monogyne natural habitat colonies to large, polygyne urban colonies resembles the invasions of more traditional invasive ants. Thus, *T. sessile* represents a unique opportunity to determine the factors driving these trait differences, which may provide insights into their evolutionary trajectory and broaden our understanding of the mechanisms linking them to species invasions.

Here, we conducted a large-scale analysis of the population and colony structure of *T. sessile* across the four geographic clades uncovered within the United States (Menke et al., 2010). For each of the four clades, we performed a paired sampling of one urban and one natural habitat in close geographic proximity (except for the Mountain clade in Colorado – see Methods). We first investigated the breeding structure of these populations to test for consistent transitions of monogyne colonies in natural habitats to polygyne colonies in urban areas, by assessing the number of queens per nest and the relatedness among nestmate workers. We then evaluated the colony structure of *T. sessile* in each locality by genetically inferring whether different nests belong to the same polydomous colony, testing for unicoloniality within urban habitats and multicoloniality within natural habitats. We also analyzed whether workers from different nests recognize each other as colony-mates through behavioral assays, testing for reduced aggression between non-nestmate workers in urban habitats compared to natural habitats, and assessed whether this discrimination toward non-nestmate workers is mediated through chemical cues. In addition, we investigated the dispersal ability of *T. sessile* by testing for an isolation-by-distance pattern in each locality and habitat. Finally, we discuss the potential evolutionary

mechanisms enabling urban invasions by a native ant species, comparing these mechanisms with the life history traits shared by most invasive ant species.

## **5.2 Methods**

### *5.2.1 Study sites and sampling*

Nests of *T. sessile* were collected from July of 2018 to August of 2020 in four localities across the United States: Bloomington, Indiana; Bay Area, California; Little Rock, Arkansas; and Boulder, Colorado (Figure 1a). These four localities correspond to the four geographic clades previously elucidated by Menke et al. (2010). For each locality, two sites in close geographic proximity were identified – one comprising the urban environment (residential or commercial areas) and the other comprising the natural environment, with fifteen nests collected in each habitat. No nests were found in the urban environment of Boulder, Colorado. Therefore, our total collection consisted of 104 nests across the four localities and seven total sites. Although *T. sessile* inhabits a variety of natural habitats, all natural collections were carried out within forests. As previous observations of the ant across several natural habitats are suggestive of a consistent natural disposition (Fellers, 1989; Kimball, 2016; Menke et al., 2010; Milford, 1999), we refer to our forest collections as natural colonies for the remainder of the paper.

In both habitats, entire nests were sampled to ensure a reliable count of queens and that ants collected belonged to the same nest, and no minimum collection distance was used between nests in order to not preclude the detection of polydomous colonies. The nests were transported to the laboratory and kept under standard conditions ( $28 \pm 2^\circ\text{C}$ , 12:12 h light period, and fed with an artificial ant diet (Dussutour & Simpson, 2008)). For

each nest, eight workers were separately placed in 200  $\mu$ l hexane for chemical analysis, while a subset of workers, queens and males were directly stored in 96% ethanol at 4°C for genetic analysis.

### 5.2.2 Genetic analyses

The genomic DNA of eight workers and up to eight queens and males from each nest was extracted following a modified Gentra Puregene extraction method (Gentra Systems Inc.). Species-specific microsatellite primers do not exist for *T. sessile*; instead, we tested 39 markers shown to amplify in closely related species (Berman, Austin, & Miller, 2014; Butler, Siletti, Oxley, & Kronauer, 2014; Krieger & Keller, 1999; Zheng, Yang, Zeng, Vargo, & Xu, 2018; Zima, Lebrasseur, Borovanska, & Janda, 2016). Forward primers were affixed with an M13 tail to enable PCR multiplexing via fluorescent labelling with 6-FAM, VIC, PET, and NED (Boutin-Ganache, Raposo, Raymond, & Deschepper, 2001). PCR reactions were performed on a Bio-Rad thermocycler T100 (Bio-Rad). Multiplex PCR products were run on an ABI 3500 capillary sequencer (Applied Biosystems) along with the LIZ500 standard. Geneious v.9.1 was used for scoring alleles (Kearse et al., 2012). Of the 39 markers tested, 21 were discarded due to nonamplification or monomorphic amplification. The linkage disequilibrium (LD) for each pair of loci was tested for each locality separately using GENEPOP v4.7 (Rousset, 2008), with *p*-values corrected via the Holm method to account for multiple comparisons (Holm, 1979). Loci exhibiting linkage disequilibrium were discarded from further analysis. Overall, the final data set includes 831 workers genotyped at 12 polymorphic microsatellite loci.

Sequencing of the cytochrome oxidase 1 (COI) mitochondrial gene was performed on at least one worker from each nest, with multiple workers from a nest sequenced if microsatellite genotypes suggested they originated from different queens (n = 145). Gene sequences were amplified using primers LepF1 and LepR1, targeting a 658-bp fragment (Hajibabaei, Janzen, Burns, Hallwachs, & Hebert, 2006; Hebert, Penton, Burns, Janzen, & Hallwachs, 2004). PCR products were purified with EXOSAP-it PCR purification kit (Affymetrix) and sequenced using the ABI BigDye Terminator v.3.1 Cycle Sequencing Kit on an ABI 3500 Genetic Analyzer (Applied Biosystems). Base calling and sequence reconciliation were performed using CodonCode Aligner (CodonCode Corporation).

### *5.2.3 Population and colony structure analyses*

A Bayesian phylogenetic tree and a haplotype network were constructed to assign each nest into one of the four clades previously described by Menke et al. (2010). In addition, the mitochondrial dataset was used to test for the presence of multiple haplotypes within nests, which would indicate the reproduction of multiple unrelated queens. MrBayes v.3.2 was used to construct the tree (Ronquist et al., 2012), using the generalized time reversible model with gamma-distributed rate variation across sites and a proportion of invariable sites as the evolutionary model. Two simultaneous MCMC simulations ran for 2 x 10<sup>6</sup> generations using four chains (three heated and one cold), with each run sampled every 500 generations. The mitochondrial network was produced via the median-joining method (Bandelt, Forster, & Röhl, 1999) implemented in POPART (Leigh & Bryant, 2015). The COI gene was extracted from the complete mitochondrial genome sequence of *T. melanocephalum* to use as an outgroup for both analyses (Du, Song, Yu, & Lu, 2019).



Additionally, sequence divergence was compared within and between habitats and populations of *T. sessile* using the Kimura 2-parameter model (Kimura, 1980) in MEGA v. 10.2.2 (Kumar, Stecher, Li, Knyaz, & Tamura, 2018; Tamura et al., 2011). The number and frequency of alleles,  $F$ -statistics (Weir & Cockerham, 1984), and observed and expected heterozygosity (Nei, 1987) were calculated for each microsatellite marker, and for each locality and habitat (as well as overall values) using FSTAT v2.9.4 (Goudet, 2003). For the overall dataset, the hierarchical partitioning of the genetic diversity between localities, between habitats within localities, between nests within habitats, between individuals within nests, and within individuals was assessed using an analysis of molecular variance (AMOVA) implemented in the ade4 R package (Dray & Dufour, 2007; R Core Team, 2020) via Poppr (Kamvar, Tabima, & Grünwald, 2014).

Three complementary approaches were used to determine whether workers collected from different nests belonged to the same colony. First, genotypic differentiation between each pair of nests within localities was tested using the log-likelihood  $G$  test implemented in GENEPOP v.4.7 (Rousset, 2008). Nests were considered distinct colonies if genotypic differentiation was found to be significantly different using Fisher's test together with the Holm method to account for multiple comparisons (Holm, 1979). Second, population structure was visualized with a principal component analysis (PCA) using the dudi.pca function in the adegenet R package (Jombart, 2008; R Core Team, 2020). Third, the presence of genetic structure was tested using the Bayesian clustering method implemented in STRUCTURE v.2.3.4 (Falush, Stephens, & Pritchard, 2003; Pritchard, Stephens, & Donnelly, 2000). Simulations were run for  $K$  (*i.e.*, genetic clusters)

ranging from 1 to the maximum number of nests per data set, with each run of  $K$  replicated 20 times. The analyses were run under the admixture model with correlated allele frequencies enabled. Each run was initiated with a 50,000 burnin period, followed by 100,000 iterations of the MCMC. The most likely number of genetic clusters ( $K$ ) was inferred using the methods of both Evanno, Regnaut, and Goudet (2005) and Puechmaille (2016), with the output visualized via CLUMPAK (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015), as implemented in the web-based software StructureSelector (Y.-L. Li & Liu, 2018). The method of Puechmaille (2016) aims at unraveling finer partitioning in the data, whereas the Evanno et al. (2005) method aims at describing the primary partitioning. Finally, isolation-by-distance analyses were performed with Mantel tests using the vegan R package (Oksanen et al., 2020; R Core Team, 2020), between matrices of genetic differentiation ( $F_{ST}$ ) and geographic distance. The PCA, STRUCTURE and isolation-by-distance analyses were first performed for the overall data set, then for each locality, and finally for each habitat within each locality.

#### *5.2.4 Breeding structure analyses*

We estimated the number of queens per nest and the genetic relatedness among nestmate workers for each locality and habitat. We also explored the possibility that queens use thelytokous parthenogenesis for the production of new queens, as this strategy was previously reported in several invasive ant species (Fournier et al., 2005; Percy, Goodisman, et al., 2011; Rabeling & Kronauer, 2013). The presence (or lack thereof) of multiple queens per nest was first determined directly from field observations. For each nest, polygyny was confirmed genetically through the presence of multiple mitochondrial

haplotypes and through the composition of worker microsatellite genotypes. Polygyny was inferred when worker genotypes could not be reliably assigned to a single queen (all workers carrying one of the two alleles of the mother queen at all microsatellite markers).

Relatedness coefficients ( $r$ ) among nests were estimated using coancestry v.1.0.1.9 (Jinliang Wang, 2011), following algorithms described by Queller and Goodnight (1989). As differences in allele frequencies may exist between localities, relatedness coefficients were calculated separately for each of the localities. Additionally, relatedness coefficients were estimated at three separate levels – (1) between workers, (2) between queens, and (3) between workers and queens. Finally, we evaluated whether queens produce new queens through thelytokous parthenogenesis by comparing the heterozygosity level and relatedness between castes in each locality. As automictic thelytokous parthenogenesis generally increases homozygosity over time (Pearcy, Hardy, & Aron, 2006; Pearcy, Hardy, & Aron, 2011; Rey et al., 2011), a decrease in observed heterozygosity and increase in relatedness should be present in the parthenogenetically produced queens when compared against sexually produced workers. For these and all further comparative analyses, figures were generated using the ggstatsplot R package (Patil, 2021).

#### *5.2.5 Chemical analyses*

Chemical differentiation between nests was determined by analyzing eight randomly chosen workers per nest using GC-MS. Individual ants were knock-downed for 1 min at  $-20^{\circ}\text{C}$  and extracted in 200  $\mu\text{l}$  hexane for 5 min with intermittent gentle mixing. Extracts were evaporated under a stream of high-purity nitrogen, redissolved in 35  $\mu\text{l}$  of hexane and transferred to a 100  $\mu\text{l}$  insert in a 1.5 ml autoinjection vial. A volume of 2  $\mu\text{l}$  was

injected in splitless mode using a 7693B Agilent autosampler into a HP-5MS UI column (30 m × 0.250 mm internal diameter × 0.25 μm film thickness; Agilent) with ultrahigh-purity helium as carrier gas (0.75 ml/min constant flow rate). The column was held at 50°C for 1 min, increased to 320°C at 10°C/min, and held at 320°C for the last 10 min. The overall chemical profile of each individual was investigated by calculating the relative abundance of each compound. All compounds occurring in at least 10 samples were used to calculate the chemical profile of individuals, but we did not aim at identifying the different chemical compounds. The chemical profile of individuals was compared between nests, localities and populations.

We performed a PCA using *ade4* (Dray & Dufour, 2007; R Core Team, 2020) in order to visualize the variation within and between nests. Additionally, we estimated the pairwise cuticular hydrocarbon (CHC) differentiation between each nest through the calculation of the Euclidean distance between nest centroids. We then assessed the level of CHC variation within each nest by calculating the average Euclidean distance between each of the eight workers and the centroid of the nest. The between nest and within nest calculations were performed on the first two PC's from the PCA. We first tested whether the level of CHC variation within nests differs between native and urban environments, as well as between monogyne and polygyne nests. Finally, we tested whether this level of CHC variation within a nest increases with genetic diversity (using expected heterozygosity as a proxy), and whether the level of CHC differentiation between nests increases with genetic differentiation or geographic distance, with significance determined using Student's *t*-distribution for Pearson's correlation coefficient.

### 5.2.6 Behavioral assays

Aggression assays were performed by randomly selecting a single worker from two distinct nests and placing them together in a 5 cm diameter petri dish for 5 min. The sides of the petri dish were coated with Fluon to prevent the ants from escaping, and the bottom of the petri dish was covered with filter paper that was changed between trials to prevent odor transfer between trials. The subsequent behavioral interactions were scored on a four-level scale of escalating aggression (Suarez, Tsutsui, Holway, & Case, 1999): (1) touch (contacts that included prolonged antennation), (2) avoid (contacts that resulted in one or both ants quickly retreating in opposite directions), (3) aggression (lunging, biting, and pulling legs or antennae), or (4) fight (prolonged aggression between individuals). For each trial, the highest level of aggression was recorded, with the mean of 10 trials for each nest pairing used to calculate an average aggression score between nests. Pairs of nests assigned to the same colony based on microsatellite markers were used as a control for this experiment. Twenty-five nest combinations were tested in Arkansas, California and Indiana (10 urban-urban, 10 natural-natural and 5 urban-natural) and 10 were tested in Colorado (10 natural-natural; no urban colonies found) for a total of 850 trials (85 nest combinations x 10 trials). Nests were matched across a short (minimum 0.001 km) to long-distance (maximum 26 km) gradient to identify whether geographic distance influenced aggression between nests. Similarly, aggression was compared against both genetic and chemical differentiation. The significance of all three relationships was evaluated using Student's *t*-distribution for Pearson's correlation coefficient. Finally, aggression levels among and between urban and natural nests were compared using Kruskal-Wallis tests,

with Dunn's test utilized to elucidate significant pairwise relationships and  $p$ -values adjusted by the Holm method to account for multiple comparisons.

### **5.3 Results**

#### *5.3.1 Population and colony structure analyses*

A total of 68 mitochondrial haplotypes were identified, of which 36 were shared between individuals. The mean genetic distance between localities was 0.079, while it was only 0.039 within localities. Mitochondrial haplotypes were rarely shared between localities (*i.e.*, a single one shared between Arkansas and Indiana). Yet, the topology of the tree did not align completely with geography and therefore did not concur entirely with the four geographic clades previously uncovered by Menke et al. (2010) (Figure 1b). Notably, a substantial portion of the eastern US samples (*i.e.*, Indiana and Arkansas) intermix with one another and appear on three distinct branches of the tree. Additionally, two samples from California were located nearest to the two basal eastern US branches, while samples from Colorado were split across two clades.

Our results further confirmed the finding of Menke et al. (2010) that mitochondrial haplotypes were commonly shared between monogyne and polygyne social structures ( $n = 12$  – dispersed across all localities). However, haplotypes were rarely shared between natural and urban habitats, as only a single haplotype was shared between the two (in Indiana; Figure 1b), suggesting that little genetic exchange occurs between habitats. Finally, the presence of multiple haplotypes within a nest was rare ( $n = 7$  nests – only in the eastern US), suggesting polygyne colonies primarily develop via the association of related queens.

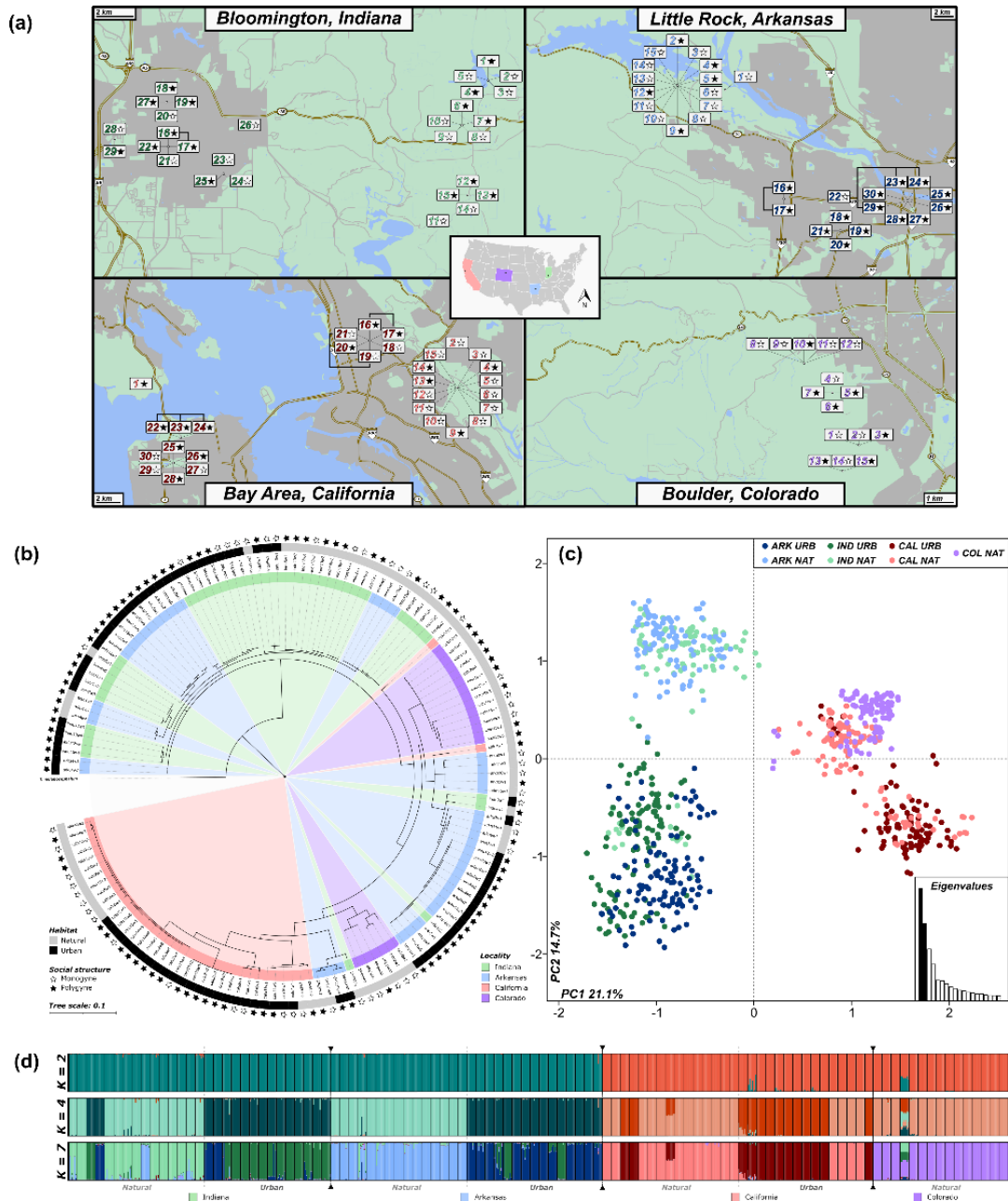


Figure 17: (a) Sampling locations of *Tapinoma sessile* across the United States. For each locality, nests were sampled in both natural and urban environments, depicted as light-colored (natural) and dark-colored (urban) numbers in the figure. The thickened black lines connecting some nests represent nests that were found to belong to the same colony. Additionally, the stars next to each number denote the social structure of the colony - white and black stars represent monogyne and polygyne colonies, respectively. Note that for each locality, the counting always begins with the first natural nest; also, note that no urban nests were found in Colorado. (b) Bayesian inference tree based on 145 COI sequences of *T. sessile* across the four localities, with one *T. melanocephalum* sequence as an outgroup. (c) PCA based on the microsatellite data of each individual from each nest sampled in the overall data set (dots represent individuals). (d) STRUCTURE analysis based on the microsatellite data across four values of  $K$ , which correspond to the levels of hierarchy present within the overall data set ( $2 = \text{habitat}$ ;  $4 = \text{locality}$ ;  $7 = \text{habitat} \times \text{locality}$ )

The 12 microsatellite markers used in this study contained an average of 12.6 alleles (range = 3-49). When split by habitat, the natural and urban datasets contained an average of 9.8 (range = 3-38) and 9.2 (range = 3-37) alleles, respectively. Therefore, the allelic diversity was not significantly different between natural and urban habitats (Mann-Whitney  $U = 4.31$ ,  $p = .907$ ). Furthermore, the allelic diversity was not significantly different between any of the four localities (Kruskal-Wallis  $H = 1.72$ ,  $p = .633$ ).

Table 2: The number and average number of alleles, observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity, inbreeding coefficient ( $F_{IS}$ ) and fixation index ( $F_{ST}$ ) for each locality and habitat across the 12 microsatellite loci.

<b>Location</b>	<b>Alleles</b>	<b>Average</b>	<b><math>H_O</math></b>	<b><math>H_E</math></b>	<b><math>F_{IS}</math></b>	<b><math>F_{ST}</math></b>
<i>Natural</i>						
Indiana	69	5.75	0.367	0.273	-0.344	0.375
Arkansas	53	4.42	0.309	0.240	-0.291	0.308
California	51	4.25	0.371	0.240	-0.542	0.519
Colorado	53	4.42	0.310	0.228	-0.360	0.391
Overall	117	9.75	0.339	0.245	-0.384	0.591
<i>Urban</i>						
Indiana	64	5.33	0.363	0.329	-0.101	0.370
Arkansas	57	4.75	0.306	0.316	0.031	0.363
California	75	6.25	0.233	0.240	0.033	0.568
Overall	110	9.17	0.299	0.295	-0.015	0.555
All	144	12.00	0.322	0.266	-0.211	0.601

In the overall data set, the AMOVA analysis revealed slight genetic diversity partitioned between localities (10.6%), with more substantial levels partitioned between habitats within localities (23.9%), between nests within habitats (27.2%), and within individuals themselves (45.5%). The difference between localities is mostly driven by a clear separation of the eastern US samples (*i.e.*, Indiana and Arkansas) from the western localities (*i.e.*, Colorado and California) observed at  $K = 2$  (Figure 1c,d). Consistently, Mantel tests identified significant isolation-by-distance when analyzing localities as a



whole, contrasting the results obtained by the mitochondrial marker, where eastern and western populations did not segregate into two clearly distinct clades. Interestingly, at  $K = 4$ , the eastern US samples grouped by habitat rather than by locality, despite being geographically distant (Figure 1d). To a lesser extent, a similar pattern can be seen in the western localities, as the natural habitats of California and Colorado mostly grouped together (Figure 1d). At  $K = 7$ , both the localities and the habitats within localities

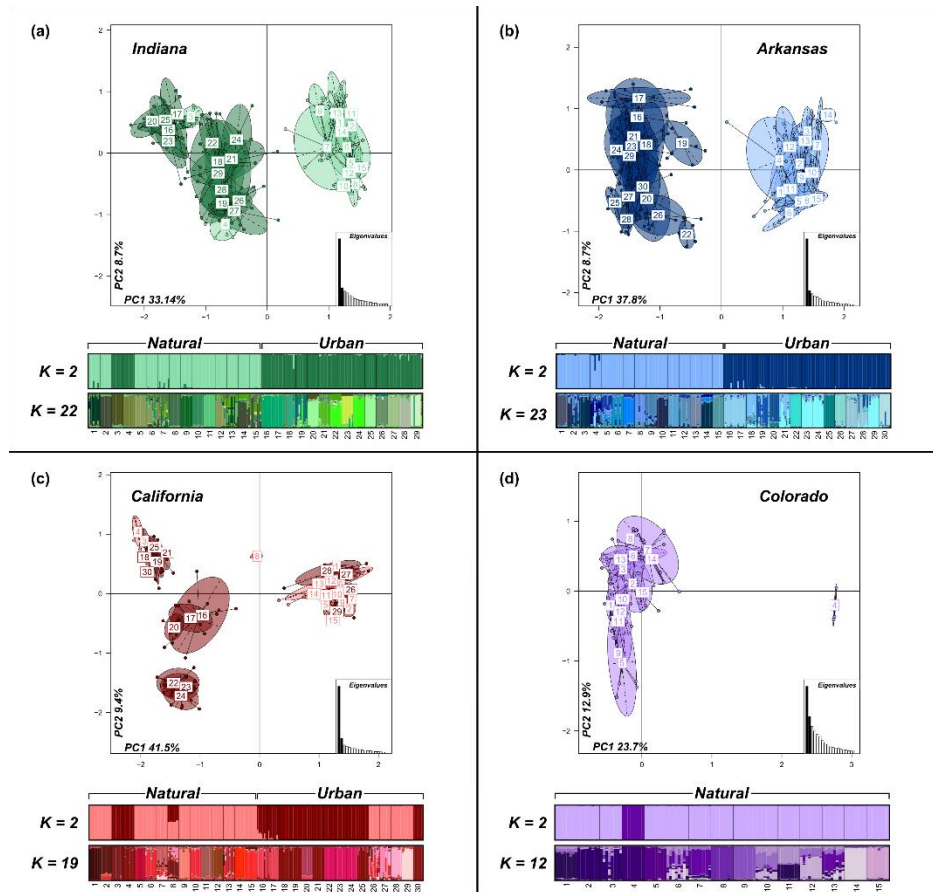


Figure 18: Population structure of *Tapinoma sessile* across the United States. Clustering of nests in (a) Indiana, (b) Arkansas, (c) California and (d) Colorado using a PCA and STRUCTURE on the microsatellite markers. For each locality, the light-shaded and dark-shaded ellipses in the PCA represent natural nests and urban nests, respectively. Additionally, two runs of STRUCTURE are shown for each locality, which correspond to best  $K$  (*i.e.*, genetic clusters) as inferred by two different methods (Evanno above and Puechmaille below)

clustered independently (Figure 1d), highlighting that the overall distribution of genetic variability is strongly influenced by both geographic distance and habitat.

Within localities, strong differentiation (*i.e.*, high  $F_{ST}$ ) was found between almost every nest (Table 1). Similarly,  $G$  tests revealed that most nests represented a single genetic entity. Of the nest pairs that could not be differentiated, 11 were in the urban environment and two were in the natural environment. STRUCTURE analyses using the method of Puechmaille (2016) (*i.e.*, finer partitioning) produced corroborating results, with best  $K$  mostly segregating each nest as its own genetic cluster (Figure 2). However, two trios of geographically adjacent nests were not genetically different from each other in urban habitats within California (nests 22-24) and Arkansas (nests 23-24, 29; Figures 1a and 2b,c). These two trios of nests also clustered together when urban habitats were analyzed separately from the natural habitat.

Remarkably, the Evanno et al. (2005) method (*i.e.*, primary partitioning) consistently depicted clear separation between urban and natural habitats (Figure 2).  $K = 2$  best explained the structure in the data for each locality and mostly segregated urban and natural colonies into two distinct clusters (Figure 2). This strong dichotomy between urban and natural habitats was also highlighted using PCAs within each locality (Figure 2). No isolation-by-distance was found when analyzing each habitat separately within

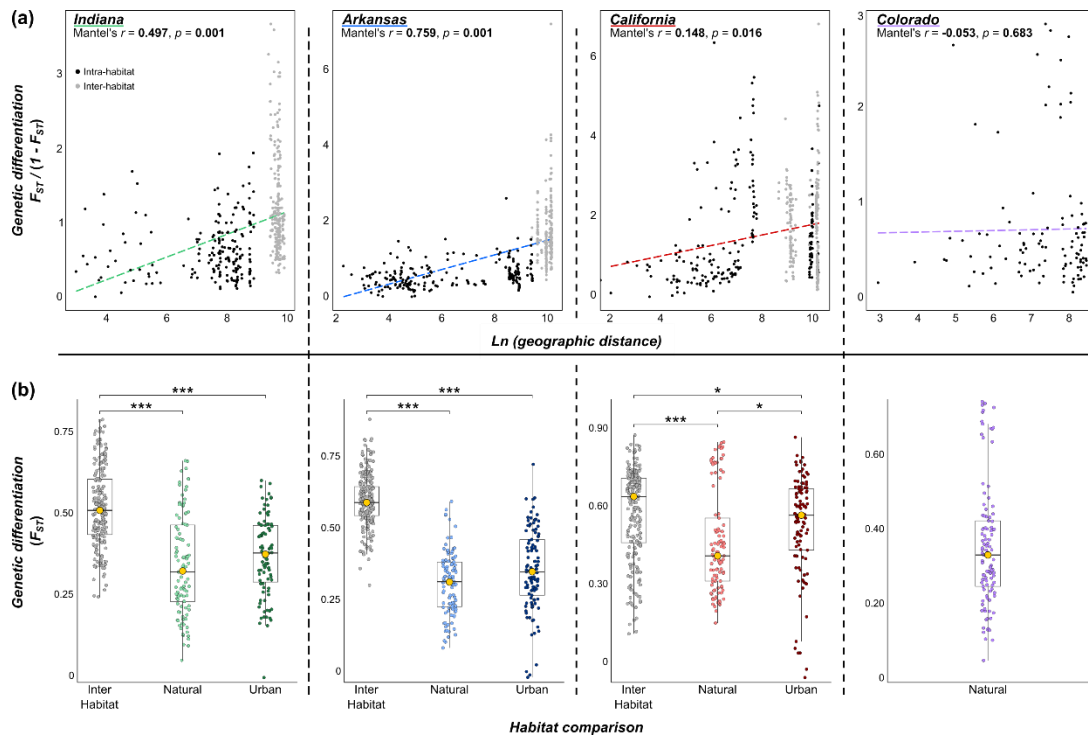


Figure 19: (a) Isolation-by-distance plots for each locality. (b) Comparisons of genetic differentiation ( $F_{ST}$ ) between each pair of nests, both between and within habitats. Each gold dot on a boxplot represents the mean of the group, and only significant pairwise comparisons are shown (as determined by Dunn's test with  $p$ -values adjusted according to the Holm method; \* $p < .05$ , \*\* $p < .01$ , \*\*\* $p < .001$ ). Note that Colorado contains only intrahabitat comparisons for both (a) and (b), as nests were only found in natural habitats

localities (all  $p > .05$ ); however, isolation-by-distance was significant when comparisons between habitats within localities were considered ( $p = .001, .001$  and  $.016$  for Indiana, Arkansas and California respectively; Figure 3a). Indeed, the interhabitat genetic differentiation between nests was always higher than the differentiation between nests within a habitat (Figure 3b). However, the genetic differentiation between nests mostly did not differ across the two habitats (Figure 3b). AMOVAs for each locality were similar to the overall data set, with most genetic diversity partitioned within individuals (avg. = 50%), and substantial amounts partitioned between habitats (avg. = 26%) and nests within

each habitat (avg. = 31%). Taken together, these results suggest that most nests sampled across the four localities represent distinct colonies. They also highlight the substantial differentiation between urban and natural populations and support the continent-wide observation that colonies of *T. sessile* grouped by habitat rather than by locality within the eastern and western populations.

### 5.3.2 *Breeding structure analyses*

Overall, the urban environment contained significantly more polygyne nests (67%) than the natural environment (38%,  $p = .002$ ; Figure 4a). Although both social structures were found in both habitats, the number of queens collected per nest was significantly higher in urban habitats (mean  $\pm$  SD =  $13.00 \pm 15.70$ , up to 62) than natural habitats (mean  $\pm$  SD =  $2.61 \pm 3.76$ , up to 21; Figure 4a). This pattern was found for the overall data set ( $p < .001$ ), as well as for each locality separately (despite being non-significant for Indiana,  $p = .117$ ).

Accordingly, the coefficient of relatedness between workers was significantly higher in the natural environment ( $R_{w-w} = 0.74$ ) than in the urban environment ( $R_{w-w} = 0.65$ ). This association was found significant for the overall dataset ( $p < .001$ ), as well as for each locality separately (all  $p < .05$ ; Figure 4b). However, the relatedness among workers was surprisingly high considering the number of queens present in each nest. This is especially true for urban nests (mean  $R_{w-w} = 0.61, 0.72$  and  $0.61$  for the urban habitats of Arkansas, California and Indiana, respectively), as they usually contained a high number of queens. No values of relatedness were found close to zero (lowest value was 0.22), which is expected under a random association of a high number of queens or with the free movement of individuals among nests across the population.

Although natural nests were significantly more outbred (*i.e.*, lower negative  $F_{IS}$ ) than their urban counterparts ( $p < .001$ ; Table 1), the high relatedness within urban nests does not appear to stem from inbreeding (mean  $F_{IS} = -0.015$ ). This suggests that queens

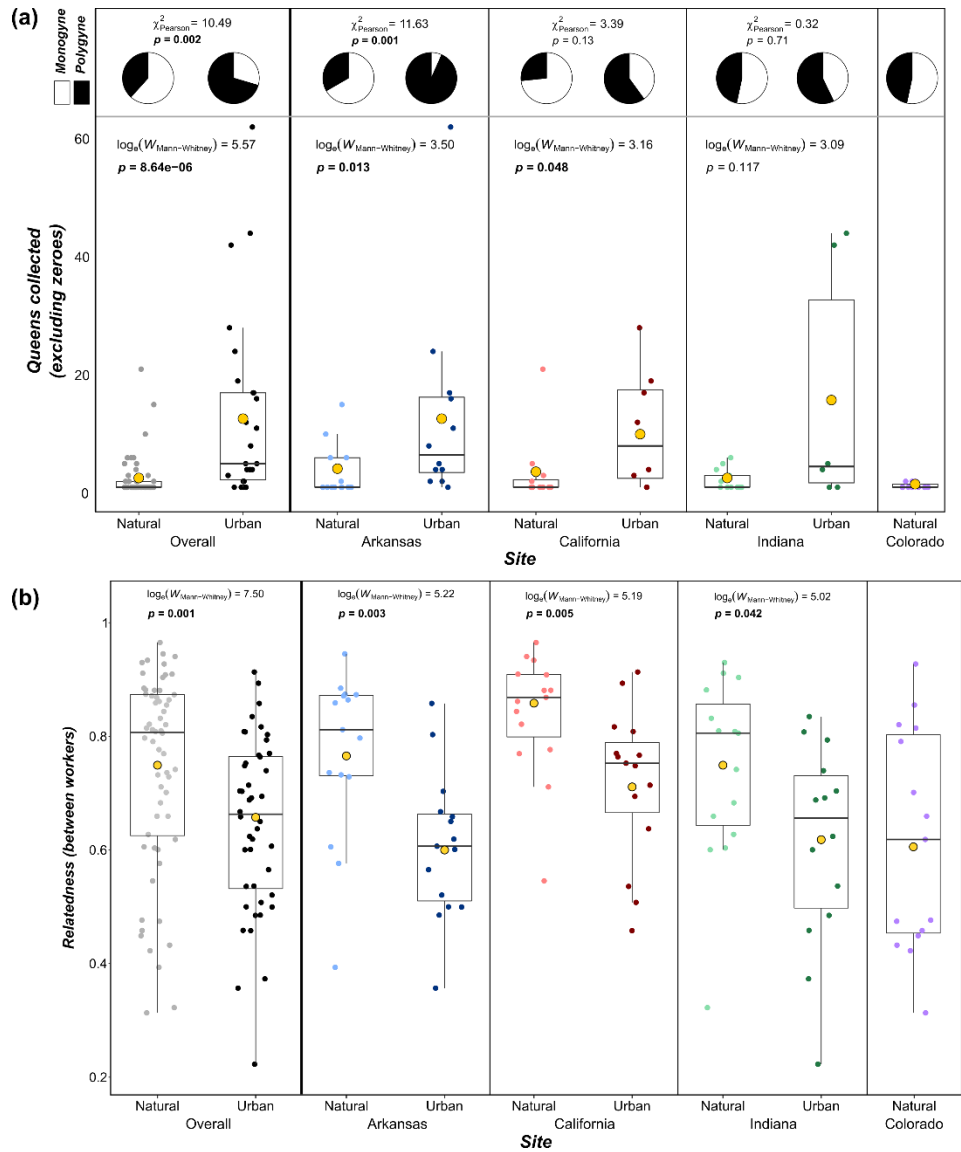


Figure 20: Breeding structure of *Tapinoma sessile* overall and across the four localities. (a) The percentage of monogyne and polygyne nests in natural and urban habitats, as well as the number of queens collected in nests where at least one queen was found. (b) The average relatedness among workers within a nest in urban and natural habitats. For both (a) and (b), each smaller dot represents a nest, and each gold dot denotes the mean of the habitat

do not exclusively participate in intranidal mating, although some level is likely considering the high relatedness values (and has been thought to occur in *T. sessile* – see Kanno (1959)). Finally, the relatedness among queens within nests was also high ( $R_{Q-Q} = 0.68$  and  $0.66$ , for natural and urban polygyne nests, respectively), an uncommon finding for a polygynous ant and indicative of daughter queens being retained within their natal nest. However, this relatedness was not significantly higher and the observed heterozygosity not significantly lower when compared to the worker caste in any of the localities, suggesting that new queens are not produced asexually.

### 5.3.3 Chemical analyses

Population clustering based on the CHCs yielded similar results to those of the genetic analyses. At the overall scale, substantial chemical differentiation was found between localities, with the eastern US, California, and Colorado samples appearing distinct from one another. Consequently, CHC differentiation was significantly positively correlated with both geographic distance and genetic differentiation at the overall level. The Colorado samples were completely separate from the California samples, despite appearing genetically similar to samples from the California natural habitat. Interestingly, the urban and natural habitats from Arkansas, as well as the urban habitat from Indiana, clustered together chemically.

The chemical segregation of nests between natural and urban habitats became clearer when clustering analyses were performed at the locality level (Figure 5). Like the genetic differentiation, the chemical differentiation was mostly higher between nests from distinct habitats than between nests from the same habitat within a given locality (Figure

5). Consequently, the chemical differentiation between nests is associated within both genetic differentiation and geographic distance within most localities. Interestingly, nests 3 and 4 from the natural habitat of Indiana clustered with the urban habitat both genetically (Figure 2a) and chemically (Figure 5a). Overall, these findings suggest that chemical differentiation is influenced by both genetic and environmental factors, and therefore by the clear effect of habitat on the genetic differentiation mentioned above.

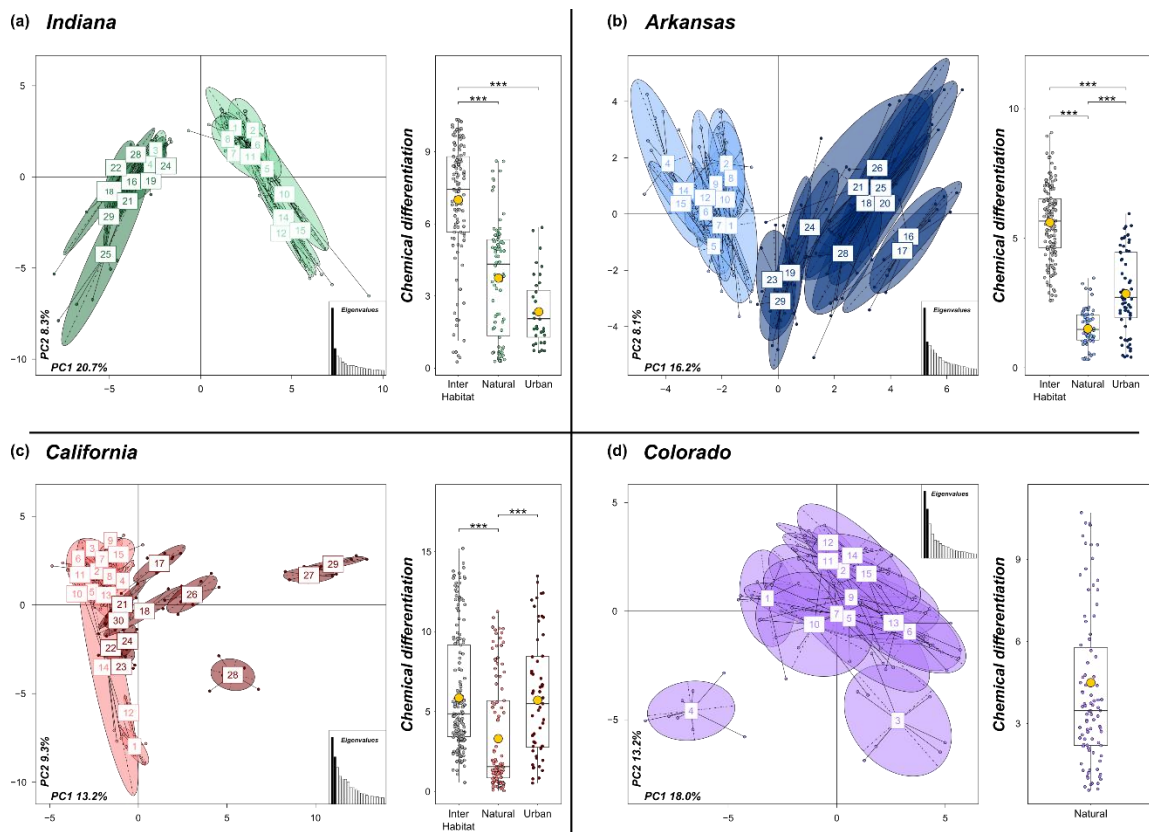


Figure 21: Clustering of nests in (a) Indiana, (b) Arkansas, (c) California and (d) Colorado using a PCA on each nest's CHC profile. For each locality, the light-shaded and dark-shaded ellipses in the PCA represent natural nests and urban nests, respectively. Additionally, the boxplots illustrate the CHC differentiation of nests among and between habitats. Each smaller dot on the boxplots represents the difference between a pair of nests, while each gold dot denotes the mean of the group. Only significant pairwise comparisons are shown (as determined by Dunn's test with  $p$ -values adjusted according to the Holm method;  $*p < .05$ ,  $**p < .01$ ,  $***p < .001$ ).

Interestingly, the within-nest CHC variation was not significantly different between monogyne and polygyne nests in any locality or for the overall dataset. However, the within- nest variation was significantly different between natural and urban nests, with natural nests having increased variation at the overall level; a similar, but not significant, pattern was also observed within each locality. No significant correlations between CHC variation and genetic diversity were found within any locality.

#### *5.3.4 Behavioral analyses*

Aggression assays further demonstrated that most nests appear to be distinct colonies. The overwhelming majority of pairings obtained aggression scores of 3 or 4, with significant differences between groups mainly driven by slight fluctuations in avoidance/aggressive behaviors (Figure 6). The notable exception to these aggressive behaviors occurred in the California urban plot, where a more even distribution of aggression scores resulted from the lack of aggression between the trio of nests collected in San Francisco, California (nests 22-24; Figures 1a and 6). A similar trio of nests without aggression were also collected from the urban environment in Little Rock, Arkansas (nests 23-24, 29; see Figure 1a). Given that the genetic analyses above also support that the trio of nests in both cities comprise a single genetic entity, these two sites potentially represent supercolonies, albeit geographically limited (approximately 7500 and 3600 m<sup>2</sup> in California and Arkansas, respectively).



The correlation analyses revealed little link between aggression and geographic distance, genetic differentiation or chemical differentiation, most probably driven by the high aggression that was observed in most aggression assays. Again, the notable exceptions here were the urban San Francisco and Little Rock sites mentioned above, as low genetic differentiation and aggression between some nests at these sites led to a significant positive correlation between genetic differentiation and aggression.

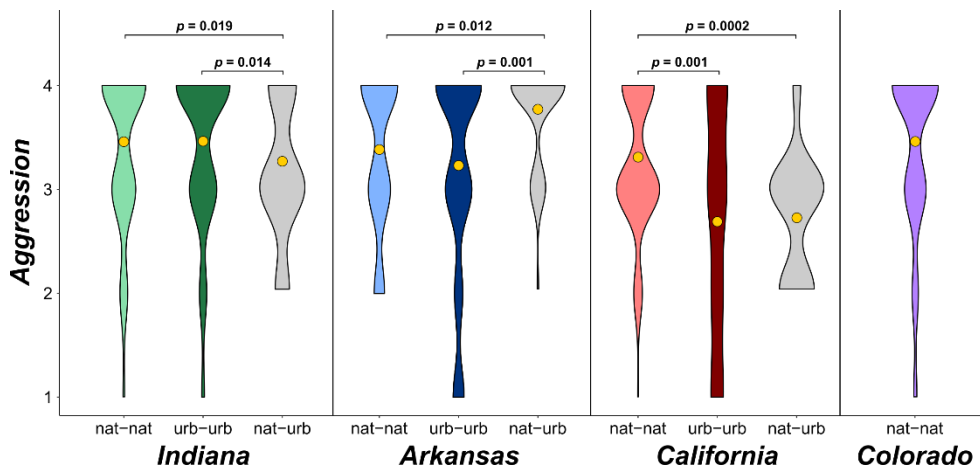


Figure 22: Violin plots of aggression between nests, with aggression compared within and among habitats for each locality (except Colorado). The gold dot on each violin represents the mean, and only significant pairwise comparisons are shown (as determined by Dunn's test with  $p$ -values adjusted according to the Holm method)

#### 5.4 Discussion

Our extensive phylogenetic, chemical and behavioral study revealed several insights into the colony and breeding structure of *T. sessile* across the United States. First, we confirmed that the social structure of a colony appears to be a plastic trait found in both natural and urban habitats; however, we did find monogyny prevalent in the natural habitat and polygyny more common in urban habitats. Furthermore, the extent of polygyny within nests was far greater in the urban habitat; however, differentiation was present between

almost every nest in each locality and habitat. This finding, supported by our chemical and behavioral analyses, suggests that urban colonies mostly do not consist of large supercolonies. Yet, two trios of nests in two separate urban habitats lacked genetic, chemical and behavioral differentiation. As no such polydomous colonies were found in any natural habitat, this suggests only the urban environment harbors the necessary conditions for supercolony formation in *T. sessile*. Interestingly, our results uncovered clear genetic and chemical differentiation between natural and urban populations. This clear separation was observed within each locality, and also at a large geographic scale as nests clustered by habitat rather than by locality within the eastern and western clades. These findings suggest that urbanization acts as a strong barrier to gene flow in this species, with the heterogeneity of suitable habitat within cities potentially limiting immigration and emigration. Additionally, the clustering of nests by habitat rather than by locality may denote a strong signature of selection for the urban environment, with specific genotypes most probably found within cities. Overall, these results provide further support for urbanization as an intense driver of evolution.

Modified ecological interactions within the urban environment are known to affect the composition of communities and have been specifically shown to promote the abundance of arthropods (Faeth, Bang, & Saari, 2011), including ants (Vonshak & Gordon, 2015). Therefore, certain characteristics of urban habitats may have allowed for *T. sessile* to achieve larger colony sizes than their natural conspecifics. For one, the abundance of traditionally limited resources may have stabilized, or even increased, colony productivity within urban habitats (Faeth, Warren, Shochat, & Marussich, 2005;

Shochat, Warren, Faeth, McIntyre, & Hope, 2006). For example, such a release and subsequent increase in productivity within urban environments has been shown to lead to larger body sizes in both mammals (Hantak, McLean, Li, & Guralnick, 2021) and guppies (Santana Marques et al., 2020). Additionally, increases in temperature in the winter (Arnfield, 2003; Oke, 1982) may extend the foraging and worker production season for urban colonies (Shochat et al., 2010; Shochat et al., 2006). Furthermore, fluctuations between seasons are lessened and extreme climate events buffered, which have been documented to prolong urban breeding seasons (H. Lowry, Lill, & Wong, 2013; Shochat et al., 2006). Therefore, these highly productive environments may be providing favorable breeding conditions throughout most of the year, possibly contributing to the large colony sizes *T. sessile* attains in cities.

However, although urbanization may reduce resource variation through time, resource variation in space may be intensified compared to natural habitats. Urban landscapes are often associated with a patchy distribution of resources (Cadenasso, Pickett, & Schwarz, 2007), which might have profound consequences on the evolution of dispersal traits in urban populations. Generally, costs of dispersal (*e.g.*, loss of propagules, energetic demands) represent strong selective forces against dispersal (Bonte et al., 2012; Bowler & Benton, 2005). This is exemplified by the reduction in dispersal capability of island bird populations because a lack of predators nullifies the benefit flight offers as a predator-avoidance technique (Carlquist, 1966a; McNab, 1994; Wright, Steadman, & Witt, 2016), as well as in island plant and insect populations due to the high risk of landing in the ocean (Carlquist, 1966a, 1966b; Cody & Overton, 1996; Roff, 1990). Similarly, the

fragmented nature of urban environments may increase the failure rate of dispersers, and therefore select against dispersal. For example, urban populations of the pavement weed *Crepis sancta* produce a higher proportion of non-dispersing plants compared to their unfragmented natural populations (Cheptou, Carrue, Rouifed, & Cantarel, 2008). Finally, frequent disturbances within urban environments may increase the success of dependent colony foundation (*i.e.*, shorter dispersal) rather than decrease the success of independent foundation (*i.e.*, longer dispersal). In ants, Tsuji and Tsuji (1996) demonstrated that dependent colony foundation should be favored in environments where the intrinsic rate of natural increase ( $r$ ) is high because the generation time of a queen is shortened. Urban areas are frequently disturbed habitats, which probably represent such high  $r$ -inducing environments (Tsuji, 2006). Further ecological models based on spatial structure found that dependent foundation was favored over long-distance independent foundation in frequently disturbed habitats of relatively small spatial scale, despite its inherent costs (*e.g.*, short dispersal distance, reduced production of differentiated colonies (Schultner et al., 2016)) (Nakamaru, Beppu, & Tsuji, 2007; Nakamaru et al., 2014). In short, frequent, local-scale ecological disturbances can create free space near the natal nest, which can then be more rapidly occupied by queens dispersing on foot through budding than by queens dispersing on wing. Overall, reduced nest-site availability due to habitat patchiness and/or frequent disturbance within urban environments may be selecting for reduced dispersal of new queens and favoring the establishment of new colonies via budding.

Notably, our results revealed surprisingly high levels of relatedness within urban colonies, including highly polygyne ones. These high relatedness values (~0.6) not only

suggest that queens are retained during colony growth, but also that some form of colony inheritance by one maternal lineage takes place every generation. The retention and subsequent inheritance of daughter queens from the same lineage would prevent the drastic loss of relatedness that the integration of new unrelated queens would otherwise cause, as well as prevent colony boundary collapse through a subsequent loss of non-nestmate discrimination. Interestingly, the development of large polydomous colonies observed in two urban localities was also not associated with a loss of relatedness among nestmate workers when compared to the genetic background of the locality. This finding supports the hypothesis that large polygyne/polydomous colonies arise through the extreme growth of a single colony (Helanterä, Strassmann, Carrillo, & Queller, 2009), probably through a combination of retaining daughter queens and the dependent colony foundation mentioned above. This hypothesis of supercolony development in invasive species supposes that polygyny and polydomy are pre-existing traits of the species within its native range (*e.g.*, (Fournier et al., 2012; Pedersen, Krieger, Vogel, Giraud, & Keller, 2006)); indeed, we found that these social traits were plastic in the natural habitat of *T. sessile*. This supercolony formation pathway allows for the coexistence of several competitive supercolonies within a given introduced/urban locality, a pattern found in many different invasive ant species (Abbott, 2005; Espadaler, Tartally, Schultz, Seifert, & Nagy, 2007; Giraud, Pedersen, & Keller, 2002). Our results contrast with the two other hypotheses proposed to explain supercolony development in invasive ants. These hypotheses suggest that supercolonies arise from (i) a loss of nestmate recognition through a loss of diversity at the recognition loci or (ii) the selection for reduced aggression within

densely populated areas (Helanterä et al., 2009). These two scenarios would both be accompanied by a loss of relatedness and reduced aggression among all urban nests, which was not found in our study.

Interestingly, we did not find significant isolation-by-distance within any habitat, although it is expected under dependent colony foundation. Rather, strong genetic differentiation was observed between nests regardless of geographic distance. The lack of isolation-by-distance within the urban habitat has several possible explanations. For one, urban populations of *T. sessile* may have retained their dispersal capability despite facing a potentially increased cost of dispersal in patchy urban environments because the benefits of dispersal (*e.g.*, inbreeding avoidance) outweigh the costs (*e.g.*, not finding a suitable nesting site; (Bowler & Benton, 2005)). However, each urban population in the study was significantly more inbred than their respective natural population despite not experiencing a reduction in genetic diversity, suggesting a shift in dispersal strategy between the habitats. Therefore, a second explanation for the lack of isolation-by-distance, considering the increased inbreeding within urban populations, may be sex-biased dispersal. As reduced dispersal may increase rates of inbreeding, male-biased dispersal could be selected for as a mechanism to avoid sib-mating (Bowler & Benton, 2005). Indeed, both simulation models (Henry, Coulon, & Travis, 2016; Perrin & Mazalov, 2000) and empirical studies (Gauffre, Petit, Brodier, Bretagnolle, & Cosson, 2009; Oklander, Kowalewski, & Corach, 2010; Stow, Sunnucks, Briscoe, & Gardner, 2001) have found that polygynous systems select for male-biased dispersal, especially in fragmented habitats (*e.g.*, cities). As dependent colony foundation in ants often results in a pattern of

isolation-by-distance (Schultner et al., 2016), males of *T. sessile* may disperse far from their natal nest, rendering urban habitat isolation-by-distance nonsignificant. Therefore, dependent colony foundation combined with male-biased dispersal within urban populations may explain the elevated level of inbreeding compared to natural populations, as well the strong genetic sub-structure between most nests. However, another possible explanation may be that structure does exist within the urban environment and was simply not detected because we did not perform a focused transect study. If this is the case, perhaps enhanced female philopatry and dependent colony foundation alone could explain the elevated levels of inbreeding within urban environments.

Isolation-by-distance was found to be significant when analyzing both habitats together within a locality, highlighting the stark differentiation present between natural and urban populations. The minimal amount of gene flow between the two habitats suggests some selective force acting against interhabitat colonization, as well as implies selection for certain traits within each environment. A genetic signature of rapid anthropogenic evolution has been purported for many species (Hendry, Farrugia, & Kinnison, 2008), although a more recent review found a lack of conclusive evidence for many studies (Lambert, Brans, Des Roches, Donihue, & Diamond, 2020). Rates of phenotypic change have been shown to be elevated in urban systems (Alberti et al., 2017), which are potentially driven by changes in the underlying genotypes. Variation in ecological conditions can certainly drive evolutionary shifts in species' life history traits, but whether phenotypic shifts are primarily the result of genetic adaptations to urban environments via selection or simply phenotypic plasticity is unclear (Palkovacs &

Hendry, 2010). Such a phenotypic shift has occurred in urban populations of the acorn ant *Temnothorax curvispinosus*, with urban workers exhibiting higher heat tolerance and diminished cold tolerance compared to natural populations (Diamond, Chick, Perez, Strickler, & Martin, 2018; Diamond, Chick, Perez, Strickler, & Zhao, 2018). A similar divergence was found between field-born workers and workers raised for two generations in the lab, indicating that such differences between habitats is driven by evolutionary divergence through selection rather than simply plasticity (Martin, Chick, Yilmaz, & Diamond, 2019). Reciprocal transplant experiments corroborated these results, with *T. curvispinosus* colonies experiencing higher survival in natal habitats compared to novel habitats, and local colonies displaying higher survival rates than foreign colonies (Martin, Chick, Garvin, & Diamond, 2021).

Interestingly, the two localities in the eastern United States grouped genetically by habitat rather than by locality. While this result makes sense for natural populations given the few barriers to gene flow between these regions of the country, it runs counter to several recent studies in other species which found heightened differentiation among distinct urban populations (Björklund, Ruiz, & Senar, 2010; Lourenço, Álvarez, Wang, & Velo-Antón, 2017; Munshi-South, Zolnik, & Harris, 2016). However, gene flow between isolated urban populations may be sustained through human-mediated dispersal (Crispo, Moore, Lee-Yaw, Gray, & Haller, 2011). Such a scenario has been coined the “urban facilitation model”, whereby human-facilitated gene flow reduces differentiation between urban populations and may actually increase urban genetic diversity through the introduction of novel alleles (Miles, Johnson, Dyer, & Verrelli, 2018; Miles, Rivkin,



Johnson, Munshi-South, & Verrelli, 2019). Indeed, invasion rates of ants have been shown to strongly correlate with waves of human globalization (Bertelsmeier et al., 2017), and a recent study on the tiny acorn ant *Temnothorax nylanderii* identified no significant differentiation between populations in distinct cities across France (Khimoun et al., 2020), highlighting that ants may be prime candidates for human-mediated dispersal. Considering selective pressures probably differ between the urban and nonurban habitats, connections between urban sites may facilitate the evolution of an “urban ecotype” (Schapira & Boutsika, 2012; Yakub & Tiffin, 2017). A more in-depth sampling scheme across the eastern United States is needed to test for such a gene flow assisted convergence in *Tapinoma sessile*.

## **5.5 Conclusion**

Convergent selection between distinct urban populations has been found across wide variety of taxa (Johnson, Prashad, Lavoignat, & Saini, 2018; Reid et al., 2016; Theodorou, Baltz, Paxton, & Soro, 2021; Yakub & Tiffin, 2017), suggesting attributes of the anthropogenic environment impart a homogenous influence upon evolution. Our study not only demonstrated the repeated life history shifts between natural and urban populations of *T. sessile*, but also highlighted the presence of significant genetic differentiation between these populations. However, as mentioned previously, all natural collections for this study took place within forests, which may not fully represent the natural phenotype. Future sampling throughout the many natural habitats occupied by *T. sessile* may elucidate potential habitat-associated variation. Additional studies are also needed to further characterize the influence of urbanization on this ant. For example, transect sampling may

be performed to test for the presence of isolation-by-distance within urban environments and to evaluate whether the reduction of gene flow along the natural-to-urban habitat gradient is progressive or abrupt. Also, a genomic approach could help identify consistent genomic regions under selection to the urban environment across different US cities. Overall, these results reinforce the need for multifaceted approaches in identifying signatures of local adaptation (Rivkin et al., 2019) and thereby the potential drivers of selection within cities (Lambert et al., 2020), as well as underscore the urban landscape as a powerful evolutionary force.

## 6 SUMMARY

### 6.1 Overview

As a whole, my dissertation research examined biological invasions perpetrated by both native and non-native social insect invaders. For my first chapter, I characterized general patterns of non-native termite influx into the US using 100 years' worth of port of entry data. The most noteworthy finding indicated that bridgeheads were/are likely involved in invasions of non-native termites to the US, providing further evidence [*e.g.*, Bertelsmeier et al. (2018)] towards the importance of bridgeheads as significant drivers of global dispersal for invasive species. For my second and third chapters, I retraced the invasions of two globally distributed invasive termites (*C. formosanus* and *R. flavipes*) using robust genetic data and ABC. The US invasion of *C. formosanus* was found to have been shaped by putative multiple introductions from eastern Asia (both to Hawaii and directly to the US mainland), as well as a bridgehead introduction event from Hawaii into the southeastern US. These multiple introductions may have buffered the termite against a reduction in genetic diversity, as the diversity present within each US population approximated the levels observed in most native populations. The global invasion of *R. flavipes* was also found to have been propelled by multiple introductions and bridgehead events. However, in contrast to the clear geographic genetic structuring of the native *C. formosanus* populations, significant admixture was identified across the native range of *R. flavipes*, concealing its exact invasion history. This high degree of admixture is likely the result of frequent and recent human-mediated jump dispersal, which may have benefited

*R. flavipes* during its invasion by enhancing the amount of genetic diversity brought to the invasive range. For my last chapter, I investigated the urban invasion of the ‘native invasive’ *T. sessile* in four localities across the US using behavioral, chemical, and genetic data. Notably, strong differentiation between urban and natural populations was identified in each locality where colonization of the urban environment occurred, suggesting specific adaptations may be necessary to persist and thrive within cities.

## **6.2 Future research directions**

While I am still interested in general invasion biology, my work on *T. sessile* sparked an interest in working in the field of urban evolutionary biology. Given the rapid expansion of urban habitat throughout the world and humankind’s construction of a global transportation network, these two fields appear to be inextricably linked. Indeed, cities are now recognized as hotspots for biological invasions (Gaertner et al., 2017; Seebens et al., 2018). Urban environments therefore offer a fantastic setting in which to study the plastic and evolutionary responses of organisms to novel habitats (Miles, Carlen, Winchell, & Johnson, 2021; Rivkin et al., 2019), as urbanization induces marked transformations to more natural environments [*e.g.*, impervious surface cover, pollution, and/or habitat degradation and fragmentation (Grimm et al., 2008)]. These repeated and consistent environmental modifications contain the capacity to substantially impact evolutionary patterns and processes (Hendry, Gotanda, & Svensson, 2017; Johnson & Munshi-South, 2017). ‘Urban fragmentation’ and ‘urban facilitation’ are thought to be the two main ways urbanization influences genetic drift and gene flow within and between populations (Miles et al., 2019). Through urban fragmentation, suitable habitat and resources may be lost for

many organisms, and populations may become isolated from one another due to inhibited dispersal (Storfer, Murphy, Spear, Holderegger, & Waits, 2010). Therefore, urban fragmentation is expected to lead to differentiation between populations as genetic drift is increased and gene flow is reduced (Miles et al., 2019). Significant differentiation may even arise on the scale of a single city; for example, the highly differentiated urban populations of the fire salamander *Salamandra salamandra* across Oviedo, Spain (Lourenço et al., 2017) and the white-footed mouse *Peromyscus leucopus* across New York City (Munshi-South & Kharchenko, 2010). Conversely, under urban facilitation urbanization may actually promote gene flow between populations via human-mediated transport (Crispo et al., 2011), especially for organisms that are highly mobile or thrive in cities [*i.e.*, urban ‘exploiters’ (Shochat et al., 2006)]. Therefore, this sustained dispersal and gene flow can reduce genetic drift and limit differentiation between populations (Miles et al., 2019). For instance, distinct urban populations (*i.e.*, cities) of the Western black widow spider *Latrodectus hesperus* in the United States (Miles et al., 2018) and the tiny acorn ant *Termnothorax nylanderi* in France (Khimoun et al., 2020) display minimal geographic structure.

Overall, most research on urban evolution has focused either on the non-adaptive processes mentioned above (*i.e.*, population genetic structure) or on the characterization of phenotypic differences between urban and natural environment conspecifics. Despite the general narrative that adaptive urban evolution should be commonplace (Lambert et al., 2020) and that genotypic and phenotypic changes in populations across rural-to-urban gradients are evident across a wide range of taxa (Alberti et al., 2017), rigorous evidence

supporting adaptive urban evolution remains scarce. Indeed, it is difficult to discern whether (1) genotypic differentiation reflects adaptive evolution or merely reduced gene flow and subsequent genetic drift (Lambert et al., 2020; Vasemägi, 2006) or (2) phenotypic differences are the result of adaptive evolution or solely phenotypic plasticity (Diamond & Martin, 2016; Merilä & Hendry, 2014). A prolonged and significant reduction in gene flow between natural and urban environments may help to facilitate local adaptation (North, Pennanen, Ovaskainen, & Laine, 2011), but snapshots captured by pure genetic studies may be unable to distinguish such adaptation from genetic drift. Similarly, without knowledge of the populations' underlying genetics, phenotypic differences uncovered between two populations through a purely observational approach may lack the power to differentiate plasticity from adaptive evolution (Lambert et al., 2020). Therefore, while observational and molecular approaches represent separate starting points in the search for urban adaptation (Diamond & Martin, 2021), integrated approaches combining the two will likely be necessary to tease apart the fine line between genotypic/phenotypic differentiation and true adaptive evolution. Whereas observational approaches have always been possible given enough manpower, the exorbitant costs of sequencing in the past may have inhibited complementary or robust molecular studies. Thankfully, the rapidly declining cost of sequencing (Wetterstrand, 2021) and availability of powerful analytical methods [*e.g.*, low-coverage whole genome sequencing combined with SNP imputation and genotype likelihood estimation (Lou, Jacobs, Wilder, & Therkildsen, 2021)] make it more convenient than ever to study the genomes of non-model species. For *T. sessile*, this may enable the characterization of genomic regions associated with colony

structure [*e.g.*, the social chromosome in *S. invicta* (John Wang et al., 2013)] and/or genomic regions associated with the transition to urban life.

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APPENDIX A. COLLECTION LOCATIONS FOR *COPTOTERMES FORMOSANUS*

<b>Country</b>	<b>Locality</b>	<b>N</b>
USA	Florida	46
USA	Louisiana	16
USA	Hawaii	14
USA	Texas	4
USA	Mississippi	1
Japan	Honshu	20
Japan	Okinawa	18
China	Beihai	20
China	Fuzhou	20
China	Guilin	20
China	Hangzhou	20
China	Hengyang	20
China	Hong Kong	20
China	Nanning	20
China	Wenzhou	20
China	Xiamen	20
China	Jieyang	15
China	Hainan	14
China	Lufeng	7
China	Changsha	3
China	Xinyu	1
Taiwan	Mainland	20

APPENDIX B. COLLECTION LOCATIONS FOR *RETICULITERMES FLAVIPES*

<b>Country</b>	<b>Locality</b>	<b>N</b>
USA	Florida	24
USA	Louisiana	24
USA	North Carolina	10
USA	Texas	9
USA	Wisconsin	7
USA	Georgia	6
USA	Mississippi	6
USA	Virginia	6
USA	Indiana	5
USA	Maryland	5
USA	Mississippi	5
USA	Missouri	4
USA	New Orleans	4
USA	South Carolina	4
USA	Arkansas	3
USA	Illinois	3
USA	Kentucky	3
USA	Massachusetts	3
USA	Nebraska	3
USA	New York	3
USA	Ohio	3
USA	Alabama	2
USA	Delaware	2
USA	Tennessee	2
USA	Arkansas	1
USA	Kansas	1
USA	Oklahoma	1
Uruguay	Uruguay	2
Germany	Hamburg	1
France	Île-de-France	18
France	Charente-Maritime	8
France	Gironde	6
France	Indre et Loire	6
France	Pyrénées-Atlantiques	4
France	Tarn	3
France	Dordogne	2
France	Ile de France	2
France	Tarn et Garonne	2
France	Vendée	2

France	Bouches-du-Rhône	1
France	Eure	1
France	Haute Garonne	1
France	Loiret	1
France	Lot et Garonne	1
France	Maine et Loire	1
France	Seine-Maritime	1
Chile	Santiago Metropolitana	16
Chile	Valparaiso Region	13
Canada	Ontario	12
Bahamas	West End	4