





Effect of social structure and introduction history on genetic diversity and differentiation

Sylvia M. Flucher¹  | Patrick Krapf¹  | Wolfgang Arthofer¹ | Andrew V. Suarez^{2,3} | Ross H. Crozier⁴ | Florian M. Steiner¹  | Birgit C. Schlick-Steiner¹ 

¹Molecular Ecology Group, Department of Ecology, University of Innsbruck, Innsbruck, Austria

²Department of Evolution, Ecology and Behavior, University of Illinois at Urbana-Champaign, Urbana, IL, USA

³Department of Entomology, University of Illinois at Urbana-Champaign, Urbana, IL, USA

⁴School of Marine and Tropical Biology, James Cook University, Townsville, QLD, Australia

Correspondence

Sylvia M. Flucher, Molecular Ecology Group, Department of Ecology, University of Innsbruck, Innsbruck, Austria.
Email: sylvia.flucher@gmail.com

Funding information

Austrian Science Fund, Grant/Award Number: J 2642 and P 30861

Abstract

Invasive species are a global threat to biodiversity, and understanding their history and biology is a major goal of invasion biology. Population-genetic approaches allow insights into these features, as population structure is shaped by factors such as invasion history (number, origin and age of introductions) and life-history traits (e.g., mating system, dispersal capability). We compared the relative importance of these factors by investigating two closely related ants, *Tetramorium immigrans* and *Tetramorium tsushimae*, that differ in their social structure and invasion history in North America. We used mitochondrial DNA sequences and microsatellite alleles to estimate the source and number of introduction events of the two species, and compared genetic structure among native and introduced populations. Genetic diversity of both species was strongly reduced in introduced populations, which also differed genetically from native populations. Genetic differentiation between ranges and the reduction in microsatellite diversity were more severe in the more recently introduced and supercolonial *T. tsushimae*. However, the loss of mitochondrial haplotype diversity was more pronounced in *T. immigrans*, which has single-queen colonies and was introduced earlier. *Tetramorium immigrans* was introduced at least twice from Western Europe to North America and once independently to South America. Its monogyny might have limited genetic diversity per introduction, but new mutations and successive introductions over a long time may have added to the gene pool in the introduced range. Polygyny in *T. tsushimae* probably facilitated the simultaneous introduction of several queens from a Japanese population to St. Louis, USA. In addition to identifying introduction pathways, our results reveal how social structure can influence the population-genetic consequences of founder events.

KEYWORDS

age of introduction, genetic paradox of invasions, monogyny, polygyny, *Tetramorium immigrans*, *Tetramorium tsushimae*

Florian M. Steiner and Birgit C. Schlick-Steiner have contributed equally as senior authors.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2021 The Authors. *Molecular Ecology* published by John Wiley & Sons Ltd.

1 | INTRODUCTION

Modern biodiversity distributions are driven by historical biogeography and contemporary human-mediated transport of non-native species. Introduced species that penetrate natural ecosystems and affect native organisms directly or indirectly are considered “invasive” (Holway et al., 2002; Mack et al., 2000) and are of major concern for conservation biology (Jenkins, 1996). Management of invasive species can benefit from understanding the history and source of introductions, a strategy facilitated by genetic research comparing introduced and native populations. From a biogeographical point of view, invasions are colonization events, useful for testing population-genetic theory pertaining to founder events and their subsequent expansion.

Sufficient genetic diversity should be a precondition for the success and persistence of introduced species, particularly for adaptation to variable environmental conditions in a new environment (Frankham, 2005a). Subsequently, successful introductions are seen as a “genetic paradox” because founder effects and bottlenecks reduce the genetic diversity of invasive populations and are suspected to decrease short- and long-term viability (Allendorf & Lundquist, 2003; Frankham, 2005b). While most studies show reduced genetic diversity in invasive populations compared with native populations (Dlugosch & Parker, 2008; Hardesty et al., 2012; Tsutsui et al., 2000; Uller & Leimu, 2011), increased genetic diversity can also result from the admixture of genotypes from different populations in the native range (Kolbe et al., 2004). Despite large reductions in diversity, the success of introduced species may stem from the presence of pre-adapted traits, purging of deleterious alleles and relief from natural enemies (Allendorf & Lundquist, 2003; Sakai et al., 2001).

In addition to the number and origin of introduction events, the age of an introduction and the organism's mating system and dispersal capability can shape population genetic structure. Dlugosch and Parker (2008) found that the relationship between genetic variation and the age of an introduction follows a u-shaped curve: genetic drift and selection initially act to reduce diversity, which eventually increases with population size and migration among separately introduced populations. However, at least for vertebrates, such a recovery of genetic variability has not been confirmed (Uller & Leimu, 2011). In social insects such as ants, mating system and dispersal capability can take a variety of shapes and are often linked with colony structure (Hakala et al., 2019; Steiner et al., 2009; Wilson, 1971).

Many species are transported to new locations, but not all of them become invasive. Identifying species' traits responsible for their success is a long-standing goal (Kolar & Lodge, 2001). However, we do not know what the mechanisms of success are for many taxonomic groups, and they also probably differ among taxa. In ants, for example, combinations of character states such as the potential to form supercolonies, an omnivorous diet and a preference for disturbed habitats are overrepresented in many of the highly invasive species (Bertelsmeier et al., 2017; Holway et al., 2002; Rabitsch, 2011; Tsutsui & Suarez, 2003). Supercoloniality describes a social system consisting of highly polygynous and polydomous “colonies”

where individual workers can move freely between multiqueen nests (Helanterä et al., 2009). Within a supercolony, there is no aggression among workers, but they show high levels of aggression towards individuals from different supercolonies (Holway et al., 1998). Queens are usually small, mate close to or inside the nest (sometimes even with their brothers), and form new colonies with the help of workers from their maternal nest. This process, called colony budding, leads to high local colony densities which may promote invasion success (Rabitsch, 2011). The presence of numerous queens per nest also increases the probability that humans transport a viable nest fragment (containing one or several queens) to another area (Rabitsch, 2011). While there are prominent examples of supercolonial invasive ants, such as the Argentine ant (*Linepithema humile*), other successful ant invaders do not exhibit these character states (Holway et al., 2002). Closely related introduced ants that vary in their social system, such as *Tetramorium immigrans* Santschi, 1927 (formerly *T. caespitum*) and *Tetramorium tsushimae* Emery, 1925, both introduced to North America, provide unique opportunities to examine how variation in life-history traits (i.e., queen number, dispersal mechanism) affects invasion dynamics. These two species, commonly known as the pavement ant and Japanese pavement ant, respectively, belong to the same species complex (Schlick-Steiner et al., 2006; Wagner et al., 2017; but note that the relationships within the complex are not yet entirely clear, Wagner et al., 2018).

Being closely related, *T. immigrans* and *T. tsushimae* share a number of characteristics. For example, the two species are omnivorous and live in habitats with similar mean annual temperature, in particular grasslands and urban areas (Cicconardi et al., 2020; Hosoishi et al., 2019; Penick et al., 2015; Sanada-Morimura et al., 2006; Schlick-Steiner et al., 2006; Wagner et al., 2017). However, they differ in characteristics of their social system. *Tetramorium immigrans* has single-queen colonies (Cordonnier et al., 2020; Schlick-Steiner et al., 2006) and queens probably disperse and found new nests independently after nuptial flights. In contrast, *T. tsushimae* is polygynous and colonies can contain several hundred queens (Sanada-Morimura et al., 2006). In its native range, *T. tsushimae* colonies are polydomous (Sanada-Morimura et al., 2006), while in its introduced range, they form a single supercolony and disperse through colony budding (Reuther, 2009).

In addition to their different life-history traits, their introduction events differed, which is important for a comprehensive understanding of their invasion genetics. *Tetramorium immigrans* is native to the Western Palearctic and was probably introduced to North America in the 1800s or earlier, being first reported in Tennessee and Nebraska in 1895 (Brown, 1957). Currently, *T. immigrans* is established throughout large parts of North America and in some locations in South America (Guénard et al., 2017). Introduced populations are primarily restricted to urban habitats, but *T. immigrans* can also be found in natural ecosystems and is therefore classified as invasive (Steiner, Schlick-Steiner, et al., 2008). *Tetramorium tsushimae* is native to Eastern Asia and was first reported from St. Louis, Missouri (USA), in 1988. Its introduction may have occurred in the 1980s or earlier (Steiner et al., 2006), and it is now found in a few

states in the Central and Eastern United States (Guénard et al., 2017; Steiner et al., 2006). Where both species co-occur, *T. tsushimae* displaces *T. immigrans* (Steiner et al., 2006).

Diverse life-history and invasion-history characteristics are reflected in population and invasion genetics (Crozier & Pamilo, 1996; and earlier in the Introduction), which we examined in the two *Tetramorium* species using mitochondrial DNA (mtDNA) sequences and microsatellite alleles. We thus used key characteristics (queen number and dispersal mechanism as life-history traits, suspected number of introductions and current distribution of invasive populations as invasion-history traits) to predict species-specific patterns (see also Table S1 for an overview of the hypotheses including the concrete references supporting the arguments). With regard to these traits, we expect the following. (1) Loss of genetic diversity in their introduced range relative to their native range is severe in both species but less extreme in *T. tsushimae* due to its polygyny. (2) Levels of inbreeding in introduced relative to native populations are lower in the monogynous *T. immigrans* than in the polygynous *T. tsushimae* where intranidal mating is suspected (Reuther, 2009). Furthermore, we propose three patterns for genetic differentiation. (3a) It is relatively weak in native populations of *T. immigrans*, due to nuptial flights and independent colony foundation, compared with *T. tsushimae*, which exhibits nest budding and of which parts of the native population are located on an island. (3b) Within the introduced range, genetic differentiation is expected to be weak in both species due to their relatively recent introductions, their high dispersal capacity (e.g., nuptial flights in *T. immigrans* and human-facilitated jump dispersal in both species), and the small invaded area in *T. tsushimae*. (3c) Between native and introduced ranges, we predict strong differentiation for both species, as only few introduction events are suspected. Finally, (4) we predict introduced populations arose from one or few introduction events from a narrow portion of the native range of both species, in line with an ecological niche modelling study (Steiner, Schlick-Steiner, et al., 2008).

2 | MATERIAL AND METHODS

2.1 | Sample collection and DNA extraction

We sampled 164 nests from native and introduced ranges of *Tetramorium immigrans* (36 Western Palearctic, 43 Nearctic nests and one Neotropical nest from Argentina; Figure S1A) and *Tetramorium tsushimae* (43 Eastern Palearctic and 41 Nearctic nests; Figure S1B) resulting in similar sample sizes across species and ranges. All sampled nests were also investigated in Steiner, Schlick-Steiner, et al. (2008), who performed ecological-niche modelling based on the distribution records. The samples were obtained through a collector network and stored in 96% ethanol at -20°C . DNA of one worker per nest was extracted from whole animals using the GenElute Mammalian Genomic DNA Extraction kit (Sigma-Aldrich) and used for mtDNA and microsatellite analyses. *Tetramorium immigrans* and *T. tsushimae* species' identity was ascertained using mtDNA

sequencing and morphometrics; for *T. immigrans*, additionally amplified fragment length polymorphism was used (Steiner, Schlick-Steiner, et al., 2008; Wagner et al., 2017).

2.2 | mtDNA sequencing and analyses

A 1113-bp sequence of the mitochondrial COI gene had already been sequenced for species delimitation and identification in earlier projects (Schlick-Steiner et al., 2006; Steiner et al., 2006; Steiner, Schlick-Steiner, et al., 2008). Haplotypes with singleton mutations were later confirmed by reanalysis using 1718f (Simon et al., 1994) or COI_f (Steiner et al., 2005) as forward primer and PAT (Simon et al., 1994) as reverse primer. Briefly, the mitochondrial COI stretch was PCR (polymerase chain reaction)-amplified in reaction volumes of 10 μl with 1 \times Rotor-Gene probe PCR Mix (Qiagen) using 0.2 μM forward and reverse primers and MilliQ water. Reactions were run on a UnoCycler (VWR) using cycling conditions of 95 $^{\circ}\text{C}$ for 30 s, 50 $^{\circ}\text{C}$ for 1 min, 72 $^{\circ}\text{C}$ for 2 min for 35 cycles with an initial denaturation step at 95 $^{\circ}\text{C}$ for 3 min, and a final extension step at 72 $^{\circ}\text{C}$ for 10 min. PCR products were purified enzymatically with 0.1 μl Exo1 and 0.1 μl FastAP in a reaction volume of 10 μl , incubated for 15 min at 37 $^{\circ}\text{C}$ and for 15 min at 80 $^{\circ}\text{C}$, and Sanger sequenced in both directions by a commercial provider (Eurofins). For three sequences showing unambiguous base state and one synonymous mutation each, the haplotypes were considered plausible without verification. For nine individuals, no sufficient sequence quality could be obtained even after repetition. In total, 155 mtDNA sequences were used: 36 and 44 sequences for the native and invasive range of *T. immigrans*, respectively, and 40 and 35 sequences for the native and invasive range of *T. tsushimae*, respectively (GenBank accession numbers specified in Table S2).

In addition to the main data set, fully overlapping mtDNA sequences of 169 individuals of *T. immigrans* (Schlick-Steiner et al., 2006; Wagner et al., 2017) and 23 individuals of *T. tsushimae* (Steiner et al., 2006) were downloaded from GenBank (see Table S3 for GenBank accession numbers) and three recently discovered occurrences of *T. immigrans* in Denmark (Sheard et al., 2020) were sequenced. The haplotypes of the three samples were identified using 0.2 μM 1718f and PAT primers (Simon et al., 1994), 1 \times OneTaq reaction buffer and 0.125 U OneTaq polymerase (New England Biolabs) in 10- μl reaction volumes. PCR, purification and sequencing were performed as reported above.

Haplotype networks were constructed using the minimum spanning method (Bandelt et al., 1999) in POPART version 1.7 (<http://popart.otago.ac.nz>). The number of haplotypes per species and range was counted, and a ratio between the number of invasive and native haplotypes was calculated. Haplotype and nucleotide diversity were calculated in R version 3.6.1 (R Core Team, 2019) using the package PEGAS version 0.11 (Paradis, 2010). Differences between the native and invasive ranges were tested by Welch's *t* test in R.

To allow a comparison with a recent study of the North American populations of *T. immigrans* (Zhang et al., 2019), the GenBank entries

from that study were downloaded, and sequences were reduced to haplotypes using *FABOX* version 1.5 *DNACOLLAPSER* (Villesen, 2007), and manually aligned to the invasive haplotypes used in this study. This resulted in a 348-bp overlapping sequence, for which the mutations were compared directly by eye.

2.3 | Microsatellite genotyping and analyses

One worker per nest was genotyped at 16 microsatellite loci (51a, 51b, 51d, 51h, 51o, 52a, 52k, 53a, 53b, 54e, 54g, 56d, 56j, 58i, 59f, 59j; Steiner et al., 2008). PCR was done in a 5- μ l reaction volume with 0.5 μ l template DNA, 1 \times reaction buffer, 0.2 μ l primers (the forward primers were fluorescently labelled), and 0.125 U MyTaq polymerase. Reactions were performed on an MJ thermocycler (Marshall Scientific) with cycling conditions of 94 °C for 30 s, 60 °C for 1 min, 72 °C for 45 s for 32 cycles with an initial denaturation step at 94 °C for 5 min and a final extension step at 68 °C for 20 min. Fragment analysis was carried out by INGENETIX (<https://www.ingenetix.com/>) using an ABI PRISM 310 genetic analyser (Applied Biosystems) and GeneScan 500-TAMRA as an internal size standard. The traces were processed using *GENESCAN* and *GENOTYPER* software (Applied Biosystems).

The number of alleles (N_a), number of private (i.e., unique) alleles ($N_{private}$), effective number of alleles (N_e), and observed and expected heterozygosity (H_o , H_e) were calculated using *GENALEX* version 6.502 (Peakall & Smouse, 2012). Allelic richness was computed with *POPGENREPORT* version 3.0.4 (Adamack & Gruber, 2014). Mean squared distance between alleles (d^2) and multilocus heterozygosity (MLH) were calculated manually (Coulson et al., 1998; Hansson, 2010). Differences between native and introduced ranges were tested by Welch's *t* test in R. Isolation by distance (IBD) was tested using a Mantel test with 10,000 permutations comparing geographical distance in kilometres and Cavalli-Sforza and Edwards Chord distance (Takezaki & Nei, 1996) between individuals using *GEOSPHERE* version 1.5-10, *HIERFSTAT* version 0.04-22, and *ADE4* version 1.7-13 (Chessel et al., 2004; Goudet & Jombart, 2015; Hijmans, 2019) and visualized as a density plot with *MASS* version 7.3-51.4 (Venables & Ripley, 2002). Being an outlier in the southern hemisphere, the Argentine sample was excluded from this analysis.

As a nonmodel-based method, a principal coordinate analysis (PCoA) was calculated from the Euclidean distances of scaled allele frequencies using *ADEGENET* version 2.1.1 (Jombart, 2008). Additionally, a discriminant analysis of principal components (DAPC) was calculated with *ADEGENET*. For DAPC, the native samples were separated into geographical regions (see Figure S1): *T. immigrans* was separated into four groups with equal sample sizes (Western Europe, Central Europe, Eastern Europe and Caucasus; each $n = 9$); for *T. tsushimae*, all Japanese samples were grouped ($n = 17$), and the continental Asian samples were divided into two regions (Huang and Yangtse plains [$n = 15$] and Manchuria and Korea [$n = 11$]). DAPC was calculated from scaled allele frequencies with 19 and 24 principal components for *T. immigrans* and *T. tsushimae*, respectively,

accounting for 70% of the variance found in the data. For introduced individuals, the highest group-membership probability to the native clusters was calculated based on the DAPC results. Bayesian cluster analyses were performed separately for each species using *STRUCTURE* version 2.3 (Pritchard et al., 2000) for $K = 1$ to 10 using an admixture model, 1,000,000 generations burn-in, 4,000,000 MCMC (Markov chain Monte Carlo) generations, and 10 replicates. Following this, cluster identities of all replicates were aligned with *CLUMPP* version 1.1.2 (Jakobsson & Rosenberg, 2007) using *Greedy* algorithm for $K = 2$ to $K = 6$ and *LargeKGreedy* algorithm for $K = 7$ to $K = 10$ and 500 repetitions each. Additionally, the mean group-membership probability across replicates was calculated. Data visualization and ΔK statistics (Evanno et al., 2005) were done with *POPHELPER* version 2.3.0 (Francis, 2017).

2.4 | Phylogenetic diversity

For phylogenetic reconstructions using mtDNA, model selections were performed for both species separately using *JMODELTEST* version 0.1.1 (Posada, 2009). Using the cumulative Akaike information criterion resulted in the TPM2uf + G as the best fit for each of the two species. The models were used in the maximum-likelihood (ML)-based phylogenetic reconstructions as implemented in *MEGA-X* version 10.0.4 (Kumar et al., 2018) using the settings subtree-pruning-regrafting = extensive (SPR level 5), make initial tree = automatically (NJ) and branch swap filter = very strong. *Tetramorium caespitum*, *Tetramorium capitale*, and either *T. immigrans* or *T. tsushimae* were used as outgroups (GenBank accession numbers: see Table S2). Phylogenetic reconstructions using the microsatellite alleles scored were performed for the two species separately. Neighbour-joining (NJ) trees were created using the method *neighbour* in the software package *PHYLIP* version 3.695 (<http://evolution.genetics.washington.edu/phylip/>). The resulting mtDNA and microsatellite phylogenetic trees were used to calculate the phylogenetic diversity (PD) and to create rarefaction curves for *T. immigrans* and *T. tsushimae* separately. Phylogenetic diversity and rarefaction curves were created using the functions *phyloRare* and *phyloCurve* in R, respectively (Nipperess & Matsen, 2013). Differences between the native and introduced ranges were tested by Welch's *t* test in R.

3 | RESULTS

3.1 | mtDNA

We found 18 mtDNA haplotypes for *Tetramorium immigrans* (Figure 1): 16 in the native range and four in the introduced range (three in North America and one in Argentina) resulting in a ratio of 4:16 (0.25) of invasive to native haplotypes. Within the native range, the greatest haplotype diversity was found in the Caucasus region. The most frequent haplotype in North America was also found to be widespread in the native range occurring from Western to Eastern

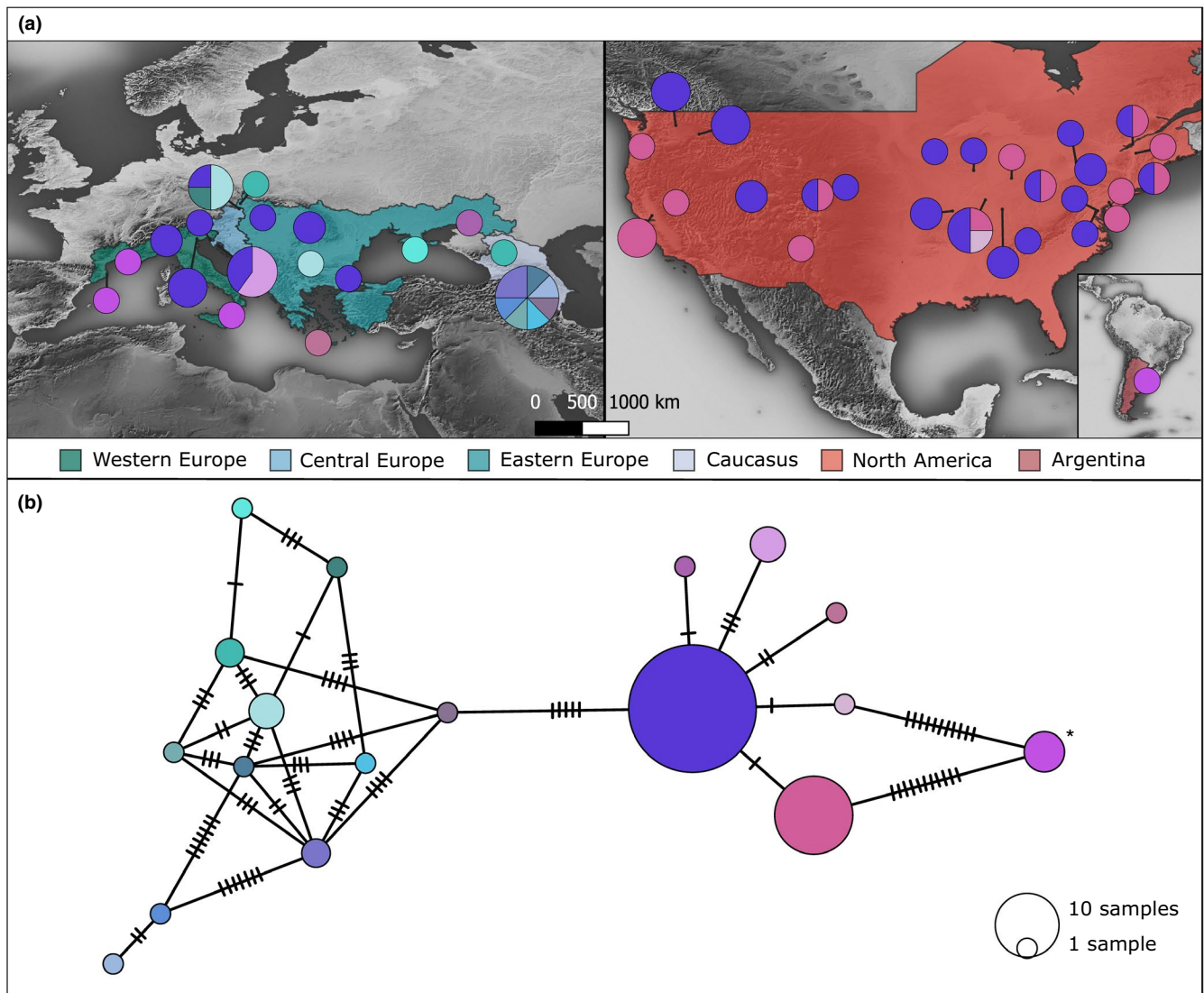


FIGURE 1 Eighteen mtDNA haplotypes of *Tetramorium immigrans* using a 1113-bp sequence of the COI gene: (a) distribution of haplotypes in the native (16 haplotypes) and invasive ranges (four haplotypes), one pie per country/state/region of similar size (see Figure S1), position according to the geographical centre of samples, colours are haplotypes and sizes relative to sample size, background colours signify geographical regions; (b) minimum spanning network. Colours correspond to haplotypes in the maps (in a). The haplotype which was found in Denmark is marked with an asterisk

Europe. The other two North American haplotypes were similar to the most frequent, with only one mutation each. They were not sampled in the native range in the main data set, although the more widespread one of these two was found in the additional sequence data set in France and Italy (Table S3, Figure S2). The two common North American haplotypes and the three downloaded North American haplotypes from Zhang et al. (2019) had an identical overlapping 348-bp segment. The third North American haplotype found in this study had one mutation in the overlapping sequence segment but was sampled only once in Illinois. The haplotype present in Argentina is only distantly related to all other haplotypes. It occurs also in Western Europe and was recently found in Denmark.

In *Tetramorium tsushimae*, we found 31 haplotypes with a ratio of 12:21 (0.57) between invasive and native haplotypes (Figure 2). All

invasive haplotypes are closely related, with one or two mutations difference to the next related haplotypes and a maximum of five mutations difference. Only two of the haplotypes found in North America were also found in the native samples. The most widespread one in the introduced range also occurs throughout all regions of the native range. The other haplotype present in both ranges was, in the native range, only found on the island Honshu, in Japan. None of the introduced haplotypes not sampled in the native range were detected in the additional samples (Figure S3).

Haplotype diversity, nucleotide diversity and PD were significantly reduced in the introduced range compared with the native range for both species (Figures 3a–c and 4a,c; test statistics: see Table S4). The relative reduction of mtDNA diversity in the invasive range was stronger in *T. immigrans* compared with *T. tsushimae*.

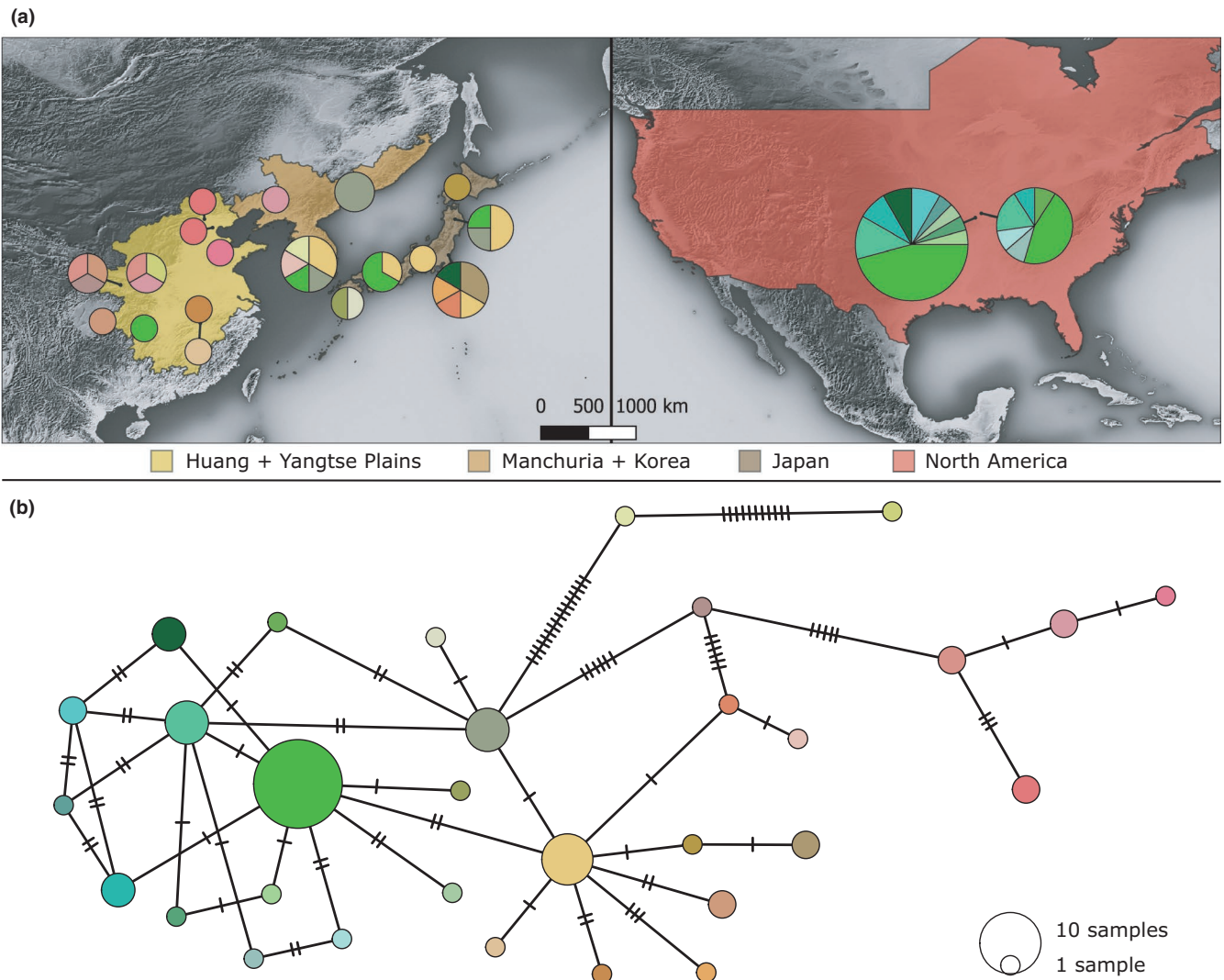


FIGURE 2 Thirty-one mtDNA haplotypes of *Tetramorium tsushimae* using a 1113-bp sequence of the COI gene: (a) distribution of haplotypes in the native (21 haplotypes) and invasive ranges (12 haplotypes), one pie per country/state/region of similar size (see Figure S1), position according to the geographical centre of samples, colours are haplotypes and sizes relative to sample size, background colours signify geographical regions; (b) minimum spanning network. Colours correspond to haplotypes in the maps (in a)

3.2 | Microsatellite data

N_a , $N_{private}$, N_e and allelic richness were significantly reduced in the introduced ranges compared with native ranges for both *T. immigrans* and *T. tsushimae* (Figure 3d–f; test statistics see Table S4). The reduction was more pronounced in *T. tsushimae*; for example, the ratio of mean N_a over loci between the invasive and native ranges was 12.69:21.25 (0.60) for *T. immigrans* and 7.19:22.31 (0.32) for *T. tsushimae*. PD was significantly reduced in *T. tsushimae* but not in *T. immigrans* (Figure 4b,d; test statistics see Table S4). In both species, H_E , H_O , MLH and d^2 were reduced in the invasive range relative to the native range (Figure 3g–i; test statistics see Table S4). In both species, H_O was lower than H_E ; however, the ratio $H_O:H_E$ was higher in *T. immigrans* (native: 0.839, invasive: 0.866) than in *T. tsushimae* (native 0.697, invasive 0.670).

Statistically significant patterns of IBD were found in the native and introduced range of *T. immigrans* and in the native but not the introduced range of *T. tsushimae* (Figure 5; test statistics see Table S4). In the native ranges, genetic distances between samples and their correlation to the geographical distances were similar in both species. For both species, genetic distances were lower among introduced range samples compared with the native ranges, but particularly in *T. tsushimae*.

PCoAs revealed that North American samples formed a cluster separate from the native samples in both species (Figure 6a,b). This separation was more distinct in *T. tsushimae*. The Argentine sample lay within the native samples. In the native ranges, geographical regions did not form distinct clusters. In *T. immigrans*, the Caucasus region revealed a strong variation, and Western European samples plotted closely together. The variation of Western European samples

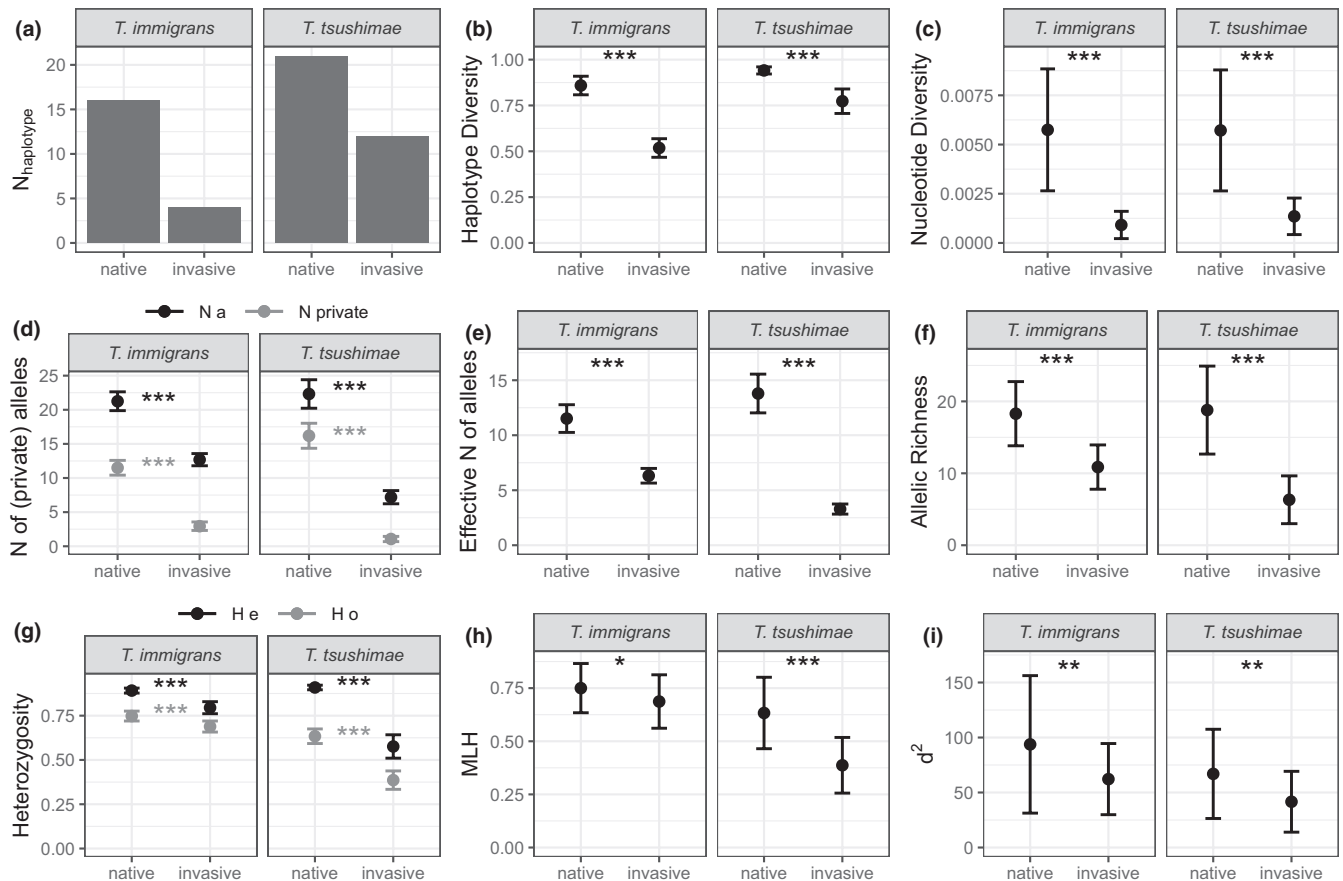


FIGURE 3 Various diversity measures of *Tetramorium immigrans* and *Tetramorium tsushimae*. mtDNA diversity: (a) number of haplotypes, (b) haplotype diversity, (c) nucleotide diversity; microsatellite diversity: (d) number of alleles (N_a , black dots) and number of private alleles ($N_{private}$, grey dots), (e) effective number of alleles (N_e), (f) allelic richness, (g) expected and observed heterozygosity (H_e , black dots; H_o , grey dots), (h) multilocus heterozygosity (MLH) and (i) d^2 . Dots represent mean values \pm SD (over loci for N_a , $N_{private}$, N_e , allelic richness, H_e , H_o ; and over individuals for MLH and d^2), asterisks represent significance level of Welch's t test (* $p < .05$, ** $p < .01$, *** $p < .001$)

was a subset of the Pan-European variation, which again was a subset of the Caucasian variation.

Similarly, using DAPC, the native regions were only partially separated and formed overlapping clusters (Figure 6c,d). When the invasive samples were plotted on the same axes, they clustered to the origin of coordinates. The invasive samples of *T. immigrans* showed the highest group-membership probabilities for Western Europe and those of *T. tsushimae* for Japan.

The method according to Evanno et al. (2005) proposed $K = 2$ to be the best K value in Bayesian clustering with STRUCTURE for both *T. immigrans* and *T. tsushimae* (Figures S4 and S5). Nevertheless, we consider also higher values of K for the interpretation of further substructure and genetic variation. In *T. immigrans*, Bayesian clustering showed a separation of European and North American samples, and the Argentine sample clustered with European samples (Figure 7a). At $K = 2$, four native samples from Spain, Italy and Slovenia showed slight probabilities for the North American cluster. Two of the Californian samples partially clustered with native samples at $K = 2$, but all three Californian samples formed their own cluster at $K = 4$. Probabilities for this cluster were also present in a fraction of other North American samples and stable for

higher K values (Figure S6). The native range differentiated into at least two clusters, in which the Argentine sample clustered mainly with Western and Central European samples. Invasive samples of *T. tsushimae* formed a homogeneous cluster, which clearly differed from native samples at $K = 2$ (Figure 7b). Further increases in K detected variation in the native but not in the introduced range samples (Figure S7).

4 | DISCUSSION

4.1 | Loss of genetic diversity during introduction

A loss of diversity resulting from founder effects during introduction is observed in most invasions (Uller & Leimu, 2011) and was expected for both *Tetramorium immigrans* and *Tetramorium tsushimae* (Table S1). Our results revealed a reduction of mitochondrial and nuclear genetic diversity in *T. immigrans* and *T. tsushimae* comparable with that seen in other invasions (Table S5; Puillandre et al., 2008), including those of social insects such as the red imported fire ant (*Solenopsis invicta*; Ascunce et al., 2011; Ross & Shoemaker, 2008),

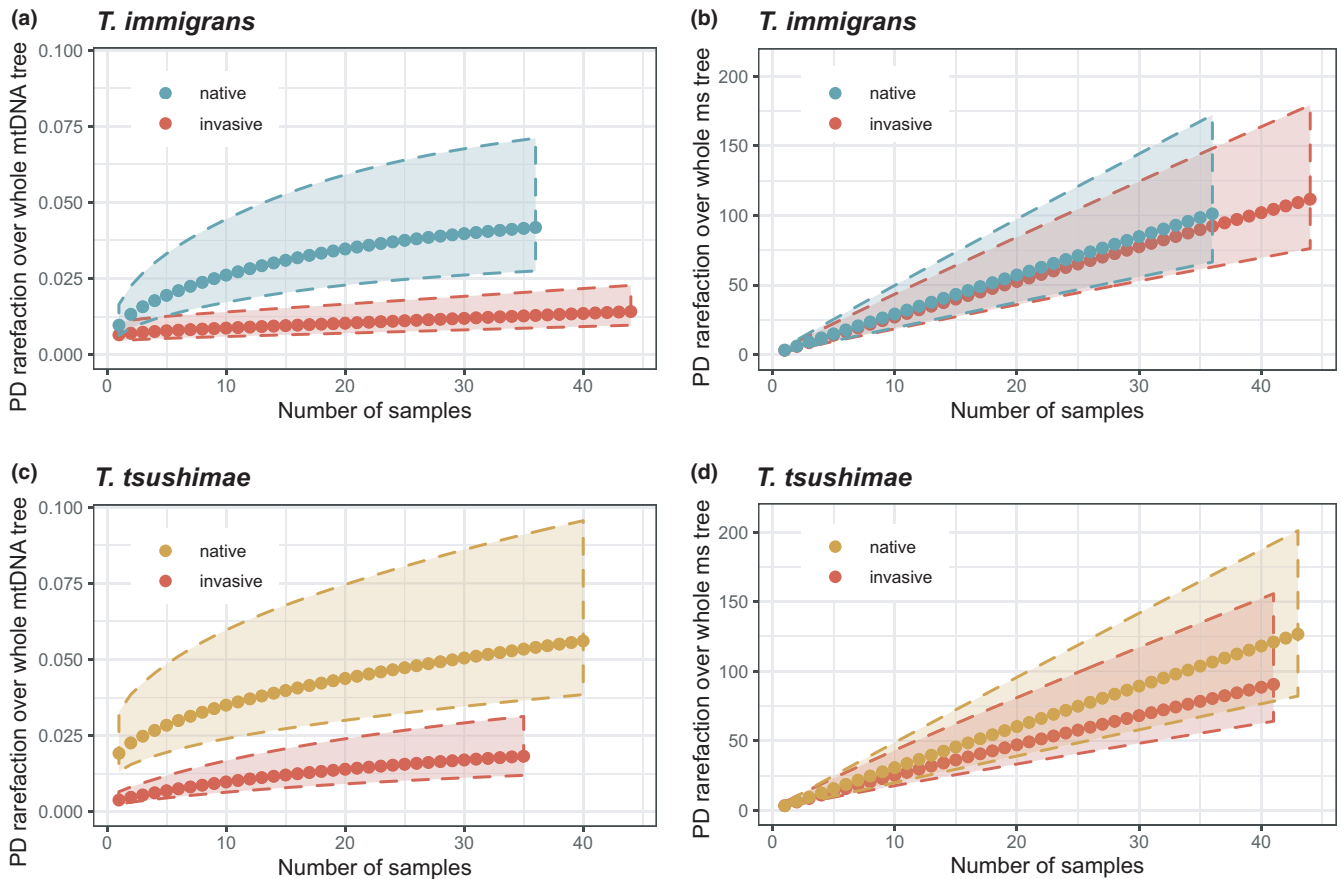


FIGURE 4 Phylogenetic diversity rarefaction curves of mtDNA (a, c) and microsatellite (ms) (b, d) trees for (a, b) *Tetramorium immigrans* and (c, d) *Tetramorium tsushimae*

the little fire ant (*Wasmannia auropunctata*; Fournier et al., 2005) and a solitary bee (*Lasioglossum leucozonium*; Zayed et al., 2007).

Comparing species, the reduction in mtDNA diversity was larger in *T. immigrans*, whereas the loss of microsatellite diversity was more pronounced in *T. tsushimae*. We had expected a larger reduction in haplotypes in the monogynous *T. immigrans*. In contrast, *T. tsushimae*, with up to several hundred queens in one nest could, at least theoretically, have been initially established with a large amount of genetic diversity (Table S1). The pattern of microsatellite diversity in *T. immigrans* could result from a large founding population, repeated introduction events or possibly the formation of new alleles after introduction. Microsatellite mutation rates range between 10^{-2} and 10^{-6} mutations per locus and generation (Ellegren, 2000; Seyfert et al., 2008). This makes the formation of at least some new alleles probable for *T. immigrans*, which has been established in North America at least since the 1800s. The appearance of a new microsatellite mutation has been observed in the congener *T. alpestre* in locus 51d (Krapf et al., 2018). In contrast, mtDNA has mutation rates of 10^{-8} per site and generation (Haag-Liautard et al., 2008), which makes new mutations rather unlikely. The strong reduction of mtDNA diversity in *T. immigrans* might be a consequence of the wide distribution of a few haplotypes in Europe (Figure 1). Thus, a large variety of nuclear information may well have been introduced to North America by several queens of the same successful

mitochondrial haplotypes. Furthermore, the loss of mtDNA diversity in *T. tsushimae* could have been underestimated because of uneven sampling density. Introduced populations of *T. tsushimae* occur over a relatively small area, resulting in higher sampling density in the invasive range compared with the native range (Figure S1). The different results for the two marker types emphasize the importance of comparing several markers; similar to phylogenies affected by incomplete lineage sorting (Pamilo & Nei, 1988), a single gene does not necessarily tell the whole story.

4.2 | Inbreeding in invasive range relative to native range

For *T. immigrans*, a monogynous and usually outbreeding species, only weak inbreeding was expected in the invasive range (Table S1). A low F_{IS} value in a single colony even indicated the absence of inbreeding (Zhang et al., 2019). In contrast, in polygynous species like *T. tsushimae*, intranidal mating can be common (Seifert, 2010), and elevated levels of inbreeding have already been suspected in the invasive range of *T. tsushimae* due to low heterozygosity (Reuther, 2009). If queens within a supercolony are related, intranidal mating might increase the level of inbreeding (Trontti et al., 2005), which is more probable in an introduced population that has recently gone

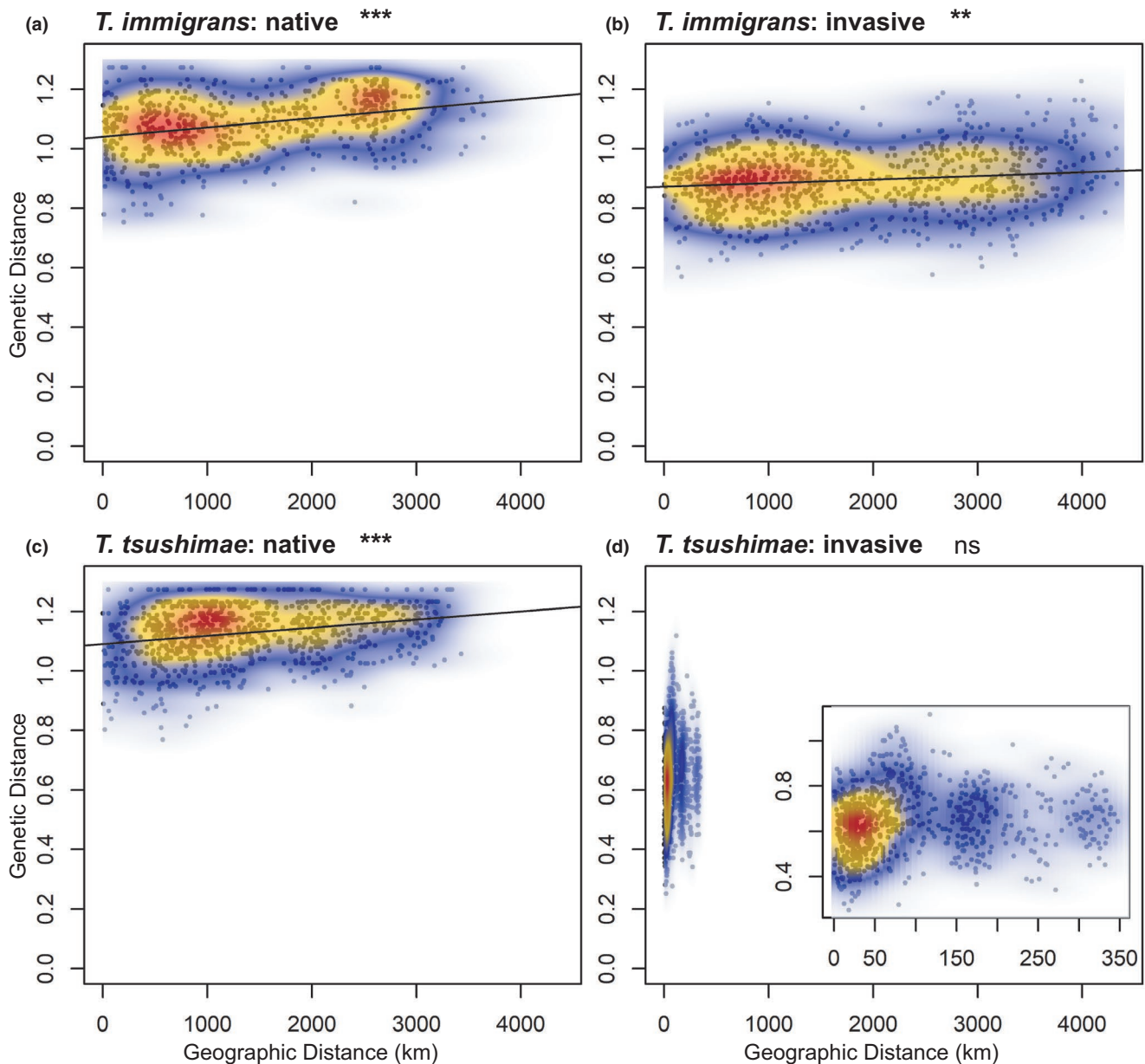


FIGURE 5 Isolation by distance using Cavalli-Sforza and Edward Chord's distance for (a) native and (b) invasive *Tetramorium immigrans* (excluding Argentina) and for (c) native and (d) invasive *Tetramorium tsushimae*. Asterisks represent significance level of Mantel test (ns $p > .05$, ** $p < .01$, *** $p < .001$)

through a bottleneck. Yet, in large supercolonies, where queens move freely among nests, intranidal mating might not necessarily lead to increased levels of inbreeding (Pamilo, 1985; Pedersen et al., 2006). We found that heterozygosity was reduced in introduced ranges compared with native ranges of *T. immigrans* and *T. tsushimae*, indicating a population bottleneck (Figure 3g). The ratio $H_O:H_E$ was similar between ranges, which suggests that the degree of inbreeding is not increased in the invasive range. However, $H_O:H_E$ differed between species—it was lower in *T. tsushimae*, which in general indicates greater inbreeding in this species. Besides being an indicator for higher levels of inbreeding, a low ratio $H_O:H_E$ can also result from population subdivision as described by the Wahlund effect (Wahlund, 1928). In contrast, both MLH and d^2 were lower in the

introduced ranges of both species (Figure 3h,i), which would support that the bottleneck during introduction also increased levels of inbreeding, and this effect was more distinct in *T. tsushimae*.

4.3 | Genetic differentiation within and across native and invasive ranges

Genetic differentiation is a consequence of restricted gene flow among populations that are isolated by geographical, ecological or behavioural factors. We expected weak differentiation in the native range of *T. immigrans*, due to independent colony foundation and a continuous distribution throughout Europe (Table S1). We

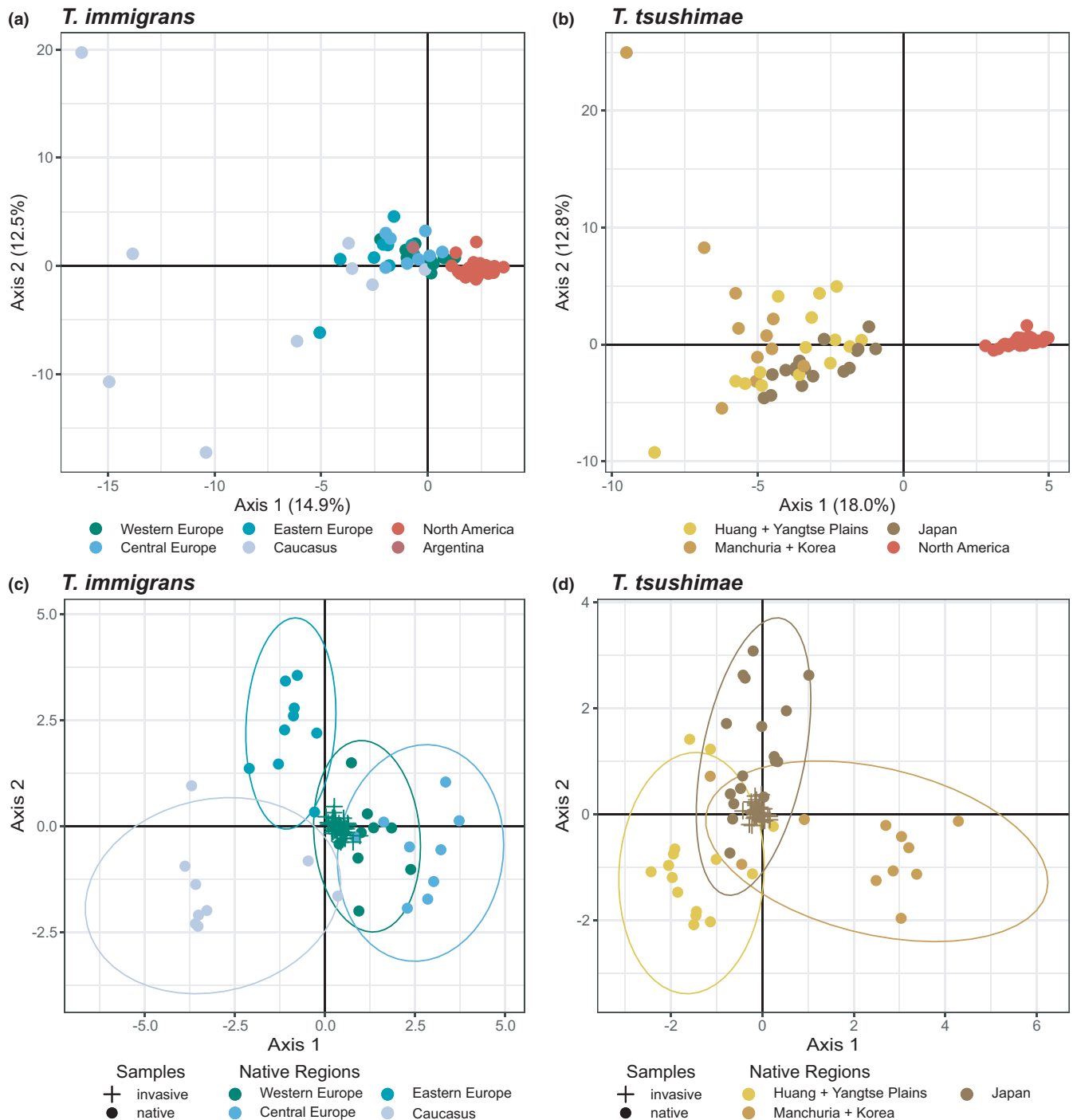


FIGURE 6 Principal coordinate analysis of (a) *Tetramorium immigrans* and (b) *Tetramorium tsushimae* microsatellite data; discriminant analysis of principal components (DAPC) of native regions of (c) *T. immigrans* and (d) *T. tsushimae*; invasive samples (+) are plotted to the native clusters with the highest predicted group membership probability based on DAPC results. Geographical distribution of samples in regions are depicted in Figure S1

found low genetic variation in Western Europe but high mitochondrial and microsatellite diversity in the Caucasus region (Figures 1 and 6a). This pattern may result from a historical expansion through Europe from an origin in the Caucasus, a plausible scenario as the Caucasus was an important ice-age refuge (Hewitt, 2000). During range expansion, drift or selection may have reduced genetic variability from east to west. The occurrence of some geographical

differentiation is also supported by a pattern of IBD and a rough west/east clustering in *STRUCTURE* (Figures 5a and 7a). The recently detected occurrence in Denmark (Sheard et al., 2020; Table S2) might account for a current expansion from Western Europe to more northern locations in Europe (Figure 1) but could be limited to urbanized habitats with warmer environmental conditions (Gippet et al., 2017). Since *T. tsushimae* spreads at least partly

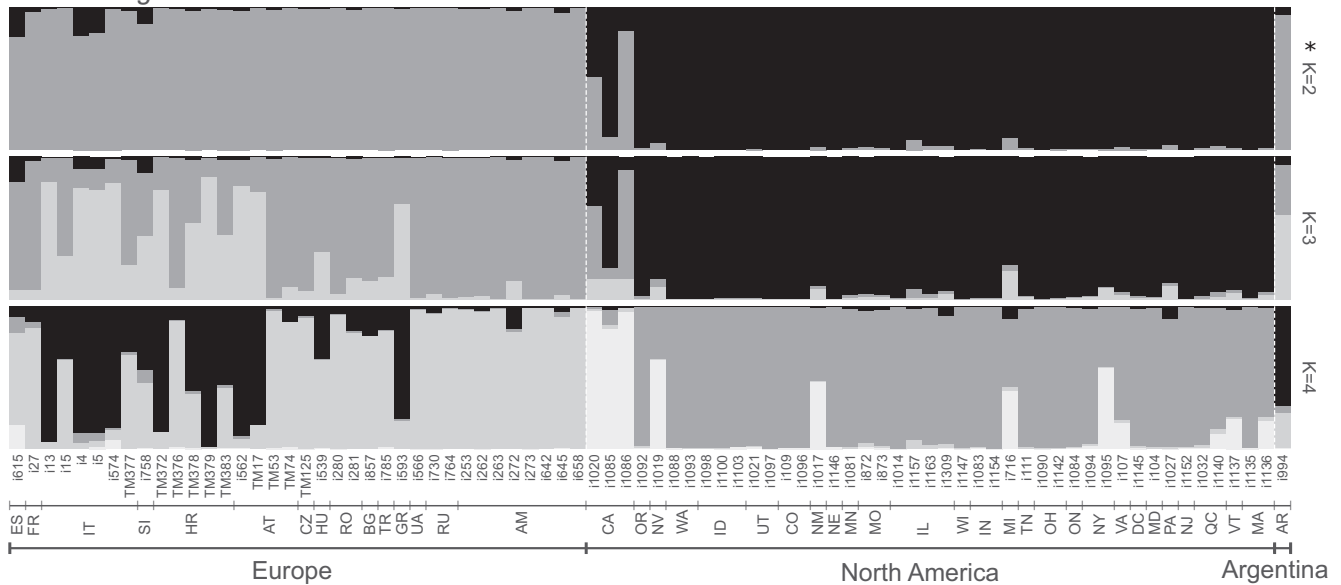
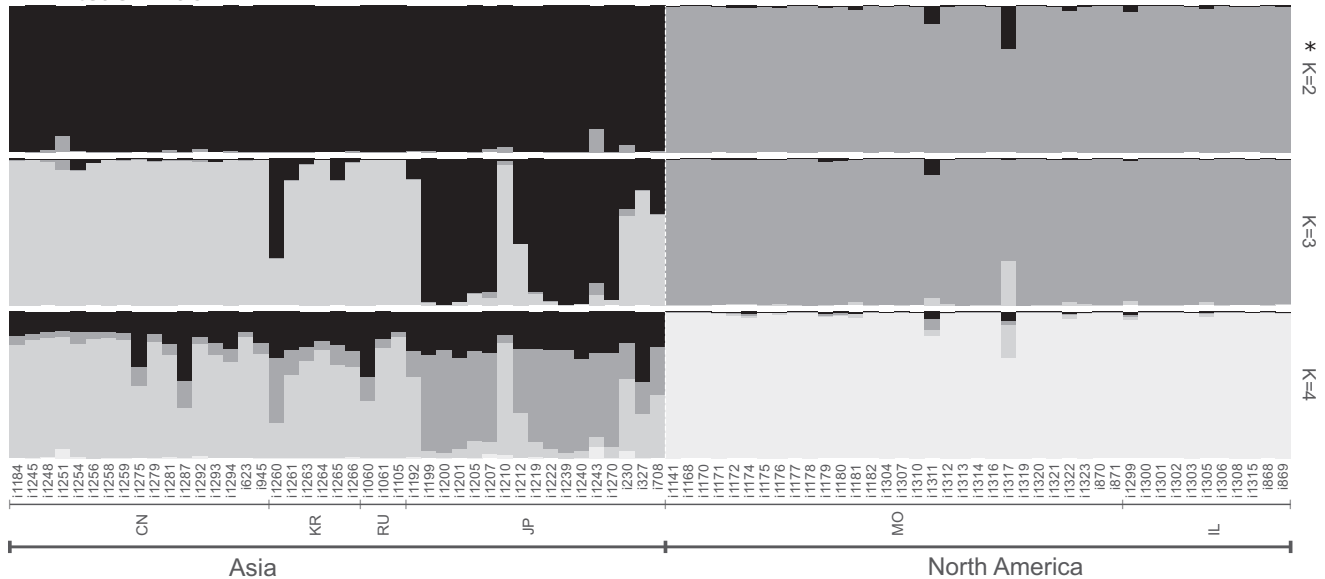
(a) *T. immigrans*(b) *T. tsushimae*

FIGURE 7 STRUCTURE barplots for (a) *Tetramorium immigrans* and (b) *Tetramorium tsushimae* for $K = 2$ to $K = 4$, sorted by continents, within continent sorted by country/state, roughly in west-east order. The best K following Evanno et al. (2005) is identified by asterisks (see also Figures S4 and S5). Europe and Asia are the native ranges, North America (plus Argentina) the invasive range. Two letter abbreviations for countries/states are: ES = Spain, FR = France, IT = Italy, SI = Slovenia, HR = Croatia, AT = Austria, CZ = Czech Republic, HU = Hungary, RO = Romania, BG = Bulgaria, TR = Turkey, GR = Greece, UA = Ukraine, RU = Russia, CA = California, OR = Oregon, NV = Nevada, WA = Washington, ID = Idaho, UT = Utah, CO = Colorado, NM = New Mexico, NE = Nebraska, MN = Minnesota, MO = Missouri, IL = Illinois, WI = Wisconsin, IN = Indiana, MI = Michigan, TN = Tennessee, OH = Ohio, ON = Ontario, NY = New York, VA = Virginia, DC = District of Columbia, MD = Maryland, PA = Pennsylvania, NJ = New Jersey, QC = Quebec, VT = Vermont, MA = Massachusetts, AR = Argentina, CN = China, KR = South Korea, JP = Japan

via nest budding (Reuther, 2009) and the native range comprises mainland Asia (China, Korea and Russia) and the islands of Japan, we expected stronger genetic differentiation in this species (Table S1). An IBD pattern in Asia along with parts of Japan forming a separate cluster in STRUCTURE confirms substantial genetic differentiation in *T. tsushimae* (Figures 5c and 7b). However, Japan shared mtDNA haplotypes with the mainland, and PCoA lacked strong

differentiation across native regions (Figures 2 and 6b), indicating that the island location of Japan might not represent such a strong barrier to gene flow as formerly anticipated.

Within the introduced ranges, genetic structure is patterned by the introduction history of species comprising the number, origin(s), and the time of introduction(s) as well as the rate of population growth shortly after introduction (affecting the occurrence

of genetic drift), and natural and human-mediated dispersal within the newly colonized area (Bélouard et al., 2019; Cristescu, 2015; Keller & Taylor, 2008; Peischl & Excoffier, 2015; Suarez et al., 2001). For *T. immigrans*, a high dispersal capacity as well as the long time after its first introduction made weak genetic structuring in North America likely (Table S1) as was indeed found by Zhang et al. (2019). We also found little geographical structuring in mitochondrial data and PCoA (Figures 1 and 6a). Nevertheless, evidence for IBD and the formation of a mainly Californian cluster in STRUCTURE reveal that the populations are not completely admixed (Figures 5b and 7a). Isolation of the Pacific coast population has already been suspected based on the absence of the social parasite *Tetramorium atratum* (= *Anergates atratulus*; Ward et al., 2015) in those populations (Helms et al., 2019). The Argentine sample differed from the North American samples in all analyses indicating an independent introduction. The examined invasive range of *T. tsushimae* is very small, with a maximum distance among sampling sites of 350 km. Therefore, admixture of the whole population seems plausible, which is also supported by the absence of IBD and the formation of a homogeneous cluster in PCoA and STRUCTURE (Figures 5d, 6b and 7b). However, IBD and three genetically distinct subpopulations were detected by Reuther (2009) in an extended data set of 52 invasive nests, including two nests found further east in Illinois. These two nests also formed one of the three subpopulations and thus might have shaped these differing results.

Genetic differentiation between native and invasive ranges is also determined by their introduction history. For both species, only one or a few introduction events have been suggested, and therefore strong differentiation was expected (Table S1). We found only a few shared mtDNA haplotypes between native and invasive samples indicating differentiation between the ranges (Figures 1 and 2). Yet, the mutation rate of the COI gene is low (Haag-Liautard et al., 2008), and consequently, the uniqueness of haplotypes to the invasive range is more likely to be caused by nonexhaustive sampling in the native range than by new mutations. This is especially true in *T. tsushimae*, where many haplotypes were found only in the invasive range. Microsatellite analyses also support a genetic differentiation between ranges, as the invasive samples formed separate clusters with little or no overlap with native regions (Figures 6a,b and 7). An exception to this is the Argentine sample, which always clustered with the European samples indicating a recent introduction directly from Europe, which has not yet experienced genetic drift. Overall, the genetic distance between ranges, as seen in the PCoA, was more pronounced in *T. tsushimae* than in *T. immigrans*. This might be due to a strong founder effect as the invasive population of *T. tsushimae* probably originated from a single introduction event and experienced a complete lack of gene flow between ranges thereafter. In contrast, *T. immigrans* might have experienced several recurrent or independent introductions. As a result, one-way gene flow could have retained a higher degree of genetic similarity to the native range.

4.4 | Number of introductions

Both the number of introduction events and the number of introduced queens influence the amount and geographical distribution of genetic diversity in the invasive range. One or few introduction events have been proposed for *T. immigrans* due to weak genetic structuring and also for *T. tsushimae* because of reduced dispersal in the invasive range (Table S1; Reuther, 2009; Steiner, Schlick-Steiner, et al., 2008; Zhang et al., 2019). In *T. immigrans*, we found three mitochondrial haplotypes and two STRUCTURE clusters in North America and a different haplotype and cluster in Argentina (Figures 1 and 7a). This suggests a minimum of two introductions to North America, with one of them to California and the other(s) to the Central or Eastern United States (the East Coast has been proposed by Marlatt, 1898), plus an independent introduction to Argentina. However, the large amount of N_a and allelic richness found in the invasive range accounts for 0.60 of the native diversity. This could stem from new mutations in microsatellites in the many years of establishment in the invasive area, polyandry and/or the cryptic introduction of queens with the same mtDNA haplotype. Multiple mating of queens as known from *T. immigrans* (Cordonnier et al., 2020) can increase the introduced diversity; however, this might only partly explain the high diversity observed. Introductions of several queens of the same mitochondrial haplotype might also include several geographical origins in the native range, which offers a probable explanation for the high microsatellite diversity in North America.

Twelve haplotypes of *T. tsushimae* were found in the invasive range, which is probably the consequence of the introduction of at least 12 queens (Figure 2). Nevertheless, the weak genetic differentiation in the invasive range, the species' polygyny and the suspected introduction with plants for the Japanese Pavilion at the World's Fair in St. Louis (Steiner et al., 2006) make the introduction of several queens in one event plausible. To determine the number of introduced nests, the characterization of intranest variation might help to elucidate how much diversity can be found within one polygynous and polydomous colony.

4.5 | Origin of introductions

The origin of the most widely spread invasive mtDNA haplotype of *T. immigrans* could be located in any of the European regions except the Caucasus (Figure 1), the second most common haplotype was probably introduced from France or Italy (additional samples; see Figure S2, Table S3). The origin of the third North American haplotype remained unclear as it was not sampled in the native range. However, this haplotype was only found once in North America and could also account for a rare but not excludable mutation. The microsatellite data show only little overlap between native and invasive samples (Figures 6a and 7a) indicating difficulties to infer the origin of introduction. Interestingly, by plotting the invasive samples to DAPC axes, the highest group-membership probabilities were

predicted for the Western European region (Figure 6c). In STRUCTURE, also four samples from Spain, Italy and Slovenia showed small but noticeable probabilities for the invasive cluster (Figure 7a), supporting a Western European origin of the North American *T. immigrans*. Having a haplotype primarily found in Spain and France, the Argentine population was probably introduced from Western Europe, which is also supported by DAPC prediction. Altogether, this indicates that Western Europe is possibly one origin of the introduction of *T. immigrans*, but it might not be the only one. Where within the Western European region the introductions originated can only be speculated. Since the introduction of ants often occurs with soil or plants (Rabitsch, 2011), it seems probable that *T. immigrans* was imported with potted crop plants or decorative shrubbery before strong regulations on transport were implemented or as stowaways in soil or rock ballast as used by ships until the mid-1900s, when using water ballast became standard practice (Carlton, 1992). As they lie within the suspected Western European origin, and strong population movements to America took place, we suggest Spain as a potential origin of the Argentine occurrence (in accordance with mtDNA) and Italy of the North American populations (in accordance with mtDNA and STRUCTURE).

In *T. tsushimae*, one mtDNA haplotype probably originates from the island of Honshu, in Japan, and one could come from any native region (Figure 2). All other invasive haplotypes were not sampled in the native range but closely related to each other, suggesting the evolutionary proximity of their origins. Microsatellite data clearly did not overlap with native data; however, DAPC predicts Japan as the most likely origin for the invasive samples (Figures 6b,d and 7b). As we had expected (Table S1), our data suggest Honshu, Japan, as the most likely origin of invasion, which is in accordance with a distribution- and niche-modelling approach and the presumed introduction history (Steiner et al., 2006; Steiner, Schlick-Steiner, et al., 2008). Yet, additional areas which have not been sampled cannot be ruled out as origins.

The uncertainties in predicting the geographical origin with microsatellite data could come from homologies due to fast marker evolution. To test this, we repeated the analyses excluding the four most variable markers per species (exclusion criterion: 29 or more alleles), which did not change the overall results (data not shown). The difficulties in inferring introduction pathways with microsatellite data we met in this study might be due to multiple or unsampled sources or drift during and after introduction (Estoup & Guillemaud, 2010). The problem of unsampled sources becomes apparent when looking at the mtDNA haplotypes, of which many invasive haplotypes were not present in the samples from the native ranges, especially in *T. tsushimae*. That an increased sampling in the native range can reveal additional information on the origins was shown with the additional mtDNA sequences of *T. immigrans* downloaded from GenBank (Table S3). Within this extended data set, several new haplotypes were found including one that was previously only known from the invasive range (Figure S2). For *T. tsushimae*, increasing the sampling density in the native regions might lead to a successful detection of the invasive haplotypes, which were closely related to each other. However, the discovery of presently unknown occurrences might also help to understand the

geographical distribution of native diversity. We cannot tell to what extent an increased sampling would have changed the microsatellite ordination and clustering results in these species. However, in two extensive studies of the ants *Solenopsis invicta* and *Solenopsis geminata* with larger sample sizes and more genetic markers, microsatellites demonstrated to be helpful to infer introduction histories (Ascunce et al., 2011; Gotzek et al., 2015). In other ant studies with similar numbers of markers as in this study, microsatellites were combined with other marker types for the reconstruction of introduction history (*L. humile*; Vogel et al., 2010), or clonal reproduction strongly reduced variability in the invasive range (*W. auropunctata*; Foucaud et al., 2010).

4.6 | Social structure and age of introduction

Many aspects of the invasion and the resulting genetic patterns of *T. immigrans* and *T. tsushimae* can be explained by their differences in social structure and the age of their introductions. Nevertheless, other species' characteristics such as slightly different habitat preferences and dietary differences might play a role in their invasion success, which we could not disentangle from the effects of queen number and colony structure. To test the robustness of our findings, future studies should focus on invasive ant species that harbour intraspecific variation in key characteristics and/or additional pairs of closely related species. The monogynous life form with independent colony formation and strong dispersal capacity of *T. immigrans* and the long time since its first introduction have led to expansion throughout its niche in North America (Steiner, Schlick-Steiner, et al., 2008). Several, probably independent introductions have occurred and added to the introduced gene pool as well as new mutations emerging over the time since its first introduction. However, the invasive populations did not diverge strongly from the native populations, which could be an indication of gene flow maintained between the continents.

In contrast, *T. tsushimae* forms polygynous supercolonies, which mainly disperse through colony budding and occur in high nest densities. This social structure might be decisive for its strong competitive ability and dominance over several species, including *T. immigrans* (Steiner et al., 2006). Though natural dispersal is limited through the absence of mating flights in the invasive range, transport by humans permits long-distance dispersal and colonization of more distant areas (Reuther, 2009). By now, the possibility of expansion throughout its niche in North America has not been exhausted (Steiner, Schlick-Steiner, et al., 2008), which might be due to the younger age of introduction. This could change in the future, and *T. tsushimae* could become a harmful pest, but the small, introduced gene pool could limit the adaptive ability to a narrower niche.

5 | CONCLUSION

We found that the amount and distribution of genetic diversity in the ants *Tetramorium immigrans* and *Tetramorium tsushimae* in their

native and invasive ranges can be explained by a combination of life-history traits (in particular their social structure), biogeographical and population genetic background, as well as the species' unique invasion histories. The diversity loss during the introduction was distinct in both species, but the severity differed across marker types and species. The inbreeding situation compared across the native and invasive ranges remained uncertain. Genetic differentiation was weak but noticeable within the native ranges of both species and in the invasive range of *T. immigrans* but undetectable in the invasive range of *T. tsushimae*. However, differentiation between ranges was substantial, especially in *T. tsushimae*. *Tetramorium immigrans* was probably introduced from Western Europe to North America in at least two independent events, whereas for *T. tsushimae*, several queens were probably introduced in one introduction event from Honshu, Japan.

This example of two congeners with different life-history traits shows that the invasion success of ants is influenced but not ultimately determined by social structure. As even closely related species can differ considerably in a multitude of invasion-relevant aspects, our study highlights once more that the investigation of a wide variety of species, including less prominent ones, is needed to deduce overall conclusions and robust predictions in invasion biology. These insights are urgently needed to evaluate the status of and advance control measures against invasive species.

ACKNOWLEDGMENTS

We thank N. Aktac, G. Alpert, M. Balint, N. Blacker, G. Bracko, A. Buschinger, A. Carniel, E. Carroll, C. Chantal, W. H. Clark, K. Clarke, S. Dash, L. R. Davis, C. DeHeer, X. Espadaler, R. Fagerlund, C. Feery, D. Fontaneto, C. Guofa, R. Güsten, L. D. Hansen, M. Hayashi, J. Heinze, S. Hosoishi, K. J. Howard, J. Huang, K. Ivanov, V. E. Kipyatkov, D. S. Kjar, V. Kochnev, Y. Kochnev, R. S. Larsen, T. Linksvayer, T. Ljubomirov, J. Longino, E. B. Lopatina, D. Lubertazzi, D.-P. Lyu, M. Maruyama, H. Mori, T. Nuhn, R. Pereira, J. Pitts, E. Provost, F. Qian, L. Quiang, A. Radchenko, L. Rericha, K. Reuther, F. Rigato, H. Sakai, A. San Juan, S. Sanada, M. Sanetra, R. Schultz, A. Schulz, W. Shao, J. K. Stahlhut, H. M. Steiner, G. Takahashi, E. Tikhonov, J. Trager, Y. Tsuneoka, D. Ward, P. S. Ward, A. Wild, D. D. Williams, M. Wuermli, S. Yamane, J. Zefeng and S. Zhou for sample donations; M. Schilling for cluster computational advice; Subject Editor Rosemary Gillespie and three anonymous reviewers for constructive criticism of earlier versions of the manuscript. The computational results presented here have been achieved (in part) using the LEO HPC infrastructure of the University of Innsbruck. F.M.S. was supported by the Austrian Science Fund (J 2642, P 30861).

AUTHOR CONTRIBUTIONS

A.V.S., R.H.C., F.M.S. and B.C.S.-S. designed the research; A.V.S., F.M.S. and B.C.S.-S. provided samples; S.M.F., W.A., F.M.S. and B.C.S.-S. performed the research; S.M.F. and P.K. analysed the data; S.M.F. wrote the paper; P.K., A.V.S., F.M.S. and B.C.S.-S. revised the manuscript. R.H.C. passed away before completion of the study.

DATA AVAILABILITY STATEMENT

Sampling locations, DNA sequences (GenBank accessions): Tables S2 and S3. Microsatellite genotypes: Dryad (<https://doi.org/10.5061/dryad.ns1rn8prg>).

ORCID

Sylvia M. Flucher  <https://orcid.org/0000-0002-9162-4892>

Patrick Krapf  <https://orcid.org/0000-0002-1625-4473>

Florian M. Steiner  <https://orcid.org/0000-0003-2414-4650>

Birgit C. Schlick-Steiner <http://orcid.org/0000-0003-4026-5778>

REFERENCES

- Adamack, A. T., & Gruber, B. (2014). PopGenReport: Simplifying basic population genetic analyses in R. *Methods in Ecology and Evolution*, 5(4), 384–387. <https://doi.org/10.1111/2041-210X.12158>
- Allendorf, F. W., & Lundquist, L. L. (2003). Introduction: Population biology, evolution, and control of invasive species. *Conservation Biology*, 17(1), 24–30. <https://doi.org/10.1046/j.1523-1739.2003.02365.x>
- Ascunce, M. S., Yang, C.-C., Oakey, J., Calcaterra, L., Wu, W.-J., Shih, C.-J., Goudet, J., Ross, K. G., & Shoemaker, D. (2011). Global invasion history of the fire ant *Solenopsis invicta*. *Science*, 331(6020), 1066–1068. <https://doi.org/10.1126/science.1198734>
- Bandelt, H. J., Forster, P., & Rohlf, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, 16(1), 37–48. <https://doi.org/10.1093/oxfordjournals.molbev.a026036>
- Béluard, N., Paillisson, J. M., Oger, A., Besnard, A. L., & Petit, E. J. (2019). Genetic drift during the spread phase of a biological invasion. *Molecular Ecology*, 28(19), 4375–4387. <https://doi.org/10.1111/mec.15238>
- Bertelsmeier, C., Ollier, S., Liebhold, A., & Keller, L. (2017). Recent human history governs global ant invasion dynamics. *Nature Ecology & Evolution*, 1(7), 0184. <https://doi.org/10.1038/s41559-017-0184>
- Brown, W. L. J. (1957). Is the ant genus *Tetramorium* native in North America? *Breviora*, 72, 1–8.
- Carlton, J. T. (1992). Blue immigrants: The marine biology of maritime history. *The Log of Mystic Seaport Museum*, 44, 31–36.
- Chessel, D., Dufour, A. B., & Thioulouse, J. (2004). The ade4 package - I: One-table methods. *R News*, 4(1), 5–10.
- Cicconardi, F., Krapf, P., D'Annessa, I., Gamsch, A., Wagner, H. C., Nguyen, A. D., Economo, E. P., Mikheyev, A. S., Guénard, B., Grabherr, R., Andesner, P., Wolfgang, A., Di Marino, D., Steiner, F. M., & Schlick-Steiner, B. C. (2020). Genomic signature of shifts in selection in a subalpine ant and its physiological adaptations. *Molecular Biology and Evolution*, 37(8), 2211–2227. <https://doi.org/10.1093/molbev/msaa076>
- Cordonnier, M., Escarguel, G., Dumet, A., & Kaufmann, B. (2020). Multiple mating in the context of interspecific hybridization between two *Tetramorium* ant species. *Heredity*, 124(5), 675–684. <https://doi.org/10.1038/s41437-020-0310-3>
- Coulson, T. N., Pemberton, J. M., Albon, S. D., Beaumont, M., Marshall, T. C., Slate, J., Guinness, F. E., & Clutton-Brock, T. H. (1998). Microsatellites reveal heterosis in red deer. *Proceedings of the Royal Society B: Biological Sciences*, 265(1395), 489–495. <https://doi.org/10.1098/rspb.1998.0321>
- Cristescu, M. E. (2015). Genetic reconstructions of invasion history. *Molecular Ecology*, 24(9), 2212–2225. <https://doi.org/10.1111/mec.13117>
- Crozier, R. H., & Pamilo, P. (1996). *Evolution of social insect colonies: Sex allocation and kin selection*. Oxford University Press.
- Dlugosch, K. M., & Parker, I. M. (2008). Founding events in species invasions: Genetic variation, adaptive evolution, and the role of

- multiple introductions. *Molecular Ecology*, 17(1), 431–449. <https://doi.org/10.1111/j.1365-294X.2007.03538.x>
- Ellegren, H. (2000). Microsatellite mutations in the germline: Implications for evolutionary inference. *Trends in Genetics*, 16(12), 551–558. [https://doi.org/10.1016/S0168-9525\(00\)02139-9](https://doi.org/10.1016/S0168-9525(00)02139-9)
- Estoup, A., & Guillemaud, T. (2010). Reconstructing routes of invasion using genetic data: Why, how and so what? *Molecular Ecology*, 19(19), 4113–4130. <https://doi.org/10.1111/j.1365-294X.2010.04773.x>
- Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: A simulation study. *Molecular Ecology*, 14(8), 2611–2620. <https://doi.org/10.1111/j.1365-294X.2005.02553.x>
- Foucaud, J., Orivel, J., Loiseau, A., Delabie, J. H. C., Jourdan, H., Konghouleux, D., Vonshak, M., Tindo, M., Mercier, J.-L., Fresneau, D., Mikissa, J.-B., McGlynn, T., Mikheyev, A. S., Oettler, J., & Estoup, A. (2010). Worldwide invasion by the little fire ant: Routes of introduction and eco-evolutionary pathways. *Evolutionary Applications*, 3(4), 363–374. <https://doi.org/10.1111/j.1752-4571.2010.00119.x>
- Fournier, D., Foucaud, J., Loiseau, A., Cros-arteil, S., Jourdan, H., Orivel, J., Le breton, J., Chazeau, J., Dejean, A., Keller, L., & Estoup, A. (2005). Characterization and PCR multiplexing of polymorphic microsatellite loci for the invasive ant *Wasmannia auropunctata*. *Molecular Ecology Notes*, 5(2), 239–242. <https://doi.org/10.1111/j.1471-8286.2005.00889.x>
- Francis, R. M. (2017). pophelper: An R package and web app to analyse and visualize population structure. *Molecular Ecology Resources*, 17(1), 27–32. <https://doi.org/10.1111/1755-0998.12509>
- Frankham, R. (2005a). Genetics and extinction. *Biological Conservation*, 126(2), 131–140. <https://doi.org/10.1016/j.biocon.2005.05.002>
- Frankham, R. (2005b). Resolving the genetic paradox in invasive species. *Heredity*, 94(4), 385. <https://doi.org/10.1038/sj.hdy.6800634>
- Gippet, J. M. W., Mondy, N., Diallo-Dudek, J., Bellec, A., Dumet, A., Mistler, L., & Kaufmann, B. (2017). I'm not like everybody else: Urbanization factors shaping spatial distribution of native and invasive ants are species-specific. *Urban Ecosystems*, 20(1), 157–169. <https://doi.org/10.1007/s11252-016-0576-7>
- Gotzek, D., Axen, H. J., Suarez, A. V., Helms Cahan, S., & Shoemaker, D. (2015). Global invasion history of the tropical fire ant: A stowaway on the first global trade routes. *Molecular Ecology*, 24(2), 374–388. <https://doi.org/10.1111/mec.13040>
- Goudet, J., & Jombart, T. (2015). *hierfstat: Estimation and tests of hierarchical F-statistics*. Retrieved from <https://cran.r-project.org/package=hierfstat>
- Guénard, B., Weiser, M. D., Gómez, K., Narula, N., & Economo, E. P. (2017). The Global Ant Biodiversity Informatics (GABI) database: Synthesizing data on the geographic distribution of ant species (Hymenoptera: Formicidae). *Myrmecological News*, 24, 83–89. https://doi.org/10.25849/myrmecol.news_024:083
- Haag-Liautard, C., Coffey, N., Houle, D., Lynch, M., Charlesworth, B., & Keightley, P. D. (2008). Direct estimation of the mitochondrial DNA mutation rate in *Drosophila melanogaster*. *PLoS Biology*, 6(8), 1706–1714. <https://doi.org/10.1371/journal.pbio.0060204>
- Hakala, S. M., Seppä, P., & Helanterä, H. (2019). Evolution of dispersal in ants (Hymenoptera: Formicidae): A review on the dispersal strategies of sessile superorganisms. *Myrmecological News*, 29, 35–55. https://doi.org/10.25849/myrmecol.news_029:035
- Hansson, B. (2010). The use (or misuse) of microsatellite allelic distances in the context of inbreeding and conservation genetics. *Molecular Ecology*, 19(6), 1082–1090. <https://doi.org/10.1111/j.1365-294X.2010.04556.x>
- Hardesty, B. D., Le Roux, J. J., Rocha, O. J., Meyer, J. Y., Westcott, D., & Wiczorek, A. M. (2012). Getting here from there: Testing the genetic paradigm underpinning introduction histories and invasion success. *Diversity and Distributions*, 18(2), 147–157. <https://doi.org/10.1111/j.1472-4642.2011.00832.x>
- Helanterä, H., Strassmann, J. E., Carrillo, J., & Queller, D. C. (2009). Uniclonal ants: Where do they come from, what are they and where are they going? *Trends in Ecology and Evolution*, 24(6), 341–349. <https://doi.org/10.1016/j.tree.2009.01.013>
- Helms, J. A., Ijelu, S. E., & Haddad, N. M. (2019). Range expansion in an introduced social parasite-host species pair. *Biological Invasions*, 21(8), 2751–2759. <https://doi.org/10.1007/s10530-019-02011-y>
- Hewitt, G. (2000). The genetic legacy of the quaternary ice ages. *Nature*, 405(6789), 907–913. <https://doi.org/10.1038/35016000>
- Hijmans, R. J. (2019). *geosphere: Spherical trigonometry*. Retrieved from <https://cran.r-project.org/package=geosphere>
- Holway, D. A., Lach, L., Suarez, A. V., Tsutsui, N. D., & Case, T. J. (2002). The causes and consequences of ant invasions. *Annual Review of Ecology and Systematics*, 33(1), 181–233. <https://doi.org/10.1146/annurev.ecolsys.33.010802.150444>
- Holway, D. A., Suarez, A. V., & Case, T. J. (1998). Loss of intraspecific aggression in the success of a widespread invasive social insect. *Science*, 282(5390), 949–952. <https://doi.org/10.1126/science.282.5390.949>
- Hosoishi, S., Rahman, M. M., Murakami, T., Park, S.-H., Kuboki, Y., & Ogata, K. (2019). Winter activity of ants in an urban area of Western Japan. *Sociobiology*, 66(3), 414–419. <https://doi.org/10.13102/sociobiology.v66i3.4374>
- Jakobsson, M., & Rosenberg, N. A. (2007). CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, 23(14), 1801–1806. <https://doi.org/10.1093/bioinformatics/btm233>
- Jenkins, P. T. (1996). Free trade and exotic species introductions. *Conservation Biology*, 10(1), 300–302. <https://doi.org/10.1046/j.1523-1739.1996.10010300.x>
- Jombart, T. (2008). adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24(11), 1403–1405. <https://doi.org/10.1093/bioinformatics/btn129>
- Keller, S. R., & Taylor, D. R. (2008). History, chance and adaptation during biological invasion: Separating stochastic phenotypic evolution from response to selection. *Ecology Letters*, 11(8), 852–866. <https://doi.org/10.1111/j.1461-0248.2008.01188.x>
- Kolar, C. S., & Lodge, D. M. (2001). Progress in invasion biology: Predicting invaders. *Trends in Ecology and Evolution*, 16(4), 199–204. [https://doi.org/10.1016/S0169-5347\(01\)02101-2](https://doi.org/10.1016/S0169-5347(01)02101-2)
- Kolbe, J. J., Glor, R. E., Rodríguez Schettino, L., Lara, A. C., Larson, A., & Losos, J. B. (2004). Genetic variation increases during biological invasion by a Cuban lizard. *Nature*, 431(7005), 177–181. <https://doi.org/10.1038/nature02807>
- Krapf, P., Russo, L., Arthofer, W., Möst, M., Steiner, F. M., & Schlick-Steiner, B. C. (2018). An Alpine ant's behavioural polymorphism: Monogyny with and without internet aggression in *Tetramorium alpestre*. *Ethology Ecology and Evolution*, 30(3), 220–234. <https://doi.org/10.1080/03949370.2017.1343868>
- Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Mack, R. N., Simberloff, D., Lonsdale, W. M., Evans, H., Clout, M., & Bazzaz, F. A. (2000). Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecological Applications*, 10(3), 689–710.
- Marlatt, C. L. (1898). *House Ants (Monomorium pharaonis et al)*. United States Department of Agriculture, Division of Entomology.
- Nipperess, D. A., & Matsen, F. A. (2013). The mean and variance of phylogenetic diversity under rarefaction. *Methods in Ecology and Evolution*, 4(6), 566–572. <https://doi.org/10.1111/2041-210X.12042>
- Pamilo, P. (1985). Effect of inbreeding on genetic relatedness. *Heredity*, 103(2), 195–200. <https://doi.org/10.1111/j.1601-5223.1985.tb00501.x>
- Pamilo, P., & Nei, M. (1988). Relationships between gene trees and species trees. *Molecular Biology and Evolution*, 5(5), 568–583. <https://doi.org/10.1093/oxfordjournals.molbev.a040517>

- Paradis, E. (2010). pegas: An R package for population genetics with an integrated-modular approach. *Bioinformatics*, 26(3), 419–420. <https://doi.org/10.1093/bioinformatics/btp696>
- Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics*, 28(19), 2537–2539. <https://doi.org/10.1093/bioinformatics/bts460>
- Pedersen, J. S., Krieger, M. J. B., Vogel, V., Giraud, T., & Keller, L. (2006). Native supercolonies of unrelated individuals in the invasive Argentine ant. *Evolution*, 60(4), 782–791. <https://doi.org/10.1111/j.0014-3820.2006.tb01156.x>
- Peischl, S., & Excoffier, L. (2015). Expansion load: Recessive mutations and the role of standing genetic variation. *Molecular Ecology*, 24(9), 2084–2094. <https://doi.org/10.1111/mec.13154>
- Penick, C. A., Savage, A. M., & Dunn, R. R. (2015). Stable isotopes reveal links between human food inputs and urban ant diets. *Proceedings of the Royal Society B: Biological Sciences*, 282(1806), 20142608. <https://doi.org/10.1098/rspb.2014.2608>
- Posada, D. (2009). Selection of models of DNA evolution with jModelTest. In D. Posada (Ed.), *Bioinformatics for DNA sequence analysis. Methods in Molecular Biology (Methods and Protocols)* (537, pp. 93–112). Humana Press. https://doi.org/10.1007/978-1-59745-251-9_5
- Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959.
- Puillandre, N., Dupas, S., Dangles, O., Zeddam, J.-L., Capdevielle-Dulac, C., Barbin, K., Torres-Leguizamon, M., & Silvain, J.-F. (2008). Genetic bottleneck in invasive species: The potato tuber moth adds to the list. *Biological Invasions*, 10(3), 319–333. <https://doi.org/10.1007/s10530-007-9132-y>
- R Core Team. (2019). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. <https://www.r-project.org/>
- Rabitsch, W. (2011). The hitchhiker's guide to alien ant invasions. *BioControl*, 56(4), 551–572. <https://doi.org/10.1007/s10526-011-9370-x>
- Reuther, K. D. (2009). *Early invasion dynamics of the Japanese pavement ant, Tetramorium tsushimae, in the St. Louis, Missouri metropolitan area* (Doctoral dissertation). Saint Louis University.
- Ross, K. G., & Shoemaker, D. D. W. (2008). Estimation of the number of founders of an invasive pest insect population: The fire ant *Solenopsis invicta* in the USA. *Proceedings of the Royal Society B: Biological Sciences*, 275(1648), 2231–2240. <https://doi.org/10.1098/rspb.2008.0412>
- Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With, K. A., Baughman, S., Cabin, R. J., Cohen, J. E., Ellstrand, N. C., McCauley, D. E., O'Neil, P., Parker, I. M., Thompson, J. N., & Weller, S. G. (2001). The population biology of invasive species. *Annual Review of Ecology and Systematics*, 32(1), 305–332. <https://doi.org/10.1146/annurev.ecolsys.32.081501.114037>
- Sanada-Morimura, S., Satoh, T., & Obara, Y. (2006). Territorial behavior and temperature preference for nesting sites in a pavement ant *Tetramorium tsushimae*. *Insectes Sociaux*, 53(2), 141–148. <https://doi.org/10.1007/s00040-005-0849-2>
- Schlick-Steiner, B. C., Steiner, F. M., Moder, K., Seifert, B., Sanetra, M., Dyreson, E., Stauffer, C., & Christian, E. (2006). A multidisciplinary approach reveals cryptic diversity in Western Palearctic *Tetramorium* ants (Hymenoptera: Formicidae). *Molecular Phylogenetics and Evolution*, 40(1), 259–273. <https://doi.org/10.1016/j.ympev.2006.03.005>
- Seifert, B. (2010). Intranidal mating, gyne polymorphism, polygyny, and supercoloniality as factors for sympatric and parapatric speciation in ants. *Ecological Entomology*, 35(Suppl. 1), 33–40. <https://doi.org/10.1111/j.1365-2311.2009.01136.x>
- Seyfert, A. L., Cristescu, M. E. A., Frisse, L., Schaack, S., Thomas, W. K., & Lynch, M. (2008). The rate and spectrum of microsatellite mutation in *Caenorhabditis elegans* and *Daphnia pulex*. *Genetics*, 178(4), 2113–2121. <https://doi.org/10.1534/genetics.107.081927>
- Sheard, J. K., Sanders, N. J., Gundlach, C., Schär, S., & Larsen, R. S. (2020). Monitoring the influx of new species through citizen science: The first introduced ant in Denmark. *PeerJ*, 8, e8850. <https://doi.org/10.7717/peerj.8850>
- Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., & Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, 87(6), 651–701. <https://doi.org/10.1093/aesa/87.6.651>
- Steiner, F. M., Arthofer, W., Schlick-Steiner, B. C., Crozier, R. H., & Stauffer, C. (2008). Twenty four new microsatellite markers in two invasive pavement ants, *Tetramorium* sp. E and *T. tsushimae* (Hymenoptera: Formicidae). *Conservation Genetics*, 9, 757–759. <https://doi.org/10.1007/s10592-007-9385-0>
- Steiner, F. M., Crozier, R. H., & Schlick-Steiner, B. C. (2009). Colony structure. In L. Lach, C. Parr, & K. Abbott (Eds.), *Ant ecology* (pp. 177–193). Oxford University Press. <https://doi.org/10.1093/acprof:oso/9780199544639.003.0010>
- Steiner, F. M., Schlick-Steiner, B. C., Sanetra, M., Ljubomirov, T., Antonova, V., Christian, E., & Stauffer, C. (2005). Towards DNA-aided biogeography: An example from *Tetramorium* ants (Hymenoptera, Formicidae). *Annales Zoologici Fennici*, 42(1), 23–35.
- Steiner, F. M., Schlick-Steiner, B. C., Trager, J. C., Moder, K., Sanetra, M., Christian, E., & Stauffer, C. (2006). *Tetramorium tsushimae*, a new invasive ant in North America. *Biological Invasions*, 8(2), 117–123. <https://doi.org/10.1007/s10530-004-1249-7>
- Steiner, F. M., Schlick-Steiner, B. C., VanDerWal, J., Reuther, K. D., Christian, E., Stauffer, C., Suarez, A. V., Williams, S. E., & Crozier, R. H. (2008). Combined modelling of distribution and niche in invasion biology: A case study of two invasive *Tetramorium* ant species. *Diversity and Distributions*, 14(3), 538–545. <https://doi.org/10.1111/j.1472-4642.2008.00472.x>
- Suarez, A. V., Holway, D. A., & Case, T. J. (2001). Patterns of spread in biological invasions dominated by long-distance jump dispersal: Insights from argentine ants. *Proceedings of the National Academy of Sciences of the United States of America*, 98(3), 1095–1100. <https://doi.org/10.1073/pnas.98.3.1095>
- Takezaki, N., & Nei, M. (1996). Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics*, 144(1), 389–399.
- Trontti, K., Aron, S., & Sundström, L. (2005). Inbreeding and kinship in the ant *Plagiolepis pygmaea*. *Molecular Ecology*, 14(7), 2007–2015. <https://doi.org/10.1111/j.1365-294X.2005.02529.x>
- Tsutsui, N. D., & Suarez, A. V. (2003). The colony structure and population biology of invasive ants. *Conservation Biology*, 17(1), 48–58. <https://doi.org/10.1046/j.1523-1739.2003.02018.x>
- Tsutsui, N. D., Suarez, A. V., Holway, D. A., & Case, T. J. (2000). Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences*, 97(11), 5948–5953. <https://doi.org/10.1073/pnas.100110397>
- Uller, T., & Leimu, R. (2011). Founder events predict changes in genetic diversity during human-mediated range expansions. *Global Change Biology*, 17(11), 3478–3485. <https://doi.org/10.1111/j.1365-2486.2011.02509.x>
- Venables, W. N., & Ripley, B. D. (2002). *Modern Applied Statistics with S*, 4th ed. Springer-Verlag New York. <http://www.stats.ox.ac.uk/pub/MASS4>
- Villesen, P. (2007). FaBox: An online toolbox for fasta sequences. *Molecular Ecology Notes*, 7(6), 965–968. <https://doi.org/10.1111/j.1471-8286.2007.01821.x>
- Vogel, V., Pedersen, J. S., Giraud, T., Krieger, M. J. B., & Keller, L. (2010). The worldwide expansion of the Argentine ant. *Diversity and Distributions*, 16(1), 170–186. <https://doi.org/10.1111/j.1472-4642.2009.00630.x>

- Wagner, H. C., Arthofer, W., Seifert, B., Muster, C., Steiner, F. M., & Schlick-Steiner, B. C. (2017). Light at the end of the tunnel: Integrative taxonomy delimits cryptic species in the *Tetramorium caespitum* complex (Hymenoptera: Formicidae). *Myrmecological News*, 25, 95–129. https://doi.org/10.25849/myrmecol.news_025:095
- Wagner, H. C., Gamisch, A., Arthofer, W., Moder, K., Steiner, F. M., & Schlick-Steiner, B. C. (2018). Evolution of morphological crypsis in the *Tetramorium caespitum* ant species complex (Hymenoptera: Formicidae). *Scientific Reports*, 8, 12547. <https://doi.org/10.1038/s41598-018-30890-z>
- Wahlund, S. (1928). Zusammensetzung von Populationen und Korrelationserscheinungen vom Standpunkt der Vererbungslehre aus betrachtet. *Hereditas*, 11(1), 65–106. <https://doi.org/10.1111/j.1601-5223.1928.tb02483.x>
- Ward, P. S., Brady, S. G., Fisher, B. L., & Schultz, T. R. (2015). The evolution of myrmicine ants: Phylogeny and biogeography of a hyperdiverse ant clade (Hymenoptera: Formicidae). *Systematic Entomology*, 40(1), 61–81. <https://doi.org/10.1111/syen.12090>
- Wilson, E. O. (1971). *The insect societies*. Belknap Press of Harvard University Press.
- Zayed, A., Constantin, S. A., & Packer, L. (2007). Successful biological invasion despite a severe genetic load. *PLoS One*, 2(9), e868. <https://doi.org/10.1371/journal.pone.0000868>
- Zhang, Y. M., Vitone, T. R., Storer, C. G., Payton, A. C., Dunn, R. R., Hulcr, J., McDaniel, S. F., & Lucky, A. (2019). From pavement to population genomics: Characterizing a long-established non-native ant in North America through citizen science and ddRADseq. *Frontiers in Ecology and Evolution*, 7, 453. <https://doi.org/10.3389/fevo.2019.00453>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Flucher SM, Krapf P, Arthofer W, et al. Effect of social structure and introduction history on genetic diversity and differentiation. *Mol Ecol*. 2021;30:2511–2527. <https://doi.org/10.1111/mec.15911>