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Positive selection has shaped the evolution of Argentine ant immune genes both in native and introduced supercolonies

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Abstract

The highly invasive Argentine ant (Linepithema humile) started its colonisation from the species’ native range in South America approximately 150 years ago and has since become one of the major pests in the world. We investigated how the shifts into new ranges have affected the evolution of Argentine ants’ immune genes. To the best of our knowledge, this is the first broadscale population genetic study focusing on ants’ immune genes. We analysed comprehensive targeted-seq data of immune and non-immune genes containing 174 genes from 18 Argentine ant supercolonies covering the species’ native and introduced ranges. We predicted that the immune gene evolution of introduced supercolonies differs from that of the native supercolonies and proposed two different, non-mutually exclusive hypotheses for this: 1) the enemy release hypothesis and 2) the higher pathogen pressure hypothesis – both of which seem to explain the observed evolutionary patterns on their behalf. Our results show that the introduced supercolonies were targeted by weaker selection than natives, but positive selection was evident among supercolonies of both ranges. Moreover, in some cases, such as the antiviral RNAi genes, introduced range supercolonies harboured a higher proportion of positively selected genes than natives. This observation was striking, knowing the recent demographic history and the detected generally lower selection efficacy of introduced supercolonies. In conclusion, it is evident that pathogen pressure is ubiquitous and strongly affects the immune gene evolution in Argentine ants.

Keywords: innate immunity, social insects, ants, population genetics, invasive species

Introduction

The highly invasive Argentine ant Linepithema humile (Hymenoptera: Formicidae) has spread from its original habitat in northern Argentina and the surrounding areas in South America to all continents except Antarctica during the past 150 years (Wetterer et al., 2009). Like many other invasive ant species, Argentine ants form polygynous (i.e., multi-queen) and polydromous (i.e., multi-nest) societies called supercolonies (Pedersen et al., 2006). These supercolonies can be enormous, especially in introduced ranges where the European Main supercolony spans over 6,000 km along Southern Europe’s Mediterranean and Atlantic coastline (Giraud et al., 2002). Supercolonies constitute large functional units where individuals move between nests without aggression and territorial boundaries (Suarez et al., 1999; Tsutsui et al., 2000). In addition, matings occur inside these supercolonies so that they form closed breeding units, and colonies expand through budding as new connected nests are constructed (Aron, 2001; Krieger & Keller, 2000; Sunamura et al., 2011).

Typically, bottlenecked introduced populations suffer from founder effects with lower genetic diversity and reduced effective population size compared to native populations. Depletion in genetic variability challenges the newly established populations in many ways, deteriorating selection efficacy and amplifying genetic drift (Nei et al., 1975; Prentis et al., 2008). Nevertheless, Argentine ants are extremely successful among introduced ranges where enormous population densities might provide a competitive advantage over local species (Giraud et al., 2002; Vogel et al., 2010). However, high population densities have drawbacks, providing a great opportunity for pathogens to spread. Indeed, social insects and, particularly supercolonial ants, are presumed to evolve under higher pathogen pressure than solitary species (Boomsma et al., 2003; Schmid-Hempel, 1998; Tragust et al., 2015; Ugelvig & Cremer, 2012). Immune genes are generally assumed to evolve rapidly due to continuous co-evolutionary arms races between host defence mechanisms and pathogens (Kimbrall & Beutler, 2001; Paterson et al., 2010), which might be caused by positive or balancing selection. While positive selection leads to the fixation of beneficial alleles, balancing selection preserves variability.

In the introduced populations, there are two contrasting but not mutually exclusive outcomes for the evolutionary patterns of immune genes. First, according to the enemy release hypothesis, after expanding into new ranges, the introduced populations are released from their natural

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enemies of the native ranges, leading to relaxed pathogen pressure and immune gene evolution under less-intensive positive selection compared to the native populations (Colautti et al., 2004; Torchin et al., 2003). Second, the introduced populations may face new pathogen fauna in the new ranges, leading to strong pathogen pressure and immune gene evolution under intense positive selection compared to the native populations (Colautti et al., 2004). Both types of changes in pathogen exposure have received empirical support as both a decline in viral and bacterial diversity and an acquisition of new range-specific taxa have been detected across introduced Argentine ant supercolonies (Felden et al., 2019; Lester et al., 2017).

All insects lack adaptive immunity and rely solely on innate immunity, constituting cellular and humoral responses. These defence mechanisms target pathogens that pass the insects’ exoskeleton and intrude on the body. Cellular responses include phagocytosis, melanisation, and encapsulation of pathogens via specialized hemocytes. Humoral responses comprise chains of events of different pathways (Toll, Imd, RNAi, JAK/STAT, and JNK), producing effector molecules, mainly antimicrobial peptides (Ferrandon et al., 2007; Lemaitre & Hoffmann, 2007). Besides physiological immunity, social insects (ants, termites, some bees, and wasps) have developed a wide array of behavioural defence mechanisms, i.e., social immunity (Cremer et al., 2007). Social immunity includes many kinds of hygienic behaviour, for instance, allogrooming, social fever, the inclusion of antimicrobial compounds into nest material or diet, transfer of immune factors, and exclusion of infested individuals (Chapuisat et al., 2007; Christe et al., 2003; Hamilton et al., 2011; Heinez & Walter, 2010; Rissansen et al., 2022; Starks et al., 2000; Traniello et al., 2002).

Pathogen-mediated evolution is universally strong (Fumagalli et al., 2011; Kimbrell & Beutler, 2001; Shultz & Sackton, 2019). Indeed, immune genes have experienced high rates of evolution, and positive selection has been observed across diverse taxa, e.g., insects, birds, and mammals (Roux et al., 2014; Sackton et al., 2007; Shultz & Sackton, 2019). In Drosophila, positive selection has specially shaped the evolution of recognition, signalling, and RNAi genes (Jiggins & Kim, 2007; Obbard et al., 2009; Sackton et al., 2007; Schlenke & Begun, 2003). High evolutionary rates (much higher than in Drosophila) have also been characteristic of immune genes in social insects (Harpur & Zayed, 2013; Viljakainen et al., 2009). However, this rapid evolution seems to be predominantly due to the effects of relaxed purifying selection rather than positive selection (Harpur & Zayed, 2013; Viljakainen et al., 2009).

Although the scarcity of observed positive selection, for example, antimicrobial peptides have been positively selected among social insects in both Hymenoptera and Isoptera (Bulmer & Crozier, 2004; Bulmer et al., 2010; Harpur & Zayed, 2013; Viljakainen & Pamilo, 2008), while this is not the case among non-social dipterans (Drosophila and Anopheles) in which positive selection seems, in general, to be more pervasive (Jiggins & Kim, 2005; Lazzaro & Clark, 2003; Parmakelis et al., 2008; Simard et al., 2007). However, several other immune genes, such as recognition and RNAi genes, have been the targets of positive selection both in social insects and non-social dipterans (Bulmer & Crozier, 2006; Bulmer et al., 2010; Palmer et al., 2018; Viljakainen et al., 2009). Indeed, despite the accumulation of knowledge outside the model species Drosophila melanogaster, there is still much work to get a comprehensive picture of the evolution of the immune system among insects, especially among social insects in which physiological immunity interplays with social immunity, and in different ecological contexts such as introductions into new ranges.

The aim of this study was to investigate how shifts into new ranges have affected the evolution of Argentine ants’ immune genes. While a previous comparative study detected that ant immune genes have evolved under positive selection over a long evolutionary time (millions of years) (Roux et al., 2014), this is, to the best of our knowledge, the first broadscale population genetic study focusing on the recent evolution of ant immune genes. For this purpose, we utilized targeted sequence data of 91 immune genes and the same amount of non-immune, i.e., control genes obtained from 18 Argentine ant supercolonies from 13 different localities covering the species’ native and introduced ranges. We performed selection analyses to evaluate selection efficacy and positive selection on each immune and control gene. We predicted that the immune gene evolution of introduced supercolonies differed from that of the native supercolonies and proposed two different, non-mutually exclusive hypotheses for this: (a) enemy release hypothesis: introduced supercolonies have left behind their natural pathogens, which is seen as less-intensive positive selection in their immune genes compared to the native supercolonies; and (b) higher pathogen pressure hypothesis: introduced supercolonies have faced new pathogens, which is seen as a stronger positive selection in their immune genes compared to native supercolonies. In the first place, the results of this study will unveil how introductions into new ranges impact immune gene evolution in newly established populations, which might be necessary for engineering invasion biology and management strategies against the Argentine ants, one of the world’s most harmful pest species, as well as against other invasive species. In addition, these results will add knowledge on the evolution of social insects’ immune systems, which interplays with social immunity.

Materials and methods

Sampling

Argentine ants were collected from 18 supercolonies in 13 localities (Supplementary Table S1). Ten diploid workers, one per nest from 10 nests, were collected from each supercolony. Half of the collected samples originated from the native Argentinian supercolonies (Corrientes S1, Corrientes S2, Otamendi S2, Otamendi S4, Otamendi S6, Otamendi S9, Boca S1, Boca S2, and Santa Coloma) in four localities (Wild, 2004). The other half originated from supercolonies (La Rioja, Tucumán, Catalonia, New Zealand, South Africa, Japan S1, European Main, Australia, and Chile) on nine localities of the species’ introduced range from different parts of the world, spanning the worldwide distribution of this invasive ant (Vogel et al., 2010; Wetterer et al., 2009). In addition, 10 diploid workers, one per nest from 10 nests, were collected from a population of the Argentine ant’s sister species, Linepithema obloungum, to serve as an outgroup in the analyses (Supplementary Table S1, Wild, 2007, 2009). The nest samples were a subset of those used in Vogel et al. (2010), but worker specimens were different. All studied introduced supercolonies have originated from separate primary introductions from different sources of the native range,
except that the European Main and Australian populations presumably have a common origin in the native range, and New Zealand is a secondary introduction from Australia (Brandt et al., 2009; Vogel et al., 2010). The Argentine ants spread into the Mediterranean and South Africa at the end of the 19th century. The first records from Australia originated in the 1930s, while the spread into other Oceanic regions (New Zealand) and Asia (Japan) was later around the 1990s (Wetterer et al., 2009).

Immune genes
The 91 target genes were chosen based on annotated immune genes in the Argentine ant reference genome (Linepithema humile Genome Assembly version 1.0., Smith et al., 2011). These genes have key roles in insect innate immune defence, both in cellular response as well as in pathways of the humoral response, spanning various functional categories, including effector, modulator, recognition, RNAi, and signalling genes. In addition, control genes with no known immune function were chosen from the same scaffolds as the immune genes, with a distance of about 100 kb between the immune and control genes to exclude physical linkage.

DNA extraction and sequencing
Genomic DNA was extracted individually from each ant using DNeasy Blood & Tissue Kit (QIAGEN). Equal amounts of DNA from 10 individuals per supercolony were pooled to represent the final population-specific sample. Primers for the PCR amplification of selected genes were designed based on the Argentine ant reference genome (Linepithema humile Genome Assembly version 1.0., Smith et al., 2011), and target genes were amplified in 5 kb fragments using NimbleGen SeqCap EZ Choice Library Kit (Roche). Genes longer than 5 kb were amplified in overlapping 5 kb fragments. BGI Genomics carried out the library preparation and paired-end sequencing of the amplified fragments using Illumina HiSeq 2000 sequencer, with a read length of 90 bp. The clean reads provided by BGI Genomics were filtered to have an average minimum base quality of 20 and a minimum read length of 70 bp using the trimfastq.pl script from the basic pipeline of PoPoolation (version 1.4). The quality encoding of the obtained sequenced raw reads was converted from the obsolete Phred 64 to Phred 33 using the Seqtk tool (version 1.3) (Seqtk, 2018). After this, the raw reads were filtered to filter only biallelic sites with a mean depth of at least 20, a minor allele count of at least 1 (except for outgroup, this argument was replaced by a minor allele frequency of at least 0 to avoid losing the data), and accepting at the most 25% of data missing (Danecek et al., 2011). Variants were also detected with the ploidy per sample set to 20 (the number of haploid genomes in the pooled sample—a recommendation for pooled data), otherwise keeping the parameters same as for the first run with the ploidy set to two. As VCFtools is unsuitable for filtering polyploid VCF files, BCFtools’ (version 1.16) isec command was used to retain desired data in the 20-ploid VCF files (Danecek et al., 2021). Thus, positions in the 20-ploid VCF files having corresponding records with VCFtools’ filtered diploid VCF files were kept, and these 20-ploid VCF files were used in the subsequent analyses.

SNP call
Variants were detected separately for each population using GATK’s (version 4.3.0.0) HaplotypeCaller, a tool suitable for pooled data (Poplin et al., 2017; van der Auwera & O’Connor, 2020). To enable variant filtering with VCFtools, variants were first detected with the ploidy per sample set to two. In addition, the maximum number of alternate alleles to genotype was set to two, the minimum base quality to consider base for calling was set to 20, and the maximum number of reads was set to 250 on the start position to retain the read for alignment. The resulting VCF files were filtered using VCFtools (version 0.1.16) to keep only biallelic sites with a mean depth of at least 20, a minor allele count of at least 1 (except for outgroup, this argument was replaced by a minor allele frequency of at least 0 to avoid losing the data), and accepting at the most 25% of data missing (Danecek et al., 2011). Variants were also detected with the ploidy per sample set to 20 (the number of haploid genomes in the pooled sample—a recommendation for pooled data), otherwise keeping the parameters same as for the first run with the ploidy set to two. As VCFtools is unsuitable for filtering polyploid VCF files, BCFtools’ (version 1.16) isec command was used to retain desired data in the 20-ploid VCF files (Danecek et al., 2021). Thus, positions in the 20-ploid VCF files having corresponding records with VCFtools’ filtered diploid VCF files were kept, and these 20-ploid VCF files were used in the subsequent analyses.

Selection analyses
Genes evolving under natural selection were identified separately for each population using the full Bayesian implementation of SnIPRE (Selection Inference using Poisson Random Effects), a McDonald–Kreitman-type analysis, with the settings provided in the authors’ example script (Eilertson et al., 2012). The input “MK-tables” containing non-synonymous and synonymous polymorphic and divergent sites and the total non-synonymous and synonymous sites surveyed for each gene were created using the mktest.R script (available at: https://github.com/ajshultz/comp-pop-gen/tree/master/mk_tests/mk_sjsw). For this, the non-synonymous and synonymous SNPs were extracted from the annotated 20-ploid VCF files using the parser_nov.py script, and per-site missingness information was parsed from diploid VCF files (due to the unsuitability of polyploid VCF files for VCFtools) using the VCFtools’ missing-site command.

The gene-specific selection effect values provided by SnIPRE estimate the selection strength acting on each gene relative to neutrality. The effects of different factors and their interaction on selection effect values were examined with a two-way analysis of variances (ANOVA) using aov() function in R (version 4.2.2) (R Core Team, 2022). In the first model,
the first factor, population range, had two levels, native versus introduced, and the second factor, gene group, had two levels, control versus immune (ANOVA 1: selection effect ~ population range (native vs introduced) * gene group (control vs immune)). In the second model, the first factor, population range, was the same as in the first model. The second factor, the gene category, had six levels, control, effector, modulator, recognition, RNAi, and signalling, based on the gene’s putative function in the immune pathway (ANOVA 2: selection effect ~ population range (native vs introduced) * gene category (control vs effector vs modulator vs recognition vs RNAi vs signalling)). The pairwise differences of each immune gene category versus controls were further investigated with Tukey’s honest significant difference method using the TukeyHSD() function in R. We further examined how the age of the introduced supercolonies affects the selection effect strength with the covariance analysis using lm() function in R. The model included introduced supercolonies; the first factor was the age of the introduced supercolony, and the second factor, gene group, was the same as in ANOVA 1 (lm 1: selection effect ~ age + group (control vs immune)).

Moreover, we examined the proportion of genes classified as positively selected, i.e., genes with credible intervals (CIs) of selection effect estimates above zero. The relationship between the gene class (classified as positive or not positive, i.e., neutral or negative, based on selection effect values) with population ranges and gene groups or categories and their interaction was examined with logistic regression using glm() function in R. In the first model, the factors were the same as used in the first ANOVA model (glm 1: gene class ~ population range (native vs introduced) * gene group (control vs immune)). In the second model, the factors were the same as used in the second ANOVA model (glm 2: gene class ~ population range (native vs introduced) * gene category (control vs effector vs modulator vs recognition vs RNAi vs signalling)).

Results

Properties of the data

The results were based on 174 (89 immune and 85 control genes) genes from 18 Argentine ant supercolonies (Supplementary Table S2). Eight genes (2 immune and 6 control genes) were dropped from the original data set due to low-quality or missing data (Supplementary Table S3). Most of the included genes were present in the data of all populations, but there might be a slight difference due to population-specific variant call and filtering steps. Each gene’s length, nucleotide content, and average number and standard deviation of SNPs are presented in Supplementary Table S2.

Immune genes have been shaped by positive selection in both native and introduced supercolonies

Selection effects were, on average, higher among natives (mean = 0.326, SD = 0.230) compared to introduced (mean = 0.238, SD = 0.226) supercolonies (Figure 1, ANOVA 1: \( F = 110, p\text{-value} < .001 \)). In addition, immune genes (mean = 0.299, SD = 0.228) had, on average, higher selection effects compared to controls (mean = 0.264, SD = 0.236) (ANOVA 1: \( F = 17.3, p\text{-value} < .001 \)). However, the interaction term did not explain selection effects (ANOVA 1: \( F = 0.5, p\text{-value} = .48 \)). As seen in Supplementary Figure S5, immune genes also seem to have, on average, higher selection effects than controls while considering each population separately. Moreover, the age of the introduced supercolonies seems to affect the selection effect values; the older the supercolony, the stronger the selection effects tend to be (lm 1: \( \beta =0.001, T = 4.28, p\text{-value} < .001 \); see Supplementary Appendix S6).

The odds of genes being positively selected did not differ between ranges (13.25% and 12.43% of genes were positively selected among natives and introduced supercolonies, respectively; glm 1: OR = 0.747, 95% CI [0.538, 1.037], p-value = .082) nor gene groups (11.23% and 14.38% of genes were positively selected among controls and immune genes, respectively; glm 1: OR = 1.103, 95% CI [0.818, 1.489], p-value = .520), and further interaction could not be detected (glm 1: OR = 1.471, 95% CI [0.952, 2.273], p-value = .082). However, when the interaction was left out from the model (glm 1 without interaction), immune genes were more likely to be positively selected than controls (glm 1 without interaction: OR = 1.328, 95% CI [1.069, 1.649], p-value = .010; see Supplementary Appendix S7).

RNAi and signalling genes have experienced the strongest positive selection

When immune genes were split into functional categories, natives (mean = 0.326, SD = 0.230) had higher selection effects compared to introduced (mean = 0.238, SD = 0.226) supercolonies (ANOVA 2: \( F = 112.22, p\text{-value} < .001 \); see Supplementary Appendix S4). Moreover, selection effects varied between gene categories (RNAi: mean = 0.370, SD = 0.231; signalling: mean = 0.318, SD = 0.232; recognition: mean = 0.287, SD = 0.198; modulator: mean = 0.269, SD = 0.197; control: mean = 0.264, SD = 0.236; effector: mean = 0.187, SD = 0.250) (Figure 2, ANOVA 2: \( F = 17.24, p\text{-value} < .001 \)). Again, the interaction term did not explain selection effects (ANOVA 2: \( F = 0.15, p\text{-value} = .98 \)). In pairwise comparisons between immune gene categories and controls, the selection effects of RNAi, signalling and effector gene categories differed from controls (Tukey’s HSD results—see Supplementary Appendix S4). RNAi genes had, on average, the highest selection effects (Tukey’s test: \( \text{diff} = 0.106, 95\% \text{ CI}[0.059, 0.153], p\text{-value} < .001 \), but also
signalling genes had, on average, higher selection effects than controls (Tukey’s test: diff = 0.053, 95% CI [0.023, 0.084], p-value < .001). Effectors had, on average, the lowest selection effect, even lower than controls (Tukey’s test: diff = -0.077, 95% CI [-0.131, -0.022], p-value < .001). The order of gene categories based on the magnitude of the selection effect was almost identical across ranges (Figure 2).

The odds of genes being positively selected again did not differ between ranges (see the percentages above) (glm 2: OR = 0.747, 95% CI [0.538, 1.037], p-value = .082). RNAi genes were more likely positively selected than controls (glm 2: OR = 1.969, 95% CI [1.190, 3.258], p-value = .008). The odds of being positively selected for the rest of the gene categories or the interactions did not differ from controls (results—see Supplementary Appendix S7). If the interaction was left out from the model (glm 2 without interaction), also signalling genes were more likely positively selected than controls (glm 2 without interaction: OR = 1.560, 95% CI [1.197, 2.034], p-value = .001), whereas effectors were less likely to be positively selected than controls (glm 2 without interaction: OR = 0.495, 95% CI [0.247, 0.989], p-value = .046).

RNAi and signalling genes had the highest selection effects and were most often positively selected (Figures 2 and 3, Supplementary Table S8). Moreover, RNAi, signalling, and recognition categories seemed to have a higher proportion of positively selected genes among introduced supercolonies than natives (Figure 3). Instead, modulator and effector categories follow the same pattern detected in controls, i.e., natives have a higher proportion of positively selected genes than introduced supercolonies. Overall, there was considerable variation in the selection effects and proportions of positively selected genes between supercolonies, especially among the native range (Supplementary Figures S5 and S9). Native Otamendi S4 was extreme, with zero positively selected genes. Despite discrepancies, some positively selected immune genes, especially RNAi and signalling genes, were shared across supercolonies (see Discussion, Supplementary Figure S10).

Discussion

The aim of this study was to investigate how the shifts into new ranges have affected the recent evolution of Argentine ants’ immune genes. For this purpose, we analysed comprehensive immune and non-immune gene sets in 18 Argentine ant supercolonies covering the species’ native and introduced ranges. We tested two different, non-mutually exclusive hypotheses to explain the immune gene evolution of introduced supercolonies: (a) the enemy release hypothesis and (b) the higher pathogen pressure hypothesis. The enemy release hypothesis with less-intensive positive selection in introduced range supercolonies fit our observations: while immune genes had higher selection effects and were more often positively selected than control genes in supercolonies of both ranges, the introduced supercolonies had, on average, lower selection effects and thus experienced weaker selection than the natives as expected in the hypothesis after release from natural pathogens. However, as the weaker selection was observed in both immune and control genes of the introduced supercolonies, it likely reflects demographic effects (genetic bottleneck and subsequent small effective population size). Moreover, the higher pathogen pressure hypothesis might explain some of the detected evolutionary patterns as well: we were able to detect positive selection in the evolutionary young introduced range supercolonies (the age of the oldest introduced population is about 100 generations) and notably, some of the gene categories harboured proportionally more positive selection in the introduced range supercolonies compared to natives. The observation of increasing selection effect with the age of the introduced supercolonies could indicate that the effect of enemy release is short-term, and as local pathogens evolve to their new hosts, they create pathogen pressure that is seen as amplifying positive selection in the host immune genes over time.

RNAi gene category harboured the highest proportion of positively selected genes; intriguingly, this proportion was higher among introduced supercolonies than natives. RNA interference (RNAi) is a major defence mechanism against viruses and transposable elements (TEs) in insects (Bronkhorst & van Rij, 2014; Obbard, Gordon, et al., 2009). Argentine ants contain many viruses and TEs (Gruber et al., 2017; Viljakainen et al., 2018, 2023), and a majority of the viruses cause active infections triggering the host RNAi response (Viljakainen et al., 2023). Thus, it is not surprising that the RNAi genes have been the most common targets of positive selection. Key RNAi pathway genes similar to r2d2, AGO2, armi, and Loqs2-like1 were positively selected in at least half of the studied supercolonies covering both native and introduced ranges. In addition, aub, Dcr2, and Dcr1 were positively selected in a couple of supercolonies. In Drosophila Dcr2, AGO2, and r2d2 encode the key components of the antiviral small interfering RNAi (siRNA) pathway (Bronkhorst & van Rij, 2014; van Rij et al., 2006).

The rapid evolution and positive selection in RNAi genes seem to be a widespread phenomenon across insects (Bronkhorst & van Rij, 2014; Obbard, Gordon, et al., 2009; Obbard, Welch, et al., 2009; Palmer et al., 2018). Consistent with our results, the siRNA genes (AGO2, Dcr2, and r2d2) and piRNA genes (aub and armi) were positively selected among many other insects, including Drosophila, Anopheles, Apis, Bombus, Megachile, Bombyx, and Heliconius. In addition to these shared targets, lineage- or species-specific positive selection has been detected among RNAi genes (Barribeau, 2009; Obbard, Welch, et al., 2009; Palmer et al., 2018).
et al., 2015; Kolaczkowski et al., 2011; Obbard et al., 2006, 2011; Obbard, Welch, et al., 2009; Palmer et al., 2018).

Many viruses contain genes that actively suppress RNAi (Nayak et al., 2010; van Mierlo et al., 2014), and it has been suggested that these viral suppressors of RNAi (VSRs) are the main drivers of the antagonistic co-evolutionary arms races between viruses and host RNAi pathway genes leading to high evolutionary rates (Kolaczkowski et al., 2011; Obbard et al., 2006; Obbard, Gordon, et al., 2009; Obbard, Welch, et al., 2009). Viljakainen et al. (2023) showed that Argentine ants contain many infection-causing viruses and discussed that some might produce VSRs whilst direct evidence is still absent. Our results support this hypothesis, and future studies focusing on virus gene evolution may shed more light on this issue.

All six Toll genes (Toll1a, Toll1b, Toll1c, Toll1-like1, Toll1-like2, and 18w [or Toll2]) included in this study were positively selected in 6–16 supercolonies from both ranges. This is in contrast to other insects: neither the Toll receptor nor other Toll pathway genes in the honeybee Apis mellifera showed any evidence of positive selection (Harpur & Zayed, 2013). This was the central observation in studies of Drosophila as well (Sackton et al., 2007), despite some limited evidence of positive selection in Toll1 (Han et al., 2013; Schlenke & Begun, 2003), and the fact that there is plenty of evidence for positive selection acting on Toll-like receptors in mammals and birds (Areal et al., 2011; Grueber et al., 2014; Huang et al., 2011).

The prevalent positive selection in the Argentine ant Toll genes could be due to recent duplications since at least Toll1 seems to have been duplicated several times, and all these paralogs have evolved under positive selection. Similarly, lineage- or species-specific duplications followed by neo- or sub-functionalization events have been suggested to underlie the rapid evolution of lineage-specific copies of Toll-3/4 in Drosophila willistoni (Levin & Malik, 2017; Lima et al., 2021). Drosophila Toll receptors are transmembrane proteins that activate the downstream signalling pathway leading to the transcription of target genes, mainly antimicrobial peptides (Valanne et al., 2011, 2022). In addition to immunity, the Toll pathway functions in developmental processes. Levin and Malik (2017) hypothesize that lineage-specific duplicates of Tolls across insects have been released from their developmental roles, relaxing their selective constraint similarly to the Toll 3/4 receptors in Drosophila, allowing rapid evolution in response to pathogens. Furthermore, the species-specific TollI1 paralogs have offered host protection against the Plasmodium parasite in Anopheles gambiae mosquitoes (Redmond et al., 2015).

The effector gene category had the least positively selected genes, even fewer than in the control genes. Moreover, in contrast to RNAi and signalling genes, effectors followed...
the pattern seen in controls: having more positively selected genes among natives than in introduced ranges. Each immune pathway produces effectors that act against pathogens (Lin et al., 2020). The best-known effectors are antimicrobial peptides (AMPs) produced by Toll and Imd pathways (Hanson & Lemaître, 2020; Myllymäki et al., 2014; Valanne et al., 2011, 2022). AMPs have responses against bacteria and fungi, but the antiviral role is also evident. Phagocytosis and autophagy are other host defence mechanisms against viruses, and autophagy-associated genes (Atgs) are key components in the antiviral response (Nakamoto et al., 2012; Rosendo Machado et al., 2021). Atg7 was positively selected in five Argentine ant supercolonies covering native and introduced ranges, while other effectors (Atg5, hymenoptacin, Lys-3, and PPO) were each positively selected only in one supercolony. The AMP defensin has evolved under positive selection in ants and honeybees (Harpur & Zayed, 2013; Viljakainen & Pamilo, 2008; Viljakainen et al., 2009). In addition, termicin was positively selected among termites (Bulmer & Crozier, 2004; Bulmer et al., 2010). Intriguingly, defensin or other AMPs have not been detected to evolve under positive selection in Drosophila or other dipterans, while balancing selection is evident (Jiggins & Kim, 2005, 2007; Obbard, Welch, et al., 2009; Parmakelis et al., 2008; Sackton et al., 2007; Simard et al., 2007). However, few Atgs were positively selected among D. melanogaster but not in Drosophila simulans (Im & Lazzaro, 2018). It seems that lineage- or species-specific evolutionary patterns are typical for effectors, at least when considering positive selection. In future studies, the role of balancing selection shaping the evolution of effectors in Argentine ants could be interesting as variability might be crucial for molecules that directly respond to pathogens.

Conclusion
In conclusion, we showed that the release from natural pathogen of the native range, exposure to new pathogens in the introduced range, demographic effects, and reduced genetic variation all have likely affected the recent evolution of Argentine ant immune genes in the introduced range leading to reduced intensity of positive selection. However, it is noteworthy that pathogen pressure in the introduced range seems to be extremely strong as positive selection, even though less intensive, was also evident among the evolutionary young bottlenecked introduced supercolonies and more often in immune genes than in control genes. RNAi genes had the highest proportion of positively selected genes in both ranges and intriguingly, this proportion was higher among introduced supercolonies compared to natives. This highlights viruses’ prominent role in shaping their host populations’ evolution. Moreover, RNAi against the Argentine immune genes Spazelle and Dicer1 has already been tested as a pest control method (Felden et al., 2023), and our results may help design new gene targets for similar methods in the future.

Supplementary material
Supplementary material is available at Journal of Evolutionary Biology online.

Data availability
Raw sequence data are submitted to NCBI under BioProject PRJNA961754.

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Conflicts of interest
There are no conflicts of interest.

Ethical statement
There is no need for ethical approval while studying insect species.

References


