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Positive selection on sociobiological traits in invasive fire ants

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Abstract

The fire ant *Solenopsis invicta* and its close relatives are highly invasive. Enhanced social cooperation may facilitate invasiveness in these and other invasive ant species. We investigated whether invasiveness in *Solenopsis* fire ants was accompanied by positive selection on sociobiological traits by applying a phylogenomics approach to infer ancient selection, and a population genomics approach to infer recent and ongoing selection in both native and introduced *S. invicta* populations. A combination of whole-genome sequencing of 40 haploid males and reduced-representation genomic sequencing of 112 diploid workers identified 1,758,116 and 169,682 polymorphic markers, respectively. The resulting high-resolution maps of genomic polymorphism provide high inference power to test for positive selection. Our analyses provide evidence of positive selection on putative ion channel genes, which are implicated in neurological functions, and on vitellogenin, which is a key regulator of development and caste determination. Furthermore, molecular functions implicated in pheromonal signalling have experienced recent positive selection. Genes with signatures of positive selection were significantly more often those overexpressed in workers compared with queens and males, suggesting that worker traits are under stronger selection than queen and male traits. These results provide insights into selection pressures and ongoing adaptation in an invasive social insect and support the hypothesis that sociobiological traits are under more positive selection than nonsocial traits in such invasive species.

KEYWORDS
adaptation, behavior/social evolution, genomics/proteomics, insects, invasive species, molecular evolution

1 | INTRODUCTION

Many invasive species possess a capacity for rapid population growth and high dispersal, as well as a tendency and capability for quickly spreading into new areas within their native ranges (Lee, 2002; Sakai et al., 2001). For example, so-called tramp bird species quickly repopulate islands after volcanic explosions (Diamond, 1974) and tramp ant species repopulate suitable areas following floods or human inflicted disturbance (Tschinkel, 2006). While the capacity to invade new areas exists in the native range of many species, populations may undergo additional adaptations following their introduction into new areas. Our current understanding of the main factors contributing to local adaptation and the evolution of invasiveness in many species remains poorly understood (Bertelsmeier & Keller, 2018).

Ants are notorious invaders; the list of "100 of the World's Worst Invasive Alien Species" published by the IUCN Invasive Species Specialist Group contains five ant species, among a highly diverse collection of invasive animals, plants and fungi (Lowe, Browne, Boudjelas, & De Poorter, 2000). While the ancestral social

structure of ants is thought to be monogyny (i.e., a colony contains a single reproductive queen) (Hughes, Oldroyd, Beekman, & Ratnieks, 2008), the most highly invasive ant species typically form polygyne colonies (i.e., a colony contains multiple reproductive queens). Additional traits associated with ant invasiveness include polydomy (multiple nests per colony), colony budding, preference for disturbed habitats, habitat/nesting generalism, and reduced intraspecific aggression and competition among colonies (Bertelsmeier, Ollier, Liebhold, & Keller, 2017; Holway, Lach, Suarez, Tsutsui, & Case, 2002; Passera, 1994; Rabitsch, 2011). Extreme examples of the latter occur in several ant species that form large supercolonies consisting of multiple nests over large areas that function as a single large interbreeding unit (Giraud, Pedersen, & Keller, 2002; Tsutsui, Suarez, Holway, & Case, 2000; Vogel, Pedersen, Giraud, Krieger, & Keller, 2010). A key feature of supercolony structure is reduced or lack of territoriality among colonies, a feature that likely is advantageous because it can lead to increased nest densities and rapid population growth. A population genetic study of Argentine ants (*Linepithema humile*), which form supercolonies in many introduced ranges, demonstrated that supercolonies exist within the native range as well, but are considerably smaller, most likely as a result of both competition with neighbouring conspecific supercolonies and with predators and competitors (Vogel et al., 2010).

Another highly successful ant invader is the fire ant *Solenopsis invicta*. This ant species was inadvertently introduced to Alabama in the 1930s from its native South American range (Buren, Allen, Whitcomb, Lennartz, & Williams, 1974; Caldera, Ross, DeHeer, & Shoemaker, 2008; Ross & Shoemaker, 2008). Subsequently, it has spread throughout most of Southeastern USA, and was introduced into California, several Caribbean islands, the Virgin Islands, Australia, New Zealand, China, Taiwan and Japan (Ascunce et al., 2011; Davis, Vander Meer, & Porter, 2001; Moloney & Vanderwoude, 2002; Morrison, Porter, Daniels, & Korzukhin, 2004; Pascoe, 2001; Yang et al., 2009; Zhang, Li, Liu, & Porter, 2007). Population genetic analyses inferred the source of the original US introduction to be a region at or near Clorinda, Herradura and Formosa in Northern Argentina, whereas all subsequent invasions apparently stem from the United States (Ascunce et al., 2011).

Solenopsis invicta and its close relatives exhibit a social polymorphism whereby some colonies are monogyne and others are polygyne (Weeks, Wilson, Vinson, & James, 2004). This social polymorphism is found in both the native range and introduced areas (Mescher, Ross, Shoemaker, Keller, & Krieger, 2003). Multiple sociobiological traits aside from queen number differ between the two social forms, including mode of reproduction and dispersal and levels of internest aggression, which give rise to different colony and population structures within the two social forms (Gotzek & Ross, 2007; Ross & Keller, 1995). Remarkably, the polygyne/monogyne social polymorphism is expressed as a simple Mendelian trait determined by a 13-Mbp-long, nonrecombining region on the so-called "social chromosome" (Wang et al., 2013). Two distinct haplotype groups, *SB* and *Sb*, exist: monogyne queens always have an *SB*/*SB* genotype, whereas polygyne queens are always *SB*/*Sb* or *Sb*/*Sb* (*Sb* alleles in introduced areas and some native areas act as recessive lethal in queens). *SB* and *Sb* haplotypes diverged from each other hundreds of thousands of years ago (Wang et al., 2013) and are present in native *S. invicta* populations (Z. Yan, D. Gotzek, D. Shoemaker, K. Ross and L. Keller, unpublished). This nonrecombining "supergene" contains more than 500 protein-coding genes, one or more of which being responsible for determining colony social form. Recent studies suggest genes within this region, along with other genes across the rest of the genome, are involved in the regulation of social behaviour and other traits related to this social polymorphism. For example, a gene expression study found 39 differentially expressed genes between *SB*/*SB* and *SB*/*Sb* workers (Wang, Ross, & Keller, 2008). Eight of the 27 genes that could be mapped to linkage groups were outside of the supergene and on other chromosomes (Wang et al., 2013). Genes belonging to functional categories involved in chemical signalling and olfactory response were overrepresented among these differentially expressed genes (five out of 39).

If variation in social behaviour is an important factor in the ecological success of this and other invasive ant species, then one would predict that local adaptation of populations may involve positive selection pressure on the genes underlying these traits. One might also predict stronger selection pressure on genes expressed in the worker caste relative to other castes because worker behaviour largely determines the fate of queens and social colony organization. In this study, we attempted to test these predictions using the invasive fire ant *S. invicta* as a study system. More specifically, we tested the hypothesis that signatures of positive selection in invasive (USA) *S. invicta* are biased towards genes underlying sociobiological traits, including developmental mechanisms underlying caste determination, and neurological and chemical communication mechanisms possibly linked to social behaviour. In addition, we also examined patterns of positive selection in native populations. One might predict fire ants are locally adapted to different geographic areas across their large native range, partly as a result of multiple, ongoing invasions into new areas after disturbances (e.g., floods). In conclusion, we considered the possibility of selection acting much earlier in the fire ant lineage, specifically, near or after the transition from the ancestral nonaggressive, noninvasive thief ants to the aggressive, invasive fire ant clade in the genus *Solenopsis*. Our results largely support our main prediction. The signature of positive selection on multiple timescales was found in genes implicated in neurological functions, pheromonal signalling and caste determination; several of these molecular pathways and functions are implicated in sociobiological functions, especially in worker-specific traits.

2 | MATERIALS AND METHODS

We conducted a population genomic survey of *S. invicta* using a combination of whole-genome sequencing and restriction site-associated DNA (RAD) sequencing, a reduced-representation sequencing method (Baird et al., 2008). RAD sequencing was applied to diploid

female samples from three locations in the native range in Northern Argentina and one location in the introduced range in Southeastern USA. Whole genomes were sequenced for haploid male samples from the native populations of Herradura and Alejandra in Argentina. Each sample was taken from a different nest. Table 1 details the locations, number of samples and the type of genomic sequencing.

2.1 | Whole-genome sequencing

Total DNA was extracted from haploid male samples using the Gentra Puregene tissue kit (Qiagen), removing RNA with RNase A. Genomic libraries were constructed using the Illumina TruSeq kit, and ten samples were multiplexed per lane of 100-bp paired-end sequencing on an Illumina HiSeq 2000 sequencer (average fragment size of 495 bp). The whole-genome sequencing produced an average coverage of 11.9× per sample (range 5.0× to 25.4×; standard deviation 5.9). Identical pairs of reads were removed. Reads were filtered based on the Illumina Chastity filter and based on the Phred quality scores using DYNAMICTRIM version 1.12 (Cox, Peterson, & Biggs, 2010). Read pairs were aligned to the reference genome of *S. invicta* (version Si_gnH; NCBI Accession no. AEAQ00000000) using BOWTIE2 (Langmead & Salzberg, 2012) (version 2.0.2; "bowtie2 -q -p 10 –end-to-end –very-sensitive –fr"). SNPs were identified and genotyped using *Varscan* (Koboldt et al., 2009). A SNP was called if two or more samples supported each allele. Erroneous SNP calling due to repetitive sequences were filtered based on excessive coverage (more than two standard deviations above the mean) and based on the finding of heterozygous genotypes, which are not expected in haploid samples (assumed repetitive sequences that were collapsed during assembly of the reference genome leading to falsely inferred heterozygosity). A total of 1,758,116 SNPs were identified across the genome after quality filtering (one SNP every 230 bp on average).

In addition, the genome of the closely related species *S. fugax* was sequenced for use as an outgroup. Whole-genome sequencing of a single haploid male was used for a draft genome assembly by SOAPDENOVO (version 1.05, $K = 63$) and GapCloser (Li et al., 2010). *S. invicta* proteins were mapped to the *S. fugax* assembly using GENBLAST (She, Chu, Wang, Pei, & Chen, 2009; She et al., 2011), in which translated BLAST hits to exons are grouped to represent a putative gene models, while stitching hits at predicted splice site junctions. Annotated protein sequences from the *S. invicta* reference genome were used as queries. Only one-to-one orthologous gene pairs were used for the SNIPRE analysis (based on reciprocal best BLAST hits). The coding sequences of orthologous genes were aligned using the program PRANK (Loytynoja & Goldman, 2008), and sites with alignment uncertainty were masked using GUIDANCE, based on *HoT* (Heads-Or-Tails) scores (Landan & Graur, 2008; Penn, Privman, Landan, Graur, & Pupko, 2010).

2.2 | RAD sequencing

DNA was extracted from diploid workers using the Gentra Puregene tissue kit (Qiagen), removing RNA with RNase A. RAD libraries were constructed as previously described (Wang et al., 2013) based on the protocols of Baird et al. (2008) and Etter, Bassham, Hohenlohe, Johnson, and Cresko (2011). In short, approximately 0.2–0.5 μg of DNA per individual was digested with *Pst*I-HF enzyme (New England Biolabs) and ligated to one of 96 barcoded P1 adapters with unique 5-bp barcodes. Ligated samples were pooled and randomly sheared (Fisher Scientific Sonic Dismembrator), and 400- to 600-bp fragments were size selected by gel purification using the MinElute Gel Extraction Kit (Qiagen). Fragments were blunted, and a 3′ dA overhang added before the P2 adaptor was ligated. Purified samples were amplified for 15 to 18 cycles and gel purified (400–600 bp). Between 31 and 68 samples were multiplexed per lane of 100-bp single-end sequencing on an Illumina HiSeq 2000 or 4000 sequencer. Sequence reads were mapped to the Si_gnH reference genome using BOWTIE2 (Langmead & Salzberg, 2012), and SNPs were identified and genotyped using STACKS (Catchen, Amores, Hohenlohe, Cresko, & Postlethwait, 2011). RAD tags had an average coverage of 30× per sample (range 3.9× to 68.0×; standard deviation 13.5). A SNP was called if two or more samples supported each allele. Erroneous SNP calling due to repetitive sequences was filtered based on excessive coverage and based on the finding of heterozygous genotypes in the same loci of the haploid male samples (as above). 71,118 RAD tags with 169,682 SNPs remained after filtering, which is one pair of adjacent RAD tags (one restriction site) every 11 kbp on average.

2.3 | Structure

STRUCTURE version 2.3.4 (Pritchard, Stephens, & Donnelly, 2000) was used to infer population structure from a random sample of 1,000

TABLE 1 Samples used for genomic sequencing

	Location	Year	Samples	Sequencing
S. invicta native range	Herradura, Formosa, Argentina (S26°29.826′, W58°18.950′)	2006	31 diploid workers	RAD sequencing
			20 haploid drones	Whole-genome sequencing
	Alejandra, Santa Fe, Argentina (S29°49.543′, W59°48.602′)	2006	38 diploid workers	RAD sequencing
			20 haploid drones	Whole-genome sequencing
	El Recreo, Santa Fe, Argentina (S31°30.303′, W60°44.455′)	2006	30 diploid workers	RAD sequencing
S. invicta introduced range	Pascagoula, Mississippi (N30°24′, W88°31′)	2009	46 diploid workers	RAD sequencing

TABLE 2 Genes inferred to have experienced positive selection in the *S. invicta* lineage according to the SNIPRE test

Gene ID	Description	Selection coefficient	Selection effect (95% credible region)
sinvm1_gene_03898	Voltage-dependent calcium channel type A subunit alpha-1	2.17	1.14 (0.56–1.79)
sinvm1_gene_07074	Serine protease	1.92	1.05 (0.37–1.76)
sinvm1_gene_08039	Probable multidrug resistance-associated protein lethal 03659-like	1.84	1.02 (0.39–1.65)
sinvm1_gene_08001	CD109 antigen	1.70	0.95 (0.32–1.68)
sinvm1_gene_07879	Carnitine O-palmitoyltransferase mitochondrial	1.69	0.97 (0.23–1.69)
sinvm1_gene_14938	Transcription factor SOX-14	1.63	0.93 (0.23–1.65)
sinvm1_gene_03085	General transcription factor 3C polypeptide 1	1.61	0.92 (0.27–1.59)
sinvm1_gene_06490	Transient receptor potential cation channel protein painless	1.59	0.91 (0.31–1.51)
sinvm1_gene_13070	Structural maintenance of chromosomes protein 6	1.57	0.91 (0.20–1.57)
sinvm1_gene_14190	Cuticular protein analogous to peritrophins 1-J precursor	1.56	0.90 (0.28–1.52)
sinvm1_gene_05982	E3 ubiquitin ligase	1.56	0.90 (0.22–1.57)
sinvm1_gene_04154	Glutamate receptor delta-2 subunit-like (**S***)	1.55	0.90 (0.33–1.55)
sinvm1_gene_01313	Set and mynd domain-containing protein 3-like	1.55	0.90 (0.15–1.65)
sinvm1_gene_08966	Cartilage oligomeric matrix protein	1.51	0.87 (0.30–1.47)
sinvm1_gene_10500	Zinc finger protein 91	1.44	0.86 (0.14–1.59)
sinvm1_gene_02665	CEPU-1	1.44	0.85 (0.13–1.57)
sinvm1_gene_01072	Mite allergen Der f 3-like	1.42	0.83 (0.24–1.47)
sinvm1_gene_11833	Glutamate receptor delta-2 subunit-like	1.40	0.84 (0.14–1.52)
sinvm1_gene_12124	N/A	1.40	0.82 (0.14–1.51)
sinvm1_gene_08333	Inorganic phosphate cotransporter-like	1.40	0.83 (0.10–1.52)
sinvm1_gene_01756	ATP-binding cassette subfamily A member 13	1.40	0.83 (0.39–1.29)
sinvm1_gene_03661	A disintegrin and metalloproteinase with thrombospondin motifs 3-like	1.39	0.82 (0.14–1.55)
sinvm1_gene_07845	SEA domain[a]	1.38	0.82 (0.16–1.49)
sinvm1_gene_00273	Gephyrin	1.37	0.83 (0.09–1.61)
sinvm1_gene_03482	ATP-dependent RNA helicase TDRD9	1.36	0.81 (0.12–1.46)
sinvm1_gene_06962	Disabled	1.35	0.80 (0.16–1.53)
sinvm1_gene_07838	Cytochrome P450	1.34	0.81 (0.13–1.54)
sinvm1_gene_06276	TWiK family of potassium channels protein 18 (**S***)	1.34	0.78 (0.28–1.34)
sinvm1_gene_04001	Voltage-dependent calcium channel type D subunit alpha-1	1.33	0.80 (0.12–1.49)
sinvm1_gene_01856	N/A	1.32	0.80 (0.04–1.59)
sinvm1_gene_14762	Epidermal growth factor-related protein 1	1.31	0.79 (0.20–1.46)
sinvm1_gene_06848	LTV1	1.31	0.79 (0.17–1.42)
sinvm1_gene_14288	Luciferin 4-monooxygenase/long-chain-fatty-acid–CoA ligase/Acyl-CoA synthetase	1.29	0.79 (0.17–1.39)
sinvm1_gene_12027	N/A	1.29	0.78 (0.08–1.47)
sinvm1_gene_09456	Alpha-tocopherol transfer	1.28	0.78 (0.01–1.59)
sinvm1_gene_14536	Uncoordinated protein 80 (unc-80)	1.26	0.77 (0.19–1.32)
sinvm1_gene_13967	tRNA (uracil-5-)-methyltransferase homolog A-like	1.26	0.77 (0.07–1.49)
sinvm1_gene_13791	Protein hunchback	1.26	0.76 (0.10–1.43)
sinvm1_gene_06753	Major facilitator superfamily domain-containing protein 6-like	1.25	0.76 (0.12–1.50)
sinvm1_gene_11128	Ryanodine receptor 44F-like	1.25	0.76 (0.22–1.37)
sinvm1_gene_15123	Uncoordinated protein 45 (unc-45)	1.25	0.76 (0.08–1.50)
sinvm1_gene_14732	N/A	1.25	0.76 (0.05–1.50)
sinvm1_gene_03932	PDZ domain; C2 domain[a]	1.25	0.76 (0.14–1.47)
sinvm1_gene_04426	Receptor-type tyrosine-protein phosphatase beta	1.24	0.76 (0.21–1.36)
sinvm1_gene_06277	Sodium channel protein nach (**S***)	1.24	0.76 (0.04–1.48)

(Continues)

TABLE 2 (Continued)

Gene ID	Description	Selection coefficient	Selection effect (95% credible region)
sinvm1_gene_14158	Cubilin	1.21	0.74 (0.30–1.18)
sinvm1_gene_08340	CLIP-associating protein	1.20	0.73 (0.08–1.47)
sinvm1_gene_14244	Cell cycle checkpoint protein RAD17	1.19	0.73 (0.01–1.48)
sinvm1_gene_01130	Apolipophorins	1.19	0.74 (0.23–1.24)
sinvm1_gene_02254	Transient receptor potential channel pyrexia	1.19	0.73 (0.14–1.40)
sinvm1_gene_05486	Long-chain fatty acid transport protein 1/4	1.18	0.74 (0.03–1.49)
sinvm1_gene_03161	Carcinine transporter/organic cation transporter	1.18	0.72 (0.03–1.42)
sinvm1_gene_09314	Zinc finger protein Xfin-like	1.17	0.72 (0.10–1.37)
sinvm1_gene_11456	Sickie	1.17	0.72 (0.05–1.43)
sinvm1_gene_08436	Chloride channel protein 2	1.15	0.70 (0.00–1.44)
sinvm1_gene_01151	Uncoordinated protein 79 (unc-97)	1.12	0.69 (0.07–1.38)
sinvm1_gene_07357	Arf-GAP with Rho-GAP domain, ANK repeat and PH domain-containing protein 2	1.10	0.68 (0.05–1.33)
sinvm1_gene_13074	Carotenoid isomerooxygenase/Beta-carotene dioxygenase and retinoid isomerase	1.07	0.67 (0.02–1.36)
sinvm1_gene_07002	Telomere-associated protein rif1	1.05	0.66 (0.04–1.34)
sinvm1_gene_08598	Sperm antigen with calponin homology and coiled-coil domains 1-like	1.04	0.65 (0.05–1.28)
sinvm1_gene_08965	DNA methyltransferase 3B	1.03	0.65 (0.03–1.27)
sinvm1_gene_06773	N/A	1.02	0.64 (0.02–1.27)
sinvm1_gene_05061	Ribosome biogenesis protein BMS1-like protein	0.99	0.63 (0.01–1.35)
sinvm1_gene_12126	Tyrosine kinase receptor Cad96Ca	0.96	0.60 (0.03–1.17)
sinvm1_gene_14459	N/A	0.95	0.61 (0.11–1.14)
sinvm1_gene_04040	Apolipophorins	0.94	0.61 (0.13–1.14)
sinvm1_gene_14343	N/A	0.90	0.57 (0.05–1.10)
sinvm1_gene_02396	Vitellogenin 1	0.89	0.58 (0.20–0.99)
sinvm1_gene_06494	N/A	0.79	0.52 (0.08–0.95)
sinvm1_gene_06836	Myosin XV	0.76	0.50 (0.05–0.98)

Notes. **S***: Genes in the nonrecombining region of the social chromosome.
[a]Domain annotation based on InterPro scan.

SNPs from the RAD sequencing data. Four chains were run until convergence for each value of *K*. *K* = 3 was chosen because higher values of *K* did not further subdivide the samples into more than three clusters.

2.4 | SNIPRE

We used the SNIPRE algorithm (Eilertson, Booth, & Bustamante, 2012) to infer long-term selection pressures for each protein-coding gene based on the ratio of nonsynonymous to synonymous substitutions that accumulated since the divergence of fire ants from the closely related thief ant species *S. fugax*. SNIPRE (Eilertson et al., 2012) applies Bayesian inference to a generalized linear mixed model of genomewide patterns of substitutions, extending the original McDonald–Kreitman test for single loci (McDonald & Kreitman, 1991). This method assumes no particular population genetic structure and affords better performance in the detection of positive selection than other variants of the McDonald–Kreitman test (Eilertson et al., 2012). The SNIPRE test was conducted using the SNPs identified in the whole-genome sequences of the 40 haploid male samples from Herradura and Alejandra. The single whole-genome sequence of the haploid *S. fugax* male was used to identify divergence between the two species. Divergence and polymorphic sites were counted in protein-coding exons and classified as synonymous or nonsynonymous using custom Perl scripts. The full Bayesian implementation was used for the estimation of model parameters by SNIPRE. Genes were reported as positively selected when the 95% confidence interval for the posterior estimate of the selection effect was above zero. The gamma parameter is reported as the inferred selection coefficient. Enrichment tests for Gene Ontology categories (Harris et al., 2004) were performed by Fisher's exact test corrected for multiple testing (Benjamini & Hochberg, 1995).

To investigate positive selection on caste-specific genes, we intersected the list of genes under positive selection with the list of genes differentially expressed among castes, as defined by Ometto, Shoemaker, Ross, and Keller (2011). That study used a gene expression microarrays representing over 11,000 transcripts (Wang et al.,

2007) to compare gene expression among workers, queens and males. Caste-specific genes were defined as those overexpressed in one particular caste in comparison with both other castes, according to differential expression analysis by BAGEL (Meiklejohn & Townsend, 2005).

2.5 | F_{ST} outliers

F_{ST} was calculated for each SNP using the AMOVA method (Excoffier, Smouse, & Quattro, 1992) for all scaffolds and contigs longer than 100 kbp, based on the RAD sequencing data (as implemented in the STACKS pipeline). Genes were assigned the highest F_{ST} among SNPs within the genomic region of the gene and 10-kbp flanking sequence on either side. To characterize genomic regions with high F_{ST} values, a moving average was also calculated for each SNP position along genomic scaffolds of at least 100 kbp using a kernel-smooth Gaussian function with standard deviation of 150 kbp. This procedure identified genes in genomic regions with high averaged F_{ST}. These regions had no predefined size, rather they were defined by the kernel-smooth function. Enrichment tests for Gene Ontology categories (Harris et al., 2004) by the *GSEA* algorithm (Subramanian et al., 2005) were applied to the gene list ranked by the maximum smoothed F_{ST} score within each gene and corrected for multiple testing (Benjamini & Hochberg, 1995).

3 | RESULTS

3.1 | Adaptive evolution in the *S. invicta* lineage

Selection pressures on protein-coding genes acting over millions of years since the divergence of fire ants from thief ants were inferred using a variant of the McDonald–Kreitman test implemented in the SNIPRE program, based on whole-genome sequencing of 40 native *S. invicta* males, and a single *S. fugax* genome. A statistically significant signature of positive selection was detected in 70 of the 8,663 protein-coding genes analysed (selection effect significantly greater than zero according to the 95% Bayesian credible interval), and the selection coefficients of these 70 genes were estimated to be between 0.76 and 2.17 (Table 2), whereas the full distribution of selection coefficients across all analysed genes ranged between −0.71 and 2.17 (Figure 1a). Three of the 70 positively selected genes were located in the nonrecombining region of the social chromosome, which is not higher than expected by chance (Fisher's exact test p-value = 0.8): a glutamate receptor (sinvm1_gene_04154), a potassium channel protein (sinvm1_gene_06276) and a sodium channel protein (sinvm1_gene_06277).

Gene Ontology (GO) enrichment tests (Tables 3 and 4) revealed that the 70 positively selected genes are enriched for transmembrane transporters in general (Fisher's exact test, false discovery rate q-value = 0.007), more specifically ion channels and gated ion channels (q-value 0.002 and 0.049, respectively). There is also marginally significant enrichment for calcium ion channels (q-value = 0.119). Of the 70 positively selected genes, the genes annotated with the enriched GO categories include six ion channels, a component of an ion channel complex (*unc-80*), and genes involved in the transport of inorganic phosphate, alpha-tocopherol (a form of vitamin E), and long-chain fatty acids.

We also tested for enrichment of positively selected genes in genes displaying caste-specific expression, as defined by Ometto et al. (2011). Genes that were overexpressed in adult workers relative to both queens and males had larger selection coefficients than queen-biased, male-biased and non-caste-biased genes (Figure 1b; q-value = 0.031 in a GSEA test for enrichment of high values of

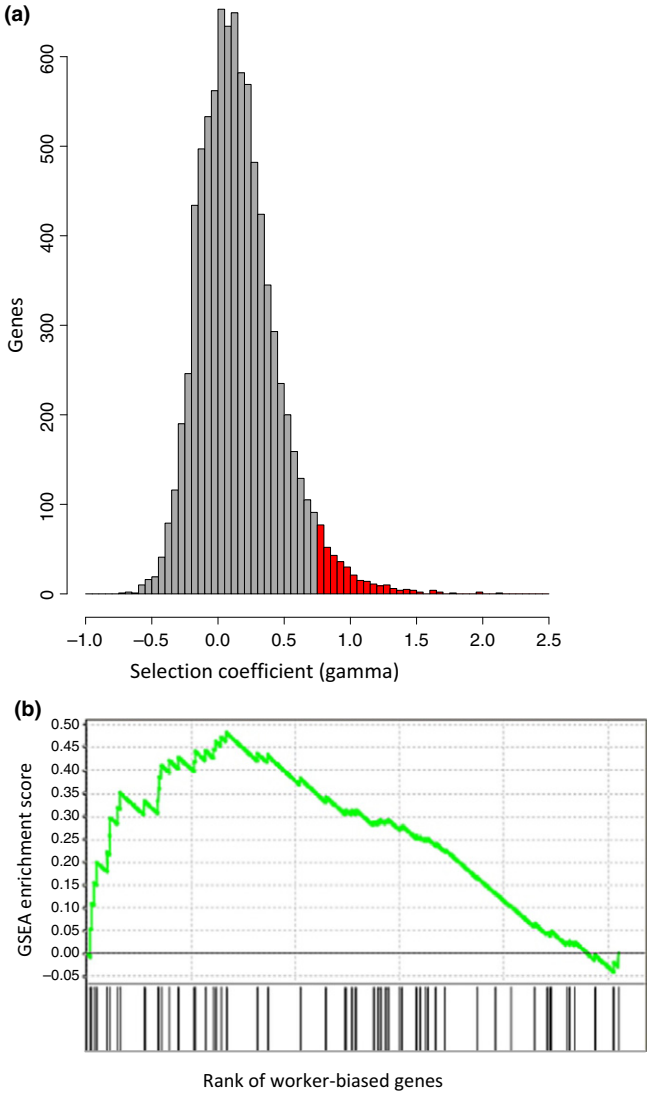

FIGURE 1 (a) Distribution of selection coefficients inferred by SNIPRE (the gamma parameter). The range coloured in red includes the 70 genes that had a statistically significant signature of positive selection. (b) GSEA enrichment plot for the 57 genes with worker-biased expression and their ranking in the full list of 1,276 genes ordered by their selection coefficients. The enrichment score reflects the over-representation of worker-biased genes in the left-hand side of the ranked list (i.e., genes having higher selection coefficient). From left to right, the plot goes up for each worker-biased gene and down for any other gene [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Categories of the Biological Process Gene Ontology database enriched for genes under positive selection in the *S. invicta* lineage according to the SNIPRE test

GO ID	GO term	Annotated	Significant	Expected	*p*-value	*q*-value
GO:0044765	Single-organism transport	561	18	5.93	3.90E−06	0.00712
GO:0006810	Transport	760	21	8.03	4.20E−06	0.00712
GO:0051234	Establishment of localization	769	21	8.13	5.10E−06	0.00712
GO:1902578	Single-organism localization	577	18	6.1	6.00E−06	0.00712
GO:0055085	Transmembrane transport	227	11	2.4	1.40E−05	0.01329
GO:0051179	Localization	829	21	8.76	1.80E−05	0.01424
GO:0098655	Cation transmembrane transport	54	5	0.57	0.00022	0.10446
GO:0098660	Inorganic ion transmembrane transport	54	5	0.57	0.00022	0.10446
GO:0098662	Inorganic cation transmembrane transport	54	5	0.57	0.00022	0.10446
GO:0070588	Calcium ion transmembrane transport	12	3	0.13	0.00022	0.10446
GO:0006811	Ion transport	269	10	2.84	0.00034	0.14676
GO:0034220	Ion transmembrane transport	65	5	0.69	0.00053	0.20970

Note. Annotated: number of genes in the genome annotated by the GO term; significant: number of genes with a significant result from SNIPRE; expected: expected number of significant genes; *p*-value: significance of enrichment according to Fisher's exact test; *q*-value: multiple testing correction for the *p*-value by the Benjamini–Hochberg method.

TABLE 4 Categories of the Molecular Function Gene Ontology database enriched for genes under positive selection in the *S. invicta* lineage according to the SNIPRE test

GO ID	GO term	Annotated	Significant	Expected	*p*-value	*q*-value
GO:0005216	Ion channel activity	114	9	1.33	5.20E−06	0.00219
GO:0022838	Substrate-specific channel activity	114	9	1.33	5.20E−06	0.00219
GO:0015267	Channel activity	115	9	1.34	5.60E−06	0.00219
GO:0022803	Passive transmembrane transporter activity	115	9	1.34	5.60E−06	0.00219
GO:0005215	Transporter activity	376	15	4.39	1.60E−05	0.00501
GO:0022892	Substrate-specific transporter activity	301	12	3.51	0.00014	0.03654
GO:0022836	Gated channel activity	76	6	0.89	0.00022	0.04922
GO:0022857	Transmembrane transporter activity	292	11	3.41	0.00044	0.08613
GO:0005261	Cation channel activity	61	5	0.71	0.00065	0.11310
GO:0005262	Calcium channel activity	16	3	0.19	0.00076	0.11902
GO:0005319	Lipid transporter activity	17	3	0.2	0.00091	0.12955
GO:0005245	Voltage-gated calcium channel activity	5	2	0.06	0.00131	0.16262
GO:0015075	Ion transmembrane transporter activity	234	9	2.73	0.00135	0.16262
GO:0015085	Calcium ion transmembrane transporter activity	23	3	0.27	0.00226	0.25279

Note. See Table 3 above.

SNIPRE's gamma parameter in a set of 57 worker-biased genes out of a total of 1,276 genes that could be included in the analysis).

3.2 | Population structure

Population structure was inferred based on single nucleotide polymorphisms (SNPs) identified by reduced-representation genomic sequencing (see Section 2.2). Clustering by *structure* (Pritchard et al., 2000) revealed two divergent population clusters in the native range: the Herradura samples and a cluster containing the Alejandra and El Recreo samples (Figure 2). All ants from the Pascagoula population in the United States belong to a single cluster, which also corresponds to a subset of the polymorphism in the Herradura population (all individuals from Herradura have membership in two clusters, with the majority membership the same as introduced ants). The same subclusters in Herradura were observed previously by Ascunce et al. (2011) and interpreted as representing the subset of polymorphism that passed the population bottleneck during the introduction and the subset that did not. Alternatively, this pattern may be the result of secondary contact of the Herradura population with another unsampled native population (Ross, Krieger, Keller, & Shoemaker, 2007). Either way, the *Structure* results support Herradura as

the most likely source of ants introduced into the United States. Therefore, we proceed below to test for population differentiation by comparing two pairs of population clusters: Herradura vs. Alejandra and El Recreo; and Herradura vs. the introduced population sample from Pascagoula.

3.3 | Population differentiation

More recent positive selection was inferred based on genomic scans for outlier F_{ST} values, which indicate specific genomic loci with differentiation between populations that surpasses the average differentiation of the rest of the genome. The genomewide average F_{ST} was 0.058 between the two native population clusters (Herradura vs. Alejandra and El Recreo) and 0.054 between the introduced population and its presumed source population in the native range (Herradura). Genomic loci with outlier F_{ST} values were identified in four distinct analyses: genes containing SNPs with high F_{ST} in the native range comparison or in the native to introduced range comparison (Tables 5 and 7); and genes located in genomic regions with high averaged F_{ST} values in the same two comparisons (Tables 6 and 8). Each of the Tables 5, 6, 7 and 8 lists the top 50 genes sorted by their F_{ST} values in the respective analyses. The genes listed for the averaged F_{ST} analyses are located in high F_{ST} regions of the genome (in genomic scaffolds of at least 100 kbp; see Section 2.5). Of these high F_{ST} regions, four had high averaged F_{ST} values in both the native range comparison and the native to introduced range comparison, which is significantly more than expected by chance if the two comparisons were independent (for 23 and 15 of the 561 genomic scaffolds that were included in the analysis; Fisher's exact test p-value = 0.002). These four regions contain an ATP-binding cassette transporter (implicated in lipid transport), three uncharacterized genes and a transposase. In the single-gene F_{ST} analyses, no genes had high F_{ST} in both native and introduced comparisons.

The 195 unique genes identified in the four analyses (Tables 5, 6, 7 and 8) include nine fatty acid synthases, a long-chain fatty acid desaturase and a reductase. The fatty acid synthase gene family was enriched for high averaged F_{ST} values in the native range comparison (GSEA q-value=0.04). The oxidoreductase GO category was also enriched for high averaged F_{ST} values (GSEA q-value=0.10). This GO category consisted of five long-chain fatty acid reductase genes annotated in the fire ant genome. There was no significant enrichment of high F_{ST} values in GO categories for the comparisons between the native and introduced ranges nor among caste-biased genes.

4 | DISCUSSION

The present study investigated the targets of positive selection in the invasive ant *S. invicta*. One prediction was that positive selection should act more frequently on genes underlying sociobiological traits than other genes. An alternative hypothesis may be that changes in the environment of native and introduced populations lead to selection on physiological, immunological, metabolic, and other traits linked to local adaptation. We also predicted sociobiological traits to be under positive selection on multiple timescales: from recent

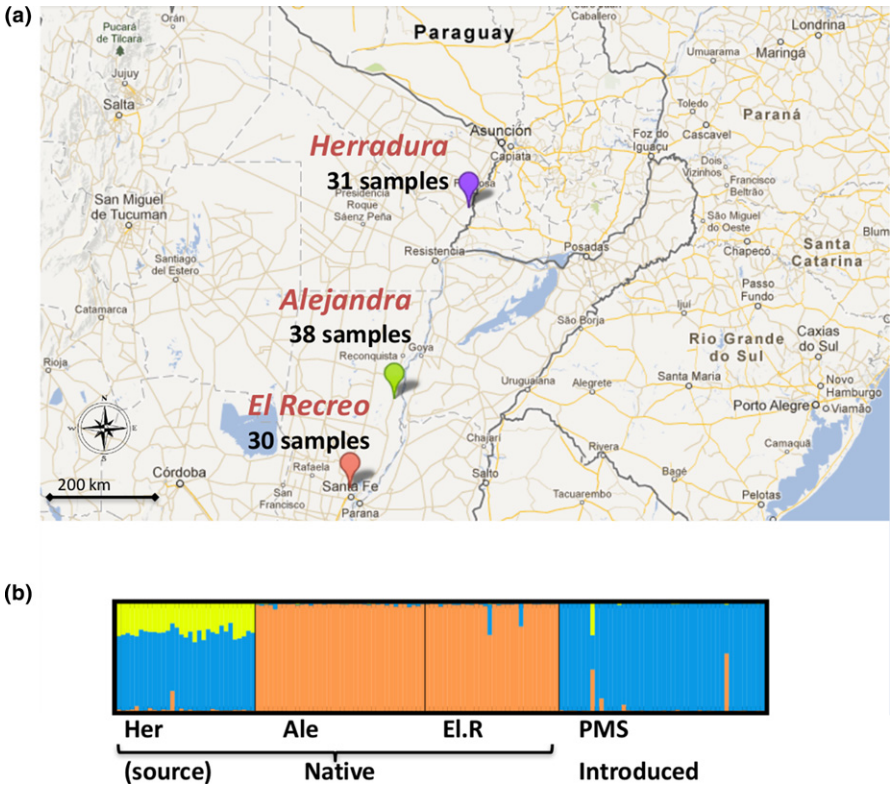

FIGURE 2 (a) Sample sites in the native range along the Paraná River in Northern Argentina. (b) Population structure inference for the native samples from Argentina (Her = Herradura, Ale = Alejandra, El.R = El Recreo) and samples from the introduced range in the United States (PMS = Pascagoula, Mississippi) based on a subsample of 1,000 SNPs. The introduced population was assigned to the blue cluster, which is also present in the putative source population in Herradura [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 5 Genes containing SNPs with highest F_{ST} scores in the native range

Gene ID	AMOVA F_{ST}	Description
SINVm1_gene_05151	1.0000	Odorant-binding protein (SiOBP17[a])
SINVm1_gene_05179	1.0000	Odorant-binding protein (SiOBP16[a])
SINVm1_gene_05180	1.0000	Domain of unknown function DUF2236[b]
SINVm1_gene_08142	1.0000	N/A
SINVm1_gene_08209	1.0000	Zinc finger MYM-type protein 1
SINVm1_gene_08445	1.0000	CD63 antigen-like
SINVm1_gene_08450	1.0000	N/A
SINVm1_gene_08454	1.0000	Nck-associated protein 5
SINVm1_gene_08855	1.0000	Serine threonine-protein kinase mark2
SINVm1_gene_13079	1.0000	Nck-associated protein 5
SINVm1_gene_13285	1.0000	Molybdopterin synthase sulphur carrier subunit-like
SINVm1_gene_14976	1.0000	N/A
SINVm1_gene_14977	1.0000	N/A
SINVm1_gene_14884	0.9743	Jerky protein homolog-like
SINVm1_gene_09598	0.9691	N/A
SINVm1_gene_07842	0.9684	Cytochrome P450
SINVm1_gene_07843	0.9684	Small subunit processome component 20 homolog
SINVm1_gene_07850	0.9684	Protein brunelleschi-like
SINVm1_gene_08266	0.9566	Lysine-specific demethylase 6A
SINVm1_gene_07103	0.9429	Luciferin 4-monooxygenase
SINVm1_gene_07104	0.9429	Ubiquitin carboxyl-terminal hydrolase isozyme L3
SINVm1_gene_04406	0.9426	Male enhanced antigen 1[b]
SINVm1_gene_04407	0.9426	N/A
SINVm1_gene_04411	0.9426	Sodium- and chloride-dependent GABA transporter 1
SINVm1_gene_04412	0.9426	Leucine-rich repeats and immunoglobulin-like domains protein 3
SINVm1_gene_08581	0.9310	Irregular chiasm C-roughest protein
SINVm1_gene_15067	0.9310	Roughest protein
SINVm1_gene_00249	0.9305	Ubiquitin carboxyl-terminal hydrolase 45-like
SINVm1_gene_06161	0.9234	Autophagy-related protein 101-like
SINVm1_gene_05170	0.9087	Glucosyl glucuronosyl transferases
SINVm1_gene_05171	0.9087	Glucose dehydrogenase
SINVm1_gene_05626	0.9055	5′-nucleotidase
SINVm1_gene_05646	0.9055	Protein 5NUC-like
SINVm1_gene_05650	0.9055	Glucose dehydrogenase
SINVm1_gene_09021	0.8844	Angiomotin, C-terminal[b]
SINVm1_gene_13221	0.8844	Heat shock protein 67B2
SINVm1_gene_15175	0.8844	Peptidyl-prolyl cis–trans isomerase-like 4-like
SINVm1_gene_05442	0.8630	WD40 repeat-containing protein smu1
SINVm1_gene_13173	0.8607	ADP-ribosylation factor-binding protein GGA1
SINVm1_gene_10131	0.8536	120.7 kDa protein in NOF-FP transposable element
SINVm1_gene_10134	0.8536	Nuclear pore glycoprotein P62
SINVm1_gene_09199	0.8516	Miniature CG9369-PA
SINVm1_gene_09216	0.8471	Aquaporin-like isoform 2
SINVm1_gene_07660	0.8303	Peroxisome proliferator-activated receptor binding protein
SINVm1_gene_07661	0.8303	Peroxisome proliferator-activated receptor binding protein
SINVm1_gene_07664	0.8303	Carbohydrate sulfotransferase 9-like
SINVm1_gene_14873	0.8303	XK-related protein 6
SINVm1_gene_09823	0.8022	Zinc finger protein
SINVm1_gene_11125	0.8000	Microtubule-associated protein
SINVm1_gene_01245	0.7920	3-hydroxyisobutyrate mitochondrial-like

[a]Naming following Gotzek, Robertson, Wurm, and Shoemaker (2011).
[b]Domain annotation based on InterPro scan.

population dynamics in both native and introduced ranges (most recent selection) to the first transition to aggressiveness and invasiveness in the *S. invicta* lineage (ancient selection). We inferred positive selection on these multiple evolutionary timescales and summarized the results in terms of molecular functions. Several molecular functions under positive selection are linked to sociobiological traits. The number of genes under positive selection in the nonrecombining region of the social chromosome is not higher than expected by chance. Thus, we interpret our results as indicating positive selection on genes underlying sociobiological traits across the genome, and not specifically within the supergene determining social form. These genes may contribute to sociobiological traits, whether related to the social polymorphism or not.

The comparison of native and introduced populations showed signatures of recent selection on multiple enzymes implicated in the synthesis of long-chain fatty acids (putative fatty acid synthases, desaturases and reductases). Long-chain fatty acids are the precursors of long-chain cuticular hydrocarbons (CHCs) and other pheromones (Blomquist & Bagneres, 2010). Long-chain hydrocarbons coat the cuticle of insects and other arthropods where they function in both prevention of desiccation and chemical signalling. It seems unlikely that selection on CHCs stems from environmental differences between the native and introduced populations because both occur in humid subtropical climates with similar ranges of temperatures and levels of precipitation. Moreover, the CHC mixture of fire ants

TABLE 6 Genes in regions with highest averaged F_{ST} in the native range

Gene ID	Smoothed F_{ST}	Description
SINVm1_gene_00255	0.2095	Tight junction protein ZO-1
SINVm1_gene_13495	0.2033	Mitochondrial glutamate carrier 1
SINVm1_gene_10131	0.1954	120.7-kDa protein in NOF-FP transposable element
SINVm1_gene_10134	0.1954	Nuclear pore glycoprotein P62
SINVm1_gene_10132	0.1949	N/A
SINVm1_gene_15368	0.1507	Fatty acid synthase
SINVm1_gene_15381	0.1474	Fatty acid synthase
SINVm1_gene_15384	0.1474	Fatty acid synthase
SINVm1_gene_15382	0.1468	Fatty acid synthase
SINVm1_gene_09823	0.1449	Zinc finger protein
SINVm1_gene_09598	0.1446	N/A
SINVm1_gene_09801	0.1446	Fatty acid synthase
SINVm1_gene_09825	0.1428	Fatty acid synthase
SINVm1_gene_15379	0.1424	Sterile alpha motif domain[a]
SINVm1_gene_15369	0.1416	Fatty acid synthase
SINVm1_gene_15370	0.1416	Copia protein
SINVm1_gene_15371	0.1416	Fatty acid synthase
SINVm1_gene_15380	0.1409	N/A
SINVm1_gene_09422	0.1336	Coiled-coil and c2 domain-containing protein 1-like
SINVm1_gene_13365	0.1332	Dual specificity protein phosphatase 3
SINVm1_gene_09888	0.1315	C11ORF46 homolog
SINVm1_gene_09889	0.1311	N/A
SINVm1_gene_13451	0.1299	N/A
SINVm1_gene_09600	0.1285	Dual specificity protein phosphatase 3
SINVm1_gene_13339	0.1278	Zinc finger mym-type protein 1
SINVm1_gene_13466	0.1273	tRNA (guanine-n)-methyltransferase
SINVm1_gene_15449	0.1268	ATP-binding cassette subfamily G member 4
SINVm1_gene_09420	0.1268	tRNA guanylyltransferase
SINVm1_gene_15266	0.1268	Vacuolar ATP synthase subunit ac39
SINVm1_gene_10088	0.1266	N/A
SINVm1_gene_14884	0.1265	Jerky protein homolog-like
SINVm1_gene_13498	0.1264	N/A
SINVm1_gene_10139	0.1254	N/A
SINVm1_gene_09505	0.1254	N/A
SINVm1_gene_09779	0.1246	Fatty acyl-reductase
SINVm1_gene_09253	0.1243	CG7120
SINVm1_gene_09657	0.1243	Sodium potassium calcium exchanger 4-like
SINVm1_gene_13381	0.1235	Sodium potassium calcium exchanger 4-like
SINVm1_gene_07722	0.1235	ATPase family AAA domain-containing protein 1A-like
SINVm1_gene_09656	0.1232	N/A
SINVm1_gene_10140	0.1223	Transposase
SINVm1_gene_09421	0.1219	DNA-directed RNA polymerase I largest subunit
SINVm1_gene_09780	0.1216	Nose resistant to fluoxetine protein 6-like
SINVm1_gene_13418	0.1211	Nose resistant to fluoxetine protein 6
SINVm1_gene_09419	0.1205	Thioredoxin-like protein
SINVm1_gene_10041	0.1199	N/A
SINVm1_gene_10042	0.1199	N/A
SINVm1_gene_09339	0.1197	Fatty acid synthase
SINVm1_gene_10100	0.1192	Zinc finger-60
SINVm1_gene_09638	0.1188	Transcription factor IIIb 90-kDa subunit-like

[a]Domain annotation based on InterPro scan.

(Eliyahu, Ross, Haight, Keller, & Liebig, 2011) and other ant species is much more complex than expected if they mainly functioned in preventing desiccation. Complex mixtures of methyl-branched alkanes are more water permeable than simple mixtures of strait alkanes, but simple mixtures contain much less information as chemical signals. This led previous authors to conclude that CHC evolution in ants is largely determined by their role as chemical signals (e.g., Hefetz, 2007). The gene family of long-chain fatty acid reductases was enriched for genes under recent positive selection in the native range. Nine fatty acid synthase genes were among the top 50 genes under selection in the native range. Two of these genes were among the top selected genes in both the comparison within the native range and the comparison of the native and introduced ranges, suggesting ongoing or repeated selection pressures on the same genes. Further evidence for recent selection pressure was found for two odorant-binding proteins, which are implicated in olfaction. However, no odorant receptors or other chemosensory receptors were found to be under positive selection, despite previous reports of widespread positive selection on these genes in ants (Engsontia, Sangket, Robertson, & Satasook, 2015; Roux et al., 2014; Zhou et al., 2015). This may be because our analyses were based on the standard automatic annotation of the *S. invicta* genome, which is known to miss many of these fast-evolving genes.

We also inferred ancient selection pressures after the divergence 25 ± 4 million years ago (Ward, Brady, Fisher, & Schultz, 2015) of the fire ant lineage represented by *S. invicta* from thief ants represented by the lineage of *S. fugax*, the life history of which most closely resembles their common ancestors. These analyses revealed signatures of positive selection on ion channels, and various other

TABLE 7 Genes containing SNPs with highest F_{ST} scores in the comparison of introduced and native ranges

Gene ID	AMOVA F_{ST}	Description
SINVm1_gene_01935	1.0000	Ubiquinone biosynthesis protein
SINVm1_gene_01980	1.0000	N/A
SINVm1_gene_03686	1.0000	Facilitated trehalose transporter TRET1-like
SINVm1_gene_03715	1.0000	Nuclear valosin-containing
SINVm1_gene_03779	1.0000	Carnitine O-palmitoyltransferase
SINVm1_gene_03834	1.0000	N/A
SINVm1_gene_04492	1.0000	Dystrophin isoform D
SINVm1_gene_05187	1.0000	Roundabout-like protein 1
SINVm1_gene_06499	1.0000	N/A
SINVm1_gene_07608	1.0000	Solute carrier family 12 member 6
SINVm1_gene_11030	1.0000	Slit homolog 3
SINVm1_gene_11208	1.0000	DNA-binding protein EWG
SINVm1_gene_13119	1.0000	Connector enhancer of kinase suppressor of RAS 3
SINVm1_gene_13835	1.0000	NADPH oxidase 5
SINVm1_gene_13836	1.0000	NADPH oxidase 5
SINVm1_gene_13943	1.0000	Protein shifted
SINVm1_gene_14260	1.0000	Sterol regulatory element-binding protein cleavage-activating protein
SINVm1_gene_15041	0.7955	Nucleolar protein 8
SINVm1_gene_07426	0.7889	AC transposable element-derived protein 4
SINVm1_gene_07427	0.7889	Transposase yabusame-W
SINVm1_gene_07439	0.7889	Golgin subfamily A member 6-like protein 1
SINVm1_gene_07441	0.7889	N/A
SINVm1_gene_08400	0.7880	ATP-dependent RNA helicase DHX33-like
SINVm1_gene_15035	0.7880	Major facilitator superfamily domain-containing protein 1
SINVm1_gene_08086	0.7780	RAP1 GTPase-activating protein 2-like
SINVm1_gene_07175	0.7714	OFD1 protein
SINVm1_gene_07981	0.7714	Fibrillin-2-like
SINVm1_gene_09538	0.7630	Enoyl-delta isomerase mitochondrial-like
SINVm1_gene_09542	0.7630	Leucine-rich repeat-containing protein 16A
SINVm1_gene_05854	0.7414	N/A
SINVm1_gene_04941	0.7347	DGMP-dependent protein isozyme 1
SINVm1_gene_13460	0.7347	N/A
SINVm1_gene_13461	0.7347	Neurogenic protein big brain
SINVm1_gene_15382	0.7222	Fatty acid synthase
SINVm1_gene_15384	0.7222	Fatty acid synthase
SINVm1_gene_09172	0.7143	Tudor domain-containing protein 5
SINVm1_gene_09468	0.7105	Parathyroid hormone parathyroid hormone-related peptide receptor-like
SINVm1_gene_13327	0.7105	N/A
SINVm1_gene_06096	0.7083	Cytosolic endo-beta-N-acetylglucosaminidase
SINVm1_gene_08865	0.6923	Chromosome 9 open reading frame 80
SINVm1_gene_08866	0.6923	Methyltransferase-like protein 5
SINVm1_gene_08868	0.6923	Alpha-aminoadipic semialdehyde dehydrogenase
SINVm1_gene_09384	0.6818	Golgin subfamily A member 1
SINVm1_gene_13498	0.6777	N/A
SINVm1_gene_06112	0.6691	N/A
SINVm1_gene_06117	0.6691	N/A
SINVm1_gene_08206	0.6667	Prenylcysteine oxidase-like
SINVm1_gene_05527	0.6444	N/A
SINVm1_gene_01579	0.6410	AMP dependent COA ligase
SINVm1_gene_04568	0.6410	Mitochondrial ATP synthase F chain

genes also implicated in neuronal functions (i.e., *gephyrin*, *disabled*, *unc-79*, *unc-80*, *CEPU-1* and two glutamate receptors). The neurological functions under selection may underlie both social and other behaviours. Distinguishing between these alternatives would require in-depth study of the neurological mechanisms in which these genes function. Evidence for ancient positive selection also was found in *vitellogenin 1*, one of the four *vitellogenin* paralogs in *S. invicta* that is overexpressed in workers relative to queens. Vitellogenin and juvenile hormone form a regulatory feedback loop that plays a major role in the regulation of caste determination in social insects (Libbrecht et al., 2013). In the honeybee, the single orthologous *vitellogenin* gene, a juvenile hormone esterase, and a juvenile hormone acid methyltransferase were also positively selected (Harpur et al., 2014), suggesting that genes implicated in the regulation of caste determination are targets of positive selection in multiple social insect lineages.

The finding of larger selection coefficients for genes overexpressed in workers than in queens and males is in line with a gene expression comparison between *S. invicta* and *S. richteri*, which revealed more extensive evolutionary changes in the expression of worker-biased genes relative to other caste-biased and non-caste-biased genes (Ometto et al., 2011). Studies in honeybees also inferred stronger positive selection on worker traits related to the regulation of colony growth and development, caste determination and division of labour (Harpur et al., 2014; Kent, Issa, Bunting, & Zayed, 2011; Vojvodic et al., 2015; Zayed & Whitfield, 2008). Harpur et al. (2014) inferred consistently stronger positive selection on honeybee worker-biased genes relative to queen-biased genes, as in our study. Vojvodic et al. (2015) observed the same pattern in genes that are overexpressed in worker-destined relative to queen-destined

TABLE 8 Genes in regions with highest averaged F_{ST} in the comparison of introduced and native ranges

Gene ID	Smoothed F_{ST}	Description
SINVm1_gene_13498	0.1587	N/A
SINVm1_gene_09910	0.1493	G-protein-coupled receptor MTH
SINVm1_gene_09911	0.1491	G-protein-coupled receptor MTH
SINVm1_gene_09908	0.1460	N/A
SINVm1_gene_09912	0.1460	G-protein-coupled receptor MTH
SINVm1_gene_09914	0.1391	G-protein-coupled receptor MTH
SINVm1_gene_09915	0.1391	G-protein-coupled receptor MTH
SINVm1_gene_09541	0.1263	N/A
SINVm1_gene_15293	0.1263	Acyl-delta desaturase
SINVm1_gene_10102	0.1247	Cytochrome P450 4AA1
SINVm1_gene_10103	0.1247	N/A
SINVm1_gene_10104	0.1245	AP-like endonuclease reverse transcriptase
SINVm1_gene_10105	0.1241	Cytochrome P450 4AA1
SINVm1_gene_13503	0.1241	Cytochrome P450 4AA1
SINVm1_gene_09540	0.1236	Gamma-tubulin complex component 4
SINVm1_gene_09545	0.1236	UPF0532 protein CG3570-like
SINVm1_gene_09546	0.1236	Vesicle transport protein USE1-like
SINVm1_gene_09539	0.1235	Dual oxidase maturation factor 1
SINVm1_gene_09544	0.1234	Splicing arginine serine-rich 7-like
SINVm1_gene_09543	0.1228	Transmembrane protein 208-like
SINVm1_gene_09538	0.1222	Enoyl-delta isomerase mitochondrial-like
SINVm1_gene_15294	0.1222	Leucine-rich repeat-containing protein 48
SINVm1_gene_09542	0.1216	Leucine-rich repeat-containing protein 16A
SINVm1_gene_15449	0.1194	ATP-binding cassette subfamily G member 4
SINVm1_gene_09537	0.1166	Dynein heavy chain
SINVm1_gene_09760	0.1086	N/A
SINVm1_gene_13415	0.1086	Zinc finger MYM-type protein 1
SINVm1_gene_15354	0.1086	FERM, RhoGEF and pleckstrin domain-containing protein
SINVm1_gene_09759	0.1084	FERM, RhoGEF and pleckstrin domain-containing protein
SINVm1_gene_10088	0.0999	Zinc finger, CCHC-type superfamily[a]
SINVm1_gene_09716	0.0992	Glutamine and serine-rich protein 1
SINVm1_gene_15340	0.0992	Glutamine and serine-rich protein 1
SINVm1_gene_15341	0.0992	Glutamine and serine-rich protein 1
SINVm1_gene_13396	0.0988	Glutamine and serine-rich protein 1
SINVm1_gene_09717	0.0987	N/A
SINVm1_gene_09715	0.0985	N/A
SINVm1_gene_10044	0.0924	Sugar transporter sweet1-like
SINVm1_gene_13488	0.0921	Ribonucleoside-diphosphate reductase large subunit
SINVm1_gene_10043	0.0920	Protein-cysteine N-palmitoyltransferase rasp-like
SINVm1_gene_09714	0.0895	Epidermal cell surface receptor
SINVm1_gene_09713	0.0891	Phospholipid-hydroperoxide glutathione peroxidase
SINVm1_gene_09712	0.0888	Regulatory factor x domain-containing protein 2
SINVm1_gene_15464	0.0885	Transposable element TC3 transposase
SINVm1_gene_09706	0.0884	N/A
SINVm1_gene_10139	0.0883	N/A
SINVm1_gene_09711	0.0880	Thioredoxin domain-containing protein 11
SINVm1_gene_13508	0.0880	Aminopeptidase N
SINVm1_gene_13510	0.0876	Aminopeptidase N
SINVm1_gene_15339	0.0872	ADP-ribosylation factorlike protein 4C-like
SINVm1_gene_09004	0.0868	Peroxisome biogenesis factor 10-like

[a]Domain annotation based on InterPro scan.

larvae. Several of the positively selected genes identified in the study by Harpur et al. (2014) are implicated in division of labour in honeybees: *vitellogenin*, the *foraging* gene, genes of the insulin/Tor pathway, juvenile hormone metabolizing enzymes and genes coding for Major Royal Jelly Protein. Enrichment of positively selected genes was also reported for functional categories related to behaviour, cognition, nervous system development, metabolism and steroid hormones. The genes and functional categories under selection support our prediction of selection on sociobiological traits, but we cannot rule out the possibility that stronger selection on worker traits relative to queen traits could also be due to their greater exposure to changing environmental conditions or factors.

In contrast to our study indicating adaptive evolution on both synthesis and olfaction of pheromones, a recent study in the honeybee *Apis mellifera* found no evidence of positive selection on chemical communication genes (Harpur et al., 2014). That study used comparable methodologies to our study to infer positive selection since the divergence of *A. mellifera* from the Asian honeybee *A. cerana*, between 17 and 33 million years ago (Cardinal, Straka, & Danforth, 2010), and more recent positive selection by comparisons among *A. mellifera* populations. Another study of the socially polymorphic halictid bee *Lasioglossum albipes* revealed positive selection on two odorant receptors and one cuticular protein in a dN/dS analysis comparing a genome from a social population and a genome from a solitary population (Kocher et al., 2013).

In comparison with the relatively recent positive selection pressures we analysed in this study, positive selection studies in ants

and bees on a larger evolutionary scale detected different molecular functions under selection. Several phylogenomics studies used genomes from different genera to infer long-term selection pressures that generally characterize the evolution of ants (Roux et al., 2014) and social bees (Kapheim et al., 2015; Woodard et al., 2011). The studies in bees found evidence for positive selection on genes implicated in signal transduction, gene regulation, carbohydrate metabolism and gland development. The analyses of ant genomes detected positive selection on genes implicated in mitochondrial functions, which was suggested to underlie the evolution of queen longevity. Additional genes under positive selection in ants were implicated in development of imaginal and genital discs, egg development, neuropeptide hormone activity, rhythmic behaviour and regulatory mechanisms including alternative splicing and post-translational protein modification. Thus, analyses on different evolutionary timescales detect different molecular functions under selection, suggesting different evolutionary processes acting on different traits in the early evolution of sociality relative to more recent evolution in particular eusocial lineages.

A caveat of our study is that we only have data from a single introduced population. In an unfortunate manner, all *S. invicta* populations studied to date appear to be secondary introductions from the introduced US population (Ascunce et al., 2011). Thus, the genes under positive selection following the first introduction may be specific to the conditions of this geographic region. It would be interesting to conduct a similar study with other species that were independently introduced to new ranges to identify whether specific genes or molecular functions repeatedly experience positive selection in different environmental conditions.

In conclusion, we found evidence for positive selection on genes implicated in sociobiological traits in all evolutionary timescales examined: from the early divergence between the more invasive *Solenopsis* fire ant and thief ant lineages to recent selection in native and introduced populations. Positive selection on worker-biased genes and genes implicated in neurological functions, pheromonal signalling and caste determination supports the hypothesis that sociobiological traits are a target for positive selection in invasive fire ants, more so than traits solely related to environmental factors in new ranges. Combined with previous studies in honeybees and halictid bees, these results suggest that natural selection targets similar molecular pathways and functions that underlie worker traits in different social insect lineages and that sociobiological traits are also under positive selection an invasive social insect.

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DATA ACCESSIBILITY

A new version of the *S. invicta* genome assembly was submitted to NCBI (gnH; Accession no. AEAQ00000000). The genome annotation can be found in the DRYAD database, https://doi.org/10.5061/dryad.n60k3t5. The *S. fugax* genome assembly gnA was submitted to NCBI (Accession no. QKQZ00000000). The population sequencing data was submitted to the NCBI SRA database: Accession no. PRJNA450756 for the 40 whole-genome sequenced male samples, and Accession no. PRJNA448217 for the RAD-sequenced samples.

AUTHOR CONTRIBUTION

EP, DS, and LK designed the study. DS collected samples and constructed RAD libraries. ORG assembled the S. invicta genome. EP, PC, and ABC analyzed the data. EP, DS, and LK wrote the manuscript.

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