Population Genetic and Social Structure Survey of Solenopsis geminata in Thailand

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Fire ants have long been known to be a major pest and have recently attracted renewed widespread attention due to the invasion of Solenopsis species, especially S. invicta, into many countries in Asia and Australia. Here, we surveyed fire ant specimens in Thailand with the aims of studying their colony biology and population structure. We sampled 38 colonies distributed in agricultural and urban areas throughout Thailand for species identification and found that all were S. geminata. We further genotyped 13 microsatellite loci from 576 workers from 23 of these colonies. Analysis of this genetic data revealed that all colonies were polygynous with only a few queens. Queens from the same colonies were highly genetically related. Population structure was partitioned into two clusters. Pairwise $F_{ST}$ values revealed very high genetic differentiation between colonies suggesting low gene flow among populations. This result suggests that queens were locally mated and founded colonies by a budding strategy. Isolation-by-distance among local populations was not significant.

**Key words:** Polygyne, Social form, Relatedness, Microsatellite, Metapopulation model.


BACKGROUND
Fire ants are one of the most important pest species distributed around the world (Tschinkel 2006). They often form large colonies at high densities in both urban and rural areas, and thus reduce crop production and harvest, damage electrical machinery, create health problems through their painful stings, and cause economic and biodiversity loss (McDonald 2006; Gutrich et al. 2007; Jetter et al. 2002). Because of their importance, fire ants have been extensively studied for its biology, distribution and application for pest control (Tschinkel 2006).

Most studies have focused on the red-imported fire ant, *Solenopsis invicta*, due to its high invasiveness and aggressiveness. In the Asia-Pacific region, it has recently invaded Australia, China, Hong Kong, Taiwan and Japan (Ascunce et al. 2011; Kyodo 2017; Kikuchi 2017). It remains unclear whether *S. invicta* has also invaded Thailand since systematic and extensive sampling throughout the country is lacking. Compared to *S. invicta*, the tropical fire ant, *Solenopsis geminata*, is less aggressive but actually has a greater worldwide invasive distribution (Wetterer 2011; Gotzek et al. 2015). So far, only *S. geminata* has been reported in Thailand (Bourmas et al. 2001; Hasin 2008; Sakchoowong et al. 2008; Jongjitvimol 2010; Etterer 2011; Wetterer 2011).

Colony social form is an important factor for the biology of social insects (Bourke and Franks 1995). Individuals from a monogyne colony, especially with once-mated queens, are always more related to each other than those from a polygyne colony (Hamilton 1964; Hölldobler and Wilson 1990). Consequently, monogyne colonies are more stable according to kin selection theory than polygyne colonies, which can have queen-queen competition (Bourke and Franks 1995). However, monogyne colonies have a higher risk of colony death though queen loss while polygyne colonies can tolerate the loss of some queens (Keller 1995). The two social forms are tightly associated with queen morphology, mode of colony foundation, and ecological constraints (Bourke and Franks 1995; Keller 1995; Wcislo 1995; Ross and Keller 1995; Cronin et al. 2013). For instance, monogyne queens are usually bigger and more fecund than polygyne queens, and thus they can found colonies independently, thereby rapidly occupy empty niches. In contrast, under some ecological circumstances such as high nest density, polygyne colonies are more fit. In *S. invicta*, monogyne workers are also bigger and more aggressive compared to polygyne colonies (Ross and Keller 1995; Araujo and Tschinkel 2010). This indicates that social form influences the biology, behavior, and life history trait of the social insects (Keller 1995; Ross and Keller 1995).

These characters were shown to be under genetic regulation in *S. invicta*, presented previously as a single gene with two alleles (Gp-9B and Gp-9b) (Keller 1995; Keller and Ross 1998, 1999; Ross and Keller 1998; DeHeer et al. 1999; Goodisman et al. 1999; Gotzek and Ross 2007; Huang and Wang 2014), but later on it was found to be a supergene (with SB and Sb alleles) containing ~600 genes and composed of multiple large inversions (Wang et al. 2013; Huang et al. 2018; Stolle et al. 2019; Yan et al. 2020). In addition, the SB and Sb alleles of the supergene are
also present in six additional related species in the South American clade of fire ants (Stolle et al. 2019; Yan et al. 2020). Altogether, the evolution of the genes controlling social structure in this clade is likely conserved (Krieger and Ross 2002; Gotzek et al. 2007; Manfredini et al. 2013; Stolle et al. 2019; Yan et al. 2020).

In contrast to the socially polymorphic South American fire ants, a different mode of social form evolution has likely occurred in *S. geminata*. The two social forms have identical *Gp-9* genotypes suggesting the absence of a supergene (Krieger and Ross 2002), thus, we cannot use this locus as a proxy for social form in *S. geminata*. Also, the polygyne form has been proposed to arise because of loss of allelic diversity at genes controlling queen acceptance after a genetic bottleneck (Mackay et al. 1990; Ross et al. 2003). Interestingly, polygyny in one population in Florida is also associated with facultative asexuality; while workers are produced normally by mating, queens are clonal offspring (Lacy et al. 2019). Here, we studied the social form and population structure of *S. geminata* in Thailand using microsatellite genotyping of 576 workers from 23 colonies distributed in all parts of Thailand. We analyzed colony social form, relatedness among queens and workers within a colony, and determined the genetic structure of *S. geminata* in Thailand. As this species has long been known to be an important pest in crop fields, we focused in this study on the biology of *S. geminata* in Thailand, with respect to its distribution, population structure, and colony social form.

**MATERIALS AND METHODS**

**Sample collection and identification**

We collected a total of 38 fire ant colonies from land adjacent to different crop fields (e.g., corn, rice, banana, lime, santols) and urban areas in all six parts of Thailand (i.e., Northern, Western, Eastern, Southern, Northeastern and Central regions). Of these, eight colonies came from five provinces in the Central region; five colonies from three provinces in the Eastern region; six colonies from four provinces in the Northeastern region; four colonies from two provinces in the Northern region; five colonies from three provinces in the southern region; and 10 colonies from three provinces in the Western region of Thailand. We also recorded the position of the colonies, crop types, and interaction of fire ants with other insects (Table 1, Fig. 1.)
## Table 1. Coordinates and information for *S. geminata* colonies sampled

<table>
<thead>
<tr>
<th>Part of Thailand</th>
<th>Province</th>
<th>Colony</th>
<th>Genotyped?</th>
<th>Area information</th>
<th>Coordinate</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Chiang Mai</td>
<td>Sge04</td>
<td>Yes</td>
<td>corn farm</td>
<td>18°47′44.6″N 98°57′35.3″E</td>
</tr>
<tr>
<td>N</td>
<td>Chiang Mai</td>
<td>Sge06</td>
<td>Yes</td>
<td>University area</td>
<td>18°48′13.4″N 98°57′10.8″E</td>
</tr>
<tr>
<td>N</td>
<td>Lampang</td>
<td>Sge08</td>
<td>No</td>
<td>urban area</td>
<td>18°17′23.0″N 99°28′28.8″E</td>
</tr>
<tr>
<td>N</td>
<td>Lampang</td>
<td>Sge17</td>
<td>Yes</td>
<td>vegetable farm</td>
<td>18°17′57.9″N 99°27′35.0″E</td>
</tr>
<tr>
<td>NE</td>
<td>Kalasin</td>
<td>Sge20</td>
<td>No</td>
<td>Cassava farm</td>
<td>16°32′08.9″N 103°25′41.9″E</td>
</tr>
<tr>
<td>NE</td>
<td>Loei</td>
<td>Sge19</td>
<td>Yes</td>
<td>near rambutan trees</td>
<td>17°28′53.7″N 101°37′44.6″E</td>
</tr>
<tr>
<td>NE</td>
<td>NakonRatchasima</td>
<td>Sge01</td>
<td>Yes</td>
<td>vegetable farm</td>
<td>14°21′57.8″N 101°53′28.9″E</td>
</tr>
<tr>
<td>NE</td>
<td>NakonNakhon</td>
<td>Sge16</td>
<td>Yes</td>
<td>rice farm</td>
<td>17°23′01.4″N 104°06′14.4″E</td>
</tr>
<tr>
<td>NE</td>
<td>NakonNakhon</td>
<td>Sge22</td>
<td>No</td>
<td>urban area</td>
<td>17°23′24.8″N 104°06′14.4″E</td>
</tr>
<tr>
<td>NE</td>
<td>NakonNakhon</td>
<td>Sge23</td>
<td>No</td>
<td>rice farm</td>
<td>17°23′01.4″N 104°06′08.0″E</td>
</tr>
<tr>
<td>C</td>
<td>Bangkok</td>
<td>Sge05</td>
<td>Yes</td>
<td>University area</td>
<td>13°50′33.6″N 100°34′17.4″E</td>
</tr>
<tr>
<td>C</td>
<td>Chai Nat</td>
<td>Sge36</td>
<td>Yes</td>
<td>Integrated farming (e.g., rice, santols)</td>
<td>15°24′05.0″N 100°05′25.7″E</td>
</tr>
<tr>
<td>C</td>
<td>Chai Nat</td>
<td>Sge37</td>
<td>No</td>
<td>Integrated farming (e.g., rice, santols)</td>
<td>15°24′05.0″N 100°05′25.7″E</td>
</tr>
<tr>
<td>C</td>
<td>Chai Nat</td>
<td>Sge38</td>
<td>No</td>
<td>banana and lime trees</td>
<td>15°14′12.0″N 100°05′20.2″E</td>
</tr>
<tr>
<td>C</td>
<td>KamphaengPhet</td>
<td>Sge33</td>
<td>No</td>
<td>bamboo trees</td>
<td>16°38′27.9″N 99°19′53.9″E</td>
</tr>
<tr>
<td>C</td>
<td>KamphaengPhet</td>
<td>Sge34</td>
<td>Yes</td>
<td>sugar apples</td>
<td>16°29′43.4″N 99°40′14.4″E</td>
</tr>
<tr>
<td>C</td>
<td>Phitsanulok</td>
<td>Sge18</td>
<td>Yes</td>
<td>vegetable farm</td>
<td>16°55′03.8″N 100°12′22.3″E</td>
</tr>
<tr>
<td>C</td>
<td>Supanburi</td>
<td>Sge39</td>
<td>Yes</td>
<td>vegetable farm</td>
<td>14°26′20.3″N 100°09′38.4″E</td>
</tr>
<tr>
<td>E</td>
<td>Chanthaburi</td>
<td>Sge29</td>
<td>No</td>
<td>mangoestees</td>
<td>12°38′06.5″N 102°00′14.0″E</td>
</tr>
<tr>
<td>E</td>
<td>Chanthaburi</td>
<td>Sge30</td>
<td>Yes</td>
<td>barn of rambutans, longazones, and mangoestees</td>
<td>12°38′16.8″N 101°59′52.2″E</td>
</tr>
<tr>
<td>E</td>
<td>Rayong</td>
<td>Sge31</td>
<td>Yes</td>
<td>barn of rambutans, longazones, and mangoestees</td>
<td>12°40′06.5″N 101°23′04.9″E</td>
</tr>
<tr>
<td>E</td>
<td>Trat</td>
<td>Sge27</td>
<td>Yes</td>
<td>barn of rambutans, longazones, and mangoestees</td>
<td>12°21′46.0″N 102°26′26.8″E</td>
</tr>
<tr>
<td>E</td>
<td>Trat</td>
<td>Sge28</td>
<td>Yes</td>
<td>palm tree near the lime trees</td>
<td>12°21′43.4″N 102°26′24.0″E</td>
</tr>
<tr>
<td>S</td>
<td>Chumphon</td>
<td>Sge13</td>
<td>Yes</td>
<td>seaside</td>
<td>9°57′11.4″N 99°09′30.7″E</td>
</tr>
<tr>
<td>S</td>
<td>Chumphon</td>
<td>Sge21</td>
<td>No</td>
<td>urban area</td>
<td>9°44′33.1″N 99°06′06.6″E</td>
</tr>
<tr>
<td>S</td>
<td>SuratThani</td>
<td>Sge24</td>
<td>No</td>
<td>urban area</td>
<td>8°55′37.9″N 99°16′30.5″E</td>
</tr>
<tr>
<td>S</td>
<td>Trang</td>
<td>Sge14</td>
<td>No</td>
<td>urban area</td>
<td>7°37′35.2″N 99°33′54.0″E</td>
</tr>
<tr>
<td>S</td>
<td>Trang</td>
<td>Sge15</td>
<td>Yes</td>
<td>seaside</td>
<td>7°20′28.9″N 99°22′23.1″E</td>
</tr>
<tr>
<td>W</td>
<td>Kanchanaburi</td>
<td>Sge25</td>
<td>Yes</td>
<td>near vegetable farm</td>
<td>14°07′23.6″N 99°19′10.0″E</td>
</tr>
<tr>
<td>W</td>
<td>Kanchanaburi</td>
<td>Sge26</td>
<td>No</td>
<td>corn farm</td>
<td>14°07′11.8″N 99°19′00.1″E</td>
</tr>
<tr>
<td>W</td>
<td>Phetchaburi</td>
<td>Sge02</td>
<td>Yes</td>
<td>lime tree</td>
<td>12°44′47.4″N 99°42′38.7″E</td>
</tr>
<tr>
<td>W</td>
<td>Phetchaburi</td>
<td>Sge03</td>
<td>Yes</td>
<td>corn farm</td>
<td>12°44′32.9″N 99°42′42.4″E</td>
</tr>
<tr>
<td>W</td>
<td>Phetchaburi</td>
<td>Sge08</td>
<td>No</td>
<td>urban area</td>
<td>12°43′40.2″N 99°45′20.6″E</td>
</tr>
<tr>
<td>W</td>
<td>Phetchaburi</td>
<td>Sge09</td>
<td>Yes</td>
<td>vegetable farm</td>
<td>12°44′31.9″N 99°42′46.0″E</td>
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<tr>
<td>W</td>
<td>Phetchaburi</td>
<td>Sge10</td>
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<td>vegetable farm</td>
<td>12°44′34.7″N 99°42′43.3″E</td>
</tr>
<tr>
<td>W</td>
<td>Phetchaburi</td>
<td>Sge11</td>
<td>No</td>
<td>peanut farm</td>
<td>12°44′35.9″N 99°42′43.3″E</td>
</tr>
<tr>
<td>W</td>
<td>Phetchaburi</td>
<td>Sge12</td>
<td>No</td>
<td>marigold flower farm</td>
<td>12°44′49.7″N 99°42′38.4″E</td>
</tr>
<tr>
<td>W</td>
<td>Tak</td>
<td>Sge32</td>
<td>Yes</td>
<td>urban area</td>
<td>16°41′14.6″N 99°16′38.8″E</td>
</tr>
</tbody>
</table>
Fig. 1. Locations of the 38 *S. geminata* colonies collected in this study. Colonies names are colored to indicate if samples were genotyped (blue) or not genotyped (brown).
To obtain colonies with queens to be observed in the lab, we dug up fire ant mounds from the field and placed them into buckets. These buckets were dripped overnight to separate fire ants from soil. Ants and brood were then placed into plastic boxes coated with fluon to prevent ant escape and supplied with an artificial nest made from petri dishes containing moistened plaster. Ants were fed with insects, tuna and honey using standard methods for colony rearing (Jouvenaz et al. 1977). The number of reproductive queens were observed after the establishment of the colonies for at least a week. If dealate queens were present, we reared them with some workers and brood for a longer period to test if the queens were inseminated and laid fertilized eggs. However, we only obtained a single fertile queen from one colony from Chanthaburi (Sge30). We reared this subcolony (Sge30M) in the laboratory for almost a year and finally collected her workers for genotyping. From the remaining 37 nests we collected only workers and extracted DNA from 24 workers per colony. Worker samples were put in 95% ethanol for species identification and DNA extraction follow by genotyping to determine the number of queens in each colony.

For species identification, we examined worker shape from multiple individuals. Some *Solenopsis geminata* workers have with large square-shaped heads while *S. invicta* workers lack such square-shaped heads. We then verified the *S. geminata* species identification based on the technical key criteria (Sarnat 2008; AntWeb 2017): the presence of a vertex with a deep groove on the head, black mandibles without teeth, no antennal scrobes, absence of a petiole process, unsculptured heads and bodies, and disproportionately large and square-shaped head that were present on at least ten major and minor ants of each colony.

**Microsatellite genotyping**

We extracted DNA using the CTAB method (modified from Doyle, JJ and Doyle, 1987) and amplified 19 microsatellite loci (Table S1) developed by Ascunce et al. (2009), Chen et al. (2003) and Krieger and Keller (1997). Due to technical difficulties for PCR amplification and non-specific, monomorphic, or ambiguous patterns at some loci, only 13 loci were chosen for genotyping in the main analysis. We genotyped 24 workers from each colony from a total of 23 colonies distributed throughout Thailand. We used a modified primer labeling method developed by (Blacket et al. 2012) except for the two primers, M-II and M-V, which were directly labeled. PCR reactions were done in a 10 µL reaction mixture containing PCR buffer, 2 mM MgCl₂, 0.2 mM dNTP mix, 0.5 µM of each F and R primer, 0.25 µM of M13 primer (except for M-II and M-V), 1 ng of template DNA, and 0.5 U of Taq polymerase (Apsalagen® for most loci, and Qiagen for C147, C367, C485 and Sol-55). All loci were amplified using the same standardized cycling profile with Eppendorf Thermocyclers: initial denaturation step at 94°C for 3 min, followed by 35 cycles at 94°C (45 s),
55-60°C (30 s) and 72°C (30 s), and a final elongation step at 72°C (5 min). Samples of the PCR products (4 µL) were visualized on 2% agarose gels for an initial check before genotyping by fragment analysis using an ABI 3730XL DNA analyzer (Applied Biosystem). All genotypes were called using Peak Scanner Software (Applied Biosystem).

Social form determination

The numbers of queens and males that were the parents of the genotyped workers were determined by Sibship Reconstruction (Wang 2004) and Sibship Inference (Wang and Santure 2009) implemented in Colony software (Jones & Wang, 2010) using the Full Likelihood (FL) analysis method with updated allele frequencies and no prior parameter setting. As Solenopsis queens typically mate only once (Ross and Fletcher 1985a) and although multiple matings occasionally occur in some populations (Lawson et al. 2012), we set the parameter as female monogamous. We used the predicted queen genotypes from this analysis for sibship evaluation to test if polygyne queens were full siblings.

Population analysis

We calculated the number of alleles as well as the observed and expected heterozygosity (H\textsubscript{O} and H\textsubscript{E}) values for each colony using the Microsatellite Toolkit (Park 2001). At some loci, we only obtained the genotype from a few individuals. Therefore, to avoid bias in calculating the average H\textsubscript{O} and H\textsubscript{E} for each colony, we decided to use only loci with \geq 70\% (14 individuals) of the 24 individuals having scorable genotyping data. We also calculated polymorphic information content (PIC) from all colonies. We calculated the genetic differentiation (F\textsubscript{ST}) between S. geminata colonies using FSTAT (Goudet 1995). Significant differentiation between colonies was determined based on the “genic differentiation” test using Genepop (Rousset 2008). Sequential Bonferroni correction (Holm 1979) was also applied to correct for multiple testing.

We identified population structure using STRUCTURE v.2.2 software (Hubisz et al. 2009) and the best clustering (i.e., best K) was chosen based on the delta K method (Evanno G, Regnaut S 2005). Isolation-by-distance (IBD) was determined using the Mantel test implemented in GenAlEx (Peakall and Smouse 2012). The statistical significance of the parameter estimates was obtained based on 999 permutations.

Relatedness analysis
We used the Related software (Pew et al. 2015) to calculate relatedness between pairs of queens and workers using the method from Queller and Goodnight (1989). The two-sided Wilcoxon Rank-Sum Test was used to test the differences between the relatedness values between queens within the same colonies and between queens of different colonies. Box plots were drawn in R (R Development Core Team 2010).

RESULTS

Fire ant species and nest structure

All *Solenopsis geminata* nests found in this study were flat compared to the more domed mounds of *S. invicta* in spring (*e.g.*, in the USA) and were often found in open areas with dry soil. We rarely found *S. geminata* colonies in the shaded areas under the trees. The colonies usually occupied an area > 1 m in diameter and could be > 50 cm deep depending on the nature of the soil. They often made the nest in a very safe place from flooding, or disturbance by human, *e.g.*, under the concrete of buildings or under the roots of living or dead trees (Figs. S1–S2). Thus, it was very difficult to get queen(s) from the mature colonies. In addition, during our surveys of fire ants, we did not find any colonies of *S. invicta*.

Crop plants associated with fire ants

*S. geminata* colonies were rarely found associated with big trees, *e.g.*, palm trees, rubber plants, and mangos; but were often found nearby small plants, *e.g.*, vegetable farms, rice or corn farms (Table 1). We also did not find *S. geminata* near the forest or uphill areas. However, it was often difficult to find them in crop fields, mostly likely, because of insecticide usage. We found them around the areas growing rice, corns, peanuts, rambutan, mangosteens, lanzones, vegetables (*e.g.*, morning glory, tomato, limes), and marigold flowers. In some parts of Thailand (*i.e.*, Northeastern) we could find *S. geminata* easily, possibly because they are likely more abundant there. This is unlikely an association with specific plant crops because we did not find them in the Northern areas growing the same plant crops. For the Southern part of Thailand, we did not find *S. geminata* in the mangosteen and rambutan fields but rather found them at the urban and seaside areas. We occasionally found that *S. geminata* tended aphids (*i.e.*, peanut fields) and caused problems for some crops, *e.g.*, stealing vegetable seeds, biting tomato seedlings, causing damage on
the flower of lanzone trees and thus depressing fruit yield, and causing burn spots on rambutans. *S. geminata* were always a nuisance to farmers due to their painful stings.

**Colony social form and genetic relatedness**

All of the 38 collected colonies were *S. geminata*. We determined the social form for 23 of the colonies by genotyping 24 workers per colony, followed by sibship analysis using Colony software (Jones and Wang 2010). This analysis revealed that all 23 were polygynous with the minimum number of queens ranging from two to seven (Table 2). Of these 23 colonies, four colonies had two queens, seven colonies had three queens, eight colonies had four queens, three colonies had five queens and only one colony had seven queens. The average number of queens in the colonies was 3.5.

**Table 2.** Number of queens, relatedness, observed heterozygosity (H\textsubscript{O}), expected heterozygosity (H\textsubscript{E}) and average number of alleles per locus for each *S. geminata* colony

<table>
<thead>
<tr>
<th>Colony</th>
<th>No. of queens</th>
<th>Average queen relatedness</th>
<th>Average worker relatedness</th>
<th>H\textsubscript{O}</th>
<th>H\textsubscript{E}</th>
<th>No. of Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sge04</td>
<td>4</td>
<td>0.97 ± 0.037</td>
<td>0.912± 0.064</td>
<td>0.16 ± 0.03</td>
<td>0.14 ± 0.053</td>
<td>1.57 ± 0.535</td>
</tr>
<tr>
<td>Sge06</td>
<td>4</td>
<td>0.97 ± 0.036</td>
<td>0.913± 0.064</td>
<td>0.34 ± 0.037</td>
<td>0.23 ± 0.112</td>
<td>1.86 ± 0.9</td>
</tr>
<tr>
<td>Sge17</td>
<td>3</td>
<td>0.68 ± 0.211</td>
<td>0.808± 0.132</td>
<td>0.35 ± 0.039</td>
<td>0.29 ± 0.1</td>
<td>1.86 ± 0.69</td>
</tr>
<tr>
<td>Sge19</td>
<td>3</td>
<td>1 ± 0</td>
<td>0.934± 0.049</td>
<td>0.4 ± 0.041</td>
<td>0.25 ± 0.102</td>
<td>1.83 ± 0.753</td>
</tr>
<tr>
<td>Sge01</td>
<td>3</td>
<td>0.67 ± 0.035</td>
<td>0.838± 0.101</td>
<td>0.59 ± 0.032</td>
<td>0.39 ± 0.062</td>
<td>2.2 ± 0.632</td>
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<tr>
<td>Sge16</td>
<td>3</td>
<td>0.80 ± 0.078</td>
<td>0.883± 0.050</td>
<td>0.46 ± 0.032</td>
<td>0.3 ± 0.07</td>
<td>1.8 ± 0.632</td>
</tr>
<tr>
<td>Sge05</td>
<td>3</td>
<td>0.60 ± 0.160</td>
<td>0.806± 0.148</td>
<td>0.52 ± 0.04</td>
<td>0.35 ± 0.075</td>
<td>1.86 ± 0.378</td>
</tr>
<tr>
<td>Sge36</td>
<td>2</td>
<td>0.83</td>
<td>0.866± 0.115</td>
<td>0.4 ± 0.038</td>
<td>0.29 ± 0.104</td>
<td>1.71 ± 0.756</td>
</tr>
<tr>
<td>Sge34</td>
<td>5</td>
<td>0.90 ± 0.043</td>
<td>0.841± 0.141</td>
<td>0.41 ± 0.044</td>
<td>0.3 ± 0.095</td>
<td>2.17 ± 0.408</td>
</tr>
<tr>
<td>Sge18</td>
<td>2</td>
<td>0.92</td>
<td>0.956± 0.042</td>
<td>0.28 ± 0.042</td>
<td>0.17 ± 0.107</td>
<td>1.4 ± 0.548</td>
</tr>
<tr>
<td>Sge39</td>
<td>5</td>
<td>0.61 ± 0.163</td>
<td>0.720± 0.183</td>
<td>0.63 ± 0.042</td>
<td>0.48 ± 0.08</td>
<td>2.83 ± 0.753</td>
</tr>
<tr>
<td>Sge30</td>
<td>4</td>
<td>0.95 ± 0.039</td>
<td>0.878± 0.093</td>
<td>0.36 ± 0.043</td>
<td>0.25 ± 0.108</td>
<td>1.83 ± 0.753</td>
</tr>
<tr>
<td>Sge30\textsuperscript{M}</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td>0.23 ± 0.029</td>
<td>0.12 ± 0.074</td>
<td>1.33 ± 0.5</td>
</tr>
<tr>
<td>Sge31</td>
<td>4</td>
<td>0.44 ± 0.272</td>
<td>0.911± 0.114</td>
<td>0.5 ± 0.043</td>
<td>0.29 ± 0.103</td>
<td>2 ± 1.095</td>
</tr>
<tr>
<td>Sge27</td>
<td>7</td>
<td>0.93 ± 0.061</td>
<td>0.961± 0.078</td>
<td>0.09 ± 0.044</td>
<td>0.08 ± 0.083</td>
<td>1.5 ± 0.707</td>
</tr>
<tr>
<td>Sge28</td>
<td>4</td>
<td>0.63 ± 0.306</td>
<td>0.897± 0.161</td>
<td>0.07 ± 0.036</td>
<td>0.06 ± 0.062</td>
<td>1.5 ± 0.707</td>
</tr>
<tr>
<td>Sge13</td>
<td>4</td>
<td>0.83 ± 0.072</td>
<td>0.891± 0.075</td>
<td>0.35 ± 0.038</td>
<td>0.26 ± 0.101</td>
<td>1.71 ± 0.756</td>
</tr>
<tr>
<td>Sge15</td>
<td>4</td>
<td>0.87 ± 0.138</td>
<td>0.954± 0.044</td>
<td>0.17 ± 0.025</td>
<td>0.11 ± 0.057</td>
<td>1.4 ± 0.516</td>
</tr>
<tr>
<td>Sge25</td>
<td>4</td>
<td>0.93 ± 0.027</td>
<td>0.944± 0.052</td>
<td>0.27 ± 0.031</td>
<td>0.17 ± 0.073</td>
<td>1.44 ± 0.527</td>
</tr>
<tr>
<td>Sge03</td>
<td>5</td>
<td>0.83</td>
<td>0.863± 0.121</td>
<td>0.23 ± 0.034</td>
<td>0.19 ± 0.075</td>
<td>1.71 ± 0.488</td>
</tr>
<tr>
<td>Sge09</td>
<td>2</td>
<td>1</td>
<td>0.846± 0.200</td>
<td>0.74 ± 0.067</td>
<td>0.44 ± 0.07</td>
<td>2 ± 0</td>
</tr>
<tr>
<td>Sge10</td>
<td>3</td>
<td>1 ± 0</td>
<td>0.967± 0.064</td>
<td>0.05 ± 0.026</td>
<td>0.04 ± 0.043</td>
<td>1.33 ± 0.577</td>
</tr>
<tr>
<td>Sge02</td>
<td>2</td>
<td>1</td>
<td>0.951± 0.053</td>
<td>0.24 ± 0.027</td>
<td>0.15 ± 0.062</td>
<td>1.64 ± 0.505</td>
</tr>
<tr>
<td>Sge32</td>
<td>3</td>
<td>0.96 ± 0.032</td>
<td>0.954± 0.037</td>
<td>0.45 ± 0.037</td>
<td>0.26 ± 0.097</td>
<td>1.63 ± 0.744</td>
</tr>
</tbody>
</table>

± indicates standard deviation.
Colony Sge30 (Chanthaburi), which was predicted to have four queens, was the only one where we were able to capture a queen. We isolated this queen with some workers and maintained this subcolony (Sge30\textsuperscript{M}) for about one year before collecting her workers for genotyping. Sibship analysis predicted that these workers were derived from one singly-mated queen, as expected.

Queens were unrelated between nests, with the average values for all pairwise comparisons of -0.037 ± 0.34. In contrast, queen relatedness values were high within nests, ranging from 0.58 ± 0.15 (Sge31) to 1 (Sge09, Sge10, Sge02 and Sge19) (Table 2) with the average value for all colonies of 0.85 ± 0.15. The genetic relatedness values of queens within nests were significantly different from that of queens between nests (\(p\)-value < 0.001, two-sided Wilcoxon Rank-Sum Test) (Fig. 2). Given the higher within nest relatedness values, we considered the possibility that queens might be sisters. Sisters would share the same paternal alleles. Detailed examination of the predicted queen genotypes revealed, that indeed, this might be the case for most queens (55 of the total of 82 queens from 20 colonies; Table S2). Similarly, the values of average within-nest relatedness of workers were also very high (> 0.7) in all colonies and the average across all colonies was 0.89 ± 0.06.

**Fig. 2.** Genetic relatedness (Queller and Goodnight 1989) of queens within nests was significantly different (\(p\)-value < 0.001, two-sided Wilcoxon Rank-Sum Test) from relatedness between nest.
Allelic diversity and population structure

We found that genetic diversity in S. geminata in Thailand was lower than the native range. The average number of alleles per locus across all colonies was 5.15 (Table 3). Within each colony, average numbers of alleles per locus ranged between 1.33 and 2.83 (mean = 1.755) (Table 2) compared to the native populations which was 5.27 (Gotzek et al. 2015). In contrast with the low allelic diversity, the average observed heterozygosity found in this study was not different from that of native populations. The observed heterozygosity (H\textsubscript{O}) in each colony ranged between 0.05 (Sge10) and 0.74 (Sge09), and expected heterozygosity (H\textsubscript{E}) ranged between 0.04 (Sge10) and 0.48 (Sge39) (Table 2). The averages of H\textsubscript{O} and H\textsubscript{E} across all loci were 0.33 and 0.49, respectively (Table 3), while the average H\textsubscript{O} and H\textsubscript{E} in native range is 0.358 and 0.587, respectively (Gotzek et al. 2015). The locus with highest heterozygosity (H\textsubscript{O} = 0.564, H\textsubscript{E} = 0.732) and highest polymorphic information content (PIC, 0.682) was C367, and the locus with lowest heterozygosity (H\textsubscript{O} = 0.0418, H\textsubscript{E} = 0.3441) and lowest PIC (0.301) was Sol-49.

<table>
<thead>
<tr>
<th>Locus</th>
<th>No. of alleles</th>
<th>H\textsubscript{O}</th>
<th>H\textsubscript{E}</th>
<th>PIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>C368</td>
<td>5</td>
<td>0.5648</td>
<td>0.4695</td>
<td>0.3953</td>
</tr>
<tr>
<td>C334</td>
<td>4</td>
<td>0.4153</td>
<td>0.361</td>
<td>0.3028</td>
</tr>
<tr>
<td>C121</td>
<td>6</td>
<td>0.4368</td>
<td>0.7078</td>
<td>0.6713</td>
</tr>
<tr>
<td>C367</td>
<td>4</td>
<td>0.5639</td>
<td>0.7324</td>
<td>0.6822</td>
</tr>
<tr>
<td>C485</td>
<td>7</td>
<td>0.1234</td>
<td>0.4888</td>
<td>0.4216</td>
</tr>
<tr>
<td>Sol-11</td>
<td>7</td>
<td>0.364</td>
<td>0.5098</td>
<td>0.4678</td>
</tr>
<tr>
<td>Sol-42</td>
<td>3</td>
<td>0.4947</td>
<td>0.4427</td>
<td>0.3508</td>
</tr>
<tr>
<td>Sol-49</td>
<td>3</td>
<td>0.0418</td>
<td>0.3441</td>
<td>0.3009</td>
</tr>
<tr>
<td>Sol-55</td>
<td>7</td>
<td>0.2787</td>
<td>0.4834</td>
<td>0.461</td>
</tr>
<tr>
<td>M-II</td>
<td>6</td>
<td>0.1787</td>
<td>0.6828</td>
<td>0.6347</td>
</tr>
<tr>
<td>M-III</td>
<td>4</td>
<td>0.5395</td>
<td>0.5046</td>
<td>0.4436</td>
</tr>
<tr>
<td>M-IV</td>
<td>5</td>
<td>0.0952</td>
<td>0.4605</td>
<td>0.3963</td>
</tr>
<tr>
<td>M-V</td>
<td>6</td>
<td>0.1941</td>
<td>0.2779</td>
<td>0.2674</td>
</tr>
</tbody>
</table>

Despite a low number of alleles found in the S. geminata populations, genetic differentiation between colonies was very high in general (Table S3). Most colony pairs (>90%) had F\textsubscript{ST} values > 0.25. The minimum F\textsubscript{ST} value was 0 (Sge10 vs. Sge30) and the maximum was 0.919 (Sge02 vs. Sge10). To examine if the colonies were genetically structured, we also conducted STRUCTURE analysis. We found that the 23 colonies including subcolony Sge30\textsuperscript{M} were clustered into two groups shown in red and green color in the figure 3. We found two clusters of the populations in almost all parts of Thailand, except in the Northern and Northeastern part of Thailand (Fig. 4). To examine if
genetic distance ($F_{ST}$) correlates with geographic distance we examined Isolation-by-distance (IBD) but did not observe a significant IBD signal among local populations ($R_{XY} = 0.113; P = 0.171$)

**Fig. 3.** Population structure of 23 colonies of *S. geminata* in Thailand was clustered into two groups, cluster 1 (green) and cluster 2 (red).

**Fig. 4.** Locations of the 23 fire ant colonies analyzed in this study. Pie charts mapped onto the Thailand map indicate population structure proportions from figure 3.
DISCUSSION

Colony social form

We have conducted the first study, to our knowledge, on the social form and population structure of *Solenopsis geminata* in Thailand. We found that all *S. geminata* populations in Thailand were polygynous with only a few queens (2–7 queens). In comparison, previous studies in northwest Gainesville, Florida, USA and in Veracruz, Mexico reported queen numbers ranging from 16-31 (Adams et al. 1976; Mackay et al. 1990). This may be partly because of an underestimated number of queens from genotyping only 24 workers per colony, colonies in Thailand are not highly polygynous, or reproductive skew, a situation where a subset of queens contribute disproportionately more progeny (Ross 1988). Our observations are similar to those from the Galapagos Island (Williams and Whelan 1991) and the common observation of polygyny in invasive population in the old world by Gotzek et al. (2015), but contrasts with finding only the monogynie social form of *S. geminata* in Taiwan (Lai et al. 2015). Polygyny level correlates with nest density for *S. invicta* (Ross and Keller 1995). If this is also the case for *S. geminata*, the low number of queens found in Thailand may be because of much lower nest density (> 20 m² per nest) compared to the native population such as in Brazil with 2,500–6,000 nests per hectare (0.6 m² per nest) (Mackay et al. 1990).

Polygynous colonies could be formed by budding or via pleometrosis, where unrelated queens cooperate in colony founding (Keller 1995; Ross and Keller 1995). We found very high within-nest relatedness among queens within a colony suggesting that *S. geminata* in Thailand may found colonies by the budding strategy rather than by pleometrosis. This is also consistent with the low population densities found in this study as pleometrosis is favored in high population density situations (Tschinkel and Howard 1983). Our observations are similar to those found for the *S. geminata* populations in the USA (Ross et al. 2003).

The very high relatedness values among queens within a colony could indicate that many queens were sisters (Table S2). A second potential explanation for the high relatedness values observed is that this is a consequence of the low number of alleles at each locus obtained in this study. Many alleles could be shared between unrelated individuals within each colony, potentially artificially inflating relatedness.

Ecology of *S. geminata*
We found that *S. geminata* preferred to nest in dry, open, sunny areas rather than wet areas, which is similar to a previous study (Harris et al. 2005). *S. geminata* is often found in urban areas and many crop fields in Thailand, including e.g., rice, corns, peanuts, rambutan, mangosteens, lanzones, morning glories, tomato, limes, and marigold flowers. We also found that *S. geminata* is a pest for humans as well as agricultural plants as has previously been reported (Risch and Carroll 1982; Way and Khoo 1992). We rarely found many *S. geminata* nests in the same area, again indicating low population densities of *S. geminata* in Thailand compared to its populations in native areas (Mackay et al. 1990). This may be due to the wide and extensive usage of pesticides in agricultural areas in Thailand.

**Population structure**

We found very low genetic variation for *S. geminata* in Thailand. The average number of alleles per locus for each colony / population was very low (mean 1.755) compared to the native population (mean 5.267) (Gotzek et al. 2015). However, we could not compare each locus in this study to the result from Gotzek et al. (2015) due to the unavailability of the raw data but we could compare our result to the previous studies for some particular loci. For example, for loci C368, C121, C367 and C485, the numbers of alleles in the *S. geminata* native population were 13, 15, 8, and 12, respectively (Ascunce et al. 2009), while our study had only 5, 6, 4, and 7, respectively. This may be because as an invasive species in Thailand (Gotzek et al. 2015), *S. geminata* has presumably experienced a genetic bottleneck (Harris et al. 2005). Low genetic diversity of *S. geminata* in Thailand was also correlated with our results that only two clusters of population were found (Fig. 2), which is similar to only a few clusters of invasive populations of *S. invicta* found in Taiwan, China and Australia (Ascunce et al. 2011). Because our surveyed colonies were all polygynous, an additional reason for low genetic variation could lie with the proposed evolutionary origin of polygyne *S. geminata*. At least for one US population, the model is that they are derived from monogyne populations that have gone through extensive genetic bottlenecks and reproductive isolation (Ross et al. 2003). Not finding any monogyne colonies also implies that polygyny probably evolved outside of and prior to the invasion of Thailand.

Despite the low number of alleles in the *S. geminata* populations, heterozygosity was high in most populations (Table 2), which was similar to the study in native populations (Gotzek et al. 2015). High heterozygosity was found to be associated with the haplodiploid sex determination system, specifically those using a complementary sex determination system, (Ross and Fletcher 1985b; Ross 1993). Two colonies, Sge28 and Sge10, did display lower heterozygosity, which may
be due to low allelic diversity at most loci, and a low number of loci (3 loci) successfully genotyped. Additionally, this may be partly due to some technical problems on DNA quality in these colonies and lower signal from indirect labeling.

The $F_{ST}$ values were also generally very high between colonies (Table S3) suggesting high genetic differentiation and low gene flow among colonies in this study (Wright 1921 1922). This is likely due in part because most colonies were in different provinces with very long geographic distances. Our results contrast with a study by Ross et al. (2003) who found low genetic differentiation between polygyne colonies, however a major difference was their samples were not far away (all within 15.6 miles) compared to our study (the average geographic distance among colonies at 430 Km.; Table S3). Although some colonies were in the same province (e.g., Sge02, Sge03, Sge09, and Sge10), the colonies we excavated were not close to each other (>50 m).

If *S. geminata* in Thailand were a continuous population with limited dispersal, relatedness values ($F_{ST}$) would be negatively correlated with the geographical distance, i.e., IBD. However, we did not observe IBD. Thus, we suggest that perhaps Thailand populations undergo metapopulation dynamics with local extinctions and recolonizations (Levins 1969; Hanski 1998). In line with metapopulations, *S. geminata* is a small insect frequently found occupying fragmented areas (e.g., near crop fields or edges of urban areas). They appear to have low dispersal patterns, as indicated by colony founding presumably through a budding strategy often composed of sister queens (Table S2), constraining them to their locality. Given their low population densities, and exacerbated by competition with native species and by pesticide use, subpopulations are likely prone to extinction. Recolonization would then occur through rare long distance dispersal by mating flights and, more probably, via widespread human transport of agricultural products or soil.

Another possible, and perhaps complementary, explanation for our population genetics results (i.e., two genetic clusters, significant $F_{ST}$, lack of IBD, and apparent limited dispersal) is that multiple invasions of *S. geminata* into Thailand has occurred. This is a distinct possibility as *S. geminata* is commonly intercepted by quarantine officials at the borders of many countries (Ward et al. 2006; Bertelsmeier et al. 2018; Suhr et al. 2019; Wylie et al. 2020). Further studies will shed light on the number of invasions into Thailand.

Despite their low density and patchy distribution, we did find *S. geminata* throughout the country, and thus, the invasion of *S. geminata* in Thailand can be considered successful. Importantly, a closely related species, *S. invicta*, which is far more aggressive and is now invading many Asian countries anew, draws our attention because if it were to invade successfully, it could spread throughout the country and result in greater ecological problems. The regular monitoring for *S. geminata* and other alien *Solenopsis* species, especially *S. invicta*, are thus recommended for the protection of Thailand’s native species and ecosystems.
CONCLUSIONS

Our study is the first study to explore colony structure of *S. geminata* in Thailand and our finding of low genetic diversity supports previous studies that *S. geminata* is invasive in South-East Asia. Further study on social forms and niches of *S. geminata* in South-East Asia would be important for understanding the ecology of this invasive species.

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Authors’ contributions: MN acquired funding and wrote the manuscript. MN and SH performed the fieldwork. SH confirmed morphological identification of fire ant samples. JW aided for grant proposal submission, optimized lab protocol and data analysis. MN and AS analyzed microsatellite data. All authors contributed to revising and approved the manuscript.

Competing interests: MN, SH, AS, and JW declare that they have no conflict of interest.

Availability of data and materials: Raw data can also be obtained upon request.

Consent for publication: Not applicable

Ethics approval consent to participate: Not applicable

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Supplementary Materials

**Fig. S1.** Characteristics of *S. geminata* nests. Nests are flat and have many entrances scattered around (A-B). Nests can be deeper than 30cm (C–D). Red arrows indicate larval chambers. (download)

**Fig. S2.** Fire ant nests are often found in a very secure place, *e.g.*, under hard soil (A), in the roots of palm trees (B), roots of dead trees (C), and in the concrete of buildings (D). (download)

**Table S1.** Microsatellite primers used in this study. (download)

**Table S2.** Full-sib analysis of the predicted queens from the same colonies. (download)

**Table S3.** Genetic differentiation as $F_{ST}$ (above diagonal) and geographic distance (km, below diagonal) between the 23 colonies of *S. geminata*. Population pairs where genetic differentiation test was significant (after sequential Bonferroni correction) are in bold. (download)