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Final report

Project Title: Colony's queen number of the invasive tropical fire ant, *Solenopsis geminata* in Thailand and some ecological factors associated with social form

By Ms. Mingkwan Nipitwattanaphon

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Contract No. TRG5780279

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บทคัดย่อ

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ชื่อโครงการ: จำนวนราชินีในรังมดคันไฟ *Solenopsis geminata* ในประเทศไทย และนิเวศวิทยาที่เกี่ยวข้องกับจำนวนราชินีในรัง

นางสาวมิ่งขวัญ นิพิฐวัธนะผล ภาควิชาพันธุศาสตร์ คณะวิทยาศาสตร์ มหาวิทยาลัยเกษตรศาสตร์

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ระยะเวลาโครงการ: 2 ปี

บทคัดย่อ:

มดคันไฟเป็นมดรุกรานซึ่งกำลังแพร่กระจายไปทั่วทุกทวีปของโลก และก่อให้เกิดความเสียหายต่อระบบนิเวศอย่างร้ายแรง โดยเฉพาะอย่างยิ่ง *Solenopsis invicta* มดคันไฟมีชีววิทยาที่น่าสนใจโดยเฉพาะการมีพันธุกรรมที่เกี่ยวข้องกับจำนวนราชินีภายในรังซึ่งเป็นปัจจัยหลักในการแพร่กระจาย มดคันไฟที่พบทั่วไปในประเทศไทยคือ *S. geminata* ซึ่งพันธุกรรมที่เกี่ยวข้องกับจำนวนราชินีภายในรังยังไม่เป็นที่ทราบดี ในการศึกษาครั้งนี้ ผู้วิจัยได้สำรวจและเก็บตัวอย่างมดคันไฟในจังหวัดต่าง ๆ ครอบคลุมทุกภาคของประเทศไทยทั้งหมด 38 โคโลนี (รัง) และศึกษานิเวศวิทยาที่เกี่ยวข้องกับมดคันไฟในพื้นที่พบว่า มดคันไฟมีการกระจายตัวอยู่ตามพื้นที่โล่ง แดดจัด ซึ่งพบได้ทั้งบริเวณรอบ ๆ บ้านและในพื้นที่การเกษตรหลายชนิด ได้แก่ ข้าว ข้าวโพด ถั่วลิสง เงาะ มังคุด ลองกอง และผักต่าง ๆ รวมถึงแปลงดอกไม้ ผู้วิจัยได้ใช้เครื่องหมายดีเอ็นเอชนิดไมโครแซทเทลไลท์ในการศึกษาจีโนไทป์ของมดคันไฟทั้งหมด 23 โคโลนีจากทุกภาคของประเทศไทยในการหาจำนวนราชินีภายในรัง และพบว่ามดคันไฟในประเทศไทยมีทั้งแบบที่มีจำนวนราชินีเพียงหนึ่งตัว (monogyne) และแบบที่มีราชินีหลายตัว (polygyne) ในรัง ซึ่งผลการวิจัยครั้งนี้เป็นที่น่าสนใจต่อการศึกษาพันธุกรรมที่เกี่ยวข้องกับการกำหนดจำนวนราชินีในมดคันไฟชนิดนี้ต่อไปเนื่องจากมดคันไฟชนิดนี้มีระบบการควบคุมจำนวนราชินีที่แตกต่างจากมดคันไฟชนิดอื่น ๆ ในสกุลเดียวกัน

คำหลัก : monogyne, polygyne, population structure, pest

Abstract

Project Code : TRG5780279

Project Title : Colony's queen number of the invasive tropical fire ant, *Solenopsis geminata* in Thailand and some ecological factors associated with social form

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Abstract:

The invasive fire ants, *Solenopsis invicta*, have been reported to have significant ecological damage in many parts of the world yet the presence of this species in Thailand should be investigated. A closely related species, *S. geminata*, is a major pest but little is known about their biology and ecology on colony structure. Colony social form is a fundamental unit of social insect societies. A colony can have either a single queen or many queens depending on their genetic background. To understand the biology of social form of *S. geminata* in Thailand, we observed the ecology of 38 colonies from six different parts of Thailand and found that *S. geminata* were likely to be opened to the sun, warm and not too humid. Plant crops that they were associated were rice, corns, peanuts, rambutan, mangosteens, lanzones, vegetables, and marigold flowers. We used genetic data to investigate number of queens in 23 colonies and found that both single and multiple queens were co-occurred. These two types of colonies encourage for future research on genetic of colony social form in *S. geminata*, which uses different genetic system from other *Solenopsis* species.

Keywords : monogyne, polygyne, population structure, pest

Executive summary

1. Introduction

It is incontrovertible that ants have great ecological consequence since they occupy about 30% of the biomass and they are the foremost predators and scavengers. The fire ant, *Solenopsis invicta*, is the top most studied species (http://myrmecos.net/2009/01/25/the-most-studied-ant-species-are-either-trampy-or-european/) because of its interesting biology and its ecological importance. *S. invicta* successfully compete other native ants due to their aggressiveness and a painful sting. The sting of fire ants composed of venom that could cause allergy to human e.g. itching, swelling, redness of the skin and pus. Fire ants also tend aphids. Thus, they are considered as a major pest in many continents around the world (Tschinkel 2006). However, due to they are important predator of insects, fire ants have a potential to control other pests (Diaz *et al.* 2002; Kaplan & Eubanks 2002; Galarraga 2003).

There are many species of fire ants (e.g. *S. daguerrei, S. fugax, S. geminata, S. invicta Buren, S. richteri, S. saevissima, S. silvestrii, S. solenopsidis, S. wagneri Santschi, S. xyloni*), but the most well known is the *S. invicta* because it is the most nuisance species that greatly affect the diversity of other insect species and replace other native ant species (Tschinkel 2006). However, the only fire species so far found in Thailand is *S. geminata* (Bourmas *et al.* 2001; Hasin 2008; Sakchoowong *et al.* 2008; Jongjitvimol 2010; Etterer 2011; Noitubtim *et al.* 2012). The fire ant *S. invicta* and *S. geminata* both natively presence in tropical region of the New world, i.e. South America and then later introduced to the southern United States and other parts of the world. Thus, both species are considered as invasive ants. While *S. geminata* has long been known as a global pest around the world, *S. invicta* was recently reported to spread in Australia and in Asia (in China, Hong Kong and Taiwan) (Ascunce *et al.* 2011). Although *S. invicta* is still less common compared to *S. geminata*, *S. invicta* is more dangerous as if it is introduced, it can even replace the *S. geminata* and very difficult to eradicate (Etterer 2011).

Fire ants also posses interesting biology, especially variation of social forms. A colony can be headed by a single queen (monogyne) or many queens (polygyne), depending on their genetic background (Keller & Ross 1998; Ross & Keller 1998), which is recently known as a supergene containing about 616 genes (Wang *et al.* 2013). This supergene has two variants, SB and Sb, containing the two variants of an odorant binding protein gene called *Gp-9*, $Gp\text{-}9^{B}$ and $Gp\text{-}9^{b}$ allele. Queens in monogyne colonies are always SB/SB genotype (previously known as $Gp\text{-}9^{BB}$), while queens in polygyne colonies are always SB/Sb genotype (previously known as $Gp\text{-}9^{Bb}$). SB/Sb workers are invariably accept more reproductive queens but only if they are SB/Sb genotype, while they always kill any reproductive SB/SB queen that has entered in the colony. Thus, the process of queen recognition and the odor produced by queens must be associated with genes in this supergene.

The two social forms, monogyne and polygyne colonies also differ in many aspects, such as the mode of colony founding (Monogyne queens found colony alone without workers while polygyne queens found colony by colony budding) and the level of between-colony aggression (greater in monogynes) (Ross & Keller 1995;

DeHeer *et al.* 1999; Goodisman *et al.* 2000; DeHeer 2002). All differences between monogyne and polygyne colonies are perfectly associated with a suite of morpho- logical, physiological and life history differences between individuals with different supergene genotypes, including the amount of fat accumulated by maturing queens, queen fecundity (Keller & Ross 1999), the proportion of saturated hydrocarbons on the queen's cuticle (Eliyahu *et al.* 2011), worker size, which are all greatest in SB/SB females (Goodisman *et al.* 1999) and sperm count (greater in SB haploid males) (Lawson *et al.* 2012).

Like *S. invicta*, *S. geminata* also have two social forms, and the differences in mode of colony founding and life history traits between individuals of the two social forms seems to be similar to of *S. invicta*. However, genetic associated with the two social form seem to evolve independently as individual of the two social forms have identical *Gp-9* genotype (Mackay *et al.* 1990; Ross *et al.* 2003). However, there is no report about genes associated with social form in *S. geminata* so far. It would be interesting to find if they also have a supergene, or if they don't have a supergene, would other genes in the supergene of *S. invicta* also control social form in *S. geminata*? It is thus interesting to work on genetic controlling social form of *S. geminata* if we have both social forms in Thailand. However, since nothing is known about the social form of *S. geminata* in Thailand yet, we propose make a first study of social form investigation in this study.

If *S. geminata* in Thailand do not have both social forms, it is still interesting to work on other biological aspects, for example, sex-determination genes, ploidy effects in males of fire ants, and genetics of queen dimorphism. In ants, sex is determined by the genotype at the sex-determining locus, i.e. heterozygous individuals develop into females while homozygous or hemizygous individuals develop into males. Thus, in general, males are haploid because they develop from unfertilized eggs and females are diploid because they develop from fertilized eggs. However, diploid males are occasionally found if the queens were match mated. This sex-determining system is called "complementary sex-determining locus" and the sex-determining gene is thus called "complementary sex-determining gene (*CSD* gene)" (Crozier 1977). This sex-determining system is although universal in Hymenoptera but the mechanism is complex and the *CSD* gene is highly diverge among species. So far the *CSD* gene is only found in honeybees (Beye *et al.* 2003; Hasselmann *et al.* 2008; Gempe *et al.* 2009). We have been working on diploid males in *S. invicta* and found some candidate genes that might be *CSD* gene (unpublished) and we are going to do some functional approach test *S. invicta*. We are interested to find this gene and do some functional tests in other fire ant species. Thus, *S. geminata* would be one of the species in this study. In addition, diploid male fire ants have interesting biology. These males are morphologically identical to haploid males but they are larger and sterile. They are thus useless and are a major of cause of colony perishing especially in the monogyne social form. Given that they are useless, apparently workers cannot recognize them and eliminate them like diploid drones in honeybees (Herrmann *et al.* 2005). We have found some interesting candidate genes involved in the differences between biology of diploid vs. haploid males (submitted, in review) that would be interesting to investigate further in related species (i.e. *S. geminata*) if these genes are universally differentially expressed between males of different ploidy levels. In addition, the effects of ploidy on these diploid males are still unclear and thus better to investigate further. Finally, it has been reported that there are two types of queens, macrogynes and microgynes, in *S. geminata* and suggested that this could be genetic basis

(McInnes & Tschinkel 1995). It is interesting to investigate genes and environmental effects that regulate queen phenotypes.

Together, it is interesting to do a preliminary ecological study of *S. geminata* in Thailand, i.e. social form variations, seeds and crop field that they are associated, other insects that they are interact with (e.g. if they tend aphid and if this a major problem for agriculture; if they are parasitized by other insects; if they have an impact on predating other insects). We believe that this preliminary study will be useful for future studies for at least six aspects: 1) the genetics of social form in fire ants; 2) the genetics of sex-determination in ants; 3) the genetics associated with ploidy effects in fire ants; 4) the control of fire ants in Thailand (if they need to be controlled); 5) the use of fire ants to control other insects or other weed or glass in the farm; 6) the genetics underlying interactions between fire ants and other insects. We also believe that this study would also help us to investigate if *S. invicta* has settled in Thailand because this is a highly invasive species which recently has been introduced into many Asian countries (Malaysia, Philippines, South China and Taiwan), Australia and New Zealand (Etterer 2011; Ascunce *et al.* 2011) and has large impact on biodiversity losses, electrical damages, heath, and agriculture (Gutrich *et al.* 2007).

2. Literature review

3. Objective

1) To investigate if both types of social form, monogyne and polgyne, exist in *S. geminata* in Thailand.

2) To find ecological factors associated with the two social forms if both social forms exist in Thailand, e.g. mound shape, colony size, worker size,

3) To observe if there are colonies of *S. invicta* in Thailand.

4) To observe what crop fields that *S. geminata* likely to be associated and to determine the problems and/or benefits caused by *S. geminata*.

5) To observe insects that have interactions with *S. geminata* for future biological control studies.

4. Research methodology

4.1 sample collection

We collected a total of 38 **fire ants colonies from surrounding different crop fields (e.g. corn, rice, banana, lime, santols) and urban area in all six parts of Thailand (i.e Northern, Western, Eastern, Southern, Northeasthern and Central parts).** Of these, eight colonies came from five provinces in the Central part; five colonies were from three provinces at the Eastern part; six colonies were from four provinces at the Northeastern part; four colonies were from two provinces at the Northern part; five colonies were from three provinces at the southern part; and ten colonies were from three provinces at the Western part of Thailand (Table 1).

4.2 Ecological data associated with colonies

We observed the mound characteristics, e.g. shape, size, density and correlate this data to colony social form. We also recorded the position of the colonies, crop types, and interaction of fire ants with other insects. We dug up the mounds from the field and collected them into buckets. **These buckets were dripped overnight to separate fire ants from soils. Colonies were set up in the laboratory by using plastic boxes covered with fluon to prevent ant escape and artificial nest made from petridishes containg moisted plaster. Ants were fed with insects, tuna and honey using stadard method of colony rearing conditions (Jouvenaz *et al.* 1977). Number of reproductive queens were observed after the establishment of the colonies for at least a week. If there were any dealate queens, we rared them with some workers and brood for a longer period to ensure that the queens were inseminated and laid fertilized eggs. However, we did not obtained the firtile queens except one colony from Chanthaburi. So, we collected only workers from those colonies and extracted DNA from 50 workers per colony. For the colony that we obtained a fertile queen, we rared this colony in the laboratory for almost a year and finally collected workers for genotyping. Worker samples were put in 95% ethanol for species identification and DNA extraction follow by genotyping to confirm number of queens.**

Table 1. Coordinates of ant colonies that we have collected samples

No.	Province	Part of Thailand	Colony	Area information	Coordinate
1	Bangkok	C	Sol05	University area	13.842678863972,100.571508742869
2	Chai Nat	C	Sol36	Integrated farming (e.g. rice, santols) nest1	15.401400566101074,100.090476989746094
3	Chai Nat	C	Sol37	Integrated farming (e.g. rice, santols) nest2	15.401400566101074,100.090476989746094
4	Chai Nat	C	Sol38	banana and lime trees	15.236668586730957,100.072273254394531
5	Kamphaeng Phet	C	Sol33	bamboo trees	16.641086578369141,99.331634521484375
6	Kamphaeng Phet	C	Sol34	sugar apples	16.495395660400391,99.670669555664062
7	Phitsanulok	C	Sol18	vegetable farm	16.917720794677734,100.206192016601562
8	Supanburi	C	Sol39	07.09.2015	14.438979148864746,100.160659790039062
9	Chanthaburi	E	Sol29	mangosteens	12.635225296020508,102.003890991210938
10	Chanthaburi	E	Sol30	barn of rambutans, longazones, and mangosteens	12.637989044189453,101.997833251953125
11	Rayong	E	Sol31	vegetable farm	12.668457984924316,101.384704589843750
12	Trat	E	Sol27	barn of rambutans, longazones, and mangosteens	12.362780570983887,102.440780639648438
13	Trat	E	Sol28	palm tree near the lime trees	12.362063407897949,102.439987182617188
14	Kalasin	NE	Sol20	Cassava farm	16.535808563232422,103.428306579589844
15	Loei	NE	Sol19	near rambutan trees	17.481569290161133,101.629066467285156
16	Nakhon Ratchasima	NE	Sol01	vegetable farm	14.3660632,101.8913489
17	Sakon Nakhon	NE	Sol16	rice farm	17.383708953857422,104.102233886718750
18	Sakon Nakhon	NE	Sol22	urban area	17.390222549438477,104.104003906250000
19	Sakon Nakhon	NE	Sol23	rice farm	17.383708953857422,104.102233886718750
20	Chiang Mai	N	Sol04	corn farm	18.795713424682617,98.959815979003906
21	Chiang Mai	N	Sol06	University area	18.803651,98.953009
22	Lampang	N	Sol08	urban area	18.289726257324219,99.474670410156250
23	Lampang	N	Sol17	vegetable farm	18.299419403076172,99.459732055664062
24	Chumphon	S	Sol13	seaside	9.953162193298340,99.158515930175781

25	Chumphon	S	Sol21	urban area	9.734181404113770,99.101844787597656
26	Surat Thani	S	Sol24	urban area	8.927205085754395,99.275146484375000
27	Trang	S	Sol14	urban area	7.626449108123779,99.565010070800781
28	Trang	S	Sol15	seaside	7.341351985931396,99.373092651367188
29	Kanchanaburi	W	Sol25	near vegetable farm	14.123216629028320,99.319435119628906
30	Kanchanaburi	W	Sol26	corn farm	14.119938850402832,99.316696166992188
31	Phetchaburi	W	Sol02	lime tree	12.746504783630371,99.710754394531250
32	Phetchaburi	W	Sol03	corn farm	12.742479324340820,99.712272644042969
33	Phetchaburi	W	Sol08	urban area	12.727828979492188,99.755722045898438
34	Phetchaburi	W	Sol09	vegetable farm	12.742182731628418,99.712776184082031
35	Phetchaburi	W	Sol10	vegetable farm	12.742959022521973,99.712036132812500
36	Phetchaburi	W	Sol11	peanut farm	12.743312835693359,99.712013244628906
37	Phetchaburi	W	Sol12	marigold flower farm	12.747143745422363,99.710670471191406
38	Tak	W	Sol32	urban area	16.687381744384766,99.277435302734375

4.3 Social form identification by molecular method

Microsatellite genotyping

We use microsatellite data to determine number of queens. We first extract DNA using CTAB method (modified from Doyle, JJ and Doyle, 1987). We used the primers of 19 microsatellite loci (Table 2) that have been developed for fire ants to genotype 24 workers from each colony (Krieger & Keller 1997; Chen *et al.* 2003; Ascunce *et al.* 2009) but we later removed some primers due to difficulty for amplifying in most samples, non-specific or monomorphic. We used the labeling method developed by (Blacket *et al.* 2012) except for the two primers, M-II and M-V. PCR condition were done in a 10 µL reaction mix ture containing PCR buffer, 2 mM $MgCl_2$, 0.2 µg/µL dNTPmix, 0.5 µM of each F and R primer but 0.025 U for M13 primers (Blacket *et al.* 2012), 1 µL 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 on Eppendof 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 final elongation step at 72 °C (5 min). Samples of the PCR products (4 µL) were visualized on 2% agarose gel for initial checking before genotyping by fragment analysis using 23 ABI 3730XLs (Applied Biosystem). All genotyped were inspect using Peak Scanner Software (Applied Biosystem).

Queen determination and population structure analysis

Numbers of queens and males that were the father of the workers we genotyped were determined by Sibship Reconstruction (Wang 2004) and Sibship interference (Wang & Santure 2009) software (Colony software; Jones & Wang, 2010) using Full Likelihood (FL) analysis method with updated allele frequencies and no prior parameter setting.

We calculated number of alleles and observed and expected heterozygosity (H_o and H_e) of each colony by using Microsatellite Tool Kits (Park 2001). Since in some loci, we only obtained the genotype from a few individuals and to avoid the biased from only a few individual, we used the data from each locus each colony to calculate the average H_o and H_e only when >=70% (14 individuals) of the 24 individuals had readable genotyping data. We also calculated polymorphic information content (PIC) from all colonies. We tested if genetic variation of monogyne and polygyne colonies in terms of heterozygosity, number of alleles, and PIC, were different by using t-test (R Development Core Team 2010). We calculated the genetic differentiation between population of *S. geminata* colonies using FSTAT (Goudet 1995).

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 delta K (Evanno G, Regnaut S 2005). PCA GEN v.1.2 (Goudet 2005) was used for PCA analysis of microsatellite data.

Table 2. Microsatellite primers used in this study

No.	Primer name	T_a	Size range (bp)	Type of M13	Fluorescent dye	Remark
1	C147	60	-	M13 A	Fam	Genotyped but cannot read
2	C536	60	225	M13 A	Fam	Removed
3	C368	60	209-217	M13 C	Cy3	Genotyped
4	C334	60	225-229	M13 C	Cy3	Genotyped
5	C121	60	199-211	M13 B	Hex	Genotyped
6	C367	60	132-135	M13 D	Rox	Genotyped
7	C485	60	131-148	M13 A	Fam	Genotyped
8	C259	60	163	M13 A	Fam	Removed
9	C294	55	95-105	M13 C	Cy3	Removed
10	C216	55	236	M13 B	Hex	Removed
11	Sol-11	55	170-192	M13 A	Hex	Genotyped
12	Sol-42	55	150-154	M13 A	Hex	Genotyped
13	Sol-49	55	170-176	M13 C	Cy3	Genotyped
14	Sol-55	55	171-185	M13 C	Fam	Genotyped
15	M-I	55	-	M13 B	Rox	Removed
16	M-II	55	334-350	Direct labeling	Fam	Genotyped
17	M-III	55	220-224	M13 A	Fam	Genotyped
18	M-IV	55	332-358	M13 A	Cy3	Genotyped
19	M-V	55	330-386	Direct labeling	Hex	Genotyped

5. Results

5.1 Ecological data associated with colony social form

Nest structure.

Solenopsis geminata nests were flat compared to *S. invicta* and often found in an opened area, dry soil, rather than in the shadow area under the trees. Their nests have many holes on the top. The colony usually occupy >1m in diameter and could be >50cm depth depending on the nature of the soil. They often make the nest in a very safe place from flooding, or disturbing by human, e.g. under the concrete of the building, or under the roots of the trees or dead trees (Figure 1-2). Thus, most of the time we could not get queen(s) from the mature colonies. We did not observed any obvious differences between monogyne and polygyne nests.

Figure 1 Characteristics of fire ants nests. Nests are flat and have many entrances scatter around (A-B). Nests can be deeper than 30cm (C-D). Red arrows indicate larval chambers.

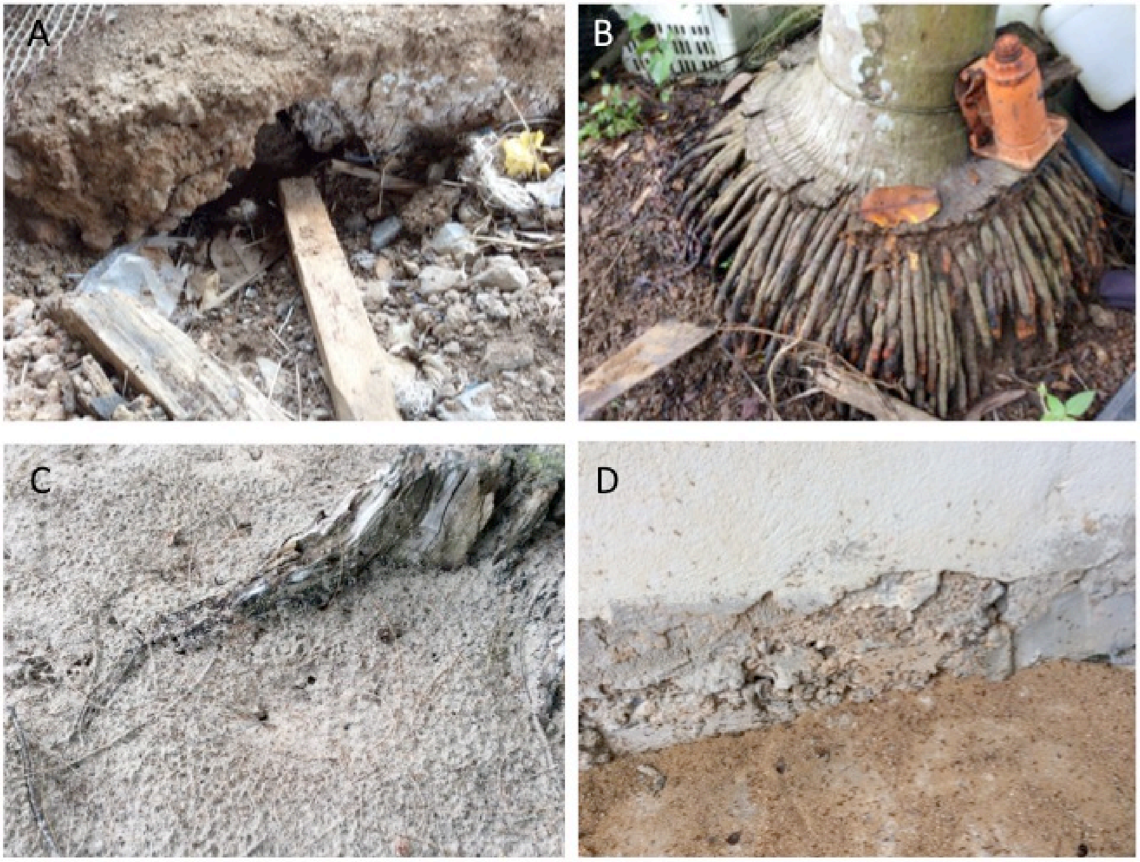

Figure 2 Fire ant nest are often found in a very secure place, e.g. under the hard soil (A), in the palm root (B) or dead tree's root (C), and in the concrete of the building (D).

Crop plants that they were associated

Fire ants were found to be unlikely to associated with big trees, e.g. palm trees and rubber plants, and mangos and prefer small plants e.g. vegetable farms, rice or corn farm (Figure 3-4, Table 1). We also did not find fire ant near the forest or uphill areas. However, it was often difficult to find them in agricultural areas because the usage of insecticide. We found them around the area growing rice, corns, peanuts, rambutan, mangosteens, lanzones, vegetables (e.g. morning glories, tomato, limes), and marigold flowers. In some parts of Thailand (i.e. Northeastern) fire ant might be prevalence as we can easily find them. This may not due to the plant crop that they are associated because we cannot find them in the area growing the same plant crops in the Northern part. For Southern part of Thailand, we did not find fire ants in the mangosteen and rambutan farm but rather found them on the urban area and seaside. Thus, we collected some colonies of urban areas and seaside instead.

Figure 4 Example of field crops where fire ants were found: A and B vegetable farm; C. Marigold flower; D corn.

Problem of fire ants on agriculture

We did not observe interaction of fire ants with other insects except in the peanut farm in Petchaburi where fire ants were tending aphids (Figure 5). Farmers usually want to eliminate fire ants if they found them in their farm because of their stings. Moreover, fire ants can cause problems in some crops, e.g. fire ants steal vegetable seeds, bite tomato seedling, cause damage on the flower of lanzone trees and then affect the fruit yield, and cause burn spots in rambutans.

Figure 5 Fire ants were tending aphids in the peanut farm, ChangHuaMan, Petchaburi District.

Species identification and observation of *S. invicta*

We have done the species identification for all samples we have collected so far. We did not find any *S. invicta* in Thailand.

Mating type and colony social form

For all 38 colonies we collected. All of them were *S. geminata* and we found both monogyne and polygyne form *S. geminata* in Thailand. Of a total of 23 colonies we genotyped, there were 9 colonies that had only a single queen and 14 colonies that had multiple queens. We found 2-3 queens were detected to be mothers of at least one of the 24 workers that were genotyped and each queen of both monogyne and polygyne form mated with one to five males. For the colony No. 9.1 (Table 3) which is the same colony as the No. 9 (Sol30) that we had obtained a single queen and reared it for at least 6 months, we also found that the Colony program predicted accurately that there was only a single queen. We found only polygyne form in the Northern and Southern part of Thailand whereas in other parts both monogyne (circle symbols) and polygyne (square symbols) forms existed (Figure 1).

Table 3. Number of queens and males that were the parents of workers for each colony.

No.	Colony	Type	No. of queens	No. of males	No. of males mated with queen
1	Sol03	Monogyne	1	5	5
2	Sol04	Polygyne	2	4	1-3
3	Sol05	Polygyne	2	5	1-4
4	Sol06	Polygyne	3	6	1-3
5	Sol09	Monogyne	1	3	3
6	Sol10	Polygyne	2	3	1-2
7	Sol27	Polygyne	2	3	1-2
8	Sol28	Polygyne	2	5	1-3
9	Sol30	Polygyne	2	5	1-4
10	Sol31	Polygyne	2	3	1-2
11	Sol34	Monogyne	1	5	5
12	Sol39	Polygyne	2	6	2-4
13	Sol17	Polygyne	2	4	2
9.1*	Sol30M	Monogyne	1	1	1
15	Sol01	Polygyne	2	5	1-4
16	Sol02	Monogyne	1	2	2
17	Sol15	Polygyne	2	4	1-3
18	Sol16	Monogyne	1	6	6
19	Sol18	Monogyne	1	2	2
20	Sol19	Monogyne	1	4	4
21	Sol13	Polygyne	2	7	3-4
22	Sol25	Monogyne	1	4	4
23	Sol32	Monogyne	1	5	5
24	Sol36	Polygyne	2	4	2

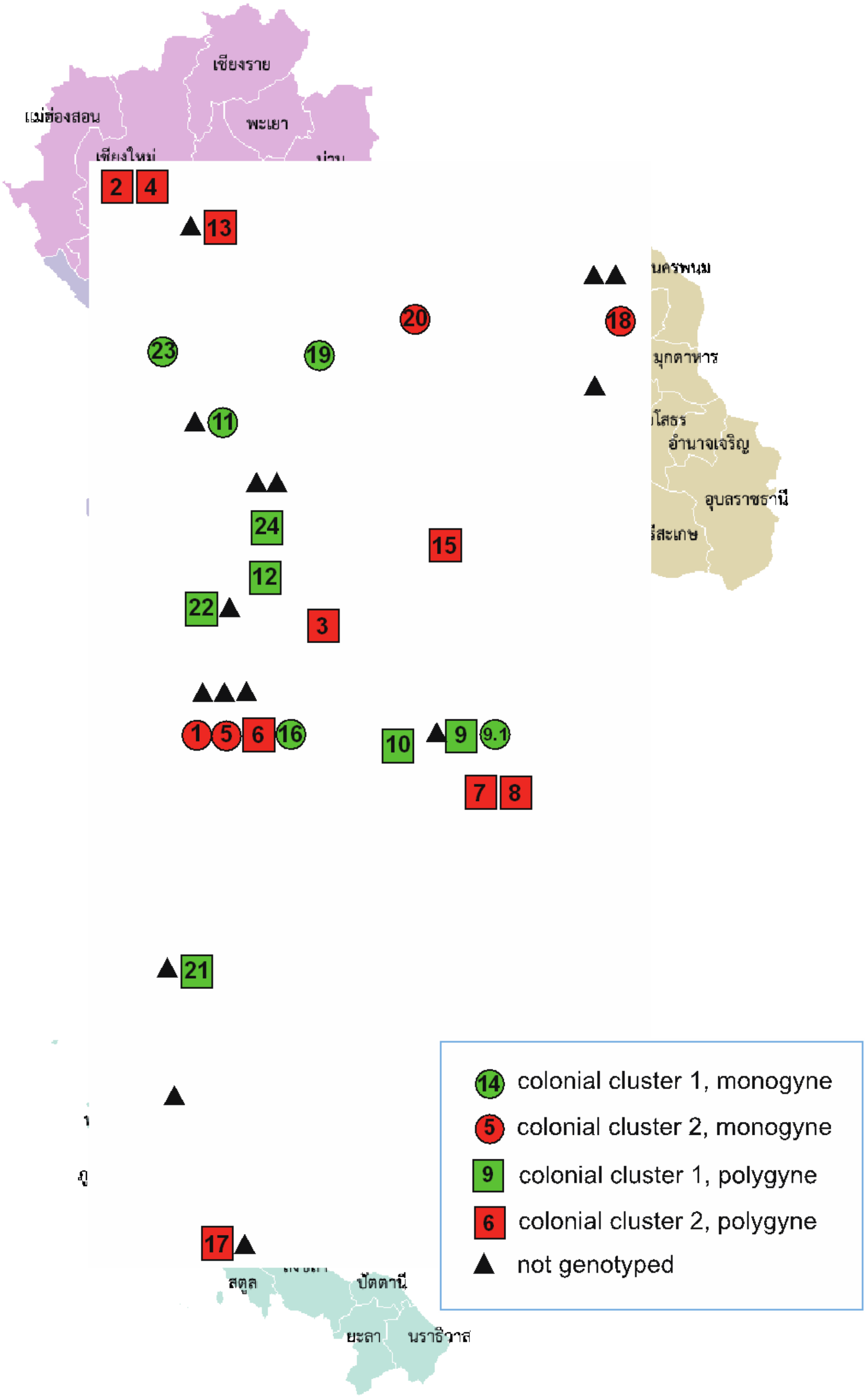

Figure 6 Show provinces from different parts (different colors) of Thailand where *S. geminata* colonies were collected. Monogyne colonies were marked with circles whereas polygyne colonies were marked with squares. Red and green indicate population structure, which was clustered into two groups, cluster 1 (green) and cluster 2 (red).

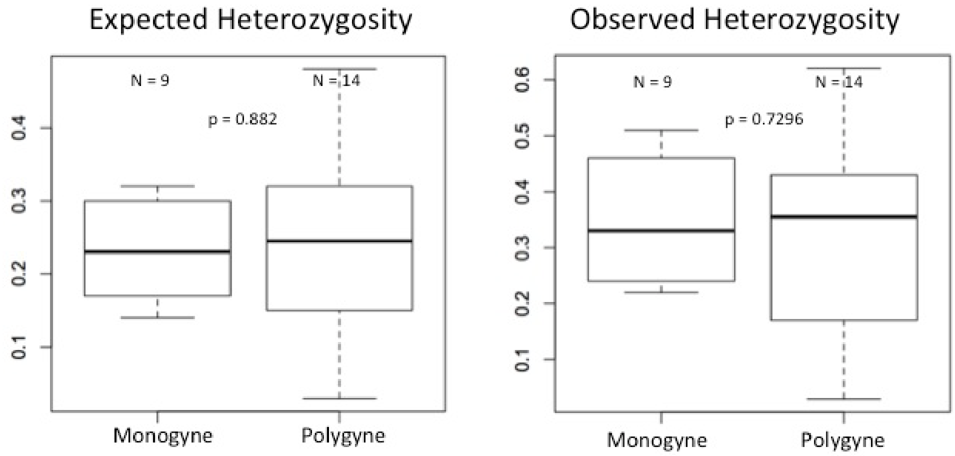

Figure 7 The average observed and expected heterozygosity was not different between the two social forms.

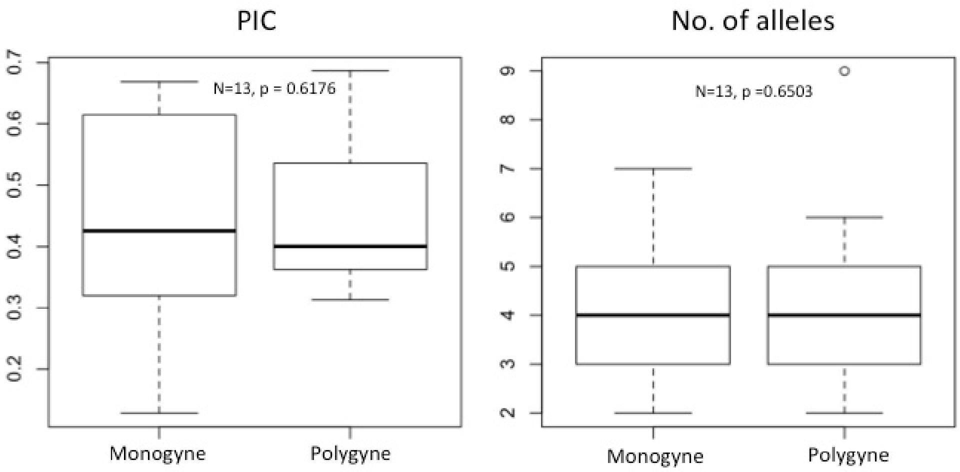

Figure 8 Number of alleles and PIC of monogyne and polygyne colonies were not different.

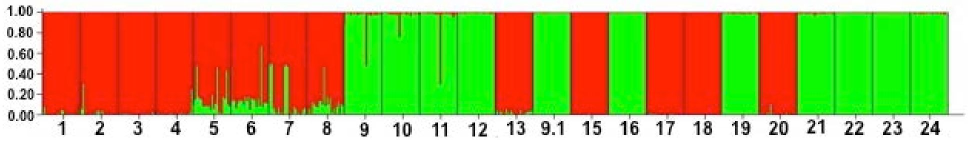

Figure 9 Population structure of 23 colonies of *S. geminata* in Thailand was clustered into two groups, cluster 1 (green) and cluster 2 (red). Colony numbers in the figure correspond to the data in the Table 3.

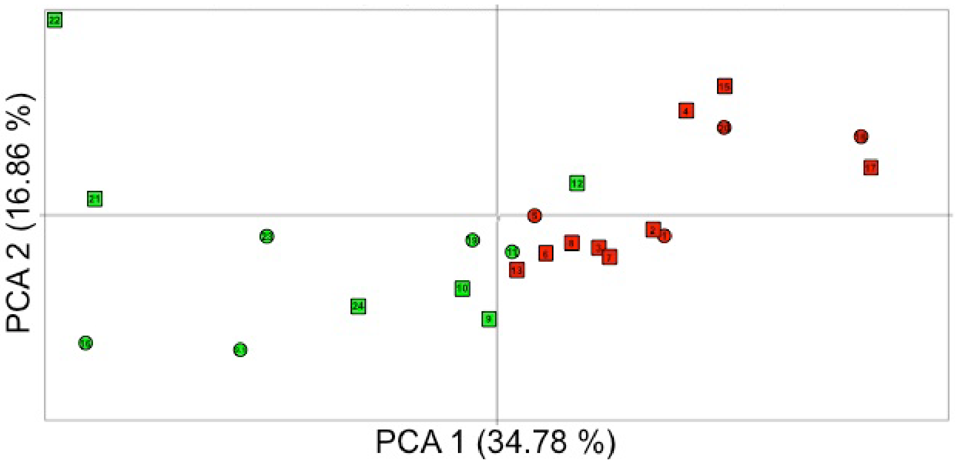

Figure 10 PCA analysis of the genotype data of 23 *S. geminata* colonies in Thailand. Green and red represent the clusters of the colonies. Monogyne and Polygyne colonies were indicated by circle and square dots. Numbers of colonies were put according to the Table 3.

Table 4. Observed heterozygosity, expected heterozygosity and average number of alleles per locus of each colony of *S. geminata.*

Colony	Social form	Ho	He	No. of alleles
Sol03	M	0.23	0.22	1.80
Sol09	M	0.49	0.32	1.80
Sol34	M	0.41	0.30	2.09
Sol30M	M	0.20	0.11	1.30
Sol02	M	0.22	0.14	1.58
Sol16	M	0.46	0.30	1.91
Sol18	M	0.28	0.17	1.57
Sol19	M	0.33	0.23	1.56
Sol25	M	0.24	0.16	1.42
Sol32	M	0.51	0.29	1.60
Sol04	P	0.16	0.17	1.60
Sol05	P	0.53	0.32	2.00
Sol06	P	0.33	0.23	1.91
Sol10	P	0.03	0.03	1.40
Sol27	P	0.06	0.06	1.40
Sol28	P	0.18	0.15	1.75
Sol30	P	0.33	0.24	1.82
Sol31	P	0.43	0.25	1.92
Sol39	P	0.62	0.48	2.44
Sol17	P	0.41	0.35	1.78
Sol01	P	0.59	0.38	2.09
Sol15	P	0.17	0.11	1.55
Sol13	P	0.38	0.32	1.58
Sol36	P	0.40	0.31	1.90

Table 5. Number of alleles and polymorphic information content (PIC) for each microsatellite marker

Locus	No. of alleles			PIC		
	M	**P**	**all**	**M**	**P**	**all**
C368	3	3	3	0.265	0.314	0.391
C334	2	2	2	0.616	0.686	0.296
C121	6	5	5	0.647	0.680	0.671
C367	4	4	4	0.479	0.362	0.682
C485	5	9	9	0.425	0.489	0.442
Sol-11	6	6	6	0.320	0.360	0.468
Sol-42	2	3	3	0.251	0.349	0.351
Sol-49	3	3	3	0.615	0.628	0.298
Sol-55	7	6	6	0.668	0.536	0.653
M-II	4	5	5	0.430	0.375	0.635
M-III	3	3	3	0.366	0.417	0.406
M-IV	4	4	4	0.128	0.379	0.396
M-V	4	4	4	0.373	0.401	0.267

Population structure

We found that genetic variation of *S. geminata* in Thailand was very low. When we analyzed by separated population according to the colonies, the observed (H_o) ranged between 0.03 (Sol10) to 0.62 (Sol39), and expected heterozygosity (H_e) ranged between 0.03 (Sol10) to 0.48 (Sol39) (Table 4) with the average of 0.333 and 0.235. The average numbers of alleles per locus for each colony ranged between 1.3-2.44 and the average was 1.74. When we analyzed all colonies as a single population in Thailand, we found that the H_o was 0.2961 and the H_e was 0.4449. The average number of allele per locus for all colonies was 4.76. We also found that the heterozygosity (H_o and H_e), number of alleles and PIC were not significantly different between monogyne and polygyne colonies (Table 5, Figure 7 and 8).

Despite very low genetic diversity and low number of alleles found in the *S. geminata* population, the genetic differentiation between colonies was very high in general (Table 6). The minimum *FST* value was 0.115 and the maximum was 0.929. The populations of all 23 colonies including a subcolony (No. 9.1) were clustered into two groups shown in red and green color in the Figure 9. The PCA analysis also supported the population structure and showed that most (21 from 23 colonies) were separated by PCA1 (Figure 10). However, the PCA could not separate the monogyne and polygyne colonies. We also found two clusters of the populations in almost all parts of Thailand except in the

Northern and Northeastern part of Thailand and both clusters were found to be both monogyne and polygyne forms (Figure 6).

Table 6. Genetic differentiation (*FST*) between the 23 colonies of *S. geminata*.

	Sol03	Sol04	Sol05	Sol06	Sol09	Sol10	Sol27	Sol28	Sol30	Sol31	Sol34	Sol39	Sol17	Sol30M	Sol01	Sol02	Sol15	Sol16	Sol18	Sol19	Sol13	Sol25	Sol32	Sol36
Sol03	0.000																							
Sol04	0.026	0.000																						
Sol05	0.237	0.298	0.000																					
Sol06	0.336	0.365	0.409	0.000																				
Sol09	0.066	0.093	0.153	0.179	0.000																			
Sol10	0.570	0.684	0.079	0.533	0.333	0.000																		
Sol27	0.524	0.579	0.351	0.426	0.362	0.492	0.000																	
Sol28	0.376	0.459	0.121	0.290	0.272	0.195	0.256	0.000																
Sol30	0.507	0.551	0.376	0.558	0.253	0.00	0.398	0.144	0.000															
Sol31	0.414	0.479	0.193	0.488	0.261	0.058	0.304	0.102	0.271	0.000														
Sol34	0.347	0.384	0.316	0.362	0.196	0.430	0.402	0.301	0.261	0.222	0.000													
Sol39	0.493	0.526	0.436	0.413	0.375	0.628	0.482	0.430	0.475	0.417	0.372	0.000												
Sol17	0.350	0.403	0.107	0.491	0.250	NA	NA	NA	0.461	0.333	0.421	0.598	0.000											
Sol30M	0.669	0.693	0.540	0.679	0.497	0.777	0.682	0.676	0.647	0.583	0.536	0.706	0.394	0.000										
Sol01	0.453	0.474	0.273	0.335	0.144	0.456	0.394	0.346	0.580	0.467	0.451	0.474	0.396	0.633	0.000									
Sol02	0.615	0.648	0.454	0.680	0.516	0.919	0.815	0.791	0.572	0.447	0.514	0.685	0.305	0.266	0.608	0.000								
Sol15	0.660	0.702	0.556	0.514	0.534	0.827	0.664	0.678	0.774	0.673	0.615	0.671	0.449	0.742	0.582	0.762	0.000							
Sol16	0.377	0.407	0.274	0.281	0.178	0.371	0.319	0.246	0.518	0.448	0.375	0.519	0.305	0.602	0.408	0.638	0.349	0.000						
Sol18	0.614	0.620	0.598	0.555	0.198	0.601	0.419	0.357	0.478	0.533	0.470	0.387	0.513	0.236	0.483	0.418	0.574	0.387	0.000					
Sol19	0.485	0.533	0.513	0.433	0.585	0.833	0.643	0.652	0.681	0.578	0.532	0.576	0.407	0.737	0.469	0.733	0.669	0.460	0.633	0.000				
Sol13	0.476	0.519	0.427	0.504	0.426	0.788	0.690	0.654	0.639	0.506	0.476	0.614	0.261	0.372	0.490	0.301	0.699	0.542	0.311	0.596	0.000			
Sol25	0.608	0.638	0.532	0.525	0.428	0.792	0.710	0.680	0.659	0.555	0.500	0.592	0.526	0.666	0.561	0.597	0.762	0.601	0.468	0.670	0.354	0.000		
Sol32	0.590	0.614	0.478	0.506	0.477	0.702	0.629	0.615	0.575	0.482	0.484	0.463	0.552	0.573	0.599	0.514	0.689	0.608	0.476	0.672	0.453	0.582	0.000	
Sol36	0.548	0.568	0.392	0.571	0.389	0.602	0.510	0.489	0.485	0.399	0.472	0.502	0.507	0.505	0.528	0.469	0.705	0.561	0.515	0.655	0.463	0.617	0.371	0.000

Conclusion and Discussion

Colony social form

The fact that we did not find *S. invicta* in Thailand may be due to the population of *S. invicta* contaminated from goods were still small and might not have conditions suitable for colony establishing in Thailand because of lots of native and invasive ant species (e.g. *Tapinoma*, *S. geminata*) that could be out compete with them (McGlynn 1999; Krushelnycky *et al.* 2010; Chen *et al.* 2011). We found that *S. geminata* in Thailand has both monogyne and polygyne form and both form co-occurred in the same area excepted in the Northern and Northeastern part of Thailand which has only polygyne form. It is possible that these two parts of Thailand may also have monogyne form if we had sampled more colonies. In the polygyne colonies, we found that there were only 2-3 queens compared to the previous report that the number of queens might be 16-31 (Adams *et al.* 1976; Mackay *et al.* 1990). This may be due to our underestimated number of queens from the genotypic data of 24 workers. Since polygyny level correlates with the nest density (Ross & Keller 1995), number of queens in a polygyne colony would be higher in the high nest density areas. The low number of queens found in Thailand may be because of low nest density (>20 m^2 per nest) compared to the native population such as in Brazil that 2,500-6,000 nests per hectare (0.6 m^2 per nest) were found (Mackay *et al.* 1990).

The co-occurrence of polygyne and monogyne colonies with no genetic differentiation between the two social forms is concordant with the fact that social forms in *S. geminata* is not controlled by the supergene, SB/Sb, like what is found in *S. invicta* and other *Solenopsis* species (Ross *et al.* 1996; Keller & Ross 1998; Ross & Keller 1998; Krieger & Ross 2002; Wang *et al.* 2013; Nipitwattanaphon *et al.* 2013). This is supported by the study of Ross *et al.* (2003) that the gene presumably responsible for social form recognition (an odorant binding pheromone, *Gp-9*) in the genus *Solenopsis* was functional lost in *S. geminata* because both monogyne and polygyne possess the same allele and polygyny originated from the founder event.

Ecology of *S. geminata*

We found that *S. geminata* prefer to nest in the open sun, rather dry than wet areas, which is similar to the report by (Harris *et al.* 2005). *S. geminata* is often found in the urban area and may agricultural area in Thailand, including e.g. rice, corns, peanuts, rambutan, mangosteens, lanzones, morning glories, tomato, limes, and marigold flowers. This is also similar to the report by (Harris *et al.* 2005). We also found that *S. geminata* is a pest for human as well as agricultural plants as it has been reported (Risch & Carroll 1982; Way & Khoo 1992). However, we found no obvious differences of colony size or shape in the field when compared between monogyne and polygyne forms unlike what is found in *S. invicta* (Chirino *et al.* 2012). We rarely found many *S. geminata*'s nests in the same area suggesting low density of population of *S. geminata* in Thailand compared to native populations (Mackay *et al.* 1990).

Mating behaviors

We found that most queens of *S. geminata* in Thailand mated multiply with the average number of males of 2.42. This surprising result is contrary to the previous studies that many *Solenopsis* species mate singly (Ross *et al.* 1988; Strassmann 2001; Chen *et al.* 2003). Multiple mating has been reported to be advantageous in some Hymenopteran species, e.g. honeybees and many ant species (Strassmann 2001), because it increases genetic diversity and reduce parasite infection. On the other hand, in some species, males do not provide enough sperm and thus multiple mating is needed. In the fire ant, *S. invicta*, also found that certain genotype of males (*b*), do not provide enough sperm and thus females mate with these males need extra mating (Lawson *et al.* 2012). However, insufficient sperm in *S. geminata* males is unlikely, as they do not possess the social chromosome that could affect many traits in males, such as smaller size or lighter weight leading to producing insufficient sperm (Goodisman *et al.* 1999; Lawson *et al.* 2012; Nipitwattanaphon *et al.* 2014).

Population structure

We found very low genetic variation of *S. geminata* in Thailand. The average number of allele per locus in all loci was very low but similar to the previous studies (Krieger & Keller 1997; Chen *et al.* 2003; Ascunce *et al.* 2009), except for the loci, C368, C121, C367 and C485 that the numbers of alleles in native population were 13, 15, 8, and 12, respectively (Ascunce *et al.* 2009), compared to our study that had only 3, 5, 4, and 9, respectively. Similarly, the loci Sol-11, Sol-42 and Sol-49 in the *S. geminata* native populations had a total of 25-32 alleles compared to our populations with had only 9 alleles. This may be due to the fact that *S. geminata* is an invasive species in Thailand (Harris *et al.* 2005). Low genetic diversity of *S. geminata* in Thailand also correlated with our results that only two clusters of population were found (Figure 9), which is similar to a few clusters of invasive populations of *S. invicta* found in Taiwan, China and Australia (Ascunce *et al.* 2011).

Despite low number of alleles in the *S. geminata* populations, the heterozygosity was high in most populations (Table 4), which was similar to the study in native population **(Ross *et al.* 2003)**. High heterozygosity was found to be associated with insects with fire ant mating system to avoid and rule out any homozygosity associated with complementary sex determining locus (Ross & Fletcher 1985; Ross 1993). However, lower heterozygosity found in the colony Sol09, Sol10, Sol27 and Sol28 may be due to low number (2-3) of loci were genotyped in these four colonies. The *FST* values were also generally very high between colonies suggesting high genetic differentiation and low gene flow among colonies in this study (Wright 1921, 1922)**. However, this result is contrary to the study by Ross *et al.* (2003) whose claimed that polygyne colonies has low genetic differentiation between colonies because of limited dispersion**. The high genetic differentiation between polygyne colonies in our study may de to the fact that most colonies were in different provinces. Although some of which were in the same province (e.g. Sol02, Sol03, Sol09, and Sol10), the colonies we excavated were not closed to each other (>20 m^2). This also correlates with the results that we found no significant differences of genetic differentiation (in terms of H_o, H_e, PIC and number of alleles) between polygyne and monogyne populations.

In conclusion, no *S. invicta* was found in Thailand so far. The only fire ant species found in Thailand is *S. geminata*. The genetic variation in the populations was low but differentiation between colonies was very high suggesting lower gene flow between colonies and supported by low nest density found in Thailand. We found various kinds of agricultural plants were associated with *S. geminata*, e.g. rice, corns, peanuts, rambutan, mangosteens, lanzones, vegetables (e.g. morning glories, tomato, limes), and marigold flowers. *S. geminata* cause various problems by their behavior of stinging, tending aphids, destroying seedlings, stealing seeds and damaging fruits. Since two social forms of *S. geminata* were found, further study on the genetic basis of social form in this species and the pest control studies would be encouraged.

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