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臺灣地區入侵紅火蟻巢內親族關係及小地理尺度族群遺傳結構之研究

Colony relatedness and microgeographical genetic structure of red imported fire ant *Solenopsis invicta* in Taiwan

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中文摘要

入侵生物於入侵前後的改變一直是為有趣的研究課題。在入侵紅火蟻(*Solenopsis invicta*)的研究上，許多報導都證實了其在入侵美國後產生了顯著的遺傳結構變異。這些轉變的存在雖然普遍被接受，但卻缺乏了直接的證據以及其他入侵地區的資料。因此台灣在近年來遭火蟻入侵的情況下，提供了一個得以驗證這些改變的絕佳機會。本研究利用 7 組微衛星體基因座(microsatellite loci)對於全部 22 個採集點進行階層式分析，結果顯示了在最小層級的巢內遺傳結構上，單蟻后社會型的巢內親族關係的量測數值約為 0.75 。這樣的情況與原產地族群及美國入侵族群極為相似，顯現了單蟻后的巢內結構具有穩定不受入侵事件影響的特性。然而在多蟻后採樣點的調查中卻呈現高(0.2)與低(0)的兩型性結果。進一步審視其中一個高數值採集點，發現了親族關係隨著不同採集時間而呈現了下降的趨勢。這個現象暗示了相較於高數值樣點，量測值為零的樣點可能遭受了較長時間的入侵與定殖。類似於美國研究的數值變化形式同樣的支持了零量測值可能是由於生態上的限制使得棲地飽合導致蟻巢隨機收養初生蟻后的機會增加，而逐漸打破蟻巢原有的高親族關係。在族群遺傳結構的層級上，利用不同遺傳類型的分子標記顯現了共域的兩社會形間在粒線體(mitochondria) DNA 上產生了顯著的分化，而微衛星體的則無。符合了兩社會型間的基因交流主要為雄蟲所媒介的模式。社會形內的分析指出了多蟻后族群在各個樣點間具有顯著的遺傳分化，並呈現了距離隔離(isolation by distance) 模式，而在單蟻后的分析上，族群內卻缺乏了分化以及地理隔離。這些分子資料對於兩兩社會型間的生態差異給予了間接的證據：多蟻后的區域性婚配(local mating)與分巢(budding)的擴散模式限制了區域間基因交流的發生;相反的情形則發生在單蟻后的區域之間，以公里為尺度的長距離婚飛與擴散的能力使得單蟻后蟻后(monogyne queen)具有均質化區域間遺傳結構的能力。此研究在族群遺傳結構上面，藉由分子的角度對於兩社會型火蟻的生物學差異給予了間接的證據。在巢內遺傳結構中，則接觀測到親族關係在時間上的

改變，這樣的變異符合了生態限制假說(ecological constraint hypothesis)，顯現火蟻在入侵台灣後的數年間，呈現了快速的社會性演化。

關鍵詞：入侵紅火蟻、入侵物種、微衛星體 DNA、巢內親族關係、族群遺傳

Abstract

Remarkable genetic changes of *Solenopsis invicta* have been reported in US. The post-invasion turnover seems unfailing but lacks further evidence from other introduced areas. Two social forms of fire ant in Taiwan provide a great opportunity to test the genetic change in different levels of hierarchical structure. At colony level, data from multiple microsatellite loci reveal that nestmate relatedness for monogyne invariably overlaps with 0.75 and is similar to those in US as well as its native range South America, suggesting that social organization of this form remains stable whether the population is native or introduced. In contrast, the nestmate relatedness of polygyne tends to be binomial; that is, one group possesses much higher value while the other one overlaps with zero. By keeping surveying one "higher" site, the significant decline of relatedness observed during successive collections from gives a direct evidence that sites belong to the "zero" group might have been invaded much longer than others from ''higher'' group. This pattern somehow parallels patterns in US and might be explained by adoption of unrelated alates driven by ecological constraints (e.g. habitat saturation) as the habitat ages. At microgeographical level, significant genetic differentiation is seen between sympatric forms in mtDNA but not microsatellite, which can be explained by the limited male mediated interform gene flow model. Subsequent genetic analyses show significant differentiation and strong isolation by

distance (IBD) among polygyne sites but not monogyne, indicating restricted inter-site gene flow by polygyne queens, who usually expand by budding or local mating. On the other hand, the ability of monogyne queens to conduct distant mating flights appears to be the force homogenizing the genetic structure in a kilometer-scale. Results from the present study in population genetic give a indirect evidence to the form-specific biology of different *Solenopsis invicta* social organization. In colony level, the observation of changes in relatedness is generally consistent with ecological constraint hypothesis and provide direct evidence that the invasive fire ants did underwent rapid social evolution associated with invasion given they were estimated to have arrived into Taiwan within the last decade.

Key words: *Solenopsis invicta*, invasive species, microsatellite DNA, nestmate relatedness, population genetics

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1. Introduction

Native to South America, the red imported fire ant, *Solenopsis invicta*, has inadvertently been introduced into the US around 1930s (Lofgren, 1986). With aid of increasing human commerce, this pest ant species has subsequently been introduced into Australia in 2001 (Nattrass and Vanderwoude, 2001; Vanderwoude *et al.*, 2004) and countries in Asia, such as Hong Kong in 2004 and China in 2005 (Zhang *et al.*, 2007). *Solenopsis invicta* colonies occur in two distinct social organizations: monogyne form possesses a single reproductive queen and polygyne form with more than one queen. In Taiwan, the occurrence of *S. invicta* was first reported in 2003; however, analysis of the size and the distribution of colonies suggest that it likely arrived some 3-5 years earlier (Huang *et al.*, 2004; Chen *et al.*, 2006). Previous studies have shown that both social form of *S. invicta* are present in the two infected areas in Taiwan. Yang *et al.* (2008) studied the genetic structure between two population in Taiwan and reported that two population (Taoyuan and Chiayi) are highly genetically differentiated in both nuclear and mitochondrial DNA genomes, and distinctive mtDNA haplotype possessed by the two population suggest that they are most likely derived from two separate introduction into Taiwan. According to the record from National Red Imported Fire Ant Control

Center (NRIFACC) in Taiwan, *S. invicta* is still expanding its invasion in recent years, especially in north Taiwan, where infestation alter have been frequently announced by counties surrounding Taoyuan.

S. invicta in well-known for having two distinctive social organization: monogyne and polygyne. In addition to having different number of queens in a colony, the body size of polygyne workers is smaller than that of monogyne (Greenberg *et al.*, 1985; Porter 1992). Related studies have shown that social form of this and other fire ant species depends on the presence of specific coding region variants of the single gene *Gp-9* within a colony (Gotzek *et al.*, 2007). Two class of variants, designated as *B*-like and b-like allele occur in *S. invicta*. Monogyne colonies contain only the *B*-like allele whereas polygyne colonies contain the b-like allele as well as *B*-like allele (Ross, 1997; Ross and Keller, 1998; Krieger and Ross 2002). Under the genetic regulation, reproductive queens with $Gp\text{-}9^{Bb}$ are only found in polygyne colony, whereas queen in monogyne colonies are all $Gp\text{-}9^{BB}$ homozygous, and only produce $Gp\text{-}9^{BB}$ workers. Further research followed by the discovery of *Gp-9* gene points out, in laboratory, the acceptance of new queen associate with the colony social form: in polygyne colonies, workers welcome all the Gp-9^{Bb} fertile queens and 50% of newly mated $Gp\text{-}9^{Bb}$ queens but kill all the $Gp\text{-}9^{BB}$ queens.

Meanwhile, colony workers of monogyne (*Gp-9*^{BB}) kill all the foreign sexual despite the genotype (Ross and Keller, 1998). It shows more aggression and discrimination toward non-nestmate of monogyne form. However lab experiment also shows that in the queen-less condition, monogyne workers quickly decrease aggression toward non-nestmate and become able to accept the newly mated queen (Vander Meer and Alonso, 2002). Actually, natural queen replacement happen in queen-less orphaned colony (Tschinkel and Howard, 1978) where colony workers adopt foreign newly mated alate as new queen (Tschinkel, 1996).

The rule of *Gp-9* gene seems simple: *Gp-9*^{BB} allele is absent in the monogyne colony. This simple genetic difference further controls many social traits, thus the two forms differ in other major aspects including phenotype, reproductive strategies of young queen, modes of colony founding (Ross and Keller, 1995; 1998), and dispersal strategy (DeHeer *et al.*, 1999). Those traits are actually correlated with each other. In the phenotypic trait, *Gp-9*^{BB} alates from monogyne colony weight 14 to 15 mg when mature. Because of the heterozygotic genotype of *Gp-9*^{Bb} queen, alates from polygyne colonies come in three genotypes, as expect from a *Gp-9*^{Bb} mother. Alates in a polygyne colony carry the *Gp-9*^{BB} weight about 13 mg, *Gp-9*^{Bb} alates weight only 11.4

mg and *Gp-9*^{bb} alates barely move the scale at 8.6 mg. By the time the female alates are ready for their nuptial flight, the whole body weight of an individual from monogyne colony is almost 50% heavier than those from polygyne colonies and the gaster part is 70% heavier. The difference in weight of those alates almost resides in the gaster's metabolic reserves (Keller and Ross, 1993a).

Among ant, variation of dispersal strategies is relatively common and is often associated with variation of social organization of their colonies. In *S. invicta*, it is correlated with body weight of newly mated queen and *Gp-9* associated dispersal polymorphism (DeHeer *et al.*, 1999). Monogyne nests produce heavier queens that accumulate large fat reserve and exhibit rapid oogenesis, whereas polygyne nests mostly produce lighter queens with lesser fat reserves and more gradual oogenesis (Keller and Ross, 1993a). The phenotype differences are adaptive to two forms. Fuller nutrition content of a monogyne queen is used as an energy reserve of independent colony founding while the rapid oogenesis serves the need of founder queen, without workers, to quickly produce her offspring. In the polygyne form, in contrast, a new queen tends to seek for adoption by an existing polygyne colony (Ross and Keller, 1998).

The dispersal of both forms is also related to the physical property. After mating, a monogyne queen either descends immediately to the ground or flies for a distance before descending, probably with the aid by wind (Rhoades and Davis, 1967). In contract with polygyne queens, the body reserve of a monogyne queen enable her to fly a longer distance (Keller and Passera, 1989; Stille, 1996). Previous research in *S. invicta* reveals that the flight distance of newly mated queen ranges from a hundred meters to maximum 16 kilometers (Markin *et al.*, 1971). The statement suggests that new queens can disperse for longer distance from their natal nest during mating flights then found colonies alone without the assistance of workers. In addition, queens produced from polygyne colonies show a polymorphism in genotype (*Gp-9*^{BB}, *Gp-9*^{Bb}, *Gp-9*^{bb}) which further control their body weight and other behavior. It is common that heavier *Gp-9*^{BB} alates are executed by nestmate workers before they mature (Keller and Ross, 1993b; 1998), however some alates can still survive and attempt to participate in mating flight with the potential to disperse far and found colonies as the mode of monogyne alates (DeHeer *et al.*, 1999). Lighter *Gp-9*^{Bb} queens are most common and exhibits mixed dispersal strategy: some disperse as long as *Gp-9*^{BB} queens after mating and look for chances to found new colony, while others remain close to their natal nest before entering the

existing polygyne colony (DeHeer *et al.*, 1999; Goodisman *et al.*, 2000a). The third lightest queens with *Gp-9*^{bb} genotype appear to expire before reaching reproductive maturity (Ross, 1997; DeHeer *et al.*, 1999; Goodisman *et al.*, 2000a). Although polygyne shows considerable diversity in reproductive and dispersal mode, less potential in initiate colony of queen with *Gp-9*^{Bb} genotype (DeHeer, 2002) let the "remain at original site" to be the typical mode of polygyne form. Beside this, other studies in both *S. invicta* and other ant species also show the philopatry toward natal subpopulation in polygyne form (DeHeer *et al.*, 1999).

In polygyne form, the spreading of colony is most likely via budding (Keller, 1991; Vargo and Porter, 1989). In polygyne form, the adoption of newly mated queen produced within the same area is just the mechanism of proliferation colony queen (Glancey and Lofgren, 1988; Porter, 1991). Previous study shows that the rate of spread of polygyne is about 1 km per year (Ross and Shoemaker, 1997), which appears local compared with the mating of monogyne that may span several kilometer wild. In ant species, limited dispersal from their birth place results in population viscosity, which leads to the genetic differentiation between geographical distant populations (Hamilton, 1964). Population genetics study of *S. invicta* in the US (Ross and Shoemarker,

1997; Shoemarker and Ross, 1996; Shoemarker *et al.*, 2006; Goodisman and Ross, 1998) and South America (Ross *et al.*, 1997) of polygyne *S. invicta* using mitochondria DNA (mtDNA) marker reveals the significant geographic structure between subpopulations of extensive population. In those studies, mtDNA was chosen as a typical marker in the detection of limited female dispersal, and the conformation of genetic consequence to the biological characters makes the molecular analysis a valuable way to prove the ecology and behavior of this ant species.

Social Hymenoptera invariably live in some sort of family group, whose genetic structure is often the focus of study (Ross, 1988). Genetic analysis of ant is a useful method to examine various issues, including colony ecology and biology, social behavior, variation within and among population. The trace of changes within the genetic level along time and ecological variation also helps to answer questions regarding social evolution, especially in the invasive ant. Recently, many studies on social insects have shown that the introduced populations are affected by genetic bottlenecks, following the initial founding, therefore they revel relatively little genetic diversity compared with the source population (Nei *et al.*, 1975; Barrett and Kohn, 1991; Davis *et al.*, 1999). *Solenopsis invicta* is an infamous invasive ant, listed in the “One Hundred of the

World's Worst Invasive Alien Species" (http://www.issg.org/); two forms of *S. invicta* are successful invader of North America (Tsutsui and Suarez, 2003) and Taiwan (Chen *et al*., 2006) and recent work suggests that the invasiveness is associated with the change in colony structure following introduction (Tsutsui and Suarez, 2003). The intra-specific diversity of *S. invicta* leads us to expect that the genetic structure, especially at colony level, of the two forms may be different given the fact that reproductive system is the major force to shape the contemporary genetic architecture of a population. Furthermore, genetic structure of invasive social insect species may change when introduced into new areas, as a result of the bottleneck effect, combined with exponential population density growth attributed to releasing from natural enemy in new environment (Orr *et al.*, 1995; Ross *et al.*, 1996; Porter *et al.*, 1997).

Ant genetic studies have to focus primarily at a lower level because theoretical work suggests that genetic correlation between individuals are likely to be an important factor in social evolution (Hamilton, 1964; 1972; Michod 1982; Pamilo, 1982a). Colony genetic structure has been usually referred to genetic relatedness (coefficient of relatedness), which is estimated by the proportion of genes that are identical in two individual because of common ancestors. While genetic relatedness between nestmate workers is

approximately 0.75 if the given colony is headed by a single queen mated with a haploid male (Keller, 1995), the relatedness generally drops and varies with species when considering polygyne form. Previous studies have revealed that the colony genetic structure of *S. invicta* changes dramatically during introduction: in native population of South America, relatedness of polygyne form is moderately higher than zero, which reduced to zero when introduced to the US (Ross *et al.*, 1996, 1997). It reveals a decreasing of relatedness along with invasiveness.

However, in some newly invaded countries, such as Australia (Henshaw *et al.*, 2005) and Taiwan (Yang *et al.*, 2008), the genetic relatedness of polygyne colonies does not decrease like those in the North America, but shows a pattern similar to those in their native range. It is possible that studies in Australia and Taiwan yield results different from those in the US due to different sampling time: Australia and Taiwan were invaded by *S. invicta* in recent years, while data in the US were sampled around 70 years after introduction. Under the supposition, the discrepancy is worthy of further study.

Another phenomena raised from the invasion of *S. invicta* was the origin of diploid male. In North America the diploid male is common in natural polygyne population that 80-95% of males in polygyne are diploidy (Ross and

Fletcher, 1985b; Hung *et al.*, 1974; Glancey *et al.*, 1976). In contrast, it is absent in monogyne colony and rare among haplodiploid genetic system of Hymenoptera insect (Ross and Fletcher, 1986). Sex of Hymenoptera is determined at one or more polymorphic loci that females are heterozygous and males hemizygous or homozygous at sex loci (Crozier, 1977). The production of diploid male results from the matched mating in which a queen mates with a male carrying a sex allele identical to one of her alleles (Adams *et al.*, 1977). Thus, large proportion of diploid male in introduced area is attributed to the increasing occurrence of matched mating and diploid male producing queen (DMQ) following by the loss of genetic diversity after funder event (Nei *et al.*, 1975). The DMQ produce diploid male and female offspring in 1:1 ratio since the queen of *S. invicta* is singly inseminated (Crozier, 1977). In natural condition, diploid male is limited to the polygyne population because half of offspring producing by a monogyne *Gp-9*^{BB} DMQ are male and result in insufficient worker force in the incipient period of colonization, which makes it hard for the queen to establish a long-lived colony (Ross and Fletcher, 1986). This postulated mechanism makes some appearance in introduced area where (1) large amount of male bred in polygyne colonies are diploidy and (2) successful mating occurs when either polygyne queens or monogyne queens

mate with male from monogyne colonies while mass of male from polygyne colonies are diploidy and sterile (Shoemaker and Ross, 1996). But, the discovery of triploidy worker in US (Krieger *et al.*, 1999) and Taiwan (Yang *et al.*, 2008) and further researches revealed that about 2.4% of diploid male were functional and produce diploid sperm (Krieger *et al.*, 1999) that caused the origin of triploid individual. Therefore, colony with triploid can be seen as an indicator of the present of DMQ in it.

Until now, the explorations of *S. invicta* were mainly done in the US. The late timing of the investigation, however, missed the chance to provided direct evidence of the genetic changes in recent year. The invasion of *S. invicta* into Taiwan therefore provides a great opportunity to study the genetic change of this pest ant species. In this study, hierarchical approach was used to investigate the genetic structure of *S. invicta* in Taiwan. Beginning with the lowest colony level, the colony relatedness was estimated for the two forms of *S. invicta* from each of the invaded site in Taiwan and the biological factors from reproductive system would be tested to see whether it can make influence on colony relatedness or not. Also, I compared the relatedness with pattern in the same population previously described by Yang *et al.* (2008), the existing data from Australia, the US and South America. Comparisons among these

areas may shed light on social evolution of *S. invicta* as well as present direct evidence verifying the previous hypotheses on the shift in colony genetic structure of *S. invicta* when introduced into new areas.

The genetic variation on the population level was highlight in several levels through out this study. The first level was the between social form structure. Because of the genetic determination social organization and the consequence of loss of allele at sex determination locus, the gene interflow between both forms would probably be constructed by specific barrier or corridor. Hence, the molecular variance between monogyne and polygyne were implemented in this study to reveal the genetic divergence. Second level was the kilometer-scale microgrograpgical genetic structure within polygyne and monogyne forms. While the form specific dispersal ability could further alter the genetic structure that I suppose limited gene flow between polygyne sites result from the restricted dispersal potential in polygyne alates but absent among the monogyne sites. By studying of the degree of genetic differentiation between either forms occupied subpopulations, the difficulty in discerning the property of reproductive and dispersal strategy through direct mean could be gleaned through the signature left in the population genetic structure (Avise, 1994; Mitton, 1994; Slatkin, 1994).

Two kind of molecular marker were used in this study: microsatellite with highly polymorphism was suitable in the invasive species which encounter the bottleneck thus lead to loss of genetic diversity (Davis *et al.*, 1999; Fonseca *et al.*, 2000; Ingram and Gordon, 2003). Another one was the mitochondria DNA (mtDNA), although the low level of polymorphism of that marker was present in Taiwan (Yang *et al.*, 2008); it was included in parts of analysis that attempt to provide the higher resolution. Moreover, due to the difference in hereditary property, more pronounced structure may be detected with mtDNA (Hoelzer *et al.*, 1994) and may give us hints that can not be exhibited in nuclear marker.

2. Materials and methods

2.1 Sample collection

Collections of colonies of *S. invicta* from several sites in Taiwan were from May, 2005 to April 2009 with the aid of colleagues of lab308 of Department of Entomology, National Taiwan University. In entire study, collection area spread all over the north Taiwan, include fourteen locations in Taoyuan County: four sites at Shinwu, two sites at Longtan, Dayuan, Chongli and each one site at Dashi, Luzhu and Yangmei; five locations in Taipei County: three sites in Sanshia and each one at Tamsui, Yingo and Fuhsing; one location in Hukou, Hsinchu County. Besides, two collection sites in Chingpu, Chiayi

County were also included in for colony relatedness study. In total 22 collection site, we collected four to fifteen colonies which separated from each other by at least 2 m to avoid collecting mounds inhabited by the same colony (Goodisman and Ross, 1997; 1998). Social form of each colony was initially determined by visual inspection according to the mound density, size and the height of mound, size of colony worker (Greenberg *et al.*, 1985); number of queen inside the nest, the level of intra-specific aggression (Morel *et al.*, 1990). All nests site were excavated as possible and recorded the GPS position, all the wingless queens were collected by systematically searching the nest after bring to laboratory. Hundreds of *S. invicta* workers and all queens were preserved into 95% alcohol for DNA extraction.

2.2 DNA extraction and social form determination by *Gp-9* multiplex PCR

Total genomic DNA was extracted using Puregene DNA Purification Kit. The social form was determined from the modified of *Gp-9* polymerase chain reaction (PCR) protocol described by Valles and Porter (2003), total 25ul of PCR reaction buffer was put into thermal machine perform with the condition below: initial temperature at 95℃ for 10 min followed by the 35 cycles of 94℃ for 15s, 55℃ for 15s and 68℃ for 30s, and final extension at 68℃ for 5 min. After amplification of the specific fragment, separation on 1.2% agarose gels

and photographing under UV light, if all individual in a single colony displayed only *Gp-9*^{BB} homozygous, the colony was recognized as monogyne form; otherwise, individuals with *Gp-9*^{Bb} heterozygous were recognized as the polygyne form. In this step, for amplifying *Gp-9* allele from multiple individuals at one time, 10-15 workers from one colony were bulk extraction. A single worker DNA was extracted additionally from each colony for other genetic marker analysis described below. 6-8 workers from each colony were randomly selected for DNA isolation.

2.3 Microsatellite analysis

DNA was extracted from 6 and 8 individuals from monogyne and polygyne colonies, respectively. In total 1,544 workers from 206 colonies as well as 84 queens from 11 colonies were genotyped for seven microsatellite loci (*Sol-11, Sol-20, Sol-42, Sol-49, Sol-55, Sol-M3* and Sol-*M4*) which were developed for *S. invicta* from previous researches (Krieger and Keller, 1997; Chen *et al.*, 2003). To score the individual microsatellite genotypes effectively, two sets of multiplex PCR were performed (as described by Lai *et al*., 2009) with the fluorescent labeled primers. All the PCR products were visualized on ABI 3100 genetic analyzer by using laser detection. Genotype scoring was operated in molecular software GENESCAN 3.1.2. Microsatellite genotype

data was used as genetic marker for all tests in this study.

2.4 Mitochondrial DNA haplotype analysis

Yang *et al.* (2008) pointed out that 3 mitochondrial DNA (mtDNA) haplotypes distributed in Taiwan (H22 in Chiayi population, H36, H5 in Taoyuan population). I amplified a 920-bp fragment of the mitochondrial DNA (mtDNA) that included portions of the CO Ⅰ and CO Ⅱ using the primers C1-J-2195 (Simon *et al.*, 1994) and DDS- CO Ⅱ-4 (Ross and Shoemaker, 1997). The PCR product was digested with restriction enzyme Taq1 and Msp1 (Ross and Shoemaler, 1997), than electrophoreses in 1.2% agarose gel and photographing under UV light. Haplotype was defined as the presence or absence of restriction site.

2.5 Social form reconfirmation from genetic allele

In addition to the *Gp-9* method, I also analyzed the allele data of microsatellite and mtDNA analysis to present the family structure of each colony and to compare with the result of *Gp-9* allele. In microsatellite method analysis, monogyne colony was verified that colony workers came from a single family, means that at least one same allele in every locus need to be carried by all sampled nestmate worker. On the contrary, colony with workers possess multiple matrilines was considered as monogyne colony. In mtDNA

method, because of the maternal heredity, if workers from one colony carry different haplotype disobey the rule of monogyne, they were recognized as polygyne.

2.6 Triploid estimates

I estimated the portion of individual with three alleles at microsatellite loci from monogyne and polygyne forms of each collection site. The triploid could be easily determined following by the genotype result. If there is any locus with three alleles, the individual is considered as the triploid.

2.7 Genetic analysis

2.7.1 Variation within population and test for Hardy-Weinberg equilibrium

Genetic variation is revealed as the number of allele, allele frequency, observed (H_O) and expected (H_E) heterozygosities across each collection site with the program Microsatellite Analyzer (MSA) 3.00 (Dieringer and Schlotterer, 2003). Probability significant deviation from Hardy-Weinberg equilibrium was computed through a Markoc chain method by 5000 permutation within GENEPOP 3.3 (Raymond and Rousset, 1995). The deficiency or excess of heterozygote was implemented with score test for evaluation the departure from Hardy-Weinberg equilibrium when significance level set as 0.05.

2.8 Genetic structure analysis

2.8.1 Colony genetic structure

We performed the colony genetic structure as the relatedness (R) of colony-mates of all monogyne sites (N=13) and polygyne sites (N=11). All genetic data (microsatellite & mtDNA) sampled from colony workers (range 6-10) was taken to present the value of one colony. Colony relatedness between nestmate workers were calculated for both social forms (monogyne, n=76; polygyne, n=130) and value from queens-queens were estimated for Polygyne form only (n=16). Relatedness was calculated using computer program RELATEDNESS 4.2 (Queller and Goodnight, 1989; Goodnight and Queller 1999). The basic equation of calculation is:

$$R = \frac{\sum_{x}\sum_{k}\sum_{l}\left(P_y - P^*\right)}{\sum_{x}\sum_{k}\sum_{l}\left(P_x - P^*\right)}$$

where X indexes individuals in the data set, k indexes loci and l indexes allelic position. The ratio P_x is the frequency within the current x individual of the allele found at x's locus k and allelic position l. P_y means the frequency of the same allele in the set of 'partners' of x. P^* indicates the frequency of the allele in the population at large, with all putative relatives of x excluded (Queller and Goodnight, 1989). Standard errors were obtained by jackknifing over nest (or other group), and 95% confidence intervals (CIs) were constructed by assuming

the t-distribution. The overall relatedness of one site was calculated by the DEMES option in RELATEDNESS 4.2. Relatedness values were considered significantly different when no overlap of the confidence intervals occurs (Ross and Fletcher, 1995). Sample from all collection sites with at least 3 colonies in whole collection sites (North and South Taiwan) were included in this analysis.

2.8.2 Correlation between nestmate queens and colony relatedness

In the polygyne ant, breeding system of ant itself is the most frequently mentioned factors that construct intra-colony relatedness (Ross, 1993); and the queen number inside the nest is one of the elements of breeding system. To verifying the correlation between nestmate queen and colony relatedness, I compared the colony relatedness with the (1) queen number and (2) nestmate queen relatedness and the correlation of these two factors.

There are two way to estimate average number of queen per nest: harmonic queen number (N_h) and effective queen number (N_e). Harmonic queen number indicates the direct count of collected queen and the effective queen number is estimate indirectly with the genetic variation (Ross *et al.*, 1996). The N_e is estimated from the equation proposed by Ross (1993):

$$N_e = \frac{4r_s - r_q}{4r_w - r_q}$$

where N_e is estimated from is the relatedness of nestmate workers (r_w), relatedness of nestmate queen (r_q) and the relatedness of daughters of single queen (r_s). r_w and r_q was calculated with genotypic data describe above, and because queen of *S. invicta* mate only once (Ross, 1992; Ross and Fletcher, 1985a; Shoemaker *et al.*, 1992), the r_s value is fixed at 0.75.

2.8.3 Inbreeding within population

I estimated of the degree of inbreeding among nonnestmates in neighboring nests within each collection site. Estimate of the inbreeding coefficient was obtained from allele and genotype frequency by calculating the F_{is} value from Wright's F statistics. Estimates were based on all microsatellite loci and mtDNA haplotype survey in 22 collection sites with the program FASTAT (Goudet, 2001). No significant of inbreeding was gauged when the 95% confidence interval overlap with 0 (Ross *et al.*, 1996).

2.9 Population genetic structure

Because there were no more than two collection sites and short of monogyne sample (1 colony) in South Taiwan, only samples from North Taiwan (Taipei, Taoyuan, Hsinchu) were including in the population genetic analysis with the molecular data. In order to avoid the influence of family structure, 3 individuals from each polygyne colony were randomly selected and

one individual from monogyne colony. Population genetic structure was estimated from two levels, (1) between social forms structure and (2) microgeographical structure within social form with the molecular analysis method as described below.

2.9.1 Analysis of molecular variance

The overall analysis of molecular variance (AMOVA) is a hierarchal analysis of the genetic variance partitioning the total variance into covariance component due to intra-individual differences, inter-individual differences and inter-population differences (Excoffier *et al.*, 1992). In order to examine the distribution of genetic variation at different level, AMOVA test was perfumed by program *ARLEQUIN* (Excoffier *et al.*, 1992; Excoffer *et al.*, 2005) with microsatellite genotype or mtDNA sequence data. To specify the genetic structure of *S. invicta* from all collection sites in north Taiwan, we constructed the hierarchal analysis with three dimensions to see the genetic differentiation between each group: (1) between social forms in north Taiwan for testing the genetic differentiation among monogyne and polygyne; (2) between collection sites within both polygyne and monogyne forms in north Taiwan; (3) between individuals within collection site. Besides, because most of the collection sites were occupied by only one form except 4 sympatric sites, another hierarchical

structure base on the variation within sympatric sites was also performed to see the genetic differentiation among social forms when two forms were sympatric. In the program calculation setting, colonies from one social form from each collection site were set as the basic unit of calculation, so those sites contain two social form have to be separated into two unit (ex: polygyne colonies from Dayuan A was set as DYaP, monogyne colonies is DYaM).

2.9.2 Pairwise F_{st} test

Pairwise F_{st} is the value to estimate the genetic variance between populations (Hartl and Clark, 1989). The F_{st} value among collection site within social form was estimated with the microsatellite data and quantified the magnitude of genetic differentiation among sites. Total ten sites with polygyne colonies and thirteen sites with monogyne colonies were analyzed separately to calculate the F_{st} value. Computer program *ARLEQUIN* (Excoffer *et al.*, 2005) was used in estimation with the population comparison settings, significance in pairwise comparisons of F_{st} value were evaluated base on the permutation test with 10000 iterations. Significant level was set at alpha=0.001.

2.9.3 Genetic distance

Beside F_{st} value, Nei's genetic distance (Hart and Clark, 1989) also calculated as the estimation of level of genetic differentiation among

populations base on the distribution of the allele frequency between two populations. The Nei's genetic distance was calculated between collection sites of two forms. The analysis was performed in program MSA 3.00 (Dieringer and Schlotterer, 2002).

2.9.4 Isolation by distance

Isolation by distance indicates that higher genetic similarity between individuals in close geographic proximity than in distantly separated location (Garnier *et al.*, 2004). It is revealed as the correlation of Nei's genetic distance on the real geographic distance. Two social forms were examined in the test to specify the probable dispersal mode. Detection of isolation by distance was achieved by the Mantel test with program GENEPOP 3.3 (Raymond and Rousset, 1995). Significant level was 0.05.

3. Result

3.1 Social form of *Solenopsis invicta*

The detailed number of colonies of two social forms in each collection site is listed in the Table 1. In total 22 collection sites, 8 sites which contain all *Gp-9*^{Bb} colony was considered as pure polygyne site, 10 were pure monogyne site only with *Gp-9*^{BB} colony; and 4 sites with both *Gp-9*^{Bb} and *Gp-9*^{BB} colonies occur as two forms sympatric site. In total 207 colonies, 77 was determined as

monogyne which contain only *Gp-9*^{B} allele, and 130 colonies with *Gp-9*^{b} allele was designated as putative polygyne colony. Ascertainment of social form by both methods was consistent (*Gp-9* multiplex PCR, microsatellite genotype and mtDNA haplotype distribution) except 19 colonies that include 16 colonies which is indicated with more than one matriline but represent all *Gp-9*^{BB}; and 3 colonies which harboring *Gp-9*^{b} allele but represent single family monogyne structures (Table 2). As the above result, 21% of *Gp-9*^{BB} monogyne colony was headed by more than one queen, and 2.36% colony contain *Gp-9*^{b} allele present single family structure.

3.2 Proportion of triploids from microsatellite genotype

Laser detection of the genotyping analysis presents a certain allele when a peak shows in certain fragment. Individuals were recognized as triploid when three peaks appear in a single locus. No triploid individual was found in monogyne colony; and in polygyne form, 28 colonies contain triploidy individuals were identified suggesting that there was diploid male produce queen (DMQ) existing in those colonies. Among them, 11 colonies exist in the Chiayi and 17 colonies exist in North Taiwan. From the scale of collection site, the proportion of colony contains triploid in each site with polygyne colonies range from 6.25% to 55% as show in Table 3. Those sites were all seen as pure

polygyne site after social form survey except one site in Chiayi (CPb) which include one monogyne colony among 12 colonies.

3.3 Genetic variation within population

All seven microsatellite loci are polymorphic in which variability is 2 to 6 per locus. Calculation of H_O and H_E and allele frequency of two social forms in 23 collection sites are listed in Table 4 and Table 5. Test of Hardy-Weinberg equilibrium shows all the collections site departure from Hardy-Weinberg equilibrium (< 0.05) were the polygyne sites. Through score test, all deviated from Hardy-Weinberg equilibrium have been cause by heterozygote deficiency.

3.4 Colony genetic structure

3.4.1 Intra-colony relatedness of *Solenopsis invicta* in Taiwan

Relatedness among workers of monogyne colonies was statistically non-significant from 0.75, a typical value for all collection sites as expected suggesting that colony genetic structure remains consistent irrespective of native or introduced populations. In contrast, mean value of relatedness estimated from all confirmed P colony site ranges from 2 to 0. According the estimated data, all value from collection sites can be roughly divided into two groups: in first group, the relatedness values were approximate to or non-significantly different from zero; and in the other group, all value were

higher than 0, ranges from 0.156 to 0.224 (Fig.2). Estimates for relatedness of polygyne colonies overlap with zero indicates that workers in most colonies are distant relatives or even unrelated. And another characteristic worthwhile mention was the correlation between relatedness value and its variance. Fig.1 showed that that the CIs of the relatedness value of each site were two-typed. Those sites with high relatedness were also with higher variance, on the other hand, in the 0 relatedness group, the variance seemed more constant than higher group.

3.4.3 Decline in relatedness in one collection site

In this study, the site YM was sampled for three times, and for further investigating the relatedness change under the time scale, the value was calculated separately according to the collection date. The interval of each collection date in about 3.5 months: first on Aug, 29th 2008, second on Dec, 18th 2008 and third on Apr, 1st 2009. Result revealed a tendency of decline in relatedness showed the value seemed keeping decreasing in these period (Fig 3); beside, significant difference in relatedness value in the first (0.19 ± 0.097) and third (0.03 ± 0.021) collection date also be found. The range of CI of relatedness narrow from beginning to final suggesting the value was keeping stabilizing. According to the data, the significant decline happens in the period

about 7 months.

3.4.3 Correlation between nestmate queens and colony relatedness

Colony queens were caught only in 14 colonies. Harmonic (count directly), effective queen number and the nestmate queen relatedness is shown in Table 6. Among them, 10 colonies showed relatedness between colony queens were indistinguishable from 0, suggesting that queens in most of the colony were unrelated. The scatter plot of colony relatedness to the breeding factors (queen number and queen relatedness) shows there was no significant correlation between colony relatedness either to harmonic queen (P=0.1402) number or queen relatedness (P=0.2789); no significant correlation between queen number and queen relatedness (P=0.5226) also found in those colonies. In 14 colonies, most of the indirectly estimated queen number (N_e) was higher than the harmonic number (N_h); this result was similar as the research in native South America population and introduced North America population (Ross *et al.*, 1996).

3.4.4 Inbreeding and relatedness

Inbreeding is one of the elements of breeding system that can influence the colony relatedness (Ross, 1993). The comparison of F_{is} inbreeding coefficient estimate with the relatedness value of collection site is shown in

Table 7. Among 11 polygyne sites, the inbreeding coefficient of DY, SWd, SSa, YG, YM and CLb were significant different from 0; and in those 6 sites, 4 of them (DY, SWd, YG and YM) are the belonging to the site with higher relatedness value and other 2 are zero relatedness sites. However, due to sampling data was too few; statistic analysis could not be performed with the result to test the correlation between inbreeding and relatedness.

3.5 Population genetic structure of *Solenopsis invicta*

3.5.1 Between social forms structure

Analysis of Molecular variance (AMOVA) was performed mainly in the between social forms variance analysis. First, all the collection sites in North Taiwan were combined to test the between social form structure. The overall analysis of the hierarchical AMOVA showed the significant genetic variation between polygyne and monogyne social forms (P=0.01941; Table 8). Beside this, for further investigating the genetic differentiation between the sympatric social forms, I also picked three two forms sympatric sites (DYa, SSb and YG) out to perform the AMOVA again. In analysis with microsatellite, no significant (P=0.23722, Table 9) differentiation between two forms indicating that two forms were genetically similar and weakly nuclear differentiated. But significant differentiation between forms was found in mtDNA analysis

(P<0.00001, Table 9). Different result derived from different genetic marker implies that the genetic interflow between two forms within sympatric were restricted to some degree. The estimated pairwise F_{st} value between social forms in three sympatric sites (DYa: F_{st}=0.02177, P=0.29052 and SSb: F_{st}=0.01855, P=0.13362; YG: F_{st}=0.03538, P=0.16193) also showed no significant differentiation between two forms conforms to the result of AMOVA.

3.5.2 Microgeographical structure within social form

In this study, the term "micro" is in contrast with the large isolation and differentiation between two main *S. invicta* populations (Taoyuan and Chiayi) in Taiwan. I focused on the sites belonging to the putative north Taiwan population and the actual geographical distance between my collection sites was less than 30 km.

Estimate of population pairwise F_{st} among monogyne (Table 10) and polygyne (Table 11) collection site revealed a noticeable difference. In the total 45 comparisons of all polygyne containing sites, most of the pairs (n=35) showed a significant difference between each other except 10 pairs. The huge amount of the significance implies that the lack of gene flow between polygyne sites. However in the 10 non-significant comparisons, 9 of them were

belonging to the pair which at least one of paired site is DYa or YG; and these two sites are known as two forms sympatric. On the contrary, the comparison of the monogyne showed the lack of genetic differentiation between sites except one site TS. The TS was differentiation between all other site, it could be caused by the large geographic distance between TS other site (at least 30 km) and topographic barrier. If limitation TS, the result conformed to dispersal ability of monogyne queens that could carry their gene out for a distance make the genetic differentiation unobvious.

3.5.3 Isolation by distance within social form

I plotted the Nei's genetic distance against the real geographic distance of monogyne (Fig 4) and polygyne form (Fig 5). The result showed that there was a significant correlation between polygyne sites (Spearman Rank correlation coefficient, r=X, Mantel test, P<0.00001) but not monogyne sites (Spearman Rank correlation coefficient, r=X, Mantel test, P=0.83). Isolation by distance presented between polygyne sites implied the strong and significant genetic viscosity existed. And no isolation by distance in monogyne form was also as expect for the extensively distant dispersal performed by both sexes during mating flight. Test of isolation by distance was compatible with the significance test of pairwise F_{st} in both forms.

4. Discussion

4.1 Social form determination by *Gp-9* locus

Determination of social form has been performed with advance of *Gp-9* allele-specific multiplex PCR by Valles and Porter (2003). Because of its convenience and rapid diagnosis, this method has somehow has been widely employed in many recent related studies and shown its accuracy, at least when dealing with introduced populations (Oi and Williams, 2003; Chen *et al.*, 2006; Oi, 2006; Goodisman *et al.*, 2007; Yang *et al.*, 2008, 2009). We had, however, approximately 10% of nests of which the results of *Gp-9* multiplex PCR turned out to be disconcordant with inspection by family structure analysis (see below). The discrepancy between two social forms determination methods involved two scenarios where putative polygyne colonies revealed by *Gp-9* multiplex PCR (*Gp-9*^{Bb}-harboring) possess single family structure and putative monogyne colonies (*Gp-9*^{BB}-harboring) showed multiple matrilines.

The first scenario may account for the sequence variations in *Gp-9* gene, a result of the fact that Valles and Porter's (2003) assay could not reach this resolution as proposed by Gotzek *et al.* (2007) and Ascunce *et al.*, (2009). This is because that expression of polygyne phenotype requires *b*-like residue at all three necessary amino acid codons at *Gp-9* gene (42, 95 and 139) and some

colonies from South America shows monogyne phenotype (single family structure revealed by microsatellite genotypes) despite one of two codons are *B*-like (Gotzek *et al.*, 2007). Consequently, Valles and Porter's (2003) assay for amplification of *b*-like allele (16bAS and 26 bS) were designed for codon 95 only and likely mislead the diagnosis result if examined colonies bear *b*-like residue on codon 95 but not other two as observed in the present study. The finding of codon variation in *S. invicta* in Taiwan may suggest the invading species may have originated from South American, given the fact that none of these bizarre codon compositions have yet been found in the US populations (Shoemaker and Marina, 2009). On the other hand, it's possible the codon variation do exist in the US but remains cryptic because interpretation made by Shoemaker and Marina (2009) was based on relatively small sample size. If so, it can not be rule out that the *S. invicta* in Taiwan were introduced from the US, China or Australia.

In the latter scenario c.a. 21% of $Gp\text{-}9^{BB}$ colonies showing multiple family structures might result from failure in amplifying $Gp\text{-}9^{b}$ allele during PCR. However, the repeated PCRs eliminated this possibility given all the positive controls showed *b*-allele band on the agarose gel. Besides, some additional possibilities may explain that putative monogyne colony harbor more than one

matriline. Firstly, although independent founding is the typical nesting strategy described in the monogyne society, some exceptions have been reported in the reproductive biology of *S. invicta* (Tschinkel, 1998). Several monogyne queens are likely to cooperate either digging the hole or rearing the first brood during the initial founding period. After the first brood eclosion, all but one queen will be executed by workers' choice. At the end of claustral period, the colony will contain only a single queen (Tschinkel and Howard, 1993). Such founding process could generally be recognized as pleometrosis and most likely take place in the region where newly mating queens (from monogyne colonies) are dense after mating flight. Hence, it seems to hold true since some areas in Taoyuan do represent a higher population density of *S. invicta* which increases the chance of pleometrosis. However, all the colonies collected for the present study are mature nests and the influence from pleometrosis tend to be less possible given that several researches reported that the imprint of pleometrotical founding fire ant is negligible in a mature monogyne colony (Wilson, 1966; 1971).

Secondly, the inconsistency of two social forms determination assays may refer to turnover of colony queen. Studies in US showed a large number of monogyne founder alates that are typically reared and join mating flight during late spring and early summer (Markin *et al.*, 1972; Tschinkel, 1993). After that, small numbers of

female alates are produced in fall and participate mating flight in next early spring (Fletcher and Blum, 1983). These overwintered alates lack enough metabolic reserve (35% loss of dry weight as compared to normal summer flying alates) and are unable to found their colony independently. Instead, they randomly seek for existing colonies and attempt to gain entry. Successful establishment of these queens generally occurs when a given colony is orphaned (Tschinkel, 1996). Once adopted, monogyne queens begin laying eggs and have their own workers, and two different family structures will present within the colony. However, the collection record in the present study showed that only 4 of 16 colonies were collected in early spring, timing theoretically for occurrence of queen turnover. Moreover, a previous empirical study showed that the rate of queen turnover is extremely low (0.7% per year, DeHeer and Tschinkel, 1998), indicating that queen turnover might not be the only attributing factor.

The third possibility may be multiple mating of queen that gives rise to more than one patriline that violates the single family structure. Although this mechanism has been well described in many ant species, for instance, leafcutter ant, *Atta spp*. (Fjerdingstad and Boomsma, 1998) and *Formica* ant (Pamilo, 1982b), no direct evidence showing multiple mating exist in both social forms has been found in *S. invicta* (Ross and Fletcher 1985; Ross 1992; Shoemaker *et al.*, 1992). However, a recent study in the US suggests that some frequency of multiple mating of polygyne

queens may exist as revealed by genotyping the sperm extracted from queen's spermatheca (Fritz *et al.*, 2006), suggesting multiple mating can't be ruled out as well.

In summary, no possible factors (cooperative founding, queen replacement & multiple mating) can be eliminated since many studies have documented dramatic evolution of other invasive ant species after invasion (Tsutsui and Case, 2001). The finding of such pattern in Taiwan remains an open question and is worth of future studies.

4.2 Colony genetic structure

Intra-colony relatedness of monogyne colonies are all around 0.75, the expected value of a colony headed by a single once-mated queen. In native polygyne population, the colony relatedness of *S. invicta* is moderately high but has reduced to indistinguishable from zero after being introduced into the US (Ross *et al.*, 1996). The surveys for relatedness in recently introduced areas showed that the colony relatedness of samples collected from Taoyuan and Chiayi counties in Taiwan (Yang *et al.*, 2008) as well as from two populations in Australia (Henshaw *et al.*, 2005) is moderate high, lying between the US and native populations in South America (Ross *et al.*, 1996). In contrast, the present study showed a binominal data distribution that could be roughly separated into two groups: higher relatedness group (around 2) and zero relatedness group.

The differences in relatedness patterns among these studies may be due to different sampling strategies implemented. Studies from Yang *et al* (2008) and Henshaw *et al.* (2005) mainly focused on population level; however, more comprehensive sampling and micro-scaled site-specific estimate in the present study not only give much more resolution revealing the relatedness pattern but also minimize the effects of population substructures that do exist in the studied population. Also, the sample strategy in the present study is consistent to those implemented to estimate the relatedness in the US and South American populations (Ross *et al.*, 1997; Ross and Shoemaker, 1997), and allows the comparison of relatedness patterns to make more biological and statistical senses.

Another interesting finding is that the statistic variation of relatedness of polygyne sites differs between groups. Variance of those sites from "higher group" tended to be higher; in contrast, it's more constant in the zero group. Standing on these two points, a provisional hypothesis that relatedness value declines as time goes by might be feasible. The hypothesis predicts that in the early stage of population colonization the relatedness might remain high because of just few reproductive individuals and then continue decreasing and eventually stabilize several generations later. This hypothesis is further

supported by Tschinkel and Nierenberg' (1983) mathematic model that simulates the relatedness dynamic with the assumption that only few female was originally introduced. The model showed that relatedness decreases from high to low and eventually remains at equilibrium level in fewer than 10 generations. In the present study, continuous sampling in site ''YM'' provides direct evidence that conforms the hypothesis of relatedness value decreasing. Suggesting the colony relatedness of polygyne *S. invicta* in Taiwan is currently at an unstable phase. Furthermore, significant decline of relatedness in YM was detectable in only 7 months, which is much shorter than what Tschinkel and Nierenberg (1983) expected. This pattern enforces the rapid evolution and great trait plasticity of invasive social insect species when introduced into novel area.

Introduced populations of polygyne *S. invicta* possesses different within-colony structure compared with native populations where colonies of given social form are generally presenting higher nestmate relatedness value as a result of coexistence of several closely related queens (Ross *et al.*, 1996). Ecological constrain has been believed to be the most favorable hypothesis to illustrate the change in colony relatedness: at the time of introduction, the lack of natural enemies allows the population of *S. invicta* to expand rapidly (Orr *et*

al., 1995; Porter *et al.*, 1997) and saturated their habitats. Under the condition, habitat saturation serves as constraint to newly mated queens to found new colonies and facilitates the adoption of additional queens into existing colonies (Nonacs, 1993). Furthermore, random adoption of newly mated queens, instead of returning to their natal colonies, is even more forced by high density of existing colonies. Adoption of these unrelated queens and production of new workers gradually break down the nestmate relatedness and allow the polygyne colony to become an open society in the introduced area (Ross *et al.*, 1996; Goodisman and Ross, 1997).

Variation of relatedness in Taiwan somehow parallels the declining relatedness as *S. invicta* was introduced from native range to the US (Ross *et al.*, 1996). However, unlike the stable persistence of the US population, all the collection sites in the present study may involve repeated extinction and colonization since the efforts of eradication have been constant. Under given circumstance, every colonization event in any collection site in the present study can be viewed as a bottleneck effect to *S. invicta*. Thus, the influence to ant population from new colonization is in some degree similar to the invasion from one region to another. It can explain the high variation of relatedness value within Taiwan after been invaded for years.

Study of colony structure of social insect was original impetus from the theory of kin selection as an explanation for the evolution of sociality (Holldobler and Wilson, 1990). The attention in the relatedness study is not only in describing them but also elucidate the factors responsible for generating them. Breeding system as the most mentioned factors which construct the relatedness (Wade, 1985). Elements of the breeding system that influence colony relatedness can be categorized into three groups: (1) matrilines inside the nest, (2) patrilines inside the nest, (3) both matrilines and matrilines which indicate inbreeding inside the nest (Ross, 1993). Matrilines inside the colony is affected by the number of the egg laying queens, the relationship among queens (queen relatedness) and the apportionment of maternity. Patrilines factor is similar as matrilines but it only works with the assumption of multiple mating.

In this study we examine the correlation between relatedness to matriline factors and inbreeding. However, there is no significant relationship been found between them, which implies that the breeding system of *S. invicta* itself could not be an important factor to construct colony relatedness in the introduced population. The zero relatedness among nestmate queens in some colonies in Taiwan suggests that they are unrelated as seen in the US (Ross *et al.*, 1996; Goodisman and Ross, 1997; Chen *et al.*, 2003). In fact, some colony

queens might not be completely recovered when collected in the field; therefore queen data should be handled in a more conservative manner. However, data revealed by portion of colony queens does not contradict previous result, as reported in the US (Ross et al, 1996; Goodisman and Ross, 1997), that increase in number of unrelated colony queens comes together with decrease in colony relatedness. Decline of relatedness in polygyne colonies in Taiwan seems to be correspondent with this ecological constrain model given that population density does increase in a constant rate. And in *S. invicta*, the importance of kinship as a stabilizing factor in the evolution of polygyne while other social insects appears to decrease in the natural enemy-free new area (Goodisman and Ross, 1997).

Recent work suggests that the successful invasion may stem from the change in colony structure during or following introduction (Tsutsui and Suarez, 2003). Research in invasive Argentine ant (*Linepithema humile*) indicates the decreasing of relatedness when introduced from native range into novel areas (Tsutsui and case, 2001). Such pattern is similar to what found in *S. invicta* but along with a visible supercolony that transited from small colony type which normally exists in native population. Although different mechanisms are likely to be involved in the construction of colony structure,

these two invasive ant species both encountered founder event leading to loss of genetic diversity. It's possible that breaking down of colony structure might be one of the mechanisms that maintain their gene pool and rescue the harmful effects of gene loss when populations meet bottleneck.

4.3 Proportion of triploid

In this study, colonies with triploid share similar characters: (1) all colonies were collected from the site dominated by polygyne form, (2) those sites are pure polygyne sites or somehow distantly isolated from monogyne sites. SWa, SWb and YM are the pure polygyne sites, and HK is in the mountain area of Hsinchu County where was considered a newly invaded site isolated from the *S. invicta* prevailing core area (Taoyuan County). CPb is the only site that both forms have been found sympatrically, but it belongs to the Chiayi population where extremely low number of monogyne form have been shown in previous survey (Yang *et al.*, 2008, 2009).

Diploid male produced queens (DMQ) are major source of production of triploidy individuals, and in natural condition the triploids are produced by the polygyne population only because the diploid male production is a significant colony mortality factor to incipient monogyne colony (Ross and Fletcher, 1986). Queens close to the monogyne population have greater chance to be

fertilized by monogyne males whose reproductive system is functional (Ross and Fletcher, 1985b, 1986; Ross and Keller, 1995). In contrast, if the given polygyne sites are distant from functional monogyne males, queens participating mating flight usually mate with polygyne males that are almost diploid and sterile. Only a small proportion of these diploid male possess well-developed testis that gives rise to triploidy female offspring. Hence, the finding here is consistent with higher proportion of colonies with occurrence of triploid within site which it is more isolated from monogyne colonies.

4.4 Between forms structure and male mediated gene flow

Its worthwhile noting that significant differentiation between two social forms revealed by AMOVA might result from the structure among geographically isolated collection sites. However, if we consider three sites with two forms sympatry, analysis reveals no nuclear differentiation but significant mtDNA differentiation within those sites, indicating that mtDNA gene glowing between two forms is limited despite the sympatric condition,. Earlier researches in US showed similar pattern for sympatric conditions using same classes of genetic pattern.

Theoretically speaking, there are four possible routes for gene flow between two forms: either a monogyne or monogyne queen establish

themselves in the colonies of alternative social type or either a monogyne or polygyne male mate with an alternative type of queen (Shoemaker and Ross, 1996). Strong genetic differentiation in mtDNA suggests that queens reproduce in the same social lineage (Shoemaker and Ross, 1996; Ross and Shoemaker, 1997; Ross and Keller, 1998, 2002). Under the circumstance, the absence of differentiation in nuclear suggests the interform gene flow is largely unidirectional and mediated through males only. Other studies estimating the genetic variation of two nuclear loci (*Gp-9* & *Pgm*-3) showed entirely different allele frequency in two social forms. Combined with the fact that large proportions of polygyne male are sterile, it indicates the only common possible route is for a monogyne male to mate with a polygyne queen (Ross, 1992; Ross and Shoemaker, 1993; Shoemaker and Ross, 1996).

4.5 Microgeographical genetic structure within social form

Significant genetic differentiation among polygyne sites but not monogyne sites (Ross and Shoemaker, 1997; Ross *et al.*, 1997; Shoemaker *et al.*, 2006) confirms the form-specific dispersal strategy. In polygyne, the social form which is normally not good at long distance flight, newly mated queens tend to conduct local mating within natal place that leads to population viscosity (Goodisman and Ross, 1998). In contrast, monogyne queens with

their great flight ability can act as the mediator homogenizing the genetic structure among sites. Analysis of isolation by distance also supports the idea. Significant isolation by distance of polygyne but not monogyne implies that the efficiency of queen dispersal do affect gene flow among sites (Liautard and Keller, 2001).

There is another message hiding in the occurrence of geographical restriction in genetic structure of polygyne form. In fact, the polygyne males presumably travel long distance (Vargo and porter, 1989; Ross and Shoemaker, 1997) with the potential to act as the force to dilute the differentiation among sites. Under the situation, among-site structure and IBD could be expected. However, the mass production of sterile males weakens this potential. Furthermore, while between-form gene flow may decrease and homogenize the structure (Ross and Shoemaker, 1997), the limitation in the unidirectional route that only monogyne males copulate with polygyne queens does not seems sufficient to unify the genetic makeup among them (Ross *et al.*, 1999).

TS (Tamsui) is the only monogyne site that highly differentiated from all other sites with the same form. There are several possible explanations, for instance, TS might be a recently invaded sites from somewhere aboard, or it has been isolated from other sites for so long that its genotype distribution is

unusual. The survey of allele variation among all collection sites show that TS harbors the least number of alleles and no unique allele exists in it (Table 5) , ruling out the first possibility. The second possibility might be more probable given that Tamsui River and Taipei Basin may act as barriers and cause the unique allele distribution of TS after its long term of isolation without gene flow from other sites.

Another noticeable finding is that the result of pairwise F_{st} of polygyne sites showed several undifferentiated pairs, most of them are composed with at both forms (Table 11), suggesting some connections between those sympatric sites and other sites do exist despite restricted dispersal registered by polygyne form. It is still possible that a polygyne queen is able to disperse distantly from her original site (DeHeer et al., 1999; Goodisman et al., 2000b) and probably unify the genetic structure. However, this phenomenon may not explain completely why differentiation only occurs in sites with mixed social forms. Another explanation is that female may seldom mediate inter-form gene flow (DeHeer, 2002). In sympatric sites (for example as DYa), monogyne Gp-9^{BB} queens mate with polygyne Gp-9b males within the sites, before dispersing distantly to other sites (those found undifferentiated with DYa) and founding colonies independently. All the offspring of the given queen is the Gp-9^{Bb},

which tolerates multiple queens and form polygyne colonies (Ross and Keller, 1998). This mechanism makes the monogyne produce queens able to bear genes of sympatric polygyne to other polygyne site and deliver them to polygyne population. Undoubtedly, one could expect the phenomenon above is fairly rare because most polygyne males are sterile.

The polygyne-derived monogyne colony is another potential route for female mediated inter-form gene flow (DeHeer, 2002). Under genetically controlled social system, 2.3% of Gp-9^{BB} queens escape the execution by workers, successfully exit the polygyne colony and participate the mating flight (DeHeer et al., 1999). These Gp-9^{BB} queens do have potential to independently found monogyne colonies if they mate with Gp-9^{B} males. A supporting evidence could be that the set of alleles found in monogyne in mixed sites (such as YG, CPb) is a subset of those found in sympatric polygyne.

5. References:

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Table 1 List of collection sites, from Taipei, Taoyuan, Hsinchu and Chiayi County

Source of collection	Collection site*	No. of colony	No. of polygyne	No. of monogyne	Social type* within site
Taipei	Sanshia A (SSa)	13	13	0	P
	Sanshia B (SSb)	13	4	9	Sym
	Sanshia C (SSc)	3	3		
	Tamsui (TS)	8	0	8	M
	Yingo (YG)	12	9	3	Sym
	Fuhsing (FS)	4	0	4	M
Taoyuan	Shinwu A (SWa)	10	10	0	P
	Shinwu B (SWb)	16	16	0	P
	Shinwu C (SWc)	8	0	8	M
	Shinwu D (SWd)	5	0	5	M
	Longtan A (LTa)	5	0	5	M
	Longtan B (LTb)	5	0	5	M
	Dayuan A (DYa)	8	4	4	Sym
	Dayuan B (DYb)	7	0	7	M
	Chongli A (CLa)	6	0	6	M
	Chongli B (CLb)	13	13	0	P
	Dashi (DS)	6	0	6	M
	Luzhu (LZ)	6	0	6	M
	Yangmei (YM)	27	27	0	P
Hsinchu	Hukou (HK)	9	9	0	P
Chiayi	Chingpu A (CPa)	11	11	0	P
	Chingpu B (CPb)	12	11	1	Sym

* Social type: P, polygyne; M, monogyne; Sym, both social forms sympatric.

* Symbols in parenthesis represent the code of collection sites.

Table 2 Ratio of discord in two methods of social form determination

	Colony with all *Gp-9*^{B} allele (Monogyne)	**Colony contain *Gp-9*^{b} allele (Polygyne)**
Colony with multiple matrilines	**16 (21%)**	127
Colony with single matriline	60	**3 (2.3%)**
Total colony	76	130

Table 3 Ratio of triploids in six collection sites

Collection site	No. of colony	No. of colony with triploid	% of triploid	Site type
SWa	10	2	20	P
SWb	16	1	6.25	P
YM	27	9	33	P
HK	9	5	55	P
CPa	11	7	63	P
CPb	12	4	33	Sym*

SW (Shinwu, Taoyuan), YM (Yangmei, Taoyuan), HK (Hukou, Hsinchu), CP (Chingpu, Chiayi).

Social type: P, polygyne; M, monogyne; Sym, both social forms sympatric.

*Only one monogyne colony in entire 12 colonies.

Table 4 Statistic genetic diversity of the polygyne form *Solenopsis invicta* population in Taiwan base on seven microsatellite loci

Source of collection	Collection site	Sample size	Mean No. of allele	N_A	H_E	H_O
Taipei	**SSa**	39	4	28	0.638	0.606
	SSb	12	3.428	24	0.547	0.548
	SSc	9	3.428	24	0.647	0.625
	YG	27	4.143	29	0.637	0.260
Taoyuan	**SWa**	30	3.587	27	0.615	0.661
	SWb	48	3.714	26	0.638	0.654
	DYa	12	3.57	25	0.682	0.621
	CLb	39	3.857	27	0.665	0.626
	YM	81	4.57	32	0.636	0.539
Hsinchu	**HK**	27	3.857	27	0.567	0.556
Chiayi	**CPa**	33	3.857	27	0.599	0.645
	CPb	33	4.143	29	0.595	0.539

SS: Sanshia, YG :Yingo, SW: Shinwu, DY: Dayuan, CL: Chongli, YM: Yangmei, HK: Hukou, CP: Chingpu.

N_A: denotes observed number of alleles found at each locus from each collection site; H_E: expected heterozygosity; H_O: observed heterozygosity

Table 5 Statistic genetic diversity of the monogyne form *Solenopsis invicta* population in Taiwan base on seven microsatellite loci

Source of collection	Collection site	Sample size	Mean No. of allele	N_A	H_E	H_O
Taipei						
	SSb	9	3.28	23	0.604	0.667
	TS	8	2.714	19	0.638	0.666
	YG	3	2.857	20	0.638	0.667
	FS	4	3	21	0.612	0.643
Taoyuan						
	SWc	8	3	21	0.524	0.589
	SWd	5	3	21	0.564	0.629
	LTa	5	3.429	24	0.673	0.628
	LTb	5	2.857	20	0.587	0.6
	DYa	4	3	21	0.668	0.643
	DYb	7	3.143	22	0.623	0.592
	CLa	6	2.857	20	0.569	0.476
	DS	6	3.429	24	0.619	0.5
	LZ	6	2.857	20	0.508	0.490

SS: Sanshia, TS: Tamsui, YG: Yingo, FS: Fuhsing, SW: Shinwu, LT: Longtan, DY: Dayuan, CL: Chongli, DS: Dashi, LZ: Luzhu.
N_A: denotes observed number of alleles found at each locus from each collection site; H_E: expected heterozygosity; H_O: observed heterozygosity.

Table 6 Nestmate relatedness and number of nestmate queen in polygyne colony of *Solenopsis invicta*

Colony	R_q	R_w	N_h	N_e
Si314	-0.00061	0.005425	5	11
Si315	-0.0877	-0.23934	5	11
Si316	0.061561	-0.43477	6	19
Si317	-0.02349	0.011306	9	18
Si318	0.031472	0.055506	10	31
Si319	0.206431	-0.02255	6	6
Si320	0.211338	-0.04046	5	21
Si324	0.116079	0.167301	21	8
Si325	0.011004	0.081031	4	71
Si335	0.234068	0.238498	9	8
YDN1	0.042476	0.010867	16	28
YDN9	0.003373	-0.04237	8	24
YDN11	-0.02745	0.040305	8	6
YDN13	-0.1044	-0.0307	5	17

R_q: relatedness between queens, R_w: relatedness between workers, N_h: harmonic queen number, N_e: effective queen number.

Table 7 Estimate inbreeding coefficient (F_{is}) of polygyne collection sites

Collection site	F_{is}	95% CI	R
CPa	-0.04333	0.051773	0.060994
CPb	-0.00631	0.040368	0.031503
DY	**0.181155**	**0.056403**	0.206568
SWc	-0.02142	0.031181	-0.00039
SWd	**0.115411**	**0.0296**	0.22388
HK	0.016028	0.023706	0.039182
SSa	**0.093142**	**0.030612**	0.021255
SSb	-0.00065	0.038654	0.208286
YG	**0.137358**	**0.033326**	0.209434
YM	**0.048677**	**0.037626**	0.155669
CLb	**0.121965**	**0.033037**	-0.00469

Symbol type indicates the F_{is} is significant different from 0.

Table 8 Analysis of molecular variance (AMOVA) showing different genetic variation in the polygyous and monogyous forms of *Solenopsis invicta* between two social forms in the North Taiwan population

Source of variation	Variance	% total	-statistic	*P*-value
Polygyne vs. Monogyne (overall collection)				
Between social forms	0.01727	0.80147	0.00801	0.01941
Within social form between collection sites	0.12575	5.48891	0.05533	<0.00001
Within collection sites between individuals	2.17914	93.70961	0.06290	<0.00001

Table 9 Analysis of molecular variance (AMOVA) estimate with different genetic markers showing different genetic variation in the polygyous and monogyous forms of *Solenopsis invicta* between two social forms within the two form sympatric site in the North Taiwan population

Source of variation	Variance	% total	-statistic	*P*-value
Both forms sympatric sites (Microsatellite)				
Between collection sites	0.00422	0.18603	0.00186	0.23772
Between social forms within collection sites	0.05329	2.35153	0.02356	0.09455
Within social form between individuals	2.20880	97.46243	0.02583	0.00545
Both forms sympatric sites (mtDNA)				
Between collection sites	-3.36345	-37.04	-0.37040	1
Between social forms within collection sites	5.31288	58.51	0.21468	0.00020
Within social form between individuals	7.13115	78.53	0.42694	0.00224

Table 10 Pairwise estimates of genetic difference of *Solenopsis invicta* between monogyne collection sites of North Taiwan

	SWc	SWd	CLa	LTa	LTb	DYb	DYa	LZ	YG	DS	FS	SSb	TS
SWc	—	0.30874	0.50267	0.40703	0.33387	0.33335	0.60831	0.51605	0.51605	0.39073	0.26415	0.66547	0.50822
SWd	0.07003*	—	0.47717	0.39304	0.38996	0.40435	0.49532	0.43078	0.43078	0.35667	0.38777	0.6194	0.39768
CLa	0.13828*	0.08577	—	0.38514	0.35328	0.31178	0.43491	0.45864	0.45864	0.5565	0.36914	0.67545	0.53264
LTa	0.05672	0.04632	0.05135	—	0.37773	0.2782	0.40234	0.27115	0.27115	0.36865	0.37729	0.70805	0.46005
LTb	0.06433	0.13285	0.12356	0.02823	—	0.26804	0.38996	0.50046	0.50046	0.39613	0.31178	0.63693	0.40547
DYb	0.09374*	0.02902	0.13241	0.05621	0.13655	—	0.37903	0.30691	0.30691	0.29886	0.32581	0.73748	0.38121
DYa	0.1253	0.11246	0.08021	0.0656	0.10293	0.11278*	—	0.44694	0.44694	0.48005	0.42348	0.60841	0.4617
LZ	0.04297	0.09059	0.1486	0.07552	0.08877	0.19497*	0.15052	—	0.41173	0.45676	0.4873	0.75037	0.68072
YG	0.0182	0.06286	0.07288	0.00839	0.02202	0.07683	0.02256	0.02754	—	0.37167	0.3721	0.75253	0.56982
DS	0.04097	0.0258	0.05832	0.04713	0.10421	0.04008	0.07576	0.09091	0.0393	—	0.55546	0.74033	0.56248
FS	0.00074	0.06598	0.08514	0.01543	0.03545	0.02304	0.08352	0.13126	0.0119	0.00045	—	0.64825	0.49899
SSb	0.0067	0.00357	0.07514	0.00722	0.04002	0.04155	0.0428	0.05398	0.03538	0.00473	0.00464	—	0.52022
TS	0.26268*	0.25881*	0.22344*	0.20926	0.28099*	0.22072*	0.13506*	0.30167*	0.19677*	0.23574*	0.25267*	0.24341*	—

$P < 0.001$.

SW (Shinwu, Taoyuan), CL (Chongli, Taoyuan), LT (Longtan, Taoyuan), DY (Dayuan, Taoyuan), LZ (Luzhu, Taoyuan), YG (Yingo, Taipei), DS (Dashi, Taoyuan), FS (Fuhsing, Taipei), SS (Sanshia, Taipei), TS (Tamsui).

Upper diagonal, Nei's genetic distance; lower diagonal, pairwise F_{st}.

Table 11 Pairwise estimates of genetic difference of *Solenopsis invicta* between polygyne collection sites of North Taiwan

	DYa	**SWa**	**SWb**	**HK**	**SSa**	**SSb**	**SSc**	**YG**	**YM**	**CLb**
DYa	—	0.06092	0.04589	0.06545	0.03247	0.05715	0.07558	0.02615	0.036	0.0509
SWa	0.03895*	—	0.03828	0.06345	0.04602	0.06735	0.12042	0.0426	0.04046	0.02992
SWb	0.02746*	0.04084*	—	0.05918	0.03638	0.09147	0.07561	0.04572	0.03671	0.03347
HK	0.05676*	0.05143*	0.05115*	—	0.06898	0.09458	0.10965	0.07949	0.06438	0.05982
SSa	-0.00031	0.04581*	0.04794*	0.06914*	—	0.04478	0.05121	0.01452	0.02689	0.04404
SSb	0.03362	0.06544*	0.09384*	0.09515*	0.03197*	—	0.08761	0.05644	0.08141	0.085
SSc	0.00378	0.07808*	0.04477*	0.07252*	0.00894	0.04021*		0.06147	0.08108	0.10769
YG	-0.00336	0.04635*	0.06348*	0.09066*	0.00063	0.03965*	0.01836	—	0.01747	0.03851
YM	0.0082	0.04018*	0.04199*	0.06063*	0.01786*	0.07765*	0.03528*	0.0097	—	0.0385
CLb	0.01331	0.02815*	0.04161*	0.05214*	0.03776*	0.08818*	0.05694*	0.0303*	0.03311*	—

$P < 0.001$.

DY (Dayuan, Taoyuan),SW (Shinwu, Taoyuan), HK (Hukou, Hsinchu), SS (Sanshia, Taipei), YG (Yingo, Taipei), YM (Yangmei, Taoyuan), CL (Chongli, Taoyuan).

Upper diagonal, Nei's genetic distance; lower diagonal, pairwise F_{st}.

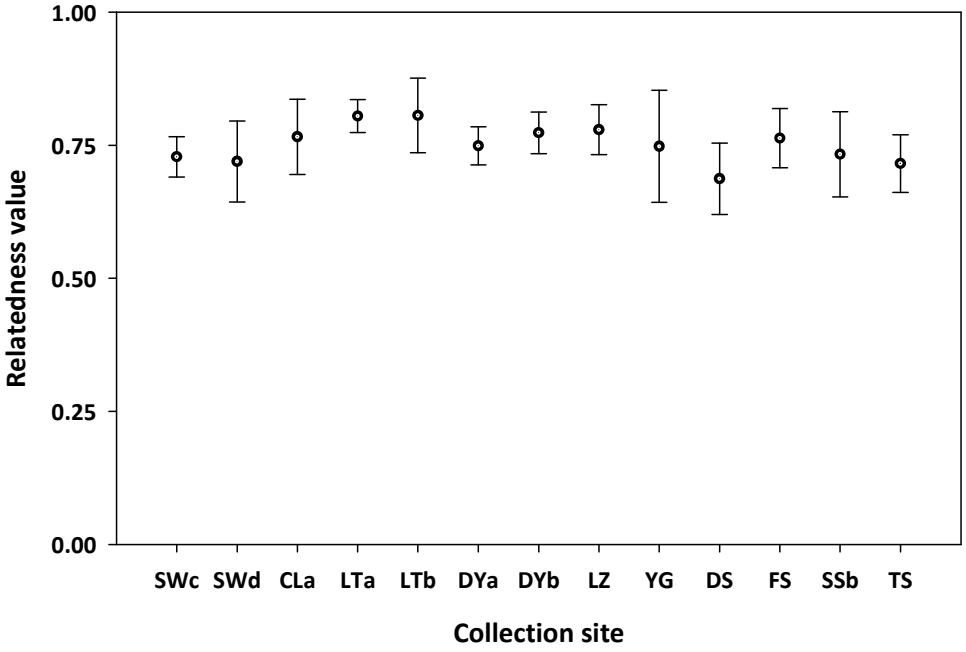

Figure 1 Average relatedness (±95 confidence interval) values for nestmate worker in monogyne form of *Solenopsis invicta* from 13 collection sites of Taiwan based on seven microsatellite loci and mtDNA haplotype. Collection site code: SW (Shinwu, Taoyuan), CL (Chongli, Taoyuan), LT (Longtan, Taoyuan), DY (Dayuan, Taoyuan), LZ (Luzhu, Taoyuan), YG (Yingo, Taipei), DS (Dashi, Taoyuan), FS (Fuhsing, Taipei), SS (Sanshia, Taipei), TS (Tamsui)

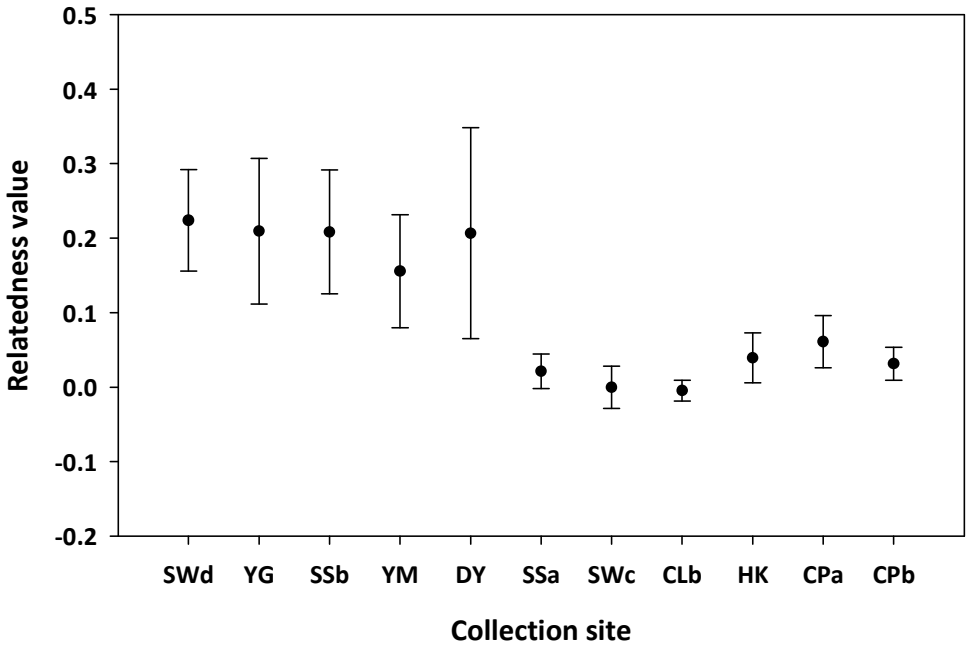

Figure 2 Average relatedness (±95 confidence interval) values for nestmate worker in polygyne form of *Solenopsis invicta* from 11 collection sites of Taiwan based on seven microsatellite loci and mtDNA haplotype. Collection site code: SW (Shinwu, Taoyuan), YG (Yingo, Taipei), SS (Sanshia, Taipei), YM (Yangmei, Taoyuan), DY (Dayuan, Taoyuan), CL (Chongli, Taoyuan), HK (Hukou, Hsinchu), CP (Chingpu, Chiayi)

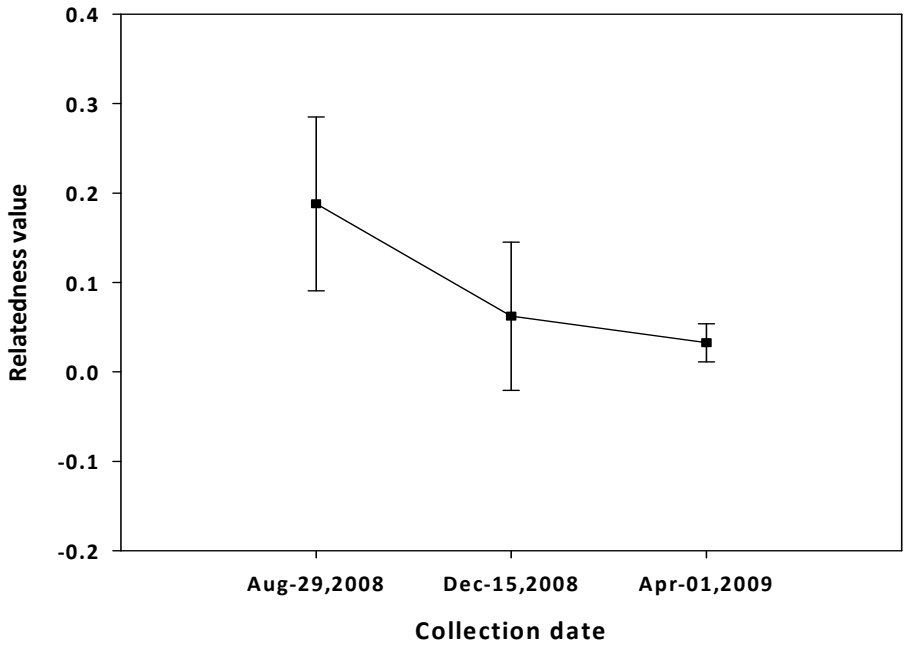

Figure 3 Variation of average relatedness value of *Solenopsis invicta* within three collection dates in YM (Yangmei, Taoyuan).

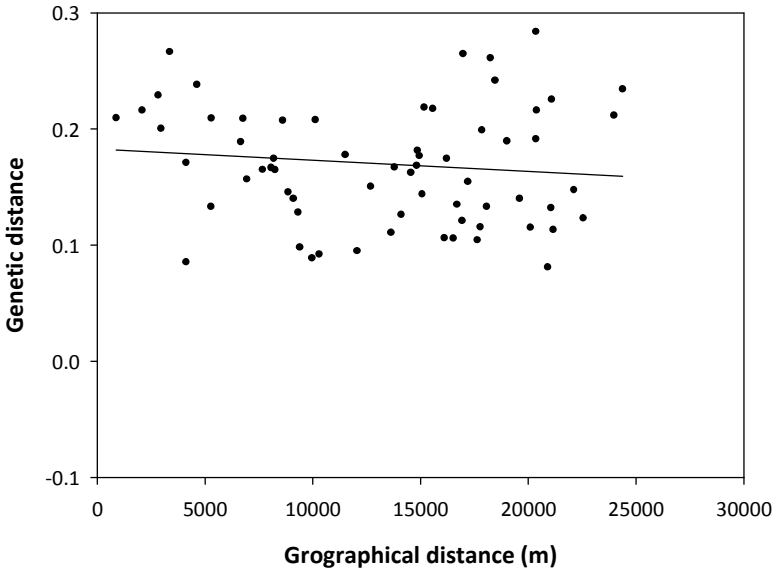

Figure 4 Correlation between the genetic distance and geographical distance measured by pairwise genetic distance and geographical distance of polygyne *Solenopsis invicta***.** No significant isolation by distance exhibited between polygyne form collection sites of *Solenopsis invicta* in North Taiwan (r = -0.1142, Mantel test: P = 0.83).

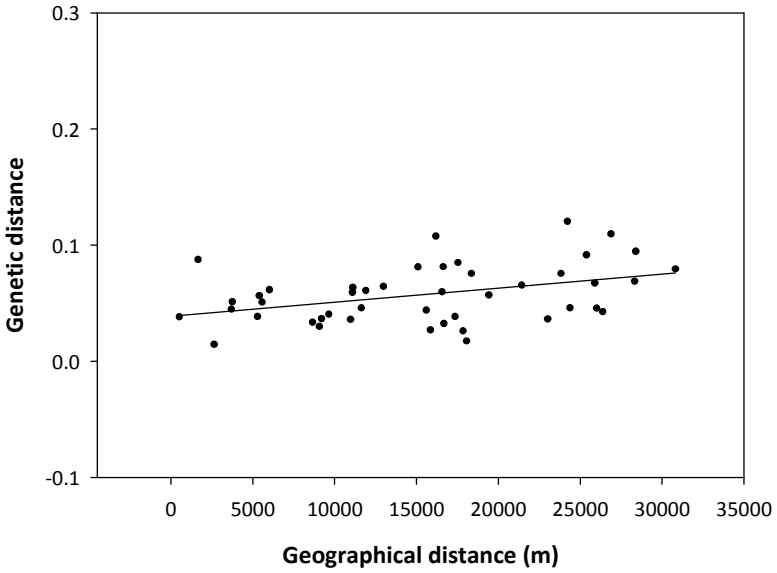

Figure 5 Correlation between the genetic distance and geographical distance measured by pairwise genetic distance and geographical distance of monogyne *Solenopsis invicta***.** Significant isolation by distance exhibited between monogyne form collection sites of *Solenopsis invicta* in North Taiwan (r = 0.402, Mantel test: $P < 0.0001$).

Appendix 1 Allele frequencies estimate from colonies of two *Solenopsis invicta* forms from both social forms sympatric collection sites

	Sanshia B		Yingo		Dayuan A		Chingpu B	
	P (N=9, n= 72)	M (N=8, n=64)	P (N=9,n=72)	M (N=4, n=32)	P (N=4, n=32)	M (N=4, n=32)	P (N=11, n=88)	M (N=1, n=8)
Microsatellite loci								
Sol－20								
102	0.1197	0.0833	0.0897	—	0.0345	0.0625	0.1023	—
104	0.4085	0.4643	0.4808	0.3750	0.4655	0.4063	0.2955	0.5625
106	0.2535	0.1905	0.1923	—	0.2096	0.3281	0.1364	0.4575
122	0.2183	0.2619	0.2372	0.6250	0.2931	0.2031	0.4659	—
Sol－M4								
264	0.2847	0.1548	0.2250	0.2083	0.2500	—	0.5000	0.5625
276	0.1042	0.2143	0.1563	0.1875	0.1250	0.2500	0.0570	—
278	0.6111	0.6310	0.6188	0.6042	0.6250	0.7500	0.4716	0.4378
284	—	—	—	—		—	0.0227	—
Sol－55								
176	0.2639	0.2195	0.4313	0.0833	0.3594	0.1774	0.4545	0.0625
178	0.069	—	—	—	—	—	0.1761	—
180	0.2431	0.0854	0.1750	0.2292	0.1250	0.1516	0.2102	0.4375
182	0.2569	0.3049	0.2188	0.7667	0.3438	0.3226	0.1193	0.3125
186	0.2292	0.3902	0.1750	0.5208	0.1719	0.0484	0.0398	0.1875
Sol－11								
141	0.0972	0.2143	0.2938	0.4375	0.3750	0.2696	0.0852	0.1875
143	0.0972	0.2381	0.2563	0.3542	0.3438	0.3750	0.0057	—
145	0.0139	—	0.0250	—	—	—	0.0170	—
149	0.0764	0.2381	0.0938	—	0.0938	—	0.5114	0.5000
153	0.7135	0.3095	0.3313	0.2083	0.1875	0.3281	0.3807	0.3125

Appendix 1 (continued) Allele frequencies estimate from colonies of two *Solenopsis invicta* forms from both social forms sympatric collection sites

	Sanshia B		Yingo		Dayuan A		Chingpu B	
	P	M	P	M	P	M	P	M
	(N=9, n= 72)	(N=8, n=64)	(N=9,n=72)	(N=4, n=32)	(N=4, n=32)	(N=4, n=32)	(N=11, n=88)	(N=1, n=8)
Microsatellite loci								
Sol－M3								
206	0.2431	0.2500	0.1625	0.1250	0.2581	0.6875	0.4773	0.1875
208	—	—	0.0563	0.0563	0.0645	—	0.3130	0.7500
210	0.7569	0.7500	0.7813	0.7813	0.6774	0.3125	0.1477	0.0625
218	—	—	—	—	—	—	0.0739	—
Sol－49								
137	0.1042	—	0.0750	0.1667	0.0165	—	0.0682	—
145	0.2708	0.2976	0.3188	0.1667	0.2500	0.3594	0.0795	—
157	0.3681	0.4762	0.2875	0.3333	0.3438	0.3281	0.3011	0.5000
159	0.0486	—	0.0875	0.3333	0.0781	0.2500	0.2829	0.5000
161	0.0069	—	0.0063	—	—	—	—	—
163	0.2014	0.2262	0.2250	—	0.3125	0.0625	0.2614	—
Sol－42								
115	0.1250	—	0.1438	0.3125	0.0469	0.3906	0.7443	0.5000
117	0.6250	0.6310	0.5000	0.5208	0.5781	0.5000	0.1705	0.3750
119	0.0556	0.0595	0.1125	0.1667	0.1875	—	0.0284	—
129	0.1944	0.3095	0.2438	—	0.1875	0.1094	0.0568	0.1250
mtDNA haplotype								
A	0.8333	0.2857	0.7821	0.6667	1	0.5	—	—
B	0.1667	0.7143	0.2179	0.3333	—	0.5	—	—
C	—	—	—	—	—	—	1	1

Appendix 2 (continued) Allele frequencies estimate from *Solenopsis invicta* polygyous colonies from polygyous collection sites

	Sanshia A (N=9, n= 72)	Sanshia c (N=8, n=64)	Shinwu A (N=8, n=64)	Shinwu B (N=9,n=72)	Chongli B (N=4, n=32)	Yangmei (N=4, n=32)	Hukou (N=4, n=32)	Chingpu A (N=4, n=32)
Microsatellite loci								
Sol－20								
102	0.0347	0.1087	0.1750	0.0547	0.0735	0.1028	—	0.0625
104	0.3960	0.3043	0.1938	0.3627	0.3480	0.3606	0.5347	0.4063
106	0.2228	0.3696	0.1500	0.3243	0.3725	0.2019	0.2778	0.3281
120	0.0099	—	—	—	0.0049	— —	—	—
122	0.3366	0.2174	0.4813	0.2539	0.2010	0.3005	0.1875	0.2031
124	—	—	—	—	—	0.0288	—	
Sol－M4								
264	0.2692	0.3333	0.1000	0.3164	0.2402	0.2569	0.2113	—
274	—	—			0.0098	0.0023	—	
276	0.1835	0.1042	0.0857	0.0781	0.2206	0.0926	0.0211	0.2500
278	0.5769	0.5625	0.6500	0.6055	0.5245	0.6296	0.7183	0.7500
282	—	—	—	—	—	0.0046	0.0211	—
284	—	—	—	—	0.0049	0.0139	0/0282	—
Sol－55								
176	0.4038	0.4375	0.4851	0.5244	0.4100	0.5257	0.5352	0.1774
178	—	0.0208	—	0.0041	—	—	—	—
180	0.1202	0.1458	0.2687	0.0854	0.2500	0.1098	0.0986	0.1516
182	0.2019	0.1458	0.1119	0.1138	0.1050	0.1285	0.1831	0.3226
186	0.2740	0.2500	0.1343	0.2724	0.2350	0.2360	0.1831	0.0484
Sol－11								
141	0.2163	0.0833	0.3750	0.3047	0.2059	0.3611	0.0493	0.2696
143	0.2452	0.1667	0.1688	0.3672	0.3376	0.2755	0.3380	0.3750
145	0.0370	0.0417	—	0.0039	—	0.0301	0.0070	—
149	0.1010	0.0625	0.1125	0.1250	0.2059	0.1088	0.3592	—
153	0.4038	0.6458	0.1992	0.1992	0.2006	0.2245	0.2465	0.3281

Appendix 2 Allele frequencies estimate from *Solenopsis invicta* polygyous colonies from polygyous collection sites

	Sanshia A (N=9, n= 72)	Sanshia c (N=8, n=64)	Shinwu A (N=8, n=64)	Shinwu B (N=9,n=72)	Chongli B (N=4, n=32)	Yangmei (N=4, n=32)	Hukou (N=4, n=32)	Chingpu A (N=4, n=32)
Microsatellite loci								
Sol－M3								
206	0.2596	0.4167	0.4500	0.6260	0.3155	0.2453	0.6875	0.6875
208	0.0337	—	0.0250	0.0039	0.0243	0.0140	0.0071	—
210	0.7069	0.5833	0.5250	0.3701	0.6602	0.7407	0.3071	0.3125
Sol－49								
137	0.0769	0.2292	0.0536	0.0625	0.1068	0.0234	0.0143	—
145	0.2837	0.1042	0.3750	0.1797	0.2913	0.1986	0.3143	0.3594
157	0.3942	0.2500	0.4063	0.3750	0.3350	0.2944	0.5214	0.3281
159	0.0385	0.1250	0.0188	0.0313	0.1262	0.1215	0.0429	0.2500
161	—	—	—	—	—	0.0070	—	—
163	0.2067	0.2917	0.1438	0.3516	0.1408	0.3528	0.1071	0.0625
165	—	—	—	—	—	0.0023	—	—
Sol－42								
115	0.1402	0.2083	0.0438	0.1181	0.0219	0.0403	0.0071	0.3906
117	0.5481	0.5833	0.3625	0.2638	0.3883	0.5213	0.6000	0.5000
119	0.0721	0.0625	0.3875	0.2717	0.4320	0.2275	0.3017	—
123	—	—	—	—	—	0.0261	—	—
129	0.2356	0.1458	0.2036	0.3465	0.1505	0.1848	0.0857	0.1094
mtDNA haplotype								
A	0.9519	0.1	0.1	0.6220	0.7468	0.9911	1	—
B	0.0481	—	—	0.3780	0.2532	0.0089	—	—
C	—	—	—	—	—	—	—	1

Appendix 3 Allele frequencies estimate from *Solenopsis invicta* monogyous colonies from monogyous collection sites

	Tamsui (N= 8, n=48)	Fuhsing (N=4, n=24)	Shinwu C (N=8, n=48)	Shinwu D (N=5, n=30)	Longtan A (N=5, n=30)	Longtan B (N=5, n=30)	Chongli A (N=7, n=42)	Dashi (N=6, n=36)	Luzhu (N=6, n=42)
Microsatellite loci									
Sol－20									
102	—	0.1667	—	0.1167	0.1000	—	0.4028	—	—
104	0.1548	0.4375	0.4344	0.3167	0.2167	0.1667	—	—	0.1389
106	0.6786	0.0208	0.0246	0.4500	0.2333	—	0.2778	0.3056	0.1667
120	—	—	—	—	—	—	—	0.4861	—
122	0.1667	0.3750	0.5410	0.1167	0.4500	0.8333	0.3194	0.2083	0.6944
Sol－M4									
264	0.1875	0.2292	0.1875	0.2667	0.2333	0.2667	0.0972	0.2222	0.0833
274	—	0.0208	—	—	—	—	—	0.0417	—
276	—	—	0.1797	0.3367	0.1833	0.3333	0.0833	0.1389	0.2619
278	0.7500	0.7500	0.5938	0.3367	0.583	0.4000	0.8194	0.5972	0.6548
284	0.0625	—	0.0391	—	—	—	—	—	—
Sol－55									
176	—	—	0.2500	0.4167	0.4833	0.3000	0.5556	0.3571	0.5238
180	0.3125	0.2500	0.1694	—	0.1000	0.1000	0.0833	0.1429	0.2143
182	0.6875	0.2917	0.3226	0.2167	0.2333	0.1500	0.2083	0.2714	0.1786
186	—	0.4583	0.2581	0.3667	0.1833	0.4500	0.1528	0.2286	0.0833
Sol－11									
141	0.1458	0.1875	0.1587	0.4000	0.1000	0.0167	0.2778	0.2500	0.0119
143	0.7292	0.4375	0.3730	0.2000	0.4333	0.6667	0.5000	0.1806	0.1190
149	0.1250	0.1250	0.1429	0.0167	0.1167	0.1167	—	—	0.6310
153	—	0.2500	0.3254	0.3833	0.3500	0.2000	0.2222	0.5694	0.2381

Appendix 3 (continued) Allele frequencies estimate from *Solenopsis invicta* monogyous colonies from monogyous collection sites

	Tamsui (N=9, n= 72)	Fuhsing (N=8, n=64)	Shinwu C (N=9,n=72)	Shinwu D (N=4, n=32)	Longtan A (N=4, n=32)	Longtan B (N=4, n=32)	Chongli A (N=11, n=88)	Dashi (N=1, n=8)	Luzhu (N=11, n=88)
Microsatellite loci									
Sol－M3									
206	0.6979	0.0417	0.0873	0.2333	0.1667	0.3833	0.3333	0.2222	0.2381
208	0.0833	—	—	0.2000	—	0.0167	—	0.0833	—
210	0.2188	0.9583	0.9127	0.2667	0.8333	0.6000	0.6667	0.6944	0.7619
Sol－49									
137	—	0.1250	0.0243	0.2667	0.2167	0.1000	—	0.0833	0.1190
145	0.2500	0.5208	0.4453	0.2833	—	0.2000	0.1667	0.3333	0.2619
157	0.0521	0.1042	0.3125	0.4500	0.1833	0.4000	0.3611	0.3889	0.6190
159	0.6771	—	0.1250	—	0.1000	—	—	—	—
163	0.0208	0.2500	0.0938	—	0.5000	0.3000	0.4722	0.1944	—
Sol－42									
115	—	0.0417	0.0789	—	0.0667	—	0.0278	0.0694	1
117	0.8438	0.5000	0.8421	0.6167	0.7167	0.5833	0.6250	0.5972	—
119	0.1042	0.3333	0.0175	0.1500	—	—	0.3472	0.2222	—
123	—	—	0.0439	—	—	0.0167	—	—	—
129	0.0524	0.1250	0.0175	0.2333	0.2167	0.4000	—	0.1111	—
mtDNA haplotype									
A	0.5000	—	1	—	0.4000	—	0.1667	0.5	0.5238
B	0.5000	1	—	1	0.6000	1	0.8333	0.5	0.4762