Monogynous and Polygynous Red Imported Fire Ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), in Taiwan

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ABSTRACT The social forms of the red imported fire ant, initially discovered in Taoyuan County in 2003, were determined based on Gp-9 allele identification. Both polygynous and monogynous colonies were found. It was also found that, in almost all of townships surveyed, there was tendency to have more polygynous nests than monogynous nests. The monogynous nests were indeed authentic monogynous nests without contamination of the cryptic b-prime allele from South America. Sequence comparison revealed that polygynous colonies in Taiwan contained either the Gp-9^{B1} or Gp-9^{B3} variant (*Sinv.B1* and *Sinv.B3* in GenBank) of the B allele, whereas some of the monogynous colonies contained a Gp-9^{B2}–like variant of the B allele (*Sinv.B2* in GenBank). Gp-9^{B1} also was found in some monogynous colonies. The scenario of a single introduction by a founding polygynous colony with all of the monogynous nests being descendents of this polygynous nest was also ruled unlikely because of the presence of the monogyne-specific B2 allele. These findings favor multiple origins of the red imported fire ant in Taiwan.

KEY WORDS Gp-9, monogyny, polygyny, multiplex polymerase chain reaction, red imported fire ant

RED IMPORTED FIRE ANTS, *Solenopsis invicta* Buren, are notorious invasive pests originating from South America. They recently were introduced to Taiwan (Drees 2004, Huang et al. 2004), with the first confirmed case in Taoyuan City in October 2003 (Huang et al. 2004). Based on the pattern of spread at the time and the size of the mounds, it was estimated that this species could have arrived in Taiwan as early as 2002. It also was suspected that the fire ants arrived by cargo crate containers near the international airport in Taoyuan (Shi 2005). Fire ants are thought to be spreading on the island mostly by human commerce such as the transportation of nursery stock and soil (Huang et al. 2004). Fire ants may also spread by mating flights, colony budding, and floods (Taber 2000, Huang et al. 2004).

Because research on red imported fire ants in Taiwan is only beginning, no published data and refereed articles about their basic biology exist (McCubbin 2004). Such research is important even though much has been done on the fire ants elsewhere in the world (Taber 2000), because the same species of organism may devise different strategies in different environments, and the environments in Taiwan differ drastically from those in the southern United States, where the ant is established (Drees 2004).

An important aspect of fire ant biology is the social form. The two distinct social forms of *S. invicta* are polygyny and monogyny (Glancey et al. 1973, 1975). These two social forms differ in many aspects of behavior, physiology, and genetics (Krieger and Ross 2002, Fritz and Vander Meer 2003, Krieger 2004). A gene known as *general protein-9* (*Gp-9*), which belongs to the family of odorant-binding proteins, is a major marker of each social form (Krieger and Ross 2002, Krieger 2004). Whereas polygynous queens are Gp-9^{Bb} heterozygotes, monogynous queens are Gp-9^{BB} homozygotes. There are three known variants of the B allele of this gene. B1 is found commonly in both social forms, whereas B2 and B3 are found only in monogynous and polygynous fire ants, respectively (Krieger and Ross 2002). Because of the specificity of B2 and B3 allelic variants to their respective social forms, sequences of the Gp-9 B allele variants may be used to determine the origins of monogyny in Taiwan. Monogyny may arise either from preexisting monogyny or when descendants are produced from the mating of a polygynous queen to a haploid B male. Such haploid males almost always come from preexisting monogynous colonies because most of the males in polygynous nests are sterile diploids (Krieger 2004). Nevertheless, the mating of polygynous queens to fertile haploid B males from polygynous nests can occur, but such males are a small minority (<10% of all males) in a polygynous nest. If monogyny in Taiwan arose from polygyny, all of the monogynous nests should contain B1B1 homozygotes, and no B2 variant of the allele should be found anywhere, because the

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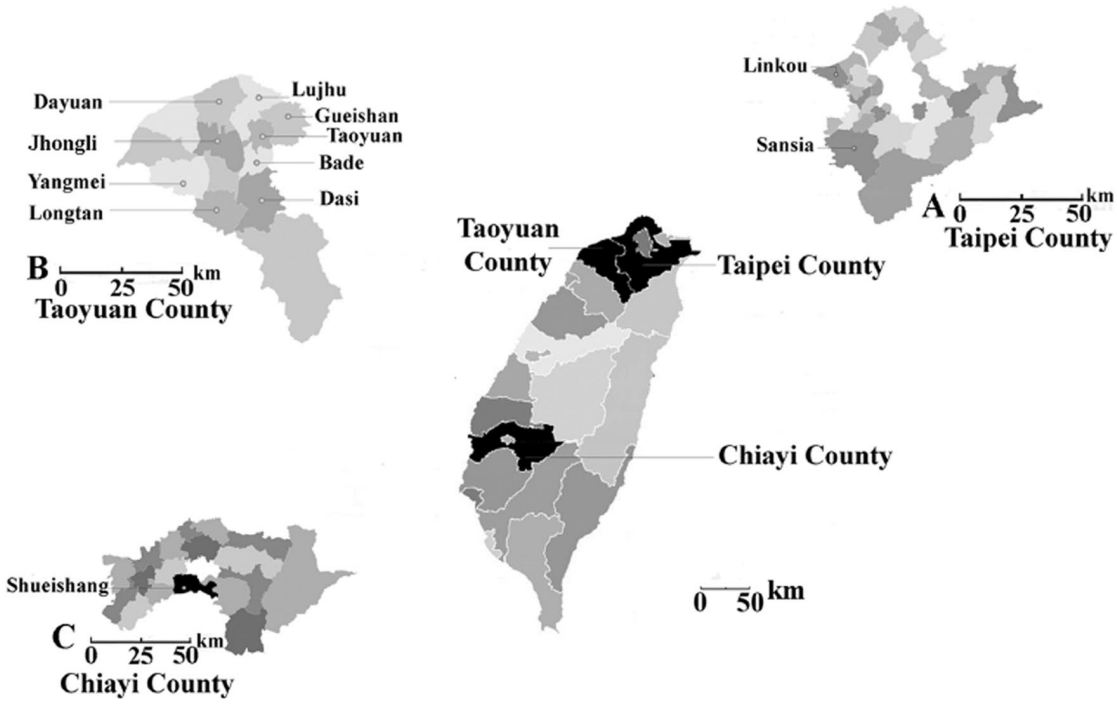

Fig. 1. Map of Taiwan and the distribution of the red imported fire ant. Counties with fire ant infestation are in black. Each infested county is enlarged to show townships within it surveyed for the social form of the fire ants. (A) Taipei County. (B) Taoyuan County. (C) Chiayi County. In all cases, only townships and cities surveyed are named. Maps are adapted from the Ministry of Interior, Taiwan.

B2 allele is not present in polygynous colonies. However, the finding of a B2 variant would suggest that the B2-bearing monogynous nests in Taiwan might not have the same origin as the polygynous colonies.

The following paper presents evidence for the presence of both social forms in Taiwan. The percentage of each social form was estimated in each infested township. The possible presence of the cryptic b-prime allele was studied. Finally, amplified $Gp9^B$ genes were cloned and partially sequenced from several colonies to reveal allelic variants of the B allele in the two social forms. By sequencing the B allele from several localities, an attempt was made to find the B2 allele among the Taiwanese fire ants.

Materials and Methods

Workers of the red imported fire ant were collected in various locations in Taipei, Taoyuan, and Chiayi Counties, the only counties to harbor red imported fire ants (Fig. 1) between October 2003 and March 2005. Whereas most townships in Taoyuan County were infested, only a few of the townships in Taipei and Chiayi Counties were infested. In Taoyuan County, 130 nests were collected from eight townships (Table 1). Twelve nests from two townships in Taipei County were collected. In Chiayi County, six nests were collected from one township. Samples of the different sites were taken at different times during the sampling period. The collected workers were immediately transferred into 95% ethanol, following the recommendations of King and Porter (2004), and brought back to the laboratory for genetic analysis.

Homogenate from ≈20 fire ant workers, macerated in 500 μl of ddH_2O, was spotted onto FTA cards (Whatman, Florham Park, NJ) for long-term storage and archiving of DNA (Snowden et al. 2002). The FTA card–preserved DNA materials were prepared for polymerase chain reaction (PCR) according to manufacturer's instructions. Diagnostic multiplex PCR reactions from Valles and Porter (2003) modified to support FTA cards were performed to reveal the social

Table 1. Distribution of monogynous and polygynous red imported fire ants in Taiwan

County	Town/city	Polygyne		Monogyne	
		No	Percent	No	Percent
Taipei	Linkou	5	33	10	67
	Sansia	7	100	0	0
Taoyuan	Bade	20	74	7	26
	Jhongli	0	0	5	100
	Dayuan	8	89	1	11
	Gueishan	10	77	3	23
	Longtan	3	100	0	0
	Lujhu	6	43	8	57
	Taoyuan	27	73	10	27
	Dasi	4	40	6	60
	Yangmei	6	100	0	0
Chiayi	Shuishang	5	83	1	17
Total		101	66	51	34

identities of the colonies. Multiplex PCR was performed with an Eppendorf Mastercycler (Brinkmann Instruments, Westbury, NY). The reactions were run in 25-μl volumes. PCR products (5 μl) were separated on a 1% agarose gel and visualized by ethidium bromide staining. For all experiments, negative controls were run alongside the DNA samples. Fifty-microliter volumes were run for the samples that were to be sequenced.

Because the PCR protocols of Valles and Porter (2003) were unable to differentiate b-prime–positive colonies from monogynous colonies (Krieger and Ross 2002), and because the possibility of a South American introduction of the fire ants must be considered, primers based on Mescher et al. (2003) were used, These authors investigated *Gp-9* genes in the South American fire ants, and their primers and amplification protocols were able to differentiate between monogynous and b-prime–positive colonies. Only the colonies scored as "monogynous" by the method of Valles and Porter were tested, because b-prime polygynous colonies would generate the same banding patterns as monogynous colonies using this PCR method. Gp-9^{Bb} polygynous colonies did not need to be retested. b-prime–positive controls from South America, provided courtesy of K. Ross, were run alongside the PCR reactions.

To sequence the *Gp9* gene, PCR amplicons of Taiwanese fire ants were eluted from the gel using the QiaQuick Gel Purification kit (Qiagen, Valencia, CA). The purified DNA amplicons were cloned onto pGEM-T Easy vector and transformed into XL1-Blue competent cells. The positive clones were sequenced by Applied Biosystems (Taipei, Taiwan).

The *Gp-*9^B gene was sequenced from 10 spatially distant monogynous colonies and 3 polygynous colonies. We used more monogynous colonies because we wished to screen for the rare *Sinv.B2* variant of the B allele. We believed that sequencing from geographically distant colonies might increase the chance of finding the B2 allele. The samples were first amplified using the primers and protocols of Valles and Porter (2003). The B amplicons were eluted and cloned as before. The sequences were aligned with reference sequences and analyzed using the AlignX module of Vector NTI software (Invitrogen, Carlsbad, CA).

Results

Both monogyny and polygyny were discovered in all three counties surveyed (Fig. 2; Table 1). Figure 2 shows gels of PCR products of North American fire ants from College Station, TX, and Taiwanese monogynous and Taiwanese polygynous fire ants side by side. Like U.S. monogynous fire ants, the Taiwanese monogynous fire ants yielded one PCR band at the same location (517 bp; Fig. 2). Polygynous populations, having both Gp-9^B and Gp-9^b alleles, are characterized by two bands at 517 and 423 bp. Taiwanese polygynous populations, as expected, yielded two PCR amplicons at the same locations (Fig. 2).

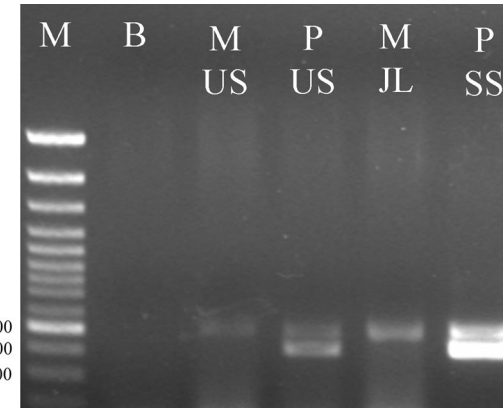

Fig. 2. Comparison of multiplex PCR banding patterns of U.S. and Taiwanese monogynous and polygynous fire ants. M, marker; B, blank; MUS, monogynous, U.S.; PUS, polygynous, U.S.; MJL, monogynous, Jhungli, Taiwan; PSS, polygynous, Sansia, Taiwan.

Comparisons of amplified DNA sequences between the U.S. and Taiwanese populations showed that they were very similar. When the sequences of the Taiwanese Gp-9 were compared on-line using BLAST (Altschul et al. 1997), the results suggest that the partial sequences were indeed those of Gp-9^B and Gp-9^b of the red imported fire ants. Because we did not use proofreading-enabled *Taq* polymerase for PCR amplification and subsequent sequencing, the data can only suggest that these were similar to the B and b alleles, and any differences in individual nucleotides may be the result of sequencing errors.

Specifically, the partial sequences of the B allele in Taiwan revealed similarity to both B1/B3 and B2 alleles. Sequences with an exact match to *Sinv.B2* were identified from some monogynous colonies (Fig. 3). Although the PCR products were only partial sequences, the amplified products nevertheless included the signature 3-bp deletion found only in the B2 allelic variant (Fig. 3). It is highly unlikely that the deletion in all of the samples were the result of amplification fidelity and sequencing errors. Sequences with an exact match to *Sinv.B1/B3* were cloned from both monogynous and polygynous colonies (Fig. 3). Although the primers used in this amplification assay were unable to differentiate between the B1 and B3 variants of the allele (Fig. 3), it is logical to suspect that the B1/B3 sequences found in monogynous colonies such as TWM_1, TWM_4, TWM_8, and TWM_10 might be the B1 allelic variant, not the B3 variant. However, differentiation between these B1 and B3 variant in polygynous colonies was beyond the scope of this paper.

Because the PCR procedure of Valles and Porter (2003) is unable to differentiate the polygynous colonies carrying the cryptic b-prime allele from monogynous colonies, all of the "monogynous"colonies scored using the procedure of Valles and Porter were retested using the protocols of Mescher et al. (2003) to reveal the possible presence of the cryptic

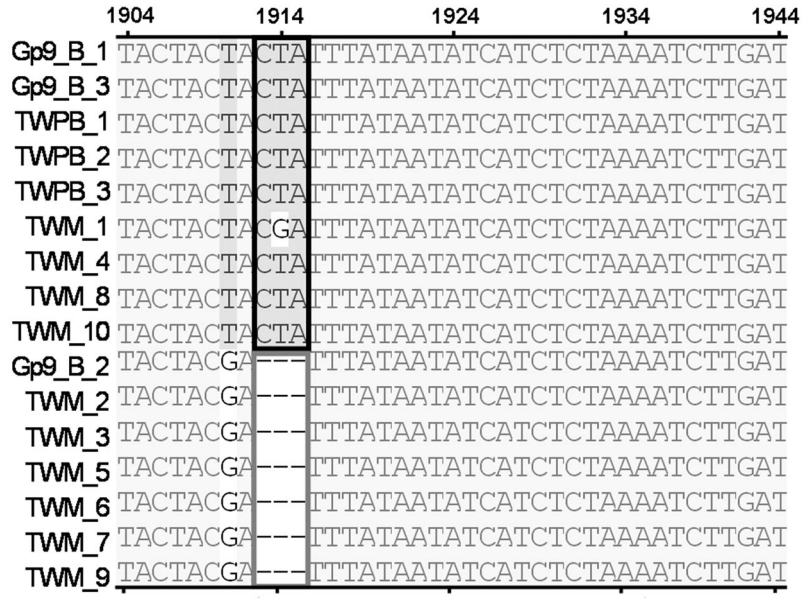

Fig. 3. Partial sequences of B alleles of fire ants in Taiwan. The gray box indicates the characteristic 3-bp deletion of the B2 allele. The black box indicates the nucleotides deleted in B2 but present in B1. rB_2, *Sinv.B2* sequences from GeneBank; rB_1, *Sinv.B1* sequences from GeneBank; rB_3, *Sinv.B3* sequences from GeneBank; TWPB_1, polygyne B allele from Sansia, Taipei County; TWPB_2, polygyne B allele from Taoyuan City, Taoyuan County; TWPB_3, polygyne B allele from Bade City, Taoyuan County; TWM_1, monogynous B allele from Shueishang Township, Chiayi County; TWM_2, monogynous B allele from Jhungli City, Taoyuan County; TWM_3, monogynous B allele from Dasi Township, Taoyuan County; TWM_4, Gueishan Township, Taoyuan County; TWM_5, Dasi Township, Taoyuan County; TWM_6, Lujhu Township, Taoyuan County; TWM_7, Bade Township, Taoyuan County; TWM_8, Taoyuan City, Taoyuan County; TWM_9, Jhungli City, Taoyuan County; TWM_10, Linkou Township: Taipei City.

b-prime allele. Results did not support the presence of the b-prime allele among the monogynous samples because the primers amplified only the positive controls from South America, whereas one of the monogynous samples from Taiwan was amplified (data not shown).

The initial discovery of *S. invicta* in Taiwan was in samples from Taoyuan City (Drees 2004, Huang et al. 2004). This sample, collected on 10 October 2003, was polygynous according to the multiplex PCR tests. The earliest sample from Chiayi in Southern Taiwan, collected in December 2003, was polygynous. The earliest monogynous samples were collected in Linkou Township in Taipei County in February 2004.

In Taoyuan County, the following townships and cities yielded fire ants of both social forms (Fig. 1; Table 1): Bade, Dayuan, Gueishan, Lujhu, Taoyuan City, and Dasi. In terms of the Taipei County, of the 13 colonies collected in Linkou, 10 were monogynous, and the remaining 3 were polygynous. The seven colonies collected in Sansia were all polygynous (Table 1). Shueishang Township in Chiayi contained fire ants of both social forms. The cities and townships in Taoyuan County with only one or the other social form included Jhungli City, Longtan Township, and Yangmei Township. Together, a majority of the collected colonies was polygynous (Table 1).

Discussion

Fire ants of both social forms were identified in Taiwan. The multiplex PCR method is 100% accurate in determining the social identity of fire ant colonies (Valles and Porter 2003). Because this PCR amplification protocol was unable to distinguish b-prime fire ants from monogynous fire ants, protocols from Mescher et al. (2003), which could distinguish between the two, were used. The lack of amplification of Taiwanese samples and the successful amplification of South American b-prime–positive sample did not provide evidence of b-prime, and hence there is no evidence of South American fire ants among the Taiwanese fire ant samples.

The three known B allele variants include B1, B2, and B3, which correspond to *Sinv.B1*, *Sinv.B2*, and *Sinv.B3*, respectively, in GenBank. Polygynous colonies carry B1 and B3, whereas monogynous colonies carry B1 and B2. B1 is the most common of the three allelic forms, whereas the other two are rarer (Krieger and Ross 2002). B2-, B1-, and B3-like alleles were cloned from Taiwanese fire ants. The protocols did not allow us to differentiate between B1 and B3 allele variants in polygynous colonies because the only difference between the B1 and B3 allelic variants is located outside of the sequenced region. However, the protocols allowed us to differentiate B2 from B1 and

B3 because the sequenced region included the signature 3-bp deletion in B2 allelic form.

The results showed that polygynous colonies carry B1/B3, whereas monogynous colonies carry B1 and B2. The single nucleotide difference in TWM_1 (from Chiayi) from the others could reflect either a sequencing error or an intrinsic nucleotide difference. Regardless, it is still classified as B1-like allele.

Monogynous Gp-9^{BB} queens may arise from preexisting polygynous colonies by the mating of Gp-9^{Bb} queens and Gp-9^{B} males (Krieger 2004). These haploid, fertile males, although present as a small minority in a polygynous nest, may nevertheless mate during the mating flight. For monogyny to have completely arisen from polygyny in Taiwan, all of the monogynous colonies in Taiwan would have to be of the B1 B1 genotype, because the B2 variant does not exist in polygynous colonies. The detection of B sequences orthologous to *Sinv.B2* suggests that these B2-bearing monogynous colonies did not arise from polygynous colonies. Therefore, the hypothesis of a single founding event by a polygynous nest, with all subsequent monogynous colonies arising from this founding polygynous colony, is supported by the data. However, these data do not preclude the possibility that some of the monogynous colonies that do not bear the B2 allele may be derived from a polygynous colony.

It is unlikely that there was a lone invasion of monogynous colonies to Taiwan followed by a subsequent mutation from Gp-9^{B} to Gp-9^{b}. In the United States, it took ≈40 yr for Gp-9^{B} to mutate to Gp-9^{b} (Glancey et al. 1973, 1975, Fritz and Vander Meer 2003), whereas the fire ants have been in Taiwan for no more than a few years (Drees 2004, Huang et al. 2004, Shi 2005). Furthermore, the b allele differs from the B allele at nine highly conserved positions (Krieger 2004). A more likely scenario would be either a cointroduction of both social forms or independent multiple introductions. A more detailed study on the population genetics of *S. invicta* is underway to reveal the manner of introduction of these ants to Taiwan.

Based on the distribution pattern of *S. invicta* in Taiwan, the county of Taoyuan must be the original port of entry (Fig. 1) for both social forms. While all of Taoyuan County's townships are infested, only a few townships on the southern fringes of Taipei County adjacent to Taoyuan County currently harbor fire ants (Fig. 1). The subsequent expansion of fire ant populations depends on mating flights, colony budding of polygynous colonies, flooding, and transport through human commerce (Taber 2000, Huang et al. 2004, Shi 2005). Flooding is one of the major mechanisms of local fire ant population expansion in Taiwan (Huang et al. 2004). One form of flooding is the local farming practice of irrigation, in which the entire field is flooded to irrigate the rice crop. This practice may also aid the spread of fire ants (Drees 2004). In the case of human transport, the major source of fire ant infestations are nurseries, particularly those in Taoyuan County, most of which are infested (Drees 2004, Huang et al. 2004). This mechanism is particularly important for spread over long distances (Taber 2000).

Chiayi County is the only county in southern Taiwan to harbor *S. invicta* (Drees 2004, Huang et al. 2004). Most of the fire ants in this county were found in the Shueishang Township (Huang et al. 2004). The four counties north of Chiayi have not yet reported fire ant invasion (Huang et al. 2004), suggesting transport over a distance much greater than the maximum 8 km that can be traversed by a queen during its mating flight (Fig. 1) (Taber 2000). In addition, the fire ants in Chiayi County were all localized in two townships surrounding the Sanjiepu Nursery (Huang et al. 2004). Together, these facts do not support the idea of a natural incursion from the north through mechanisms such as mating flight, budding, or flood. Rather, they suggest that the ants were introduced to Chiayi by human commerce from one or more infested counties in northern Taiwan, with subsequent local expansion occurring by mating flight, flooding, and colony budding.

Polygynous fire ants are potentially more harmful to the economy and the environment than the monogynous form because of their higher mound densities of polygyny (Porter et al. 1991). In Texas, polygyny was found in 54% of the sites surveyed in Texas and is not correlated to habitat and environmental conditions. The high frequency of polygyny in Texas led the authors to conclude that the fire ants are a more serious problem in Texas than previously believed (Porter et al. 1991). In the case of Taiwan, polygyny is also the majority form in most cities and townships surveyed. Although no current data exist on the effect of polygynous red imported fire ants on Taiwanese wildlife, the findings by Porter et al. (1988) may serve to predict a similar scenario occurring in Taiwan. Therefore, Taiwan may be under greater economic and ecological threat from *S. invicta* than is currently realized because of the presence of polygyny in almost all of the townships and cities surveyed.

In conclusion, both monogynous and polygynous social forms of the red imported fire ant were found in most of the townships surveyed. These data suggest either a cointroduction of both social forms to Taiwan or multiple introductions. The scenario of a single introduction of a founding polygynous colony, with all subsequent monogynous nests descending from it, is considered unlikely because of the presence of the monogyny-specific B2 allele in several monogynous colonies. Most townships have a tendency to have more polygynous colonies than monogynous colonies. This could be caused by higher mound densities, characteristic of the polygynous form. Fire ants in Chiayi are unlikely to have arrived initially by natural means, but rather they most likely arrived by human-mediated transport from the north. The lack of amplification of the South American b-prime allele using specific primers and amplification protocols does not support a South American origin of the introduced fire ants, at least in the case of the monogynous colonies sampled. Finally, the higher frequency of polygyny than monogyny in most of the townships surveyed may indicate that Taiwan is under greater economic and ecological threat than is currently realized.

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