Tracing the Origin of *Chilades pandava* (Lepidoptera, Lycaenidae) Found at Kinmen Island Using Mitochondrial COI and COII Genes

Li-Wei Wu¹, David C. Lees²,³, Yu-Feng Hsu¹*

¹Department of Life Science, National Taiwan Normal University
Taipei, Taiwan

²Department of Entomology, Natural History Museum
London, UK

³Centre de Recherche d’Orléans, Zoologie Forestière
Orléans, France

(Received: 12 October 2009, accepted: 30 October 2009)

ABSTRACT

Recent outbreaks of the Cycad Blue, *Chilades pandava*, have caused severe damage to *Cycas* plants, affecting their horticultural value. In July of 2007, this butterfly pest was recorded for the first time from Kinmen, offshore of Fujian, where it has caused substantial damage to the ornamental cycad, *Cycas revoluta* planted on this island. This Cycad Blue colony disappeared by December 2007, but irrupted again in 2008 and 2009. Because neither the Cycad Blue nor native *Cycas* plants were documented in Kinmen before ornamental cycads were introduced from Taiwan, the origin of the Cycad Blue individuals found on the island was unknown. Two possible scenarios are given herein. Firstly, Cycad Blue may have been introduced via imported *Cycas* plants from Taiwan. Secondly, Cycad Blue may have invaded from neighboring regions in mainland China, with the planted cycads providing extra resources for colonization. To trace the source of the outbreak in Kinmen, we sequenced 165 specimens covering its neighborhood regions, including Taiwan and mainland China, for the mitochondrial COI and COII genes. Five COI haplotypes and eleven COII haplotypes were found in Kinmen. Most haplotypes were also found in southeastern China, but no haplotype found in Kinmen samples was found in Taiwan. This evidence supports a mainland Chinese origin for Cycad Blue colonies at Kinmen. Our results also show that the turnover colonies of the Cycad Blue had different genetic composition in the three sampled years. In addition, the COI dataset is concordant with the COII dataset, and both of them are considered as good markers for identifying the Cycad Blue populations between mainland China and Taiwan.

Keywords: population outbreak, *Chilades pandava*, *Cycas*, range expansion, horticultural trade, COI, COII

Introduction

The Cycad Blue, *Chilades pandava* (Horsfield [1829]), which feeds on and defoliates *Cycas* plants, has become a conspicuous and serious pest on native (Gao *et al.*, 2004; Raju, 2009) and horticultural cycads (Chang, 1989; Liu *et al.*, 2003; Wu *et al.*, 2010). The natural distribution of this butterfly spans Indo-Asia, including India, Sri Lanka, Sundaland, Philippines, and Taiwan (Hsu, 2002; Igarashi and Fukuda, 2000), but now it can be found in relatively remote regions such as the Mascarenes and Madagascar (Wu *et al.*, 2010), and Guam (Moore, 2005; Moore, 2008), and can even be found in the temperate zone, such as Japan (Mitsuhashi, 1992; Hirai, 2009) and Korea (Takeuchi, 2006) through introduced horticultural cycads. The Cycad Blue has a specialized association with *Cycas* plants (Chang, 1989; Bascombe *et al.*, 1999), which are all listed under Appendix I or II of Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES). By continually causing severe damage to young leaves or soft tissue of *Cycas* spp. in horticulture but potentially also on their survival in the wild. In surveys for biological control purposes,
the first step is to understand the invasion biology of the species and to trace back its origin to determine appropriate strategies, such as the discovery and possible introduction of natural enemies (DeBach, 1964).

The biota of Kinmen, a continental island off the south-eastern coast of Fujian Province of mainland China (Figure 1), include organisms which originated both naturally and artificially from mainland China and Taiwan. Since Kinmen National Park was set up on the island in 1995, natural resources of Kinmen, such as birds (Severinghaus, 1999), reptiles (Lue et al., 1998), mammals (Chen et al., 2003), plants (Yang and Lu, 1997), and insects (Chen et al., 1999; Huang et al., 2000; Tung and Yang, 2008), have been investigated intensively. It is suggested that the flora and fauna are strongly influenced by surrounding regions (i.e. mainland China and Taiwan). For instance, 56% of the insect fauna has affinities to mainland and 42% to Taiwan among a total of 542 recorded species (Tung and Yang, 2008). Ninety-four percent of the Kinmen flora can be found in Taiwan, but the remaining 35 species of plants are found only in mainland (Yang and Lu, 1997). Sixty-eight species of butterflies were found on this small island (Huang et al., 2000; Chang, 2001).

*Chilades pandava* is the most recently discovered butterfly of Kinmen. The Cycad Blue was first recorded there in July 2007. During subsequent monitoring, occurrences persisted for six months and caused substantial damage to sago palms, *Cycas revoluta*, and then disappeared by December 2007. However, this butterfly was found again in July of 2008 and 2009, persisting for several months before disappeared again in the winter. Discovery of a new butterfly from a small region, such as Hong Kong, usually suggests a vagrant (Bascombe et al., 1999; Lo, 2005). However, it may represent an unusual case for the Cycad Blue colony to establish at Kinmen repeatedly during the warmer seasons and went extinct when larval food source went short or cold winter was in place year. Two possible origins of the Cycad Blue colony present at Kinmen are given here: 1) the Cycad Blue may have been introduced along with imported cycads into Kinmen (Wu, 2004), or 2) alternatively, the Cycad Blue may have invaded Kinmen from mainland, with *Cycas* plants introduced from Taiwan providing larval food resource.

Mitochondrial COII sequences have empirically proved their utility to trace back the origin of population outbreaks of Cycad Blue in Taiwan (Wu et al., 2010); therefore, the COII gene and additional data from the COI gene were chosen for tracing the origin of the recent outbreak of the Cycad Blue at Kinmen. The following questions are addressed herein: 1) From where were the colonies of the Cycad Blue found at Kinmen derived? 2) Is the genetic composition identical between the colonies found at Kinmen in 2007, 2008, and 2009? (3) Was the genetic signal from the COI gene concordant with that from the COII gene?

**Materials and Methods**

**Sampling**

A total of 165 individuals of *Chilades pandava* were used in the study (Table 1). In order to trace the origin of Kinmen colonies of this butterfly, 81 specimens were collected from Kinmen (Figure 1, No. 1), including 18 samples collected in 2007, 35 in 2008, and 28 in 2009 respectively. Kinmen colonies were divided according to sampled years in order to observe the effect of temporal variation on their genetic composition. Two possible source populations (southeastern China and Taiwan) were also sampled in this study: 60 specimens from Taiwan (Nos. 2-10) and 24 specimens from mainland (Figure 1, Nos. 11-15).

**Molecular technologies**
Genomic DNA was obtained from the thoracic muscle tissue or legs using the Purgene DNA Isolation kit (Genta Systems, Minnesota, USA), following the extraction protocol of the manufacturer. The precipitated DNAs were resuspended in 50 μl of dH2O and we used the primers pairs: k698 (TACAA TTTAT CGCCT AAACT CCAGC C) and Nancy (CCCGG TAAAA TTAAA ATATA AA CT); Pierre (5’-AGAGC CTCTC TTATA ATAGA ACA-3’) and Eva (5’-GAGAC CATTA CTTGC TTTCA GTCAT CT-3’) (Caterino and Sperling 1999) to amplify the partial mitochondrial cytochrome oxidase subunit I and II genes, respectively, by polymerase chain reaction (PCR). Each PCR reaction was carried out in a final volume of 30 μl with 0.32 μM dNTP, 1.5mM MgCl2, 0.2μM of each primers, 1X Taq Healthcare, Buckinghamshire, UK), and finally dH2O was added up to 30μl. PCR conditions were carried out as following standard three steps: an initial denaturation step of 94°C (2min), followed by 35 cycles consisting of denaturation at 94°C (30 sec), annealing at 55°C (30 sec), extension at 72°C (1-1.5min), and a final extension step of 72°C (7min). Different annealing temperatures (50-58°C) were used to improve the PCR quality. At the next, the products were run on 1.0% agarose gels in 1X TBE buffer to ensure that the lengths of PCR fragments were correctly amplified, and DNA sequence reactions were conducted on an ABI3730 DNA Analyzer (Applied Biosystems).

Data treatments and analyses

COI and most of COII were sequenced in this study. Some COII sequences from the specimens of Taiwan and of southeast mainland China were obtained from a previous study (Wu et al., 2010). All sequences were checked and assembled into contiguous arrays using Sequencher 4.8 (GeneCode, Boston, USA).

Neutrality of the mtDNA COI and COII genes was tested to detect any hidden positive selection (Otto, 2000). A combination of Tajima’s D (Tajima, 1989), Fu & Li’s D* (Fu and Li, 1993) and Fay and Wu’s H (Fay and Wu, 2000) was used, and statistical significance was assessed by coalescent simulations with 10,000 replicates performed by using Dnasp v5 (Librado and Rozas, 2009). Pairwise genetic distance (p-distance) and genetic differentiation (FST) were also performed to detect the differences between Kinmen and other populations. If the Kinmen colonies were derived from one source population, the values of genetic distance and differentiation should be lower than the relationship between Kinmen and non-derived populations. Other general information, such as the number of unique haplotypes, variable nucleotide positions and measured genetic diversities including nucleotide diversity (π) and haplotype diversity (h) were also described by using Dnasp v5. Conspecific populations often have lower divergences than at interspecific level (Posada and Crandall, 2001), so we performed haplotype network for studying closed relationships. Haplotype networks were constructed with the software TCS 1.21 based on the principle of parsimony (Clement et al., 2000). Each branch in the network was supported with a minimum probability threshold of 0.95 (Templeton et al., 1992).
Gene diversities and pairwise F_{ST} value and genetic distance

The overall COI gene dataset of the Cycad Blue had nucleotide diversity (\(\pi=0.0035\pm0.00084\)) similar to that of the COII gene (\(\pi=0.00315\pm0.00086\)), but the COII gene had higher haplotype diversity (\(h=0.784\pm0.02\)) than that of the COI gene (\(h=0.571\pm0.02\)). Even in each sampled population, the mean of COII haplotype diversity was more variable than that of the COI (\(t=2.953, P=0.0079\), df=20, five populations were excluded because they all showed zero haplotype diversities). Only the Taizhong population (No. 4) had a more variable haplotype diversity of the COI than that of the COII gene (Table 1). Gene diversities of Kinmen colonies showed temporal variation in the three sampled years. The colonies sampled in 2007 in Kinmen colonies showed variable haplotype diversity. The 2008 colony showed no variation in both the COI and COII genes. The 2009 showed certain degree of genetic variation, which was also found based on the COII gene (Table 2). The pairwise F_{ST} values obtained from comparing these two groups were higher than the F_{ST} values obtained within the Taiwanese populations alone (\(t=12.873, P<0.0001, df=79\)) or within the southeastern mainland Chinese populations alone (\(t=3.41, P=0.001, df=53\)). The F_{ST} values obtained from comparing Kinmen to the populations in southeast mainland China was significantly lower than the values obtained from comparing Kinmen to Taiwanese populations (based on the COI gene: \(t=14.758, P<0.0001, df=12\); based on the COII gene: \(t=2.052, P=0.06, df=12\)). This result indicates Kinmen populations have higher gene flow with the populations in southeastern China than with Taiwanese populations.

Haplotype distribution and haplotype network

There were six COI haplotypes and eleven COII haplotypes obtained from 165 specimens (Table 1). The distribution of COI haplotypes had the same distinct haplotype distribution as the COII haplotypes showed between Taiwan (COI haplotype A-E) and southeast mainland China (COII haplotype H, J, L, O, AC, and AD; Wu et al., 2010). The COI haplotypes (a, b, and e) could be found only in Taiwan, whereas the COI haplotypes (c, d, and f) were only found in southeastern China. In individuals from Kinmen, three COI haplotypes (c, d, and f) and six COII haplotypes (H, J, L, O, AC, and AD) gene were found. Interestingly, all the haplotypes found in Kinmen were also found in mainland China (Nos. 11-15), except that the COI

![Table 1. sampling locations and their genetic diversity and haplotypes.](image)
### Table 2. Pairwise FST values among each location. The FST values on the below side of the matrix are based on COI gene as the values on the upper side are based on COII gene. Gray color shows the value were compared among Taiwan region (Nos. 2-10) or southeast mainland China regions (Nos. 11-15).

<table>
<thead>
<tr>
<th></th>
<th>No.1</th>
<th>No.2</th>
<th>No.3</th>
<th>No.4</th>
<th>No.5</th>
<th>No.6</th>
<th>No.7</th>
<th>No.8</th>
<th>No.9</th>
<th>No.10</th>
<th>No.11</th>
<th>No.12</th>
<th>No.13</th>
<th>No.14</th>
<th>No.15</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.1</td>
<td>-</td>
<td>0.555</td>
<td>0.680</td>
<td>0.905</td>
<td>0.905</td>
<td>0.785</td>
<td>0.905</td>
<td>0.642</td>
<td>0.622</td>
<td>0.702</td>
<td>0.043</td>
<td>0.140</td>
<td>0.828</td>
<td>0.656</td>
<td>0.714</td>
</tr>
<tr>
<td>No.2</td>
<td>0.662</td>
<td>-</td>
<td>0.111</td>
<td>0.143</td>
<td>0.143</td>
<td>0.008</td>
<td>0.143</td>
<td>0.208</td>
<td>0.124</td>
<td>0.299</td>
<td>0.528</td>
<td>0.610</td>
<td>0.774</td>
<td>0.714</td>
<td>0.730</td>
</tr>
<tr>
<td>No.3</td>
<td>0.767</td>
<td>0.111</td>
<td>-</td>
<td>0.000</td>
<td>0.000</td>
<td>0.100</td>
<td>0.000</td>
<td>0.300</td>
<td>0.211</td>
<td>0.390</td>
<td>0.633</td>
<td>0.750</td>
<td>0.853</td>
<td>0.815</td>
<td>0.812</td>
</tr>
<tr>
<td>No.4</td>
<td>0.930</td>
<td>0.126</td>
<td>0.000</td>
<td>-</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.667</td>
<td>0.545</td>
<td>0.731</td>
<td>0.825</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>0.966</td>
</tr>
<tr>
<td>No.5</td>
<td>0.975</td>
<td>0.143</td>
<td>0.000</td>
<td>0.000</td>
<td>-</td>
<td>0.000</td>
<td>0.000</td>
<td>0.360</td>
<td>0.303</td>
<td>0.473</td>
<td>0.718</td>
<td>0.875</td>
<td>0.929</td>
<td>0.999</td>
<td>0.889</td>
</tr>
<tr>
<td>No.6</td>
<td>0.975</td>
<td>0.143</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>-</td>
<td>0.000</td>
<td>0.667</td>
<td>0.545</td>
<td>0.731</td>
<td>0.825</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>0.966</td>
</tr>
<tr>
<td>No.7</td>
<td>0.975</td>
<td>0.143</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>-</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>No.8</td>
<td>0.793</td>
<td>0.086</td>
<td>0.154</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.576</td>
<td>0.706</td>
<td>0.848</td>
<td>0.800</td>
<td>0.800</td>
</tr>
<tr>
<td>No.9</td>
<td>0.975</td>
<td>0.143</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>No.10</td>
<td>0.114</td>
<td>0.646</td>
<td>0.748</td>
<td>0.909</td>
<td>0.952</td>
<td>0.952</td>
<td>0.952</td>
<td>0.952</td>
<td>0.774</td>
<td>0.952</td>
<td>0.300</td>
<td>0.500</td>
<td>0.125</td>
<td>0.333</td>
<td>0.333</td>
</tr>
<tr>
<td>No.11</td>
<td>0.078</td>
<td>0.679</td>
<td>0.786</td>
<td>0.952</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>0.813</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>0.909</td>
</tr>
<tr>
<td>No.12</td>
<td>0.078</td>
<td>0.679</td>
<td>0.786</td>
<td>0.952</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>0.813</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>No.13</td>
<td>0.078</td>
<td>0.679</td>
<td>0.786</td>
<td>0.952</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>0.813</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>No.14</td>
<td>0.078</td>
<td>0.679</td>
<td>0.786</td>
<td>0.952</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>0.813</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>No.15</td>
<td>0.078</td>
<td>0.679</td>
<td>0.786</td>
<td>0.952</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>0.813</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

haplotype f and COI haplotype AD were only discovered in Kinmen samples (No. 1).

All the COI and COII unique haplotypes were used to construct haplotype networks (Figure 2). The COI haplotypes from southeast mainland China (Nos. 1, 11-15) were related by a one-six step connection to Taiwanese haplotypes. Only the haplotype E was considered as an introduced haplotype from Malaysian region (Wu et al., 2010). Among all COII haplotypes found, haplotype AD was a new one found only in Kinmen (No. 1) which showed a one step mutation to the most common haplotype O.

The COI haplotype network presented a similar structure to the COII haplotype network (Figure 2). The COI haplotypes of southeast mainland China also presented a distinct grouping from the Taiwanese haplotypes. The COI haplotype “e”, which presented a long connection to Taiwanese common haplotypes (a, and b), shared the same pattern as COII haplotype E. The haplotype E in Taiwan is considered to be a result of introduction with imported cycads from Malaysia (Wu et al., unpublished data). Although the number of COI haplotypes was less than that of COII haplotypes, the gap between southeastern China and Taiwan in the COI gene was larger than that in the COII gene.

### Discussion

We found that the Cycad Blue colonies found at Kinmen were more closely related to the populations from mainland China than that from Taiwan based on both COI and COII genes. This supports a scenario of a population outbreak of the Cycad Blue at Kinmen where the host plant, *Cycas revoluta*, was introduced from Taiwan, providing larval food resources for the Cycad Blue to colonize from mainland China. A prominent seasonal pattern of northward expanding can be seen in the populations of *C. pandava* of southeastern China. In this region this species first appeared in March at Xiamen (Yang, 2007), then in June and July at Fuzhou (Mr. Zeng, personal communication, 2007), and subsequently at Hangzhou (North of Fuzhou) after July (Xue, 2007). The Kinmen colonies of this butterfly seem to be linked with this chain of
expansion because they shared identical haplotypes (COI haplotype c, d, and COII haplotype H, L, O, and AC) with the populations in southeastern China. The Cycad Blues found at Kinmen often appeared after June as intermittently established colonies (from June to November). The northward pattern of seasonal expansion in southeastern China was also reflected in Taiwan, where the Cycad Blue can be seen in southern Taiwan in January and February, in central Taiwan in March, and Northern Taiwan after May (Wu et al., 2010). The butterfly was even found in Okinawa after August (Mitsuhashi, 1992). The Cycad Blue records from Japan are also interesting, as there is a native Cycad population in Japan. However, in Japan, C. pandava has only previously reported once before 2000. Hirai (2009), nevertheless, pointed out that the Cycad Blue is now common in the Okinawa archipelagos and the southern part of Honshu in Japan after July.

The Cycad Blue found at Kinmen showed distinctly different haplotype composition among the three sampled years (Table 1). The populations sampled in 2007 and 2009 possessed more than two COI and four COII haplotypes. By contrast, the 2008 samples were fixed for a single haplotype in both COI and COII genes. This pattern revealed that this species has high population dynamics, and molecular data used to infer its population structure and demographic history should be treated with caution because genetic variation is highly correlated with population dynamics (Saccheri and Hanski, 2006). Although there were only three years of genetic records, most of the haplotypes from Kinmen could be found in samples from mainland China, and no haplotype was shared with samples from Taiwan. The possibility that the Cycad Blue was introduced with horticultural cycads from Taiwan can therefore be ruled out.

Acknowledgements

We thank Miss Hsiu-Chu Chen (Kinmen National Park), Yung-Ching Su (Kinmen High School), and Mr. Ming-Chung Ng (National Taiwan Normal University) for help with collecting specimens from Kinmen. We also thank Miss Ting-Wai Chen (National Taiwan Normal University) to deal with some molecular work and Mr. Fang-Qin Zheng (Fuzhou National Forest Park) to provide the Cycad Blue information in Fuzhou. This work was supported by the grants from Council of Agriculture 98-FM2.1-RC19 and from National Science Council (NSC) NSC90-2313-B-003-002, NSC91-2313-B-003-002, and NSC92-2313-B-003-001. One of us (DCL) benefited from a STUDIUM fellowship during the writing of this paper.

Reference

Liu GH, Lu YY, Gan YH, Zeng L, Fu ML, and Pan...


Raju AJS. 2009. Nesting behaviour of the Baya Weaver bird, Ploceus philippinus (Ploceidae) and the life-cycle of the Plains Cupid butterfly, Chilades pandava (Lycaenidae) with the red-listed Cycas sphaerica and C. beddomei (Cycadaceae). JoTT Communication 1: 429-433.


Wu LW, Yen SH, Lees DC, and Hsu YF. 2010. Elucidating genetic signatures of native and introduced populations of the Cycad Blue, Chilades pandava to Taiwan: a threat both to Sago Palm and to native Cycas populations worldwide. (in press)


利用粒線體 COI 及 COII 序列追溯金門島上蘇鐵綺灰蝶（*Chilades pandava*）的來源

吳立偉 1 David C. Lees 2, 3 徐堉峰 1*

1 國立臺灣師範大學生命科學系
2 獸物學系
3 法國奧爾良森林動物研究中心

（收稿日期：2009.10.12，接受日期：2009.10.30）

摘 要

2007 年 7 月首次在金門島上發現蘇鐵綺灰蝶（*Chilades pandava*），並觀察到此蝴蝶造成園藝蘇鐵（*Cycas revoluta*）的觀葉組織嚴重損害，進而影響蘇鐵的觀賞價值。雖然蘇鐵綺灰蝶在同年 12 月後消失匿跡，但是 2008 及 2009 年 7 月又再次發現，並且持續一段數月後才消失。在 2007 年之前，雖然金門島上有少量零星園藝蘇鐵植株，島上的物種調查卻未有蘇鐵綺灰蝶的紀錄。金門島上的蘇鐵綺灰蝶的出現原因可能有二：1) 近年來金門大量栽種蘇鐵作為行道樹或美化環境之用，蘇鐵綺灰蝶隨著園藝蘇鐵從台灣引入；2) 引入栽種的蘇鐵雖然未發現蘇鐵綺灰蝶，但是卻提供大陸地區的蘇鐵綺灰蝶額外的資源並在金門建立新的族群。因此，為了瞭解金門島上蘇鐵綺灰蝶的來源，我們利用粒線體 COI 及 COII 的序列分析 165 個包括金門、大陸沿岸及台灣島上的樣本。結果顯示，大陸所具有的基因型（haplotype）明顯與台灣不同，而且兩地區所具有的基因型在網狀親緣關係中（haplotype network）也各自成群。而金門島上 81 個樣本共檢測出 5 個 COI 基因型及 11 個 COII 基因型，大部分的基因型可以在大陸地區發現，但是沒有一個基因型與台灣的相同。這個證據支持金門島上的蘇鐵綺灰蝶族群源自中國大陸。此外，採自金門島上三次不同年份的樣本，其遺傳組成比例皆不相同，顯示此蝶種在金門地區的變動性頗大。而 COI 的分析結果與 COII 相似，同為鑑別台灣及大陸地區蘇鐵綺灰蝶的良好分子標記。

關鍵詞：族群大發生、蘇鐵綺灰蝶、蘇鐵屬、族群擴張、粒線體 COI、COII