

## Geographical Diversity of Japanese *Papilio* Butterflies Inferred from the Number of Contact Chemosensillum on the Fifth Foretarsal Segments

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### Abstract

The numbers of contact chemosensillum on ventral side of fifth foretarsal segments of Japanese *Papilio* butterflies were determined by scanning electron microscope observation. *P. bianor*, *P. helenus*, *P. protenor* and *P. memnon* exhibited geographical diversity for this character among mainland Japan, Amami Islands, Okinawa Islands and Yaeyama Islands populations. This characteristic was useful for reconfirming the geographical variations proposed by the wing color patterns and genetic characteristics of each these species. This characteristic also uncovered hidden geographical variations and phylogenetic structures of *P. machaon*, *P. xuthus*, *P. maackii*, *P. macilentus* and that of *P. memnon* on mainland Japan and that of *P. protenor* on Okinawa Islands. Also discussed are possible causes for the apparent phylogeny disparities between this report and other previous studies including DNA analyses.

**Keywords:** Contact chemosensillum; Lepidoptera; *Papilio*; Phylogeny; Biodiversity; Japanese

### Introduction

In my previous publication [1], I described the foreleg tarsal morphology of Japanese *Papilio* butterflies and pointed out that these characteristics are closely related to both phylogeny and host plant selection by females. According to that analysis, I was able to divide ten Japanese *Papilio* species plus *P. polyxenes* (occurs in north America), *P. demodocus* (occurs in Africa, type species of *Princeps*) and *P. paris* (occurs in Southeast Asia, type species of *Achillides*) into five subgenera: *Papilio* (*P. machaon* and *P. polyxenes*), *Princeps* (*P. demodocus*, *P. demoleus* and *P. xuthus*), *Achillides* (*P. paris*, *P. bianor* and *P. maackii*), *Menelaides* (*P. helenus*, *P. polytes*, *P. protenor* and *P. macilentus*) and *Iliades* (*P. memnon*). This study also showed that females of *P. (Papilio)* species, *P. (Achillides) maackii*, and *P. (Menelaides) macilentus* were distinct for foretarsal morphology differences that are related to their special host plant preferences among the subgenus/genus in which these species belong. I also suggested that certain *Papilio* species might exhibit geographical diversity on the numbers of contact chemosensillum, but this could not be confirmed because of a lack of samples, in particular for *P. xuthus* from Nansei Islands and *P. protenor* from Amami Islands [1].

The geographical diversities of *Papilio* species in Japan have been mainly studied by a large number of amateur researchers. According to [2-11], the description of Japanese *Papilio* butterflies on mainland Japan (hereafter Hondo) and Nansei Islands (Amami Islands, Okinawa Islands, Daitō Islands, Miyako Islands and Yaeyama Islands) were begun by European researchers during the late 19th and early 20th century. After 1960, Japanese researchers delineated some additional subspecies primarily by their wing color schemes for the small peripheral populations. The geographic diversity of *P. bianor* was derived on the basis of host plant preference [12-20], intersubspecies hybridization [13,14,21-31], morphology [13,21,22,25-27,29,32-43],

isozyme analysis [29,44,45], DNA analysis [46-50], photoperiodism [31] and principle analysis of wing shape [51]. The geographical diversity of *P. protenor* has been studied in terms of larval color [52], photoperiodism [53,54], DNA analysis [46] and intersubspecies hybridization [26,55]. The geographical diversity of *P. memnon* has been investigated with respect to photoperiodism [56], adult wing shapes [52] and adult wing color schemes [57-59]. Some other species also have been studied in terms of the adult and/or larval color scheme [40,60-64].

After 2005, I undertook a study of the foreleg tarsus morphology of newly obtained Japanese *Papilio* specimens with a focus on the number of contact chemosensillum present on the ventral surface of the fifth segment. Here, I report these results and discuss the relationship between foreleg morphology and geographical diversity.

### Materials and Methods

New data from 127 male and 161 female forelegs of summer form Japanese *Papilio* butterflies were added to the data set used in [1] and the total data set was analyzed for this article. With inclusion of the new data, studies included a total of 53 males and 67 females of *P. machaon*, 94 males and 114 females of *P. xuthus*, 4 males and 2 females of *P. demoleus*, 80 males and 89 females of *P. maackii*, 94 males and 100 females of *P. bianor*, 81 males and 86 females of *P. helenus*, 21 males and 24 females of *P. polytes*, 88 males and 90 females of *P. protenor*, 49 males and 44 females of *P. macilentus*, and 56 males and 71 females of *P. memnon*. The method of observation was the same as that of [1]. Excised forelegs were washed in detergent water with sonication for 30 minutes, dried at room temperature, coated with 10-20 nm of gold and photographed using Scanning Electron Microscope (JEOL JSM-6301F). The number of contact chemosensillum in the fifth foretarsal segment was counted. The ventral spine numbers in the second through fifth foretarsal segments, the number of contact chemosensilla in the second through fourth foretarsal segments, and lengths and widths of the fifth foretarsal

segments were also recorded. Each species was separated into a number of small populations. For grouping of each of these species, the current taxonomy of these species, plant distribution patterns, and geographical history [65-67] were considered. When appropriate, the chemosensilla number means were tested for significant differences among populations of each species according to an F-distribution test [68,69].

## Results

I could confirm the diversity of nine Japanese *Papilio* species, *P. machaon*, *P. xuthus*, *P. maackii*, *P. bianor*, *P. helenus*, *P. polytes*, *P. protenor*, *P. macilentus* and *P. memnon*, and not of *P. demoleus*. All results were shown in Figures 1-4 and Tables 1-9.

Geographical difference of means of fifth tarsal contact chemosensilla numbers of each Japanese *Papilio* species.

<i>P. machaon</i>		
	Average	EJ
EJ	25.611	
	71.935	
WJ	26.882	1.271
	65.455	-6.480*

**Table 1:** Diversity of *P. machaon*. Upper: male, Lower: female. Abbreviations on top or left of each row/column e.g. “EJ” or “WJ” are as same as those shown in figure legends. \*: the variance between 2 populations is not equal in 5% normal significance level in F-distribution. \*\*: the variance between 2 populations is not equal in 1% normal significance level in F-distribution.

<i>P. xuthus</i>			
	Average	EJ	WJ
EJ	27.824		
	69.603		
WJ	25.6	-2.224	
	62.651	-6.952*	
SW	26.667	-1.157	1.067
	67.846	-1.757	5.195**

**Table 2:** Diversity of *P. xuthus*. Upper: male, Lower: female. Abbreviations on top or left of each row/column e.g. “EJ” or “WJ” are as same as those shown in figure legends. \*: the variance between 2 populations is not equal in 5% normal significance level in F-distribution. \*\*: the variance between 2 populations is not equal in 1% normal significance level in F-distribution.

<i>P. macilentus</i>			
	Average	EJ	
EJ	18.833		

	64.645		
WJ	18	0.833	
	57.692	-6.953*	

**Table 3:** Diversity of *P. macilentus*. Upper: male, Lower: female. Abbreviations on top or left of each row/column e.g. “EJ” or “WJ” are as same as those shown in figure legends. \*: the variance between 2 populations is not equal in 5% normal significance level in F-distribution. \*\*: the variance between 2 populations is not equal in 1% normal significance level in F-distribution.

<i>P. maackii</i>				
	Average	HK	EJ	WJ
HK	19.1			
	40.333			
EJ	22.769	3.669*		
	49.878	9.544*		
WJ	25.462	6.718**	2.692*	
	55.214	14.881**	5.337*	
9S	23.833	4.733**	-1.064	-1.628
	50.6	10.267*	0.722	-4.614

**Table 4:** Diversity of *P. maackii*. Upper: male, Lower: female. Abbreviations on top or left of each row/column e.g. “EJ” or “WJ” are as same as those shown in figure legends. \*: the variance between 2 populations is not equal in 5% normal significance level in F-distribution. \*\*: the variance between 2 populations is not equal in 1% normal significance level in F-distribution.

<i>P. bianor</i>							
	Average	HK	EJ	WJ	S9	AM	OK
HK	25						
	49.75						
EJ	21	-4.026					
	60.064	10.314*					
WJ	24	-1	3.026				
	62.056	12.306**	1.992				
S9	20.5	-4.500*	-0.474	-3.5			
	53.333	3.583	-6.73	8.722			
AM	13.333	-11.667**	-7.640*	-10.667**	-7.167**		
	38.111	-11.639**	-21.953**	-23.944**	-15.222**		
OK	13.375	-11.625**	-7.599**	-10.625**	-7.125**	0.042	
	21.857	-27.893*	-38.207**	-40.198**	-31.476**	16.254*	

YY	21.571	-3.429	0.598	-2.429	1.071	8.238 **	8.196**
	61.556	11.806 **	1.492	-0.5	8.222	23.444*	39.698*

**Table 5:** Diversity of *P. bianor*. Upper: male, Lower: female. Abbreviations on top or left of each row/column e.g. “EJ” or “WJ” are as same as those shown in figure legends. \*: the variance between 2 populations is not equal in 5% normal significance level in F-distribution. \*\*: the variance between 2 populations is not equal in 1% normal significance level in F-distribution.

<i>P. helenus</i>				
	Average	EJ	WJ	AM
EJ	19.84			
	108.625			
WJ	17.892	-1.948		
	109.378	0.753		
AM	17.429	-2.411	-0.463	
	97.182	-11.443*	-12.196	
OK	17.6	-2.24	-0.292	0.171
	93.833	-14.792**	-15.544*	-3.348

**Table 6:** Diversity of *P. helenus*. Upper: male, Lower: female. Abbreviations on top or left of each row/column e.g. “EJ” or “WJ” are as same as those shown in figure legends. \*: the variance between 2 populations is not equal in 5% normal significance level in F-distribution. \*\*: the variance between 2 populations is not equal in 1% normal significance level in F-distribution.

<i>P. protenor</i>					
	Average	EJ	WJ	AM	OK
EJ	13.719				
	135.034				
WJ	15.24	1.521			
	143.063	8.028			
AM	17.5	3.781**	2.26		
	131	-4.034	-12.063		
OK	15	1.281	-0.24	-2.5	
	131.333	-3.701	-11.729	0.333	
YY	13.667	-0.052	-1.573	-3.833**	-1.333
	125.059	-9.976	-18.004	-5.941	-6.275

**Table 7:** Diversity of *P. protenor*. Upper: male, Lower: female. Abbreviations on top or left of each row/column e.g. “EJ” or “WJ” are as same as those shown in figure legends. \*: the variance between 2 populations is not equal in 5% normal significance level in F-

distribution. \*\*: the variance between 2 populations is not equal in 1% normal significance level in F-distribution.

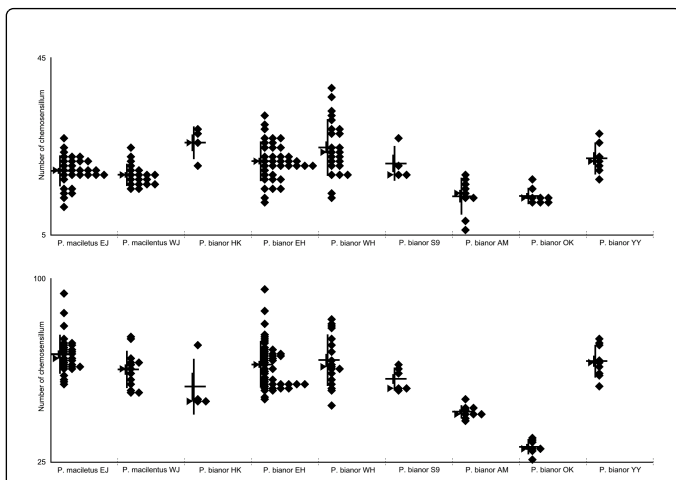
<i>P. memnon</i>			
	Average	WJ	AM
WJ	22.519		
	123.421		
AM	25.7	3.181*	
	145.1	21.679**	
OK	23.556	1.037	-2.144
	128.556	5.135	-16.544*

**Table 8:** Diversity of *P. memnon*. Upper: male, Lower: female. Abbreviations on top or left of each row/column e.g. “EJ” or “WJ” are as same as those shown in figure legends. \*: the variance between 2 populations is not equal in 5% normal significance level in F-distribution. \*\*: the variance between 2 populations is not equal in 1% normal significance level in F-distribution.

<i>P. polytes</i>				
	Average	AM	OK	MY
AM	18.667			
	83.2			
OK	15.5	-3.167		
	82.833	-0.367		
MY	19	0.333	3.5	
	83.5	0.3	0.667	
YY	17	-1.667	1.5	-2
	76.636	-6.564	-6.197	-6.864

**Table 9:** Diversity of *P. polytes*. Upper: male, Lower: female. Abbreviations on top or left of each row/column e.g. “EJ” or “WJ” are as same as those shown in figure legends. \*: the variance between 2 populations is not equal in 5% normal significance level in F-distribution. \*\*: the variance between 2 populations is not equal in 1% normal significance level in F-distribution.

*P. machaon* chemosensilla numbers showed geographical diversity among female populations. The population variances for the east Hondo (Hokkaido including Rishiri Island, Tohoku, Kanto, Chubu, south Kinki, Izu islands and Sado island) and west Hondo (north Kinki, Chugoku, Shikoku, Kyushu, Awaji island, Oki islands, Tsushima islands, Iki islands, Goto islands, Koshiki islands, Kumage islands and Tokara islands) populations were significantly different at the 5% level (Figure 1).

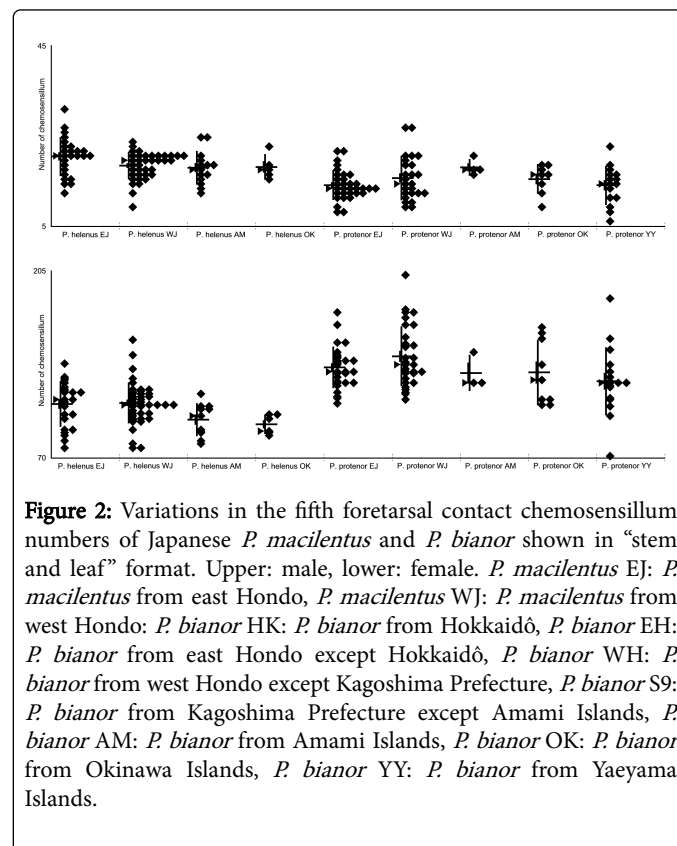


**Figure 1:** Variations in the fifth foretarsal contact chemosensillum numbers of Japanese *Papilio machaon*, *P. xuthus* and *P. maackii* shown in “stem and leaf” format. “”: datum from each specimen plotted in sideways histogram, horizontal bar: means, long vertical bar: mean+/-SD, short vertical bar: mean +/-SE, “”: median. Upper: male, lower: female. *P. machaon* EJ: *P. machaon* from east Hondo (=Hokkaidô including Rishiri Island, Tôhoku, Kantô, Chûbu, south Kinki, Izu Islands and Sado Island), *P. machaon* WJ: *P. machaon* from west Hondo (=north Kinki, Chûgoku, Shikoku, Kyûshû, Awaji Island, Oki Islands, Tsushima Islands, Iki Island, Gotô Islands, Koshiki Islands, Kumage Islands and Tokara Islands), *P. xuthus* EJ: *P. xuthus* from east Hondo, *P. xuthus* WJ: *P. xuthus* from west Hondo, *P. xuthus* SW: *P. xuthus* in the Nansei Is, *P. maackii* HK: *P. maackii* from Hokkaidô, *P. maackii* EH: *P. maackii* from east Hondo except Hokkaidô, *P. maackii* WH: *P. maackii* from west Hondo except Kyûshû, Tsushima Islands, Iki Islands, Gotô Islands, Koshiki Islands and Kumage Islands, *P. maackii* S9: *P. maackii* from Kyûshû, Tsushima Islands, Iki Islands, Gotô Islands, Koshiki Islands and Kumage Islands.

*P. xuthus* females also exhibited differences between east Hondo and west Hondo and the variances for these two populations were different at the 5% significance level. Chemosensilla variances for west Hondo females and Nansei islands females were different at the 1% significance level; in contrast, the variances for east Hondo females and Nansei islands females were not significantly different (Figure 1). The differences among Amami islands populations, Okinawa islands populations and Yaeyama islands populations were not still confirmed because of the shortage of specimen of these areas.

*P. macilentus* female showed a similar diversity pattern to those of *P. machaon* and *P. xuthus* in Hondo (Figure 2). The chemosensilla variances for the east Hondo and west Hondo populations were different at the 5% significance level. The geographical boundary of east and west populations of these three species appears to be located along Wakasa bay, Biwa lake, Yodo river and Kii channel. This boundary is also applied to *P. maackii*, *P. bianor*, *P. helenus*, *P. protenor* and *P. memnon*. *P. macilentus* consists basically of one brood in Hokkaido and I could not examine the summer form of Hokkaidô *P. macilentus* in this study. Moreover I could not detect the differences between Hokkaidô and east Hondo except Hokkaido of both *P. machaon* and *P. xuthus* even I could examine 4 males and 3 females of

summer form Hokkaido *P. machaon* and 3 males and 3 females of summer form Hokkaido *P. xuthus*.



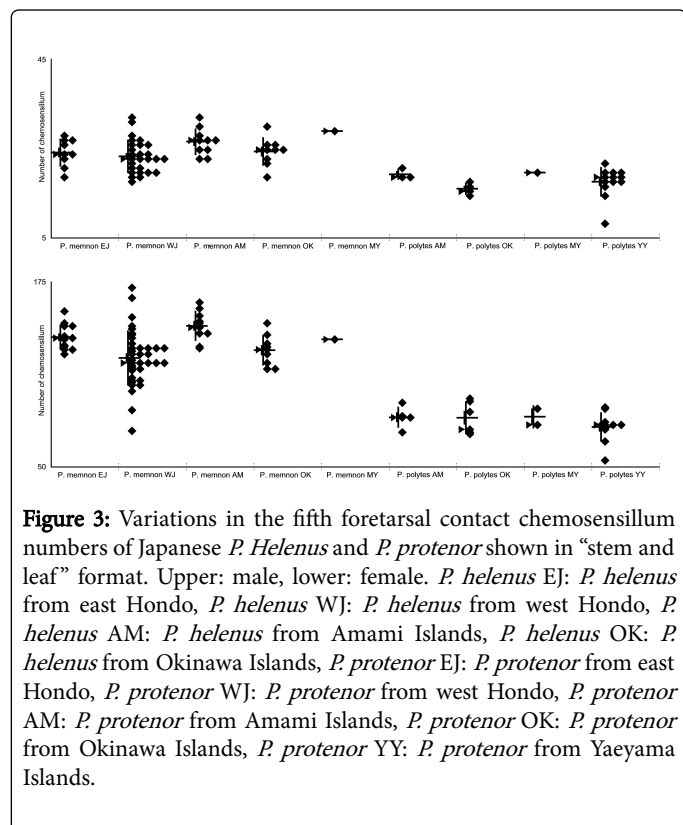
**Figure 2:** Variations in the fifth foretarsal contact chemosensillum numbers of Japanese *P. macilentus* and *P. bianor* shown in “stem and leaf” format. Upper: male, lower: female. *P. macilentus* EJ: *P. macilentus* from east Hondo, *P. macilentus* WJ: *P. macilentus* from west Hondo, *P. bianor* HK: *P. bianor* from Hokkaidô, *P. bianor* EH: *P. bianor* from east Hondo except Hokkaidô, *P. bianor* WH: *P. bianor* from west Hondo except Kagoshima Prefecture, *P. bianor* S9: *P. bianor* from Kagoshima Prefecture except Amami Islands, *P. bianor* AM: *P. bianor* from Amami Islands, *P. bianor* OK: *P. bianor* from Okinawa Islands, *P. bianor* YY: *P. bianor* from Yaeyama Islands.

For both sexes of *P. maackii*, the average contact chemosensillum numbers increased in an east-to-west pattern among the populations in Hokkaido, east Hondo except for Hokkaidô and west Hondo except for Kyushu, Tsushima Islands and Kumage islands, in contrast, the populations from Kyushu, Tsushima islands and Kumage islands did not show this tendency (Figure 1). Both male and female between Hokkaidô and West Japan except Kyushu, and male between Hokkaido and Kyushu were significantly different (1% level).

For both sexes of *P. bianor*, generally, the average contact chemosensillum numbers decreased in west Japan to Okinawa Islands, in contrast, the populations in Yaeyama Islands did not show this tendency. The male and female patterns were slightly different in some populations of *P. bianor* (Figure 2). For the females, the variance for the Okinawa Islands population was significantly different (1% level) from the other populations (Hokkaido, east Hondo except Hokkaido, west Hondo except Kagoshima Prefecture, Kagoshima Prefecture except Amami islands, Amami islands, Yaeyama islands). Similarly the Amami islands population was significantly different (1% level) from the other populations (Hokkaido, east Hondo except Hokkaido, west Hondo except Kagoshima Prefecture, Kagoshima Prefecture except Amami islands, Okinawa islands, Yaeyama islands). For males, the Okinawa Islands population was significantly different (1% level) from the other populations (Hokkaido, east Hondo except Hokkaido, west Hondo except Kagoshima Prefecture, Kagoshima Prefecture except Amami islands, Yaeyama islands) and not different from Amami islands population. The Amami islands population was different from the Kagoshima Prefecture except Amami islands and Yaeyama islands populations at the 1% significance level. The variances between Amami

islands male populations and east Hondo male populations except Hokkaido were different at the 5% significance level.

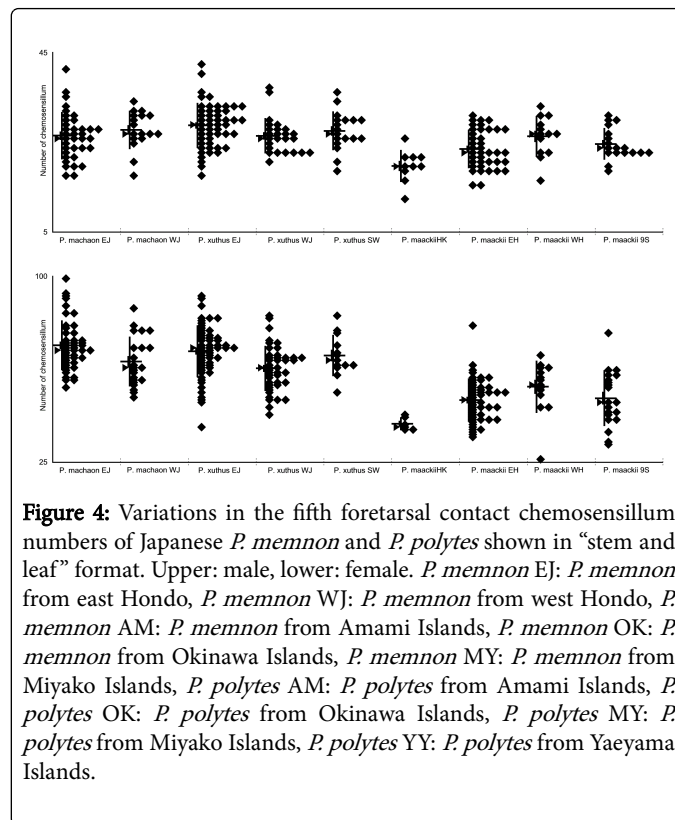
For *P. helenus*, statistical differences were observed between the female populations from Hondo and those of Amami islands and Okinawa islands. The variances for the east Hondo and Okinawa islands were not the same at the 1% significance level. In addition, comparisons of variances for the east Hondo and Amami islands and for the west Hondo and Okinawa islands were different at the 5% significance level (Figure 3).



**Figure 3:** Variations in the fifth foretarsal contact chemosensillum numbers of Japanese *P. Helenus* and *P. protenor* shown in “stem and leaf” format. Upper: male, lower: female. *P. helenus* EJ: *P. helenus* from east Hondo, *P. helenus* WJ: *P. helenus* from west Hondo, *P. helenus* AM: *P. helenus* from Amami Islands, *P. helenus* OK: *P. helenus* from Okinawa Islands, *P. protenor* EJ: *P. protenor* from east Hondo, *P. protenor* WJ: *P. protenor* from west Hondo, *P. protenor* AM: *P. protenor* from Amami Islands, *P. protenor* OK: *P. protenor* from Okinawa Islands, *P. protenor* YY: *P. protenor* from Yaeyama Islands.

For *P. protenor*, the diversity patterns of the chemosensillum numbers for all male populations as well as the female populations from east Hondo, west Hondo and Yaeyama islands formed nearly normal distribution patterns. The average numbers for males increased continuously from east Hondo to Amami islands and decreased continuously from Amami islands to Yaeyama islands. For females, the west Hondo populations have more sensilla than east Hondo populations and Yaeyama islands populations have less than both Hondo populations. In contrast, the Amami islands and Okinawa islands populations showed irregular distribution patterns with two or three peaks (Figure 3). Comparisons of chemosensillum variances between east Hondo and Amami islands males, between Amami islands and Yaeyama islands males, and between west Hondo and Yaeyama islands females were all different at the 1% significance level.

For *P. memnon*, the variances for west Hondo females and Amami islands females were different at the 1% significance level and variance comparisons between west Hondo males and Amami islands males and between west Hondo females and Okinawa islands females were different at the 5% significance level (Figure 4).



**Figure 4:** Variations in the fifth foretarsal contact chemosensillum numbers of Japanese *P. memnon* and *P. polytes* shown in “stem and leaf” format. Upper: male, lower: female. *P. memnon* EJ: *P. memnon* from east Hondo, *P. memnon* WJ: *P. memnon* from west Hondo, *P. memnon* AM: *P. memnon* from Amami Islands, *P. memnon* OK: *P. memnon* from Okinawa Islands, *P. memnon* MY: *P. memnon* from Miyako Islands, *P. polytes* AM: *P. polytes* from Amami Islands, *P. polytes* OK: *P. polytes* from Okinawa Islands, *P. polytes* MY: *P. polytes* from Miyako Islands, *P. polytes* YY: *P. polytes* from Yaeyama Islands.

*Papilio polytes* populations showed no significant differences for either sex (Figure 4).

For *P. xuthus*, *P. helenus*, *P. macilentus* and *P. memnon*, the diversity patterns of all of the male populations were the same as those of the females even though significant differences were not detected among the populations in these species. For *P. machaon* and *P. helenus*, the patterns of males were similar but not identical to those of the females.

## Discussion

### The consistency between foretarsal morphology and the current taxonomy based on other phylogenetic characteristics

*P. machaon*, *P. xuthus* and *P. macilentus* have been thought to have no geographical diversity in Japan [2-4]; however, my analysis of foretarsal morphology showed that these species can be divided into east Hondo populations and west Hondo populations, and this boundary does not correspond to any estimated biological boundary [70]. The average body sizes of both populations of each species are almost the same; thus, differences in chemosensillum numbers of these populations cannot be attributed to body size differences. The differences between Hondo and Nansei islands populations of *P. xuthus* were also first pointed out in this current of my article.

*P. maackii* was previously thought to have some geographical diversity although that had not been confirmed. *P. maackii* can be divided into four populations in Figure 1; however, the differences in the average contact chemosensillum numbers of the Hokkaido, east Hondo except Hokkaido and west Hondo except Kyushu, Tsushima islands and Kumage islands populations may be attributed to

differences in their average body sizes. The reduced chemosensillum numbers for the Kyushu, Tsushima islands and Kumage islands population, whose average body sizes are the largest of the Japanese *P. maackii*, suggests that this population is independent from the other Japanese populations. These size differences are often thought to be caused by climate differences between the localities of these populations; however, individuals of each population reared from eggs in the same laboratory maintain their own characteristics for size and wing color scheme. This suggests that these differences are already fixed genetically. Some researchers say that west Japan *P. maackii* might be separated as *P. maackii masuokai* (e.g. [11]; the type locality is Ehime Pref., Shikoku), this must be still discussed carefully because Shikoku populations of *P. maackii* are included in that of West Japan except Kyushu in my study.

The geographical variations of Japanese *P. bianor* have already been researched and according to the last minute bibliographical research [9,10] and some other evidence, Japanese *P. bianor* can be divided into four subspecies: *P. bianor dehaanii* (Hondo); *P. bianor amamiensis* (Amami islands); *P. bianor ryukyensis* (Okinawa islands) and *P. bianor okinawensis* (Yaeyama islands). [14,27,30] provided evidence of interpopulation hybridization in which, according to them, fertile hybrid females almost always occurred from crosses between adjacent island populations [71] reported that fertile F1 females originated from crosses between Hondo and Yaeyama islands populations, and this is the only exception at this point, [71] also reported that F3 females between *P. polyctor* in the Himalayas and *P. bianor* in Taiwan were fertile. In contrast, F1 females between *P. polyctor* in the Himalayas and *P. bianor* in Hondo were sterile [71]. Moreover, 5th instar larva of Amami islands and Okinawa islands populations resemble each other morphologically but are different from those of both Hondo and Yaeyama islands populations [21,26,71]. According to these findings, *P. polyctor* should be included in Taiwanese *P. bianor*, whereas, Japanese *P. bianor* might be separated into at least two subspecies groups. In this case, only *P. bianor* in Yaeyama islands would remain in *P. bianor bianor* group and the other populations might be *P. bianor dehaanii* group ([72] showed that *P. bianor* on Orchid islands and Green Islands are completely included in *P. bianor* on Main Taiwan). *P. bianor amamiensis* and *P. bianor ryukyensis* are thought to form *P. bianor ryukyensis* subspecies group and this is thought to be closer to *P. bianor dehaanii* subspecies group than *P. bianor bianor* subspecies group. My current results showed continuous reduction of the number of fifth foretarsal ventral-side contact chemosensillum from west Japan to Okinawa Islands, thus these results generally support this grouping [73] divided Japanese *Graphium doson* (Papilionidea) into Yaeyama islands subspecies (ssp *perillus*) and other Japanese subspecies (ssp *albidum*) including Amami islands, Okinawa Islands and Tsushima islands (ssp *tsushimanus*) populations, this also corresponds to my current results, [74] described these *P. bianor* subspecies groups as independent species, however, this is denied according to the interspecies hybridization results shown in [71]. The East Hondo populations, except for that of Hokkaido, may contain two different female races because two clear peaks can be seen (Figure 2); one peak is at a chemosensillum number of 49 and other at 65. The peak at 49 is similar to one of the peaks of the Kagoshima Prefecture population, except for Amami islands, and that of the Hokkaido populations. Another south Kyushu peak seems to correspond to that of west Hondo populations with the exception of Kagoshima Prefecture. The relationship between these differences and minor geographical diversities in Hondo is not clear.

*P. helenus* is known for remarkable biodiversity in terms of wing shape, body size, number of red submarginal lunules on the hindwing and number of white patches in the center of the hindwings. It is divided into a number of subspecies [43,60,75]. Although studies of the differences between Hondo and Nansei islands populations are still insufficient compared with those of *P. bianor*, *P. protenor* and *P. memnon*, those were described as the introduction, [40,63-65] have already described the differences between the Hondo and Amami-Okinawa islands populations. According to their work, the differences appear in contrast of the adult wing color, the shape of the large yellow patch on the hindwings, and average body size. And the most important difference appears in the last instar larval color scheme, where that of the Amami-Okinawa islands population does not resemble either that of Hondo [76] or Taiwan populations [77] but is similar to that of the Philippine population [64-65,78]. Currently, the Japanese *P. helenus* populations are treated entirely as *P. helenus nicconicolens*; however, it may be possible to separate Nansei populations away from the Hondo populations as *P. helenus orosius* (Type locality is Amami-Oshima). My results seem to partially support this classification, but the average chemosensillum numbers decrease from north populations to south populations so the difference between Hondo and Nansei populations remains unclear.

From the results of geographical survey, Japanese *P. protenor* is separated into two subspecies: *P. protenor demetrius* from Hondo to Amami islands and *P. protenor liukiensis* for Okinawa Islands and Yaeyama islands [3, type locality of ssp. *liukiensis* is not clear]. These subspecies are also easily distinguished from each other by the shapes of the submarginal red lunules on the upper hindwings of the females and the length of the hindwing tail of both sexes [40,42]. Moreover, including my unpublished observations, the color scheme of the 2nd-4th instar larva of *P. protenor* in Okinawa islands and Yaeyama islands does not resemble that of either *P. protenor* in Hondo [42-43], Taiwan-China or other continental Asian *Menelaides* species such as *P. helenus* and *P. nephelus* [43]. In fact, it resembles that of *P. polytes* in Philippine-Maluku, and *P. fuscus* and *P. aegaeus* in Maluku-Australasia-Melanesia [43,78-84]. In contrast, that of *P. protenor* in Hondo and Amami islands is the same as that of both *P. protenor* in Taiwan-China and other continental Asian *Menelaides* species [43]. Overall, this current classification of *P. protenor* seems to be reasonable, but it does not fit my recent results. Although, the abnormal distribution pattern of sensillum numbers shown in Fig. 3 might be caused by the shortage of examined individuals, I propose that the origin of the current *P. protenor* of the Okinawa islands populations (and probably also of the Amami islands populations) is a hybrid between *P. protenor* from Yaeyama islands and from Hondo-(ancient) Amami islands. Hybridization experiments between Honshu and Yaeyama islands individuals revealed that female color patterns and the tail length of both sexes in hybrid butterflies resembled those of Yaeyama islands rather than those of Hondo [42,43,52] which seems to support my hypothesis.

*P. memnon* also was divided into many subspecies and some peripheral populations are treated as independent species such as *P. polymnestor*, *P. mayo*, *P. lowi* and *P. rumanzovia* [43,59]. Here the geographical diversity and distribution resembled that of *P. polytes* and some other Southeast Asian butterflies [43,59,62,85-89]. The *P. memnon* female is well known to have many mimetic forms, whereas almost all Japanese females belong to f. *agenor*. The geographical variation of the Japanese *P. memnon* is not as remarkable as those of *P. bianor* and *P. protenor*, but some researchers have pointed out that in Japan the f. *agenor* females are whitest at the south end of their habitat,

and become increasingly black toward the north end. For this reason, the Japanese *P. memnon* was once divided into two subspecies, namely *P. memnon pryeri* (Okinawa islands and Amami islands) and *P. memnon thunbergii* [52]. Now all Japanese *P. memnon* are considered to be *P. memnon thunbergii* [8] because these differences are almost continuous. Japanese *P. memnon* females have one further characteristic; very few f. achates have been collected from west Hondo and Nansei islands. Fortunately I was able to obtain this form in 2004 by rearing the larva of *P. memnon* sent to me by Mr. Ito Nobuo from "Flowerpark Kagoshima", Yamagawa, Kagoshima [90]. According to the reviews of [91,92] and [93], the notes of [94-102] and my own specimen, f. achates found in Nansei Islands, Nagasaki Prefecture and Shimane Prefecture are almost the same as those in continental Southeast Asia. In contrast, those found in South Kyushu and Tsushima islands have a reduced hindwing white patch area and the overall color is darker than those in continental Southeast Asia. The poison-eating butterflies as the models of f. achates (*Pachliopta* and some taild *Parides* species) and f. agenor (*Idea leuconoe*) do not occur in Hondo, on the other, Hondo *P. memnon* males prefer black females to white females [Hashimoto, Niki, Seta, unpublished]. So I think these darker f. achates and black f. agenor are established in Japan. The male color scheme in Hondo and Nansei islands is also slightly different from that found in continental Southeast Asia. From my results, both sexes do not correspond to the results for wing color pattern. The populations of east Hondo have been established in recent years (almost after 2000) and their origin is discussed [56,103]. In Figure 4, Hondo populations were divided into east and west Hondo populations in consideration of these discussions. The average for the east Hondo populations did not correspond to the average of the west Hondo populations but rather to the Amami islands population females. This should be considered when we estimate the quick spread and establishment of *P. memnon* in east Hondo. By these reason, the data from east Hondo population were not used for analysis to significant differences among populations using an F-distribution test.

Japanese *P. polytes* has been treated as *P. polytes polytes* and is now included in *P. polytes polytes* [6] because the large diversity in female wing patterns of this species is thought to result from mimicry. No other noticeable geographical difference between the continental Southeast Asian populations and the Japanese populations has been found. My result in Japan seems to support this classification; however, we must examine a Chinese and Southeast Asia specimen to confirm this relationship, and, of course, this is also required for all other species.

The data from *P. demoleus* specimens examined this time were not useful for revealing their diversity but might be useful for estimating the origin of these butterflies.

### Relations between foreleg morphology, geographical distribution patterns and host plant preference in females of each species

When the reasons for the geographical diversity of the female foreleg morphology for every species are discussed, we must remember that chemoreception sensitivity is a chemical reaction that depends on temperature. With respect to female *P. machaon*, *P. xuthus* and *P. macilentus*, east Hondo individuals on average have more contact chemosensilla than those of west Hondo. This fact may show that east Hondo individuals of these species could retain sufficient sensitivity to detect their host plant correctly even in cooler temperature zone by increasing their chemosensilla. Even though *P. machaon* basically

established in subarctic zone, East Asia *P. machaon* populations are sometimes thought they have already specialized from subarctic *P. machaon* populations, thus the fact that Japanese *P. machaon* populations show the diversity resembling to *P. xuthus* and *P. macilentus* those are thought to established in East Asia temperature zone is reasonable. Some other geographical background also might affect these differences. The chemosensillum number diversity patterns of all Japan *P. maackii* and Hondo *P. bianor* resemble each other and are different from those of *P. machaon*, *P. xuthus* and *P. macilentus*. *P. maackii* and *P. bianor* already appear to have been well-adapted to cool-temperate zones more than East Asia *P. machaon*, *P. xuthus* and *P. macilentus*, thus the number of contact chemosensilla of these two species depend on the total body size. In contrast, the decrease in the contact chemosensilla of *P. maackii* in Kyushu and of *P. bianor* in South Kyushu seems to be caused by the high temperature in this area. In this climate, they seem to be able to maintain the sensitivity required to detect the correct host plants even though their contact chemosensilla are reduced. The temperature of Nansei islands is also high (or higher); however, in case of *P. bianor*, we should find another explanation for the variation in the chemosensillum numbers of *P. bianor* in these areas. *P. bianor* in Yaeyama islands is also known to be less selective regarding host plants [14] as occurs in Hondo. In contrast, *P. bianor* larvae on both Amami islands and especially on Okinawa islands often reject anything other than *Euodia meliifolia* as their host [15-21]. Although the average body sizes in these areas are often smaller than for either Hondo or Yaeyama islands, the noticeable reduction in female contact chemosensillum numbers in *P. bianor* Amami-Okinawa islands populations might relate to these peculiarities.

In the current study, I could not explain the difference of contact chemosensillum numbers in *P. xuthus*, *P. helenus*, *P. protenor* and *P. memnon* between Hondo and Nansei islands, or for those in *P. protenor* and *P. memnon* among Nansei islands populations, as well as those in *P. helenus*, *P. protenor* and *P. memnon* between east and west Hondo. In these cases, the sensillum number distributions in some populations showed more than one peak. Further studies of behavior and general physiology including neuronal responses by these species to plant components and their geographical history are required to resolve this issue.

Although the role of the male chemosensillum is still unknown, the similarity of chemosensillum number diversity between males and females is reasonable.

### Cause of phylogeny mismatch between DNA and other methods including my morphological work

[49,50,104] also proposed the phylogeny of *P. bianor*, *P. (Papilio)* species and *P. protenor* respectively. When DNA data are used for this purpose; however, we should consider that the accumulation of DNA mutations might depend on generation numbers rather than years. For example, every *Papilio* species in Kanto currently produces 2-4 generations per year, but in the glacial age it must have been 0.5-1 generations per year and more than 5 generations in the warmest periods. Moreover, today *P. bianor* in Hokkaido produces 0.5-2 generations per year, whereas that on Amami islands is 4 generations and on Yaeyama islands the number is increasing [42,43]. The number of generations will also be affected by wet/dry seasonal changes. In fact, [47,49] showed that *P. paris* and *P. maackii* belong to the same cluster and *P. bianor* to another cluster. These findings do not correspond to previous results [1,71] and the phylogenetic trees of *P.*

*bianor* and *P. polyctor* mentioned in [47,49] correspond better to the differences in generations per year.

Another necessary correction appears in Figures 3 and 4 found in [50] which show the phylogeny of *Menelaides* and *Iliads* species. In these figures, the relationship of Japanese “black *Papilio*” is shown as “((*P. polytes*+*P. memnon*)+(*P. protenor*+*P. macilentus*))+*P. helenus*”. This result, however seems to correspond somewhat to distribution patterns (basal metabolism amounts of these species) rather than phylogeny. As described above, the distribution areas of *P. polytes* and *P. memnon* superspecies almost overlap, especially these are the only South-East *Papilio* species those occur in both Andaman-Nicobar Islands and Maluku islands, and neither *P. protenor* nor *P. macilentus* do not occur in Malaysia where all other Southeast Asian *Papilio* species occur. The distribution area of *P. helenus* is similar to those of *P. polytes* and *P. memnon* superspecies; however, *P. helenus* does not occur in neither Andaman-Nicobar Islands nor Maluku islands. In addition, *P. helenus* has special distribution characteristics in that two sympatric species, *P. iswara* and *P. iswaroides* occur in Malay Peninsula, Borneo and Sumatra [85]. Without *P. helenus*, only *P. paris* shows a similar distribution with *P. (Achillides) karna* [85]. Moreover, the close relationship between the *P. machaon* group and *P. xuthus* shown by [50,104] also seems to be caused by the similarity of the habitats of these species rather than by their phylogenetic relationship according to hybridization study [71,105-108]. *P. xuthus* is the only *P. (Princeps)* species occurring in the Asian temperature zone with *P. machaon*. All other *Princeps* occur in the Asia-Africa tropical and subtropical zones. Because butterflies are cold blooded creatures and oxygen inhalation is a chemical reaction whose velocity depends on temperature, the amount of oxygen inhalation per unit-time becomes necessarily larger in southern individuals than in northern ones. Mitochondrial enzymes relate directly to oxygen consumption, thus the phylogenies that they exhibit appear reasonable because they must originate from the convergence of mitochondrial enzymes. Moreover, DNA mutation is also a chemical reaction itself, thus the velocity of mutation accumulation in each cold-blooded animal must depend on the environmental temperature.

The problem with mt DNA phylogeny analysis is that an appropriate correction is not provided for the DNA results. The corrections provided by [49-50,99] are solely for clock adjustment, not for dendrogram reconstruction and this might be the cause of the low bootstrap values and the “unexpected results” of the dendrograms in [49-50,99] and others. For *Papilio* butterflies, I think this correction will be more strictly required because the diversities both for numbers of generations per year and the habitat of each population of every *Papilio* species vary considerably and are influenced by many factors compared with other groups of butterflies. Fortunately, the phylogeny of genus *Papilio* has already been well estimated with many authentic methods such as interspecies hybridization or morphology as introduced in [1] and this article. Thus, we can find suitable ways for correcting DNA results by comparing these results with each other and this will be useful for the analysis of other groups of creatures.

#### Appendix of my previous article, [1]

A further survey of the literature provided the following studies of hybridization: among the *P. machaon* group [104]; between *P. machaon* and *P. xuthus* [110,111]; between *P. maackii* and *P. bianor* [112,113]; between *P. maackii* and *P. machaon* [109,110]; between *P. helenus* and *P. polytes* in the field [114,115]; between *P. protenor* and *P. polytes* [116], in the field [117] and in an insectarium [118,119];

between *P. helenus* and *P. protenor* [116]; between *P. aegeus* and *P. fuscus* [120]; between *P. castor* and *P. hipponous* in the field [121], illustrated as aberration of *P. pitmani*); between *P. memnon* and *P. rumanzovia* in the field [122]; and between *P. memnon* and *P. helenus* in the field [123]. Author of [124] succeeded to take a photo of mating *P. maackii* male and *P. bianor* female in the field. Moreover, [125] estimated the phylogeny of the *Papilionidae* of Japan from the morphology of the occipital region. All the above studies support my previous results [1] and this article.

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