Molecular taxonomic analysis of the genus Velarifictorus and V. micado species complex, and the speciation mechanism in East Asia

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(Degree)
博士（学術）

(Date of Degree)
2014-03-25

(Date of Publication)
2015-03-01

(Resource Type)
doctoral thesis

(Report Number)
甲第6029号

(URL)
https://hdl.handle.net/20.500.14094/D1006029

Doctoral Dissertation

博士論文

Molecular taxonomic analysis of the genus *Velarifictorus* and *V. micado* species complex, and the speciation mechanism in East Asia

Velarifictorus **属とツヅレサセコオロギ種群の分子系統解析と東アジアにおける種分化機構**

January 2014

平成 26 年 1 月

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CONTENT

Chapter 1 General introduction

1.1 Taxonomy

More than 26 000 species have been recorded under the Order Orthoptera in Orthoptera Species File Online (Eades *et al.*, 2013). ‘Crickets’ are the insects which belong to the family Gryllidae, Ensifera. Orthoptera insects use hind legs for jumping and females usually have a long ovipositor for laying eggs. Female-biased size dimorphism prevails in this order as in many other Orders (Hochkirch & Gröning, 2008). The subfamilies Gryllinae, Eneopterinae and Trigonidiinae contain many species (Walker & Masaki, 1989). The first fossil cricket is found from Permian. Then mole crickets and tree crickets appeared subsequently (Sharov & Rodendorf, 1971). Crickets adapted to a variety of habitats. Gryllotalpinae species live under ground nearly all their life. Trigonidiinae species dwell on the grass or bush. Phalangopsinae species evolve long legs and live in caves. The sea crickets, *Caconemobius* occur in the tidal zone and can dive into sea for several minutes (Kim, 2010). The ant crickets, Myrmecophilinae, occur in the ant nests. Normally, common crickets such as Gryllinae and Nemobiinae species can be found on the ground.

Early taxonomic studies on Orthoptera taxonomy mainly relied on morphology. However, most circkets usually have color variation within one species, which ended up with many synonyms such as *Acheta domesticus* (Linnaeus, 1758), *Gryllus bimaculatus* De Geer, 1773 and *Gryllodes sigillatus* (Walker, 1869). Then Fulton studied cricket songs and used acoustic characters for taxonomy. Chopard used male genitalia for identification. His woks on the India crickets made the foundation of the modern crickets taxonomy (Chopard, 1969). Molecular technology is a useful tool that can analyze the molecular differences in DNA sequences which can be used to research evolutionary relationships. The research history of using molecular diagnostics to distinguish cricket species and examine evolutionary divergence among lineages encompasses work from allozymes (Harrison, 1979, Howard, 1983) to amplified fragment length polymorphisms (Mendelson and Shaw 2002; Parsons and Shaw 2001) and mitochondrial and nuclear DNA sequences (Gray *et al.*, 2006). Not only genera or families, but the phylogeny of one specie has also been discussed (Ferreira & Ferguson, 2010). The mostly used DNA sequences include mitochondrial DNA (mtDNA) as 12S, 16S, ND4, COI, COII, Cytb (Cameron et al. 2006b; Dowton et al. 2009; Kômoto et al. 2011; Kim et al. 2011b) and nuclear DNA (nDNA) as 18S, 28S, H3 and wingless (wnt) (Shaw, 2002, Svenson & Whiting, 2009). Although it is better to use both mtDNA and nDNA to establish phylogenetic tree, the complete mtDNA can also give enough information as well (Plazzi *et al.*, 2011, Cameron *et al.*, 2006). There is still some debate over the phylogram by molecular marks (Whitfield & Kjer, 2008, Shaw, 2002).

1.2 Geographic distribution and life cycle

Most of the cricket species are living in moist tropic regions and fewer in the polar zones (Walker & Masaki, 1989). There are some cosmopolitan species such as *A. domesticus* (Linnaeus, 1758), *G. bimaculatus* De Geer, 1773 and *G. sigillatus* (Walker, 1869). However, most species are restricted in some areas as endemic species. The most common *Teleogryllus* species in Japan is *Teleogryllus emma* (Ohmachi & Matsuura, 1951). It is distributed in the Honshu Island, the Shikoku Island and the Kyushu Island. It is difficult for them to adapt to the cold climate in the North Hokkaido and they are not distributed in the Ryukyu Islands either (Masaki & Ohmachi, 1967). The distribution of *Teleogryllus infernalis* (Saussure, 1877) includes the Hokkaido Island and the North Honshu Island. *Teleogryllus occipitalis* (Serville, 1838) is distributed in the South Shikoku Island, the Kyushu Island and the Ryukyu Islands. Although *T. emma* has overlap areas with the other two species, hybrid offspring can be seldom found. The life cycle is the key to their distribution. *T. emma* crickets are univoltine and enter diapause at egg stage. In the next summer, the eggs hatch and nymphs emerge in fall. The cold temperature in Hokkaido forces it to develop fast in the limit warm season. The North boundary forms when time is not enough for *T. emma* to complete life cycle from egg to adult. On the other hand, the fickle weather in South may force premature egg hatch at wrong time and fail to overwinter. Although *T. occipitalis* from Shikoku is univoltine, they enter diapause at nymph stage and emerge the next summer. Adults lay eggs immediately and eggs hatch in two or three weeks. Nymphs will enter diapause until next year. Thus studying the life cycle of cricket, not only gives the biologic information, but also helps to predict their distribution.

1.3 Photoperiodic response

Crickets can sometimes adjust their develop rate and life cycle according to photoperiod and temperature in the temperature zones (Shiga and Numata 1997; Shimizu and Masaki 1997; Tanaka 1978). Sinzo Masaki has made great effort to classify this aspects using Japanese crickets (Masaki 1960; Masaki 1961; Masaki 1962; Masaki 1978) and R. S. Bigelow did with the American field crickets *Gryllus* spp (Bigelow 1960; Bigelow 1962; Bigelow 1958) by rearing them in the controlled condition. Because of the cold temperature in winter, crickets in Japan should overwinter by diapause state. Although bark crickets such as *Duolandrevus ivani* and mole crickets such as *Gryllotalpa orientalis* can overwinter at adult stage, most crickets can do so at egg or nymphal stage (Masaki & Walker, 1987). There is a trend that in Northern areas, the proportion of egg diapause crickets increases, because nymphs can not survive such a long period of cold winter. Researching on the responses to photoperiod enables to reconstruct cricket life cycle in the field (Arai *et al.*, 2004).

Masaki defined three types of photoperiodic response: long-day type, short-day type and intermediate-day type (Kidokoro & Masaki, 1978), but see other complex models (Tanaka, 1979). *T. occipitalis* and *Modicogryllus siamensis* Chopard, 1961 belong to the long-day type. With the increase of photoperiod, these species develop fast and emerge quickly (Masaki and Sugahara 1992; Tanaka et al. 1999; Taniguchi and Tomioka 2003). On the other hand, development of *T. emma*, *T. infernalis* and *Loxoblemmus doenitzi* Stein, 1881 gets accelerated with the decrease of photoperiod and this is classified as short-day type (Masaki 1963; Masaki 1966; Masaki 1967). *Dianemobius nigrofasciatus* (Matsumura, 1904) and *Polionemobius taprobanensis* (Walker, 1869) in warm area of Japan are bivoltine. Their eggs hatch in early summer and emerge quickly. The adult laid eggs immediately and eggs hatch without diapause. Since the first generation adults lay eggs for a period of time, nymphal development of the second generation must be adjusted their life to emerge together in fall. The eggs of the second generation enter diapause and hatch in the next year. This species will emerge quickly under short and long photoperiod, but do slowly under intermediate photoperiod such as LD 14:10, thus it is classified as the intermediate-day type (Masaki, 1973, Masaki, 1979). Whether they lay diapause eggs or not depends on photoperiod (Shiga & Numata, 1996).

1.4 Wing type

Wing length is ariable in some crickets. Like other insects, some lost the wing secondly (Roff, 1986, Roff, 1994, Roff, 1990). Many crickets in Gryllinae subfamily have two wing forms, macropters and brachypters (Masaki and Shimizu 1995;

Shimizu and Masaki 1993; Walker and Sivinski 1986), or even intermediate forms (Tanaka et al. 2001; Tanaka et al. 1976). The long- or short-wing are easy to distinguish, because they are usually discrete. Both genetic and environmental effects, even injury at nymph (Shimizu, 1993, Tanaka, 1985), alter the wing type (Arai 1978; Masaki and Shimizu 1995; Roff 1990b; Tanaka 1978; Tanaka et al. 1976). The long winged individuals have a peculiar well-developed metanotum and first abdominal tergite (Fulton, 1931). Needless to say, long-winged individuals can migrate but the short-winged can not. There is a trade-off between the two wing types. Short winged males can allocate energy to attract more females and short winged females to lay more eggs (Crnokrak & Roff, 1998, Tanaka, 1993). In East Asia, belonging to subfamilies such as Oecanthinae, Podoscirtinae are macropterous. On the other hand, species belonging to Mogoplistidae, Landrevinae are always brachypterous. In the remaining subfamilies, both types exist. For example, all the *Teleogryllus* crickets from East Asia are macropterous, but *Gryllus* spp. from the USA show varied wing forms (Walker & Sivinski, 1986). However, most of the species from Gryllinae in East Asia can produce long-wing type in laboratory condition including *Loxoblemmus* Saussure, 1877; *Tarbinskiellus* Gorochov, 1983; *Phonarellus* Gorochov, 1983; *Gryllodes* Saussure, 1874; *Modicogryllus* Chopard, 1961. However in wild, macropterous individuals are usually seldom formed for *Gryllodes sigillatus* (Walker, 1869).

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Chapter 2 Phylogenetic analysis of the genus *Velarifictorus* and *V. micado*

Abstract

Recent advent of molecular taxonomy has shown its power in phylogeny. This investigation challenged for classification of taxonomic status and speciation mechanism in *Velarifictorus micado* species complex based on COI sequences. *V. micado* is also an introduce species in USA since 1959. The COI gene of *Velarifictorus* species were sequenced, especially in *V. micado* collected from 33 areas, including three stocks from Newnan (Georgia), St. Louis (Missouri) and Nashville (Tennessee). Each contains both egg diapause and nymphal diapause forms. Although the original stock collected in Mobile (Mississippi) was named the Japanese burrowing cricket, the results indicates that the Newnan stock was more closely related to Chinese stocks.

2.1 Introduction

Velarifictorus micado belongs to Gryllinae, Modicogryllini. The genus *Velarifictorus* was established in 1964 (Randell, 1964). The type species is *V. micado*. However, Randell misidentified *V. aspersus* as *V. micado*. Thus the type species should be *V. aspersus*. *V. micado* is famous for the Chinese fighting crickets (Suga, 2006). *V. micado* is about 13-19 mm in length. The body is black or dark brown. A pale transverse band connects lateral ocelli. This band is usually thin, but sometimes it is thick particularly in some individuals from China (personal observation). Several pale steaks are on the head. Its original distribution ranges from Japan, Korea, China, Taiwan to Indo-china. It was first discovered in the United States in 1959 (Alexander & Walker, 1962). By 1977 it had colonized a wide range of East Coast, at least 23 counties in 6 Southeastern US states (Walker, 1977).

In north Japan and north China, *V. micado* eggs hatch in late summer and become adults in fall. After mating, females lay eggs in soil by her long ovipositor. These eggs will not hatch until next summer. On the other hand, *V. micado* in the south Japan and south China enter diapause at nymph stage. They emerge in the summer and their eggs hatch quickly. If there is enough accumulated temperature, several generations are possible. Compare with the north compatriot, the ovipositors of south individuals are shorter (Ichikawa *et al.*, 2006). But unlike the different species or subspecies are sterile as Haldane's rule (Hewitt *et al.*, 1987, Haldane, 1922), these two races can mate and have normal offspring. The different life cycle also can be found on other crickets like *Allonemobious fasciatus* and *Gryllus firmus* (Mousseau & Roff, 1989, Walker & Moore, 2000), however, usage of different overwinter stage is rare example. Ichikawa *et al*. (2006) defined the egg overwinter type as *V. micado*, while nymph overwinter type as *Velarifictorus grylloides* in Japan. In China and Korea, all individuals are defined as *V. micado* despite of diapause stage. The individuals from Vietnam are first record in this study. The individuals from USA are considered as *V. micado*.

There are four models for speciation. Allopatric, Peripatric and Parapatric are well proved. Sympatric indicates that speciation can happen without geographical isolation (Smith, 1966, Dieckmann & Doebeli, 1999). In USA, the differences between *Gryllus veletis* and *G. pennsylvanicus* were discovered after studied their life cycle (Alexander & Bigelow, 1960, Alexander, 1968). I reared the stocks of *V. micado* from Osaka and Kobe, which is just 31 km distance. In Osaka, *V. micado* is univoltine and overwinter by nymph. In Kobe, it is also univoltine but overwinter by egg stage. Morphology and calling song can not distinguish the two species. There are two hypotheses for their life cycle. One is that the sympatric speciation happened in Japan when they colonized the Japan Islands from South. Another hypothesis is that, the speciation happened out of Japan and then two different life cycles *V. micado* crickets colonized the Japan.

V. micado have two wing forms, macropterous and brachypterous. Long photoperiod will make *V. micado* develop long wings and more number of molts

(Saeki, 1966a). The population density also affects the wing form. When reared alone, *V. micado* is always brachypterous. And then the percent of macropterous will increase with the density until the maximum (Saeki, 1966b).

2.2 Methods and materials

2.2.1 Taxon sampling

The information of crickets for analyzing COI sequence is listed in Table 2.1 They were totally 36 individuals of 4 species. These samples were kept in the 99% ethanol in -30 degree for PCR experiment.

Table 2.1 Taxon information

Species	No.	Locality	Latitude and longitude	GenBank Number
V. micado	1	Shizuoka, Japan	34.98N 138.38E	KF042341
V. micado	2	Osaka, Japan	34.69N 135.50E	KF042330
V. micado	3	Okinawa, Japan	26.19N 127.71E	KF042319
V. micado	4	Banqiao, Xinbei, Taiwan	25.01N 121.45E	KF042314
V. micado	5	Puli, Taiwan	23.97N 120.97E	KF042313
V. micado	6	Qingyuan, Fujian, Chian	27.62N 119.06E	KF042312
V. micado	7	Daan, Fujian, China	27.87N 117.90E	KF042311
V. micado	8	Tongmu, Fujian, China	27.75N 117.68E	KF042310
V. micado	9	Huaping, Guangxi, China	25.54N 109.88E	KF042309
V. micado	10	Babe National Park, Vietnam	22.40N 105.61E	KF042340
V. micado	11	Siem Reap,Cambodia	13.37N 103.86E	KF042339
V. micado	12	Daegu, Korea	35.97N 128.60E	KF042338
V. micado	13	Tiantong, Zhejiang, China	29.87N 121.54E	KF042337
V. micado	14	Yangzhou, Jiangsu, China	32.39N 119.41E	KF042336
V. micado	15	Changzhou, Jiangsu, China	31.81N 119.97E	KF042335
V. micado	16	Newnan, GA,USA	33.38N 84.80W	KF042334
V. micado	17	Tianmushan, Zhejiang, China	30.32N 119.43E	KF042333
V. micado	18	Baishanzu, Zhejiang, China	27.73N 119.19E	KF042332
V. micado	19	Shenyang, Liaoning, China	41.81N 123.43E	KF042331
V. micado	20	Changchun, Jilin, China	43.90N 125.33E	KF042329
V. micado	21	Haerbin, Heilongjiang, China	45.80N 126.53E	KF042328
V. micado	22	Sayo，Hyogo，Japan	35.03N 134.36E	KF042327
V. micado	23	Kussharo Lake, Hokkaido, Japan	43.06N 141.35E	KF042326
V. micado	24	Kobe, Hyogo, Japan	34.69N 135.20E	KF042325
V. micado	25	Nashville, TN, USA	36.17N 86.78W	KF042324
V. micado	26	St. Louis, MO, USA	38.63N 90.20W	KF042323
V. micado	27	Nanjing, Jiangsu, China	32.06N 118.80E	KF042322
V. micado	28	Dongying, Shandong, China	37.43N 118.67E	KF042321
V. micado	29	Ningyang, Shandong, China	35.76N 116.81E	KF042320

V. micado	30	Shijiazhuang, Hebei, China	38.04N 114.51E	KF042318
V. micado	31	Cangzhou, Hebei, China	38.30N 116.84E	KF042317
V. micado	32	Ningshan, Shanxi, China	33.31N 108.31E	KF042316
V. micado	33	Chengdu, Sichuan, China	30.66N 104.06E	KF042315
V. flavifrons	34	Shenzhen, Guangdong, China	22.57 N 114.05 E	KF128891
V. ornatus	35	Changzhou, Jiangsu, China	31.84 N 119.98 E	KF128890
V. aspersus	36	Qingyuan, Fujian, China	27.64 N 119.06 E	KF128889

2.2.2 Rearing condition

Velarifictorus crickets were collected from East Asia during 2010-2012, with a special quarantine permission by the Kobe Plant Protection Station. These stocks were reared in 27.5℃, LD 16:8 photoperiod for laying eggs (consisting of 16 hours light phase provided by 20W fluorescent lamp controlled by an electric timer, and 8 hours dark phase). Then, the eggs were transported into a walk-in 30℃ constant temperature room (NK SYSTEM Experimental Laboratory at constant temperature and humidity, LP-1/2/3PH). The eggs hatched soon and they were reared in transparent plastic cups (10 cm in diameter × 10 cm in height) as 10 individuals per cup. As duplicate, nymphs were reared under LD 16:8 or 12:12 at 27.5℃. They were fed with rodent food (MF, ORIENTAL YEAST Co. Ltd) and water in cotton-plugged glass vials. Sufficient food and water were given until they became adults. The eggs of some species need a period of chill time for breaking their diapause. After laid in soil, the eggs were kept at 20℃ for 2 weeks for development. The eggs were transported into 4℃ for 3 months. After experiencing the cold time, the eggs were transported into 30℃. The hatching time was recorded from the day returned to 30℃.

2.2.3 DNA experiment

The total DNA was extracted from the muscles of one hind femur by using GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich Inc., www.sigmaaldrich.com). Each sample was dissolved in 200 µL elution buffer. The mitochondrial cytochrome C oxidase subunit I was amplified from the total DNA by using PCR with the primers as following primers: TW-J1301 (GTT AAW TAA ACT AAT ARC CTT CAA A) and C2- N3389 (TAC TCA TAR GAT CAR TAT CAY TG) (Simons *et al.*, 1994). PCR reaction was carried out by KOD DNA Polymerase (Toyobo Biologics Inc., www.toyobobiologics.com) in a 50 µL reaction mixture including distilled water 11 µL, 2 mM dNTPs 10 µL, 2×PCR Buffer for KOD FX Neo 25 µL, each primer 1.5 µL, KOD FX Neo 1 µL. The amplification protocol was 30 cycles of denaturation at 94 ℃ for 2 min, annealing at 40 ℃ for 30 sec, extension at 68 ℃ for 120 sec in GeneAmp® PCR System 9700 (Applied Biosystems, www.appliedbiosystems.com). The PCR product was purified by illustra™ GFX™ PCR DNA and Gel Band Purification Kit (GE healthcare, www.gelifesciences.com). Direct sequencing of the COI DNA fragment was performed by using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with the two primers mentioned above and two middle primers (ggtacaggatgaacagtttatccacc, gttaatcctgtaaataaagg). The sequence of the COI gene was determined by the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, www.appliedbiosystems.com).

2.2.4 Phylogenetic analysis

The sequence alignments were performed by using ATCG Sequence Assemble software Version 3.03 (SOFTWAE DEVELOPMENT CO., LTD). Mega Version 5.2 was used for constructing the phylogenetic tree by using maximum-likelihood method with bootstrap value of 1000 replications (Tamura *et al.*, 2011) and Mrbayes 3.2.2 software. See result 1.2, 1.3 for arameters settings. The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible mode. The tree with the highest log likelihood (-8465.0155) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 1.8033)). The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 49.1007% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 54 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 1485 positions in the final dataset.

2.3 Results

2.3.1 Phylogeny of genus *Velarifictorus*

Total length of 1485 bp, occupying most of the COI gene, was sequenced. No insert or deletion was found among different species. When translated into amino acid, all *V. micado* individuals had the same sequences, except those from Vietnam and Cambodia where were Isoleucine at 470th, substituted Valine in other populations. Other species (*V. ornatus, V. aspersus* and *V. flavifrons*) were also Isoleucine at 470th. This shared amino acid was considered as the ancient trait (see table 2.2). That *V. micado* was at the apical position of the phylogenic tree could be another evidence for that *V. micado* was the recent species split from others (see figure 2.1). Several katydids and one grasshopper were added in the tree as outgroup. The bifurcation time between Ensifera and Caelifera was set as 250 mya (Béthoux *et al.*, 2002). *V. ornatus* is the basal species separated at 73.3±9.2 mya. *V. aspersus* and *V. flavifrons* followed. *V. micado* just appeared 7.0±2.0 mya.

Table 2.2 Haploid of *Velarifictorus* spp. COI gene.

	57	97	107	127	132	168	350	396	397	401	408	459	465	470	474	476	477
micado 1	I	L	T	T	A	S	I	I	T	K	I	I	L	V	N	L	F
micado 2	.	.	.	.	.	.	.	.	.	.	.	.	.	I	.	.	.
flavifrons	V	.	S	.	.	G	V	L	M	Q	.	.	.	I	S	.	.
aspersus	.	F	.	A	.	G	.	L	.	.	L	L	.	I	K	M	S
ornatus	.	.	.	.	T	G	.	L	.	.	L	.	M	I	K	.	.

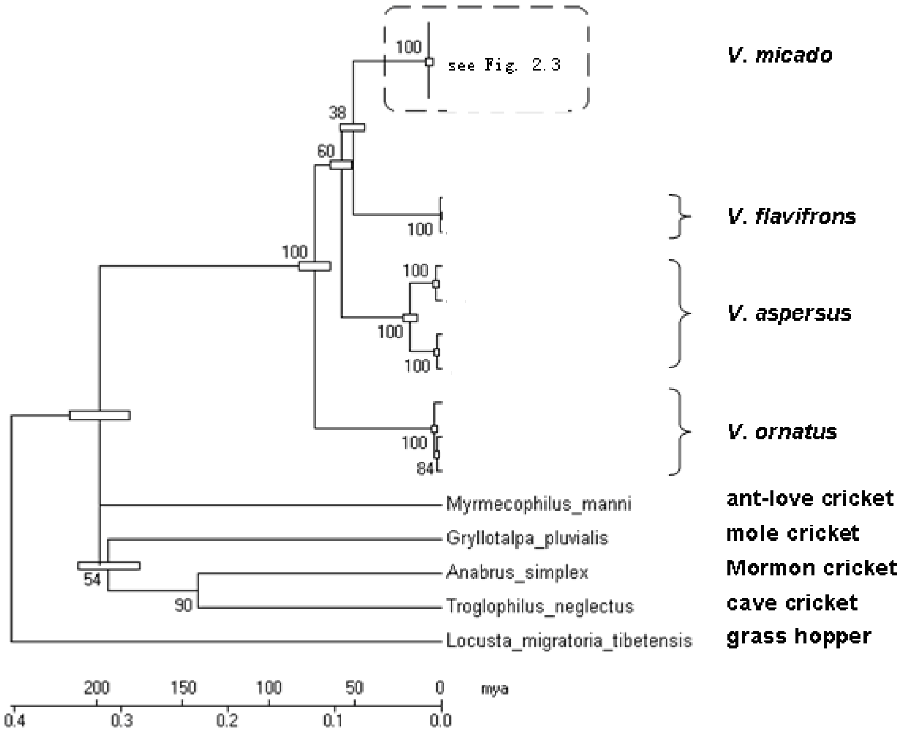

Figure 2.1 Molecular phylogenetic analysis of genus *Velarifictorus* by Maximum Likelihood method. *V. micado* is omitted for detailed description see details in Fig. 2.3.

2.3.2 Phylogeny of *V. micado*

No insert or deletion among different individuals was found. The phylogenetic tree showed that there were four main groups derived from common ancestors, including 1) South group 2) North group 3) East China group and 4) Taiwan-Japan group (Fig. 2.2, 2.3). The three later groups were closer and homology, in which South group was less homology. The USA individuals from Newnan were similar to East China group, whereas individuals from Nashville and St. Louis were similar to North group.

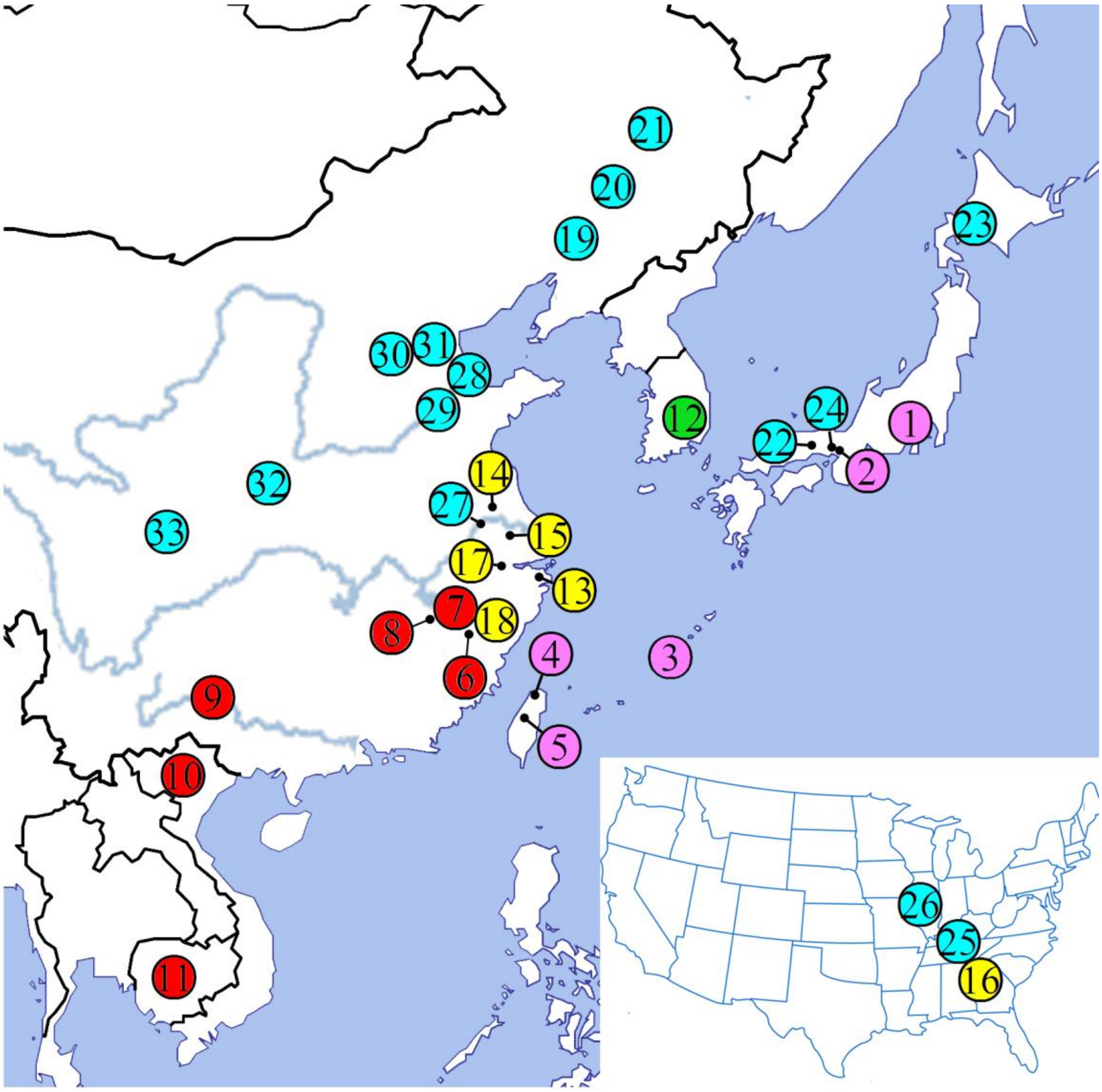

Figure 2.2 Geographic distribution of *V. micado* samples. Different groups with different color (Blue: North group, yellow: East China group, pink: Taiwan-Japan group; red: South group; green: Korea). The numbers correspond to those in table 2.1.

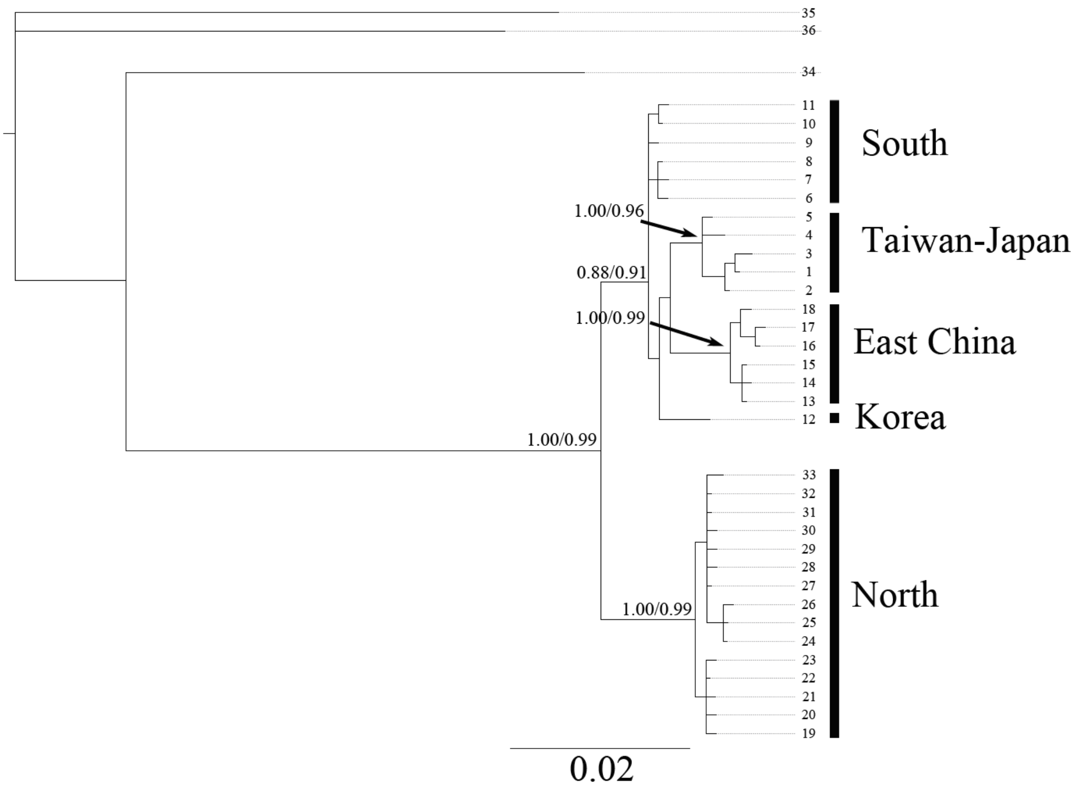

Figure 2.3 Topology from Bayesian inference of phylogeny for 33 individuals, based on COI gene. Black bars indicate distinct groups. Posterior probability (PP) values were given at main branches. Numbers accord to table 2.1. (left: Bayes and right: maximum likelihood, PP values lower than 0.7 or conflict with ML tree were not shown).

Table 2.3 A list of polymorphic sites in haplotype of *V. micado* COI gene.

	12	75	96	114	171	243	255	261	291	306	312	318	321	330	333	375	384	411	454	468	474	480	498	507	526	534	562	600	604	633	667	678	690	696	720	735	756	825	826	846	879	894	927	981	991	996	1036	1044	1074	1086	1098	1122	1221	1227	1233	1236	1251
1	T	A	A	G	T	A	A	T	A	A	C	A	T	A	T	G	A	T	T	T	T	T	T	G	C	A	T	C	C	A	T	T	C	C	C	T	T	A	T	G	C	T	G	T	C	T	T	T	C	T	A	A	T	C	T	C	A
2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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4	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.
5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.
6	.	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	A	T	.	A	.	.	.	.	.	.	.	.	.	.	T	C	.	.
7	.	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	A	T	.	A	.	.	.	.	.	.	.	.	.	.	T	C	T	.
8	.	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	A	T	.	A	.	.	.	.	.	.	.	.	.	.	T	C	.	.
9	.	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	A	T	.	A	.	.	.	.	.	.	.	.	.	.	T	.	.	.
10	.	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	A	T	.	A	.	.	.	.	.	.	.	.	.	.	T	C	.	.
11	.	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	A	T	.	A	.	.	.	.	.	.	.	.	.	.	T	C	.	.
12	C	.	.	.	.	G	.	C	G	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	G	.	.	.	T	.	.	T	.	.	.	.	T	.	A	T	.	.	.	.	.	.	C	.	.	.	.	C	T	C	.	.
13	.	.	G	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	.	C	C	.	.	.	.	.	T	.	.	.	.	T	.	.	C	.	.	C	A	T	.	.	.	.	C	.	.	.	.	.	.	.	T	C	.	.
14	.	.	G	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	.	C	C	.	.	.	.	.	T	.	.	.	.	T	.	.	C	.	.	C	A	T	.	.	.	.	C	.	.	.	.	.	.	.	T	C	.	.
15	.	.	G	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	.	C	C	.	.	.	.	.	T	.	.	.	.	T	.	.	C	.	.	C	A	T	.	.	.	.	C	.	.	.	.	.	.	.	T	C	.	.
16	.	.	G	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.	G	.	T	.	.	C	.	T	.	.	C	.	.	C	A	T	.	.	.	.	C	.	.	.	.	.	.	.	T	C	.	.
17	.	.	G	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.	G	.	T	.	.	C	.	T	.	.	C	.	.	C	A	T	.	.	C	.	C	.	.	.	.	.	.	.	T	C	.	.
18	.	.	G	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	.	C	C	.	.	.	.	.	T	.	.	C	.	T	.	.	C	.	.	C	A	T	.	.	.	.	C	.	.	.	.	.	.	.	T	C	.	G
19	.	C	.	.	.	G	G	C	.	.	T	G	.	.	C	A	G	.	.	.	C	C	C	.	T	.	.	.	.	.	.	C	T	T	.	.	C	.	.	A	T	C	A	.	T	.	.	C	.	.	G	.	.	T	C	.	.
20	.	C	.	.	.	G	G	C	.	.	T	G	.	.	C	A	G	.	C	.	C	C	C	.	T	.	.	.	.	.	.	C	T	T	.	.	C	.	.	A	T	C	A	.	T	.	.	C	.	.	G	.	.	T	C	.	.
21	.	C	.	.	.	G	G	C	.	.	T	G	.	.	C	A	G	.	.	.	C	C	C	.	T	.	.	.	T	.	.	C	T	T	.	.	C	.	.	A	T	C	A	.	T	.	.	C	.	.	G	.	.	T	C	.	.
22	.	C	.	.	.	G	G	C	.	.	T	G	.	.	C	A	G	.	.	.	C	C	C	.	T	.	.	.	.	.	.	C	T	T	.	.	C	.	.	A	T	C	A	.	T	.	.	C	.	.	G	.	.	T	C	.	.
23	.	C	.	A	.	G	G	C	.	.	T	G	.	.	C	A	G	.	.	.	C	C	C	.	T	.	.	.	.	.	.	C	T	T	.	.	C	.	.	A	T	C	A	.	T	.	.	C	.	.	G	.	.	T	C	.	.
24	.	C	.	.	.	G	G	C	.	G	T	G	.	.	C	A	G	.	.	C	C	C	C	.	T	.	.	.	.	.	.	C	T	T	.	.	C	.	.	A	T	C	A	.	T	.	.	C	T	.	G	.	.	T	C	.	.
25	.	C	.	.	.	G	G	C	.	G	T	G	.	.	C	A	G	.	.	C	C	C	C	.	T	.	.	.	.	.	.	C	T	T	.	.	C	.	.	A	T	C	A	.	T	.	.	C	T	.	G	.	.	T	C	.	.
26	.	C	.	A	.	G	G	C	.	G	T	G	.	.	C	A	G	.	.	C	C	C	C	.	T	.	.	.	.	.	.	C	T	T	.	.	C	.	.	A	T	C	A	.	T	.	.	C	T	.	G	.	.	T	C	.	.
27	.	C	.	.	.	G	G	C	.	G	T	G	.	.	C	A	G	.	.	.	C	C	C	.	T	.	.	.	.	.	.	C	T	T	.	.	C	.	.	A	T	C	A	.	T	.	.	C	.	.	G	.	.	T	C	.	.
28	.	C	.	.	.	G	G	C	.	G	T	G	.	.	C	A	G	.	.	.	C	C	C	.	T	.	.	.	.	.	.	C	T	T	.	.	C	.	.	A	T	C	A	.	T	.	C	C	.	.	G	.	.	T	C	.	.
29	.	C	.	.	.	G	G	C	.	G	T	G	.	.	C	A	G	.	.	.	C	C	C	.	T	.	C	.	.	.	.	C	T	T	.	.	C	.	.	A	T	C	A	.	T	.	.	C	.	.	G	.	.	T	C	.	.
30	.	C	.	.	.	G	G	C	.	G	T	G	.	.	C	A	G	.	.	.	C	C	C	A	T	.	.	.	.	.	.	C	T	T	.	.	C	.	.	A	T	C	A	.	T	.	.	C	.	.	G	.	.	T	C	.	.
31	.	C	.	.	.	G	G	C	.	G	T	G	.	.	C	A	G	.	.	.	C	C	C	.	T	.	.	.	.	.	.	C	T	T	.	.	C	.	.	A	T	C	A	.	T	.	.	C	.	.	G	.	.	T	C	.	.
32	.	C	.	.	.	G	G	C	.	G	T	G	.	.	C	A	G	.	.	.	C	C	C	.	T	.	.	.	.	.	.	C	T	T	.	.	C	.	.	A	T	C	A	.	T	.	.	C	.	.	G	.	.	T	C	.	.
33	.	C	.	.	.	G	G	C	.	G	T	G	.	.	C	A	G	.	.	.	C	C	C	.	T	.	.	.	.	.	.	C	T	T	.	.	C	.	.	A	T	C	A	.	T	C	.	C	.	C	G	.	.	T	C	.	.

2.4 Discussions

2.4.1 Phylogeny of the genus *Velarifictorus*

Taxonomy study includes two parts, classification and phylogeny. Orthoptera is one of the biggest order in cohort Polyneoptera (other orders Embioptera, Dictyoptera, Phasmida, Dermaptera, Notoptera, Zoraptera, Plecoptera), including over 26 000 species (Eades *et al.*, 2013, Legendre *et al.*, 2010), in which 15 000 species belong to Ensifera (katydids and crickets). The phylogeny of Ensifera is not understood well so far. Some of the previous studies focused on the morphology (Desutter-Grandcolas 2003; Desutter-Grandcolas and Robillard 2006; Gorochov 2001; Gwynne 1995; Judd 1948; Ragge 1955; Zeuner and Edwards 1939), and others employed molecular taxonomical methods (Jost & Shaw, 2006, Robillard & Desutter-Grandcolas, 2006). There are inconsitent and different phylogenetic relationships were found. The reason is probably due to the fast adaptations in Ensiferans.

Crickets belong to Grylloidea, which includes Gryllidae, Mogoplistidae, Myrmecophilidae and Gryllotalpidae. Most common crickets belong to the family Gryllidae, which includes genus *Velarifictorus*. This genus contains six species, *micado, ornatus, aspersus, flavifrons, arisanicus* and *beybienkoi* in East Asia. The *Velarifictorus* crickets in Africa probably have no relationship with Asian species, though their genitalia are similar. According to the phylogenetic tree (Fig. 2.1), genus *Velarifictorus* appeared in the end of Cretaceous. Specie *ornatus* is the original species, because its head is nearly round like genus *Modicogryllus* spp., while other

Velarifictorus crickets have longer face, especially highly developed mandible. *V. micado* is famous for the English name, Chinese fighting crickets. Males bite rivals by mandible directly, which is probably the reason for their having large mandible (Judge & Bonanno, 2008). *V. ornatus* crickets were relatively peaceful during my rearing process (HE, personal observation) and they do not have large mandibles.

By checking the amino acid translated from COI gene in Table 2.2, position 168 is G, 396 is L and 470 is I in *V. ornatus, V. aspersus* and *V. flavifrons,* while these acids are S, I, V in *V. micado* species, respectively. In the phylogenetic tree, *V. micado* located at the end, not root of this genus, which indicated that *V. micado* is the recent derived species from this genus. In my study, only one gene for phylogenetic tree was analyzed, but it still support the high homology of *Velarifictorus* genus. Also it reflects the instable root of Ensifera. No relationship could be found among other subfamilies such as mole crickets or ant love crickets. Even more samples added might not improve this situation (Legendre *et al.*, 2010). Rapid adaptation and speciation makes it difficult to find evidence for researching phylogeny of the current species.

2.4.2 Phylogeny of *V. micado*

V. micado was named as *Scapsipedus aspersus*. *Velarifictorus* was established by the genitalia differences with *Scapsipedus* (Randell, 1964). *Mocidogryllus* and *Mitius* may have the close relationship with *Velarifictorus* (Ingrisch, 1998). The phylogeny of this genus and the phylogeny of *V. micado* species have not been studied yet.

Alexander and Walker pointed out that *V. micado* might be introduced with bait sold by stores (Alexander & Walker, 1962). The present result suggests that *V. micado* in USA was introduced more than one time. One stock is from East China and another is from North group. Interestingly, distribution of genotype in USA is similar to that of East Asia. Newnan is in the south of St. Louis and Nashville. The original genotype of Newnan is in the East China group, where is in the south of North group. The North group is the origin of the genotype in St. Louis and Nashville.

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Chapter 3 Discrete modes of life cycle in *Velarifictorus micado* species complex

(This article is published on ISRN Entomology, vol. 2013, Article ID 851581, 5 pages, 2013. doi:10.1155/2013/851581)

Abstract

Different modes of climatic adaptation often lead to a split in reproductive unit and stimulate speciation between populations with different patterns of life cycle. We here examined egg development and photoperiodic adaptations in the nymphal development of *Velarifictorus micado*. We defined fast hatching populations as nymphal diapause, while slow hatching populations as egg diapause. The nymphs were reared under two photoperiods, LD 16:8 and LD 12:12 at 27.5 ℃ and the mean days of nymphal development were compared. The results indicate that the nymphal diapause populations showed slower nymphal development under LD 12:12 than under LD 16:8 and this retardation increased with the increase of original latitude. The egg diapause populations showed slower nymphal development under LD 16:8 than under LD 12:12. These features help synchronizing their overwintering stages. Gene flow from the opposite forms may disturb this synchronization mechanism and therefore natural selection should favor displacement of the two forms. This could be an incipient climatic isolation mechanism of reproductive stage.

3.1 Introduction

Crickets are omnivorous feeders, widely distributed in low and wetland habitats between 57 ° north and south latitudes [1]. Their life cycles can be divided into two basic types: one without seasonality and the other with it [2]. Crickets of the first type continue producing new generations throughout the year, so they lack synchrony in generations. Crickets of the second type usually have a unique and regular life cycle, where climate exerts synchronizing effects on their life cycle [1, 3]. Crickets in temperate zones with different life cycle show different photoperiod responses. Masaki Sinzo researched the photoperiodic response of Japanese crickets systematically [4-10] and he defined three types of photoperiodic response: long-day type, short-day type and intermediate-day type [11] (other complex models also exist [12]). Crickets belonging to long-day type develop fast and emerge synchronously under long photoperiod (LD 16:8) and they are bivoltine or multivoltine with nymph diapause [10, 13, 14]. Crickets belonging to short-day type develop fast under short photoperiod (LD 12:12) and are univoltine with egg diapause [5-7]. Intermediate-day type crickets include *Dianemobius nigrofasciatus* (Matsumura, 1904) and *Polionemobius taprobanensis* (Walker, 1869). In warm area, they are bivoltine. These two species emerge quickly in both short and long photoperiods, but emerge slowly under intermedium photoperiod (LD 14:10). The first generation emerges in summer and lay non-diapause eggs according to long photoperiod [15]. The second generation develops slowly under intermedium photoperiod in early autumn and emerges synchronously under short photoperiod [8]. But in north Japan, the same species are univoltine and only produce diapause eggs as short-day type.

Alexander and Bigelow [16] first proposed a model of allochronic speciation in field crickets. They found that *Gryllus pennsylvanicus* and *G. veletis* were very similar in morphology and overlapped in some areas. They were treated as same species, but they had different life cycles. Sympatric divergence of life cycles resulted in seasonal isolation of their offspring. However, later molecular taxonomic works ruled out the relatedness of these two species [17, 18]. Both of them have other sibling species with the similar life cycle. Their overlap seems to be the result of their migration.

Velarifictorus micado develops two diapause strategies in Japan [3]. One group is strictly univoltine, which enters an obligatory diapause at egg stage as short-day type. Their calling songs can be heard from August to October. This group distributed all over Japan, except the Hokkaido and the Ryukyu Islands. Another group, distributed in south Japan, grows slowly and overwinters at a late nymphal stage. They are univoltine and their calling songs can be heard from May to July. Masaki concluded this as climatic speciation because low temperature in winter made them have to diapause at egg or nymphal stage. Egg diapause groups were preferred in north area and nymph diapause groups were preferred in south area. Such selection will form two species after enough time [19].

Masaki cited *V. micado* as an instance for his hypothesis. He pointed out that two diapause strategies could both succeed in northern colonization and had some overlaps. However, *V. micado* are not restricted in Japan and they have a wide distribution in East Asia (Japan, Korea, China, Taiwan and Indo-china peninsula) from Haerbin, China, (45.80 °N, sometime reaching -30 ℃) to Siem Reap, Cambodia (13.37 °N, a tropical area). Their life cycles in other Asian countries are not well understood. Because of its wide distribution and probably different climatic adaptations in different areas, *V. micado* offers a good subject for climatic adaptation study. There is a need for life cycle analysis of this species and Masak's climatic speciation should be tested in a wider area. In this study, we analyzed egg and nymphal development to discuss its relevance to climatic speciation.

3.2 Methods and materials

Seven geographic populations of *V. micado* were collected from China, Japan, Korea, Taiwan and Vietnam. Their offspring were reared for this study (see details in Table 3.1, Figure 3.1). Females were allowed to lay eggs for 24 hours. These eggs oviposited into soil were incubated at 27.5 ℃. Hatching number was recorded everyday. Then, nymphs were reared in transparent plastic cups (10 cm in diameter × 10 cm in height) with 10 individuals per cup under LD 16:8 or LD 12:12 at 27.5 ℃ in a walk-in constant temperature room (NK SYSTEM Experimental Laboratory, LP–1/2/3PH). They were fed with rodent food (MF, ORIENTAL YEAST Co. Ltd.) and water in cotton-plugged glass vials until nymphs reached adult stage. The nymphs were checked everyday. Adults were removed from the culture in order to preventing cannibalism.

Table 3.1: Collection sites of populations

Population	Collection site	Latitude and longitude
Japan	Osaka, Japan	34.69^{O} N 135.50^{O} E
Taiwan	Wulai, Xinbei, Taiwan	24.87^{O} N 121.55^{O} E
S. China	Shenzhen, Guangdong, China	22.54^{O} N 114.06^{O} E
Vietnam	Tam Dao, Vietnam	21.57^{O} N 105.67^{O} E
N. China	Changchun, Jilin, China	43.90^{O} N 125.33^{O} E
Korea	Daegu, Korea	35.97^{O} N 128.60^{O} E
M. China	Tianmushan, Zhejiang, China	30.32^{O} N 119.43^{O} E

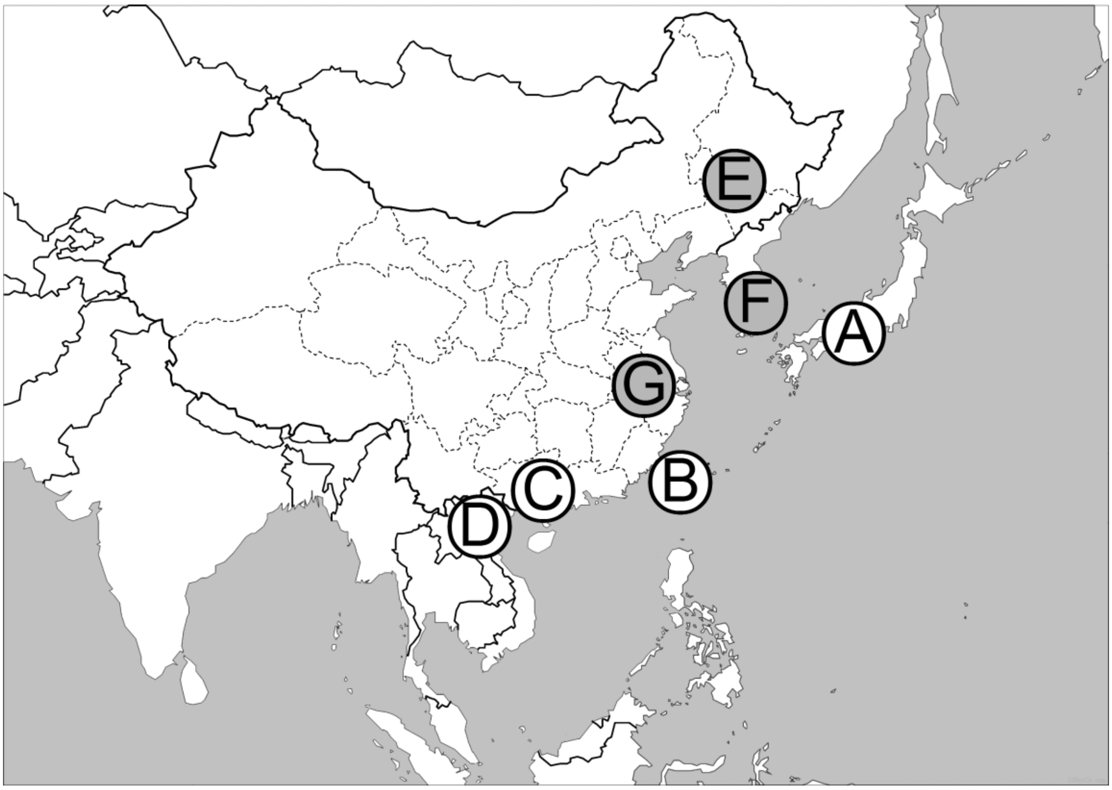

Figure 3.1: Geographic distribution of samples. Nymphal diapause, A: Osaka, Japan, B: Wulai, Taiwan, C: Shenzhen, S. China, D: Tam dao, Vietnam; egg diapause, E: Changchun, N. China, F: Daegu, Korea, G: Tianmushan, M. China.

3.3 Results

There were two types of egg hatching rate. Populations of Vietnam, S. China, Taiwan and Japan had relatively fast hatching rate. Their nymphs almost hatched in 20 days. They are defined as nymph diapause groups. Population of M. China, N. China and Korea had relatively slow hatching rate. The range of mean hatching days were from one month to two months with large standard deviation, which mean they did not hatch synchronously. In wild, eggs of these populations need low temperature to terminate diapause. If these eggs are directly incubated in high temperature, their hatching days will be varied [5]. They are defined as egg diapause group (Table 3.2).

The period of nymphal development was calculated and was compared between two photoperiods (Table 3.3, Figure 3.2). Results showed significant differences of nymphal developmental days under long and short photoperiod in all populations. In the egg diapause group, individuals under LD 12:12 developed faster than those under LD 16:8, while those of nymphal diapause group developed slower under LD 12:12 than under LD 16:8. Egg diapause populations developed faster and more synchronously. The mean developmental days of M. China population was 74 days as the longest under LD 16:8 and N. China population finished it in 50 days. On the other hand, nymphal diapause populations spent less time on egg stage, but more time on nymphal stage under short photoperiod. They developed fast (about 60-80 days) under LD 16:8, but developed slow (over 90 days) under LD 12:12. Japanese population of Osaka was an exception, their emergent days were varied from 50 to 150 days under both photoperiods.

Table 3.2: Diapause stage and egg hatching days

Population	N	Mean hatching days ±SD	Diapause stage
Japan	251	17.68 ±1.28	nymph
Taiwan	382	16.66 ±1.21	nymph
S. China	447	16.27 ±1.26	nymph
Vietnam	369	17.09 ±1.28	nymph
N. China	352	39.64 ±12.33	egg
Korea	225	56.18 ±13.65	egg
M. China	416	78.29 ±17.07	egg

Table 3.3: Durations of nymphal development under LD 12:12 and LD 16:8.

	LD	N	nymphal duration ± SD	Retardation	t-test	
Nymphal diapause						
Japan	12:12	28	113.61 ±25.06	18.41	t(66.5)=2.766	*p*=0.007
	16:8	45	95.20 ±31.36			
Taiwan	12:12	9	124.44 ±6.39	47.44	t(27)=8.169	*p*<0.001
	16:8	20	77.00 ±16.74			
S. China	12:12	63	90.67 ±13.72	28.78	t(105.5)=13.110	p<0.001
	16:8	45	61.89 ±9.08			
Vietnam	12:12	34	96.59 ±22.71	17.78	t(50.3)=4.059	*p*<0.001
	16:8	47	78.81 ±13.75			
Egg diapause						
N. China	12:12	39	41.46 ±6.97	–8.07	t(75)=-5.861	*p*<0.001
	16:8	38	49.53 ±4.89			
Korea	12:12	9	53.67 ±8.80	–13.5	t(25)=-3.348	*p*=0.003
	16:8	18	67.17 ±10.34			
M. China	12:12	25	61.88 ±13.73	–12.12	t(60)=-3.868	*p*<0.001
	16:8	37	74.00 ±9.74			

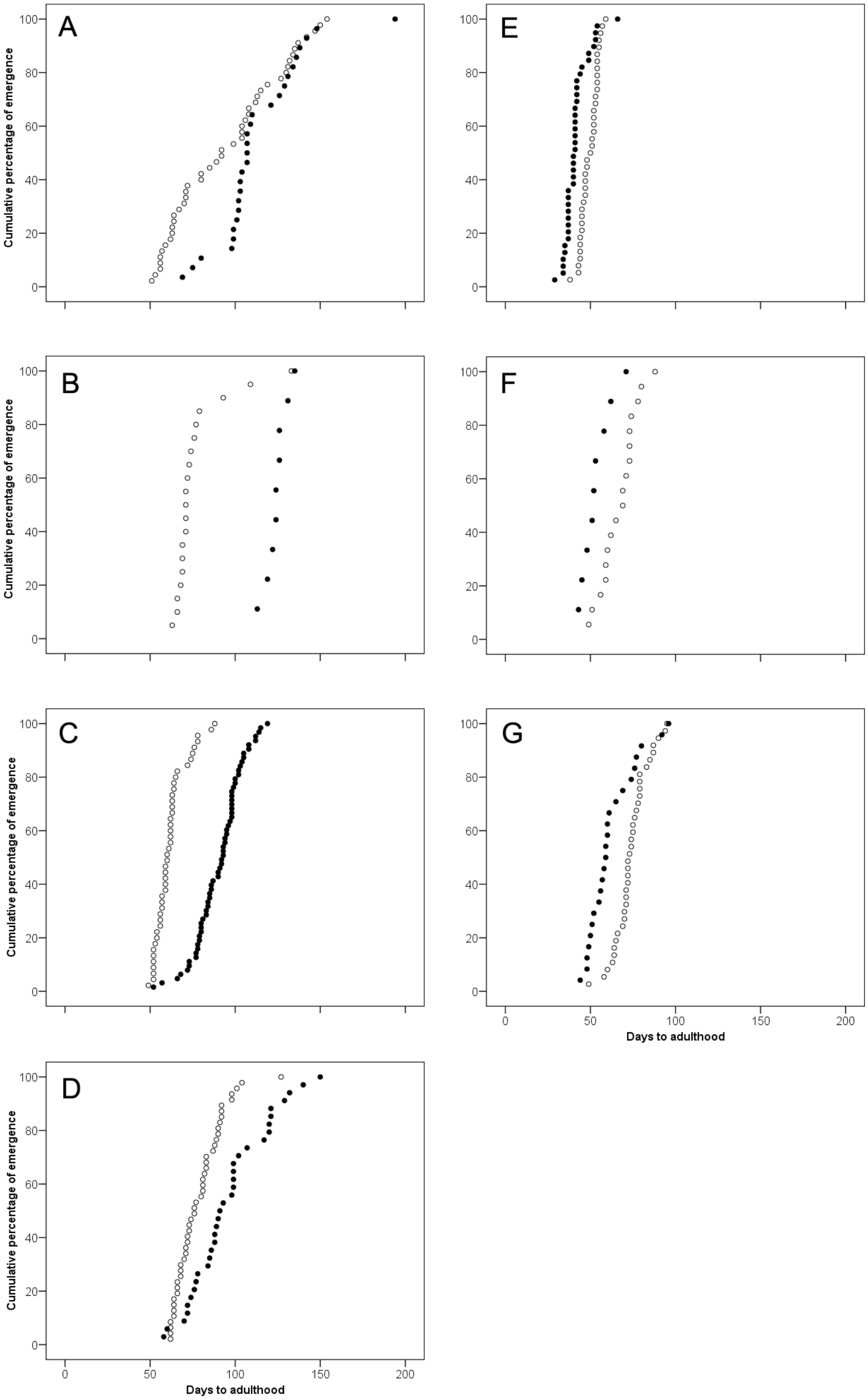

Figure 3.2: Cumulative percentage of *V. micado* emergence. Nymphal diapause, A: Osaka, Japan, B: Wulai, Taiwan, C: Shenzhen, S. China, D: Tam dao, Vietnam; egg diapause, E: Changchun, N. China, F: Daegu, Korea, G: Tianmushan, M. China. (circles, LD 16:8; solid circles, LD 12:12).

3.4 Discussions

Masaki investigated the life cycle of Japanese *V. micado* and defined these two groups with different life cycle as two species [3]. He concluded this as climatic speciation, which is similar to the allochronic speciation by Alexander and Bigelow. As winter becomes more severe in North Japan, *V. micado* must enter diapause at either egg or nymphal stage. Although two groups have not completely separated, natural selection will make the individuals entering either diapause stage strictly survive.

We investigated seven populations of *V. micado* from Vietnam, China, Taiwan, Korea and Japan. Fast egg developing populations developed slowly at nymphal stage and vice versa. Their life cycle can be deduced by these results because it is adjusted by photoperiodic response and thermal parameters for development [20]. The Vietnam population probably develops continuously, although developmental days under LD 12:12 showed slight retardation. They may overwinter mainly at nymphal stage because they face a shortage of food and cold temperature in winter. The nymphal development was less synchronous than those of other nymphal overwintering populations. In the S. China and Taiwan population, the degree of retardation increased with latitude (47 days in Taiwan population, 29 days in the S. China population and 18 days in the Vietnam populations), which suggests that these two populations enter diapause more strictly. If adults emerge in winter, it will be unfavorable for survival. After such natural selection for generations, they emerged more synchronously. The developmental rates were fast under LD 16:8, which suggests they may produce continually as bivoltine or multivoltine if heat units are enough.

The variation of Osaka population emergency was large. This could be the result of two diapause termination mechanism. Tanaka investigated *Pteronemobius nitidus* and found it had a complex photoperiod response [12]. *P. nitidus* is a univoltine species. They enter diapause at nymphal stage in winter and emerge next summer. Their first diapause stage is terminated by the decrease of photoperiod in autumn and second diapause stage is terminated by the increase of photoperiod in summer. Under stationary photoperiod, this species developed less synchronously. Our result of Osaka population followed this complex photoperiod response model and suggests that this population is univoltine and overwinters at nymphal stage.

Three egg-diapause populations all emerged synchronously and fast. They are univoltine species and enter diapause at egg stage. The egg diapause of *Teleogryllus emma* crickets has been studied by Masaki [5]. In his study, southern individuals of *T. emma* developed slowly and the developmental period was longer than that in northern individuals. If the southern individuals migrated to the north, their low growth rate would make them develop so slowly that they might remain at nymphal stage when winter came. On the other hand, if northern individuals migrated to the south, they might develop too fast that they would lay their eggs in early fall and eggs would hatch in wrong season. Our result is consistent with the above interpretation. Nymphal developmental days decreased with the increase of latitude under both photoperiods because limit warm season in north forces them to develop fast. Egg diapause populations completely follow Masaki's model.

V. micado ancient probably formed in tropical areas. They produced generations continually. When they colonized northern area, low temperature in winter became the biggest challenge. They should enter diapause to overwinter. Vietnam population developed slightly slowly under LD 12:12, which suggested the incipient of nymphal overwintering adaptation. This retardation increased with latitude reflected longer time of winter season. The developmental rates of Taiwan and S. China populations were fast and synchronous under LD 16:8, but were slow under LD 12:12. This result indicated several generations might occur before winter when enough heat units given. This diapause strategy does not fit in Osaka because warm season is not long enough to finish two generations. The Osaka population has to enter diapause at nymphal stage strictly and become univoltine. This is probably the north border of the nymphal diapause group. In egg diapause group, all populations are univoltine, which reflected their better adaptation in north area. This egg diapause strategy can be found in a wide rang of North China and Japan (though we did not have the data of Japanese *V. micado* overwintering at egg stage). Although Masaki suggested speciation occurred in south Japan, there is another possibility that the speciation might happen in south China. The egg diapause group could migrate from Korea to Japan. They met nymphal diapause group in Japan, which colonized from Taiwan and Ryukyu Islands. Because both of them should strictly enter diapause at either egg or nymphal stage, they hardly have chance to mate. If it happened, individuals with irregular life cycle will be rejected by natural selection. We consider that the speciation of *V. micado* with different life cycles were separated by sea between mainland China and Taiwan-Japan Islands, which belongs to parapatric speciation. *T. emma* and *T. occipitalis* as other examples of climatic speciation reported by Masaki also distributed in China. Further study of cricket life cycle in the area between south and middle China will help us understand the transition from nymphal diapause to egg diapause better.

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Chapter 4 Morphology differences in genus *Velarifictorus*

Abstract

The same species may exhibit distinct morphological differences due to different life cycle. Even having the same life cycle, the same species may express large differences in morphological traits, size and shape. In the genus *Velarifictorus*, *V. micado* has a wide range of distribution from tropic zones to temperature zones. Here I analyzed several body parts and compared them with each other. First, the ratio of ovipositor/hind femur length depicted the differences between two groups of *V. micado* that have different overwinter stages. The *V. micado* that overwinters at egg stage has high value of OV/FM, while those that overwinters at nymphal stage has low value. All *Velarifictorus* spp. have two wing forms, macropters and brachypters, except *V. flavifrons*. The macropterous percent is constant in *V. aspersus*, which is not affected by different photoperiods. *V. ornatus* individuals are all brachypters under LD 12:12 and many develop macropters under LD 16:8, which shows an strong environmental effect. When *V. micado* reared under LD 12:12, few individuals developed longwing, while the macropterous percent rapid increased under LD 16:8. But there were huge differences of the percent from different areas, which showed the genetic factors. Macropterous individuals have wider pronotum, which consisted of strong muscle for flying. Dimorphism also exists in forewing length. Macropterous individuals have longer forewings in all species, except male *V. aspersus*.

4.1 Introduction

Most of the cricket species are living in moist tropic regions and fewer to poles (Walker & Masaki, 1989). As discussed in Chapter 3, species with wide distribution sometimes show different life cycle patterns and some differences in relative size of body parts. Female crickets lay eggs in soil or stem of plants. If a cricket overwinters at egg stage, it may have longer ovipositor than than with nymphal overwintering. In Japan, three *Teleogryllus* species also give a good example of this adaptation. The most common *Teleogryllus* species in Japan is *T. emma* (Ohmachi & Matsuura, 1951). It is distributed in the Honshu Island, the Shikoku Island and the Kyushu Island. It is difficult for them to adapt to the cold climate in north Hokkaido and they do not inhabit in the Ryukyu Islands (Masaki & Ohmachi, 1967). The distribution of *T. infernalis* (Saussure, 1877) includes the Hokkaido Island and the Northern Honshu Island. *T. occipitalis* (Serville, 1838) is distributed in the Shikoku Island, Kyushu Island and Ryukyu Islands. *T. emma* crickets are univoltine and enter diapause at egg stage. Although *T. occipitalis* in the Shikoku Island is univoltine, they enter diapause by nymph stage and emerge the next summer. Laid eggs immediately and eggs hatch in two or three weeks. Nymphs will enter diapause until next year. The ovipositor length of *T. occipital* is the shortest because their eggs develop in warm days and there is no need for females to lay eggs in deeper. *T. emma* females have longer ovipositors compared with *T. occipital*, because their egg surfer the cold winter in Japan. *T. infernalis* exist in the northern and their ovipositors are longest (Masaki, 1961, Masaki & Walker, 1987).

Dimorphism can be found in a wide range of insects (Crnokrak & Roff, 1998). In species with wing-dimorphism, one morph with long hindwings has functional flight muscles and can fly, while the other morph with reduced hindwings can not fly. Wing-dimorphic insects are good study subjects for researching the evolution of dispersal since the nondispersing brachypterous morph is easily recognized (Roff, 1986). It has evolved independently in most of the major orders of insects (Harrison, 1980, Roff, 1994). Among crickets, some species only have short hindwing, such as bark crickets (*Duolandrevus* spp.) and scale crickets (*Ornebius* spp.). Others always have long hindwing, such as *Teleogryllus* spp. and *Xenogryllus* spp. While *Teleogryllus* spp. usually fly to light, *Xenogryllus* spp. never do that. For some species, such as *Gryllodes sigillatus*, *Modicogryllus consobrinus*, *Pteronemobius* spp., *Dianemobius* spp., *Polionemobius* spp., *Svistella* spp., *Natula* spp. and *Homoeoxipha* spp., they have two wing forms and all these species fly to light, if they have long hindwing. Otherwise they do not need to become long wing, since they allocated energy for developing wings. Individuals with long hindwing can fly, which provides them the advantage of migration (Roff, 1990). Macropterous females reproduce earlier and have higher fecundity (Roff 1986; Roff and Fairbairn 1991; Tanaka 1993). The wing type could be affected by genetic and environmental factors (Tanaka, 1993).

4.2 Methods and materials

4.2.1 Taxon sampling

The information of crickets for analyzing morphology is listed in Table 4.1 They were totally 36 individuals of 4 species.

Table 4.1 Taxon information

Species	Locality	Latitude and longitude
V. micado	Osaka, Japan	34.69N 135.50E
V. micado	Wulai, Taiwan	23.97N 120.97E
V. micado	Babe, Vietnam	22.40N 105.61E
V. micado	Daegu, Korea	35.97N 128.60E
V. micado	Newnan, GA,USA	33.38N 84.80W
V. micado	Tianmushan, Zhejiang, China	30.32N 119.43E
V. micado	Changchun, Jilin, China	43.90N 125.33E
V. micado	Sayo，Hyogo，Japan	35.03N 134.36E
V. micado	Nashville, TN, USA	36.17N 86.78W
V. micado	Shenyang, Liaoning, China	41.81N 123.43E
V. micado	Chengdu, Sichuan, China	30.66N 104.06E
V. micado	Shenzhen, Guangdong, China	22.57 N 114.05 E
V. flavifrons	Babe National Park, Vietnam	22.40N 105.61E
V. ornatus	Sayo，Hyogo，Japan	35.03N 134.36E
V. ornatus	Aridagawa, Wakayama, Japan	34.08N 135.12E
V. aspersus	Babe National Park, Vietnam	22.40N 105.61E
V. aspersus	Tianmushan, Zhejiang, China	30.32N 119.43E

4.2.2 Rearing condition

See Chapter 3.

4.2.3 Data record

Adults were removed from the culture and kept in a small plastic cup separately without food and dried at 60 ℃ overnight after they died. Vernier scale was used for measuring the maximum width of head, maximum width of pronotum, the length of fore- and hind-wings and hind femur and ovipositor. Forewing was measured by the right tegmen from the middle of the pronotum posterior to the apical of tegmen. Head width was measured from the base behind pronotum to the apical end of wing. All data was measure in 1/100 mm.

4.3 Results

Correlation of photoperiod (PHT), sex (SEX), wing type (WNG), head width (HD), pronotum width (PR), hind femur length (FM), ovipositor length (OV), forewing length (FW), hindwing length (HW) and development days (DAY) were compared with each other.

Each pat and body size showed an isometric relationship except hindwings. The forewing length was uniform in varied body size. Probably the song produced by forewing would be considerately affected by the shape and size of forewing. The hindwing was either very long or short, except that *V. flavifrons* had only brachypterous adults.

Ralationships

female $y = 0.7488x + 136.48$; male $y = 0.8531x + 74.111$. (Fig. 4.3.1)
female $y = 2.1134x + 247.53$; male $y = 1.9589x + 195.11$. (Fig. 4.3.2)
female $y = y = 1.0698x + 607.39$. (Fig. 4.3.3)
female $y = 1.1712x + 261.64$; male $y = y = 0.2801x + 725.63$. (Fig. 4.3.4)
female $y = 1.255x - 116.97$; male $y = 0.0152x + 377.73$. (Fig. 4.3.5)
female $y = 1.1944x + 574.86$; male $y = 1.1437x + 518.75$. (Fig. 4.3.6)
female $y = 0.3588x + 883.54$. (Fig. 4.3.7)
female $y = 1.2164x + 136.33$; male $y = 0.7421x + 504.12$. (Fig. 4.3.8)
female $y = 1.3739x - 206.63$; male $y = 0.4764x + 179.79$. (Fig. 4.3.9)
female $y = 0.5296x + 460.09$. (Fig. 4.3.10)
female $y = 0.6086x - 4.9372$; male $y = 0.3133x + 506.19$. (Fig. 4.3.11)
female $y = 0.4693x - 129.83$; male $y = 0.1637x + 218.85$. (Fig. 4.3.12)
female $y = 0.4019x + 243.72$. (Fig. 4.3.13)
female $y = 0.5073x - 144.55$. (Fig. 4.3.14)
female $y = 0.685x - 69.611$; male $y = 0.6957x - 188.06$. (Fig. 4.3.15)

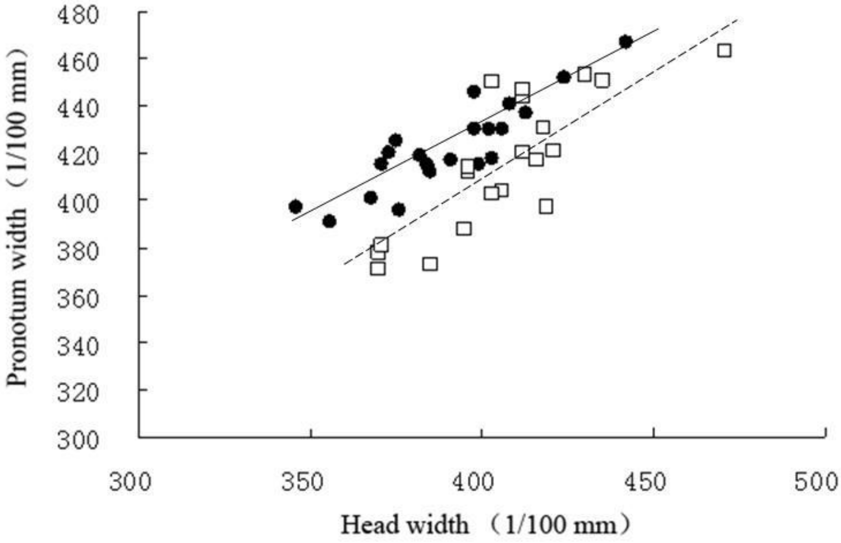


Figure 4.3.1 The width of pronotum relative to the width of head in *V. micado* from Shenzhen under LD 12:12. Open squares (dotted line), male; closed circles (black line), female.

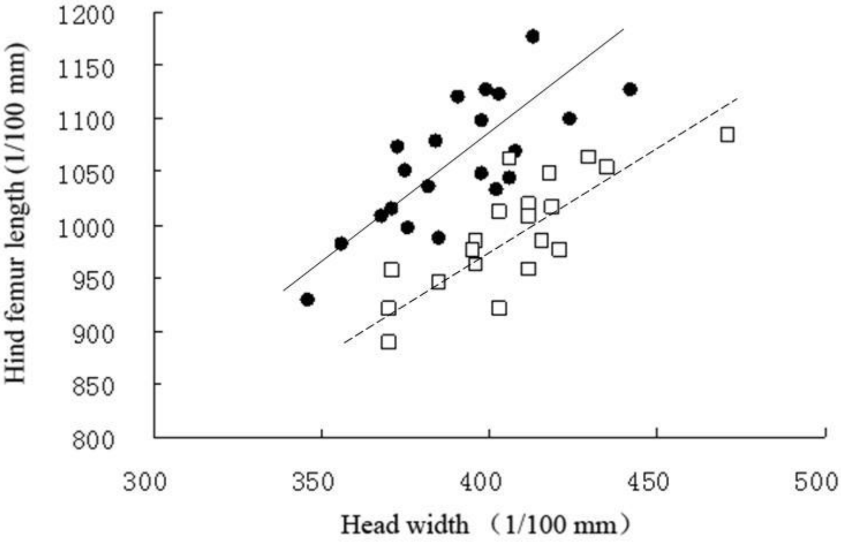


Figure 4.3.2 The width of hind femur relative to the length of head in *V. micado* from Shenzhen under LD 12:12. Open squares (dotted line), male; closed circles (black line), female.

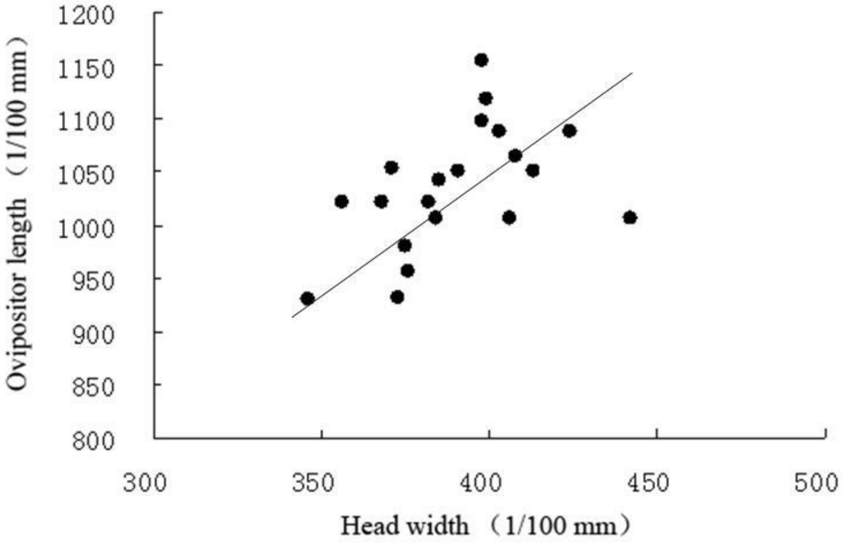


Figure 4.3.3 The length of ovipositor relative to the width of head in *V. micado* from Shenzhen under LD 12:12. Open squares (dotted line), male; closed circles (black line), female.

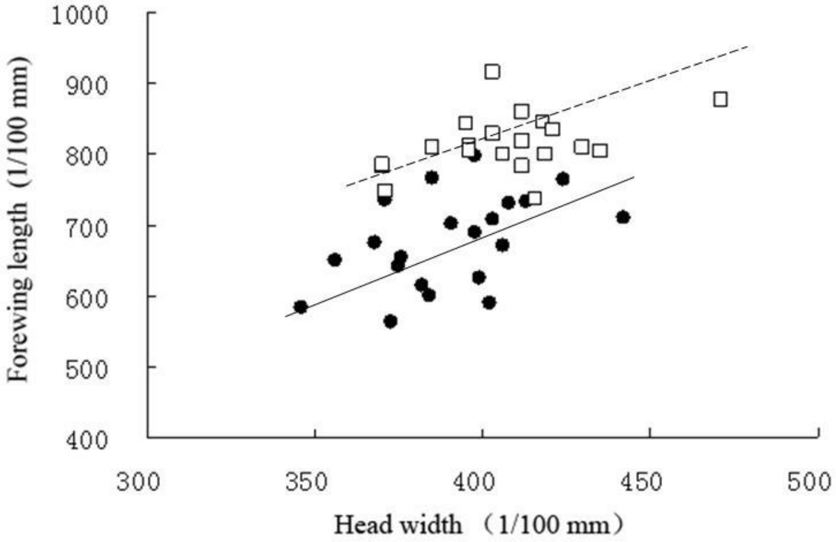


Figure 4.3.4 The length of forewing relative to the width of head in *V. micado* from Shenzhen under LD 12:12. Open squares (dotted line), male; closed circles (black line), female.

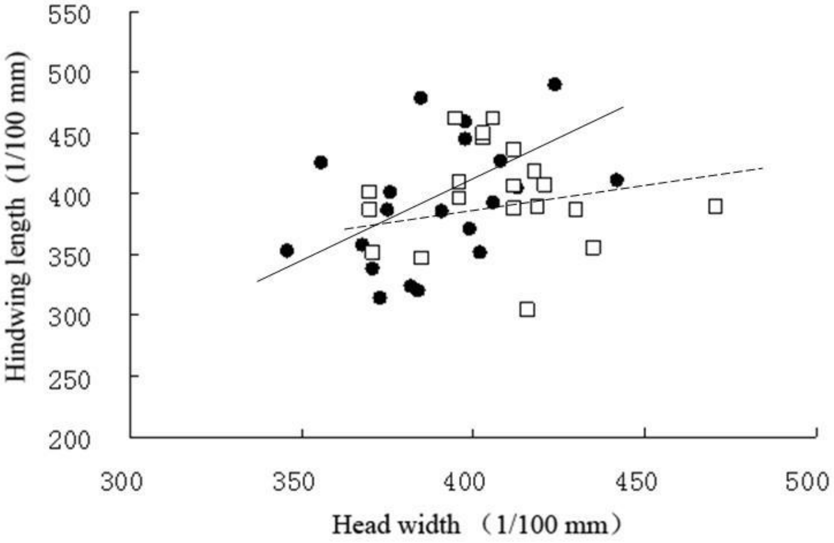


Figure 4.3.5 The length of hindwing relative to the width of head in *V. micado* from Shenzhen under LD 12:12. Open squares (dotted line), male; closed circles (black line), female.

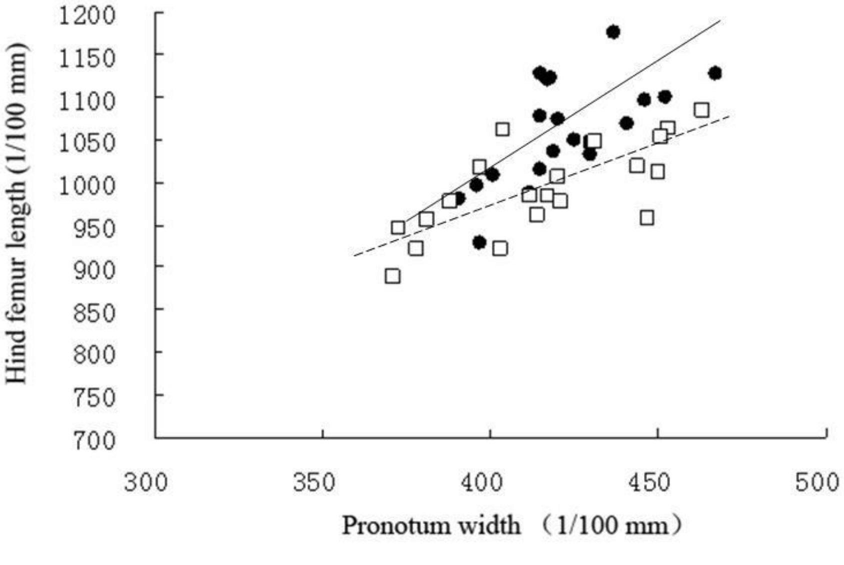


Figure 4.3.6 The length of hind femur relative to the width of pronotum in *V. micado* from Shenzhen under LD 12:12. Open squares (dotted line), male; closed circles (black line), female.

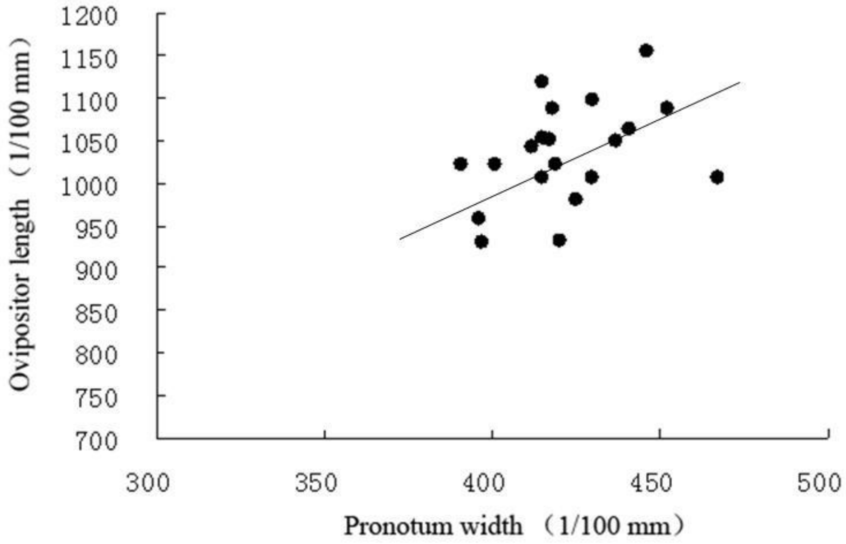


Figure 4.3.7 The length of ovipositor relative to the width of pronotum in *V. micado* from Shenzhen under LD 12:12. Open squares (dotted line), male; closed circles (black line), female.

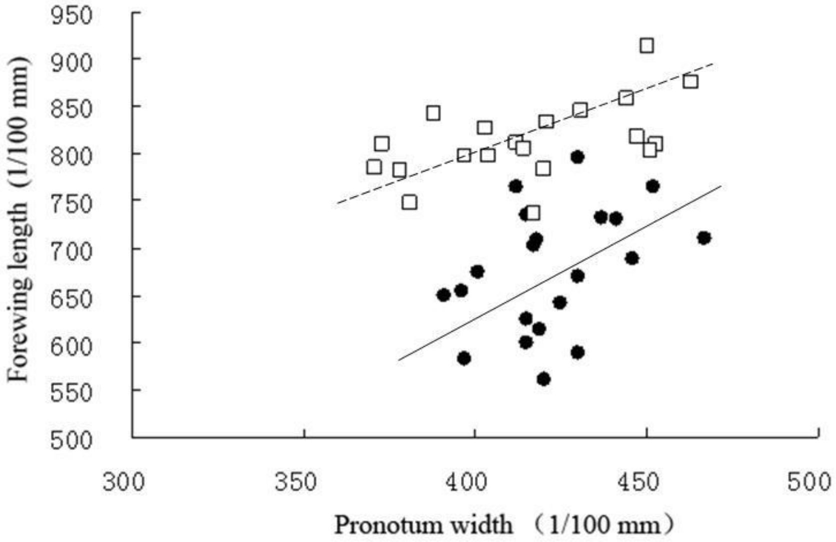


Figure 4.3.8 The length of forewing relative to the width of pronotum in *V. micado* from Shenzhen under LD 12:12. Open squares (dotted line), male; closed circles (black line), female.

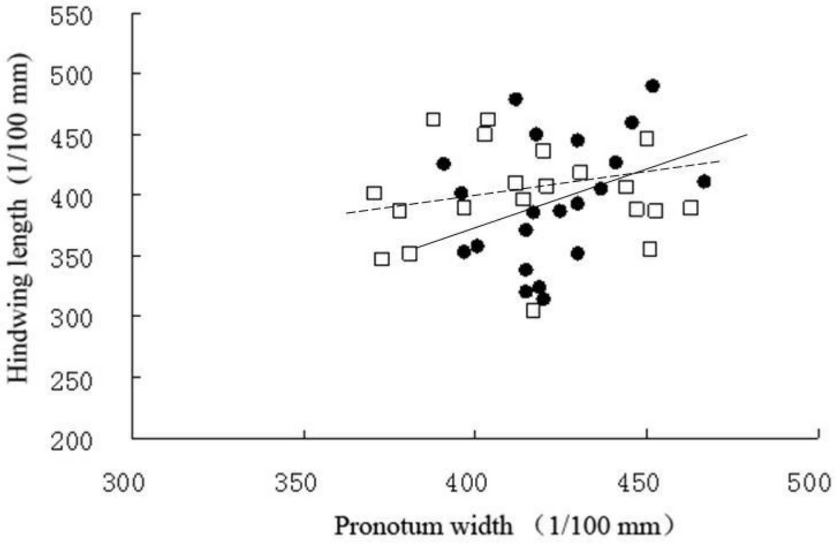


Figure 4.3.9 The length of hindwing relative to the width of pronotum in *V. micado* from Shenzhen under LD 12:12. Open squares (dotted line), male; closed circles (black line), female.

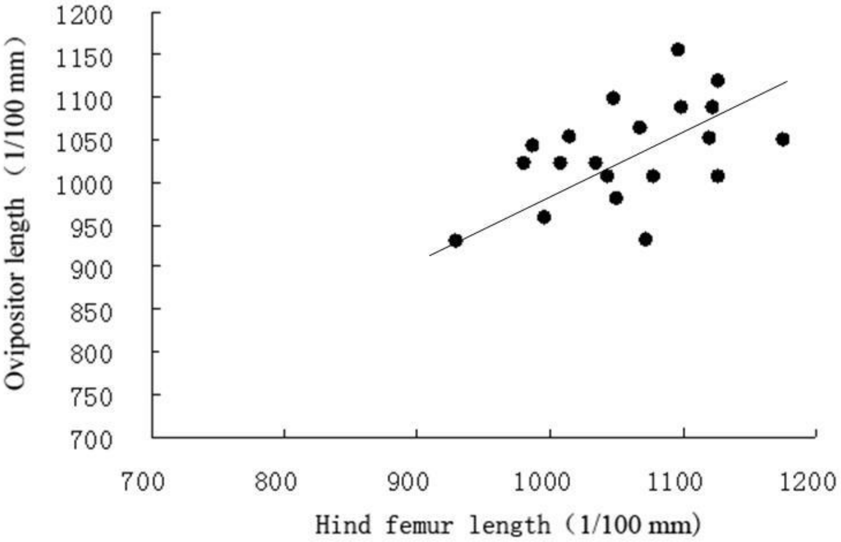


Figure 4.3.10 The length of ovipositor relative to the length of hind femur in *V. micado* from Shenzhen under LD 12:12. Open squares (dotted line), male; closed circles (black line), female.

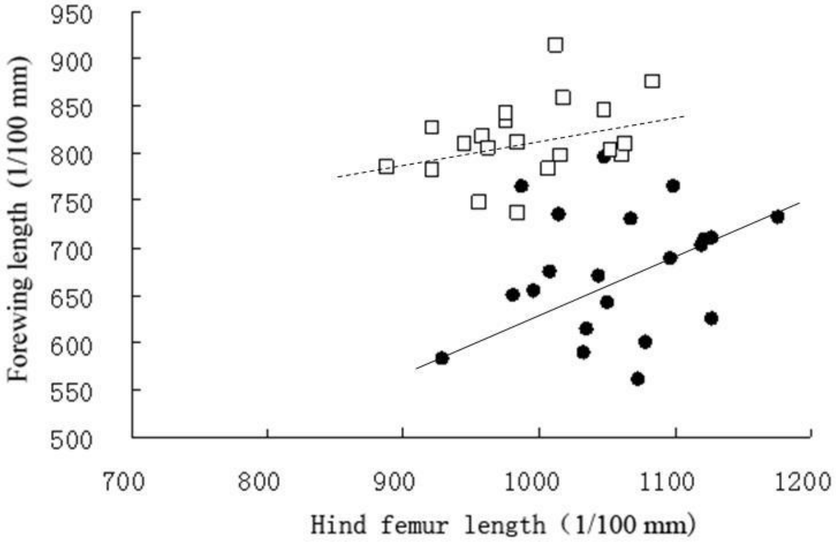


Figure 4.3.11 The length of forewing relative to the length of hind femur in *V. micado* from Shenzhen under LD 12:12. Open squares (dotted line), male; closed circles (black line), female.

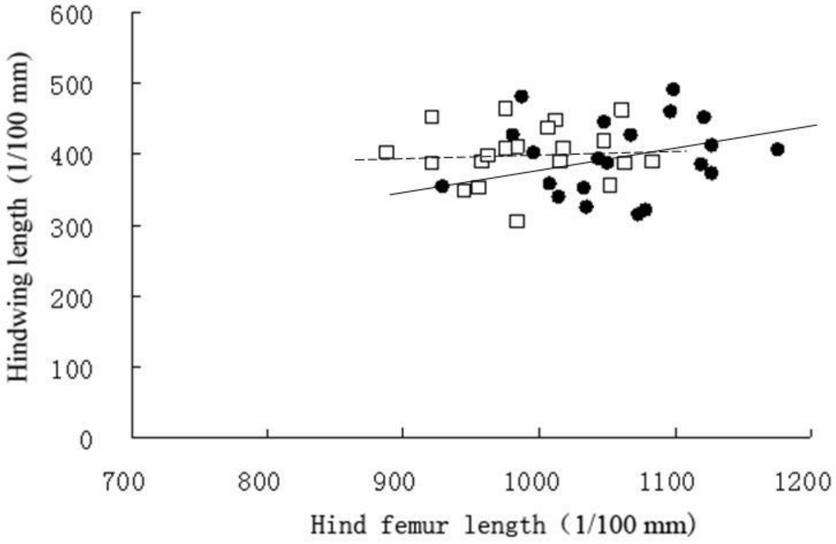


Figure 4.3.12 The length of hindwing relative to the length of hind femur in *V. micado* from Shenzhen under LD 12:12. Open squares (dotted line), male; closed circles (black line), female.

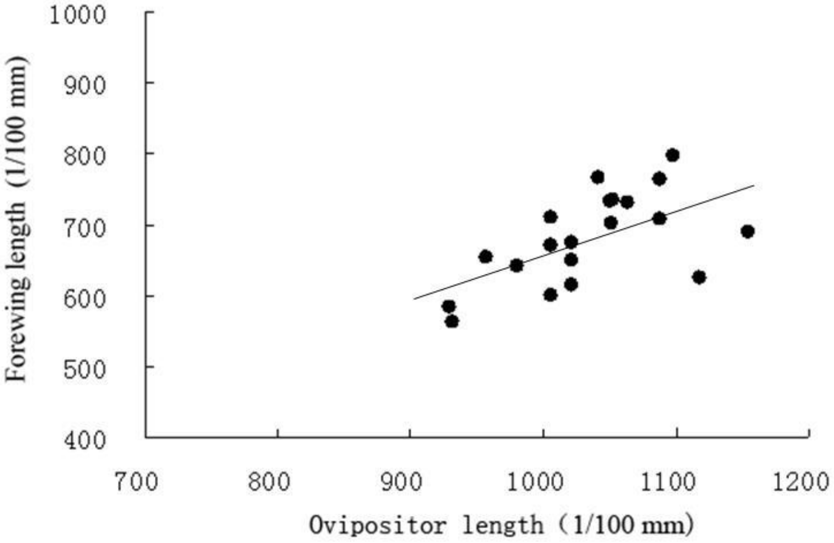


Figure 4.3.13 The length of forewing relative to the length of ovipositor in *V. micado* from Shenzhen under LD 12:12. Open squares (dotted line), male; closed circles (black line), female.

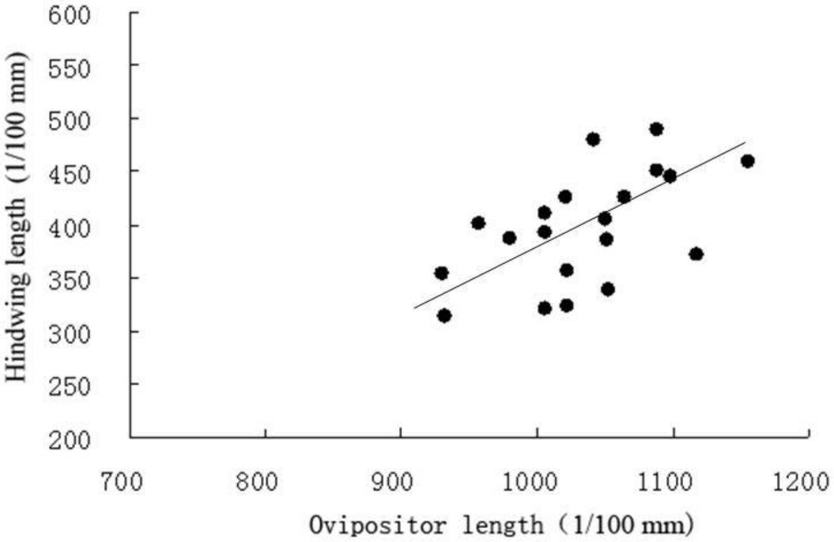


Figure 4.3.14 The length of hindwing relative to the length of ovipositor in *V. micado* from Shenzhen under LD 12:12. Open squares (dotted line), male; closed circles (black line), female.

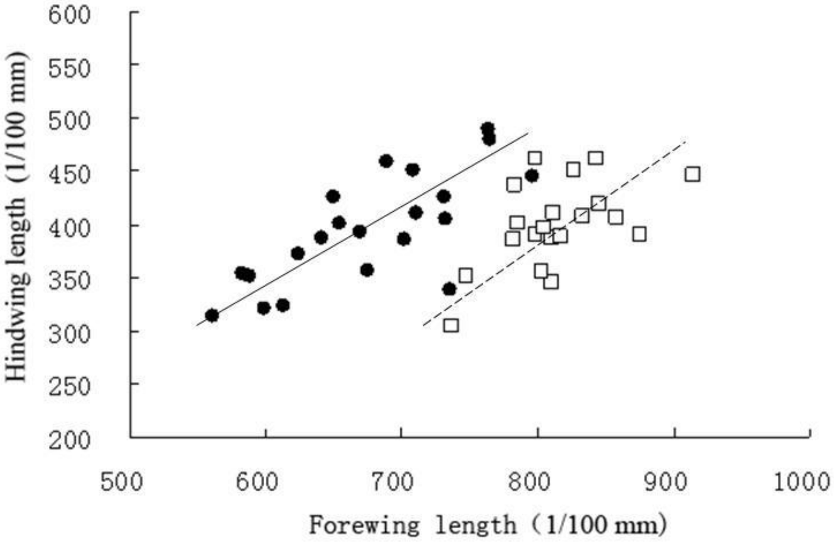


Figure 4.3.15 The length of hindwing relative to the length of forewing in *V. micado* from Shenzhen under LD 12:12. Open squares (dotted line), male; closed circles (black line), female.

In *V. micado* group (overwinter at nymph stage, Table 4.3.1-4), development days were proportion to body size. Males had bigger head (wider) than females did. Males also had longer forewing than females did. In *V. micado* group (overwinter at egg stage, Table 4.3.5-12), development days was proportion to photoperiod. Development days were proportion to body size. Males had bigger head (wider) than females did. Males also had longer forewing than females did. In *V. ornatus* group (Table 4.3.13-14), development days was proportion to photoperiod. Development days were proportion to body size. Males had bigger head (wider) than females did. Males also had longer forewing than females did. In *V. flavifrons* group (Table 4.3.15), photoperiod days had no direct effect on development. Development days were proportion to body size. Males had bigger head (wider) than females did. Males also had longer forewing than females did. In *V. aspersus* group (Table 4.3.16-17), development days was proportion to photoperiod. Development days were proportion to body size. Males had bigger head (wider) than females did. Males also had longer forewing than females did.

Table 4.3.1 Correlation of photoperiod (PHT), sex (SEX), wing type (WNG), head width (HD), pronotum width (PR), hind femur length (FM), ovipositor length (OV), forewing length (FW), hindwing length (HW) and developmental period in days (DAY) of *V. micado* from Vietnam.

Correlations

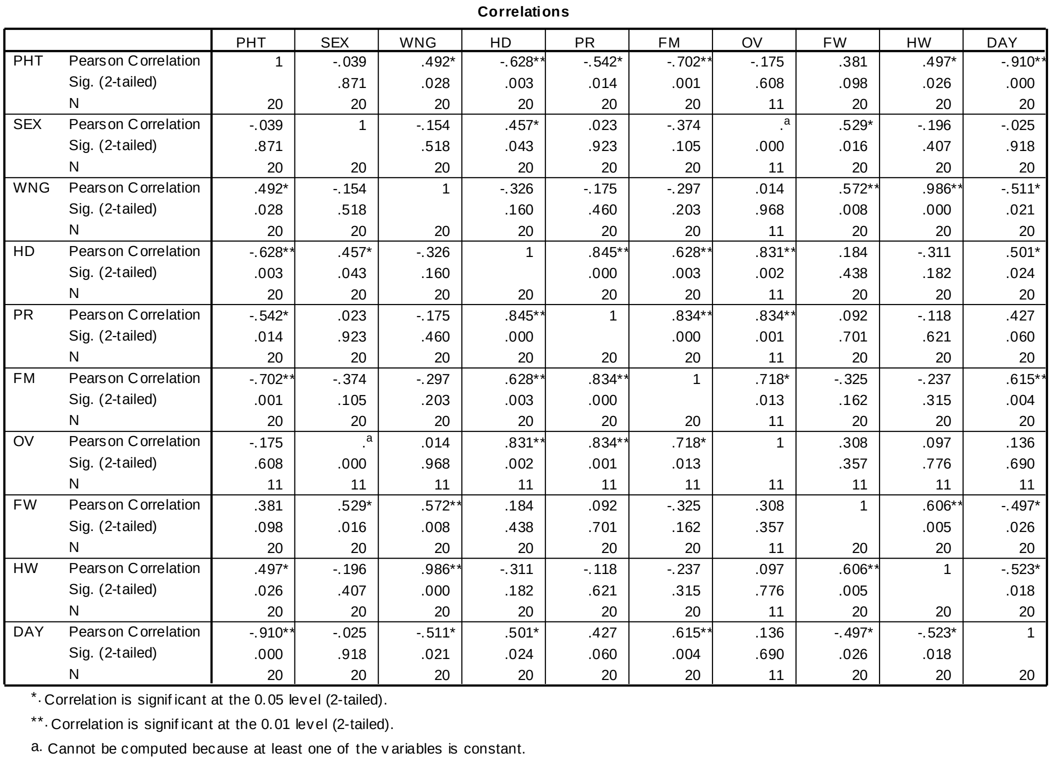
		PHT	SEX	WNG	HD	PR	FM	OV	FW	HW	DAY
PHT	Pearson Correlation	1	-.063	.255*	-.217	-.032	-.082	-.152	.042	.252*	-.324**
	Sig. (2-tailed)		.596	.029	.065	.788	.485	.319	.725	.032	.005
	N	74	74	74	73	74	74	45	74	73	74
SEX	Pearson Correlation	-.063	1	.066	.493**	.035	-.517**	.[a]	.692**	.155	-.246*
	Sig. (2-tailed)	.596		.577	.000	.770	.000	.000	.000	.191	.034
	N	74	74	74	73	74	74	45	74	73	74
WNG	Pearson Correlation	.255*	.066	1	.038	.267*	.008	.179	.504**	.952**	-.219
	Sig. (2-tailed)	.029	.577		.748	.022	.943	.239	.000	.000	.060
	N	74	74	74	73	74	74	45	74	73	74
HD	Pearson Correlation	-.217	.493**	.038	1	.667**	.315**	.608**	.582**	.104	.159
	Sig. (2-tailed)	.065	.000	.748		.000	.007	.000	.000	.386	.178
	N	73	73	73	73	73	73	44	73	72	73
PR	Pearson Correlation	-.032	.035	.267*	.667**	1	.603**	.632**	.397**	.322**	.090
	Sig. (2-tailed)	.788	.770	.022	.000		.000	.000	.000	.005	.445
	N	74	74	74	73	74	74	45	74	73	74
FM	Pearson Correlation	-.082	-.517**	.008	.315**	.603**	1	.526**	-.122	.021	.336**
	Sig. (2-tailed)	.485	.000	.943	.007	.000		.000	.299	.862	.003
	N	74	74	74	73	74	74	45	74	73	74
OV	Pearson Correlation	-.152	.[a]	.179	.608**	.632**	.526**	1	.383**	.226	.111
	Sig. (2-tailed)	.319	.000	.239	.000	.000	.000		.009	.140	.466
	N	45	45	45	44	45	45	45	45	44	45
FW	Pearson Correlation	.042	.692**	.504**	.582**	.397**	-.122	.383**	1	.566**	-.365**
	Sig. (2-tailed)	.725	.000	.000	.000	.000	.299	.009		.000	.001
	N	74	74	74	73	74	74	45	74	73	74
HW	Pearson Correlation	.252*	.155	.952**	.104	.322**	.021	.226	.566**	1	-.223
	Sig. (2-tailed)	.032	.191	.000	.386	.005	.862	.140	.000		.057
	N	73	73	73	72	73	73	44	73	73	73
DAY	Pearson Correlation	-.324**	-.246*	-.219	.159	.090	.336**	.111	-.365**	-.223	1
	Sig. (2-tailed)	.005	.034	.060	.178	.445	.003	.466	.001	.057	
	N	74	74	74	73	74	74	45	74	73	74

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Table 4.3.2 Correlation of photoperiod (PHT), sex (SEX), wing type (WNG), head width (HD), pronotum width (PR), hind femur length (FM), ovipositor length (OV), forewing length (FW), hindwing length (HW), developmental period in days (DAY) of *V. micado* from Taiwan.



Correlations

		PHT	SEX	WNG	HD	PR	FM	OV	FW	HW	DAY
PHT	Pearson Correlation	1	-.039	.492*	-.628**	-.542*	-.702**	-.175	.381	.497*	-.910**
	Sig. (2-tailed)		.871	.028	.003	.014	.001	.608	.098	.026	.000
	N	20	20	20	20	20	20	11	20	20	20
SEX	Pearson Correlation	-.039	1	-.154	.457*	.023	-.374	.[a]	.529*	-.196	-.025
	Sig. (2-tailed)	.871		.518	.043	.923	.105	.000	.016	.407	.918
	N	20	20	20	20	20	20	11	20	20	20
WNG	Pearson Correlation	.492*	-.154	1	-.326	-.175	-.297	.014	.572**	.986**	-.511*
	Sig. (2-tailed)	.028	.518		.160	.460	.203	.968	.008	.000	.021
	N	20	20	20	20	20	20	11	20	20	20
HD	Pearson Correlation	-.628**	.457*	-.326	1	.845**	.628**	.831**	.184	-.311	.501*
	Sig. (2-tailed)	.003	.043	.160		.000	.003	.002	.438	.182	.024
	N	20	20	20	20	20	20	11	20	20	20
PR	Pearson Correlation	-.542*	.023	-.175	.845**	1	.834**	.834**	.092	-.118	.427
	Sig. (2-tailed)	.014	.923	.460	.000		.000	.001	.701	.621	.060
	N	20	20	20	20	20	20	11	20	20	20
FM	Pearson Correlation	-.702**	-.374	-.297	.628**	.834**	1	.718*	-.325	-.237	.615**
	Sig. (2-tailed)	.001	.105	.203	.003	.000		.013	.162	.315	.004
	N	20	20	20	20	20	20	11	20	20	20
OV	Pearson Correlation	-.175	.[a]	.014	.831**	.834**	.718*	1	.308	.097	.136
	Sig. (2-tailed)	.608	.000	.968	.002	.001	.013		.357	.776	.690
	N	11	11	11	11	11	11	11	11	11	11
FW	Pearson Correlation	.381	.529*	.572**	.184	.092	-.325	.308	1	.606**	-.497*
	Sig. (2-tailed)	.098	.016	.008	.438	.701	.162	.357		.005	.026
	N	20	20	20	20	20	20	11	20	20	20
HW	Pearson Correlation	.497*	-.196	.986**	-.311	-.118	-.237	.097	.606**	1	-.523*
	Sig. (2-tailed)	.026	.407	.000	.182	.621	.315	.776	.005		.018
	N	20	20	20	20	20	20	11	20	20	20
DAY	Pearson Correlation	-.910**	-.025	-.511*	.501*	.427	.615**	.136	-.497*	-.523*	1
	Sig. (2-tailed)	.000	.918	.021	.024	.060	.004	.690	.026	.018	
	N	20	20	20	20	20	20	11	20	20	20

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Table 4.3.3 Correlation of photoperiod (PHT), sex (SEX), wing type (WNG), head width (HD), pronotum width (PR), hind femur length (FM), ovipositor length (OV), forewing length (FW), hindwing length (HW), developmental period in days (DAY) of *V. micado* from Osaka.

Correlations

		PHT	SEX	WNG	HD	PR	FM	OV	FW	HW	DAY
PHT	Pearson Correlation	1	-.038	.236	.038	-.069	-.050	-.431*	.016	.291*	-.222
	Sig. (2-tailed)		.797	.106	.798	.642	.738	.032	.915	.045	.130
	N	48	48	48	47	48	48	25	48	48	48
SEX	Pearson Correlation	-.038	1	-.075	.358*	.139	-.389**	.[a]	.752**	-.023	.090
	Sig. (2-tailed)	.797		.611	.013	.346	.006	.000	.000	.876	.543
	N	48	48	48	47	48	48	25	48	48	48
WNG	Pearson Correlation	.236	-.075	1	-.151	.029	.003	-.124	.216	.986**	-.108
	Sig. (2-tailed)	.106	.611		.311	.847	.982	.555	.140	.000	.464
	N	48	48	48	47	48	48	25	48	48	48
HD	Pearson Correlation	.038	.358*	-.151	1	.507**	.575**	.533**	.359*	-.091	.521**
	Sig. (2-tailed)	.798	.013	.311		.000	.000	.006	.013	.541	.000
	N	47	47	47	47	47	47	25	47	47	47
PR	Pearson Correlation	-.069	.139	.029	.507**	1	.328*	.210	.073	.045	.357*
	Sig. (2-tailed)	.642	.346	.847	.000		.023	.314	.623	.763	.013
	N	48	48	48	47	48	48	25	48	48	48
FM	Pearson Correlation	-.050	-.389**	.003	.575**	.328*	1	.716**	-.154	.020	.428**
	Sig. (2-tailed)	.738	.006	.982	.000	.023		.000	.295	.894	.002
	N	48	48	48	47	48	48	25	48	48	48
OV	Pearson Correlation	-.431*	.[a]	-.124	.533**	.210	.716**	1	.175	-.096	.461*
	Sig. (2-tailed)	.032	.000	.555	.006	.314	.000		.404	.649	.020
	N	25	25	25	25	25	25	25	25	25	25
FW	Pearson Correlation	.016	.752**	.216	.359*	.073	-.154	.175	1	.271	.002
	Sig. (2-tailed)	.915	.000	.140	.013	.623	.295	.404		.063	.990
	N	48	48	48	47	48	48	25	48	48	48
HW	Pearson Correlation	.291*	-.023	.986**	-.091	.045	.020	-.096	.271	1	-.112
	Sig. (2-tailed)	.045	.876	.000	.541	.763	.894	.649	.063		.448
	N	48	48	48	47	48	48	25	48	48	48
DAY	Pearson Correlation	-.222	.090	-.108	.521**	.357*	.428**	.461*	.002	-.112	1
	Sig. (2-tailed)	.130	.543	.464	.000	.013	.002	.020	.990	.448	
	N	48	48	48	47	48	48	25	48	48	48

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Table 4.3.4 Correlation of photoperiod (PHT), sex (SEX), wing type (WNG), head width (HD), pronotum width (PR), hind femur length (FM), ovipositor length (OV), forewing length (FW), hindwing length (HW), developmental period in days (DAY) of *V. micado* from Shenzhen.

Correlations

		PHT	SEX	WNG	HD	PR	FM	OV	FW	HW	DAY
PHT	Pearson Correlation	1	.069	.326**	-.216*	-.189	-.121	-.014	.428**	.386**	-.700**
	Sig. (2-tailed)		.533	.003	.049	.087	.278	.933	.000	.000	.000
	N	83	83	83	83	83	83	40	83	82	83
SEX	Pearson Correlation	.069	1	.037	.439**	-.108	-.134	.[a]	.392**	.011	-.207
	Sig. (2-tailed)	.533		.738	.000	.331	.227	.000	.000	.925	.060
	N	83	83	83	83	83	83	40	83	82	83
WNG	Pearson Correlation	.326**	.037	1	-.072	.158	-.122	-.231	.716**	.991**	-.330**
	Sig. (2-tailed)	.003	.738		.517	.155	.271	.151	.000	.000	.002
	N	83	83	83	83	83	83	40	83	82	83
HD	Pearson Correlation	-.216*	.439**	-.072	1	.647**	-.037	.414**	.215	-.078	.305**
	Sig. (2-tailed)	.049	.000	.517		.000	.742	.008	.051	.484	.005
	N	83	83	83	83	83	83	40	83	82	83
PR	Pearson Correlation	-.189	-.108	.158	.647**	1	-.008	.184	.191	.165	.338**
	Sig. (2-tailed)	.087	.331	.155	.000		.940	.256	.084	.138	.002
	N	83	83	83	83	83	83	40	83	82	83
FM	Pearson Correlation	-.121	-.134	-.122	-.037	-.008	1	-.042	-.246*	-.131	.160
	Sig. (2-tailed)	.278	.227	.271	.742	.940		.795	.025	.241	.149
	N	83	83	83	83	83	83	40	83	82	83
OV	Pearson Correlation	-.014	.[a]	-.231	.414**	.184	-.042	1	.031	-.186	-.091
	Sig. (2-tailed)	.933	.000	.151	.008	.256	.795		.851	.252	.579
	N	40	40	40	40	40	40	40	40	40	40
FW	Pearson Correlation	.428**	.392**	.716**	.215	.191	-.246*	.031	1	.765**	-.509**
	Sig. (2-tailed)	.000	.000	.000	.051	.084	.025	.851		.000	.000
	N	83	83	83	83	83	83	40	83	82	83
HW	Pearson Correlation	.386**	.011	.991**	-.078	.165	-.131	-.186	.765**	1	-.375**
	Sig. (2-tailed)	.000	.925	.000	.484	.138	.241	.252	.000		.001
	N	82	82	82	82	82	82	40	82	82	82
DAY	Pearson Correlation	-.700**	-.207	-.330**	.305**	.338**	.160	-.091	-.509**	-.375**	1
	Sig. (2-tailed)	.000	.060	.002	.005	.002	.149	.579	.000	.001	
	N	83	83	83	83	83	83	40	83	82	83

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

[a] Cannot be computed because at least one of the variables is constant.

Table 4.3.5 Correlation of photoperiod (PHT), sex (SEX), wing type (WNG), head width (HD), pronotum width (PR), hind femur length (FM), ovipositor length (OV), forewing length (FW), hindwing length (HW), developmental period in days (DAY) of *V. micado* from Tianmu.

Correlations

		PHT	SEX	WNG	HD	PR	FM	OV	FW	HW	DAY
PHT	Pearson Correlation	1	-.129	.083	.423*	.507**	.514**	.543*	.152	.097	.321
	Sig. (2-tailed)		.475	.646	.014	.003	.002	.016	.398	.592	.068
	N	33	33	33	33	33	33	19	33	33	33
SEX	Pearson Correlation	-.129	1	.155	.209	-.100	-.284	.[a]	.736**	.195	-.062
	Sig. (2-tailed)	.475		.389	.243	.581	.109	.000	.000	.277	.730
	N	33	33	33	33	33	33	19	33	33	33
WNG	Pearson Correlation	.083	.155	1	.130	.132	.138	.233	.503**	.993**	-.024
	Sig. (2-tailed)	.646	.389		.472	.466	.444	.336	.003	.000	.892
	N	33	33	33	33	33	33	19	33	33	33
HD	Pearson Correlation	.423*	.209	.130	1	.911**	.806**	.841**	.617**	.202	.274
	Sig. (2-tailed)	.014	.243	.472		.000	.000	.000	.000	.260	.123
	N	33	33	33	33	33	33	19	33	33	33
PR	Pearson Correlation	.507**	-.100	.132	.911**	1	.912**	.866**	.432*	.196	.281
	Sig. (2-tailed)	.003	.581	.466	.000		.000	.000	.012	.275	.113
	N	33	33	33	33	33	33	19	33	33	33
FM	Pearson Correlation	.514**	-.284	.138	.806**	.912**	1	.738**	.254	.177	.247
	Sig. (2-tailed)	.002	.109	.444	.000	.000		.000	.154	.325	.166
	N	33	33	33	33	33	33	19	33	33	33
OV	Pearson Correlation	.543*	.[a]	.233	.841**	.866**	.738**	1	.875**	.357	-.110
	Sig. (2-tailed)	.016	.000	.336	.000	.000	.000		.000	.134	.653
	N	19	19	19	19	19	19	19	19	19	19
FW	Pearson Correlation	.152	.736**	.503**	.617**	.432*	.254	.875**	1	.566**	.044
	Sig. (2-tailed)	.398	.000	.003	.000	.012	.154	.000		.001	.809
	N	33	33	33	33	33	33	19	33	33	33
HW	Pearson Correlation	.097	.195	.993**	.202	.196	.177	.357	.566**	1	-.036
	Sig. (2-tailed)	.592	.277	.000	.260	.275	.325	.134	.001		.842
	N	33	33	33	33	33	33	19	33	33	33
DAY	Pearson Correlation	.321	-.062	-.024	.274	.281	.247	-.110	.044	-.036	1
	Sig. (2-tailed)	.068	.730	.892	.123	.113	.166	.653	.809	.842	
	N	33	33	33	33	33	33	19	33	33	33

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

[a] Cannot be computed because at least one of the variables is constant.

Table 4.3.6 Correlation of photoperiod (PHT), sex (SEX), wing type (WNG), head width (HD), pronotum width (PR), hind femur length (FM), ovipositor length (OV), forewing length (FW), hindwing length (HW), developmental period in days (DAY) of *V. micado* from Nashville.

Correlations

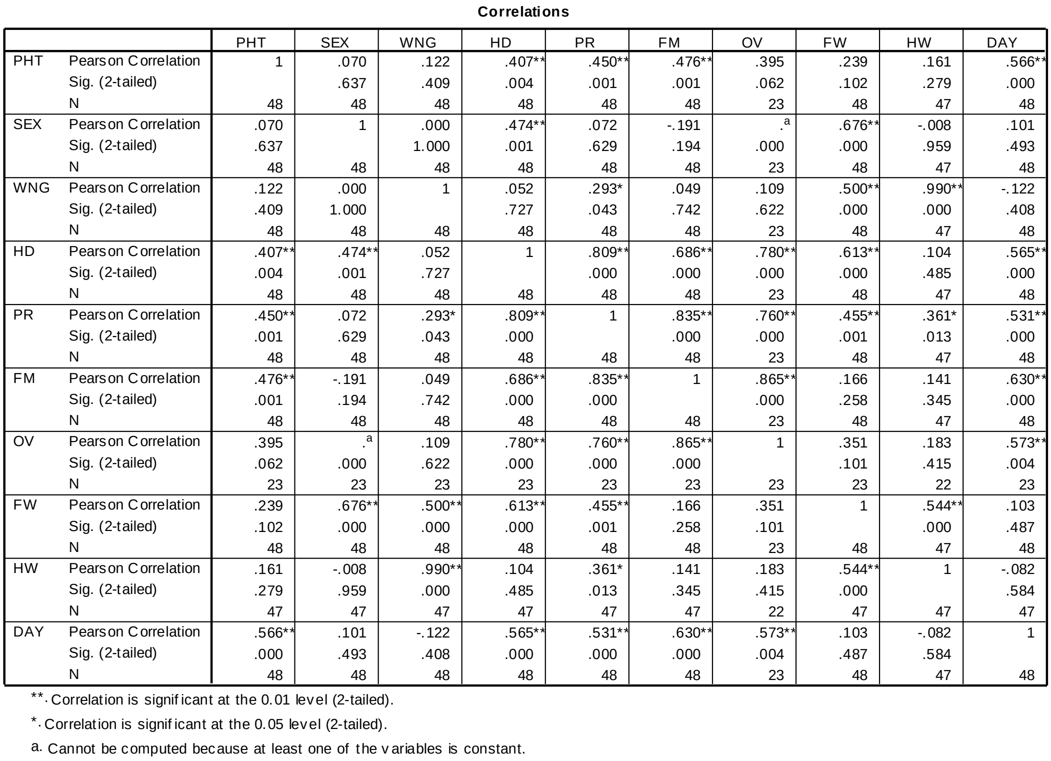
		PHT	SEX	WNG	HD	PR	FM	OV	FW	HW	DAY
PHT	Pearson Correlation	1	.182	.135	.631**	.563**	.563**	.759**	.349*	.215	.725**
	Sig. (2-tailed)		.312	.453	.000	.001	.001	.004	.050	.246	.000
	N	33	33	33	33	33	32	12	32	31	33
SEX	Pearson Correlation	.182	1	-.102	.471**	.060	-.253	.[a]	.722**	.066	.331
	Sig. (2-tailed)	.312		.571	.006	.739	.163	.000	.000	.725	.060
	N	33	33	33	33	33	32	12	32	31	33
WNG	Pearson Correlation	.135	-.102	1	-.103	.076	-.006	.070	.332	.986**	.071
	Sig. (2-tailed)	.453	.571		.567	.675	.973	.828	.064	.000	.694
	N	33	33	33	33	33	32	12	32	31	33
HD	Pearson Correlation	.631**	.471**	-.103	1	.821**	.673**	.882**	.607**	.081	.733**
	Sig. (2-tailed)	.000	.006	.567		.000	.000	.000	.000	.667	.000
	N	33	33	33	33	33	32	12	32	31	33
PR	Pearson Correlation	.563**	.060	.076	.821**	1	.865**	.859**	.354*	.207	.658**
	Sig. (2-tailed)	.001	.739	.675	.000		.000	.000	.047	.264	.000
	N	33	33	33	33	33	32	12	32	31	33
FM	Pearson Correlation	.563**	-.253	-.006	.673**	.865**	1	.891**	.122	.029	.610**
	Sig. (2-tailed)	.001	.163	.973	.000	.000		.000	.513	.878	.000
	N	32	32	32	32	32	32	12	31	30	32
OV	Pearson Correlation	.759**	.[a]	.070	.882**	.859**	.891**	1	.618*	.477	.699*
	Sig. (2-tailed)	.004	.000	.828	.000	.000	.000		.032	.138	.011
	N	12	12	12	12	12	12	12	12	11	12
FW	Pearson Correlation	.349*	.722**	.332	.607**	.354*	.122	.618*	1	.356*	.409*
	Sig. (2-tailed)	.050	.000	.064	.000	.047	.513	.032		.049	.020
	N	32	32	32	32	32	31	12	32	31	32
HW	Pearson Correlation	.215	.066	.986**	.081	.207	.029	.477	.356*	1	.191
	Sig. (2-tailed)	.246	.725	.000	.667	.264	.878	.138	.049		.302
	N	31	31	31	31	31	30	11	31	31	31
DAY	Pearson Correlation	.725**	.331	.071	.733**	.658**	.610**	.699*	.409*	.191	1
	Sig. (2-tailed)	.000	.060	.694	.000	.000	.000	.011	.020	.302	
	N	33	33	33	33	33	32	12	32	31	33

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Table 4.3.7 Correlation of photoperiod (PHT), sex (SEX), wing type (WNG), head width (HD), pronotum width (PR), hind femur length (FM), ovipositor length (OV), forewing length (FW), hindwing length (HW), developmental period in days (DAY) of *V. micado* from Newnan.



Correlations

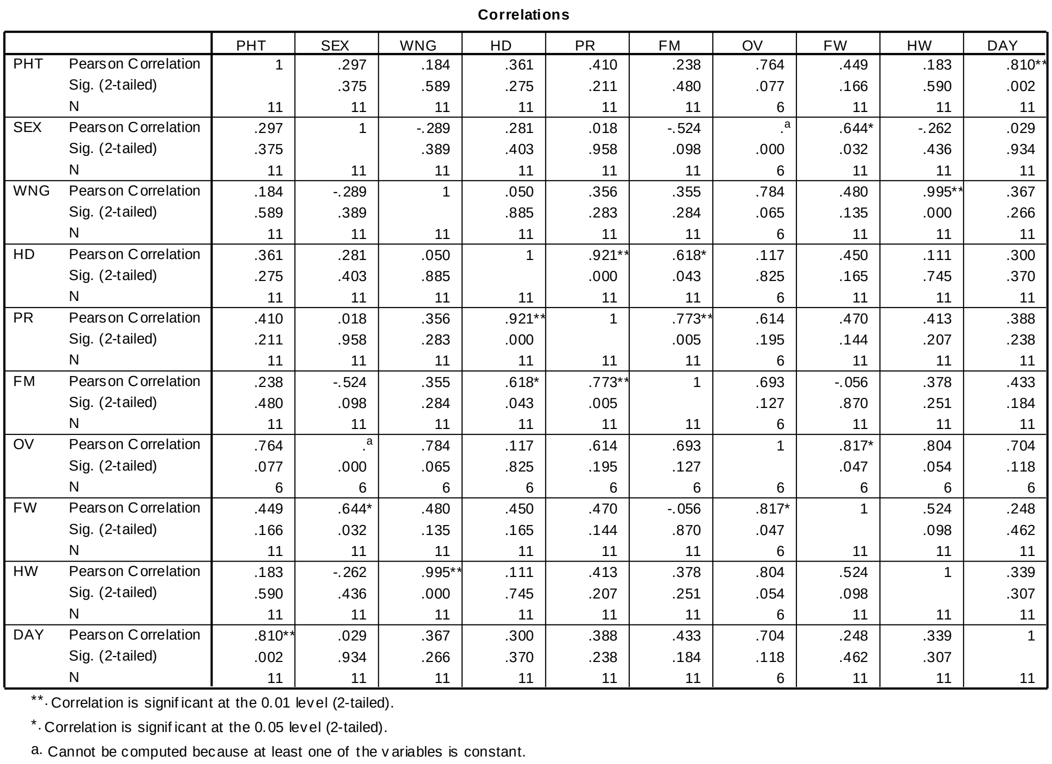
		PHT	SEX	WNG	HD	PR	FM	OV	FW	HW	DAY
PHT	Pearson Correlation	1	.070	.122	.407**	.450**	.476**	.395	.239	.161	.566**
	Sig. (2-tailed)		.637	.409	.004	.001	.001	.062	.102	.279	.000
	N	48	48	48	48	48	48	23	48	47	48
SEX	Pearson Correlation	.070	1	.000	.474**	.072	-.191	.[a]	.676**	-.008	.101
	Sig. (2-tailed)	.637		1.000	.001	.629	.194	.000	.000	.959	.493
	N	48	48	48	48	48	48	23	48	47	48
WNG	Pearson Correlation	.122	.000	1	.052	.293*	.049	.109	.500**	.990**	-.122
	Sig. (2-tailed)	.409	1.000		.727	.043	.742	.622	.000	.000	.408
	N	48	48	48	48	48	48	23	48	47	48
HD	Pearson Correlation	.407**	.474**	.052	1	.809**	.686**	.780**	.613**	.104	.565**
	Sig. (2-tailed)	.004	.001	.727		.000	.000	.000	.000	.485	.000
	N	48	48	48	48	48	48	23	48	47	48
PR	Pearson Correlation	.450**	.072	.293*	.809**	1	.835**	.760**	.455**	.361*	.531**
	Sig. (2-tailed)	.001	.629	.043	.000		.000	.000	.001	.013	.000
	N	48	48	48	48	48	48	23	48	47	48
FM	Pearson Correlation	.476**	-.191	.049	.686**	.835**	1	.865**	.166	.141	.630**
	Sig. (2-tailed)	.001	.194	.742	.000	.000		.000	.258	.345	.000
	N	48	48	48	48	48	48	23	48	47	48
OV	Pearson Correlation	.395	.[a]	.109	.780**	.760**	.865**	1	.351	.183	.573**
	Sig. (2-tailed)	.062	.000	.622	.000	.000	.000		.101	.415	.004
	N	23	23	23	23	23	23	23	23	22	23
FW	Pearson Correlation	.239	.676**	.500**	.613**	.455**	.166	.351	1	.544**	.103
	Sig. (2-tailed)	.102	.000	.000	.000	.001	.258	.101		.000	.487
	N	48	48	48	48	48	48	23	48	47	48
HW	Pearson Correlation	.161	-.008	.990**	.104	.361*	.141	.183	.544**	1	-.082
	Sig. (2-tailed)	.279	.959	.000	.485	.013	.345	.415	.000		.584
	N	47	47	47	47	47	47	22	47	47	47
DAY	Pearson Correlation	.566**	.101	-.122	.565**	.531**	.630**	.573**	.103	-.082	1
	Sig. (2-tailed)	.000	.493	.408	.000	.000	.000	.004	.487	.584	
	N	48	48	48	48	48	48	23	48	47	48

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Table 4.3.8 Correlation of photoperiod (PHT), sex (SEX), wing type (WNG), head width (HD), pronotum width (PR), hind femur length (FM), ovipositor length (OV), forewing length (FW), hindwing length (HW), developmental period in days (DAY) of *V. micado* from Chengdu.



Correlations

		PHT	SEX	WNG	HD	PR	FM	OV	FW	HW	DAY
PHT	Pearson Correlation	1	.297	.184	.361	.410	.238	.764	.449	.183	.810**
	Sig. (2-tailed)		.375	.589	.275	.211	.480	.077	.166	.590	.002
	N	11	11	11	11	11	11	6	11	11	11
SEX	Pearson Correlation	.297	1	-.289	.281	.018	-.524	.[a]	.644*	-.262	.029
	Sig. (2-tailed)	.375		.389	.403	.958	.098	.000	.032	.436	.934
	N	11	11	11	11	11	11	6	11	11	11
WNG	Pearson Correlation	.184	-.289	1	.050	.356	.355	.784	.480	.995**	.367
	Sig. (2-tailed)	.589	.389		.885	.283	.284	.065	.135	.000	.266
	N	11	11	11	11	11	11	6	11	11	11
HD	Pearson Correlation	.361	.281	.050	1	.921**	.618*	.117	.450	.111	.300
	Sig. (2-tailed)	.275	.403	.885		.000	.043	.825	.165	.745	.370
	N	11	11	11	11	11	11	6	11	11	11
PR	Pearson Correlation	.410	.018	.356	.921**	1	.773**	.614	.470	.413	.388
	Sig. (2-tailed)	.211	.958	.283	.000		.005	.195	.144	.207	.238
	N	11	11	11	11	11	11	6	11	11	11
FM	Pearson Correlation	.238	-.524	.355	.618*	.773**	1	.693	-.056	.378	.433
	Sig. (2-tailed)	.480	.098	.284	.043	.005		.127	.870	.251	.184
	N	11	11	11	11	11	11	6	11	11	11
OV	Pearson Correlation	.764	.[a]	.784	.117	.614	.693	1	.817*	.804	.704
	Sig. (2-tailed)	.077	.000	.065	.825	.195	.127		.047	.054	.118
	N	6	6	6	6	6	6	6	6	6	6
FW	Pearson Correlation	.449	.644*	.480	.450	.470	-.056	.817*	1	.524	.248
	Sig. (2-tailed)	.166	.032	.135	.165	.144	.870	.047		.098	.462
	N	11	11	11	11	11	11	6	11	11	11
HW	Pearson Correlation	.183	-.262	.995**	.111	.413	.378	.804	.524	1	.339
	Sig. (2-tailed)	.590	.436	.000	.745	.207	.251	.054	.098		.307
	N	11	11	11	11	11	11	6	11	11	11
DAY	Pearson Correlation	.810**	.029	.367	.300	.388	.433	.704	.248	.339	1
	Sig. (2-tailed)	.002	.934	.266	.370	.238	.184	.118	.462	.307	
	N	11	11	11	11	11	11	6	11	11	11

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Table 4.3.9 Correlation of photoperiod (PHT), sex (SEX), wing type (WNG), head width (HD), pronotum width (PR), hind femur length (FM), ovipositor length (OV), forewing length (FW), hindwing length (HW), developmental period in days (DAY) of *V. micado* from Jilin.

Correlations

		PHT	SEX	WNG	HD	PR	FM	OV	FW	HW	DAY
PHT	Pearson Correlation	1	-.182	.332**	.098	.288*	.189	-.005	.104	.332**	.455**
	Sig. (2-tailed)		.132	.005	.417	.016	.118	.976	.393	.006	.000
	N	70	70	70	70	70	70	35	70	68	70
SEX	Pearson Correlation	-.182	1	-.087	.250*	-.221	-.489**	.[a]	.388**	-.127	.049
	Sig. (2-tailed)	.132		.474	.037	.067	.000	.000	.001	.302	.689
	N	70	70	70	70	70	70	35	70	68	70
WNG	Pearson Correlation	.332**	-.087	1	.230	.580**	.354**	.178	.624**	.990**	.089
	Sig. (2-tailed)	.005	.474		.055	.000	.003	.306	.000	.000	.464
	N	70	70	70	70	70	70	35	70	68	70
HD	Pearson Correlation	.098	.250*	.230	1	.766**	.595**	.519**	.636**	.333**	.381**
	Sig. (2-tailed)	.417	.037	.055		.000	.000	.001	.000	.006	.001
	N	70	70	70	70	70	70	35	70	68	70
PR	Pearson Correlation	.288*	-.221	.580**	.766**	1	.825**	.505**	.629**	.686**	.284*
	Sig. (2-tailed)	.016	.067	.000	.000		.000	.002	.000	.000	.017
	N	70	70	70	70	70	70	35	70	68	70
FM	Pearson Correlation	.189	-.489**	.354**	.595**	.825**	1	.511**	.329**	.460**	.208
	Sig. (2-tailed)	.118	.000	.003	.000	.000		.002	.005	.000	.084
	N	70	70	70	70	70	70	35	70	68	70
OV	Pearson Correlation	-.005	.[a]	.178	.519**	.505**	.511**	1	.399*	.237	.133
	Sig. (2-tailed)	.976	.000	.306	.001	.002	.002		.018	.176	.448
	N	35	35	35	35	35	35	35	35	34	35
FW	Pearson Correlation	.104	.388**	.624**	.636**	.629**	.329**	.399*	1	.673**	.192
	Sig. (2-tailed)	.393	.001	.000	.000	.000	.005	.018		.000	.111
	N	70	70	70	70	70	70	35	70	68	70
HW	Pearson Correlation	.332**	-.127	.990**	.333**	.686**	.460**	.237	.673**	1	.117
	Sig. (2-tailed)	.006	.302	.000	.006	.000	.000	.176	.000		.343
	N	68	68	68	68	68	68	34	68	68	68
DAY	Pearson Correlation	.455**	.049	.089	.381**	.284*	.208	.133	.192	.117	1
	Sig. (2-tailed)	.000	.689	.464	.001	.017	.084	.448	.111	.343	
	N	70	70	70	70	70	70	35	70	68	70

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Table 4.3.10 Correlation of photoperiod (PHT), sex (SEX), wing type (WNG), head width (HD), pronotum width (PR), hind femur length (FM), ovipositor length (OV), forewing length (FW), hindwing length (HW), developmental period in days (DAY) of *V. micado* from Shenyang.

Correlations

		PHT	SEX	WNG	HD	PR	FM	OV	FW	HW	DAY
PHT	Pearson Correlation	1	-.013	-.024	.581**	.300	.401	.399	.205	.026	.346
	Sig. (2-tailed)		.957	.921	.009	.213	.089	.253	.399	.915	.147
	N	19	19	19	19	19	19	10	19	19	19
SEX	Pearson Correlation	-.013	1	-.490*	-.118	-.531*	-.612**	.[a]	.304	-.464*	-.073
	Sig. (2-tailed)	.957		.033	.630	.019	.005	.000	.206	.045	.767
	N	19	19	19	19	19	19	10	19	19	19
WNG	Pearson Correlation	-.024	-.490*	1	.229	.795**	.635**	.687*	.577**	.985**	.482*
	Sig. (2-tailed)	.921	.033		.347	.000	.003	.028	.010	.000	.037
	N	19	19	19	19	19	19	10	19	19	19
HD	Pearson Correlation	.581**	-.118	.229	1	.717**	.672**	.612	.406	.282	.381
	Sig. (2-tailed)	.009	.630	.347		.001	.002	.060	.085	.241	.108
	N	19	19	19	19	19	19	10	19	19	19
PR	Pearson Correlation	.300	-.531*	.795**	.717**	1	.864**	.713*	.514*	.813**	.528*
	Sig. (2-tailed)	.213	.019	.000	.001		.000	.021	.024	.000	.020
	N	19	19	19	19	19	19	10	19	19	19
FM	Pearson Correlation	.401	-.612**	.635**	.672**	.864**	1	.795**	.331	.663**	.339
	Sig. (2-tailed)	.089	.005	.003	.002	.000		.006	.166	.002	.156
	N	19	19	19	19	19	19	10	19	19	19
OV	Pearson Correlation	.399	.[a]	.687*	.612	.713*	.795**	1	.744*	.692*	.690*
	Sig. (2-tailed)	.253	.000	.028	.060	.021	.006		.014	.027	.027
	N	10	10	10	10	10	10	10	10	10	10
FW	Pearson Correlation	.205	.304	.577**	.406	.514*	.331	.744*	1	.652**	.323
	Sig. (2-tailed)	.399	.206	.010	.085	.024	.166	.014		.002	.178
	N	19	19	19	19	19	19	10	19	19	19
HW	Pearson Correlation	.026	-.464*	.985**	.282	.813**	.663**	.692*	.652**	1	.414
	Sig. (2-tailed)	.915	.045	.000	.241	.000	.002	.027	.002		.078
	N	19	19	19	19	19	19	10	19	19	19
DAY	Pearson Correlation	.346	-.073	.482*	.381	.528*	.339	.690*	.323	.414	1
	Sig. (2-tailed)	.147	.767	.037	.108	.020	.156	.027	.178	.078	
	N	19	19	19	19	19	19	10	19	19	19

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Table 4.3.11 Correlation of photoperiod (PHT), sex (SEX), wing type (WNG), head width (HD), pronotum width (PR), hind femur length (FM), ovipositor length (OV), forewing length (FW), hindwing length (HW), developmental period in days (DAY) of *V. micado* from Sayo.

Correlations

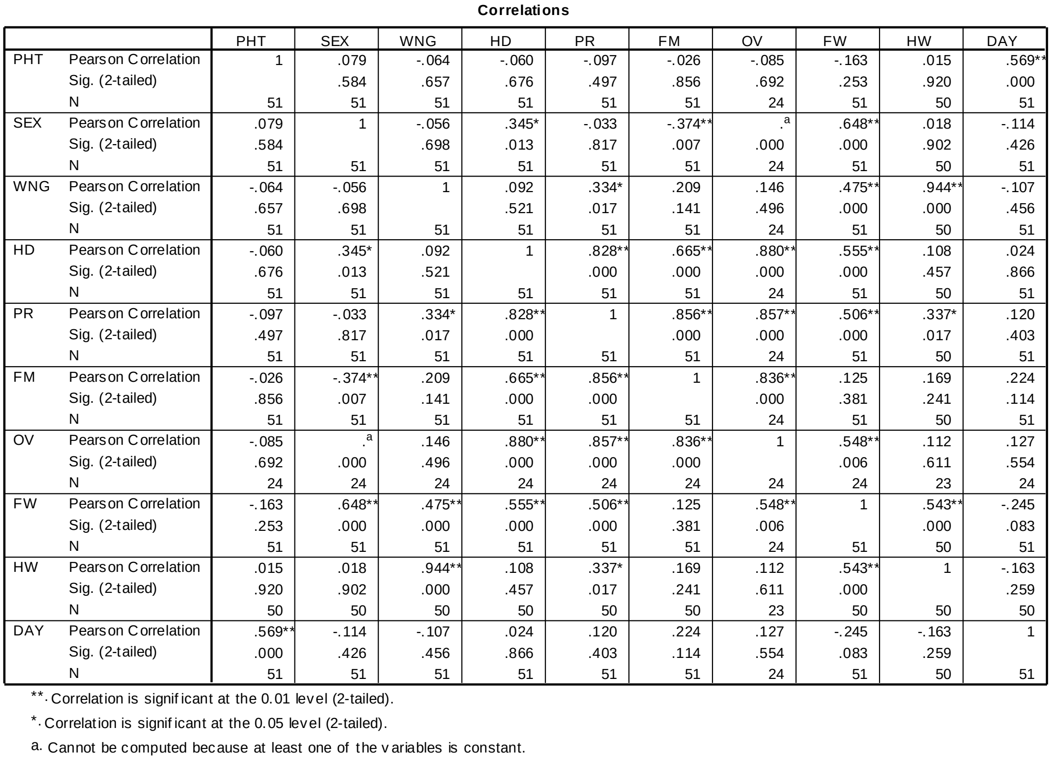
		PHT	SEX	WNG	HD	PR	FM	OV	FW	HW	DAY
PHT	Pearson Correlation	1	.049	.033	.538	.708**	.625*	.551	.303	.077	.171
	Sig. (2-tailed)		.880	.919	.071	.010	.040	.099	.338	.813	.595
	N	12	12	12	12	12	11	10	12	12	12
SEX	Pearson Correlation	.049	1	-.135	.683*	.394	.090	.[a]	.598*	-.090	.062
	Sig. (2-tailed)	.880		.676	.014	.205	.793	.000	.040	.780	.849
	N	12	12	12	12	12	11	10	12	12	12
WNG	Pearson Correlation	.033	-.135	1	.247	.421	.370	.659*	.572	.994**	-.055
	Sig. (2-tailed)	.919	.676		.438	.173	.262	.038	.052	.000	.864
	N	12	12	12	12	12	11	10	12	12	12
HD	Pearson Correlation	.538	.683*	.247	1	.912**	.766**	.943**	.883**	.322	.417
	Sig. (2-tailed)	.071	.014	.438		.000	.006	.000	.000	.307	.177
	N	12	12	12	12	12	11	10	12	12	12
PR	Pearson Correlation	.708**	.394	.421	.912**	1	.876**	.881**	.816**	.476	.361
	Sig. (2-tailed)	.010	.205	.173	.000		.000	.001	.001	.117	.249
	N	12	12	12	12	12	11	10	12	12	12
FM	Pearson Correlation	.625*	.090	.370	.766**	.876**	1	.930**	.654*	.430	.494
	Sig. (2-tailed)	.040	.793	.262	.006	.000		.000	.029	.186	.122
	N	11	11	11	11	11	11	9	11	11	11
OV	Pearson Correlation	.551	.[a]	.659*	.943**	.881**	.930**	1	.922**	.714*	.574
	Sig. (2-tailed)	.099	.000	.038	.000	.001	.000		.000	.020	.083
	N	10	10	10	10	10	9	10	10	10	10
FW	Pearson Correlation	.303	.598*	.572	.883**	.816**	.654*	.922**	1	.639*	.205
	Sig. (2-tailed)	.338	.040	.052	.000	.001	.029	.000		.025	.524
	N	12	12	12	12	12	11	10	12	12	12
HW	Pearson Correlation	.077	-.090	.994**	.322	.476	.430	.714*	.639*	1	-.016
	Sig. (2-tailed)	.813	.780	.000	.307	.117	.186	.020	.025		.960
	N	12	12	12	12	12	11	10	12	12	12
DAY	Pearson Correlation	.171	.062	-.055	.417	.361	.494	.574	.205	-.016	1
	Sig. (2-tailed)	.595	.849	.864	.177	.249	.122	.083	.524	.960	
	N	12	12	12	12	12	11	10	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Table 4.3.12 Correlation of photoperiod (PHT), sex (SEX), wing type (WNG), head width (HD), pronotum width (PR), hind femur length (FM), ovipositor length (OV), forewing length (FW), hindwing length (HW), developmental period in days (DAY) of *V. micado* from Korea.



Correlations

		PHT	SEX	WNG	HD	PR	FM	OV	FW	HW	DAY
PHT	Pearson Correlation	1	.079	-.064	-.060	-.097	-.026	-.085	-.163	.015	.569**
	Sig. (2-tailed)		.584	.657	.676	.497	.856	.692	.253	.920	.000
	N	51	51	51	51	51	51	24	51	50	51
SEX	Pearson Correlation	.079	1	-.056	.345*	-.033	-.374**	.[a]	.648**	.018	-.114
	Sig. (2-tailed)	.584		.698	.013	.817	.007	.000	.000	.902	.426
	N	51	51	51	51	51	51	24	51	50	51
WNG	Pearson Correlation	-.064	-.056	1	.092	.334*	.209	.146	.475**	.944**	-.107
	Sig. (2-tailed)	.657	.698		.521	.017	.141	.496	.000	.000	.456
	N	51	51	51	51	51	51	24	51	50	51
HD	Pearson Correlation	-.060	.345*	.092	1	.828**	.665**	.880**	.555**	.108	.024
	Sig. (2-tailed)	.676	.013	.521		.000	.000	.000	.000	.457	.866
	N	51	51	51	51	51	51	24	51	50	51
PR	Pearson Correlation	-.097	-.033	.334*	.828**	1	.856**	.857**	.506**	.337*	.120
	Sig. (2-tailed)	.497	.817	.017	.000		.000	.000	.000	.017	.403
	N	51	51	51	51	51	51	24	51	50	51
FM	Pearson Correlation	-.026	-.374**	.209	.665**	.856**	1	.836**	.125	.169	.224
	Sig. (2-tailed)	.856	.007	.141	.000	.000		.000	.381	.241	.114
	N	51	51	51	51	51	51	24	51	50	51
OV	Pearson Correlation	-.085	.[a]	.146	.880**	.857**	.836**	1	.548**	.112	.127
	Sig. (2-tailed)	.692	.000	.496	.000	.000	.000		.006	.611	.554
	N	24	24	24	24	24	24	24	24	23	24
FW	Pearson Correlation	-.163	.648**	.475**	.555**	.506**	.125	.548**	1	.543**	-.245
	Sig. (2-tailed)	.253	.000	.000	.000	.000	.381	.006		.000	.083
	N	51	51	51	51	51	51	24	51	50	51
HW	Pearson Correlation	.015	.018	.944**	.108	.337*	.169	.112	.543**	1	-.163
	Sig. (2-tailed)	.920	.902	.000	.457	.017	.241	.611	.000		.259
	N	50	50	50	50	50	50	23	50	50	50
DAY	Pearson Correlation	.569**	-.114	-.107	.024	.120	.224	.127	-.245	-.163	1
	Sig. (2-tailed)	.000	.426	.456	.866	.403	.114	.554	.083	.259	
	N	51	51	51	51	51	51	24	51	50	51

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Table 4.3.13 Correlation of photoperiod (PHT), sex (SEX), wing type (WNG), head width (HD), pronotum width (PR), hind femur length (FM), ovipositor length (OV), forewing length (FW), hindwing length (HW), developmental period in days (DAY) of *V. ornatus* from Sayo.

Correlations

		PHT	SEX	WNG	HD	PR	FM	OV	FW	HW	DAY
PHT	Pearson Correlation	1	.053	.495**	.338**	.421**	.300**	.485**	.561**	.543**	.488**
	Sig. (2-tailed)		.573	.000	.000	.000	.001	.000	.000	.000	.000
	N	116	116	116	115	116	114	51	116	109	116
SEX	Pearson Correlation	.053	1	.019	.218*	-.017	-.175	.[a]	.390**	.030	.049
	Sig. (2-tailed)	.573		.838	.019	.858	.063	.000	.000	.754	.604
	N	116	116	116	115	116	114	51	116	109	116
WNG	Pearson Correlation	.495**	.019	1	.088	.297**	.138	.329*	.562**	.925**	.120
	Sig. (2-tailed)	.000	.838		.349	.001	.143	.019	.000	.000	.198
	N	116	116	116	115	116	114	51	116	109	116
HD	Pearson Correlation	.338**	.218*	.088	1	.905**	.858**	.574**	.629**	.314**	.336**
	Sig. (2-tailed)	.000	.019	.349		.000	.000	.000	.000	.001	.000
	N	115	115	115	115	115	113	50	115	108	115
PR	Pearson Correlation	.421**	-.017	.297**	.905**	1	.903**	.640**	.649**	.493**	.381**
	Sig. (2-tailed)	.000	.858	.001	.000		.000	.000	.000	.000	.000
	N	116	116	116	115	116	114	51	116	109	116
FM	Pearson Correlation	.300**	-.175	.138	.858**	.903**	1	.674**	.499**	.327**	.349**
	Sig. (2-tailed)	.001	.063	.143	.000	.000		.000	.000	.001	.000
	N	114	114	114	113	114	114	51	114	107	114
OV	Pearson Correlation	.485**	.[a]	.329*	.574**	.640**	.674**	1	.482**	.342*	.523**
	Sig. (2-tailed)	.000	.000	.019	.000	.000	.000		.000	.016	.000
	N	51	51	51	50	51	51	51	51	49	51
FW	Pearson Correlation	.561**	.390**	.562**	.629**	.649**	.499**	.482**	1	.705**	.253**
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.000		.000	.006
	N	116	116	116	115	116	114	51	116	109	116
HW	Pearson Correlation	.543**	.030	.925**	.314**	.493**	.327**	.342*	.705**	1	.127
	Sig. (2-tailed)	.000	.754	.000	.001	.000	.001	.016	.000		.187
	N	109	109	109	108	109	107	49	109	109	109
DAY	Pearson Correlation	.488**	.049	.120	.336**	.381**	.349**	.523**	.253**	.127	1
	Sig. (2-tailed)	.000	.604	.198	.000	.000	.000	.000	.006	.187	
	N	116	116	116	115	116	114	51	116	109	116

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Table 4.3.14 Correlation of photoperiod (PHT), sex (SEX), wing type (WNG), head width (HD), pronotum width (PR), hind femur length (FM), ovipositor length (OV), forewing length (FW), hindwing length (HW), developmental period in days (DAY) of *V. ornatus* from Aridagawa.

Correlations

		PHT	SEX	WNG	HD	PR	FM	OV	FW	HW	DAY
PHT	Pearson Correlation	1	-.104	.356	.379	.444	.520	.590	.078	.359	.042
	Sig. (2-tailed)		.735	.233	.201	.129	.069	.410	.799	.252	.891
	N	13	13	13	13	13	13	4	13	12	13
SEX	Pearson Correlation	-.104	1	-.178	.384	-.022	-.178	.[a]	.642*	-.240	.023
	Sig. (2-tailed)	.735		.561	.195	.942	.561	.000	.018	.453	.939
	N	13	13	13	13	13	13	4	13	12	13
WNG	Pearson Correlation	.356	-.178	1	-.066	.321	.200	-.053	.529	.998**	-.092
	Sig. (2-tailed)	.233	.561		.831	.285	.513	.947	.063	.000	.765
	N	13	13	13	13	13	13	4	13	12	13
HD	Pearson Correlation	.379	.384	-.066	1	.788**	.746**	.144	.200	-.081	.406
	Sig. (2-tailed)	.201	.195	.831		.001	.003	.856	.513	.802	.169
	N	13	13	13	13	13	13	4	13	12	13
PR	Pearson Correlation	.444	-.022	.321	.788**	1	.830**	.763	.219	.331	.528
	Sig. (2-tailed)	.129	.942	.285	.001		.000	.237	.473	.294	.064
	N	13	13	13	13	13	13	4	13	12	13
FM	Pearson Correlation	.520	-.178	.200	.746**	.830**	1	.069	-.021	.205	.260
	Sig. (2-tailed)	.069	.561	.513	.003	.000		.931	.945	.523	.392
	N	13	13	13	13	13	13	4	13	12	13
OV	Pearson Correlation	.590	.[a]	-.053	.144	.763	.069	1	-.082	-.026	.442
	Sig. (2-tailed)	.410	.000	.947	.856	.237	.931		.918	.984	.558
	N	4	4	4	4	4	4	4	4	3	4
FW	Pearson Correlation	.078	.642*	.529	.200	.219	-.021	-.082	1	.575	-.022
	Sig. (2-tailed)	.799	.018	.063	.513	.473	.945	.918		.051	.944
	N	13	13	13	13	13	13	4	13	12	13
HW	Pearson Correlation	.359	-.240	.998**	-.081	.331	.205	-.026	.575	1	-.110
	Sig. (2-tailed)	.252	.453	.000	.802	.294	.523	.984	.051		.733
	N	12	12	12	12	12	12	3	12	12	12
DAY	Pearson Correlation	.042	.023	-.092	.406	.528	.260	.442	-.022	-.110	1
	Sig. (2-tailed)	.891	.939	.765	.169	.064	.392	.558	.944	.733	
	N	13	13	13	13	13	13	4	13	12	13

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Table 4.3.15 Correlation of photoperiod (PHT), sex (SEX), wing type (WNG), head width (HD), pronotum width (PR), hind femur length (FM), ovipositor length (OV), forewing length (FW), hindwing length (HW), developmental period in days (DAY) of *V. flavifrons* from Vietnam.

Correlations

		PHT	SEX	WNG	HD	PR	FM	OV	FW	HW	DAY
PHT	Pearson Correlation	1	-.033	.[a]	-.274	-.323*	-.294	-.493*	-.046	.001	-.160
	Sig. (2-tailed)		.838	.	.083	.039	.062	.012	.776	.993	.323
	N	41	41	41	41	41	41	25	41	41	40
SEX	Pearson Correlation	-.033	1	.[a]	.538**	.420**	.305	.[a]	.910**	.267	.524**
	Sig. (2-tailed)	.838		.	.000	.006	.052	.000	.000	.091	.001
	N	41	41	41	41	41	41	25	41	41	40
WNG	Pearson Correlation	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]
	Sig. (2-tailed)	.	.		.	.	.	.	.	.	.
	N	41	41	41	41	41	41	25	41	41	40
HD	Pearson Correlation	-.274	.538**	.[a]	1	.967**	.938**	.875**	.754**	.326*	.802**
	Sig. (2-tailed)	.083	.000	.		.000	.000	.000	.000	.037	.000
	N	41	41	41	41	41	41	25	41	41	40
PR	Pearson Correlation	-.323*	.420**	.[a]	.967**	1	.941**	.803**	.632**	.256	.800**
	Sig. (2-tailed)	.039	.006	.	.000		.000	.000	.000	.106	.000
	N	41	41	41	41	41	41	25	41	41	40
FM	Pearson Correlation	-.294	.305	.[a]	.938**	.941**	1	.845**	.568**	.259	.703**
	Sig. (2-tailed)	.062	.052	.	.000	.000		.000	.000	.102	.000
	N	41	41	41	41	41	41	25	41	41	40
OV	Pearson Correlation	-.493*	.[a]	.[a]	.875**	.803**	.845**	1	.715**	.157	.420*
	Sig. (2-tailed)	.012	.000	.	.000	.000	.000		.000	.455	.041
	N	25	25	25	25	25	25	25	25	25	24
FW	Pearson Correlation	-.046	.910**	.[a]	.754**	.632**	.568**	.715**	1	.424**	.671**
	Sig. (2-tailed)	.776	.000	.	.000	.000	.000	.000		.006	.000
	N	41	41	41	41	41	41	25	41	41	40
HW	Pearson Correlation	.001	.267	.[a]	.326*	.256	.259	.157	.424**	1	.140
	Sig. (2-tailed)	.993	.091	.	.037	.106	.102	.455	.006		.391
	N	41	41	41	41	41	41	25	41	41	40
DAY	Pearson Correlation	-.160	.524**	.[a]	.802**	.800**	.703**	.420*	.671**	.140	1
	Sig. (2-tailed)	.323	.001	.	.000	.000	.000	.041	.000	.391	
	N	40	40	40	40	40	40	24	40	40	40

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Table 4.3.16 Correlation of photoperiod (PHT), sex (SEX), wing type (WNG), head width (HD), pronotum width (PR), hind femur length (FM), ovipositor length (OV), forewing length (FW), hindwing length (HW), developmental period in days (DAY) of *Velarifictorus aspursus* from Vietnam.

Correlations

		PHT	SEX	WNG	HD	PR	FM	OV	FW	HW	DAY
PHT	Pearson Correlation	1	-.065	.000	-.086	-.022	.014	.001	-.099	.019	.305**
	Sig. (2-tailed)		.334	.996	.196	.737	.840	.994	.137	.785	.000
	N	226	226	226	226	226	225	94	225	216	226
SEX	Pearson Correlation	-.065	1	-.230**	.423**	-.094	-.118	.[a]	.332**	-.230**	-.163*
	Sig. (2-tailed)	.334		.000	.000	.161	.077	.000	.000	.001	.014
	N	226	226	226	226	226	225	94	225	216	226
WNG	Pearson Correlation	.000	-.230**	1	-.049	.190**	-.006	.345**	.302**	.993**	-.209**
	Sig. (2-tailed)	.996	.000		.467	.004	.927	.001	.000	.000	.002
	N	226	226	226	226	226	225	94	225	216	226
HD	Pearson Correlation	-.086	.423**	-.049	1	.748**	.080	.637**	.719**	-.003	.056
	Sig. (2-tailed)	.196	.000	.467		.000	.230	.000	.000	.963	.399
	N	226	226	226	226	226	225	94	225	216	226
PR	Pearson Correlation	-.022	-.094	.190**	.748**	1	.162*	.658**	.648**	.241**	.117
	Sig. (2-tailed)	.737	.161	.004	.000		.015	.000	.000	.000	.079
	N	226	226	226	226	226	225	94	225	216	226
FM	Pearson Correlation	.014	-.118	-.006	.080	.162*	1	.154	.073	.004	-.019
	Sig. (2-tailed)	.840	.077	.927	.230	.015		.137	.280	.953	.773
	N	225	225	225	225	225	225	94	224	215	225
OV	Pearson Correlation	.001	.[a]	.345**	.637**	.658**	.154	1	.610**	.357**	-.042
	Sig. (2-tailed)	.994	.000	.001	.000	.000	.137		.000	.001	.688
	N	94	94	94	94	94	94	94	93	88	94
FW	Pearson Correlation	-.099	.332**	.302**	.719**	.648**	.073	.610**	1	.343**	-.122
	Sig. (2-tailed)	.137	.000	.000	.000	.000	.280	.000		.000	.067
	N	225	225	225	225	225	224	93	225	216	225
HW	Pearson Correlation	.019	-.230**	.993**	-.003	.241**	.004	.357**	.343**	1	-.142*
	Sig. (2-tailed)	.785	.001	.000	.963	.000	.953	.001	.000		.037
	N	216	216	216	216	216	215	88	216	216	216
DAY	Pearson Correlation	.305**	-.163*	-.209**	.056	.117	-.019	-.042	-.122	-.142*	1
	Sig. (2-tailed)	.000	.014	.002	.399	.079	.773	.688	.067	.037	
	N	226	226	226	226	226	225	94	225	216	226

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Table 4.3.17 Correlation of photoperiod (PHT), sex (SEX), wing type (WNG), head width (HD), pronotum width (PR), hind femur length (FM), ovipositor length (OV), forewing length (FW), hindwing length (HW), developmental period in days (DAY) of *Velarifictorus aspursus* from Tianmu.

Correlations

		PHT	SEX	WNG	HD	PR	FM	OV	FW	HW	DAY
PHT	Pearson Correlation	1	-.047	.028	.077	.131	-.010	.397*	.207*	.109	.149
	Sig. (2-tailed)		.643	.782	.443	.190	.924	.012	.038	.312	.137
	N	101	101	101	101	101	100	39	101	88	101
SEX	Pearson Correlation	-.047	1	.083	.528**	-.053	.045	.[a]	.082	-.011	-.121
	Sig. (2-tailed)	.643		.410	.000	.599	.654	.000	.414	.920	.228
	N	101	101	101	101	101	100	39	101	88	101
WNG	Pearson Correlation	.028	.083	1	.058	.083	.058	.231	.320**	.955**	-.094
	Sig. (2-tailed)	.782	.410		.566	.409	.569	.157	.001	.000	.352
	N	101	101	101	101	101	100	39	101	88	101
HD	Pearson Correlation	.077	.528**	.058	1	.782**	.132	.662**	.556**	.239*	.036
	Sig. (2-tailed)	.443	.000	.566		.000	.190	.000	.000	.025	.721
	N	101	101	101	101	101	100	39	101	88	101
PR	Pearson Correlation	.131	-.053	.083	.782**	1	.136	.655**	.648**	.352**	.136
	Sig. (2-tailed)	.190	.599	.409	.000		.176	.000	.000	.001	.175
	N	101	101	101	101	101	100	39	101	88	101
FM	Pearson Correlation	-.010	.045	.058	.132	.136	1	.576**	.086	.095	-.035
	Sig. (2-tailed)	.924	.654	.569	.190	.176		.000	.394	.376	.727
	N	100	100	100	100	100	100	39	100	88	100
OV	Pearson Correlation	.397*	.[a]	.231	.662**	.655**	.576**	1	.499**	.409*	.001
	Sig. (2-tailed)	.012	.000	.157	.000	.000	.000		.001	.012	.996
	N	39	39	39	39	39	39	39	39	37	39
FW	Pearson Correlation	.207*	.082	.320**	.556**	.648**	.086	.499**	1	.497**	-.031
	Sig. (2-tailed)	.038	.414	.001	.000	.000	.394	.001		.000	.755
	N	101	101	101	101	101	100	39	101	88	101
HW	Pearson Correlation	.109	-.011	.955**	.239*	.352**	.095	.409*	.497**	1	-.034
	Sig. (2-tailed)	.312	.920	.000	.025	.001	.376	.012	.000		.756
	N	88	88	88	88	88	88	37	88	88	88
DAY	Pearson Correlation	.149	-.121	-.094	.036	.136	-.035	.001	-.031	-.034	1
	Sig. (2-tailed)	.137	.228	.352	.721	.175	.727	.996	.755	.756	
	N	101	101	101	101	101	100	39	101	88	101

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Macropterous individuals were produced from each population (Table 4.5.1; 3.5.3; 3.5.5) and the percent of total number (Table 4.5.2; 3.5.4; 3.5.6) was calculated. *V. flavifrons* were always brachypterous in my study. The wing length of *V. micado* was greatly affected by photoperiod. Under LD 12:12, few individuals developed long wings in both males and females, while those under LD 16:8 developed more long-winged form. Females tended to be macropterous in the North groups (Fig. 4.5.1-4).

Table 4.5.1 Number of macropterous and total number in () of *V. micado*

Sex LD	♀ 12:12	♀ 16:08	X^2	p value	♂ 12:12	♂ 16:08	X^2	p value
Newnan	3(22)	5(11)	4.043	0.044	2(12)	4(10)	1.497	0.221
Vietnam	1(18)	4(20)	1.730	0.188	1(15)	12(27)	6.439	0.011
Osaka	0(13)	2(20)	1.384	0.239	1(14)	5(25)	1.140	0.286
Jilin	3(16)	16(18)	16.902	0.000	6(23)	7(20)	0.403	0.526
Shenzhen	16(34)	20(25)	6.572	0.010	11(29)	14(20)	4.871	0.027
Tianmu	1(13)	3(24)	0.202	0.653	0(12)	0(12)	/	/

Table 4.5.2 Macropterous percent of *V. micado*

Sex LD	♀ 12:12	♀ 16:08	♂ 12:12	♂ 16:08
Newnan	13.64	45.45	16.67	40.00
Vietnam	5.56	20.00	6.67	44.44
Osaka	0	10.00	7.14	20.00
Jilin	18.75	88.89	26.09	35.00
Shenzhen	47.06	80.00	37.93	70.00
Tianmu	7.69	12.50	0	0

Table 4.5.3 Number of macropterous and total number in () of *V. aspersus*.

Sex LD	♀ 12:12	♀ 16:08	X^2	p value	♂ 12:12	♂ 16:08	X^2	p value
Vietnam	14(47)	7(52)	3.937	0.047	5(70)	8(59)	1.455	0.228
Tianmu	20(26)	19(26)	0.103	0.749	17(23)	32(42)	0.042	0.838

Table 4.5.4 Macropterous percent of *V. aspersus*.

Sex	♀	♂	♀	♂
LD	12:12	12:12	16:8	16:8
Vietnma	29.79	7.14	13.56	13.56
Tianmu	76.92	73.91	73.08	76.19

Table 4.5.5 Number of macropterous and total number in () of *V. ornatus*.

Sex	♀	♀			♂	♂		
LD	12:12	16:8	X^2	p value	12:12	16:8	X^2	p value
Aridagawa	0(5)	2(12)	0.944	0.331	0(4)	1(10)	0.431	0.512
Sayo	0(21)	33(53)	23.60	0.000	0(17)	56(87)	23.71	0.000

Table 4.5.6 Macropterous percent of *V. ornatus*.

Sex	♀	♂	♀	♂
LD	12:12	12:12	16:8	16:8
Aridagawa	0	0	16.67	10.00
Sayo	0	0	62.26	64.37

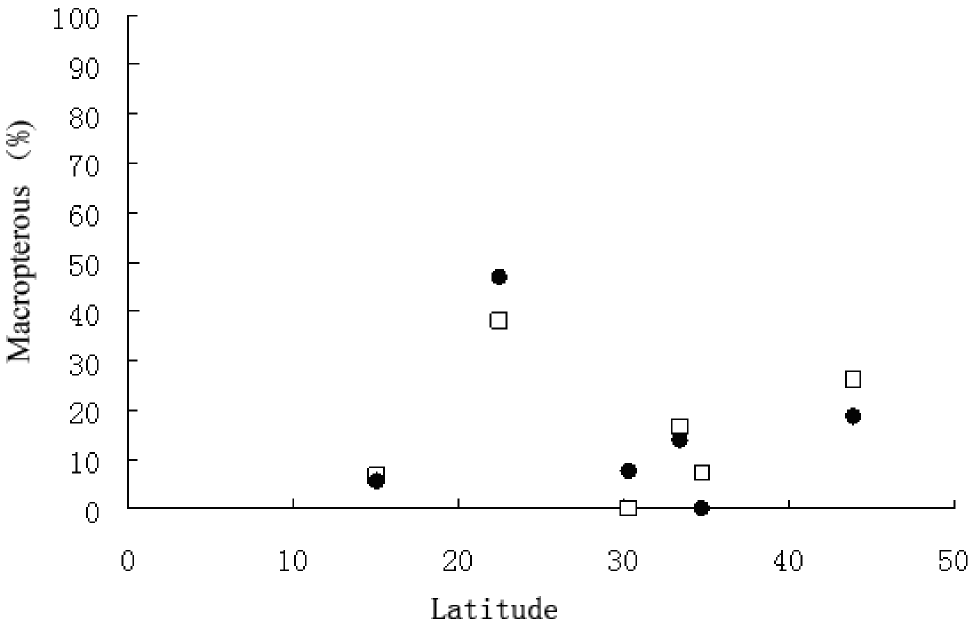


Figure 4.5.1 Geographic profile of macropterous under LD 12:12 and 27.5℃ in *V. micado*. Open squares, male; closed circles, female.

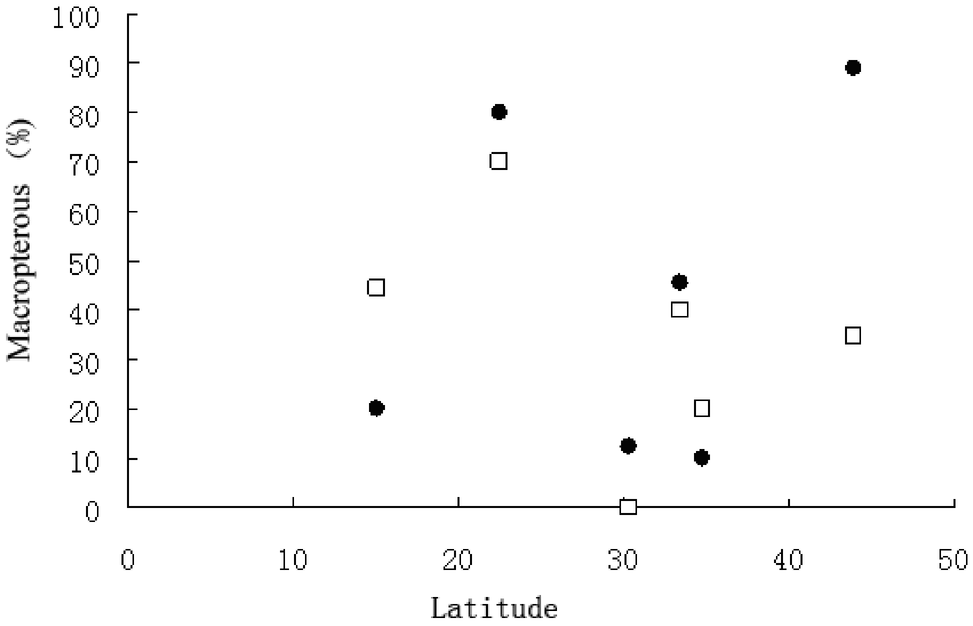


Figure 4.5.2 Geographic profile of macropterous under LD 16:8 and 27.5℃ in *V. micado*. Open squares, male; closed circles, female.

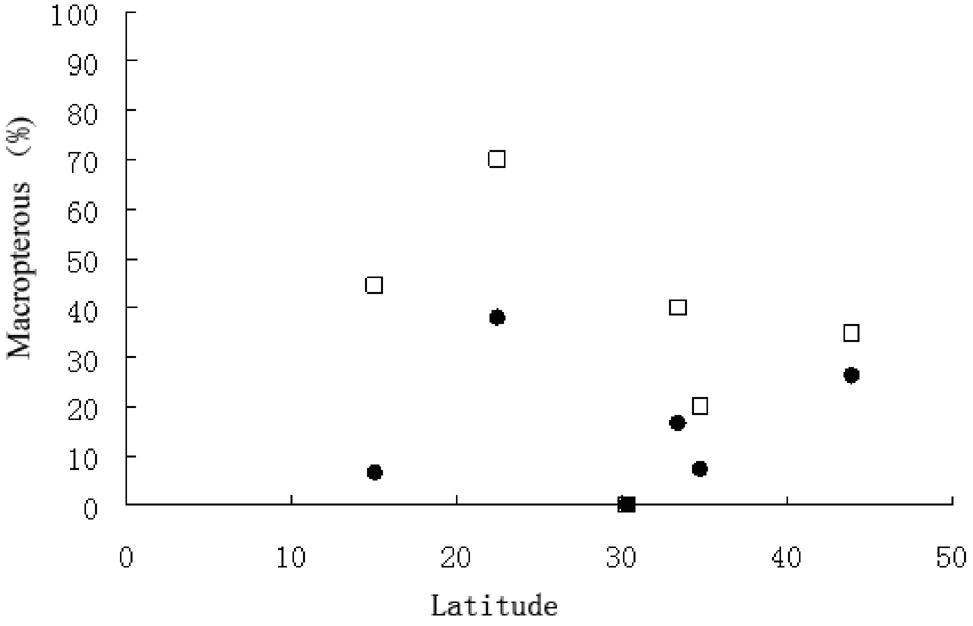


Figure 4.5.3 Geographic profile of macropterous in males at 27.5℃ in *V. micado*. Open squares, under LD 16:8; closed circles, under LD 12:12.

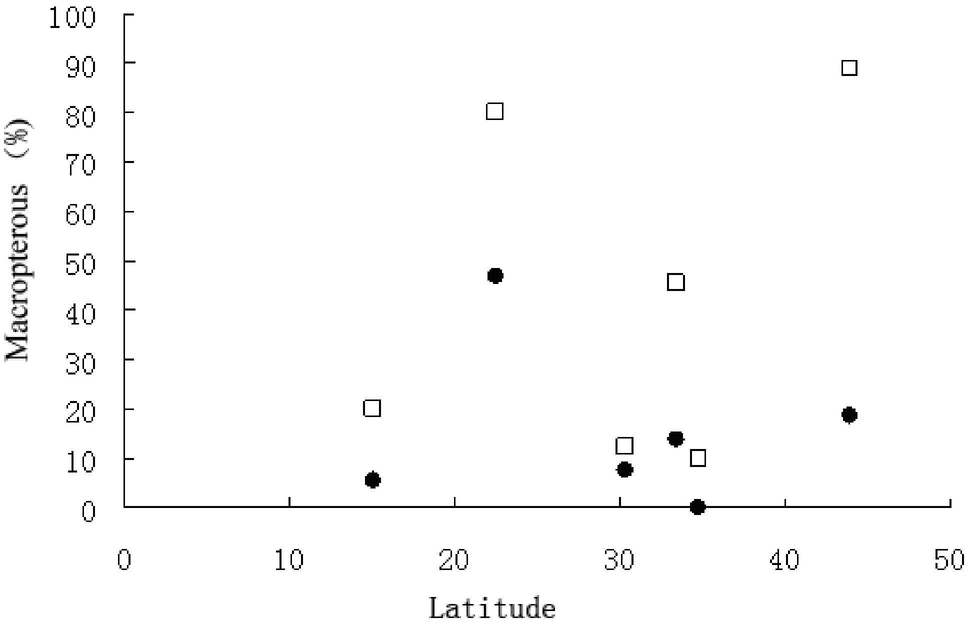


Figure 4.5.4 Geographic profile of macropterous in females at 27.5 ℃ in *V. micado*. Open squares, under LD 16:8; closed circles, under LD 12:12.

The developmental period in days was compared under LD 16:8 or LD 12:12 of *V. micado* from Shenzhen, *V. ornatus* from Sayo and *V. aspersus* from Tianmu. Both macropterous and brachypterous individuals developed at the same rate (Fig. 4.5.5-8).

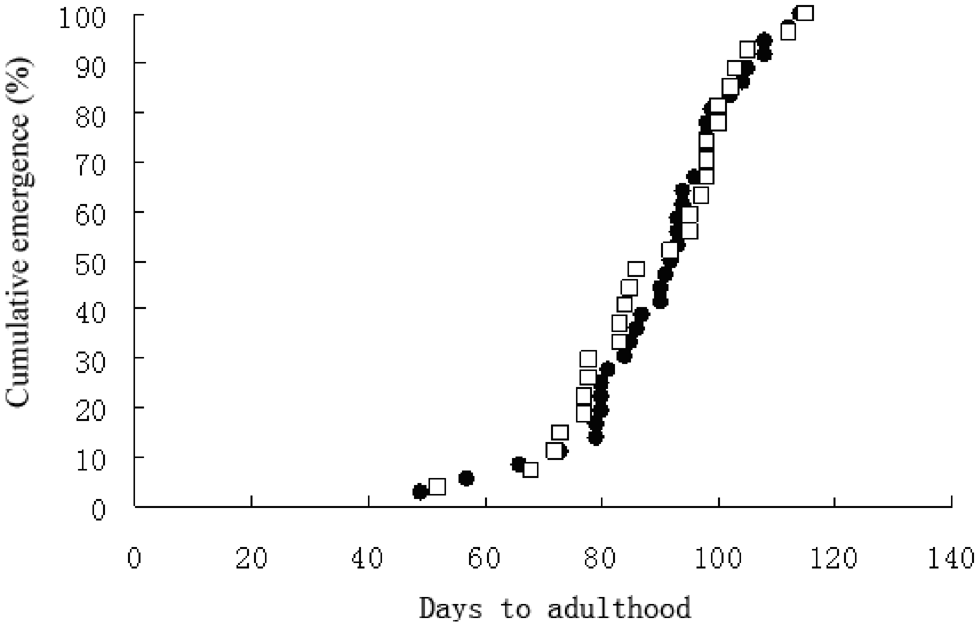


Figure 4.5.5 Cumulative emergence of *V. micado* from Shenzhen under LD 12:12 at 27.5 ℃, open squares, macropterous; closed circles, brachypterous.

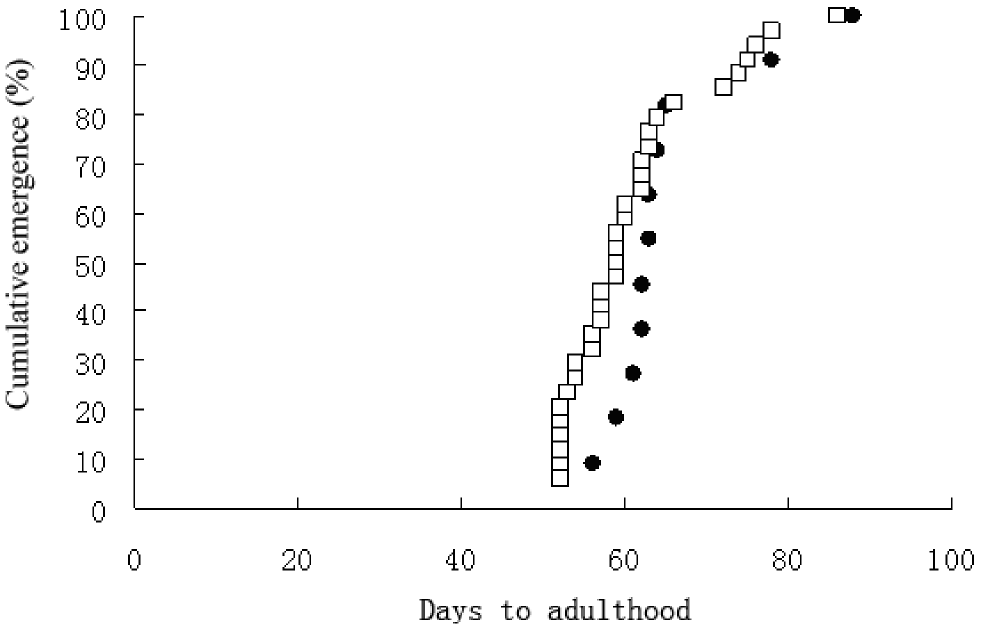


Figure 4.5.6 Cumulative emergence of *V. micado* from Shenzhen under LD 16:8 at 27.5℃, open squares, macropterous; closed circles, brachypterous.

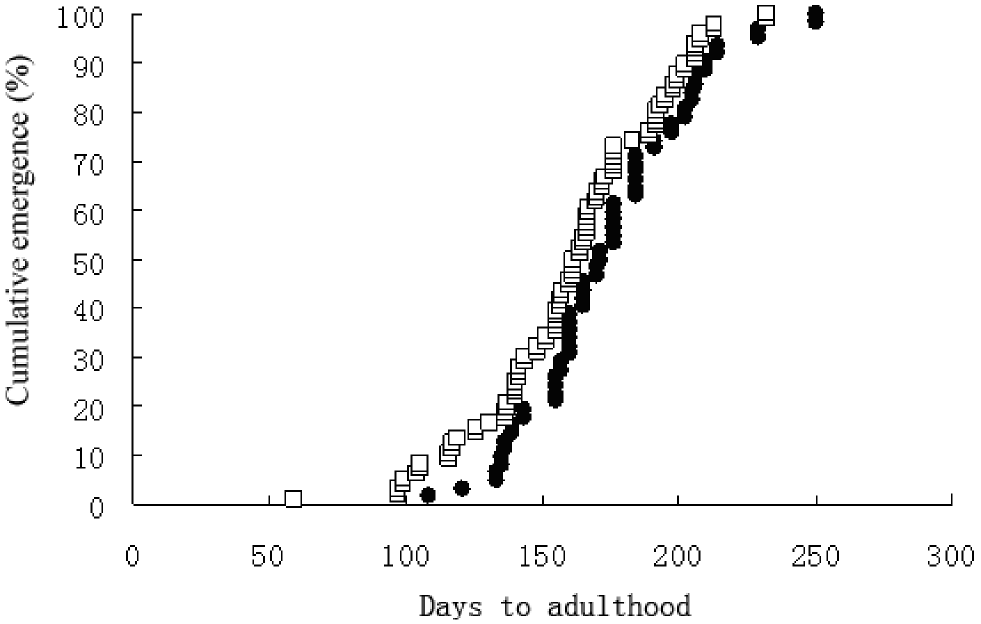


Figure 4.5.7 Cumulative emergence of *V. ornatus* from Sayo under LD 16:8 at 27.5℃, open squares, macropterous; closed circles, brachypterous.

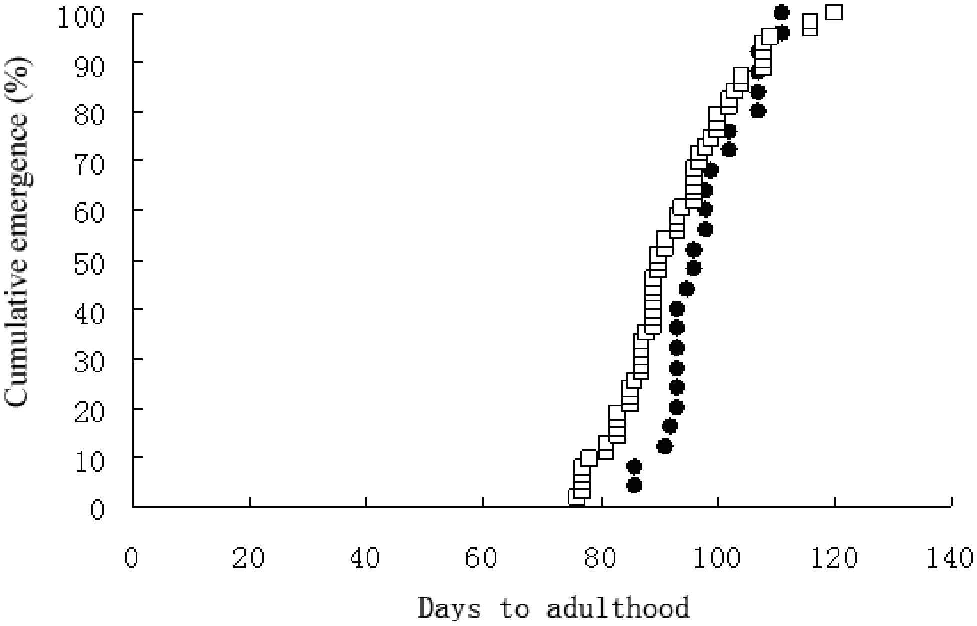


Figure 4.5.8 Cumulative emergence of *V. aspersus* from Tianmu under LD 16:8 at 27.5℃, open squares, macropterous; closed circles, brachypterous.

HD, PR and FM were compared between the two wing forms of these species. There were no significant differences between them (Table 4.5.7-15).

Table 4.5.7 A comparison of head width between macropters and brachypters of *V. micado*. B: brachypters; M: macropters.

	LD	WNG	SEX	HD±SD	t-test
Shenzhen	12:12	B	♂	418.7±22.5	F(16)=0.2 p=0.272
Shenzhen	12:12	M	♂	430.1±17.4	
Shenzhen	12:12	B	♀	395.1±19.8	F(19)=0.083 p=0.544
Shenzhen	12:12	M	♀	389.5±20.6	
Shenzhen	16:8	B	♂	391.5±29.0	F(10)=0.049 p=0.261
Shenzhen	16:8	M	♂	414.1±24.0	
Shenzhen	16:8	B	♀	388.3±34.1	F(8)=3.011 p=0.436
Shenzhen	16:8	M	♀	376.6±13.7	
Jilin	12:12	B	♂	375.8±37.6	F(16)=3.385 p=0.981
Jilin	12:12	M	♂	376.2±18.0	
Jilin	12:12	B	♀	353.8±28.8	F(10)=0.102 p=0.501
Jilin	12:12	M	♀	368.0±36.7	
Jilin	16:8	B	♂	369.0±27.4	F(7)=0.025 p=0.04
Jilin	16:8	M	♂	411.6±23.6	
Jilin	16:8	B	♀	345.0±11.6	F(12)=6.796 p=0.072
Jilin	16:8	M	♀	377.1±31.0	
Vietnam	16:8	B	♂	448.3±23.5	F(7)=4.494 p=0.914

Vietnam	16:8	M	♂	450.0±13.2	
Vietnam	16:8	B	♀	412.7±30.8	F(13)=0.425 p=0.792
Vietnam	16:8	M	♀	417.3±21.0	

Table 4.5.8 A comparison of head width between macropters and brachypters of *V. aspersus*. B: brachypters; M: macropters.

	LD	WNG	SEX	HD±SD	t-test
Tianmu	12:12	B	♂	563.3±34.9	F(14)=0.054 p=0.065
Tianmu	12:12	M	♂	521.0±32.7	
Tianmu	12:12	B	♀	435.8±35.2	F(8)=0.702 p=0.791
Tianmu	12:12	M	♀	428.7±42.8	
Tianmu	16:8	B	♂	524.6±41.9	F(25)=0.092 p=0.483
Tianmu	16:8	M	♂	538.5±39.1	
Tianmu	16:8	B	♀	436.0±32.1	F(17)=1.673 p=0.044
Tianmu	16:8	M	♀	460.9±17.8	

Table 4.5.9 A comparison of head width between macropters and brachypters of *V. ornatus*. B: brachypters; M: macropters.

	LD	WNG	SEX	HD±SD	t-test
Sayo	16:8	B	♂	417.2±27.7	F(44)=1.984 p=0.232
Sayo	16:8	M	♂	393.4±79.9	
Sayo	16:8	B	♀	387.9±35.6	F(34)=1.869 p=0.589
Sayo	16:8	M	♀	382.3±25.9	

Table 4.5.10 A comparison of pronotum width between macropters and brachypters of *V. micado*. B: brachypters; M: macropters.

	LD	WNG	SEX	HD±SD	t-test
Shenzhen	12:12	B	♂	428.8±27.5	F(16)=4.031 p=0.175
Shenzhen	12:12	M	♂	445.0±14.8	
Shenzhen	12:12	B	♀	426.2±16.7	F(19)=0.662 p=0.203
Shenzhen	12:12	M	♀	438.1±25.1	
Shenzhen	16:8	B	♂	410.0±26.1	F(18)=0.232 p=0.823
Shenzhen	16:8	M	♂	413.8±30.1	
Shenzhen	16:8	B	♀	413.0±27.4	F(10)=0.946 p=0.320
Shenzhen	16:8	M	♀	425.9±15.9	
Jilin	12:12	B	♂	373.0±31.2	F(16)=2.335 p=0.179
Jilin	12:12	M	♂	394.0±17.3	
Jilin	12:12	B	♀	380.8±25.5	F(10)=0.949 p=0.041
Jilin	12:12	M	♀	425.3±38.4	
Jilin	16:8	B	♂	374.8±21.3	F(7)=0.242 p=0.006
Jilin	16:8	M	♂	425.8±17.6	
Jilin	16:8	B	♀	369.3±19.9	F(12)=0.724 p=0.02
Jilin	16:8	M	♀	432.3±29.7	
Vietnam	16:8	B	♂	470.1±16.5	F(7)=3.092 p=0.275

Vietnam	16:8	M	♂	483.3±7.5	
Vietnam	16:8	B	♀	461.6±43.7	F(13)=1.818 p=0.193
Vietnam	16:8	M	♀	492.8±13.3	

Table 4.5.11 A comparison of pronotum width between macropters and brachypters of *V. aspersus*. B: brachypters; M: macropters.

	LD	WNG	SEX	HD±SD	t-test
Tianmu	12:12	B	♂	508.7±27.7	F(13)=0.000 p=0.293
Tianmu	12:12	M	♂	490.4±25.4	
Tianmu	12:12	B	♀	472.7±52.8	F(8)=0.301 p=0.808
Tianmu	12:12	M	♀	481.6±56.4	
Tianmu	16:8	B	♂	496.4±18.0	F(25)=1.316 p=0.719
Tianmu	16:8	M	♂	502.9±38.6	
Tianmu	16:8	B	♀	466.6±38.4	F(17)=2.704 p=0.003
Tianmu	16:8	M	♀	514.6±22.2	

Table 4.5.12 A comparison of pronotum width between macropters and brachypters of *V. ornatus*. B: brachypters; M: macropters.

	LD	WNG	SEX	HD±SD	t-test
Sayo	16:8	B	♂	406.6±28.2	F(25)=2.115 p=0.540
Sayo	16:8	M	♂	413.3±41.8	
Sayo	16:8	B	♀	397.2±33.8	F(35)=5.107 p=0.030
Sayo	16:8	M	♀	423.3±33.5	

Table 4.5.13 A comparison of femur length between macropters and brachypters of *V. micado*. B: brachypters; M: macropters.

	LD	WNG	SEX	HD±SD	t-test
Shenzhen	12:12	B	♂	1009.2±47.6	F(16)=0.197 p=0.112
Shenzhen	12:12	M	♂	1047.4±46.1	
Shenzhen	12:12	B	♀	1083.8±49.0	F(19)=0.547 p=0.560
Shenzhen	12:12	M	♀	1068.5±69.8	
Shenzhen	16:8	B	♂	987.0±43.8	F(10)=0.181 p=0.688
Shenzhen	16:8	M	♂	1003.8±53.2	
Shenzhen	16:8	B	♀	1022.3±66.5	F(8)=0.415 p=0.578
Shenzhen	16:8	M	♀	1044.4±50.8	
Jilin	12:12	B	♂	825.2±72.7	F(16)=0.885 p=0.335

Jilin	12:12	M	♂	860.8±52.0	
Jilin	12:12	B	♀	897.7±60.5	F(10)=0.083 p=0.364
Jilin	12:12	M	♀	973.7±74.3	
Jilin	16:8	B	♂	837..5±48.6	F(7)=0.099 p=0.262
Jilin	16:8	M	♂	876.8±47.6	
Jilin	16:8	B	♀	851.0±57.9	F(12)=0.587 p=0.024
Jilin	16:8	M	♀	953.3±69.7	
Vietnam	16:8	B	♂	1090.1±28.9	F(7)=0.000 p=0.848
Vietnam	16:8	M	♂	1094.3±31.5	
Vietnam	16:8	B	♀	1153.6±77.7	F(13)=2.242 p=0.998
Vietnam	16:8	M	♀	1153.8±26.3	

Table 4.5.14 A comparison of femur length between macropters and brachypters of *V. aspersus*. B: brachypters; M: macropters.

	LD	WNG	SEX	HD±SD	t-test
Tianmu	12:12	B	♂	1080.7±28.7	F(13)=1.522 p=0.119
Tianmu	12:12	M	♂	1029.4±50.3	
Tianmu	12:12	B	♀	1063.0±72.2	F(8)=0.528 p=0.763
Tianmu	12:12	M	♀	1078.8±82.0	
Tianmu	16:8	B	♂	1014.0±65.6	F(25)=0.146 p=0.069
Tianmu	16:8	M	♂	1069.7±58.0	
Tianmu	16:8	B	♀	1101.4±65.8	F(4.834)=5.107 p=0.395
Tianmu	16:8	M	♀	1130.2±35.0	

Table 4.5.15 A comparison of femur length between macropters and brachypters of *V. ornatus*. B: brachypters; M: macropters.

	LD	WNG	SEX	HD±SD	t-test
Sayo	16:8	B	♂	874.3±40.6	F(25)=0.146 p=0.069
Sayo	16:8	M	♂	882.3±67.5	
Sayo	16:8	B	♀	913.5±92.3	F(4.834)=5.107 p=0.395
Sayo	16:8	M	♀	915.5±56.2	

When the value of HD/PR was calculated, there was a significant difference between both sexes and among the three species except male *V. aspersus*. Fig 3.5.9 showed that female *V. micado* from Shenzhen had significantly different values of HD/PR (brachypters, 0.9238±0.02616, while macropters 0.8877±0.02998, F(41)=0.466 p<0.001). For males, the differences were not so big, but still significant (brachypters, 0.9801±0.03890, while macropters 0.9559±0.03696, F(47)=0.010

p=0.032, see Fig. 4.5.10). *V. micado* from Jilin had the same situation (females, brachypters, 0.9297±0.03030, while macropters 0.8683±0.02871, F(34)=0.039 p<0.001; males, brachypters, 0.9980±0.03701, while macropters 0.9553±0.03840, F(36)=1.529 p=0.002), see Fig. 4.5.11-12. In *V. ornatus* species, they had also significant differences in this value. (females, brachypters, 0.9795±0.03675, while macropters 0.9072±0.03356, F(55)=0.551 p<0.001; males, brachypters, 1.0252±0.04264, while macropters 0.9847±0.04069, F(64)=0.375 p<0.001) see Fig. 4.5.13-14. Significant differences was also observed in female of *V. aspersus* (brachypters, 0.9279±0.02964, while macropters 0.9029±0.03257, F(48)=0.123 p=0.016), but in males, there was no significant difference (brachypters, 1.0735±0.04722, while macropters 1.0737±0.03084, F(69)=10.539 p=0.980), see Fig. 4.5.15-16.

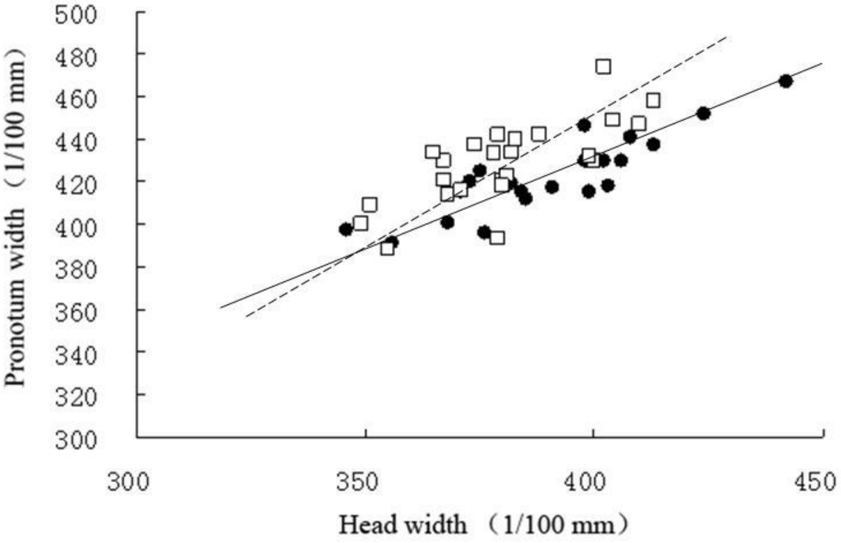


Figure 4.5.9 The width of head relative to the width of pronotum in female *V. micado* from Shenzhen. Open squares (dotted line), macropters; closed circles (black line), brachypters.

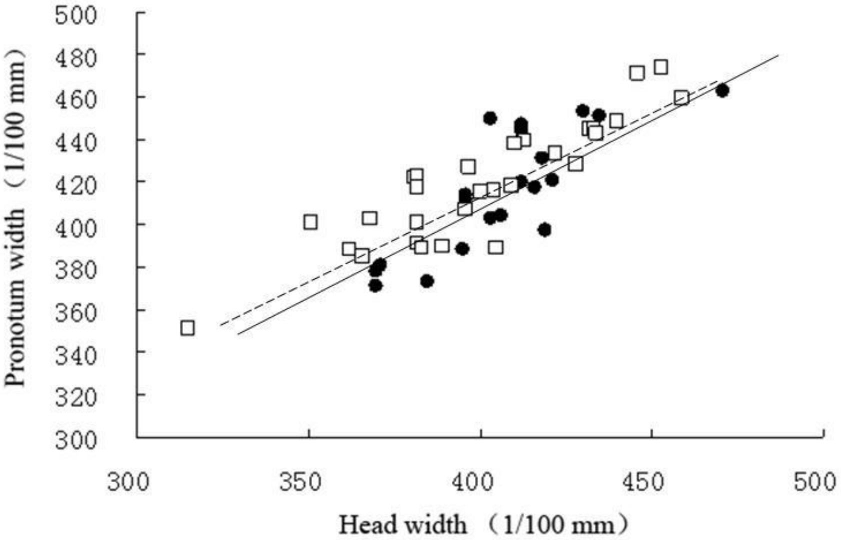


Figure 4.5.10 The width of head relative to the width of pronotum in male *V. micado* from Shenzhen. Open squares (dotted line), macropters; closed circles (black line), brachypters.

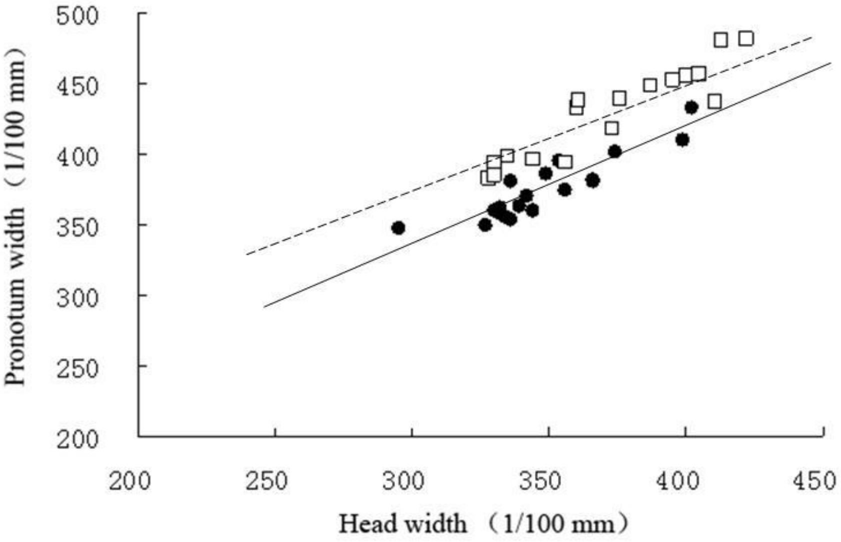


Figure 4.5.11 The width of head relative to the width of pronotum in female *V. micado* from Jilin. Open squares (dotted line), macropters; closed circles (black line), brachypters.

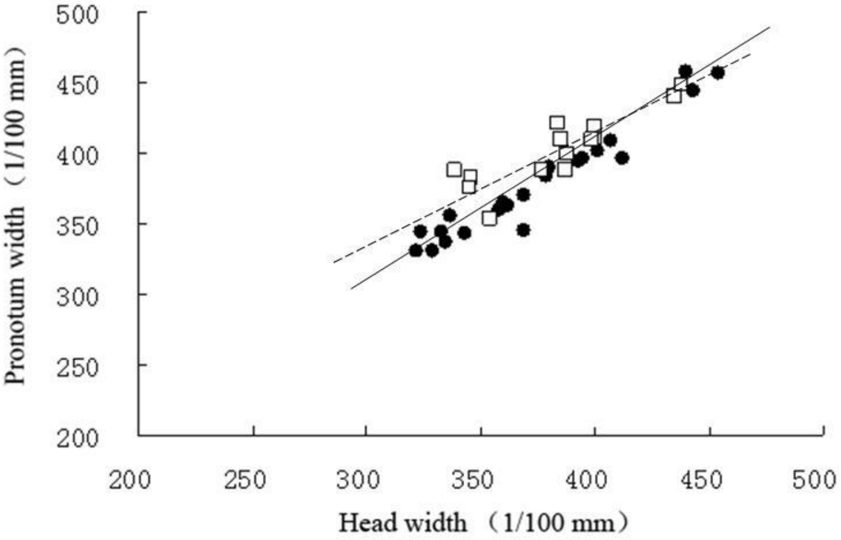


Figure 4.5.12 The width of head relative to the width of pronotum in male *V. micado* from Jilin. Open squares (dotted line), macropters; closed circles (black line), brachypters.

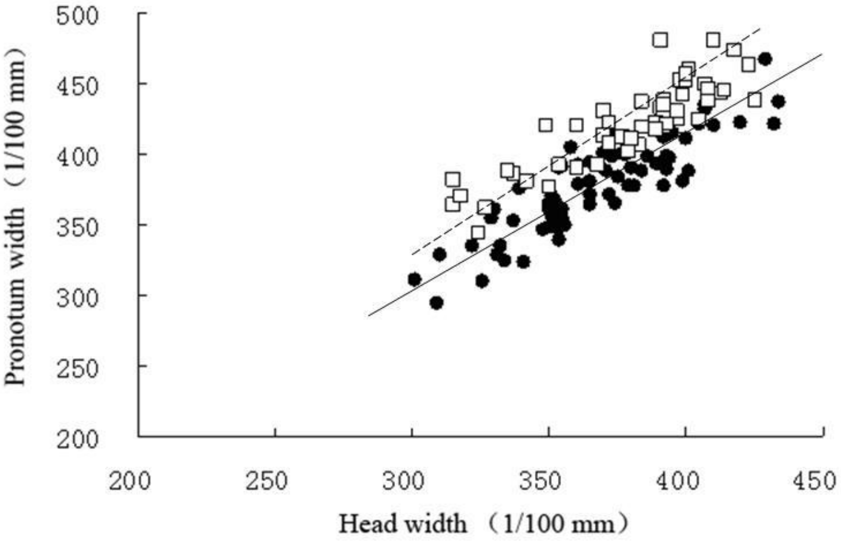


Figure 4.5.13 The width of head relative to the width of pronotum in female *V. ornatus* from Sayo. Open squares (dotted line), macropters; closed circles (black line), brachypters.

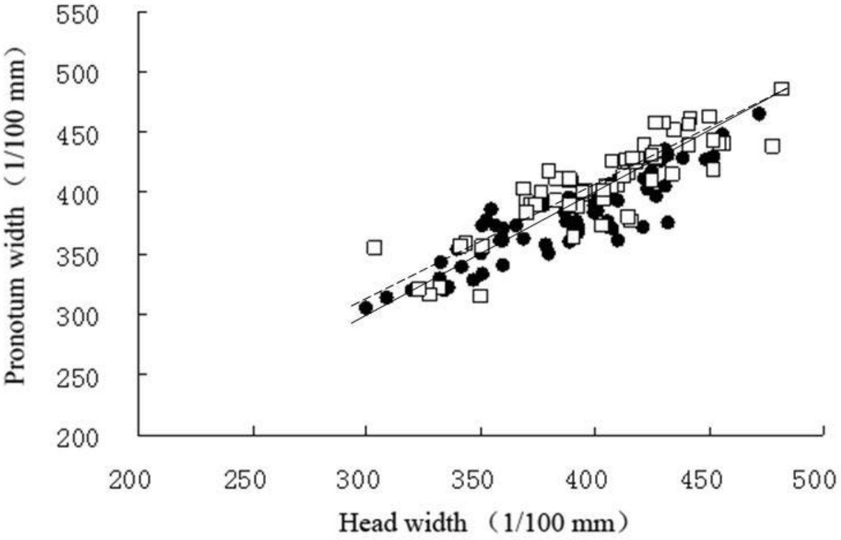


Figure 4.5.14 The width of head relative to the width of pronotum in male *V. ornatus* from Sayo. Open squares (dotted line), macropters; closed circles (black line), brachypters.

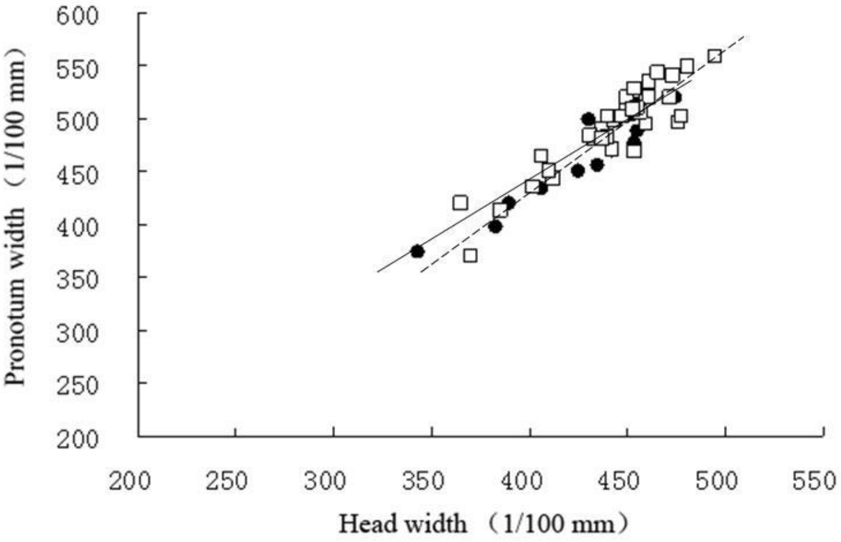


Figure 4.5.15 The width of head relative to the width of pronotum in female *V. aspersus* from Tianmu. Open squares (dotted line), macropters; closed circles (black line), brachypters.

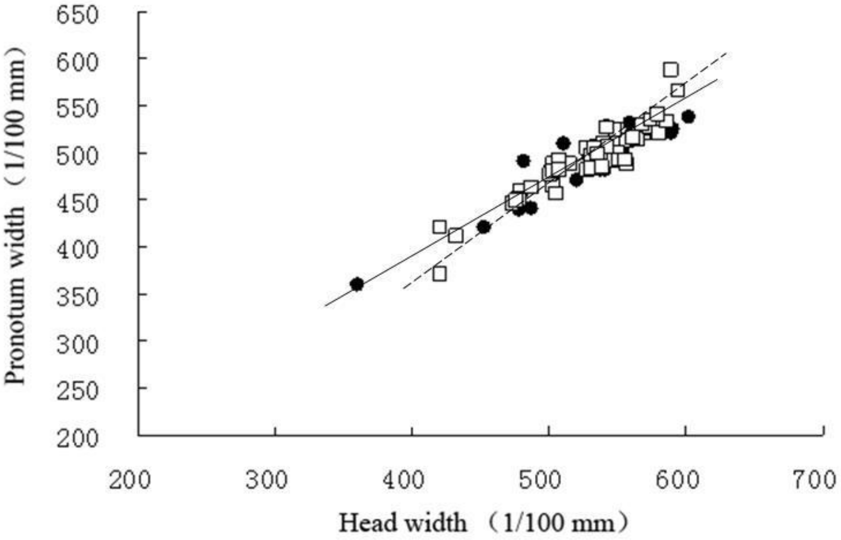


Figure 4.5.16 The width of head relative to the width of pronotum in male *V. aspersus* from Tianmu. Open squares (dotted line), macropters; closed circles (black line), brachypters.

The differences of two wing type expressed as forewing length had differences in length. Macropterous individuals of *V. micado* from Shenzhen had longer forewing than brachypterous did (females, brachypterous, 0.6441±0.06337, while macropters 0.8420±0.06308, F(42)=0.092 p<0.001; males, brachypterous, 0.8181±0.04628, while macropters 0.9125±0.06156, F(46)=0.020 p<0.001) see Fig. 4.5.17-18. The *V. micado* from Jilin had the same situation (females, brachypterous, 0.6531±0.07373, while macropters 0.8856±0.05140, F(34)=1.941 p<0.001; males, brachypterous, 0.9262±0.06169, while macropters 0.9802±0.03289, F(35.827)=4.450 p=0.001), see Fig. 4.5.19-20.

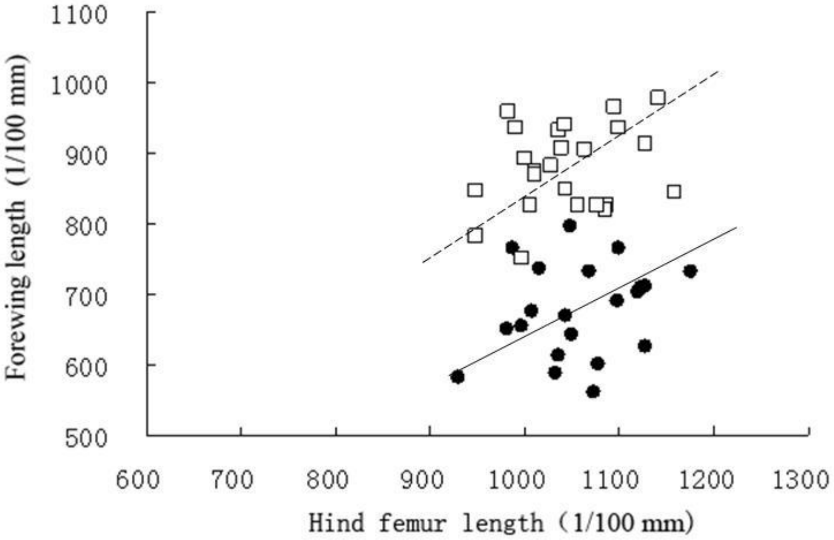


Figure 4.5.17 The length of hind femur relative to the length of forewing in female *V. micado* from Shenzhen. Open squares (dotted line), macropters; closed circles (black line), brachypters.

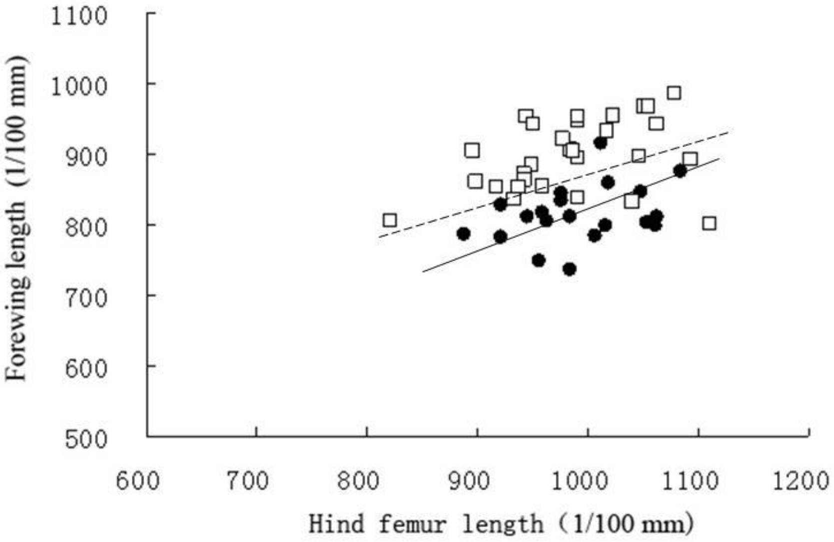


Figure 4.5.18 The length of hind femur relative to the length of forewing in male *V. micado* from Shenzhen. Open squares (dotted line), macropters; closed circles (black line), brachypters.

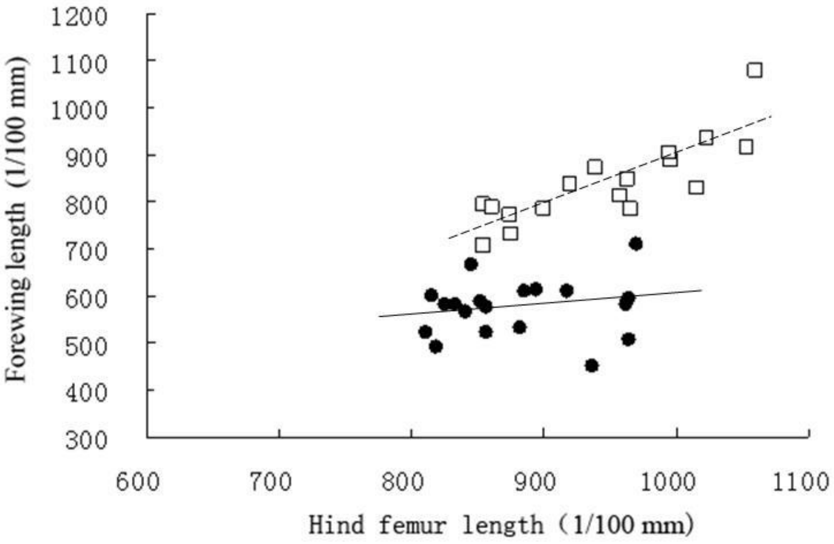


Figure 4.5.19 The length of hind femur relative to the length of forewing in female *V. micado* from Jilin. Open squares (dotted line), macropters; closed circles (black line), brachypters.

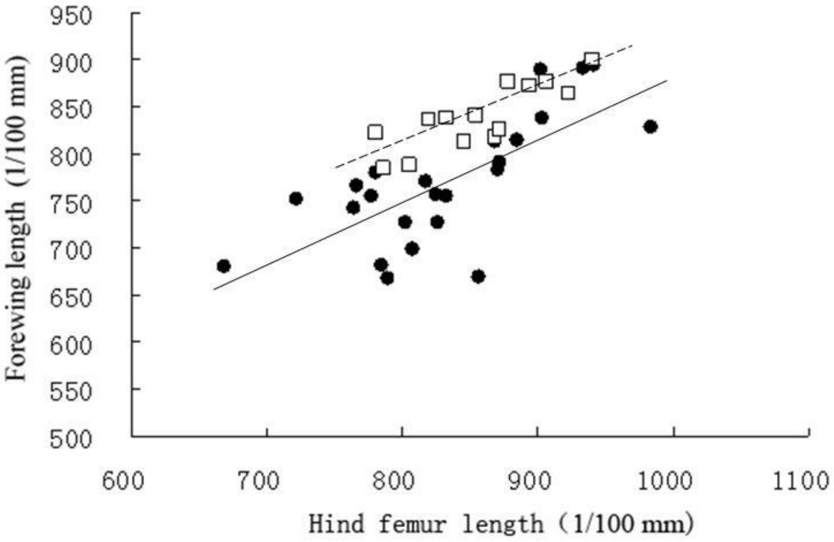


Figure 4.5.20 The length of hind femur relative to the length of forewing in male *V. micado* from Jilin. Open squares (dotted line), macropters; closed circles (black line), brachypters.

In *V. ornatus* species, they had also significant differences of this value. (females, brachypterous, 0.5243±0.08867, while macropters 0.8318±0.04837, F(114)=9.423 p<0.001; males, brachypterous, 0.8332±0.07890, while macropters 0.9185±0.05648, F(127)=4.060 p<0.001) see Fig. 4.5.21-22. And also in female *V. aspersus* (brachypterous, 0.7191±0.09661, while macropters 0.8215±0.05952, F(48)=3.909 p<0.001), but in males, there was no significant difference (brachypterous, 0.8347±0.19251, while macropters 0.8731±0.12545, F(68)=1.062 p=0.332), see Fig. 4.5.23-24.

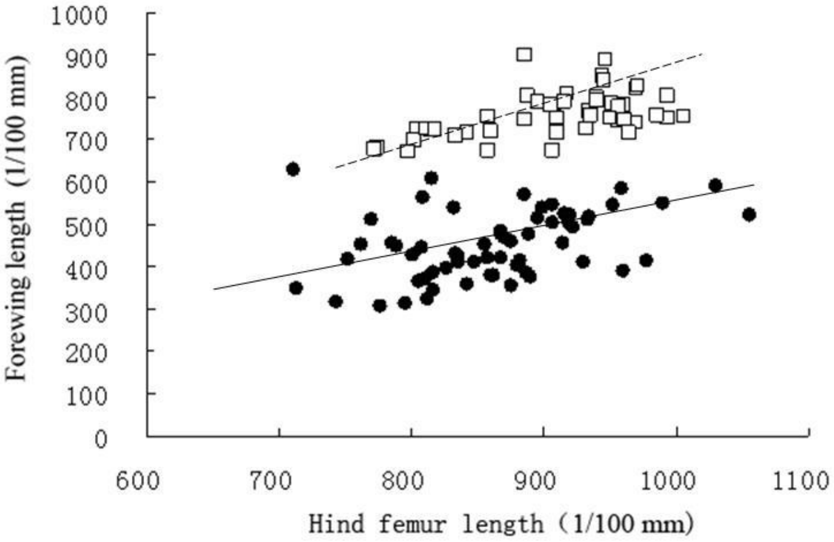


Figure 4.5.21 The length of hind femur relative to the length of forewing in female *V. ornatus* from Sayo. Open squares (dotted line), macropters; closed circles (black line), brachypters.

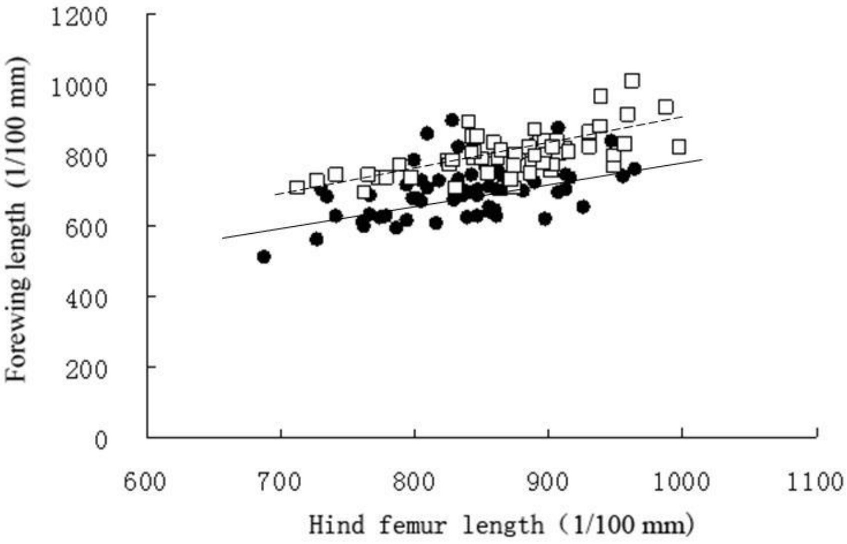


Figure 4.5.22 The length of hind femur relative to the length of forewing in male *V. ornatus* from Sayo. Open squares (dotted line), macropters; closed circles (black line), brachypters.

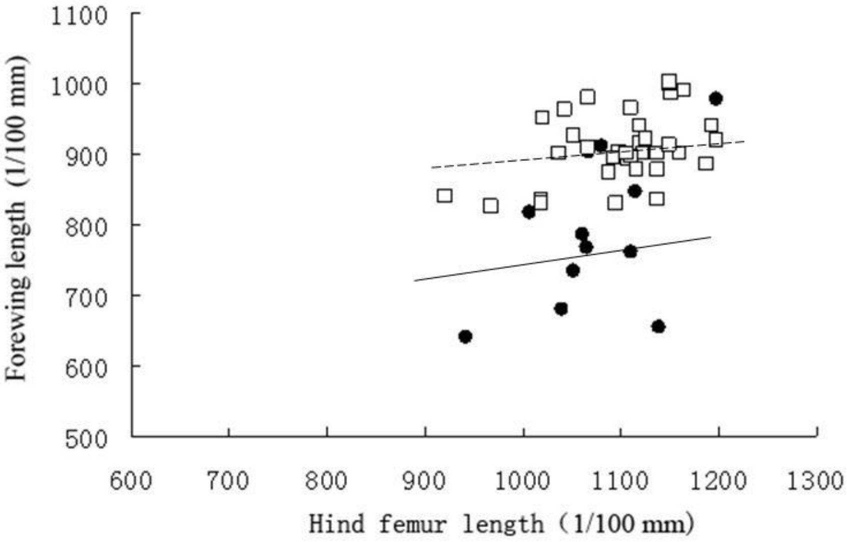


Figure 4.5.23 The length of hind femur relative to the length of forewing in female *V. aspersus* from Tianmu. Open squares (dotted line), macropters; closed circles (black line), brachypters.

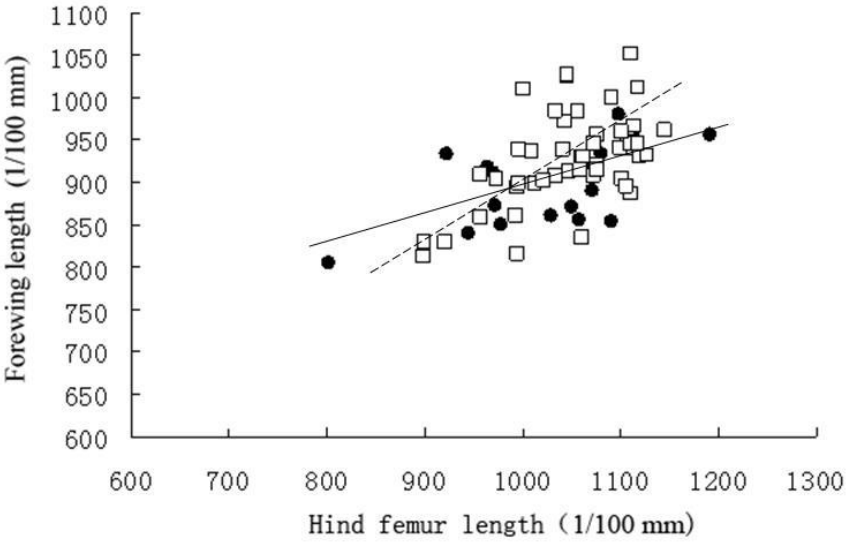


Figure 4.5.24 The length of hind femur relative to the length of forewing in male *V. aspersus* from Tianmu. Open squares (dotted line), macropters; closed circles (black line), brachypters.

Hind femur length of *V. micado* (overwinter at egg stage) was compared among different strains, see Fig. 4.6.1, Table 4.6.1. The FM increased with the decrease of latitude (male, y = -12.696x + 1363.6, R^2 = 0.734; female, y = -11.904x + 1388.9, R^2 = 0.7117), while that of *V. micado* (overwinter at nymphal stage) did not had this trend, see Fig. 4.6.2, table 4.6.2. The ovipositor length, same as hind femur, increased with the decrease of latitude, Fig. 4.6.3, table 4.6.3, but *V. micado* from Tianmu is an exception, see detail for phylogeny tree in Fig. 4.2.1-2. When compare the OV/FM value, in egg overwinter groups, this value was uniform except the ones of Tianmu and Chengdu (Fig. 4.6.4). In nymph overwinter groups, this value increased with the decrease of latitude, except for Osaka strain (Fig. 4.6.4).

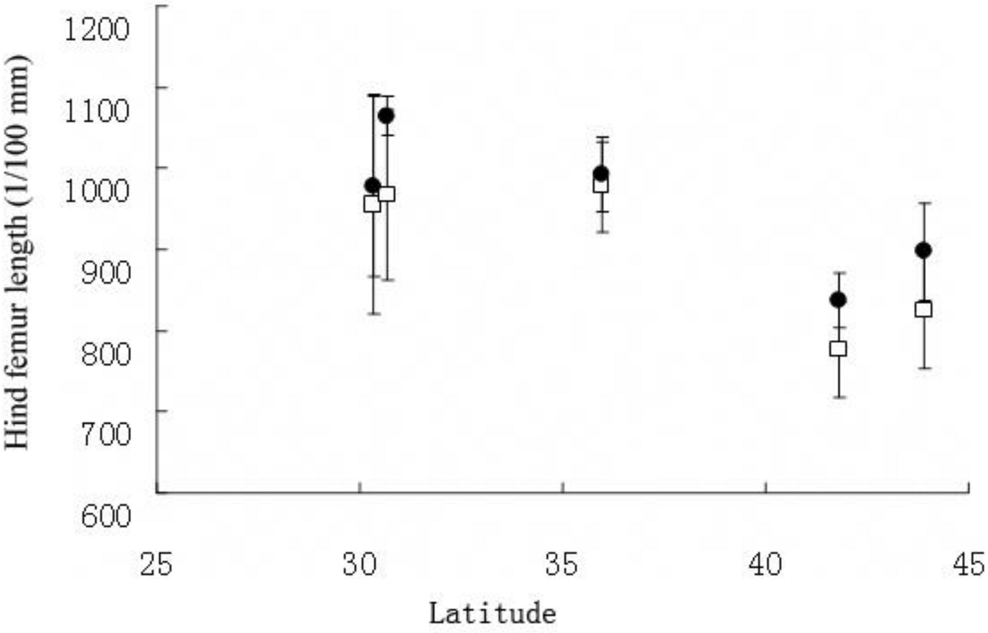


Figure 4.6.1 A geographic trend in hind femur length under LD 12:12 and 27.5℃ in *V. micado* (egg diapause type). Open squares, male; closed circles, female.

Table 4.6.1 A geographic profile in hind femur length in *V. micado* (egg diapause type) under LD 12:12 and 27.5 ℃.

	♀	♂	latitude
Tianmu	978.5±112.9	953.8±134.1	30.32 N
Shenyang	837.5±33.2	776.4±59.1	41.81 N
Jilin	897.4±60.5	825.2±72.7	43.90 N
Chengdu	1064.0±24.3	967.0±105.2	30.66 N
Korea	992.6±46.5	976.75±55.5	35.97 N

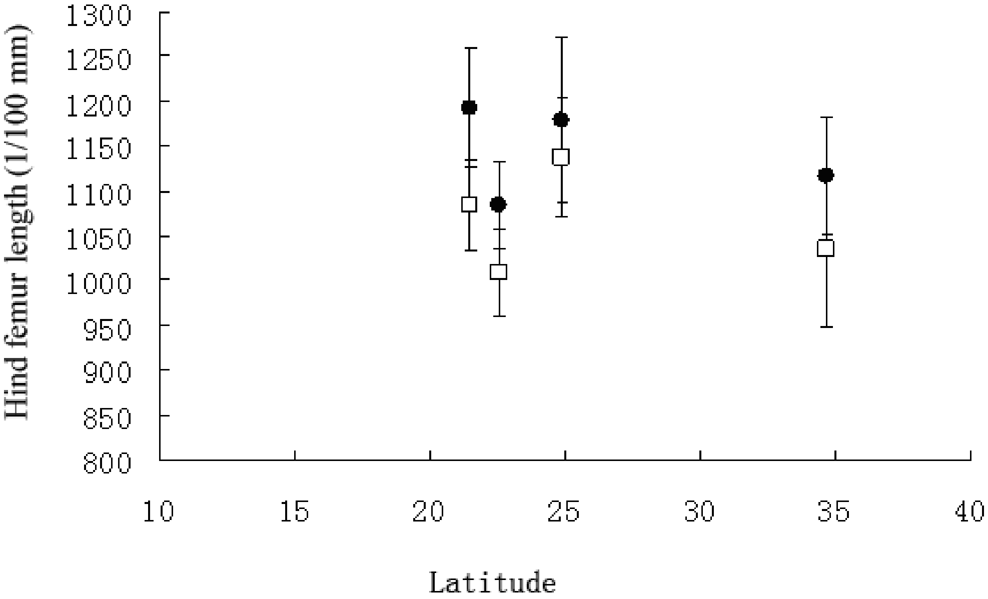


Figure 4.6.2 A geographic profile in hind femur length in *V. micado* (nymph overwinter type)under LD 12:12 and 27.5 ℃. Open squares, male; closed circles, female.

Table 4.6.2 A geographic profile in hind femur length in *V. micado* (nymph overwinter type)under LD 12:12 and 27.5 ℃.

	♀	♂	latitude
Wulai	1178.3±93.1	1137.3±66.0	24.87 N
Shenzhen	1083.8±48.9	1009.2±47.6	22.54 N
Vietnam	1192.7±67.4	1084.4±50.8	21.49 N
Osak	1117.0±65.9	1035.1±87.2	34.69 N

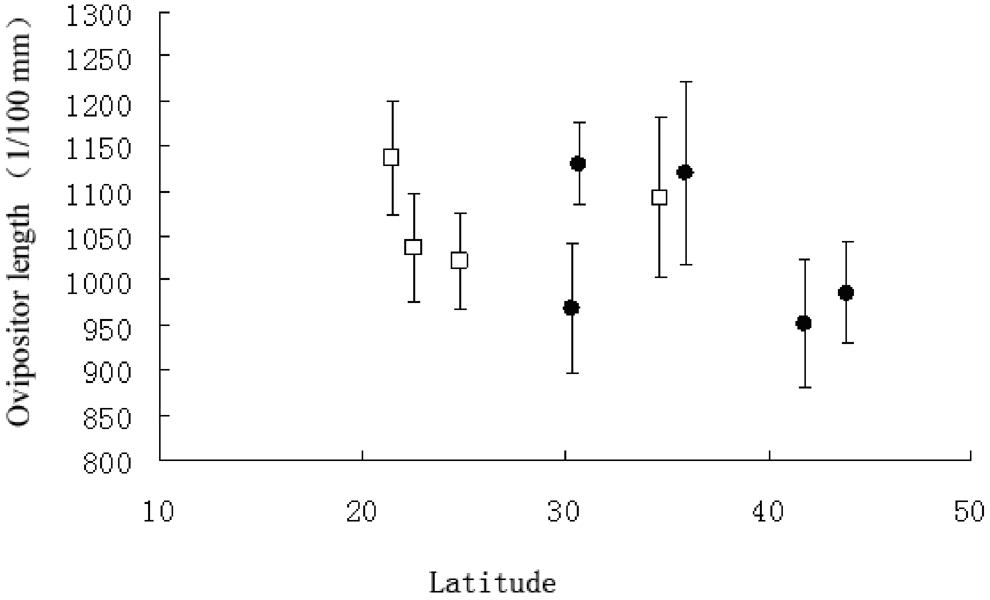


Figure 4.6.3 A geographic profile in ovipositor length in *V. micado*.under LD 12:12 and 27.5 ℃ X axis, latitude. Open squares, nymph overwinter type; closed circles, egg overwinter type.

Table 4.6.3 A geographic profile in ovipositor length in *V. micado* under LD 12:12 and 27.5 ℃.

	ovipositor±SD	latitude
Tianmu	968.8±71.7	30.32 N
Shenyang	952.0±70.7	41.81 N
Jilin	985.8±55.7	43.90 N
Chengdu	1130.0±46.0	30.66 N
Korea	1120.3±102.0	35.97 N
Wulai	1021.2±52.7	24.84 N
Shenzhen	1036.3±61.1	22.54 N
Vietnam	1136.7±63.8	21.49 N
Osaka	1091.9±89.9	34.69 N

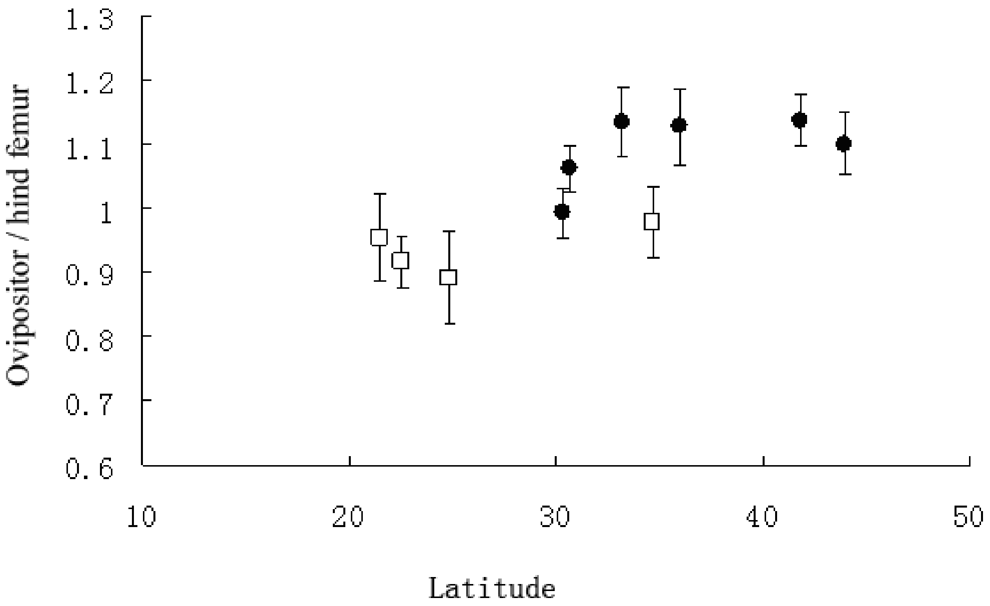


Figure 4.6.4 Geographic profile of ovipositor/hinder fumer length under LD 12:12 and 27.5 ℃ in *V. micado*. Open squares, nymph overwinter type; closed circles, egg overwinter type.

The FM was compared with OV in all four species. The dotted line (slope=1) means FM equal to OV. If the dots were on the upper side, it means that OV was longer than FM and visa versa. The dots of *V. micado* (nymph overwinter) were near the dotted line, see Fig. 4.6.5-8, *V. micado* from Tianmu was an exception, which had egg overwinter life cycle, but short ovipositor, see Fig. 4.6.9. The dots of *V. micado* (egg overwinter) were in the upper area, see Fig. 4.6.10-14. The dots of *V. aspersus* were in the lower area, see Fig. 4.6.15-16. The dots of *V. ornatus* and *V. flavifrons* were near the dotted line, see Fig. 4.6.17 and Fig. 4.6.18, respectively.

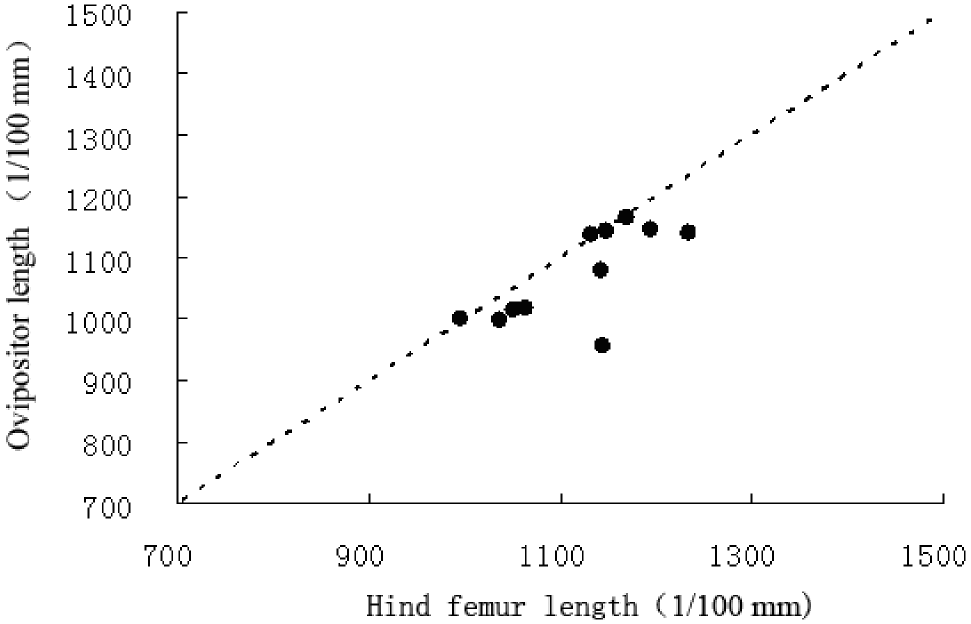


Figure 4.6.5 The length of ovipositor relative to the length of hind femur *V. micado* from Wulai. Dotted line, hind femur:ovipositor=1:1.

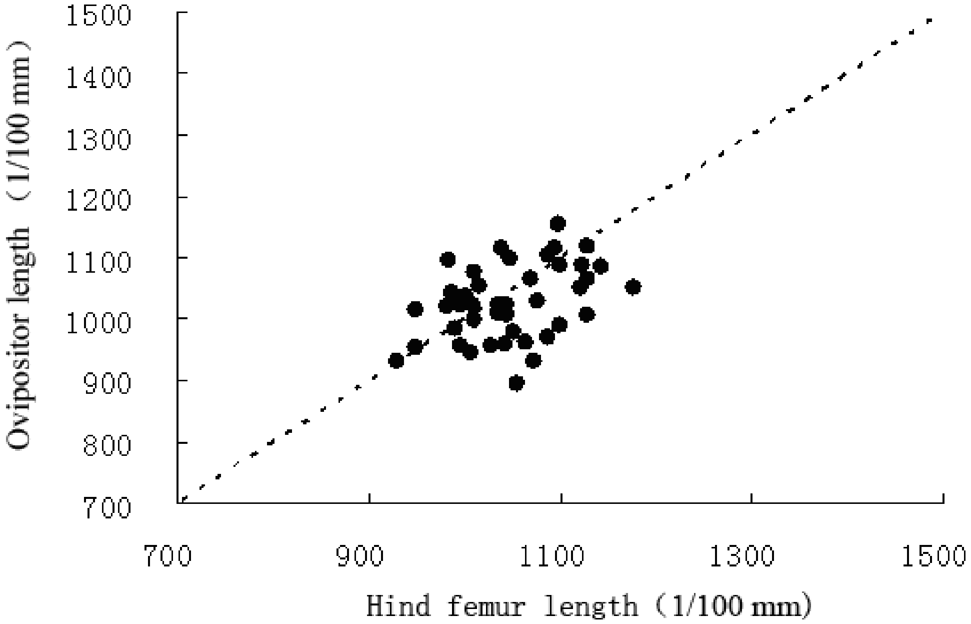


Figure 4.6.6 The length of ovipositor relative to the length of hind femur *V. micado* from Shenzhen. Dotted line, hind femur:ovipositor=1:1.

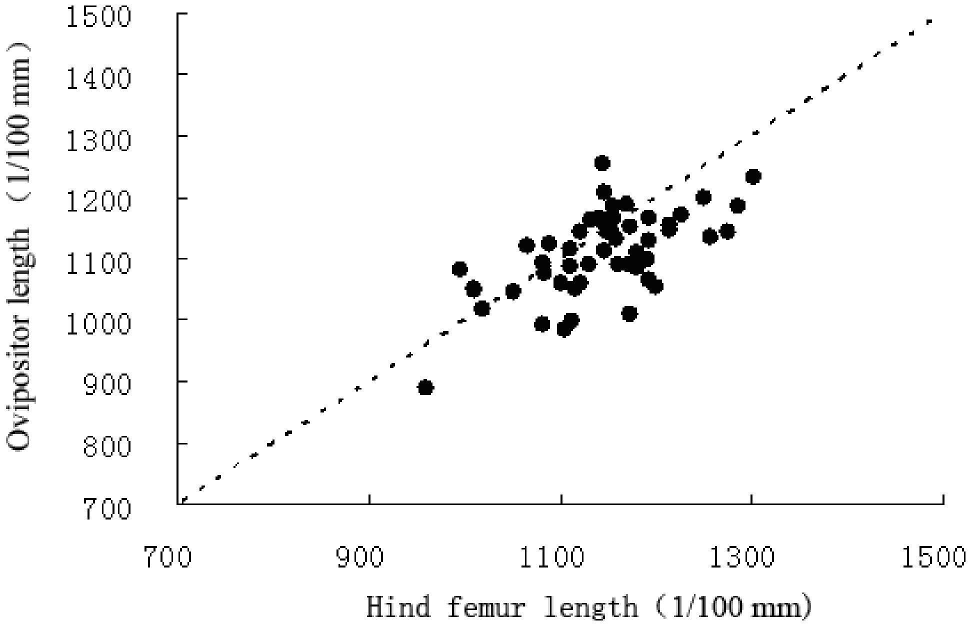


Figure 4.6.7 The length of ovipositor relative to the length of hind femur *V. micado* from Vietnam. Dotted line, hind femur:ovipositor=1:1.

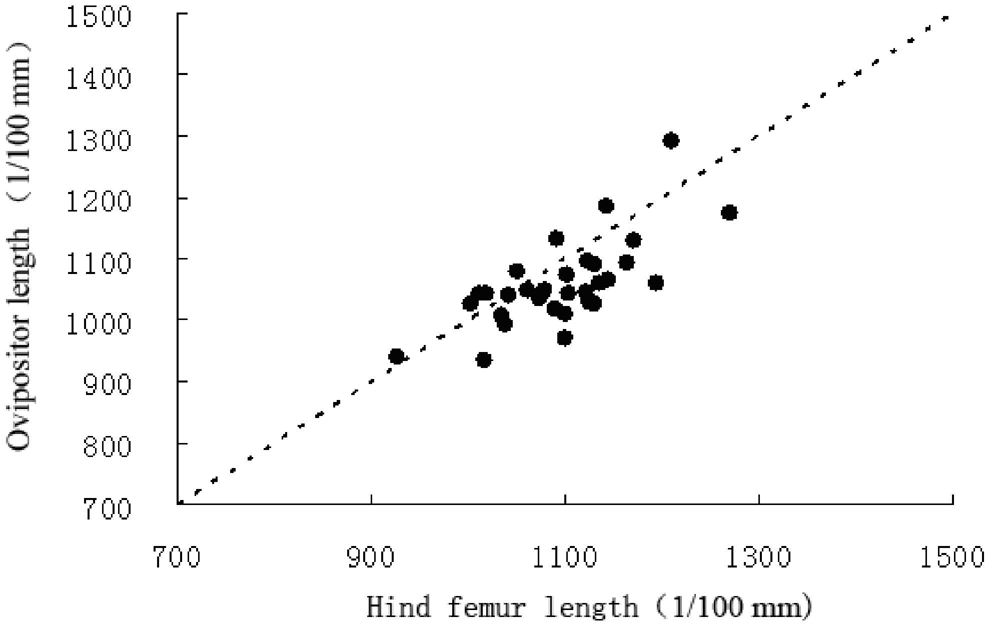


Figure 4.6.8 The length of ovipositor relative to the length of hind femur *V. micado* from Osaka. Dotted line, hind femur:ovipositor=1:1.

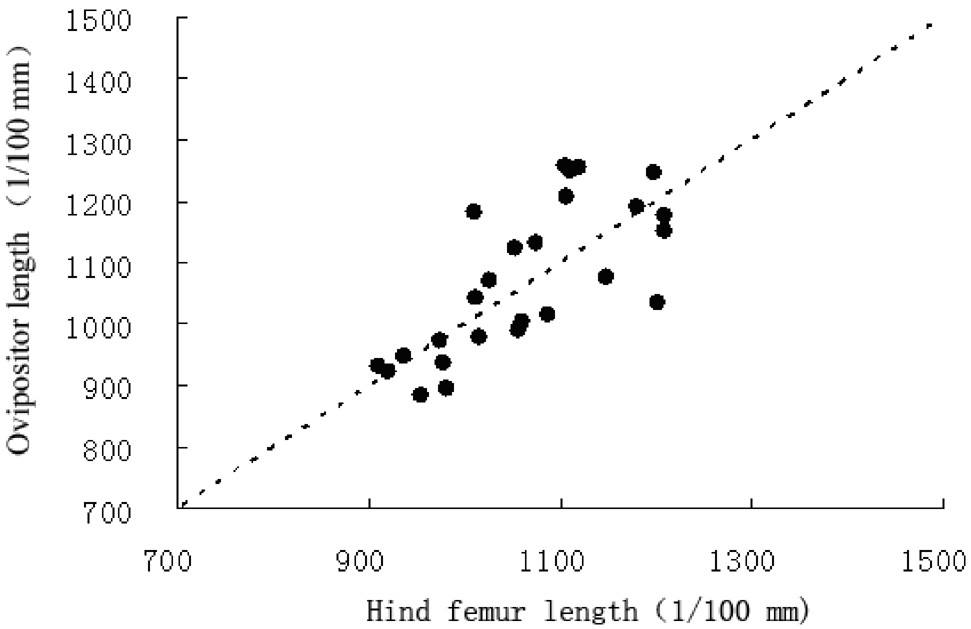


Figure 4.6.9 The length of ovipositor relative to the length of hind femur *V. micado* from Tianmu. Dotted line, hind femur:ovipositor=1:1.

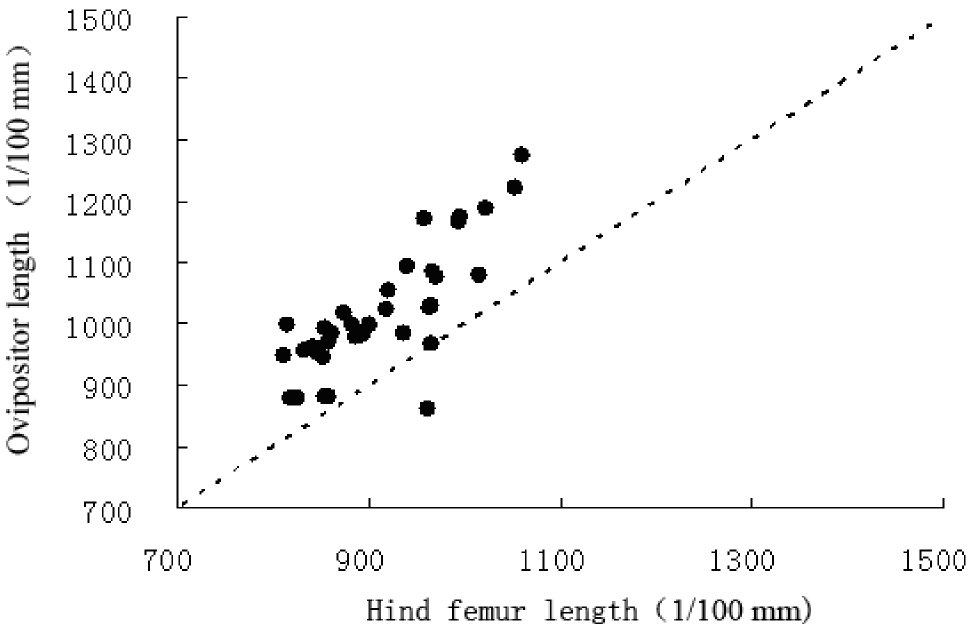


Figure 4.6.10 The length of ovipositor relative to the length of hind femur *V. micado* from Jilin. Dotted line, hind femur:ovipositor=1:1.

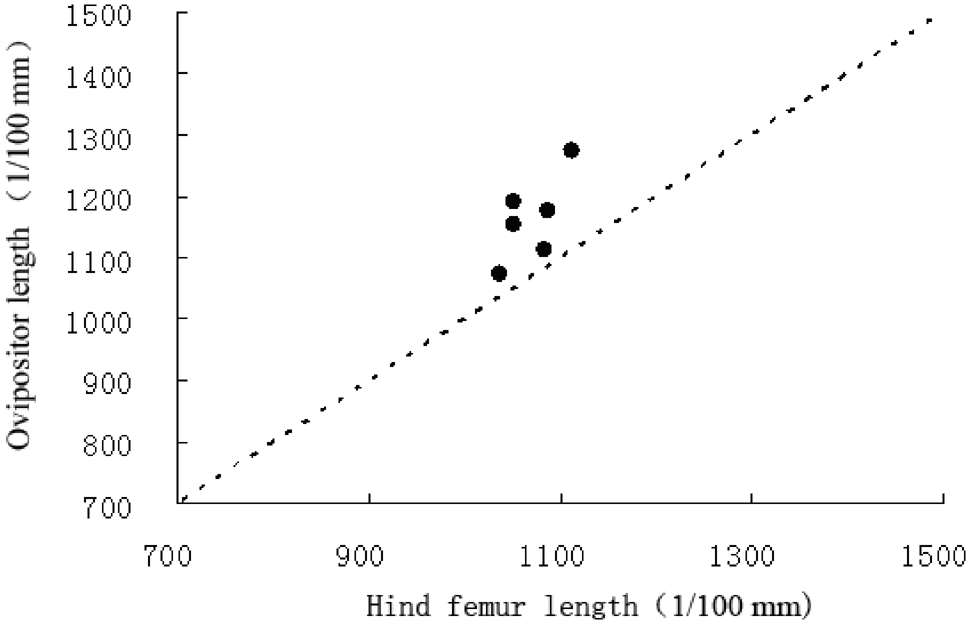


Figure 4.6.11 The length of ovipositor relative to the length of hind femur *V. micado* from Chengdu. Dotted line, hind femur:ovipositor=1:1.

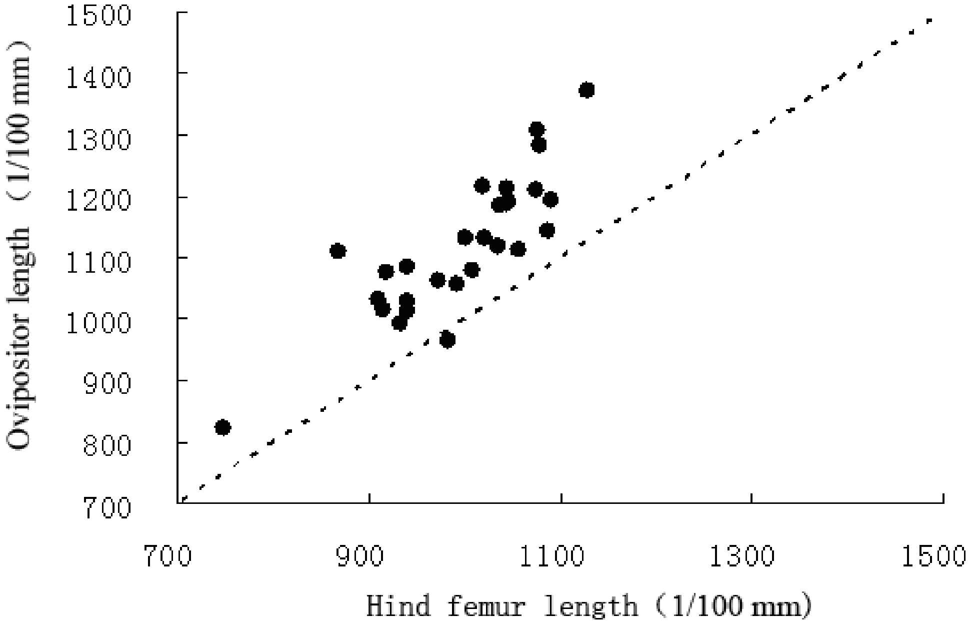


Figure 4.6.12 The length of ovipositor relative to the length of hind femur *V. micado* from Korea. Dotted line, hind femur:ovipositor=1:1.

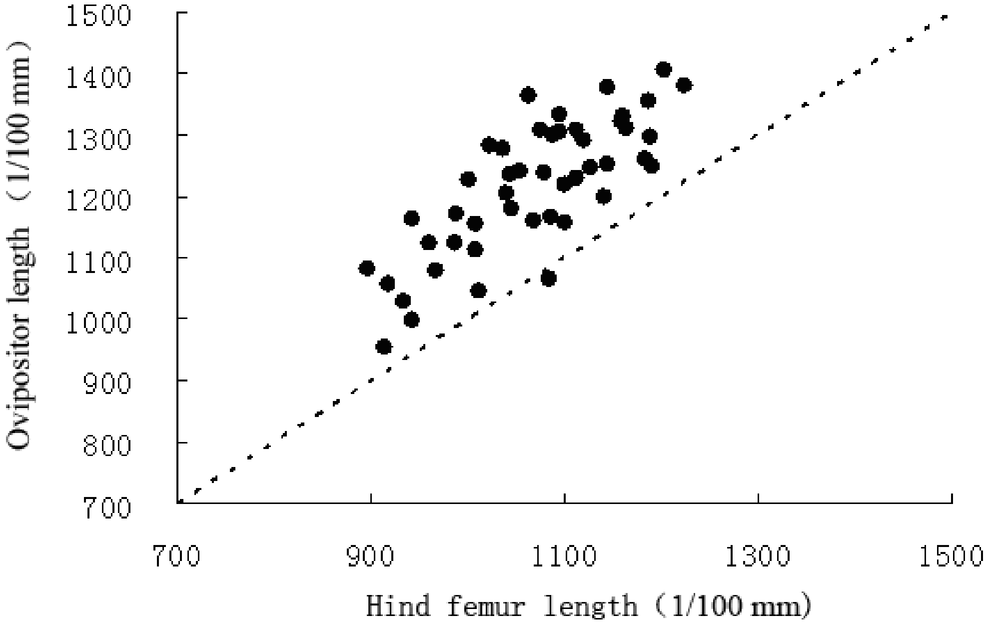


Figure 4.6.13 The length of ovipositor relative to the length of hind femur *V. micado* from Newnan. Dotted line, hind femur:ovipositor=1:1.

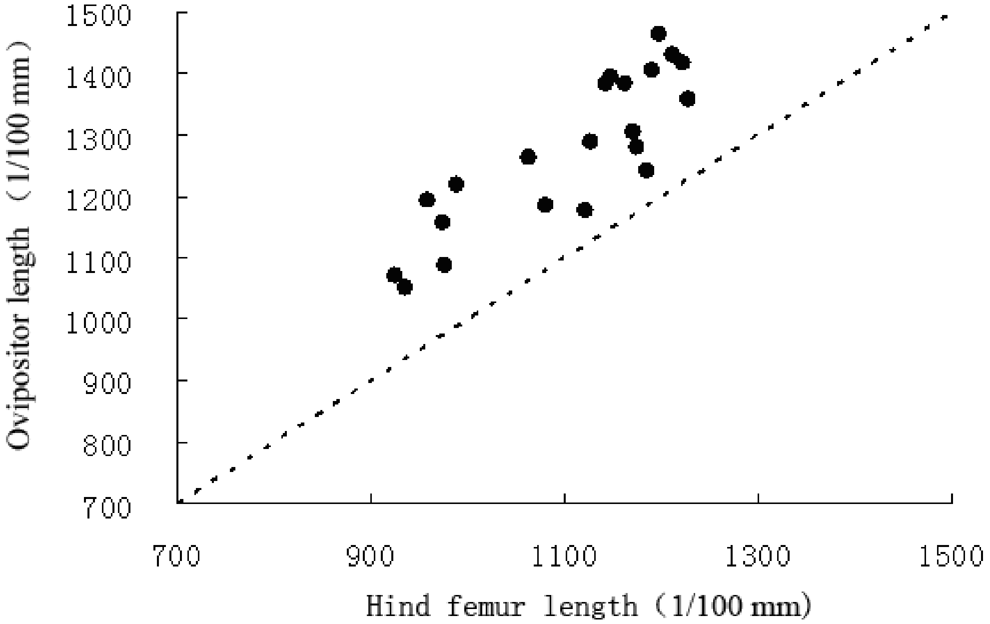


Figure 4.6.14 The length of ovipositor relative to the length of hind femur *V. micado* from Nashville. Dotted line, hind femur:ovipositor=1:1.

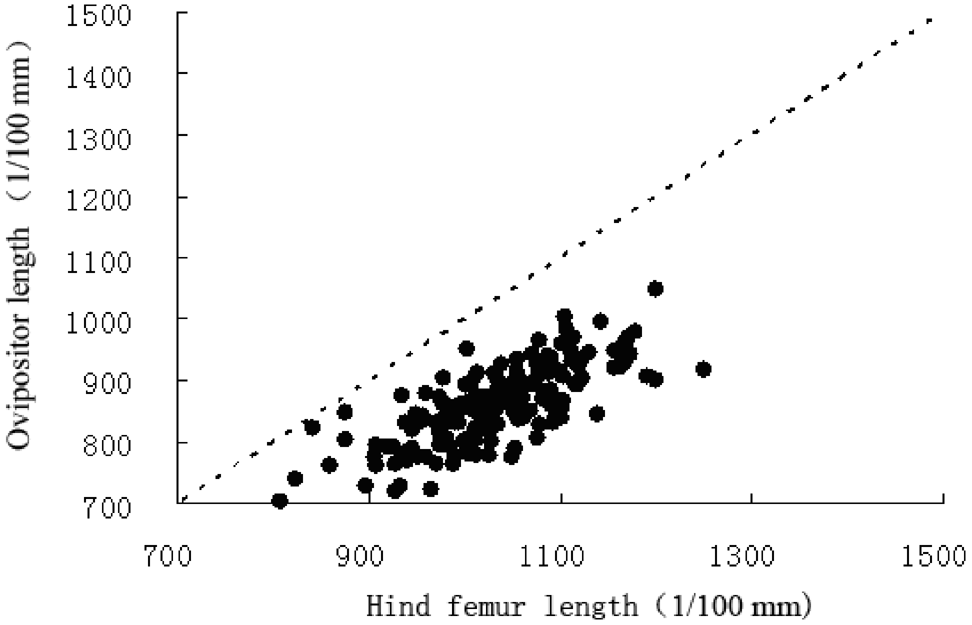


Figure 4.6.15 The length of ovipositor relative to the length of hind femur *V. aspersus* from Vietnam. Dotted line, hind femur:ovipositor=1:1.

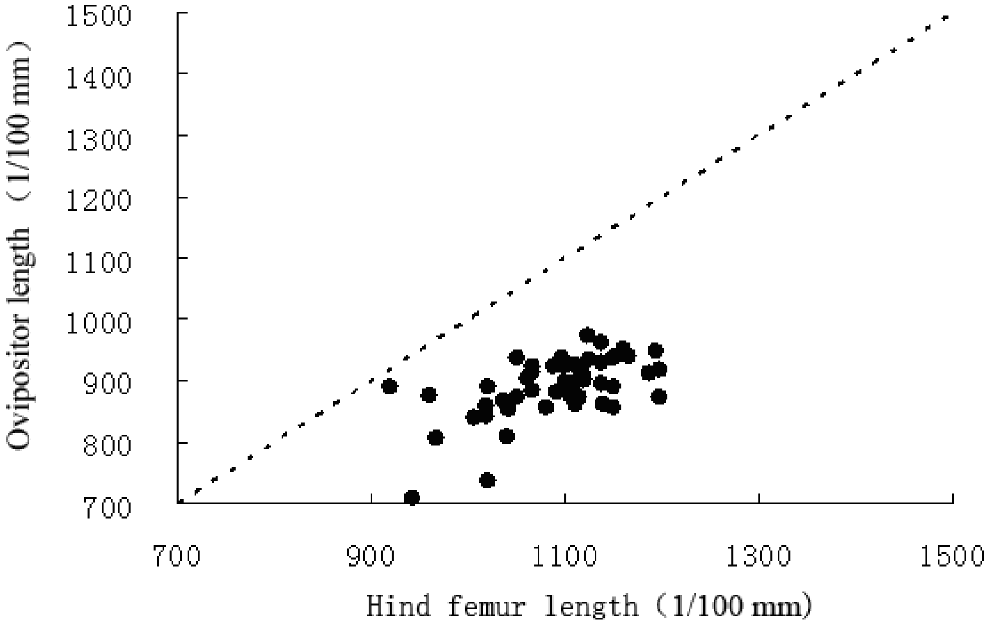


Figure 4.6.16 The length of ovipositor relative to the length of hind femur *V. aspersus* from Tianmu. Dotted line, hind femur:ovipositor=1:1.

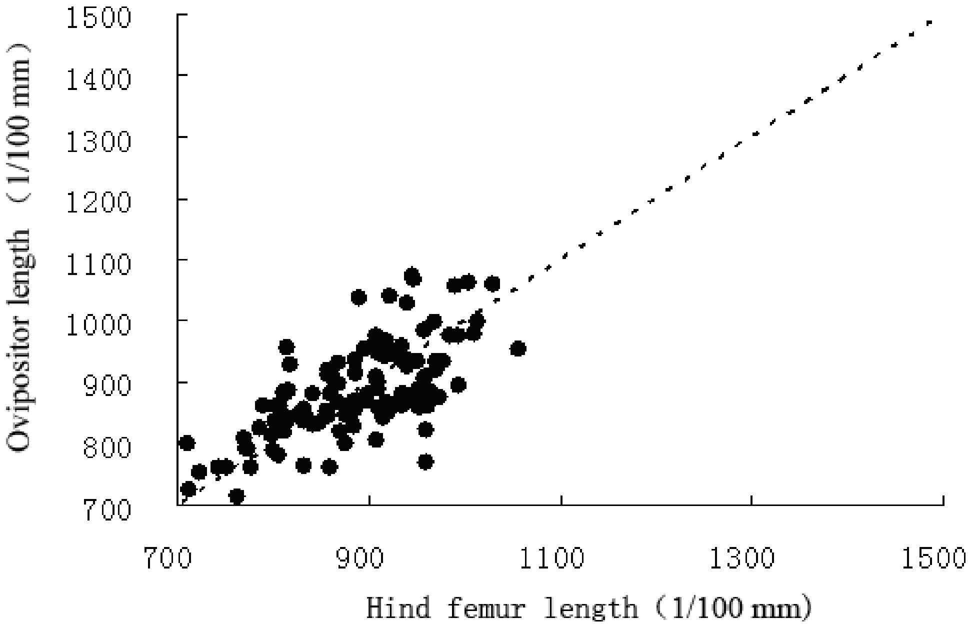


Figure 4.6.17 The length of ovipositor relative to the length of hind femur *V. ornatus* from Sayo. Dotted line, hind femur:ovipositor=1:1.

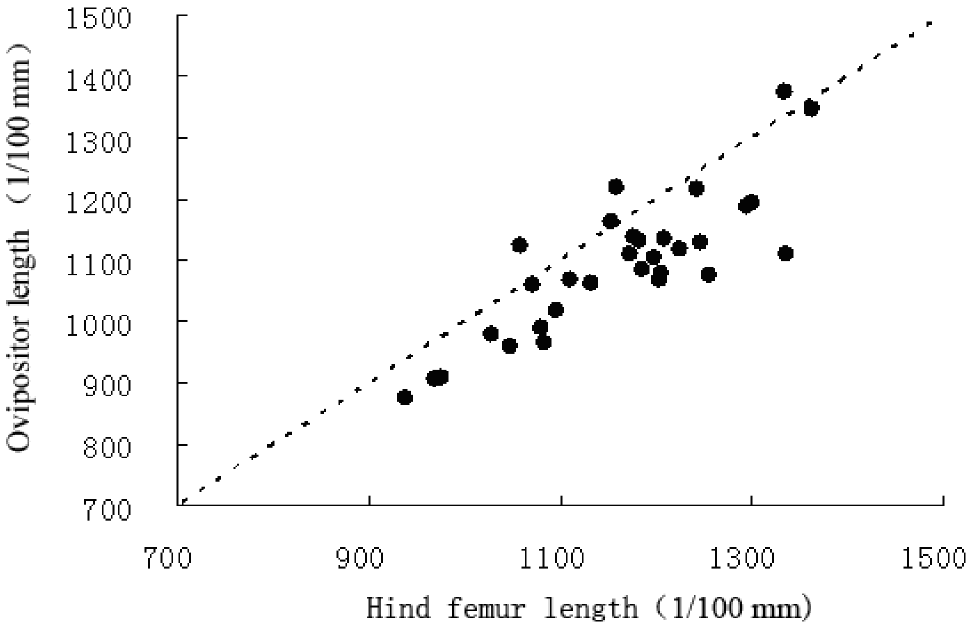


Figure 4.6.18 The length of ovipositor relative to the length of hind femur *V. flavifrons* from Vietnam. Dotted line, hind femur:ovipositor=1:1.

4.4 Discussions

Insects sometimes develop peculiar body proportions. For example, *Cyrtodiopsis whiter* (Diptera: Diopsidae) has eyes on the ends of long stalks; *Acrocinus longimanus* (Coleoptera: Cerambycidae) and *Cheirotonus jansoni* (Coleoptera: Euchirinae) have extra long fore-legs. *Cyclommatus imperator* (Coleoptera: Lucanidae) has long and serrated mandibles. *Golofa porteri* (Coleoptera: Scarabaeidae) and *Enema pan* (Coleoptera: Scarabaeidae) have horns on their thorax. They have the most bizarre looking organisms in the animal world (Emlen & Nijhout, 2000). However, in some species, not all individuals express such exaggerated morphology. Usually big individuals are prone to have these exaggerated traits and body size is affected by environmental factors, such as nutrition, temperature, photoperiod, population density and humidity. Crickets are hemimetabolous insects. They become bigger after each molt. How much food they eat during nymphal stage will largely determine how big they will be.

In the genus of *Velarifictorus*, body parts have the relative same proportion to total body size. That is to say, smaller individuals have relatively narrower head or shorter leg and vise versa. However, the ratio of these parts is constant. For example, value head width/hind femur length is constant, despite of body size. If there is significant difference of these values, it should not be individual differences, but genetic differences. These genetic differences exist not only in genus level, but also in specie level. According to the result of correlation, head width, pronotum width, hind femur length and ovipositor length have linear dependent. Forewing is longer in males in all four species. Hindwing length did not show any relationship with other body parts or sex in short wing form groups, probably because of its useless for crickets.

For the genus *Velarifictorus*, both these factors exist. Wing type of *V. aspersus* did not show difference under LD 16:8 or LD 12:12. The macropterous percent of *V. aspersus* from Tianmu was 70% under two photoperiods and it was equal in both sexes. This indicated that the initial gene frequency, which decides the wing type, affected the wing type of the offspring. On the other hand, *V. ornatus* showed great differences under different photoperiod. When they were reared under LD 12:12, no macropterous individuals appeared for both sexes. When they were reared under LD 16:8, macropters appeared. The macropterous percent of *V. ornatus* was similar in both sexes, but different strains show great differences. This is probably caused by the initial gene frequency, which is partly decided by their geographical location. For *V. flavifrons*, there is no macropters in any rearing condition. One possibility is that there is no macropterous individual in the wild either. Another possibility is that the initial parents collected did not carry the “macropterous” gene.

For *V. micado*, macropterous percent was greatly affected by photoperiod. Under LD 12:12, both females and males have relatively low percent of macropters, and this value is similar between both sexes. When reared under LD 16:8, all strains show much high percent of macropters. The hindwing will shed after a period of time, while brachypterous never shed. That *V. micado* adjusts wing type closely to photoperiod seems to be one of their success factors and makes them a wide distribution.

Emerging time macropterous and brachypterous individuals did not have difference in all three *Velarifictorus* spp. This seems to refute the conclusion above. Because macropterous individuals should spend more time for migration and they should emerge early. I consider that constant photoperiod confused the cricket system. In my experiment, all crickets were reared at 27.5 ℃, and under constant photoperiod, either 16:8 or 12:12. While in the wild, day length will change gradually. In *Modicogryllus siamensis*, macropterous females laid eggs later than brachypterous ones did (Tanaka, 1993). This indicated that macropterous females should emerge early than brachypterous ones. They allot energy to emerge fast, because if they emerge as late as brachypterous female, they do not have enough time to migrate and mate. This should be the advantage that they could colonize new habits and had more offspring. If they did not find new habits and migrated to some places where has been already colonized, the macropterous females did not have any advantage compared with local females.

I also compared the morphology differences between two wing type groups. Although head width and pronotum width did not show any significant differences in both sexes, the value of HD/PR did have significant differences. Macropterous ones have low HD/PR value compared with brachypterous ones. This could be caused by small head or widen pronotum. When I compared HD/FM value, no significant differences was found. The pronotum widened can come to a conclusion. Because flying needs strong muscle in thorax, which cause the wider pronotum in macropterous individuals (Tanaka, 1993).

The second difference is forewing length. Macropterous ones have obviously longer forewing in females. There are two possibilities. One is that there is a protect function for hindwing, because hindwing is weak and translucent. The forewing is full of pigment and harder than hindwing. Another possibility is that forewing and hindwing have some common genes for controlling their development. Because when the forewing length of males was compared, macropterous males also have longer forewing than brachypterous, though the different is not so large. This indicates that common control genes hypothesis is probably right.

Most crickets lay eggs into soil or plant stem to prevent their eggs from predators. If one species maintains a univoltione life cycle, they have less time to garner resources and to reach the diapause stage in cold area, while they are living in warm area, they have more time and become bigger (Masaki, 1967). When a species with a wide latitudinal range and has a transition in voltinism, the body size will result in a sawtooth pattern (Walker & Masaki, 1989, Masaki, 1979). Masaki documented such a pattern of *Dianemobius mikado* and *D. nigrofasciatus*. For *Velarifictorus* spp., these four species lay eggs into soil. If these species live in tropical areas, ovipositor length is not such an important thing, because eggs hatch fast. When they colonized northern areas, ovipositor length changed with latitude, and diapause intensity also following changed. For the *V. micado* (egg overwinter), their ovipositors are longer than that of

V. aspersus, *V. ornatus*, *V. flavifrons* and even than that of *V. micado* (nymph overwinter). The length increased with the latitude. However, this result seems to be opposite of the relationship of ovipositor length and temperature. Because the temperature decreases with the increase of latitude and females should lay their eggs deeper for prevent them from frozen injure. As I mentioned above, crickets keep constant ratio among body parts. Since the short warm period day in Northern areas, northern individuals are usually smaller compared with southern ones, which are both univoltine and overwinter at egg stage. Thus, I calculated the value of OV/FM for comparing the relative length. It can be found that this value was not similar in North group and South group. The Tianmu and Chengdu group overwinter at egg stage, while their value of OV/FM was lower. If Tianmu strain can be explained by the genetic difference in Fig. 4.2.2, Chengdu strain is difficult to explain. Gene drift may happen between north and south groups. Except these, North and South group do exist significant difference in the value of OV/FM, but not in OV length. North group should have longer ovipositors, but limited by their body size, their ovipositor are not longer than that of South strains. Another possibility is that *Velarifictorus* spp. females will dig soil and stretch their abdomen into that hole, which can make the eggs lay deeper by my observation.

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Chapter 5 Conclusions

Crickets are common insects, widely distributed creatures. They have been used as good materials for researching phylogeny. The COI gene of different cricket species have been sequenced. This investigated the COI sequence of *V. micado* collected from 33 areas from Indochina Penisula to Heilongjiang as well as three localities in US. The phylogenetic tree showed that *V. micado* are recently derived species, because it had some mutations different from other species and located at the apical of the tree. The phylogeny of *V. micado* showed that there were mainly four different haplotype groups. The common ancestor was from Indochina peninsula, because their 470th position of amino acid was different from *V. micado* stocks, but the same as the other *Velarifictorus* spp. The ancestor group was non-diapause species and their eggs hatched without delay. They live in tropical areas and have no different photoperiod responses. Individuals from south China also were included in this group. One *V. micado* group is distributed in Taiwan and Southern Japan. Their eggs hatch without delay, but nymph develop slowly in short photoperiod. That indicates that they probably overwinter at nymphal stage and emerge in the next year. They can be uni- or bivoltine and even multivoltine according to local climate. Another group colonized the Northern China and Japan. Nymphs develop relatively fast compared with the group mentioned above under long and short photoperiod condition, and the individuals reared under LD 12:12 grew faster. This indicates that this group emerged synchronously according to photoperiod, which can make them mate and lay eggs before winter comes. Because winter in North China is so severe that nymphal stage overwinter strategy can not make them survive here unless their eggs enter diapause before winter. Two *V. micado* groups coexist in Japan displacing each other. The rest one is East China groups and it only exist in China. They are univoltine and whether they can mate with North group is unknown. *V. micado* is introduced to USA since 1959. Because the holotype is from Japan and they have the digging habit, *V. micado* was named as Japanese burrowing cricket. Whether they were from Japan has not been certified. Three strains from Newnan, St. Louis and Nashville from USA was collected and sequenced. All of them have egg diapause. By sequencing the COI gene, Newnan individuals was similar to East China group, while St. Louis and Nashville individuals were most similar to North group. This showed that *V. micado* in USA have distinct origins.

The different photoperiodic responses of *V. micado* reflect the different life cycles. The results indicated that the nymphal-diapause form showed slower nymphal development under LD 12:12 than under LD 16:8 and this retardation increased with original latitude. The egg-diapause form showed the opposite trend, namely slower development under LD 16:8 than under LD 12:12. These features help synchronize the timing of the overwintering stage before winter comes. While *V. ornatus*, *V. aspersus* and *V. flavifrons* have the similar responses to the *V. micado*, which collected from different areas. *V. ornatus* from Aridagawa is univoltine species and overwinter at nymphal stage, which is similar to *V. micado* from Osaka. *V. aspersus* from Tianmu is univoltine species and overwinter at egg stage, which is similar to *V. micado* from Tianmu. *V. aspersus* from Vietnam develop almost continuously and it is similar to that of *V. micado* from Vietnam. The same natural selection makes *Velarifictorus* spp. develop similar life-cycle. *V. micado* developed two life-cycle in two close areas is a special and rare example of two colonization separated by sea. The different life cycle makes them have no chance of gene flow. If one strategy were better than the other and it would exclude the latter.

The value of ovipositor/hind femur length illuminated the differentiation between *V. micado* of different overwinter stage. *V. micado* overwinter at egg stage had high value of OV/FM, while those overwinter at nymphal stage had low value. This was thought to be the evidence of two different species by the Japanese taxonomies. However, *V. micado* from Tianmu has short ovipositor and lay diapause eggs. This supplied the link between two strains above. The Tianmu strain have the similar emerge rate as Vietnam strain under LD 16:8, while the emerge rate sped up under LD 12:12 in Tianmu strain but slowed down in Vietnam strain. The change of emerge rate under short photoperiod was affected by nature selection. Another possibility is that Tianmu strain was biovoltine in past warm period, which makes it short ovipositor.

Adaptive radiation also is related to fast migrate. In my study all *Velarifictorus* spp. have two wing types, macropterous and brachypterous, except *V. flavifrons*, but they do not have the same mechanism. The macropterous percent of *V. aspersus* is constant, which is not affected by different photoperiods. *V. ornatus* individuals from

Sayo are all brachypterous in LD 12:12 and developed many macropters under LD 16:8, which shows an strong environmental effect. Macropters of *V. micado* shows both environmental and genetic effect. When reared under LD 12:12, few individuals developed longwings, while the macropterous percent rapid increased under LD 16:8. There are differences of the percent from different areas, which showed the genetic factors. Some macropters had wider pronotum, which includes strong muscle for flying. Dimorphism also exists in forewing length. Macropterous individuals have longer forewing in all species, except male *V. aspersus*.

Abstract

Crickets are widely distributed omnivorous insects, but their morphology is simple. Therefore life cycle and behavioral characteristics have been extensively used for taxonomy. Also recent advent of molecular taxonomy has shown its power in phylogeny. This investigation challenged for classification of taxonomic status and speciation mechanism in *Velarifictorus micado* species complex. The COI gene of *Velarifictorus* species were sequenced, especially in *V. micado* collected from 33 areas in Asia. The phylogenetic tree showed that *V. micado* are recently split species. There were mainly four different haplotype groups in *V. micado*. The common ancestor resides in Indochina peninsula and they are non-diapause species whose eggs hatch without developmental interception. One *V. micado* group is distributed in Taiwan and Japan. Their eggs develop without interception, but nymphs develop slowly under short photoperiod. That indicates that they probably overwinter at nymphal stage and emerge in next year. They can be uni- or bivoltine and even multivoltine according to the locality of origin. Another group colonized North China and Japan. The nymphs develop relatively fast compared with the group mentioned above, and the individuals reared under short photoperiod grow faster than under long photoperiod. This indicates that short photoperiod accelerate development to emerge synchronously before winter comes. Adults lay eggs before winter and their eggs enter diapause to overwinter and hatch in the spring of the next year. Because of these different life cycles, the reproduction should b seasonally isolated in Japan. This allows *V. micado*

and its sibling species to coexist.

V. micado is also an introduce species in USA since 1959. Three stocks from Newnan (Georgia), St. Louis (Missouri) and Nashville (Tennessee) were collected and their COI sequences were determined. Although the original stock collected in Mobile Mississippi was named the Japanese burrowing cricket, the results indicates that the Newnan stock was more closely related to Chinese stocks.

The different photoperiodic responses of *V. micado* reflect the different natural selection on different strains. Different photoperiodic responses provide bi-stability in their life cycles and hybridization between the two independent stable groups destroys life cycle.

The value of ovipositor/hind femur length illuminated the differences between *V. micado* of different overwinter stages. The *V. micado* overwinter at egg stage had higher OV/FM values, while nymphal overwinters had low values. All *Velarifictorus* spp. had two wing forms, macropters and brachypters, except *V. flavifrons*, but they do not have the same mechanism, since the macropterous percent in *V. aspersus* is constant, which is not affected by different photoperiods. *V. ornatus* individuals are all brachypterous in LD 12:12 and developed macropterous individuals under LD 16:8, which shows an strong environmental effect. When *V. micado* reared under LD 12:12, few individuals developed longwings, while the macropterous percent increased under LD 16:8. There were differences of the percent from different areas, which showed the genetic factors.

Acknowledgements

During my PhD study, I owe a debt of gratitude to many people.

I thank Prof. Takeda Makio for his advices. He is an erudite professor in insects and gave me numerous suggestions. He widened my vision of entomology. I was just a taxonomist when I graduated as Master Degree student. However, now I acquire the knowledge of phylogeny, sexual behavior, growth of allometry and molecular technology.

I appreciate the help in PCR technology from Drs Muhammad Tufail and Sakamoto Katsuhiko.

I have also benefited greatly from many of students in our lab. They are Li Xiaoting, Nagaba Yoshiki, Kobayashi Takeru and Dao Bach Khoa. I especially appreciate Wang Qiushi for her helps in both life and lab.

I thank Drs Ma Libin, Hu Jiayao, Tang Liang, Lin Yixiang, Zhang Tao, Chang Wenhao, Luo Yi, Cheng Muhua, Li Maowei, Wu Shihai, Zhu Xiaoyu, Jiang Ye and Shen Yikan for collections.

I thank my parents for their support. I was greatly supported by them. They always respect my choice.

I thank my wife, Wu Wenying. I have a vivid recollection of insect collection every year in China. Without her supports, I could not finish this work.