

CHAPTER 3

MATERIALS AND METHODS

3.1 Study sites

Doi Suthep-Pui ($98^{\circ}47'-98^{\circ}56'E$, $18^{\circ}47'-18^{\circ}55'N$; 1,685 m) and Doi Inthanon ($98^{\circ}27'-98^{\circ}40'E$, $18^{\circ}19'-18^{\circ}40'$; 2,565 m) were selected for the study sites. Doi Suthep-Pui covers an area of about 261 km², with an altitude range 400-1600m. Doi Inthanon has an area of about 482 km² and altitudes between 400-2,565m. Both mountain situate on the mountain range Thanon Thong Chai. They locate in the area of Changwat Chiang Mai, Thailand.

The major geological structures of both mountains are para-gneiss (Precambrian metamorphic rock) with granite in the higher elevation area. Doi Suthep-Pui consists partially of sedimentary rocks (Baum *et al.*, 1981; Rhodes *et al.*, 2000). Both areas are protected as National Parks. Most of the forests and streams are in quite good natural condition.

Table 3.1 The major rock type on Doi Suthep-Pui and Doi Inthanon. X to XXX indicate the composition of each rock type and their relative proportion from minor to major composition (Modified from: Rhodes *et al.*, 2000).

Rock type	Doi Suthep-Pui	Doi Inthanon	Comments
Igneous	X	X	Mesozoic and Tertiary granitoids
Sedimentary	X	-	Lower Paleozoic and sedimentary and metasedimentary rocks
Metamorphic	XX	XXX	Core complex ortho- and paragneiss

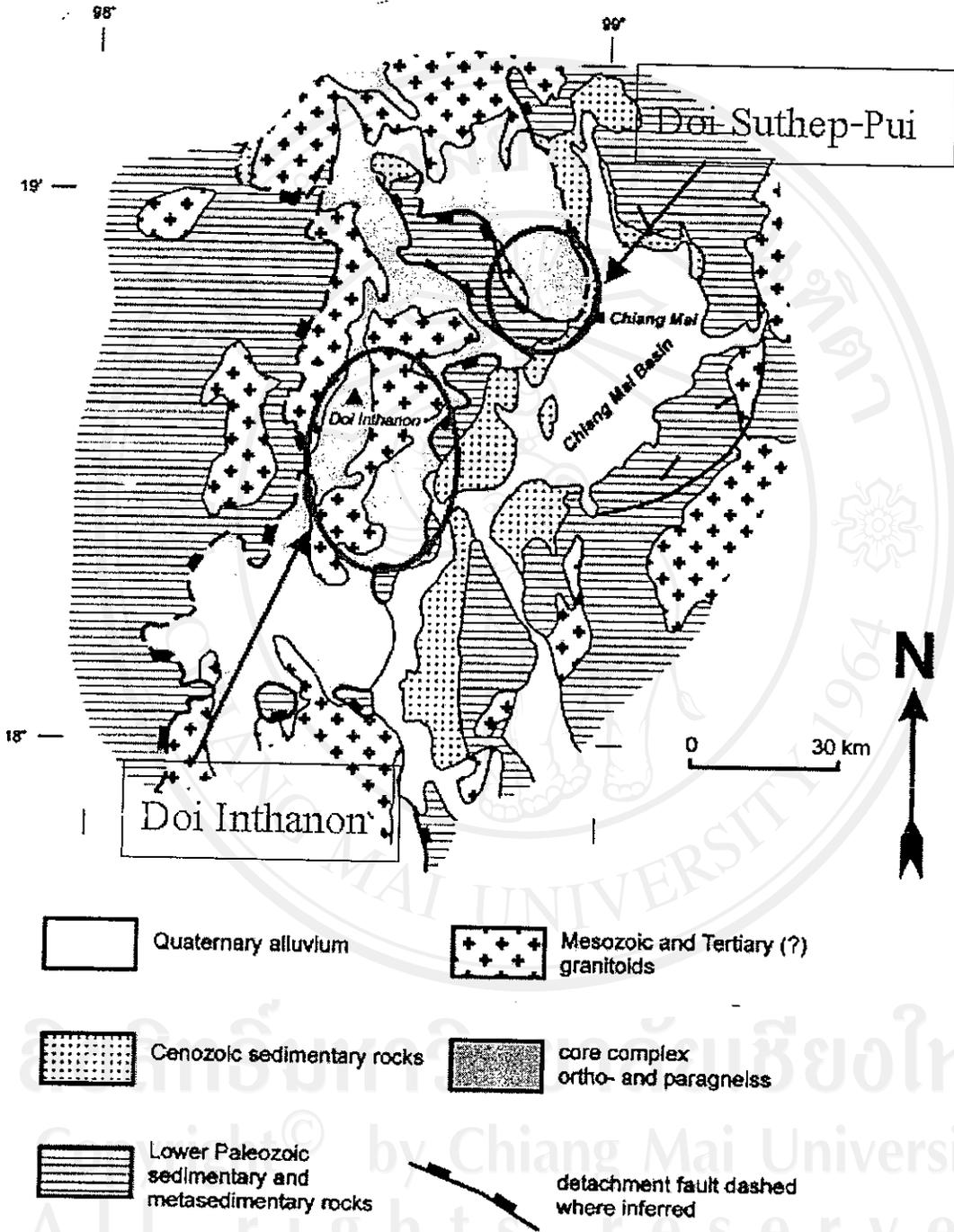


Fig 3.1 A simplified geologic map of Doi Suthep-Pui and Doi Inthanon.
 (Reference: Rhodes *et al.*, 2000)

Although Doi Suthep-Pui and Doi Inthanon occupy the same general mountain range, they differ in their relationship to the main range. Doi Inthanon is located on the middle of the range, continuously connected to the higher than 1,000 m region. In contrast, the upper parts of Doi Suthep-Pui (above 1,000 m) are separated from the main range by surrounding valleys (Fig. 3.2). This isolation causes the upper part of Doi Suthep-Pui to be relatively insular. In this study we refer to Doi Inthanon as a Himalaya-inlier and Suthep-Pui as a Himalaya-outlier mountain.

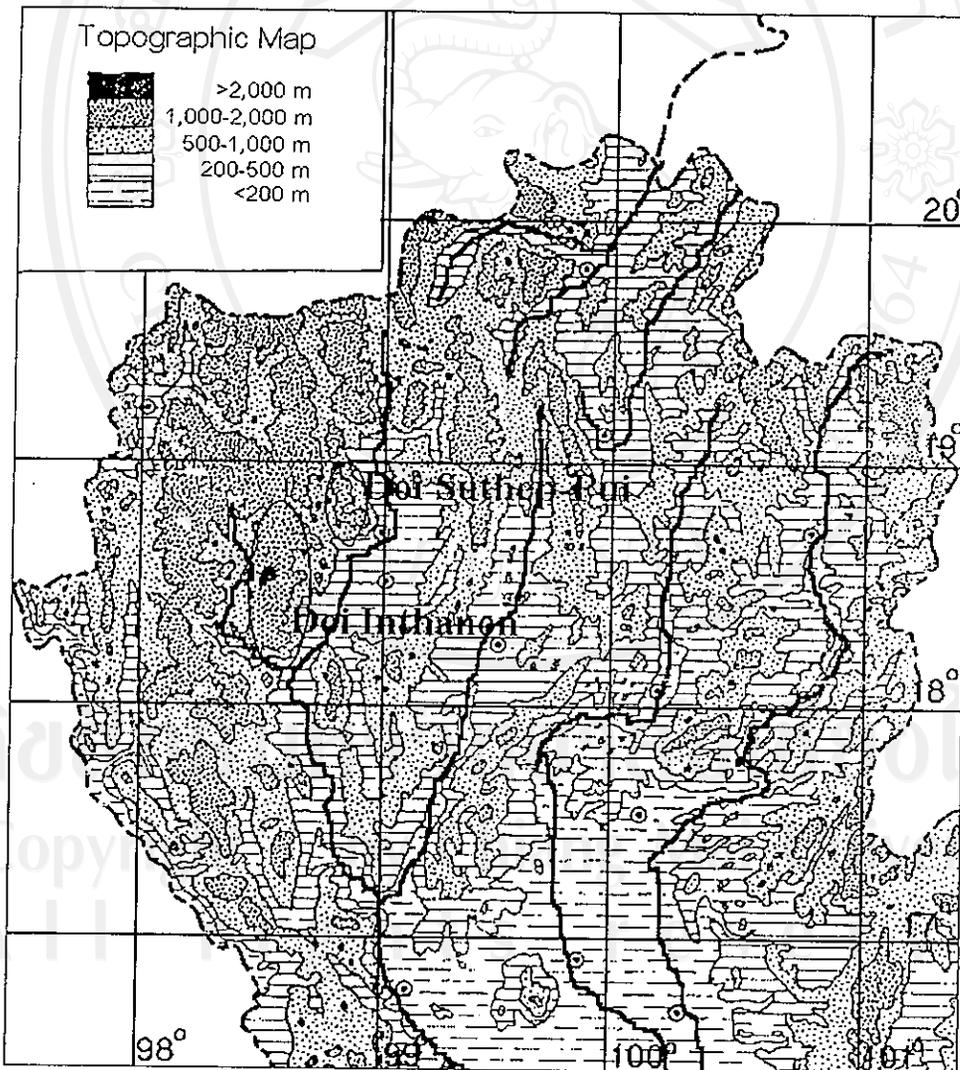


Fig 3.2 The topographic map show the elevation of Doi Suthep-Pui and Doi Inthanon.

Three riparian forest areas at the elevations of 600, 1,000 and 1,200 m were selected on Doi Suthep-Pui and Doi Inthanon. Three forest types occupying along the elevation gradient in this study. The first type is Lowland Forest, locating below 800 m. It mostly contains *Dipterocarpus*, Leguminosae, Moraceae and some bamboo. The second forest type is mixed deciduous forest and locates from 800m to 1,200 m. The major tree families are Fagaceae and Dipterocarpaceae. This habitat contains the highest diversity of flora especially in the area which are not much disturbed. There are above 1200 m, the vegetation is evergreen forest which contains with mostly of Magnoliaceae and Theaceae.

In this study site, some physico-chemical parameters were measured. The insects in these sites were also sampled using 5 study methods; light trap, sweeping net, Malaise trap, pitfall trap and beating tray methods. The facilities and equipments for this research were supported by Aquatic Insects and Biomonitoring Laboratory of Biology Department, Faculty of Science, Chiang Mai University, Thailand and the Biogeography and Conservation laboratory, School of Geography and Environmental Studies, University of Tasmania, Australia.

3.2 Environmental parameters

Some environmental parameters were measured in every study sites. These parameters were carried out from both terrestrial and aquatic ecosystems. The minimum and maximum air temperature, relative humidity, channel width and soil pH were the parameters of terrestrial ecosystems. In the other hand, the parameter in aquatic ecosystems were the stream dimension, water temperature, current velocity, discharge volume, conductivity, total dissolved solid (TDS), Dissolved Oxygen (DO),

Biochemical Oxygen Demand (BOD₅), alkalinity and the amount of nitrate, phosphate, ammonia and sulfate. The detail on the method and equipment's used is showed as follow.

3.2.1 Parameters in terrestrial ecosystem

A. Air temperature (°C)

The Min & Max thermometer was left overnight in the field in order to record the minimum and maximum value temperature during a day.

B. Relative humidity (RH; %)

The Wet & Dry thermometer (Hygrometer) was applied to measure the relative humidity in the site.

C. Channel width (m)

The tape measurer was used to measure the distance between the stream bank of both sides.

D. Soil pH

Three samples of soil were picked up in the plastic containers and returned them to the laboratory. The soil samples were filled up with distilled water to make the soil solution. After that, using the pH meter (pH scan2, waterproof, Eutech Instruments Pte Ltd, Singapore) to measure the pH in the solution.

3.2.2 Parameters in aquatic ecosystem

Some of parameters in aquatic ecosystems were measured directly in field such as stream dimension, water temperature, stream velocity, discharge volume, conductivity and total dissolved solid (TDS). The important tools for directly measuring in field was a plastic container (in this study, the 1.000 ml water sampler were applies) for fetching up the water from stream and taking a measurements of pH, temperature, conductivity and TDS.

The remaining parameters; DO, BOD₅, Alkalinity and the amount of nitrate, phosphate, ammonia and sulfate, need more complicate methods to take the measurement. Then, the 500 ml water sampler for 3 sets per site were prepared for collecting the water and returned them to analyze in the laboratory.

A. Stream dimension (m for width; cm for depth)

The width and depth of water body in stream were measured using tape measurer. The water bodies were also changed. Then the locations for taking measurements were fixed in the specific location in the sites.

B. pH of water in stream

The specified plastic container was used to draw up the water from stream. The pH meter (pH scan2, waterproof, Eutech Instruments Pte Ltd, Singapore) was dipped into the water in the container for examining the value of pH and repeated this method for 3 times.

C. Water temperature (°C)

A thermometer was dipped into the water in a specified plastic container and read the temperature value. This parameter also measured for 3 times.

D. Stream velocity (m/s)

The floating material was released into the stream. In this study, this material was allowed to travel for 3 meters. Concurrently, the stopwatch was applied to read the spending time on the specific distance. The velocity was calculated from the distance and the spending time. This parameter also measured for 3 times.

E. Discharge volume (m³/s)

This parameter was evaluated using the calculation between the cross-section of the water body and the stream velocity as the follow equation.

Discharge volume (m³/s)

= stream width (m) X stream depth (m) X stream velocity (m/s)

F. Conductivity (μS/cm)

The conductivity of water was measured using a conductivity meter (Cyberscan 300, Eutech Cybernatics, Singapore) by dipped the probe of this equipment into the water in a specified plastic container. Take a measurement for 3 times.

G. Total Dissolved Solid (TDS; mg/l)

The TDS was also measured using a conductivity meter (Cyberscan 300, Eutech Cybernatics, Singapore) then follow the same method with the conductivity.

H. Turbidity (mg/l)

The turbidity was analyzed with the spectrophotometer; HACH DR2000. (HACH Company World Headquarter, USA) under the operation program P750 at the wavelength 450 nm. The measuring of turbidity used the distilled for a blank and did not need the specified reagents.

I. Dissolved Oxygen (DO; mg/l)

The standard method applied in determination of DO was called Azide Modification. The roughly process of the method is showed as follow.

A clear BOD bottle was applied to collect water from study sites. Fill in an reagent Manganese Sulfate for 1 ml and Alkali Iodine-Azide for 1 ml in the water sample. Take the sample return to laboratory by filled with concentrated Sulfuric acid for 2 ml. Shared the supernatant for 100 ml in the flask and titrate with Sodium Thiosulphate 0.025 N and using Iodine solution as an indicator. Analyzed the data by multiple the volume of used tritrant with 2 to be a DO in mg/l. This value will be the DO_1 in the calculation of BOD_5 .

J. Biochemical Oxygen Demand (BOD₅; mg/l)

The analysis of BOD₅ also applied the same method with DO. But using a dark BOD bottle to collect a water sample from field and keep the water sample in incubator at 20°C for 5 days. After that, analyzed the DO with Azide Modification technique. The value will be the value of DO₅. Then subtract the DO₅ with DO₁ for the value of BOD₅ as follow equation.

$$\text{BOD}_5 = \text{DO}_1 - \text{DO}_2$$

K. The nutrient; nitrate, phosphate, ammonia and sulfate

These nutrients also analyzed using spectrophotometer: HACH DR2000. Each test was run under the specified program of HACH DR2000 and reaction reagents from HACH company, the detail show in Table 3.2

Table 3.2 The specified program of spectrophotometers HACH DR2000 and wavelength for analysis the concentration of sulfate, phosphate, nitrate and ammonia in water sample. The specific reagents for developing chemical reaction from HACH Company are also mentioned.

Parameters	Program	Wavelength	Reaction developing reagent
Sulfate	P685	450(nm)	SulfaVer 4 Sulfate Reagent Powder Pillow
Phosphate	P490	890(nm)	PhosVer 3 Phosphate Reagent Powder Pillow
Nitrate	P355	500(nm)	NitrateVer 5 Nitrate Reagent Powder Pillow
Ammonia	P380	425(nm)	Nessler Reagent, Mineral Stabilizer and Polyvinyl Alcohol

L. Alkalinity (mg/l)

The alkalinity of water sample was analyzed using the titration method. The sulfuric acid 0.02N was applied as the titrant and methyl orange as an indicator. The alkalinity was calculated from the volume of Sulfuric acid used multiply by 10.

3.3 Riparian insects sampling

The insects samples will be quantitatively collected using Malaise traps and light traps to collect riparian insects during the day and night time, respectively. A quantitative study using sweeping nets and beating methods will be also applied to study the abundance and collect insects that are not readily sampled by traps. Pitfall traps will be used to sample for ground-dwelling insects.

3.3.1 The sampling methods

A. Light trap

In order to trap both Trichoptera and Lepidoptera for study, dry light traps (Fig. 3.3) were left overnight in the riparian zone of each site. Light traps consisted of a 20-watt ultra-violet lamp which was operated with a 12 volt DC battery. This kind of lamp has an evidence indicates a “radius of attraction” around 50-200 m (Rickets *et al.*, 2001).

The lamp was suspended with transparent plastic over a funnel on a plastic bucket. These suspend transparent plastic also struck the attracted insects to fall down into a bucket below. In the bucket, there was a small bottle containing chloroform that was evaporated from a wick. The substance in the bucket was applied

to knock the trapped insects. Chloroform is the hazardous substance, in some country it is forbidden for sell. The ethyl acetate can be applied instead of this substance. Two dry light traps were left overnight in the field.

The trapped insects were enumerated and identified. The Trichoptera and Geometridae (Lepidoptera) were identified in the species level. The remaining insects were counted and identified in the Family level.

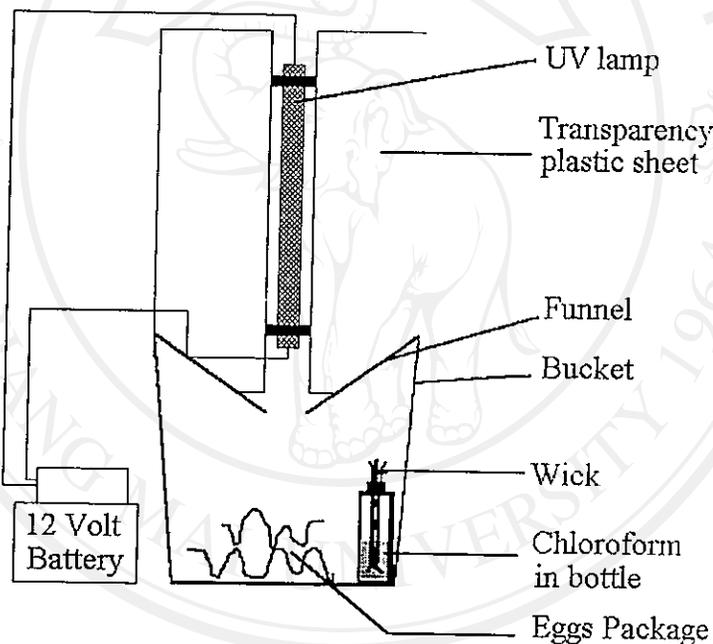


Fig 3.3 The model dry light trap. UV Lamp was operated under the DC battery 12 Volt. Chloroform in bottle was gradually evaporated pass through the wick in order to knock down the trapped insects. Egg package was applied for reduction the struggle of the trapped insects.

B. Malaise trap

This kind of trap is look like a mosquito net. It was applied to intercept mostly the day flying insect. The median screen of the trap is designed for intercept the flying insects. The trapped insects were naturally move up to the roof of the trap to find the exit, which was connected with the plastic bottle filled with alcohol. Insects were trapped down in this collecting bottle.

Therefore the limitation of the available trap, two Malaise traps were left in each mountain. The trapped insects were taken from the collecting bottle in each month. The specimens were enumerated and identified.

C. Sweeping net

The sweeping net in this study had a mouth diameter around 30 cm and the depth of net around 50 cm. This net was randomly swept over the shrub in riparian zones for 40 times. This method was done twice in each site. The captured insects were continuously kept in 70% alcohol. They were also enumerated and identified to Family level.

D. Beating trap

The plastic tray approximately size 30 X 15 cm was applied to obtain the falling insects from the tree canopies. These canopies were shaken by beating with a plastic stick. The beating frequency was standardized for 10 times per canopy, for 5 canopies for a replicate. This method was applied for 3 times in each site. The falling specimens in a tray were suddenly removed and stored with 70% alcohol solution in plastic bags. These insects were also studied.

E. Pitfall trap

The plastic container diameter size 7 cm and depth 7 cm, filled with 70% alcohol was buried as a pitfall in the riparian area. The mouth of the container had to stay at the same level of ground surface. Five of pitfall traps were left in the field overnight. The trapped insects were automatically stored in the alcohol. These captured insects were identified and enumerate.

3.3.2 Identification and preservation

All of the insect specimens from Malaise trap, sweeping net, beating trap and pitfall trap were preserved in 70% alcohol. Most of them were identified in Family level using the general insect key of Borror *et al.*, 1989.

The specimens available from dry light trap were also maintained by storage in 70% alcohol and keeping dry by pinning. Most of the mediam and large size of Lepidoptera and some of Coleoptera, Orthoptera, Megaloptera and Diptera were kept dry in insect boxes and carbinets. The Trichoptera and the other small size insects were stored in 70% alcohol.

The Trichoptera were identified to species using the Preliminary Key of Trichoptera in Thailand developed by Prof. Hans Malicky. Some of Trichoptera specimens were delivered to Prof. Hans Malicky lab, Austria in order to checking the identification.

For Geometridae, the recent revisions of many Asian genera were available (e.g. Holloway, 1993 and Inoue, 1999, Sato, 1987, Stüning, 2000) in University of

Tasmania, Australia combined with the assistance of Dr. Peter McQuillan were aided to identified the specimen from Thailand.

All specimens were deposited at the Aquatic insects Biomonitoring Laboratory Unit, Biology Department, Chiang Mai University, for future reference and studies.

3.4 The analysis of data

The main computer programs for analysis the data in this study was PATN, SPSS and Microsoft Excel.

The ecological statistics program PATN was used to analyze the insects community structure. The multivariate outputs from PATN were PCC ordination, TWINSpan and cluster analysis classified the study sites based on the difference of community characteristics in each site (Belbin, 1995).

Ordination was the suitable and popular method, which allows sites to be readily compared on the basis of their species similarity.

TWINSpan (Two-way Indicator Species Analysis) allows sites to be grouped on the basis of indicator species, which are most typical of the site. Such species, once identified, can be important in future monitoring.

The physico-chemical parameters were analyzed by Analysis of Variance in the statistical package SPSS.

3.5 Research Duration

The field monitoring was taken place during September 2001 to February 2003. The Geometridae and others Lepidoptera were delivered to study during April

to August 2003 at University of Tasmania, Australia. The suspicious specimens of Trichoptera were also brought out to Lunz am see, Austria under the taking care of Prof. Dr. Hans Malicky during November 2003 to December 2003.

3.6 Some important notes about this research

The research in national parks in Thailand requires the permission from Royal Forestry Department of Thailand. The permission progress needs a proposal written with the specific form.

All of the specimen parts and bodies sampling from National Park are occasionally brought out for the study outside Thailand, but all of them should be retrieved and deposited in Thailand. The import and export of these natural materials need the academic cooperative identified documents and the permission from Royal Forestry Department in order to present at the custom of Thailand and oversea countries. The most important the specimens must be preserved under the scientific methods in the proper condition. Finally, the species annotated under CITES treaty are forbidden to import and export.

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