

## Seasonal abundance and daily flying activity of black flies (Diptera: Simuliidae) attracted to human baits in Doi Inthanon National Park, northern Thailand

Wej CHOOCHOTE<sup>1)</sup>, Hiroyuki TAKAOKA<sup>2), 3)</sup>, Masako FUKUDA<sup>2), 3)</sup>,  
Yasushi OTSUKA<sup>2)</sup>, Chiharu AOKI<sup>2)</sup> and Nobuoki ESHIMA<sup>4)</sup>

<sup>1)</sup> Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand

<sup>2)</sup> Department of Infectious Disease Control, Faculty of Medicine, Oita University,  
Yufu City, Oita, 879-5593, Japan

<sup>3)</sup> Division of Epidemiology, Culture, and Communication, Institute of Scientific  
Research, Oita University, Yufu City, Oita, 879-5593, Japan

<sup>4)</sup> Department of Medical Information Analysis, Faculty of Medicine, Oita University,  
Yufu City, Oita, 879-5593, Japan

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**Abstract:** The seasonal abundance and daily flying activity of adult black flies were investigated at four different altitudes (400 m, 860 m, 1,360 m and 2,460 m) in Doi Inthanon National Park, northern Thailand. Collections were carried out at each altitude twice a month from 0600 to 1800 hours from August 2003 to July 2004, and adult black flies attracted to a human bait were captured by a hand net. The numbers of black flies collected were 2,599, 24,010, 7,996 and 4,496 at altitudes of 400 m, 860 m, 1,360 m and 2,460 m, respectively. A total of 39,101 adult black flies comprising 39,060 females, 37 males and 4 sex mosaics of 23 species of the genus *Simulium* Latreille s. l. were identified. Simuliid species composition and abundance varied according to altitude. Three species, i.e., *S. (Gomphostilbia) asakoe* Takaoka and Davies, *S. (Simulium) rufibasis* Brunetti and *S. (S.) nigrogilvum* Summers, were regularly attracted to a human bait in this order of predominance. *Simulium (G.) asakoe* was captured from low to medium altitudes, while *S. (S.) rufibasis* was captured mostly at high altitude, and *S. (S.) nigrogilvum* at medium altitudes. The seasonal patterns in abundance of all these predominant species varied among species even at the same altitudes and also by altitude even within the same species. Daily flying activities of these three predominant species differed in patterns and in peak hours by altitudes, by species, and also by seasons. Those of *S. (G.) asakoe* were similar in all seasons showing essentially a bimodal pattern with the first peak early in the morning and the second in the late afternoon, or vice versa; while *S. (S.) nigrogilvum* and *S. (S.) rufibasis* showed uni-, bi- and trimodal daily flying patterns. Five species, i.e., *S. (G.) asakoe*, *S. (S.) chamlongi* Takaoka and Suzuki, *S. (S.) nigrogilvum*, *S. (S.) nodosum* Puri and *S. (S.) rufibasis*, were confirmed to bite human beings.

Key words: black fly, seasonal abundance, diurnal flying activity, man-biting species, Simuliidae, northern Thailand

### INTRODUCTION

Black flies or Simuliidae (Diptera) are one of the medically and veterinary important groups of small bloodsucking in-

sects. The bites of adult females cause a wide range of problems for humans and other vertebrates. Some species serve as vectors of several pathogens, e.g., filarial nematodes of the genus *Onchocerca* to man and cattle, genus *Dirofilaria* to bear,

and blood protozoans of the genus *Leucocytozoon* to birds (Crosskey, 1990; Kettle, 1990). Moreover, black-fly bites can cause very serious problems, since they frequently inflict pain, localized swelling and inflammation accompanied by intense irritation that lasts for several days or even weeks. Repeated biting by some species can cause headaches, fevers, swollen lymph glands and aching joints (Service, 1996).

In the Oriental region, several man-biting simuliid species have been incriminated, e.g., *Simulium* (*Simulium*) *indicum* Becher, which is widely distributed at the southern foot of the Himalayas, from Pakistan in the west, through India and Nepal, to Myanmar and southern China in the east (Lewis, 1974; Datta, 1992); *S.* (*S.*) *asishi* Datta, *S.* (*S.*) *himalayense* Puri, *S.* (*S.*) *nodosum* Puri and *S.* (*Gomphostilbia*) *tenuistylum* Datta in India (Datta, 1992); and *S.* (*S.*) *japonicum* Matsumura in the Ryukyu Islands, Japan (Takaoka, 1977). Recently, *S.* (*S.*) *nigrogilvum* Summers and *S.* (*S.*) *nodosum*, which are both anthropophilic and zoophilic, were reported to be naturally infected with the third-stage larvae of different unknown filarial species in northern Thailand (Takaoka et al., 2003; Fukuda et al., 2003).

In Thailand, at least 56 black-fly species have been recorded, and classified into six subgenera in the genus *Simulium* Latreille s. l., i.e., *Asiosimulium* Takaoka and Choochote, *Deviesellum* Takaoka and Adler, *Gomphostilbia* Enderlein, *Montisimulium* Rubtsov, *Nevermannia* Enderlein, and *Simulium* Latreille s. str. (Takaoka and Choochote, 2004a, b, c, 2005a, b, c, d, e, f, g, h, i). Apart from these, significant progress has been made only in the population cytogenetic (Kuvangkadilok et al., 1998, 1999b, c, 2003) and molecular genetic studies (Otsuka et al., 2003), and in the studies on natural transmission of zoonotic filariae by the Thai simuliids (Takaoka et al., 2003; Fukuda et al., 2003).

Little is known about the biological and/or ecological aspects of black flies,

particularly the species found in Doi Inthanon National Park, northern Thailand, except for a report of larval distribution of 16 species from 12 localities in this area (Kuvangkadilok et al., 1999a). It has been generally accepted for a long time that black flies are vicious pests of humans and other vertebrates in Doi Inthanon National Park. They are found in large numbers and cause irritation to indigenous people, tourists and domestic animals. In view of their important role as a vicious biter, and lack of biological and/or ecological information, a more thorough study was needed. Therefore, the seasonal abundance and daily flying activity of adult black flies attracted to a human bait at four different altitudes in Doi Inthanon National Park are described below. Also, the proven man-biting simuliid species from this area are reported for the first time.

#### MATERIALS AND METHODS

**Study sites:** Four sites with different altitudes in Doi Inthanon National Park, Chiang Mai province, northern Thailand, i.e., Site 1, Mae Klang Waterfall (altitude 400 m); Site 2, the Fire Control Center (altitude 860 m); Site 3, Siribhume Waterfall (altitude 1,360 m); and Site 4, Ang Ka (altitude 2,460 m), were selected for adult collections (Fig. 1A, B).

**Adult collections:** Collections were carried out twice a month at each site starting from 0600 to 1800 hours, from August 2003 to July 2004. Adult black flies trying to bite or flying around a human bait positioned approximately 5 meters from perennial breeding streams were captured by a catcher using a hand-net (diameter 30 cm, depth 60 cm). The collection time for each hour was divided into 50 min catching and 10 min resting. Adult black flies caught hourly were preserved in separate vials filled with 80% ethanol, and brought to the laboratory. The species were identified using the morphological keys of Takaoka and

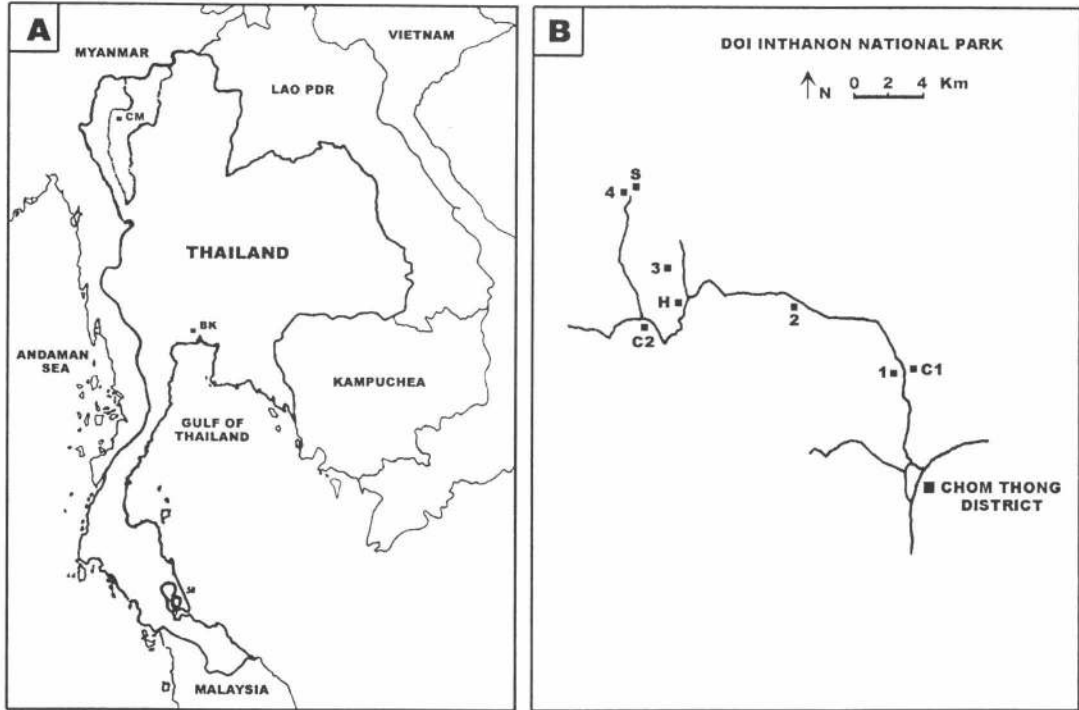


Fig. 1. A, Map of Thailand showing Doi Inthanon National Park, Chiang Mai province, Thailand (CM), where black fly collections were performed at four sites. Chiang Mai is situated at a latitude of  $18^{\circ}47'N$  and longitude  $98^{\circ}59'E$  in northern Thailand, and approximately 581 kilometers away from the capital city, Bangkok (BK), central Thailand; B, Map showing the location of four sites used for collections of adult black flies, numbers 1 to 4 indicate Site 1 (400 m in altitude), Site 2 (860 m in altitude), Site 3 (1,360 m in altitude) and Site 4 (2,460 m in altitude), respectively.

Choochote (2004b). The mean total numbers of adult females of each species per day calculated from the two-day collections of each month were used as the mean monthly numbers of adult females to express seasonal flying activities of three predominant species. On the other hand, daily flying activities of three predominant species were evaluated when the total numbers of adult females of the respective species caught per day exceeded 50. On the basis of the data obtained from the two-day collections of each month, their patterns (i.e., unimodal, bimodal and trimodal) together with the major and/or second peak activity hours were determined. For reference, air temperature and relative humidity were measured every 10 minutes from 0600 to 1800 hours by a digital thermometer and

hygrometer, respectively during the experiment. The mean monthly air temperature and relative humidity are represented by the mean daily air temperature and relative humidity calculated from the data obtained from the two-day collections of each month. Following the Thai Meteorological Department, the year is climatically divided into three seasons on the basis of rainfall and air temperature values, i.e., hot season (mid February to mid May), rainy season (mid May to mid October), and dry-cool season (mid October to mid February), which are shown in Fig. 2, together with monthly changes in rainfall, air temperature and humidity measured from August 2003 to July 2004 by the Northern Meteorological Center, situated in Muang district, Chiang Mai province, at altitude of 312 m.

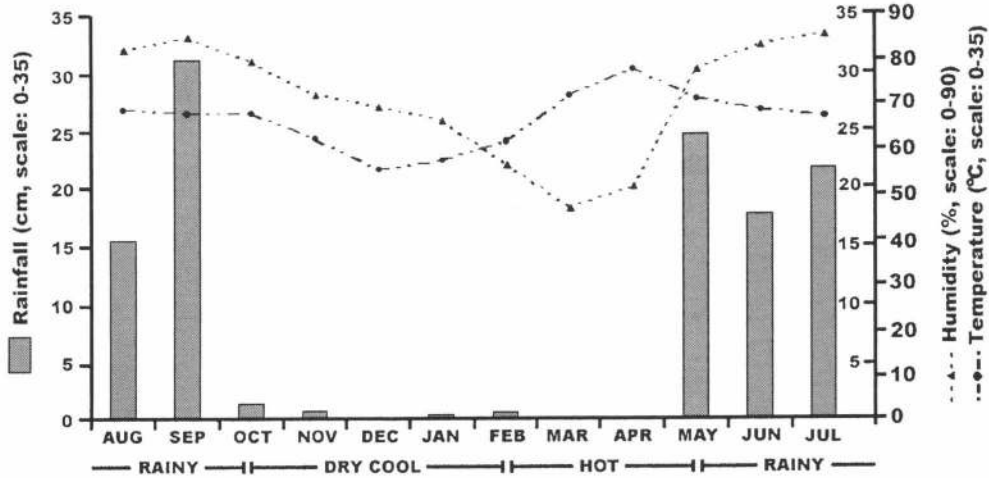


Fig. 2. Three seasons in Thailand, together with mean monthly rainfalls, air temperatures and humidities measured from August 2003 to July 2004 by Northern Meteorological Center, Muang district, in Chiang Mai province (altitude 312 m).

Man biting habit was determined by occasional observations of adult females being allowed to feed on blood of a human bait (first author) at four different altitudes.

## RESULTS

Species captured, in descending order of dominance, at four different altitudes in Doi Inthanon National Park, are shown in Table 1. A total of 39,101 adult black flies comprising 39,060 females (including 138 gravid females), 37 males and 4 sex mosaics of 23 species belonging to 4 subgenera (*Gomphostilbia*, *Montisimulium*, *Nevermannia*, *Simulium* s. str.) of the genus *Simulium* s. l. were captured. Fourteen of 23 species, i.e., *S. (S.) rufibasis*, *S. (S.) setsukoae* Takaoka and Choochote, *S. (G.) burtoni* Takaoka and Davies, *S. (G.) inthanonense* Takaoka and Suzuki, *S. (S.) rudnicki* Takaoka and Davies, *S. (S.) siripoomense* Takaoka and Saito, *S. (S.) barnesi* Takaoka and Suzuki, *S. (S.) yuphae* Takaoka and Choochote, *S. (N.) aureohirtum* Brunetti, *S. (N.) feuerborni* Edwards, *S. (S.) suchariti* Takaoka and Choochote, *S. (M.) surachaii* Takaoka and Choochote, *S. (S.) crocinum* Takaoka and Choochote and *S. (N.) caudisclerum* Taka-

oka and Davies, were captured for the first time using a human bait. Three species, i.e., *S. (G.) asakoae*, *S. (S.) rufibasis* and *S. (S.) nigrogilvum*, were regularly attracted to the human bait in order of predominance. The most predominant species, *S. (G.) asakoae*, was captured from low (400 m) to medium (860 m; 1,360 m) altitudes (Site 1 to Site 3) in air temperatures ranging from 10.9 to 44.0°C. Its flying activities were observed in air temperatures between 11.1°C and 39.9°C. Total numbers of 2,425, 22,522 and 6,757 were captured at altitudes of 400 m, 860 m and 1,360 m, respectively. *Simulium (S.) rufibasis*, the second predominant species, totaling 4,090, were captured mostly at high altitude (2,460 m) in low air temperatures (7.0–21.3°C) throughout the year. Its flying activities were recorded between 8.0°C and 21.3°C. The third predominant species, *S. (S.) nigrogilvum*, was captured mostly from medium altitudes (Sites 2 and 3) in air temperatures ranging from 10.9 to 35.8°C. Its flying activities were confirmed between 13.4°C and 29.8°C. Total numbers of 1,102 and 827 were captured at Site 2 and Site 3, respectively. The following five species were captured in satisfactory numbers at respective altitudes: *S. (G.) chumpornense* Takaoka and Kuvangka-

Table I. Numbers of adult black flies collected on a human bait at four different altitudes in Doi Inthanon National Park, northern Thailand from August 2003 to July 2004.

<i>Simulium</i> spp.	Site (Altitude, m)				Total	Female	Male	Sex mosaic
	1 (400)	2 (860)	3 (1,360)	4 (2,460)				
<i>S. (G.) asakoe</i>	2,425	22,522	6,757	1	31,705	31,667	34	4
<i>S. (S.) rufibasis</i> *	0	0	7	4,090	4,097	4,097	0	0
<i>S. (S.) nigrogilvum</i>	2	1,102	827	2	1,933	1,933	0	0
<i>S. (S.) doipuiense</i>	0	2	214	5	221	221	0	0
<i>S. (S.) setsukoe</i> *	0	0	7	203	210	210	0	0
<i>S. (G.) chumpornense</i>	159	21	26	0	206	206	0	0
<i>S. (S.) fenestratum</i>	0	3	10	189	202	202	0	0
<i>S. (S.) nakhonense</i>	5	150	12	0	167	165	2	0
<i>S. (S.) chamlongi</i>	0	30	95	1	126	126	0	0
<i>S. (S.) nodosum</i>	0	58	17	0	75	75	0	0
<i>S. (G.) siamense</i>	4	47	3	0	54	54	0	0
<i>S. (G.) burtoni</i> *	0	25	3	0	28	28	0	0
<i>S. (G.) inthanonense</i> *	4	19	2	1	26	26	0	0
<i>S. (S.) rudnicki</i> *	0	23	2	0	25	25	0	0
<i>S. (S.) siripoomense</i> *	0	4	4	0	8	8	0	0
<i>S. (S.) barnesi</i> *	0	0	4	0	4	4	0	0
<i>S. (S.) yuphae</i> *	0	4	0	0	4	4	0	0
<i>S. (N.) aureohirtum</i> *	0	0	3	0	3	3	0	0
<i>S. (N.) feuerborni</i> *	0	0	2	0	2	2	0	0
<i>S. (S.) suchariti</i> *	0	0	0	2	2	2	0	0
<i>S. (M.) surachaii</i> *	0	0	0	1	1	1	0	0
<i>S. (S.) crocinum</i> *	0	0	1	0	1	1	0	0
<i>S. (N.) caudisclerum</i> *	0	0	0	1	1	0	1	0
Total	2,599	24,010	7,996	4,496	39,101	39,060	37	4
No. of species collected	6	14	19	11	23	22	3	1

\* Species collected for the first time using a human bait.

Subgenera: *G.*, *Gomphostilbia*; *M.*, *Montisimulium*; *N.*, *Nevermannia*; *S.*, *Simulium* s. str.

dilok at 400 m, *S. (S.) nakhonense* Takaoka and Suzuki at 860 m, *S. (S.) doipuiense* Takaoka and Choochote at 1,360 m, and *S. (S.) setsukoe* and *S. (S.) fenestratum* Edwards at 2,460 m. The other 12 species, i.e., *S. (S.) chamlongi* Takaoka and Suzuki, *S. (S.) nodosum*, *S. (G.) siamense* Takaoka and Suzuki, *S. (G.) burtoni*, *S. (G.) inthanonense*, *S. (S.) rudnicki*, *S. (S.) siripoomense*, *S. (S.) barnesi*, *S. (S.) yuphae*, *S. (N.) aureohirtum*, *S. (N.) feuerborni* and *S. (S.) crocinum*, were captured mostly at altitudes of 860 m and 1,360 m, whereas *S. (S.) suchariti*, *S. (M.) surachaii* and *S. (N.) caudisclerum* were captured only at 2,460 m.

Man-biting habit was confirmed for five species, i.e., *S. (G.) asakoe*, *S. (S.) chamlongi*, *S. (S.) nigrogilvum*, *S. (S.) nodosum* and *S. (S.) rufibasis*. Among these, *S. (S.)*

*nigrogilvum* and *S. (S.) rufibasis* tended to bite and feed without hesitation once they landed on the skin of the human bait. *Simulium (G.) asakoe* seemed to take more time to land on the skin before it began to bite and was more easily disturbed while feeding as compared to the other species.

Mean monthly numbers of *S. (G.) asakoe*, *S. (S.) nigrogilvum* and *S. (S.) rufibasis* at four different altitudes expressing the seasonal abundance are demonstrated in Figs. 3, 4 and 5. For *S. (G.) asakoe* at Site 1 (400 m), the mean monthly numbers were relatively high (115–284) during the dry-cool season (from mid October to mid February), with the first peak in January and the second one in October (Fig. 3). It is interesting to note that

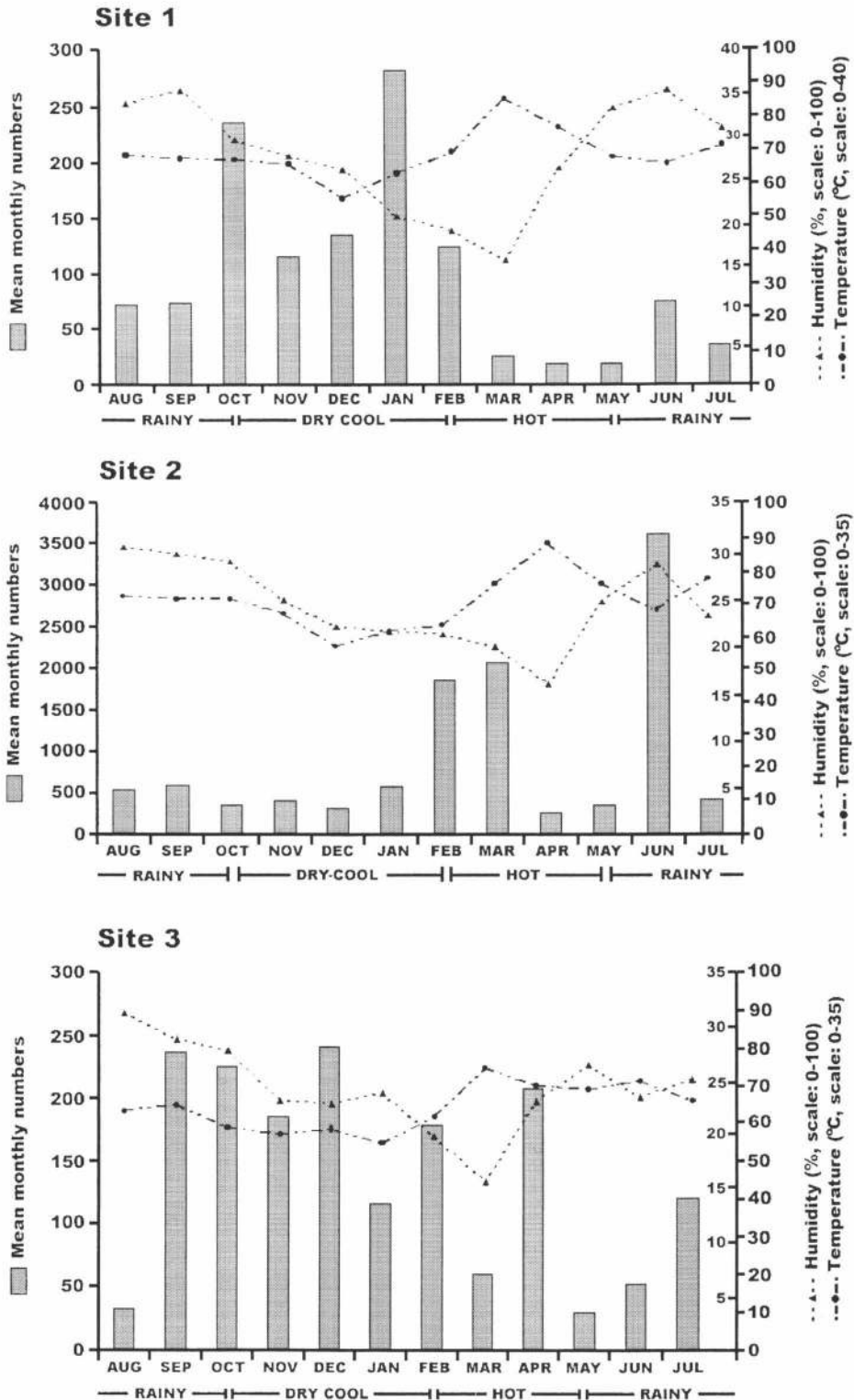


Fig. 3. Seasonal changes in abundance of *S. (G.) asokoae* attracted to a human bait at Site 1 (400 m), Site 2 (860 m) and Site 3 (1,360 m).



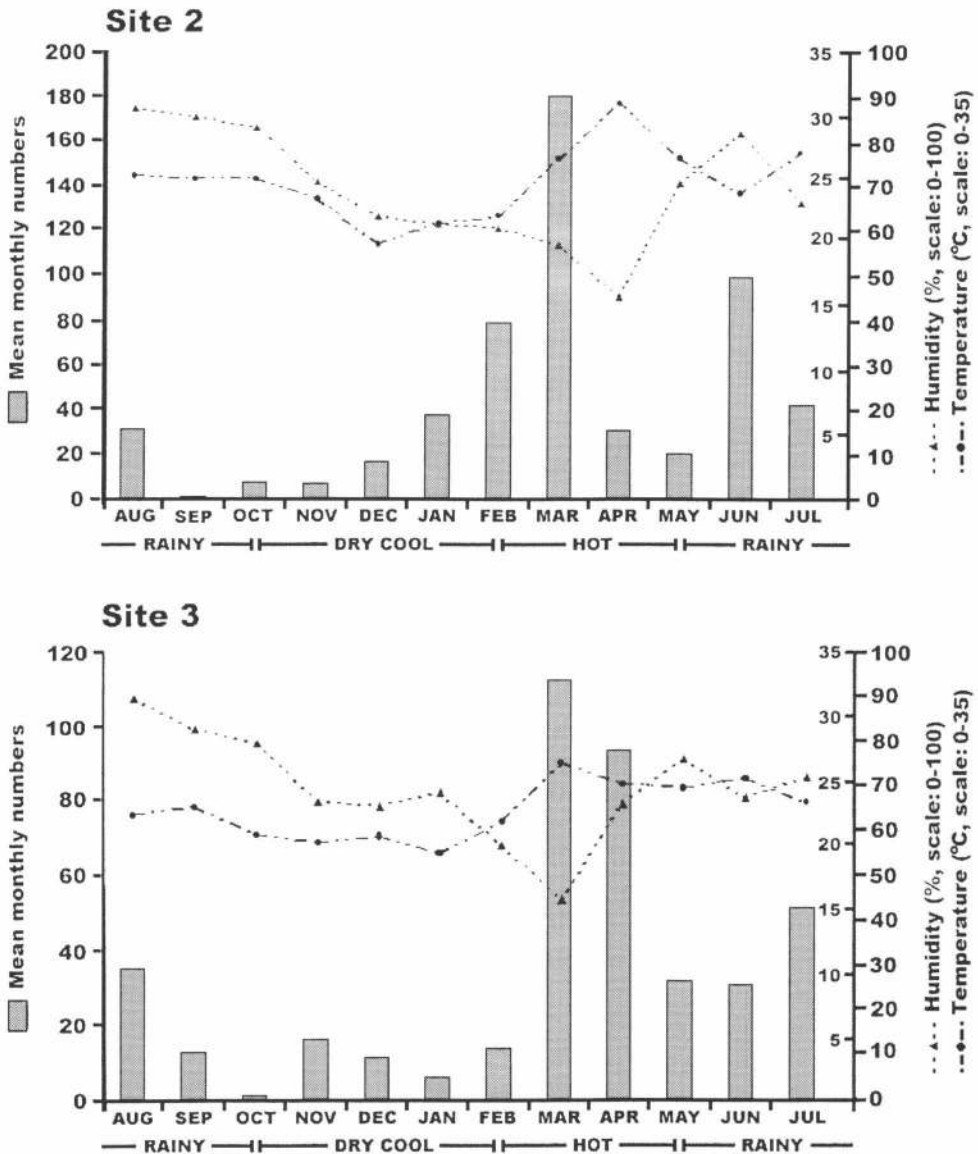


Fig. 4. Seasonal changes in abundance of *S. (S.) nigrogilvum* attracted to a human bait at Site 2 (860 m) and Site 3 (1,360 m).

during this season the mean rainfall was extremely low (0–1.28 cm), extending to April of the hot season. From March to September, the mean monthly numbers were relatively low, ranging from 18.5 to 75.5. At Site 2 (860 m), the total number of collections (22,522) was approximately three and nine times higher than those collected at Site 3 (1,360 m) and Site 1 (400 m). High numbers were obtained through-

out the whole-year or all three seasons with mean monthly numbers ranging from 225.5 to 3,598.5 (Fig. 3). Two peaks were clearly observed, i.e., the first peak in the early rainy season (June), and the second one in the early to mid hot season (February and March). At Site 3 (1,360 m), appreciable mean monthly numbers were recovered throughout the year, ranging from 61 to 483, though relatively high

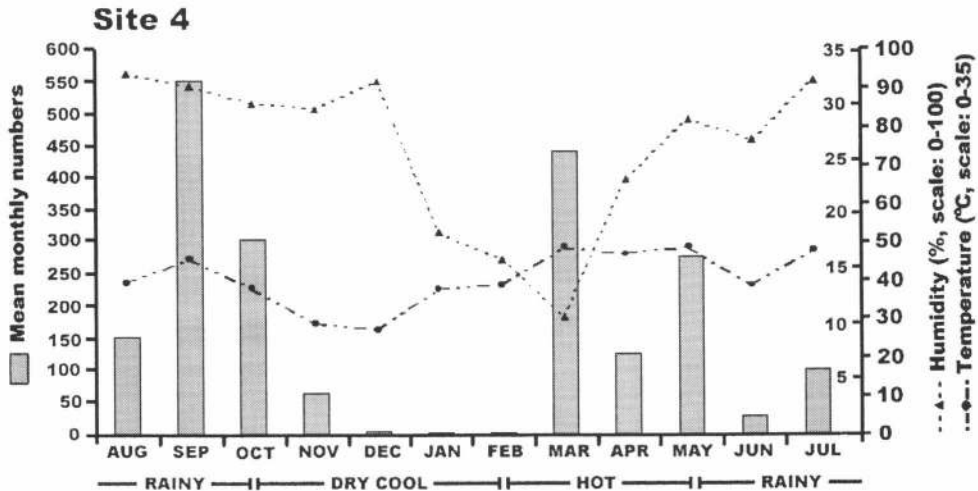


Fig. 5. Seasonal change in abundance of *S. (S.) rufibasis* attracted to a human bait at Site 4 (2,460 m).

numbers were continuously seen from the late rainy season (September) to the mid hot season (April) except those in January and March (Fig. 3).

For *S. (S.) nigrogilvum*, nearly similar patterns of mean monthly numbers were observed at both Site 2 (860 m) and Site 3 (1,360 m) (Fig. 4). There were two peaks, of which the first peak was in March of the hot season and the second one was in June (at 860 m) or July (at 1,360 m) of the mid rainy season. In the late rainy and dry-cool seasons, the mean monthly numbers recovered from both sites were very low and erratic (1–37.5 at Site 2 and 1–16 at Site 3).

A clear bimodal seasonal abundance was seen for *S. (S.) rufibasis* at Site 4 (2,460 m), i.e., the first peak was in the late rainy season (September) with a mean monthly number of 551.5, and the second peak was in the early hot season (March) with a mean monthly number of 438.5 (Fig. 5). The mean monthly numbers during the dry-cool season from December to February were extremely low (2–5.5).

The patterns and peak hours of daily flying activities of the two most predominant species at Sites 1–4 are shown in Table 2. At Site 1 (400 m), *S. (G.) asakoe* showed a clear bimodal pattern with the first peak in the morning and the second

peak in the late afternoon in the dry-cool season except in November and January when the first and second peaks were reversed. On the other hand, it showed a unimodal pattern with its peak in the early morning in the rainy season except for a bimodal pattern in one of the two collection days in August. The mean hourly air temperatures measured in the representative month of each season were 20.5–33.5°C in February, 23.2–27.9°C in June, and 15.2–31.3°C in December.

At Site 2 (860 m), *S. (G.) asakoe* also showed similar bimodal patterns throughout the year except in April of the hot season and July of the rainy season, when it showed a unimodal pattern with its peak in the early morning. In the bimodal pattern, the first and second peaks both in the morning and afternoon cycles seemed to shift by seasons i.e., in the dry-cool season, peaks in the morning cycle were delayed one to three hours while those in the afternoon cycle advanced one hour as compared with those in the rainy season. *Simulium (S.) nigrogilvum*, the second dominant species at Site 2, showed a bimodal pattern with its peaks in the early morning and in the late afternoon in February and March. However, this species also showed a unimodal pattern of daily flying activities with its peak in the late after-



Table 2. Daily flying activity patterns of three predominant simuliid species in two-collection days of each month at Sites 1-4 in Doi Inthanon National Park, northern Thailand.

Sites [Altitude]	Months and seasons											
	1	2	3	4	5	6	7	8	9	10	11	12
Species	Dry-cool		Hot			Rainy				Dry-cool		
Site 1 [400 m]												
<i>S. asakoae</i>	B 17 (7)	B 8 (17)	—	—	—	U 7	—	B 6 (15)	U 7	B 7 (17)	B 17 (7)	B 8 (16)
	B 17 (8)	B 8 (17)	—	—	—	U 7	—	U 6	—	B 7 (17)	B 7 (17)	B 8 (17)
Site 2 [860 m]												
<i>S. asakoae</i>	B 16 (9)	B 9 (17)	B 17 (8)	U 6	B 7 (17)	B 6 (17)	U 6	B 7 (17)	B 6 (17)	B 6 (13)	B 7 (16)	B 16 (7)
	B 8 (16)	B 9 (17)	B 17 (8)	U 6	B 7 (17)	B 6 (17)	U 6	B 7 (17)	B 6 (16)	B 6 (17)	B 8 (16)	B 16 (8)
<i>S. nigrogilvum</i>	—	B 7 (16)	B 7 (17)	—	—	—	—	—	—	—	—	—
	U 16	—	B 16 (7)	—	—	—	—	—	—	—	—	—
Site 3 [1,360 m]												
<i>S. asakoae</i>	B 17 (8)	B 17 (8)	B 7 (17)	B 17 (7)	—	B 6 (16)	U 6	—	B 6 (16)	B 6 (17)	B 17 (8)	B 16 (8)
	B 17 (7)	B 17 (9)	B 17 (7)	B 15 (7)	B 7 (17)	B 6 (16)	U 6	B 6 (17)	B 6 (17)	B 6 (17)	B 8 (17)	B 16 (8)
<i>S. nigrogilvum</i>	—	—	B 16 (7)	T 17 (7)	B 14 (7)	—	—	—	—	—	—	—
	—	—	B 7 (17)	B 15 (6)	—	B 17 (7)	B 17 (8)	—	—	—	—	—
Site 4 [2,460 m]												
<i>S. rufibasis</i>	—	—	U 9	B 10 (16)	T 7 (10)	—	—	—	T 9 (17)	U 15	—	—
	—	—	U 9	B 10 (16)	T 9 (17)	—	B 7 (17)	U 16	T 6 (16)	B 16 (7)	U 13	—

U, B, and T mean unimodal, bimodal and trimodal flying activity patterns, respectively  
 Numbers mean major peak time (e.g., 6, 0600-0700 hours).  
 Numbers in parenthesis mean secondary peak time (e.g., 6, 0600-0700 hours).  
 Bars indicate 'not assessed for flying activity patterns' due to small number less than 50 flies collected per day.

noon in one of the two collection days in January, which differs from that of *S. (G.) asakoae* showing a bimodal pattern with its first peak in the early morning. The mean hourly air temperatures measured at Site 2 were 18.8-30.6°C in March, 20.9-26.1°C in June and 14.0-27.6°C in December.

At Site 3, *S. (G.) asakoae* showed a clear bimodal pattern with the first peak in the late afternoon in the dry-cool and hot

seasons, and in the early morning in the rainy season. In July of the rainy season, it showed a unimodal pattern with its peak in the early morning, as in the same month at Site 2. Bimodal patterns with the first peak in the late afternoon were also obtained for *S. (S.) nigrogilvum* collected at Site 2 in the hot and early rainy seasons except in April when a trimodal pattern was seen. The mean hourly air temperatures measured at Site 3 were

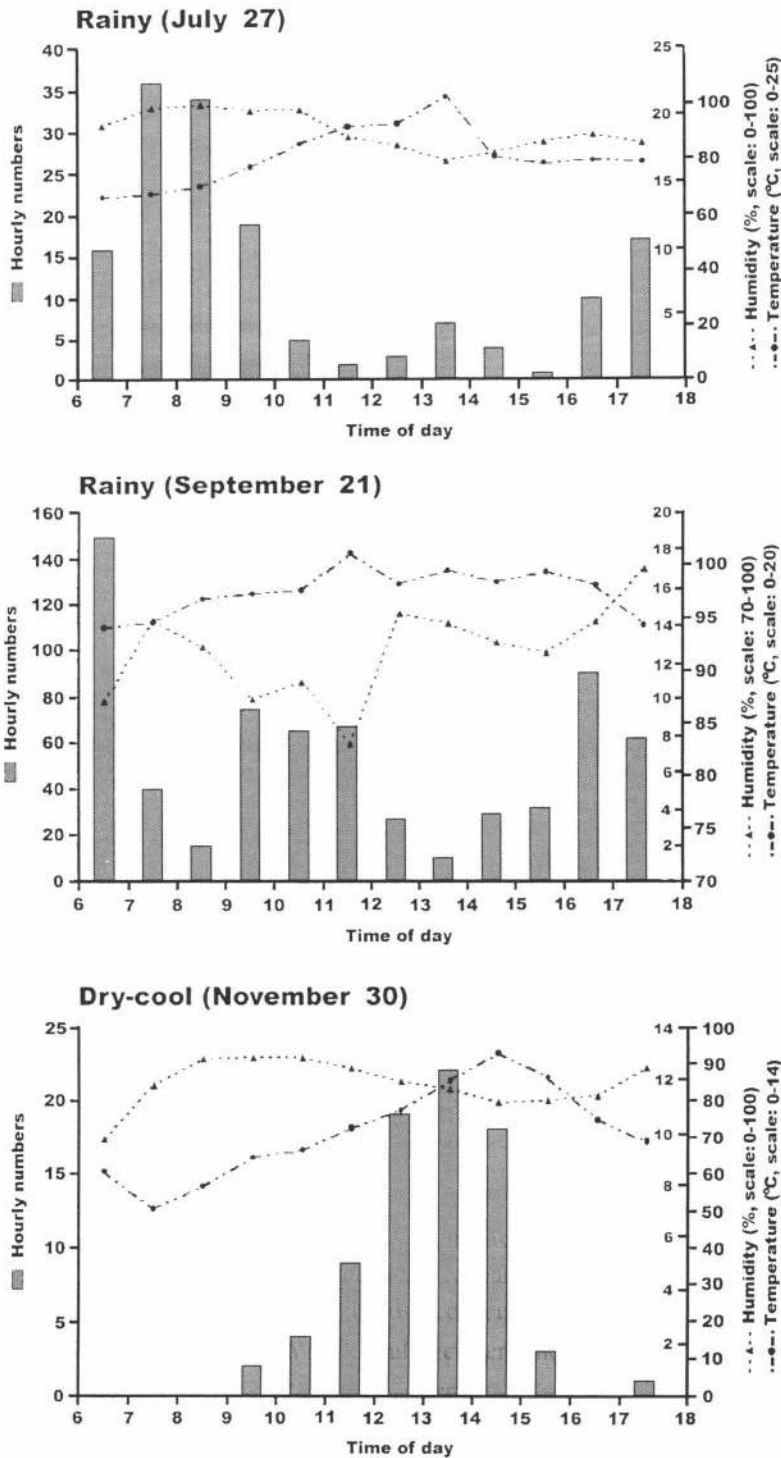


Fig. 6. Different daily flying activity patterns of *S. (S.) rufibasis* attracted to a human bait from 0600 to 1800 hours at Site 4 (2,460 m): bimodal pattern in July, trimodal pattern in September, and unimodal pattern with its peak early in the afternoon in November; all representative examples were based upon the data obtained from a single collection day.

17.9–30.1°C in March, 19.9–28.0°C in June and 12.0–27.0°C in December.

At Site 4, *S. (S.) rufibasis*, the most predominant species, most remarkably varied in daily flying patterns having all three patterns, i.e., uni-, bi-, and trimodal patterns even in the same seasons (e.g., Fig. 6). And also there is a clear difference in the peak hour even in the same unimodal patterns by seasons, i.e., 0900–1000 hours in the hot season, 1500–1600 hours or 1600–1700 hours in the rainy season, 1300–1400 hours in the dry-cool season. The mean hourly air temperatures measured at Site 4 were 12.4–20.6°C in March, 13.7–18.0°C in September, and 7.7–11.6°C in November.

#### DISCUSSION

To the best of our knowledge, this study is the first attempt to identify the black fly species attracted to or biting humans, and to measure their seasonal abundance and daily flying activity in the famous tourist area of Doi Inthanon National Park, northern Thailand.

It is clear from this study that Doi Inthanon National Park with well conserved natural forests is rich in diversity of simuliid fauna in terms of species number and variety, though only adult samples collected on a human bait were examined. The high number (23) of simuliid species collected, in part due to the altitude-specific species composition, corresponds to 41.1% of the total number of species recorded from Thailand. The richness in species variety is also evidenced by the fact that they are classified into the four phylogenetically different subgenera (Table 1). This may be a reflection of richness in various types of clean perennial and/or seasonal flowing watercourses from tiny trickles to large rivers well developed from low to high altitudes in this park. Black flies of various subgenera usually differ in their preference for the aquatic sites that are suitable for breeding of the preimaginal stages.

As already mentioned, most man-biting species belong to the subgenus *Simulium* s. str. in the Oriental region. Our results are striking in that nine of the 23 species belong to three different subgenera (i.e., five to *Gomphostilbia*, three to *Nevermannia*, and one to *Montisimulium*), all of which are thought to be ornithophilic (Crosskey, 1990). In particular, it is noteworthy that the most predominant species in low and medium altitudes was *S. (G.) asakoe*.

It is clarified that simuliid species composition and abundance varied according to altitude (Table 1). Three species, i.e., *S. (G.) asakoe*, *S. (S.) nigrogilvum*, and *S. (S.) rufibasis*, were regularly attracted to human bait, the first two being captured mostly in medium altitudes, and the last one mostly in high altitude. All these three predominant species as well as two minor ones, i.e., *S. (S.) chamlongi* and *S. (S.) nodosum*, have been proven to bite humans in this park. However it should be noted that *S. (S.) nigrogilvum* and *S. (S.) rufibasis* are the most vicious man-biter in medium and high altitudes, respectively. Three other species, in particular *S. (G.) asakoe*, are at most a hesitant man-biter, according to our field observations.

Data on the seasonal abundance of the three predominant species in this park are firstly demonstrated in Figs. 3, 4 and 5. Surprisingly, considerable numbers of *S. (G.) asakoe* were collected at an altitude of 860 m throughout the year, with mean fly numbers per day of 225.5–3,598.5 (Fig. 3, Site 2). The actual maximum total number of *S. (G.) asakoe* recorded was 4,149 in one of the two collection days of June. The seasonal patterns in abundance of *S. (G.) asakoe* were distinct but markedly differed by altitudes (Fig. 3), while those of *S. (S.) nigrogilvum* were nearly similar at two altitudes (Fig. 4) but its seasonal pattern obtained at an altitude of 1,360 m was different from that of *S. (G.) asakoe* at the same altitude (Figs. 3 and 4). Thus, the seasonal patterns in abundance appear to vary by altitude even

within the same species and also among species even at the same altitudes in the same park. The similar results were reported on the seasonal abundance of adult black flies in a Guatemalan endemic area of human onchocerciasis (Takaoka, 1981, 1982). Adult female populations of three anthropophilic species, *S. (Psilopelmia) ochraceum* Walker, *S. (S.) metallicum* Bellardi and *S. (S.) horacioi* Okazawa and Onishi, fluctuated differently from one another, when compared even at the same location; and those of *S. (P.) ochraceum* also differed from one location to another. These different seasonal patterns of abundance were presumed to be associated with seasonal variations in the availability of streams suitable for the breeding of the three species which choose differently their aquatic sites (Takaoka, 1981). The seasonal patterns of *S. (G.) asakoe*, *S. (S.) nigrogilvum* and *S. (S.) rufibasis* obtained in the present study may be at least partially explained by the seasonal variations in the availability of the larval streams. However, this should be confirmed in the future studies because we do not have any information on the biology of these three species including their preference for the types of breeding watercourses, duration of larval development, and nutrition. The seasonal abundance of adult black flies attracted to humans is not always correctly reflected by that of the larval population, which would be reflected to a certain extent by the quantity and stability of larval streams. The other factors concerned include the preference of adult females for host animals as a blood-meal source, the availability of other animals, the distance from the oviposition and pupal sites to collecting sites, and climate (air temperature, humidity, rainfall, light intensity, wind, etc.).

Results obtained on the seasonal abundance of adult females were derived from a one-year survey. The data are insufficient to be analysed in relation to three distinct seasons in this park (hot, rainy and dry-cool); however the yearly pattern

in abundance of *S. (S.) rufibasis* obtained at an altitude of 2,460 m, which is characterized by its adult flying activities occurring only during the hot and rainy seasons and lacking during the dry-cool season (Fig. 5), appears to be related to seasonal variations of air temperatures. More data will be needed to determine the seasonality in abundance of all these three predominant species in this park and to clarify their relationship to the three seasons.

Daily flying activities of the three predominant species examined in this study differed in patterns and peak hours to some extent by altitudes even in the same species, by species compared even at the same site and on the same day, and also remarkably by seasons in the same species, as shown in Table 2. It is apparent that *S. (G.) asakoe* usually shows bimodal patterns with its peaks either in the early morning or in the late afternoon, and rarely unimodal patterns with the peak in the early morning in certain months of the rainy season at three altitudes, and also varies to some extent in the peak hours from one season to another even at the same site. It is remarkable that both *S. (S.) nigrogilvum* and *S. (S.) rufibasis* showed three patterns, i.e., uni-, bi-, and trimodal (Fig. 6). So far, bimodal or unimodal pattern of the daily biting activities was observed for other species, e.g., bimodal pattern in *S. (Trichodagnia) guianense* Wise and *S. (Psaroniocompsa) oyapockense* Floch and Abonnenc in the humid Amazon between Venezuela and Brazil (Grillet et al., 2005) and *S. (Notolepria) exiguum* Roubaud s. l. in Ecuador (Vieira et al., 2005), and unimodal in *S. (Edwardsellum) yahense* Vajime and Dunbar in Liberia (Davis et al., 1994), *S. (Ps.) incrustatum* Luz in the humid Amazon between Venezuela and Brazil (Grillet et al., 2005) and *S. (P.) quadrivittatum* Loew in Ecuador (Vieira et al., 2005). The daily patterns of flying activities of adult black flies attracted to human bait are also influenced by many factors such as those

mentioned above. In our preliminary analyses (unpublished data), it is at least suggested that the daily flying activity pattern of *S. (S.) rufibasis* in November of the dry-cool season at Site 4 (2,460 m) is positively associated with the mean hourly air temperatures (Fig. 6).

During this one-year survey, a total of about two hundred biting midges and about a hundred of mosquitoes were also collected in particular in the late afternoon and/or early morning, but these were of negligible importance in comparison with the huge numbers of black flies caught in the present survey. It is proven that black flies are the most important man-biter during daytime in the Doi Inthanon National Park. It is hoped that the findings obtained in this study would be helpful as a baseline information for people living in, or visiting, this park to avoid the risk of being bitten by black flies.

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