CHAPTER - 6

RESULTS

6.1. Relatedness of colonies seasonally occupying the same nest site

Swarms occupied the window site in 1993, 1995, 1996, 1997 and 1998. These 5 colonies were used to test hypothesis-I that colonies occupying the same nest site in subsequent seasons would be more related than random colonies. Relatedness was estimated using three microsatellite loci (A88, A14 and B124) developed by Estoup et al., (1994). B124 revealed only 1 kind of queen allele (see appendix-V) whereas loci A88 and A14 revealed 2 different kinds of queen alleles. Genotypes of queens and parental drones of the 5 colonies examined are presented in Table 12. The mean number of patrilines per locus was 7.72.

Table 12. Genotypes (microsatellite length in base pairs) of queens and parental drones of five colonies (Appendix-V).

	Microsatellite locus[a]		Number of worker bees observed
	A88	A14	
	Colony-1		
Queen allele: 1	99	167	
Queen allele: 2	103	165	
Drone 1	99	167/169	5
Drone 2	99	168	1
Drone 3	99	171	1

	Microsatellite locus		Number of worker bees observed
	A88	A14	
Drone 4	105	167/165	3
Drone 5	105	168	7
Drone 6	105	171	1
Drone 7	109	167	1
Drone 8	109	168	1
		Total	20
Colony-2			
Queen allele: 1	94	167	
Queen allele: 2	111	171	
Drone-1	94	167	1
Drone-2	95	169	1
Drone-3	99	167	1
Drone-4	101	167/171	6
Drone-5	107	169	1
Drone-6	107	171	1
Drone-7	111	167/171	7
Drone-8	111	169	2
		Total	20
Colony-3			
Queen allele: 1	96	165	
Queen allele: 2	108	172	
Drone-1	96	165	2
Drone-2	96	170	1

	Microsatellite locus		Number of worker
	A88	A14	bees observed
Drone-3	98	173	1
Drone-4	100	165/172	2
Drone-5	100	167	1
Drone-6	100	173	2
Drone-7	102	165/172	1
Drone–8	108	165/172	6
Drone-9	108	167	1
		Total	20
	Colony-4		
Queen allele: 1	87	169	
Queen allele: 2	109	171	
Drone-1	87/109	169/171	5
Drone-2	102	165	1
Drone-3	117	169/171	1
Drone-4	117	173	1
Drone-5	127	169/171	1
Drone-6	147	165	1
		Total	10
	Colony - 5		
Queen allele: 1	109	171	
Queen allele: 2	117	173	
Drone-1	92	173	1
Drone-2	102	171	2

	Microsatellite locus		Number of worker
	A88	A14	bees observed
Drone-3	109	171/165	4
Drone-4	109/117	169	2
Drone-5	124	165	1
Drone-6	124	171	1
		Total	11

"a" = A88 and A14 are given as length in base pair; "/" indicates two alternative genotypes where parental and maternal alleles can not be distinguished.

The queen alleles of 5 colonies seasonally occupied the same nest sites are presented in Table 13. There is no evidence of relatedness of queens headed in these 5 colonies examined. Each colony was headed with different queen alleles (Table 15).

Table 13. Inferred queen genotypes of *A. dorsata* colonies occupying the same nest site from 1993-1998.

Colony number	Nest sites Location	Nest sites occupying Year	Microsatellite locus A88 (bp)	Microsatellite locus A14 (bp)
Colony-1	Window HCC	1993	99/103	167/169
Colony-2	Window HCC	1995	94/111	167/171
Colony-3	Window HCC	1996	96/108	165/172
Colony-4	Window HCC	1997	87/109	169/171
Colony-5	Window HCC	1998	109/117	171/173

6.2. Relatedness of aggregated colonies

Seven colonies were used to test the hypothesis that aggregated colonies on a single tree are related or not. Relatedness was estimated using two microsatellite loci

A88 and A14 developed by Estoup et al., (1994). Genotypes of queens and parental drones are presented in Table 14. The total number of patrilines observed in 7 colonies ranged from 7-16. The mean number of patrilines per locus is 10.14. In colony-1, 15 patrilines were observed despite of high sample number bees (n=72) examined. 169 bp was a most common allele presented in 7 colonies examined. However, colonies 6 and 7 had carried 165/169 bp on locus A14.

Table 14. Genotypes (microsatellite length in base pairs) of queens and parental drones of seven colonies (Appendix-VI).

	Microsatellite locus		Number of worker bees observed
	A88	A14	
	Colony-1		
Queen allele: 1	99	164	
Queen allele: 2	118	169	
Drone-1	95	164/169	1
Drone-2	95	167	1
Drone-3	97	164/169	5
Drone-4	97	167	4
Drone-5	99/118	166	3
Drone-6	99/118	167	1
Drone-7	99/118	164/169	2
Drone-8	103	164/169	23
Drone-9	103	167	8
Drone-10	103	171	5
Drone-11	109	166	2
Drone-12	109	167	7
Drone-13	109	171	7

	Microsatellite locus		Number of worker
	A88	A14	bees observed
Drone-14	118	164/169	1
Drone-15	118	167	1
		Total	71
		Colony-2	
Queen allele: 1	99	164	
Queen allele: 2	103	169	
Drone-1	95	164/169	1
Drone-2	97	164/169	4
Drone-3	99/103	164/169	12
Drone-4	105	164	1
Drone-5	105	167	1
Drone-6	105	169	1
Drone-7	109	166	3
Drone-8	109	169	2
Drone-9	118	164	1
Drone-10	136	164/169	4
Drone-11	136	171	1
		Total	31
		Colony-3	
Queen allele: 1	103	164	
Queen allele: 2	109	165	
Drone-1	93	169	3
Drone-2	93	171	1

	Microsatellite locus		Number of worker
	A88	A14	bees observed
Drone-3	95	164/165	6
Drone-4	95	167	4
Drone-5	97	167	1
Drone-6	97	169	1
Drone-7	99	164	3
Drone-8	99	169	1
Drone-9	103/109	164/165	3
Drone-10	103/118	165	1
Drone-11	103/109	167	2
Drone-12	109	164/165	2
Drone-13	109	167	1
Drone-14	118	164/165	3
Drone-15	118	167	1
Drone-16	118	169	4
		Total	32
	Colony-4		
Queen allele: 1	109	171	
Queen allele: 2	117	169	
Drone-1	92	165	1
Drone-2	102	165	1
Drone-3	102	171/169	2
Drone-4	109	165	1
Drone-5	117	167	1
Drone-6	127	171/167	1

	Microsatellite locus		Number of worker
	A88	A14	bees observed
Drone-7	127	167	1
Drone-8	136	171	1
		Total	12
	Colony-5		
Queen allele: 1	109	169	
Queen allele: 2	109	169	
Drone-1	85	167	1
Drone-2	96	171	1
Drone-3	109	167	1
Drone-4	109	169	1
Drone-5	117	169	2
Drone-6	127	166	1
Drone-7	127	167	2
		Total	10
	Colony-6		
Queen allele: 1	140	165	
Queen allele: 2	117	169	
Drone-1	103	167	2
Drone-2	109	165	1
Drone-3	109	171	2
Drone-4	117	174	2
Drone-5	117	174	1

	Microsatellite locus A88	Microsatellite locus A14	Number of worker bees observed
Drone-6	124	165	2
Drone-7	124	171	2
		Total	12
	Colony-7		
Queen allele: 1	103	165	
Queen allele: 2	140	169	
Drone-1	85	167	1
Drone-2	94	171	1
Drone-3	103/124	165/169	4
Drone-4	103/124	171	2
Drone-5	109	165/169	1
Drone-6	117	165/169	1
Drone-7	140	165/169	2
		Total	12

There is a evidence that colonies 1 and 2 had the same queen alleles 99/103 and 164/169 bp on both loci: A88 and A14 respectively. However, colony-3 had different queen alleles at both loci A88 and A14 (Table 15). Subsequently, other 5 colonies were headed by queens carrying the different alleles. Colonies 6 and 7 had 165/169 bp on the locus A14 (Table 15).

Table 15. Inferred queen genotypes of *A. dorsata* colonies aggregated on the single supports.

Colony Number	Nest sites location	Microsatellite locus A88 (bp)	Microsatellite locus A14 (bp)
Aggregation-I			
Colony-1	Water tower, MU	99/118	164/169
Colony-2	Water tower, MU	99/103	164/169
Colony-3	Water tower, MU	103/109	164/165
Aggregation-ii			
Colony-4	HCC building	109/117	171/169
Colony-5	HCC building	109/109	169/167
Aggregation-iii			
Colony-6	Tree, MTT	140/117	165/169
Colony-7	Tree, MTT	103/140	165/169

MU= Maejo University; HCC = Health care center building; MTT = Mea Tung Ting.

6.3. Seasonal migratory patterns of *A. dorsata*

The seasonal migratory pattern of *A. dorsata* colonies was studied at Chiang Mai from June 1995 to September 1998 (Figure 14). Each year two peaks of reoccupation and abandonment of nest sites were observed. Early reoccupation occurred in November and a second in January and February. Subsequently, two types of migration of *A. dorsata* with and without swarming were observed (Figure 14A). The early migration commenced in December when the ambient temperature ranged from 10-24°C and the second occurred from June-August. On 14th of August 1997, 7 queen cells observed on the particular window frame of the health care center building. One of 7 queen cells was damaged. Six queens were successfully emerged by opening caps. On 5th and 25th of August 1997, 2 colonies observed were both combless, because they did not construct combs. Two weeks later, both colonies migrated. In other case, on 28th of August 1998, 2 colonies found on small trees with small combs 0.03 m × 0.4 m. On 5th of September, both colonies had brood. During this time of the year (August-September), foreign bees were regularly visited old prominent combs to collect wax. The wax collector bees usually scarped cell wall especially from honey storing areas and deposited on her hind legs. In the rainy season (August-October), *A dorsata* nests were situated at less than 3 m on small branches of small trees, usually sheltered inside 2-3 layers of canopy. Colonies were very small comprised of about 3,000-10,000 bees. Therefore, in the rainy season, *A. dorsata* were not easily visible as they are in winter season (November-March). August-September was classified as a peak absconding (migration) period. In November, when *A. dorsata* reoccupied the old nest sites they carried honey with them. During the curtain formation, they protruded their tongues exposing a drop of honey. At that time, *A. dorsata* built a comb size 0.4 m × 0.6 m within 3 days.

Similarly, the migratory pattern of *A. dorsata* was observed in Mae Tung Ting and Mae Hong Son (colonies harvested sites) from 1996-1998 are presented in Figure 14B. In these two sites, *A. dorsata* swarms were returned at the end of February and remained till the end of April. Swarms of *A. dorsata* did not observe in the rainy season.

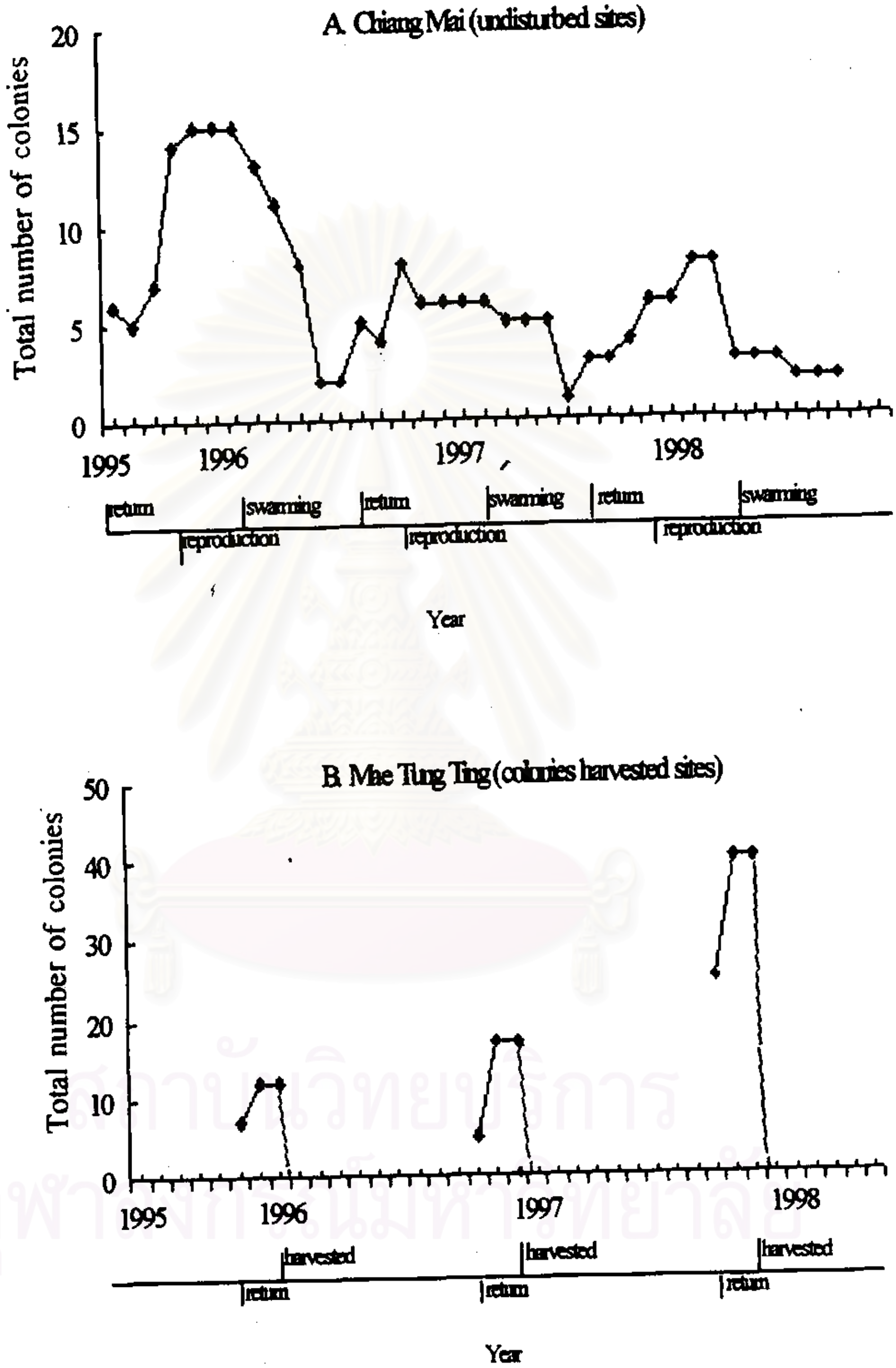

Figure 14. A-B. Seasonal migratory pattern of *A. dorsata* in Chiang Mai and Mae Tung Ting from 1995-1998.

6.4. Migration due to variable environmental factors

6.4.1. Broodnest temperature

The broodnest temperature of *A. dorsata* colonies (n = 3 nests) and ambient temperature measured in January are shown in Figure 15. Broodnest temperature followed the pattern of ambient temperature. Broodnest temperature was ranged from 22-28°C. Maximum broodnest temperature 28°C was recorded at 1500 hour and 1800 hour at 25°C air temperature. Typically, at 1500 hr the broodnest temperature was closely (1°C higher) coupled to the ambient temperature (Figure 15). The lowest broodnest temperature 22°C recorded at 0900 hours when the air temperature was 16° C. However, the broodnest temperature was 10°C higher than ambient temperature at 0600 hr and 2400 hour. The broodnest temperature shows consistently high and low with air temperature. Broodnest temperature was always 6.4°C higher than ambient temperature. The maximum difference between broodnest temperature and ambient 7° C occurred at 0900 hour. Ambient temperature ranged from 14-27°C. Average broodnest temperature recorded was 25.5±0.94 (n = 3 nests).

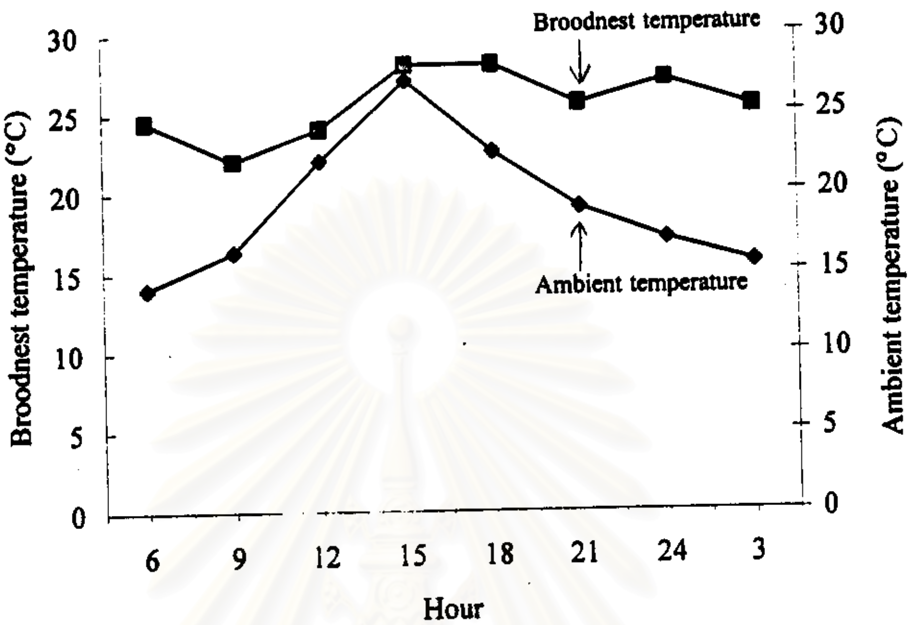

Figure 15. Broodnest temperature of *A. dorsata* colonies in winter (January).

6.4.2. Ambient temperature

On 2-13th of January 1997, 4 of 15 (26.6%) colonies occupied old nest sites in undisturbed sites when the ambient temperature was ranged from 21.6-23°C. In May maximum air temperature 42°C and extremely minimum temperature 9.5°C in December were recorded. Colony migration shown that a negative correlation with air temperature (simple linear regression $y = -0.32x + 14.6$, $r^2 = 0.06$) (Figure 16A).

6.4.3. Rain

Rainfall patterns and means of intertropical convergence zones and tropical cyclone in Thailand are presented in Figure 17. Maximum rainfall of 135.3-335.9 mm per month was occurred from June-August (Figure 16B). From May-October, the monsoon came from southwest (SW) to north. Subsequently, in winter season (October-February) monsoon came from northeast (NE) to south with thunderstorm.

In July and August maximum rain of 46.5-56.9 mm per day was recorded. The Figure 16B shows that colony migration has negative correlation ($y = -0.014x + 7.53$, $r^2 = 0.08$).

6.4.4. Wind

The main wind direction in north of Thailand is presented in Figure 18. Maximum wind speed 29-45 km per hour was occurred in April and June. In summer, wind came from south-southeast (SSE) and north (N). On 28th of June 1996, one colony of *A. dorsata* nested on the southern face of the adjacent water tower of the health care center building dislodged by the combination effect of strong wind and rain (Figure 19). The wind speed was 29 km per hour on 28th of June 1996. Similarly, in June 1997, 2 colonies nested on the south faces of the Maharaja hospital building and 1 colony on a tree near Airport Department store dislodged by strong wind. The wind speed was 45 km per hour. Colony migration shows a negative correlation with wind speed ($y = -1.02x + 15.9$ $r^2 = 0.51$) (Figure 16C).

6.4.5. Relative humidity

Maximum relative humidity (RH) 96% was recorded in August. Colony migration was also significantly greater in the low humidity than high humidity ($y = -0.145x + 16.7$, $r^2 = 0.2$) (Figure 16D).

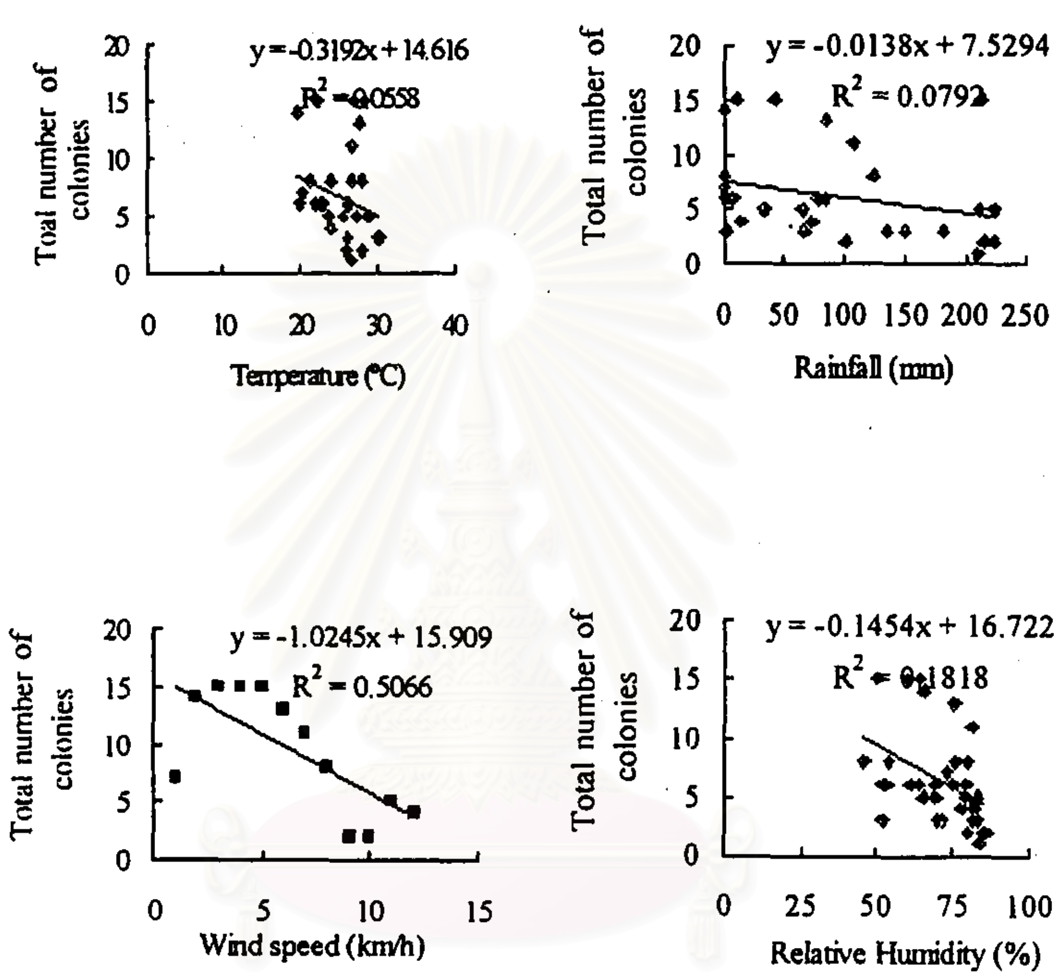

Figure 16. A-D. Plots of *A. dorsata* colonies versus climatic parameters.

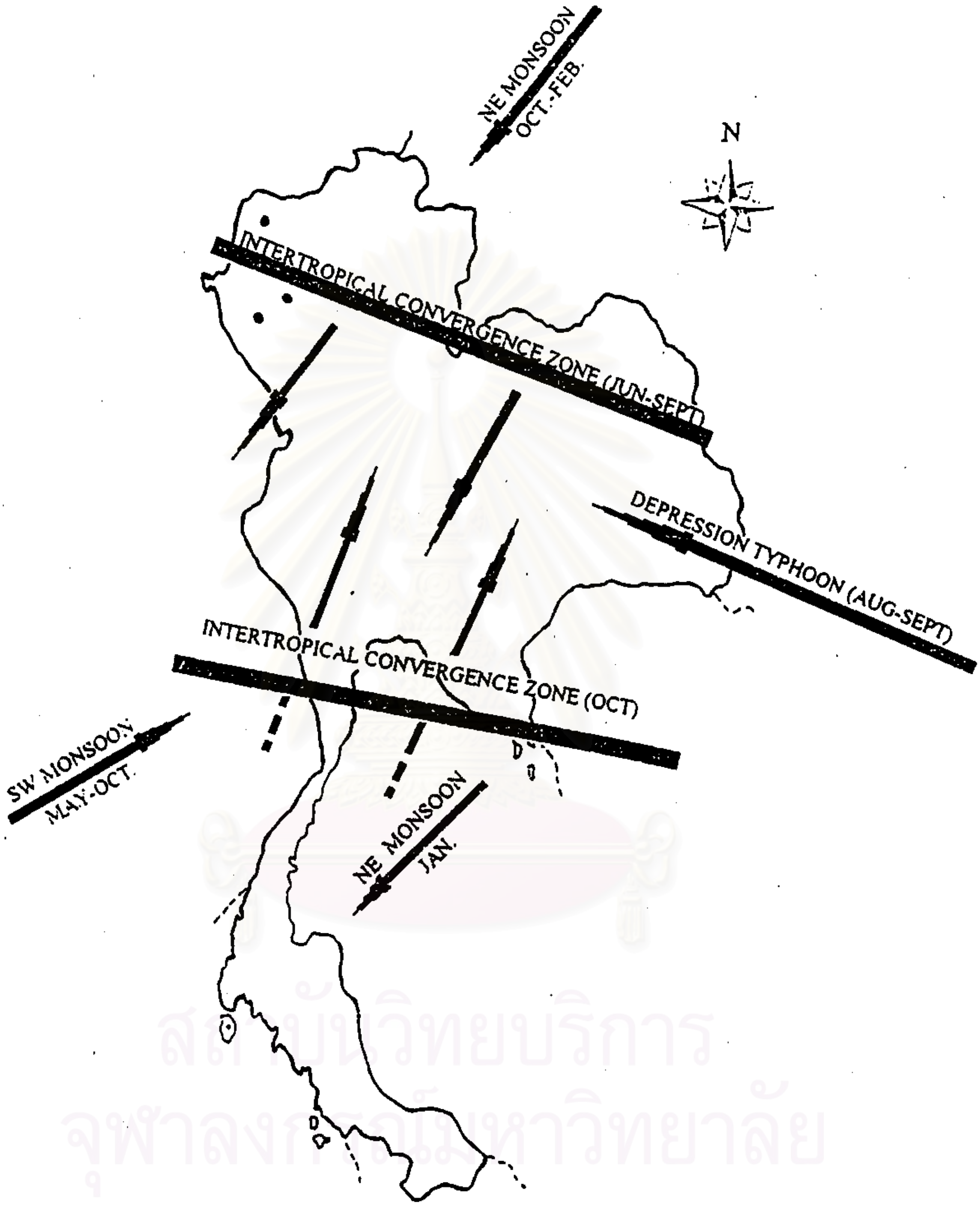

Figure 17. Rainfall patterns and means of intertropical convergence zones and tropical cyclone tracks. (Source: Meteorological department Chiang Mai)

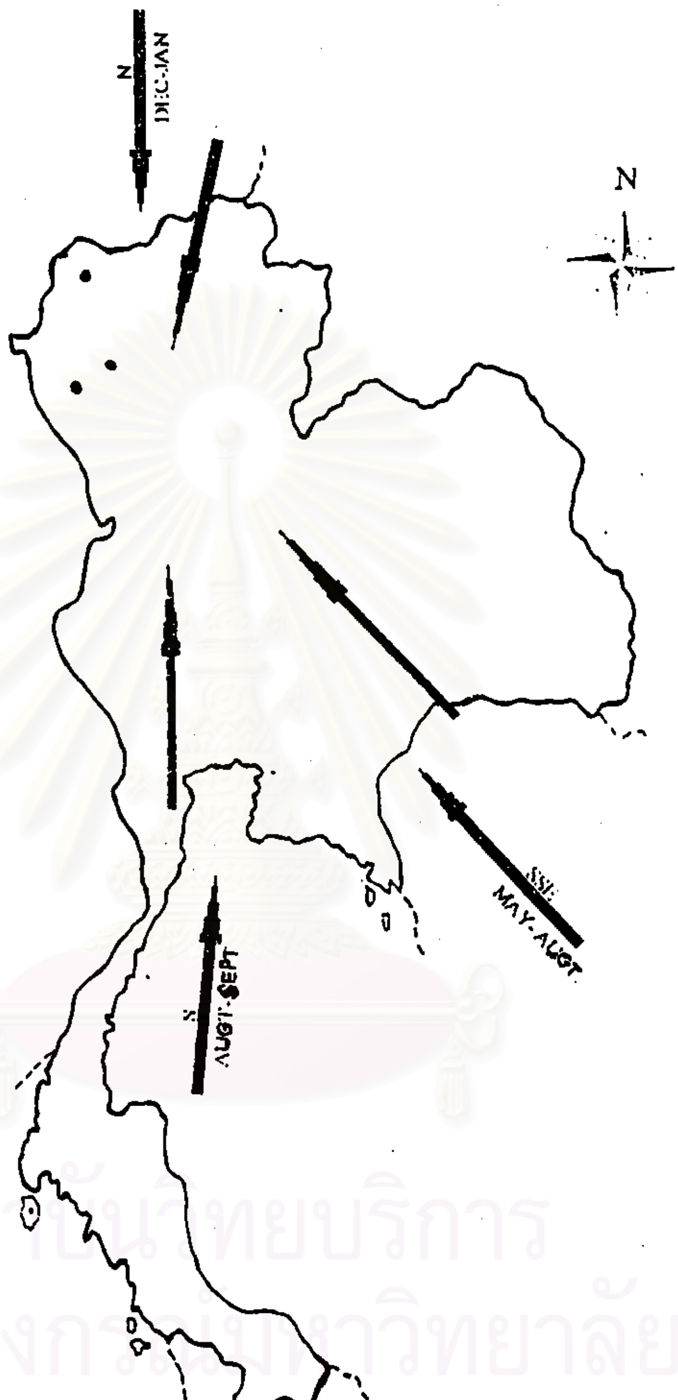

Figure 18. Wind speed patterns in northern Thailand.

(Source: Meteorological department Chiang Mai)

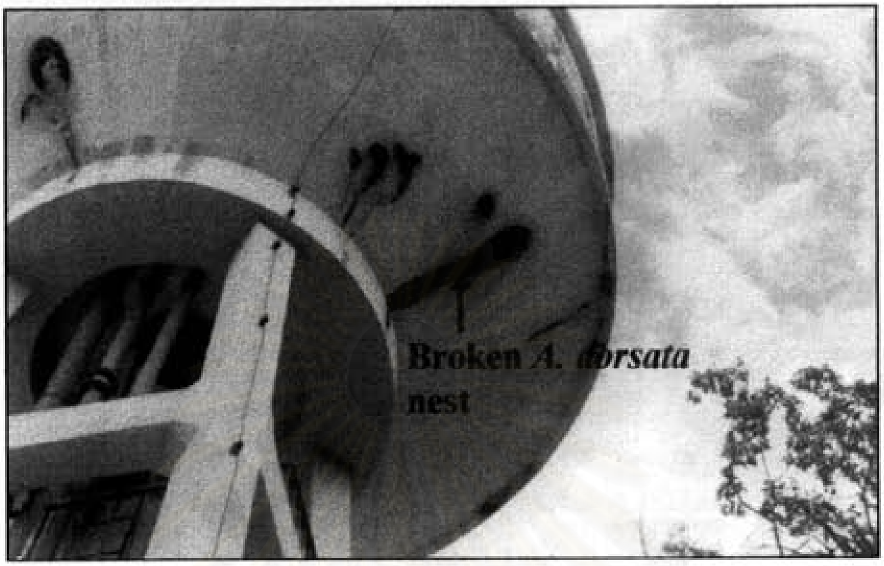

Figure 19. *A. dorsata* nest dislodged from the adjacent water tower of the health care center building by wind speed (29km/h) in June 1996.

6.5. Migration due to predator pressures

In undisturbed sites (Chiang Mai) colonies (n=235 accumulated number of colonies from 1995-1998) did not migrate during the main honey flow season. After honey flow season, 8 of 15 (53.3%) colonies were stayed 6-9 months (mean = 7.2± 1.24 months) and 3 colonies (20.0%) more than a year (14 months) (Figure 20).

In disturbed sites, in total, 62 colonies were harvested in a traditional way by burning and cutting the whole combs including brood during the major honey flow season (March-April) in Mae Tung Ting from 1996-1998. All harvested colonies (n = 62 colonies) migrated after 3-7 days. Predator pressure caused significantly higher rates of colony migration than was observed in undisturbed colonies (2×2 contingency test; χ^2 = 199.2, df = 3, $p<0.01$) and accepted the null hypothesis: colonies are regularly migrated due to predator pressure.

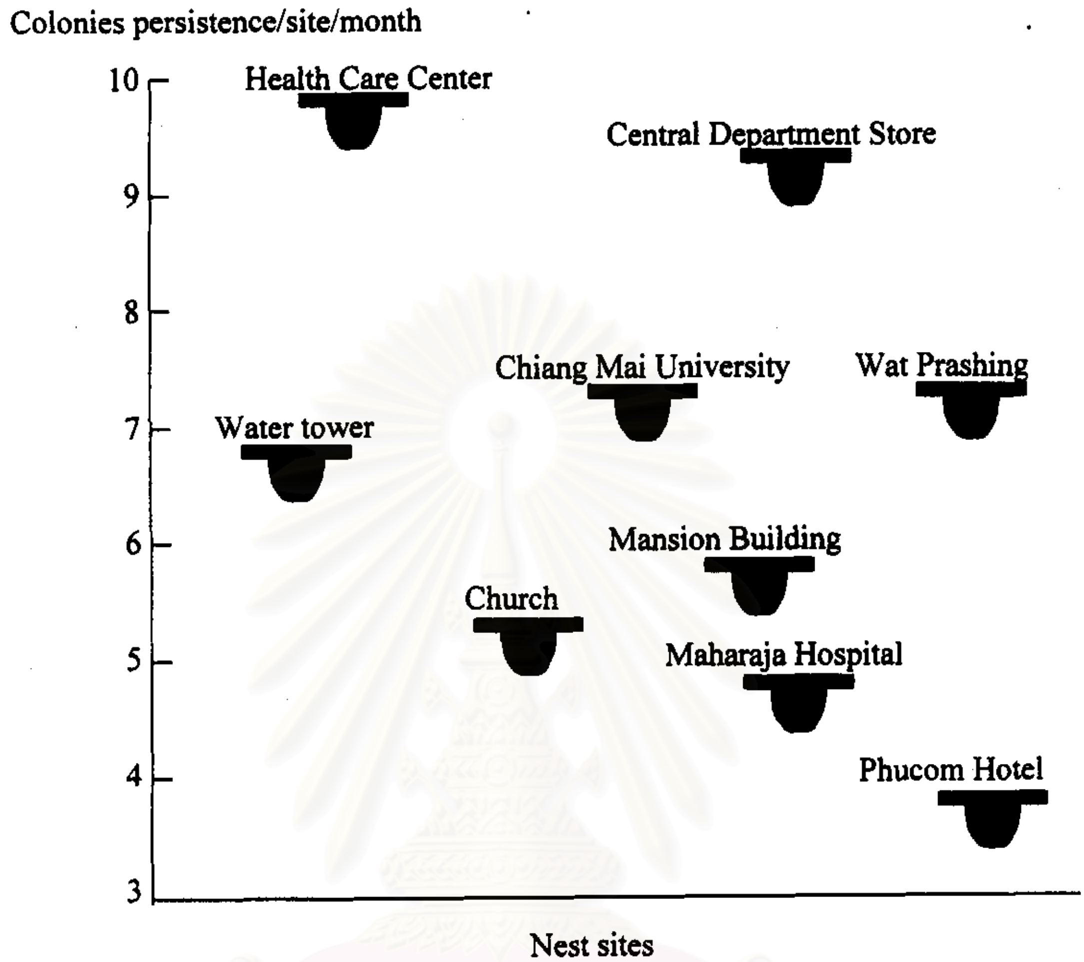

Figure 20. *A. dorsata* colonies persistence in undisturbed sites.

6.6. Migration due to parasitic mites pressure

In total, 8 colonies were examined. Only one species of ectoparasitic mite: *Tropelaelaps clareae* was detected. In total 358 bees from 8 colonies (on average 44.75 bees per colony) were examined and only 11 mites (3.10%) were found on the body of adult bees. The mite infestation rate was low (mean = 1.62; SD = 1.30). High number of mite infestation rate (11.12%) was detected in colony-2. Subsequently, 296 sealed brood of 8 combs were examined. Seven mites detected from 22 sealed brood containing dead brood in one deserted comb. Three of 8 colonies examination show the evidence of mite infestation (average 37 cells examined per comb). However, the sexes (male or female) of mites detected on the body of adult bees and brood were differentiated.