

CHAPTER V

DISCUSSION

In the first attempt to determine the specific status of *An. minimus* on Ishigaki Island, the Ryukyu Archipelago, Japan, Kanda *et al.* (1984) failed to demonstrate clear evidence of post-mating barrier with two strains of *An. minimus* from Kanchanaburi, Thailand. It is not clear whether these two Thai strains were species A or C because they established them mainly on the basis of wing characteristics which are not reliable (Green *et al.*, 1990; Van Bortel *et al.*, 1999; Chen *et al.*, 2002). Sucharit *et al.* (1988a, b) showed that their *An. minimus* P form (species C) and M form (species A) were genetically incompatible in which hybrid progeny were obtained if the female was M form. This was later confirmed by Choochote *et al.* (2002) who also reported that *An. minimus* M form and V form are the same species. Similarly, Somboon *et al.* (2001) found that only *An. minimus* species A females crossed with species E males produced hybrids. In contrast to the above, the present study found that hybrid progeny were obtained from both directions of species C x species E crosses (Table 8), probably because species C is more closely related to species E than to species A, base on DNA sequences (Fig. 3). The hybrid males obtained from either A x E or C x E cross were sterile or almost sterile with atrophied testes or abnormal spermatozoa whereas the ovaries of hybrid females were normal. By contrast, Choochote *et al.* (2002) reported that the hybrid females from the A x C

cross had abnormal ovaries whereas the hybrid males were fertile. It is therefore concluded that hybridization is a powerful means to identify species in the *An. minimus* complex.

Polytene chromosomes are another powerful means to identify mosquito species (e.g. Coluzzi *et al.*, 1979; Baimai *et al.*, 1988). Asynapsis in polytene complements in hybrids is used as one of the criteria in determining species status. In hybrids between members of the *An. minimus* complex, the chromosomes from A x C cross (Choochote *et al.*, 2002) and C x E cross in the present study are partially asynapsed, but those of A x E cross fully synapsed (Somboon *et al.*, 2001). The results of Kanda *et al.* (1984) in which complete synapsis was found in their F₁ hybrids from any direction or combinations of Japan (presumably species E) and Thai *An. minimus* strains (presumably species A or C) are ambiguous and must be treated with caution because no exact identification of species was made before hybridization. The species of mosquitoes used in the present study and of Choochote *et al.* (2002) were confirmed by DNA sequencing for the D3 region of the 28S gene of rDNA in order to avoid a mix of two species or more.

There is evidence for a significant biological difference between *An. minimus* species E and A. The size of wild Japan *An. minimus* varies seasonally, with mosquitoes being significantly larger in the winter than in other seasons. In the laboratory and under similar rearing conditions, a significant difference in the size of female mosquitoes between species E and A was found (Somboon *et al.*, 2001). No attempt was made to study this matter between species C and E because it is considered that species A and C have no significant difference in size. However, it was found that the larval period of species C was significantly shorter than species

E (Table 3). In the laboratory, the longevity of females of species C was a bit longer than species E (Table 5). By contrast, species C males had shorter longevity than species E males (Table 6). The finding in the laboratory cannot reflex their longevity in the nature because species C used in this study was the newly established colony whereas species E has adapted to laboratory conditions for years.

The ability of mosquitoes in the *An. minimus* complex free-mating in a small cage or stenogamy was demonstrated in some populations of species A (Somboon and Suwonkerd, 1997) and species E (Somboon *et al.*, 2001). These two species mate readily in 30-cm cages. *An. minimus* species C has no stenogamous behavior, but the species E males had a little success in inseminating with species C females (Table 7). This result confirms Somboon and Suwonkerd (1997) that the stenogamous behavior in *An. minimus* is male-dependent.

Differences of biological characteristics are nothing more than supportive evidence and cannot be used alone to identify species status. *An. minimus* species C is likely to be more exophagic and zoophilic than species A (Sucharit *et al.*, 1988a, b; Van Bortel *et al.*, 1999). The role of species C in malaria transmission is not clear and needs further investigations. Species E is the only species of the *An. minimus* complex found in The Ryukyu Archipelago, Japan (Somboon *et al.*, 2001; Chen *et al.*, 2002). It was the primary vector of *falciparum* malaria, particularly after World War II until 1962 when transmission was completely disrupted (WHO, 1966). A recent survey on Ishigaki Island revealed that a large number of immature stages were commonly found in the areas where cattle were available and humans were scared (P. Somboon, personal communication). This indicates that this species, in the absence of human, have adapted to feed on cattle.

In conclusion, the present study has provided strong evidence of genetic incompatibility between *An. minimus* species C and E, supporting Somboon *et al.* (2001) that species E is clearly a distinct species in the *An. minimus* complex in addition to the two known species (A and C). It also confirms that the D3 rDNA sequence is very informative of species status of the *An. minimus* complex as suggested by Walton *et al.* (1999). Of the approximately 370 base pairs of the D3 region, species E has only three bases differing from species C (Somboon *et al.*, 2001). Such a minor difference is nonetheless indicative of genetic divergence between two separate species as demonstrated by crossing experiments in the present study.