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# Genetic Diversity and Gene Flow among Stable Fly Populations, *Stomoxys calcitrans* (L.) in Thailand

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## ABSTRACT

Isozymes from five wild-caught *Stomoxys calcitrans* (L.) were compared using starch gel electrophoresis to estimate the rates of gene flow between and among *S. calcitrans* populations from five different geographic regions of Thailand. Among ten enzyme systems, 13 putative loci and 10 polymorphisms were detected. Limited genetic differentiation among the five populations was observed as indicated by the low $F_{ST}$ (0.078). The highest percentage of polymorphic loci was observed in eastern Trat province and northern Chiang Mai province (69.2%), whereas the lowest percent polymorphism was seen in south-central Saraburi province (23.1%). Gene flow between populations varied from 6.16 to 15.38 reproductive migrants per generation with no fixed genetic differences detected. Among the five population samples, no correlation was seen between genetic and geographical distances showing that sampled *S. calcitrans* fit closely in the same cluster taxa. The genetic and epidemiological ramifications of these findings are discussed.


**Key words:** *Stomoxys calcitrans*, genetics, isozyme, gene flow, Thailand

## INTRODUCTION

Stable fly (Muscidae: Stomoxyinae) in the genus *Stomoxys* contains at least eighteen described species (Zumpt, 1973). Both sexes are avid blood sucking insects and considered significant economic pests and disease vectors on livestock and other warm-blooded animals in many parts of the world (Bruce and Decker, 1958; Zumpt, 1973; Mullens *et al*., 2006). Among these, *Stomoxys calcitrans* (L.), the most cosmopolitan species of stable fly, is an aggressive, vicious biter and will readily attack humans (Harwood and James, 1979; Wall and Shearer, 1997). This species can serve as either mechanical or biological vectors of important veterinary diseases such as: *Trypanosoma evansi* (surra) in horses, camels and dogs; *Trypanosoma equium* in various domesticated ungulates in the Neotropics; several species of gastrointestinal *Habroneam* nematodes of equines; and the filarial parasite of cattle, *Setaria cervi*. It has also been implicated in the transmission of polio virus, equine infectious anemia, anthrax and fowl pox (Lehane, 1991). Although most active near livestock areas, *S. calcitrans* can be a significant nuisance insect on


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beaches and in residential areas near agricultural areas, with a typical flight range of approximately one to two km from origin (Jones *et al*., 1991). The biology of stable files has been published elsewhere (Harris *et al*., 1976; Charlwood and Lopes, 1980; Smith *et al*., 1989; Berkebile *et al*., 1994; Schofield and Brady, 1996).

In Thailand, three species of stable fly have been identified, the most prevalent being *S. calcitrans* (Masmeatathip *et al*., 2006) and they sare found in many areas of the country, mainly in the central and northeastern regions of Thailand (Sucharit *et al*., 1979; Echeverria *et al*., 1983). Recent investigations indicated that *S. calcitrans* is widespread throughout Thailand where domestic and wild animals are common (Masmeatathip *et al*., 2006). Reasons for the extensive geographical range of the non-native species are unclear but might be related to passive factors (favorable environmental conditions) and active dispersal mechanisms (e.g., search for animal hosts or transport by human conveyances). Moreover, wind-assisted active migration (appetitive flight) or passive wind-borne dispersion was reported to play a role in the movement patterns of *S. calcitrans* (Williams and Rogers, 1976; Hogsette and Ruff, 1985). Similar findings suggest that greater movement over a few kilometers appears to be a normal characteristic of *S. calcitrans* (Voegtline *et al*., 1965).

A better understanding of the biology of *S. calcitrans*, especially population genetics, is needed before initiating fly control activities. Knowledge of population structure can help estimate migration between/among different stable fly populations, provide insight into the epidemiology and transmission of pathogens, and support more responsive and effective fly control. Analysis of genetic profiles using isozymes can be used to measure the variability and levels of gene flow between *S. calcitrans* populations and provide an estimation of the natural spatio-temporal movement of genes over a wide geographical area. This study measured the genetic relationship between populations of *S. calcitrans* collected from five regions of Thailand using allele variation frequencies for estimating gene flow among these populations.

## MATERIALS AND METHODS

### Study sites

*Stomoxys calcitrans* was collected from five different provincial regions of Thailand: north, Chiang Mai (CHM); northeast, Nakhon Ratchasrima (NAK); central, Saraburi (SAR); east, Trat (TRA); and south, Surat Thani (SUR) (Figure 1). GPS coordinates and a brief description of the locations are given in Table 1.

### Collection method

At each collection site, nine 'Vavoua' traps (Laveissiere and Grebaut, 1990) were randomly placed on the ground near animals, about 10 m apart . Traps were made from blue and black cotton cloth and white polyester insect netting. Daytime (0600 to 1800 hr) collections of *S. calcitrans* occurred for two consecutive days at each site. Flies were identified to the species level and the abdomen removed to avoid blood contamination. All specimens were kept frozen (-20°C) and brought back to the laboratory at the Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand. Strict segregation of specimens was maintained to prevent sample contamination between localities.

### Starch gel electrophoresis

Horizontal starch gel electrophoresis was conducted following methods of Harris and Hopkinson (1976) and Manguin *et al*. (1995). Each fly was ground in 25 µl of grinding buffer (25 µl /2 wicks) and homogenate absorbed into a 0.4×1.4 cm cellulose polyacetate wick (Gelman Sciences Inc., Ann Arbor, MI). Ten enzyme

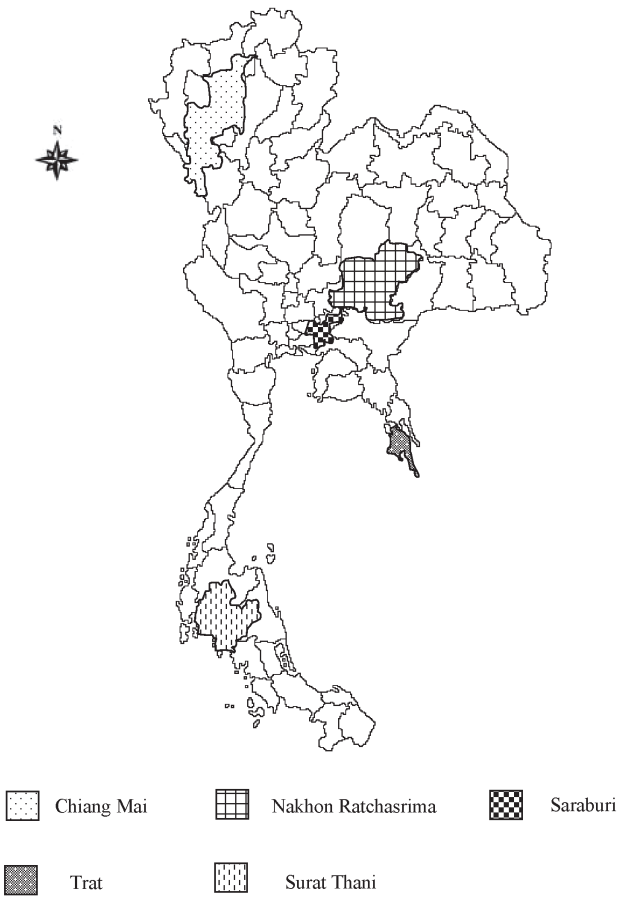


**Figure 1** *Stomoxys calcitrans* collection sites in Thailand.

**Table 1** Stable fly collection sites.

| Population | Reference points | Remarks |
|---|---|---|
| CHM | 18° 48′ N,98° 58′ E | Industrial dairy farm with approximately 80 cattle located at Mae Jo University, Chiang Mai province, northern Thailand. |
| NAK | 15° 0′ N,102° 6′ E | Local dairy farm with approximately 40 cattle located in Wang Nam Kheow District, Nakhon Ratchasima province, northern Thailand. |
| SAR | 14° 31′ N,100° 52′ E | Industrial dairy farm with > 200 cattle located in the Dairy Farming Promotion Organization of Thailand, Mauk Lek, Saraburi province, central Thailand. |
| TRA | 12° 13′ N,102° 30′ E | Local dairy farm with approximately 20 cattle located in Bo Rai District, Trat province, eastern Thailand. |
| SUR | 9° 8′ N,99° 19′ E | Local dairy farm with approximate 20 cattle located in Mueang District, Surat Thani province, southern Thailand. |

systems were analyzed with two different buffer systems: morpholine (Morph) and Tris-malate-EDTA (TMEDTA) (Pasteur *et al*., 1988), run for 6 h at a constant 16 volts/cm, stained and incubated at 37°C for 15-60 min (Table 2). Two or more alleles appearing at the same locus were defined as polymorphic. Any locus containing one allele was considered monomorphic. Different alleles of the same locus demonstrated different banding patterns, depending upon the migration speed. The most common allele was designated as "100" (Pasteur *et al*., 1988).

**Data analysis**

Chi-square tests were performed to observe any significant differences between observed and expected allelic frequencies between and among sampled populations. Analysis of allele frequencies, heterozygosity per locus, conformity to Hardy-Weinberg expectations and genetic distances were calculated using BIOSYS-1 (Swofford and Selander, 1989). Differentiation among populations was determined using *F*-statistics ($F_{ST}$). The effective migration rate ($N_em$) and exchange of genes between populations were estimated from the $F_{ST}$ values as $N_em = (1-F_{ST})/4F_{ST}$ (Nei, 1978, Wright, 1978). The GENEPOP-3.1 program was used to estimate the degree of isolation by the distance between collection samples (Raymond and Rousset, 1995; Rousset, 1997), i.e., the relationship between pairwise estimates of $F_{ST}$ and logarithms of geographical distance to determine whether geographical distance among populations served as an effective barrier to gene flow.

## RESULTS

From 10 enzyme systems, 13 putative loci were detected (Table 2). The number of polymorphic loci by populations included CHM (7), NAK (7), SAR (4), TRA (9), SUR (6), and allelic frequencies are presented in Table 3. Among all test populations, *Acp-1, Aox-1, Gpd-1* and *Pgm-1* were observed as polymorphic.

From 65 comparisons, there were 20 significant deviations from the Hardy-Weinberg equilibrium ($P < 0.05$), a value greater than 5% of expected deviations by chance alone. Ten of these significant departures occurred in the TRA population, including *Aox-1* ($\chi 2$=61.851 df=6), *Gpd-1* ($\chi 2$=57.518 df=6), *Got-1* ($\chi 2$= 59.018 df= 1), *Got-2* ($\chi 2$=59.018 df=1), *Had-1* ($\chi 2$=57.038 df=3), *Idh-1* ($\chi 2$=88.800 df=3), *Idh-*

**Table 2** Enzymes and loci used in starch gel electrophoresis on adult *S. calcitrans*.

| Enzyme system | E.C. * | No. of loci** | Buffer |
|---|---|---|---|
| *Acp* (Acid phosphatase) | 3.1.3.2 | 1 | Morph |
| *Aox* (Aldehyde oxidase) | 1.2.3.1 | 1 | Morph |
| *Mez* (Malic enzyme) | 1.1.1.40 | 1 | Morph |
| *Mdh* (Malate dehydrogenase) | 1.1.1.37 | 2 | Morph |
| *Pgm* (Phosphoglucomutase) | 2.7.5.1 | 1 | Morph |
| *Gpd* (α-Glycerophosphate dehydrogenase) | 1.1.1.8 | 1 | TMEDTA |
| *Got* (Glutamate-oxaloacetate-transaminase) | 2.6.1.1 | 2 | TMEDTA |
| *Had* (β-Hydroxyacid dehydrogenase) | 1.1.1.30 | 1 | TMEDTA |
| *Idh* (Isocritrate dehydrogenase) | 1.1.1.42 | 2 | TMEDTA |
| *6-Pgd*(6-Phosphogluconate dehydrogenase) | 1.1.1.44 | 1 | TMEDTA |
| | | 13 | |

* Enzyme Commission Number

** Number of scored bands per phenotype

*2* ($\chi$2=59.139 df=3), *Mez-1* ($\chi$2=59.018 df=1), *Pgd-1* ($\chi$2=67.797 df=3) and *Pgm-1* ($\chi$2=29.278 df= 6). Departures were found at one locus in SAR (*Gpd-1*) ($\chi$2=85.010 df=1), two loci from SUR (*Pgd-1*) ($\chi$2=8.816 df=1) and *Pgm-1* ($\chi$2=17.175 df=3), three loci from NAK (*Acp-1*) ($\chi$2=63.971 df=6), (*Idh-1*) ($\chi$2=34.043 df=3) and *Pgm-1* ($\chi$2=17.023 df=6) and four loci from CHM (*Aox-1*) ($\chi$2=15.005 df=3), (*Got-1*) ($\chi$2=39.390 df=3), (*Got-2*) ($\chi$2=35.457 df=1) and *Pgd-1* ($\chi$2=9.429 df=1). All significant deviations were associated with heterozygote deficiency.

A number of alleles were restricted to a single population: *Aox-1* (allele 138), *Gpd-1* (allele 127), *Got-1* (allele 240), *Had-1* (allele 92), *Idh-2* (allele 130), *Mez-1* (allele111) and *Pgd-1* (allele 155) in the TRA population; *Got-1* (allele 75) and *Mdh-1* (allele 200) with CHM; and *Acp-1* (allele 133), *Mez-1* (allele 86) and *Pgd-1* (allele 54) with NAK. Moreover, *Idh-1*(allele 108), *Idh-2* (allele 54), *Pgd-1*(allele 108) and *Pgm-1*(allele 43) were absent in the SAR population (Table 3). A higher percentage of polymorphic loci was observed in the CHM and TRA populations (69.2%) compared to the other three, which had values ranging from 23.1 to 53.8%. The greatest number of alleles per locus (2.8) was observed in the TRA population with the most heterozygosity (Ho=0.121±0.031) observed in the NAK population (Table 4). The lowest variability was observed in the SAR population (23.1%), with a low number of alleles per locus (1.9) and a low level of heterozygosity (Ho=0.072±0.032). Observed heterozygosities from all locations were not significantly different from the Hardy-Weinberg expected heterozygosities ($t_{0.025} = 2.306$, df $=8$, $P > 0.05$) (Table 4).

Gene flow between populations was

**Table 3** Allele frequency and sample size (*n*) of five collections of *S. calcitrans*.

| Locus | *Stomoxys calcitrans* population | | | | | |
|---|---|---|---|---|---|---|
| | Allele | CHM[1] | NAK[2] | SAR[3] | TRA[4] | SUR[5] |
| *Acp*-1 | n | 25 | 28* | 43 | 30 | 28 |
| | 75 | 0 | 0.125 | 0.174 | 0.017 | 0 |
| | 100 | 0.88 | 0.768 | 0.802 | 0.917 | 0.982 |
| | 133 | 0 | 0.036 | 0 | 0 | 0 |
| | 195 | 0.12 | 0.071 | 0.023 | 0.067 | 0.018 |
| *Aox*-1 | n | 30* | 21 | 21 | 30* | 28 |
| | 76 | 0.15 | 0.024 | 0.024 | 0.017 | 0 |
| | 100 | 0.75 | 0.905 | 0.905 | 0.783 | 0.857 |
| | 128 | 0.1 | 0.071 | 0.071 | 0.167 | 0.143 |
| | 138 | 0 | 0 | 0 | 0.033 | 0 |
| *Gpd*-1 | n | 30 | 27 | 43* | 29* | 27 |
| | 64 | 0 | 0.019 | 0 | 0.017 | 0 |
| | 100 | 0.95 | 0.852 | 0.977 | 0.845 | 0.898 |
| | 127 | 0 | 0 | 0 | 0.034 | 0 |
| | 142 | 0.05 | 0.13 | 0.023 | 0.103 | 0.111 |
| *Got*-1 | n | 30* | 22 | 43 | 30* | 28 |
| | 75 | 0.033 | 0 | 0 | 0 | 0 |
| | 100 | 0.9 | 1 | 0.988 | 0.967 | 0.964 |
| | 148 | 0.067 | 0 | 0.012 | 0 | 0.036 |
| | 240 | 0 | 0 | 0 | 0.033 | 0 |

**Table 3** Allele frequency and sample size (*n*) of five collections of *S. calcitrans*. (cont)

| Locus | *Stomoxys calcitrans* population | | | | | |
|---|---|---|---|---|---|---|
| | Allele | CHM[1] | NAK[2] | SAR[3] | TRA[4] | SUR[5] |
| *Got*-2 | n | 30* | 28 | 43 | 30* | 28 |
| | -100 | 0.9 | 1 | 0.977 | 0.967 | 0.982 |
| | -273 | 0.1 | 0 | 0.023 | 0.033 | 0.018 |
| *Had*-1 | n | 30 | 28 | 43 | 29* | 28 |
| | 92 | 0 | 0 | 0 | 0.034 | 0 |
| | 100 | 0.933 | 1 | 1 | 0.931 | 0.875 |
| | 110 | 0.067 | 0 | 0 | 0.034 | 0.125 |
| *Idh*-1 | n | 30 | 26* | 43 | 29* | 21 |
| | 86 | 0 | 0.019 | 0 | 0.034 | 0 |
| | 100 | 0.95 | 0.904 | 1 | 0.793 | 1 |
| | 108 | 0.05 | 0.077 | 0 | 0.172 | 0 |
| *Idh*-2 | n | 26 | 28 | 43 | 30* | 22 |
| | 54 | 0 | 0.036 | 0 | 0.067 | 0.068 |
| | 100 | 1 | 0.964 | 1 | 0.9 | 0.932 |
| | 130 | 0 | 0 | 0 | 0.033 | 0 |
| *Mdh*-1 | n | 28 | 27 | 43 | 30 | 22 |
| | 57 | 0 | 0.093 | 0.035 | 0 | 0.045 |
| | 100 | 0.964 | 0.907 | 0.965 | 1 | 0.995 |
| | 200 | 0.036 | 0 | 0 | 0 | 0 |
| *Mdh*-2 | n | 7 | 9 | 43 | 16 | 10 |
| | -100 | 1 | 0.889 | 0.942 | 0.906 | 1 |
| | -157 | 0 | 0.111 | 0.058 | 0.094 | 0 |
| *Mez*-1 | n | 30 | 28 | 43 | 30* | 27 |
| | 86 | 0 | 0.018 | 0 | 0 | 0 |
| | 100 | 1 | 0.982 | 1 | 0.967 | 1 |
| | 111 | 0 | 0 | 0 | 0.033 | 0 |
| *Pgd*-1 | n | 30* | 18 | 30 | 30* | 30* |
| | 54 | 0 | 0.028 | 0 | 0 | 0 |
| | 100 | 0.883 | 0.972 | 1 | 0.9 | 0.933 |
| | 108 | 0.117 | 0 | 0 | 0.067 | 0.067 |
| | 155 | 0 | 0 | 0 | 0.033 | 0 |
| *Pgm*-1 | n | 29 | 26* | 43 | 30* | 27* |
| | 43 | 0.086 | 0 | 0 | 0.017 | 0 |
| | 68 | 0.138 | 0.058 | 0.012 | 0.033 | 0.056 |
| | 87 | 0 | 0.038 | 0.012 | 0 | 0 |
| | 100 | 0.757 | 0.808 | 0.953 | 0.867 | 0.87 |
| | 135 | 0.017 | 0.096 | 0.023 | 0.083 | 0.074 |

[1] Chiang Mai, [2] Nakhon Ratchasima, [3] Saraburi, [4] Trat, [5] Surat Thani.

* Deviation from Hardy-Weinberg equilibrium ($P < 0.05$).

**Table 4** Genetic variability at 13 loci of pooled collections of *S. calcitrans*.

| Collection | Average alleles per locus | % polymorphic loci [1] | Mean heterozygosity | |
|---|---|---|---|---|
| | | | $H_{obs}$ | $H_{exp}$ [2] |
| CHM | 2.1±0.2 | 69.2 | 0.107±0.030 | 0.154±0.038 |
| NAK | 2.3±0.3 | 53.8 | 0.121±0.031 | 0.146±0.037 |
| SAR | 1.9±0.3 | 23.1 | 0.072±0.032 | 0.069±0.026 |
| TRA | 2.8±0.3 | 69.2 | 0.102±0.028 | 0.175±0.031 |
| SUR | 1.9±0.2 | 46.2 | 0.121±0.030 | 0.125±0.028 |
| | | | Average | 0.134±0.032 |
| | | | | $t_{0.025} = 2.306$ [ns] |

[1] Locus considered polymorphic when frequency of the most common allele < 0.95.

[2] Unbiased estimate and standard error (Nei, 1978).

[ns] Not significant.

**Table 5** F-statistics, effective migration rates by distance among populations of all loci at five collection samples of *S. calcitrans*.

| | CHM | NAK | SAR | TRA | SUR |
|---|---|---|---|---|---|
| CHM | Infinite | 777 | 625 | 1,011 | 1,340 |
| NAK | 0.028 | Infinite | 152 | 399 | 903 |
| | -8.68 | | | | |
| SAR | 0.038 | 0.018 | Infinite | 380 | 751 |
| | -6.33 | -13.64 | | | |
| TRA | 0.021 | 0.017 | 0.035 | Infinite | 959 |
| | -11.65 | -14.46 | -6.89 | | |
| SUR | 0.025 | 0.027 | 0.039 | 0.016 | Infinite |
| | -9.75 | -9 | -6.16 | -15.38 | |

Coefficient of determination of isolation by distance between populations $r^2 = 0.043^{ns}$.

Above diagonal: Geographic distance (km); Below diagonal: Pairwise *F*-statistics[1] and effective migration rate ($N_em$) .

[1] Genetic differentiation scale: $F_{ST} > 0.25$ Large, $0.25 > F_{ST} > 0.15$ Moderate, $0.15 > F_{ST} > 0.05$ Small, and $F_{ST} \leq 0.05$ Negligible.

[ns] Not significant.

calculated by estimating $N_eM$, where $N_e$ is the effective population size and $M$ is the migration rate between populations. As $M$ is the proportion of migrants (number of migrants/$N_e$), $N_eM$ is actually an estimate of the number of migrants regardless of population size that would be allowed, still permitting the observed degree of genetic differentiation between the test populations. Among all test populations, $N_eM$ estimated from the $F_{ST}$ (0.040) was 6.00 (data not shown). The $N_eM$ between populations ranged from 6.16 to 15.38 (Table 5). The lowest gene flow was found between SAR and SUR, whereas the highest was observed between SUR and TRA (Table 5). An analysis among all populations indicated that there was no correlation between genetic and geographical distance among the five populations of *S. calcitrans* ($r^2$= 0.054, df =8, $P >$ 0.05) (Table 5). The derived phenogram shows that the five sampled populations of *S. calcitrans* from different sites in Thailand occurred in a common genetic cluster as indicated by a low genetic distance (< 0.018) (Figure 2).

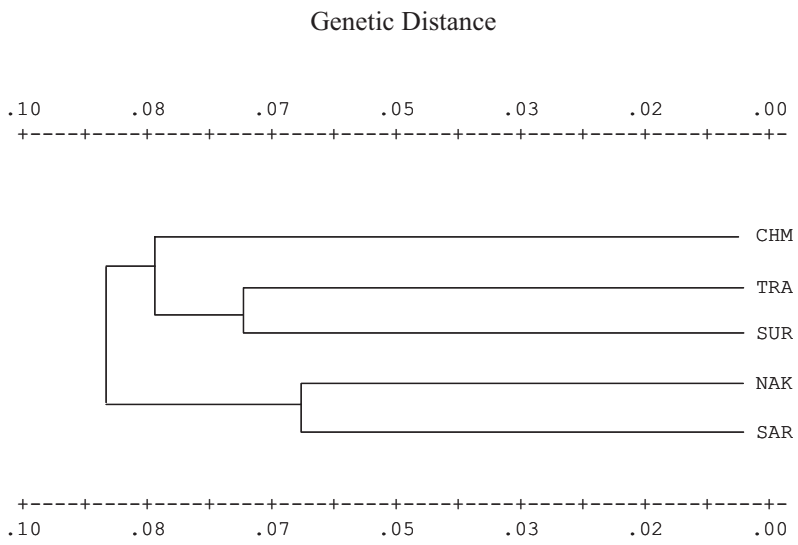


**Figure 2** An unweighted pair-group method averaging phenogram from modified Roger's distance (Wright, 1978) matrix among five populations of *Stomoxys calcitrans* in Thailand (cophenetic correlation = 0.852).

## DISCUSSION

Insect population dispersal via passive and active movements is considered an important means of natural gene flow. Dispersal by whatever means would likely influence the genetic structure and gene flow between *S. calcitrans* populations. Such information can be of epidemiological importance in understanding the insect's biology and potential to expand geographically its negative economic impact and ability to transmit disease. The temporal and spatial differences, with respect to expression of enzymes that are associated with various components of vectorial capacity for disease pathogens, may be influenced by the patterns of gene flow between and within same-species populations. For example, a correlation existed between genetic distance and variation in ability of *Aedes aegypti* to competently replicate and transmit dengue viruses (Bosio *et al*., 2000; Ocampo and Wesson, 2004). Defining the population structure of stable flies in association with capacity to transmit diseases can assist predictive modeling and timely planning for allocation of insect monitoring and application of control.

The genetic background of *S. calcitrans* populations has not previously been reported in Thailand. The genetic variation and gene flow between and among five different geographical populations of *S. calcitrans* were compared using electrophoresis and resulting allozyme allele frequencies. The percentage of polymorphic loci and mean heterozygosity were comparatively high in this study compared to findings with *S. calcitrans* in the USA (Jones *et al*., 1991; Szalanski, 1995). Several polymorphic loci in this study were not observed in others (Jones *et al*., 1987; Szalanski *et al*., 1996). However, the findings in the current study did correspond more closely to the percentage of polymorphic loci and mean heterozygosity reported by Krafsur (1993).

The low genetic variability in the SAR population (23.1 %) was due to the absence of several alleles among isozymes tested, and it is

suggested that there may be several contributing factors, namely a genetic bottleneck produced by insecticide applications. The SAR population was collected from a large, industrial-size dairy farm with frequent (once or twice a week) use of insecticides for controlling the fly. Additionally, progressive urbanization and other human activities near the SAR site have greatly reduced preferred ecological habitats and other animal hosts to sustain large fly populations with a significant impact on the genetic structure of *S. calcitrans* in the area. This finding is in concurrence with other studies on genetic differentiation of other dipterans (Failloux *et al*., 1995; Lerdthusnee and Chareonviriyaphap, 1999). In contrast, higher genetic variability was seen in populations associated with relatively open, more rural farming systems and in which fly control activity was generally quite limited and preferred breeding habitats and hosts more abundant.

Ten of the 18 significant departures from the Hardy-Weinberg equilibrium occurred in the TRA population collected near the Cambodian border and surrounded by steep mountains on the eastern side and the Gulf of Thailand to the west. *Stomoxys calcitrans* from this site exhibited significant deficiency of heterozygotes that could have arisen from a restricted, inbred, population structure or from a sampling bias, i.e., only one or a few sympatric interbreeding 'families' existed at the time of sampling, possibly the consequence of the normal prolonged rainy period (8-10 months per year) experienced in Trat province. Relative geographical isolation and less conducive weather conditions and habitats reduce reproduction success and are more prone to produce smaller, less diverse populations.

Among r-K model life history strategies, r-selection (i.e., intrinsic rate of population increase) is a common ecological strategy for many insect species, including the stable fly. R-strategists typically exist as opportunists, quickly exploiting varying environmental conditions and are characterized as producing large numbers of offspring with often high, immature mortality, and large numbers of adult insects displaying a strong predilection for dispersal during times of stress (Schowalter, 1996). Regardless of the mechanism(s) involved, any significant reduction in adult insects from generation to generation can result in genetic bottlenecks in a localized population (Futuyma, 1986). It is suspected that the prevailing environmental conditions at TRA have had a significant impact on development and expansion of *S. calticans* in the area.

Conversely, the significant deviation from Hardy-Weinberg equilibrium and heterozygosity deficiency seen in the CHM population was likely the result of dispersal and migratory behavior as both larval breeding habitats and animal hosts were readily available and no geographical barriers existed in the area. Passive transportation of immature stages in manure for agricultural use may play a significant role in population structure in the CHM site. Both active and passive movement offer possible dispersal pathways for *S. calcitrans* into and out of CHM.

The NAK population was collected from a small rural village in the Tub Lan National Park. Genetic diversity and average allele per locus were relatively high compared with the other 4 samples. This would be expected for wild populations of insects exposed to few, if any, population control activities (Sukonthabhirom *et al*., 2005). This can be contrasted with the SAR population which had very low genetic variability compared to the other four populations with a leading contributing factor being the routine use of insecticides (primarily synthetic pyrethroids) to control *Stomoxys* flies.

The $N_eM$ value among all five populations was 4.21 reproductive migrants/ generation which was lower than the $N_eM$ value obtained from Nebraska (5.85) (Szalanski, 1995) and from Reunion Island (12.25) (Gilles *et al*., 2004). However, the fact that the $N_eM$ value was greater than 4.0 indicates that sufficient gene flow

may still play a major role in the genetic structure of the insect populations (Szalanski, 1995 cited in Schnabel and Hamrick, 1990)

The unbiased genetic distances obtained indicated that there was no significant genetic difference between the five populations examined. The resulting phenogram produced two closely-related clusters: Nakhon Ratchasrima and Saraburi (NAK and SRA); and the second including Chiang Mai, Trat and Surat Thani (CHM, TRA, SUR). CHM had the greatest deviation but with no significant difference in genetic background with the others.

## CONCLUSIONS

Isozyme electrophoresis has long been used for the study of genetic and evolutionary biology of many organisms, including insects. It has been used to distinguish species and populations of organisms that are difficult to identify by standard morphological methods. This technique has demonstrated the value of the study of phylogenetic relationships to investigate species homologies and to study ecological genetic relationships.

The starch gel electrophoresis technique allowed the current study to explore dozens of genes on one set of test specimens simultaneously. In this study, starch gel electrophoresis was used to estimate the rates of gene flow between and among *S. calcitrans* populations from five different geographic regions of Thailand. Among ten enzyme systems, 13 putative loci and 10 polymorphisms were detected. Limited genetic differentiation among the five populations was observed as indicated by the low $F_{ST}$ The highest percentage of polymorphic loci was observed in eastern Trat Province and northern Chiang Mai Province, whereas the lowest percentage of polymorphism was seen in south-central Saraburi Province.

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