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THESIS

POPULATION GENETICS OF STABLE FLY, *Stomoxys calcitrans* (Linneaus) IN THAILAND

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A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
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Krajana Tainchum 2009: Population Genetics of Stable Fly, *Stomoxys calcitrans* (Linneaus) in Thailand. Master of Science (Entomology), Major Field: Entomology, Department of Entomology. Thesis Advisor: Associate Professor Pongthep Akratanakul, Ph.D. 52 pages.

Starch gel electrophoresis of isozymes was used to estimate gene flow among nine populations of *Stomoxys calcitrans* (L.) of thirteen putative loci, 10 polymorphic were detected among the ten enzyme systems examined. Limited genetic differentiation among populations was observed (F_{ST} =0.060). The highest level of polymorphism was observed in eastern Trat and northern Chiang Mai Provinces (69.2%), whereas the lowest level of polymorphism was seen in central Saraburi Province (23.1%). Gene flow between populations varied from 3.32 to 27.53 reproductive migrants per generation with out diagnostic differences. Among the nine populations, 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.

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INTRODUCTION

Many species of dipterans are known as blood sucking insect (Schofield and Torr, 2002). Previous studies on blood sucking diptera indicated that a few species are related to the occurrence of flies of medical and economic importance, including *Stomoxys calcitrans* (L.) (Szalanski and Owens, 2003).

Stable flies belong to the subfamily Stomoxyinae in the family Muscidae of the order Diptera. Around the world the genus *Stomoxys* contains at least eighteen species (Zumpt, 1973). They are blood sucking insects and considered pests of livestock and other warm-blooded animals in several parts of the world (Zumpt, 1973, Mullens *et al*., 2006; Masmeathathip *et al*., 2006). Among these, *S. calcitrans* (L.), the most cosmopolitan species, is aggressive and sometimes bite humans in extreme conditions (Wall and Shearer, 1997). Although being active at livestock farms, *S. calcitrans* is invariably a nuisance insect on beaches and in residential areas used for agricultural purposes, with a flight range of approximately one mile. The biology of stable files has been published elsewhere (Newstead, 1906; Bruce *et al*., 1958; LaBrecque *et al*., 1975; Harris *et al*., 1976; Charlwood and Lopes, 1980; Smith *et al*., 1989, Berkebile *et al*., 1994; Schofield and Brady, 1996; Gilles *et al*., 2004).

In Central of Thailand, three species of stable fly were identified (*S. calcitrans* (L.), *Stomoxys uruma* Shinonaga & Kano, *Stomoxys sitiens* Bezzi), with the most prevalent being *S. calcitrans* (Masmeatathip *et al*., 2006). Due to a favorable environment, *S. calcitrans* is found in many areas of the country, mainly in the central and northeastern parts of Thailand (Sucharit *et al*., 1979; Echeverria *et al*., 1983). Recent investigation indicated that *S. calcitrans* is now widespread throughout Thailand where domestic and wild animals are present (Masmeatathip *et al*., 2006; Muanvorn unpublished paper). Reasons for this rapid movement are unclear but it could be related to both passive (via environmental factors and manure transportation) and active

movements (via search of animal hosts). Moreover, wind-borne dispersion of active migration, passive wind-borne dispersion and a series of appetites were reported to play a role in movement pattern of *S. calcitrans* in the USA (Williams and Rogers, 1976; Hogsette and Ruff, 1985). Similar findings suggested 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 in the area of population genetics, is needed prior to launching any fly control activities. Knowledge of population structure can help estimate migration between/among stable fly populations, provide insight into the epidemiology and transmission of pathogens, and support more responsive and effective fly control. Among several techniques, 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.

OBJECTIVE

The goal of this present study was to measure the relationship between nine populations of *Stomoxys calcitrans* (L.) collected from nine different locations of Thailand using information from comparable allele variation frequencies by estimating gene flow among those populations.

LITERATURE REVIEW

1 Biology of *Stomoxys calcitrans* (L.)

The stable fly, *Stomoxys calcitrans* (L.) is a species in the family Muscidae, subfamily Stomoxiynae. This species is a largely cosmopolitan and native to Africa. The genus *Stomoxys* contains at least eighteen species that are of meaningful veterinary importance as major pests of confined and pastured livestock (Zumpt, 1973). *Stomoxys calcitrans* is known by several common names, including stable fly, biting house fly, dog fly, barn fly, and power mower fly (Hall and Smith, 1986).

Stable flies resemble house flies in appearance. Although, a stable fly can be notable by mouth parts, piercing /sucking long proboscis that points forward from under the head. This proboscis is composed of the labium, with a short labellum, the labrum, and the hypopharynx (Zumpt, 1973). The wing venation of stable fly has a slight bend upwards on vein M_{1+2} (Castro, 1967; Foil and Hogsette, 1994). Adult stable flies are 5 - 6 mm in length, dark gray in color and possess four longitudinal black stripes on the thorax and a larger checkered abdomen with a distinct pattern of black spots (Service, 1980; Hewitt, 1910).

Both sexes of stable fly are hematophagous (i.e., blood feeders), which regularly draw blood from warm-blooded animals and feed to full capacity in less than 4 minutes (Harwood and James, 1979). Adults of both sexes approach host, two to three times daily to feed (Schofield and Torr, 2002). Stable flies are diurnal feeders and they have a bimodal pattern of feeding with peaks at 10 a.m. and 4 .00 p.m. under favorable environmental conditions (Hoffman, 1968). The majority of adult stable flies prefer to feed on the lower side of large animals such as cattle, horses, pigs, sheep and donkeys. During summer, when large populations of stable flies occur, they may also feed on the sides or the backs of their hosts (Hogsette and Farkas, 2000). Female stable flies are anautogenous, meaning that they require several blood meals to complete their reproductive function (Jones *et al*., 1992). The males require at least one blood meal to produce seminal fluid and to stimulate sexual drive (Klowden, 1996). Furthermore, Jones *et al.* (1992) observed that the main energy of adult stable flies is nectar from

different flowers, however the nectar itself was deficient for reproduction if a blood meal was not available (Jones *et al*., 1985).

1.1 Breeding Habits

The habitat of the stable fly, as suggested by their common name, are almost anywhere where horses, cattle, and other agricultural animals can be found especially inside barns and stables (Bishop, 1913). They breed in a number of habitats commonly found in agricultural areas such as decaying straw, oats, rice, barley, wheat, silage, horse manure, lot manure (manure from pig farms), and cow manure. The female must be engorged for reproduction. The female never oviposits before the third feeding and, on average four engorgements are necessary before eggs can be laid. The female has a greatly extended pseudovipositor with which she deposits eggs into decaying straw where there is moisture.

1.2 Life History

The life cycles of this species consists of eggs, three larval stages (maggots), pupa, and adult (Ross *et al.*, 1982). *Stomoxys calcitrans* locates its host by sight. After a stable fly female has taken enough of a blood meal, they search for a suitable oviposition site and deposits eggs throughout the media. Oviposition sites of stable fly females are composed of decomposing grass clippings, green chop, compost piles, spilled feed, manure and urine - contaminated hay or straw, and manure especially manure over 3 weeks old (Meyer and Petersen, 1983).

Bishop (1913) reported that an egg is laid singly, or in bunches of 25 or 30. While, Lysyk (1998) explored that a female stable fly can lay between 40 and 60 eggs in each gonotrophic cycle. The life fecundity ranges between 30 and 700 eggs. The duration of this period is affected by ambient temperature, relative humidity, and how long the egg was retained by the female. During the summer months, the stable fly completes its life cycle in about 3 weeks while the house fly requires about 2 weeks. The eggs of stable flies are about 1 mm long and 0.2 mm wide and banana-like in shape (Harwood and James, 1979).The eggs hatch between 12 and 24 hours after being laid.

The first larvae stage requires 24 h for development, whereas the second stage lasts for 28 h and the third stage lasts for up to 7 days (Foil and Hogsette, 1994).

The larval stage lasts from 11 to 30 (and sometimes more) days, based upon habitat suitability and availability of food. Larvae range in size from 5 to 12 mm long. Mature larvae are yellowish white maggots, and are a cylindrical shape that tapers anteriorly. Larval habitats of stable flies in confined livestock operations are well documented, and include spilled feed, stored manure, and silage (Meyer and Peterson, 1983). It was demonstrated by Skoda *et al*. (1991) in a feedlot study that feed aprons yielded about 63% of the larvae of stable flies. However, information is limited on stable fly development in pastures. Hay wasted by cattle while feeding from large bales and mixed with manure may constitute the greatest medium for stable fly development in certain places (Foil and Hogsette, 1994). It has been demonstrated that hay wasted by cattle during winter feeding mixed with manure and remaining in the field through early spring, can become excellent habitats for the development of stable fly larvae (Broce *et al.*, 2005). Other materials not related to livestock such as compost pile containing grass clippings, and poultry litter used as fertilizer in horticultural crop production have also been shown to be ideal habitats for stable fly development (Cook *et al*., 1999).

After the third stage, it takes approximately 6 to 20 days to pupate. As with larval maturation, length of pupation is based upon food abundance and quality during larval growth. Stable fly pupal development takes place inside the puparium, which is the hardened cuticle and has a reddish-to-dark brown exterior with more or less 4 to 7 mm long. The posterior spiracles on the puparia are black with three S-shaped yellow slits, and are lightly sclerotized (Bishop, 1913). Pupal development lasts between 5 and 26 days. In tropical areas, development of larvae and pupae is fast and continuous year round. However, in temperate areas, development of larvae and pupae is slower during cooler temperatures (Service, 1980).

Once an adult emerges, it elongates and the body turns dark within 30 minutes, the wings expands, the proboscis folds forward, and then the newly emerged fly is ready to fly (Castro, 1967). Adult stable flies are generally gray body and can be identified by four characteristic longitudinal stripes across the thorax as well as several

dark spots on top of the abdomen. On the vertex and frons, there are three ocelli and two large compound eyes (Bishop, 1913). Broad frons at vertex measuring about 1/2 of eye length in female and 1/3 in male (Zumpt, 1973). The total time for development of the fly, from egg laying to emergence to be 33-36 days at 21˚c (Mitzmain, 1913).

1.3 Food Habits

On cattle, it will first land and rest on the back of the animal. As soon as the cattle is disturbed, the flies land on the outside of the forelegs; where it will bite the cow near the knees and begin feeding. On horses, *S. calcitrans* prefers feeding on the sides of the neck, lower legs, and underbelly. After feeding, *S. calcitrans* rests nearby, usually on warm, sunny sites such as fences, walls and vegetation near the hosts. When disturbed it will fly and then return to the original spot of feeding (Bishop, 1913). *Stomoxys calcitrans* is a daytime feeder, draws blood quickly and fills to full capacity in 3-4 minutes if undisturbed, but ordinary, even when undisturbed, it changes position frequently or flies from one animal to another, where the meal is continued (James and Harwood, 1969). Stable flies are not specific host insect. They prefer cattle, being less frequent on horses and pigs, under laboratory conditions, the flies consume blood of laboratory mammals and birds (Greenburg, 1971). After feeding, the stable fly is sluggish, and remains motionless near the host. After hatching, the larvae begin feeding on local microbial flora and fauna (Bishop, 1913).

2 Medical importance

Stomoxys calcitrans economically affects humans in two different ways: disease transmission and livestock reduction. However, it has been confirmed that stable flies can serve as carriers of pathogens for anthrax, brucellosis, equine infectious anemia and animal trypanosomosis, and that they also play the role of intermediate hosts of nematode worms and some stomach parasites (Greenburg, 1971, Harwood and James, 1979). For horses, ox and sheep, *S. calcitrans* is a vector of *Trypansoma vivax.* This parasite causes the disease known as souma. For ox, it is a vector of *T. pecaudia*. For domestic animals and humans, it is a vector of anthrax. This disease can cause a number of different symptoms, inculding lesions in the lungs and brain. It is also a vector for *T.*

evansi (the agent of Surra), *T. brucei*, ERF, brucellosis, swine erysipelas, equine swamp fever, African horse sickness, and fowl pox (Bishop, 1913). Lameness in horses has also been reported to be due to the continuous stomping, and swelling; and stiff joints in other animals bitten by stable flies are common (Zumpt,1973).

Campbell *et al*. (2001) and Greenburg (1971) reported that the stable fly affect the cattle industry by destroying the hides of cattle due to the holes created by the piercing of the skin for feeding. In the beef cattle and dairy cattle farm, fly feeding may result in a 10 to 15 % loss of beef cattle body weight when stable fly population is high and reduced yield of dairy cattle has been reported to be as high as 40 to 60 % in some cases, just as, Catangui *et al.* (1997) demonstrated that they can affect to weight gain reductions of 0.02 to 0.05 kg per day and feed efficiency reduction of 11 to 13 percent have been documented in feedlots. Several economic thresholds have been estimated. Mc Neal and Campbell (1981) used an economic threshold of 5 stable flies per cow's front leg, while Catangui *et al.* (1997) established an economic threshold of 7 per cow per leg.

As a result of host's defensive behaviors, stable flies make numerous visits, biting repeatedly before obtaining a full meal. Cattle attacked by stable flies, attempts to find a position within the bunch to protect their front legs, which are the favored feeding site of the flies. Considerable energy is expended by foot stamping, tail twitching, and throwing the head toward the front legs in an effort to dislodge the flies or prevent feeding. Stable flies can reduce weight gain, milk production, and feed efficiency both from their feeding and because of the bunching behavior of the cattle, which may induce or increase heat stress and hence reduce feed intake (Weiman *et al.*, 1992). Bruce and Decker (1958) estimated the effect of stable flies on the hosts increases proportionally with an increase in the number of bites. This trend eventually reaches a plateau, due to the fact that the stable fly is only a daytime feeder.

3 Distribution and Abundance

Stable fly is inhabitant in the old world. However, they were possibly found in the New World possibly during the mid 17th century (Szalanski, 1995). Stable fly is a

strong flier and has been observed to travel long distances (Hoffman, 1968). A wind assisted flight maximum range of 225 km in Florida by using mark release recapture (Hogsette and Ruff, 1985) and Eddy *et al*. (1962) recovered marked adults 5 miles from the release point within 2 hours after release. Gersabeck and Merritt (1985) intended a potential adult lifetime migration radius of 140 km. This was based on movement of 7 km per day (Bailey *et al*, 1973). Movement of stable flies in time and space is probably a function of host activity patterns, duration of feeding, and potential of the insect to fly. When provided with abundant host source, i.e., feedlots, 90% of marked flies were captured within 0.8 km of the release site (Gersabeck and Merritt, 1985). Scholl (1986) observed that 80% of dispersing flies recovered between two feedlots, of 0.8 km apart, were males. The information on the distribution and dispersal pattern of the stable fly should be an important step in developing a pest management strategy (Gersabeck and Merritt, 1985).

4 Stable Fly Genetics

The five pairs of homomorphic chromosomes in stable flies were reported (Boyes, 1967; Joslyn *et al.* 1979,). Willis *et al*. (1981) reported that the chromosomes 2, 4, and 5 are submetacentric while 1 and 3 are metacentric. Chromosome 1 is a located loci of sex chromosome. Actually, males show decrease or lack of crossing over (Joslyn *et al*., 1979). The experiment related to genetics structure of stable fly was determined by using isozymes. All of evidence found low levels of variation. Jones *et al*. (1991) reported several irrigularity. These include the observation of several rare alleles in the homozygous state with no heterozygotes, also reported the structure of G_3*pdh*, *Idh*, *Gpdh*, *Mdh*, *Ldh*, *Fba* and *Had* to be monomeric. Harris and Hopkinson (1976) reported that G_3*pdh* and *Ldh* are tetramers and *Idh*, *Gpdh*, *Mdh* and *Had* are dimers. Krafsur (1993) reported a lack of genetic differentiation between analyzed collections. Unfortunately, collection size and allelic variation were not reported. These are needed for comparatives purposes. The high frequency of alternate alleles suggested that the most polymorphisms in organism remain essentially neutral (Avise, 1994). Variation in a certain organism is controlled by a balance between mutation and extinction.

From opinions on genetic variability, evolution remains a common element. Evolution can be defined as the change in genetic composition of populations in time (Dobzhansky, 1937). Evolution depends on three components, a source of genetic variation (mutation), forces that change the frequency of alleles (selection, genetic drift), and historical components in time and space (Dobzhansky, 1937). Mutations may occur during DNA replication, resulting in changes in nucleotide sequences (Futumya, 1986). Examples certain substitutions, duplications, deletions, translocations, and inversions. Frequently, mutations are lethal, and the proportion of their establishment in a population is quite low (Hartl, 1988). However, over millions of years, mutations are incorporated into the species, resulting in genetic variation (Avise, 1994). Decline of population size (or bottle necks) and founder events may reduce the genetic variability and may create genetic differentiation between populations by drift and founder effects, but this reduction of genetic diversity is completely influenced by gene flow between populations (Gilles *et al*., 2004).

Partitioning of genetic variation within and among populations is dependent on several factors including environmental, genetic drift and population bottlenecks. Localized environmental forces can reduce migration (gene flow) among populations (Hartl, 1988). Environmental forces may include geographical barriers or selection pressures (i.e., temperature tolerance and mate preference). Genetic drift can partition variation if immigration is absent. Eventually, bottlenecks during colonization can reduce genetic variation or alter gene frequencies significantly (Hartl, 1988).

5 Prevention and control

A number of pest management strategies have been developed for the control of flies in livestock. Sanitation is made a major component of these strategies and is supplemented with the use of insecticides, traps and biological control agents as paretic wasps (parasitoids). Keeping the feedlot dry and proper management of manure constitutes the basic sanitation measures the helps reduce fly breeding.

In term of biological control agents, Petersen (1989) reported potential pathogens, predators and parasites that may control stable flies. Inundative release of

sterile males as in screw worm control cannot be applied in stable flies because both sexs are blood feeders, thus releasing males will increase nuisance problems in livestock (Buschman and Patterson, 1981). Natural enemies reported include species of beetles and mites preying on fly eggs and larvae. Some species of pteromalid wasps were found parasitoids of stable and house flies pupae. Pteromalids lay eggs inside pupae through its ovipositor, immatures then feed on the pupae and cause death (Mullen and Durben, 2004).

Trapping is also one of the components of pest management strategy to control stable flies. Efficacy of box traps has been greatly improved through the discovery of adhesive alsynite fiberglass sticky panel (Williams, 1973). Broce (1988) improved the original box trap design and use the cylindrical alsynite trap made from cheap and thin plastic with less adhesive required. Additional innovations include the use of volatile compound to enhance attractiveness of traps. Hoy (1969) found that malaise traps baited with CO_2 caught three times more than the unbaited ones. Cilek (1999) reported the use of dry ice, acetone, and octenal as powerful attractants for stable flies when using cylindrical alsynite traps. Other traps uses high voltage electrocutor grid that causes fly disintegration. This could be effective however has disadvantage of releasing bacteria and pathogen during the disintegration (Urban and Broce, 2000). Natural product attractant baited have not been proven sufficient to reduce biting flies in significant numbers (Ashworth and Wall, 1994).

Insecticides intended for fly control includes water soluble formulation of pyrethroids. Permethrin has been reported to show quick knockdown effect in adult stable flies (Mock and Greene, 1989). Pyrethrins have been shown to control house flies, adventages of which include negligible impact on the natural enemies (Geden *et al*., 1992). Treating animals with insecticides has also been used to control of flies (Foil and Hogsette, 1994). Insecticide residual spraying on walls, bunk and shelters also showed efficacious control (Campbell, 1993), though currently used insecticides showed short duration of efficacy. In a number of instances however, use of insecticides resulted to the development of resistance (Cilek and Greene, 1994) resulting to fewer available alternatives.

6 Electrophoresis

Since 1960, electrophoresis has been used to delimit phylogenetic relationships, investigate homologie between species and their ecological-genetic relationships (Jorgenson and Phillips, 1987). The method employs separation and analysis of proteins and polynucleotides to determine homologies or differences between species (Sande and Karcher, 1960).

Electrophoresis is based on the movement of soluble proteins an electric potential. The proteins migrate at different rates depending upon its net charge (Hartl, 1988). Common amino acids are neutrally charged except lysine arginine, histidine proteins which are positively charged and aspartic acid and glutamic acid proteins which are negatively charged (Avise, 1994). This mean that these charged amino acid were substituted, there will changes in their mobility that can be detected by electrophoresis. Size and shaped of the protein also affects its mobility. Size and shape vary as a result of nucleotide rearrangements resulting to a different structure.

In electrophoresis, the distance traveled is monitored thought the use of dyes. The individual protein mobility is measured as a fraction of the distance traveled by the marker dyes.

Staining acids is finding specific protein in question. Demonstrable proteins will show similar forms (isozymes) or different form (allozymes) of enzymes. Depending on the number of active polypeptide chain, an enzyme is said to be monomer with only one plynucleotide chain active protein, a dimer if two sub units are required and tetramer if four sub units are required (Ayala, 1983).

Testing observerd allelic frequiencies with Hardy Weinberg expectations confirms Mendelian inheritance (Hartl, 1988). With this, allozymes make it an ideal genetic maker to assess parentage and gene flow, thereby allowing calculation of genetic distances among populations (Avise, 1994).

MATERIALS AND METHODS

1. Study sites

Stomoxys calcitrans was collected from nine provinces of 6 different regions of Thailand. North: Chiangmai (CHM) and Lampang (LAM), Northeast: Nakhon Ratchasrima (NAK), Central: Saraburi (SAR), East: Chonburi (CHO) and Trat (TRA), West: Kanchanaburi (KAN) and South: Prachuapkhirikhun (PRA) and Surat Thani (SUR) (Appendix Figure 1). To evaluate the genetic significance of observed difference among *S. calcitrans* populations, *Stomoxys uruma* was also included in the electrophoretic data. *Stomoxys uruma* was collected from Nakhon Ratchasrima (OUT), Northeastern Thailand. GPS coordinates and a brief description of the locations are given in Table 1.

2. Collection method

At each collection site, nine 'Vavoua' traps (Laveissiere and Grebaut, 1990) were randomly placed on the ground near animals, about 10 meters apart. Traps were made from blue and black cotton cloth and white polyester insect netting (Figure 1). Daytime (0600 to 1800 hr) collections of *S. calcitrans* occurred for at least two consecutive days at each site. In addition to Vavoua traps, sweep net was used to collect the fly in order to increase the fly number. Flies were identified to species according to Zumpt (1973) and the abdomen was removed to avoid blood contamination. All specimens were kept frozen 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.

Table 1 Characteristics of stable fly collection sites

Population	Reference points	Characteristics of collection sites
CHM	18° 48′ N,98° 58′ E 312 m asl	Industrial dairy, Mae Jo University, Mae Jo District, Chaing Mai Province. Approximately 80 cows.
LAM	18° 17′ N, 99° 28′ E 1,253 m asl	The Thai elephant conservation center , Thung Kwian forest park, Hang Chat District, Lampang Province. Approximately 100 elephants.
NAK	15° 0′ N,102° 6′ E 992 m asl	Dairy, Wang Nam Kheow District, Nakhon Ratchasima Province. Approximately 40 cows.
SAR	14° 31′ N,100° 52′ E 248 m asl	Industrial dairy, Dairy Farming Promotion Organization of Thailand, Mauk Lek District, Saraburi Province. Approximately 200 cows.
CHO	13° 24′ N, 101° 0′ E 562 m asl	Khao Kheow National forest Reserves, established a Zoological Park (Khao Kheow Open Zoo) is located in Sriracha District, Chonburi Province, eastern Thailand. There are approximately 300 species, 8000 animals in this zoo.
TRA	12° 13′ N,102° 30′ E 644 m asl	Dairy, Bo Rai District, Trat Province. Approximately 20 cows.
KAN	14° 1′ N, 99° 31′ E 305 m asl	Dairy, Pu Teuy Village, Sai Yok District, Kanchanaburi Province. Approximately 25 cows.
PRA	11° 49′ N, 99° 47′ E 5 m asl	Dairy, Huai Sat Yai sub-district, Hua Hin District, Prachuap Khiri Khan Province. Approximately 30 cows.
SUR	9° 8′ N,99° 19′ E 200 m asl	Dairy, Mueang District, Surat Thani Province. Approximately 20 cows.

3. Starch gel electrophoresis

Horizontal starch gel electrophoresis was conducted following methods of Harris and Hopkinson (1976) and Manguin *et al*., (1995). To avoid blood contamination, abdomen was removed. Each fly cut that abdomen 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 systems were analyzed with two different buffer systems: morpholine (Morph) and Tris-malate-EDTA (TMEDTA) (Pasteur *et al*., 1988), run for 6 hours at a constant 16 volts/cm, stained and incubated at 37 ˚C for 15-60 minutes (Table 2). Locus considered polymorphic when two or more alleles are found in a certain locus. Different alleles of the same locus demonstrated different banding patterns, depending upon the migration speed. The most common allele is designated as "100" (Pasteur *et al*., 1988).

4. 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 $N_em = (1-F_{ST})/4\ F_{ST}$ (Nei, 1978, Wright, 1978). The GENEPOP-3.1 program was used to estimate the degree of isolation by 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 serves as an effective barrier to gene flow.

Table 2 Enzyme systems and loci used in electrophoresis on adult *Stomoxys calcitrans* (L.)

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
Pgd (6-Phosphogluconate dehydrogenase)	1.1.1.44	1	TMEDTA
		13	

** Number of scored bands per phenotype

* Enzyme commission number

RESULTS

From our results, two different types of peptide chain are produced. The first is made from two peptide chains and known as dimer, a heterozygote with three bands. The intermediate band is more intensely stained than the other two bands in dimmer form. The second is referred to monomer, the enzyme made of one polypeptide chain whereas a heterozygote produces only two bands (Pasteur *et al*., 1988). In this study, six enzymes; *Aox*, *Got*, *Gpd*, *Had*, *Mdh* and *Pgd* are found to be a dimer (Appendix figures 3 and 4), while four enzymes; *Acp*, *Idh*, *Mez* and *Pgm*, are monomer. From 10 enzyme systems, 13 putative loci were detected (Table2). The number of polymorphic loci by populations was CHM (10), LAM (11), NAK (10), SAR (8), TRA (12), CHO (8), KAN (10), PRA (11), SUR (10), and allele frequencies are presented in Table 3.

From 117 comparisons, 44 exhibited deviated from Hardy-Weinberg equilibrium ($P < 0.05$), a value greater than the 5% of expected deviations by chance alone. The number of loci for departures were found in TRA, KAN, LAM, CHM, CHO, PRA, NAK, SAR and SUR were 10, 8, 6, 5, 5, 4, 3, 2, and 2 respectively (Table 3). All significant deviations were associated with heterozygote deficiency. Several alleles were restricted to a single population: *Aox-1* (allele 138), *Gpd-1* (allele 127), and *Idh-2* (allele 130) in the TRA population; *Acp-1* (allele 133), *Mez-1* (allele 86) and *Pgd-1* (allele 54) in NAK; *Got-1* (allele 75) in CHM; *Got-2* (allele -250) in PRA and *Aox-1* (allele 40) in CHO; Moreover, the *Had*-1 (allele 110), *Idh-1*(allele 108), *Idh-2* (allele 54), *Pgd-1*(allele 108) and *Pgm-1*(allele 43) were absence in the SAR population (Table 3). Four loci from TRA population show high F_{IS} value (1.000)(*Got*-1, *Got*-2, *Idh*-1 and *Mez*-1), from CHO population also observed 3 loci (*Got*-1, *Had*-1and *Idh*-1); 2 loci (*Had*-1 and *Pgd*-1) from KAN population and 1 loci form SAR population (*Gpd-1*) and PRA population (*Idh*-1) (Table 3).

Higher percentages of polymorphic loci were observed in the CHM and TRA populations (69.2%) compared to the other seven, ranging from 23.1 to 53.8%. The greatest number of alleles per locus (2.8) was observed in the TRA population and the most heterozygosity (Ho=0.161$\pm$0.066) observed in the KAN population (Table 4). The lowest variability was observed in the SAR population (23.1%), with low allele per

locus (1.9) and level of heterozygosity (Ho=0.072±0.032). Observed heterozygosities from all locations were not significantly different from the Hardy-Weinberg expected heterozygosities ($t_{0.025} = 2.145$; df =14; $P > 0.05$) (Table 4).

The F-statistics (F_{ST}), a measure of the amount of differentiation among populations, showed an average value of 0.060 and a mean index of fixation of individuals relative to the total of subpopulations (F_{IS}) value of 0.158 when all *S. calcitrans* populations were analyzed (Table 5). Five loci, *Got-1*, *Got-2*, *Idh-1*, *Mez-1* and *Pgd-1*, among a total of 13 demonstrated great differentiation, with values of 0.554, 0.673, 0.706, 0.797 and 0.548, respectively. Significant linkage disequilibrium were detected ($P < 0.05$) for 17 of 78 pairs of loci in TRA population and LAM, CHM and KAN, SUR , PRA and NAK, SAR, CHO (11, 8,7,3, 2,1 respectively) (Table 6).

Gene flow between populations was calculated by estimating N_em where N_e is the effective population size and m is the migration rate between populations. Because 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} (0.060) was 3.92. The N_em between populations ranged from 3.32 to 27.53 reproductive migrants per generation (Table 7). The lowest gene flow was found between CHM and KAN (3.32 migrants per genration), whereas the highest was observed between LAM and SUR (27.53 migrants per generation) (Table 7). An analysis among all populations indicated that there was no correlation between genetic and geographic distance among the nine populations of *S. calcitrans* ($r^2 = 0.014$; df=35; $P > 0.05$) (Table 7). The derived phenogram shows that the nine sampled populations of *S. calcitrans* in Thailand occur in a common genetic cluster as indicated by a low genetic distance (< 0.017) (Figure 1). The comparison of *S. calcitrans* with *S. uruma* shows higher genetic divergence (Figure 2).

Table 3 Allele frequency and sample size (*n*) of nine collections of *Stomoxys calcitrans* (L.)

Locus		Allele	*Stomoxys calcitrans* populations								
			CHM	LAM	NAK	SAR	TRA	CHO	KAN	PRA	SUR
Acp-1	*n*		25	30	28*	43	30	33	19*	30	28
		75	0.000	0.000	0.125	0.174	0.017	0.106	0.105	0.050	0.000
		100	0.880	0.933	0.768	0.802	0.917	0.848	0.763	0.900	0.982
		133	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000
		195	0.120	0.067	0.071	0.023	0.067	0.045	0.132	0.050	0.018
	F_{IS}		-0.116	-0.036	0.324	-0.200	-0.058	-0.103	0.716	-0.064	-0.000
Aox-1	*n*		30*	30	21	21	30*	19*	25	27	28*
		40	0.000	0.000	0.000	0.000	0.000	0.053	0.000	0.000	0.000
		76	0.150	0.083	0.024	0.024	0.017	0.263	0.000	0.037	0.000
		100	0.750	0.833	0.905	0.905	0.783	0.684	1.000	0.833	0.857
		128	0.100	0.083	0.071	0.071	0.167	0.000	0.000	0.130	0.143
		138	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000
	F_{IS}		0.437	0.103	-0.059	-	0.362	-0.072	-	0.141	-0.149
Gpd-1	*n*		30	29*	27	43*	29*	32	25	26*	27
		64	0.000	0.034	0.019	0.000	0.017	0.000	0.040	0.000	0.000
		100	0.950	0.897	0.852	0.977	0.845	1.000	0.920	0.769	0.898

Table 3 (Continued)

Locus		Allele	*Stomoxys calcitrans* populations								
			CHM	LAM	NAK	SAR	TRA	CHO	KAN	PRA	SUR
Gpd-1		127	0.000	0.000	0.000	0.000	0.034	0.000	0.000	0.000	0.000
(Cont.)		142	0.050	0.069	0.130	0.023	0.103	0.000	0.000	0.231	0.111
	F_{IS}		-0.074	0.291	0.222	1.000	0.137	-	-0.043	-0.316	-0.083
Got-1	*n*		30*	30*	22	43	30*	32	25	30	28
		75	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		100	0.900	0.950	1.000	0.988	0.967	1.000	1.000	1.000	0.964
		148	0.067	0.033	0.000	0.012	0.000	0.000	0.000	0.000	0.036
		240	0.000	0.017	0.000	0.000	0.033	0.000	0.000	0.000	0.000
	F_{IS}		-0.036	0.663	-	0.000	1.000	-	-	-	-0.019
Got-2	*n*		30*	30*	28	43	30*	32*	25*	30	28
		-100	0.900	0.967	1.000	0.977	0.967	0.938	0.900	0.983	0.982
		-250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000
		-273	0.100	0.033	0.000	0.023	0.033	0.063	0.100	0.000	0.018
	F_{IS}		0.638	-0.000	-	0.000	1.000	1.000	0.422	-0.000	-0.000

Table 3 (Continued)

Locus		Allele	*Stomoxys calcitrans* populations								
			CHM	LAM	NAK	SAR	TRA	CHO	KAN	PRA	SUR
Had-1	*n*		30	30	28	43	29*	33*	25*	27*	28*
		92	0.000	0.033	0.000	0.000	0.034	0.000	0.000	0.019	0.000
		100	0.933	0.950	1.000	1.000	0.931	0.970	0.960	0.889	0.875
		110	0.067	0.017	0.000	0.000	0.034	0.030	0.040	0.093	0.125
	F_{IS}		-0.055	-0.024	-	-	0.487	1.000	1.000	0.281	0.201
Idh-1	*n*		30	30	26*	43	29*	33*	25	28*	21
		86	0.000	0.000	0.019	0.000	0.034	0.000	0.000	0.000	0.000
		100	0.950	0.983	0.904	1.000	0.793	0.970	1.000	0.964	1.000
		108	0.050	0.017	0.077	0.000	0.172	0.030	0.000	0.036	0.000
	F_{IS}		-0.036	-0.000	0.482	-	1.000	1.000	-	1.000	-0.019
Idh-2	*n*		26	27	28	43	30*	33	25	28	22*
		54	0.000	0.111	0.036	0.000	0.067	0.000	0.100	0.018	0.068
		100	1.000	0.889	0.964	1.000	0.900	1.000	0.900	0.982	0.932
		130	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000
	F_{IS}		-	-0.106	-0.000	-	0.293	-	-0.091	-0.000	-0.174

Table 3 (Continued)

Locus		Allele	*Stomoxys calcitrans* populations								
			CHM	LAM	NAK	SAR	TRA	CHO	KAN	PRA	SUR
Mdh-1	*n*		28	29*	27	43	30	32	25*	24	22
		57	0.000	0.017	0.093	0.035	0.000	0.000	0.180	0.104	0.045
		100	0.964	0.931	0.907	0.965	1.000	0.969	0.820	0.896	0.995
		200	0.036	0.052	0.000	0.000	0.000	0.031	0.000	0.000	0.000
	F_{IS}		-0.018	-0.024	-0.080	-0.024	-	-0.016	-0.200	-0.095	-0.022
Mdh-2	*n*		7	11	9	43	16	17	25*	9	10
		-100	1.000	1.000	0.889	0.942	0.906	0.882	0.580	0.944	1.000
		-157	0.000	0.000	0.111	0.058	0.094	0.118	0.420	0.056	0.000
	F_{IS}		-0.215	-0.000	-0.067	-0.500	-0.111	-0.103	-0.778	-0.000	-
Mez-1	*n*		30	29	28	43	30*	30	25*	24	27
		86	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000
		100	1.000	1.000	0.982	1.000	0.967	1.000	0.960	1.000	1.000
		111	0.000	0.000	0.000	0.000	0.033	0.000	0.040	0.000	0.000
	F_{IS}		-	-	-0.039	-	1.000	-	0.000	-	-

Table 3 (Continued)

Locus		Allele	*Stomoxys calcitrans* populations								
			CHM	LAM	NAK	SAR	TRA	CHO	KAN	PRA	SUR
Pgd-1	*n*		30*	18	18	30	30*	20	25*	26*	30*
		54	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.000	0.000
		100	0.883	0.983	0.972	1.000	0.900	1.000	0.960	0.885	0.933
		108	0.117	0.017	0.000	0.000	0.067	0.000	0.000	0.115	0.067
		155	0.000	0.000	0.000	0.000	0.033	0.000	0.040	0.000	0.000
	F_{IS}		0.527	-0.000	-0.000	-	0.648	-	1.000	0.635	-0.045
Pgm-1	*n*		29	28	26*	43	30*	33*	23*	19	27*
		43	0.086	0.000	0.000	0.000	0.017	0.030	0.022	0.000	0.000
		68	0.138	0.107	0.058	0.012	0.033	0.030	0.022	0.000	0.056
		87	0.000	0.000	0.038	0.012	0.000	0.000	0.087	0.000	0.000
		100	0.757	0.804	0.808	0.953	0.867	0.909	0.848	0.974	0.870
		135	0.017	0.089	0.096	0.023	0.083	030	0.022	0.026	0.074
	F_{IS}		0.118	0.068	0.097	-0.021	0.082	0.304	0.071	-0.000	0.223

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

F_{IS} = Inbreeding coefficient

Table 4 Genetic variability at 13 loci of pooled collections of *Stomoxys calcitrans* (L.)

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
LAM	2.3±0.2	61.5	0.104±0.030	0.125±0.030
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
CHO	1.9±0.3	38.5	0.088±0.039	0.110±0.039
KAN	2.2±0.3	53.8	0.161±0.066	0.172±0.044
PRA	2.1±0.2	53.8	0.122±0.037	0.135±0.032
SUR	1.9±0.2	46.2	0.121 ±0.030	0.125±0.028
			Average	
			0.135±0.033	
				$t_{0.025} = 2.145^{ns}$

[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 analysis of polymorphic loci in nine populations of *Stomoxys calcitrans* (L.)

Locus	F_{IS} [1]	F_{ST} [2]
Acp-1	0.095	0.043
Aox-1	0.132	0.063
Gpd-1	0.013	0.051
Got-1	0.554	0.035
Got-2	0.673	0.029
Had-1	0.306	0.034
Idh-1	0.706	0.063
Idh-2	-0.052	0.058
Mdh-1	-0.050	0.051
Mdh-2	-0.341	0.176
Mez-1	0.797	0.026
Pgd-1	0.548	0.044
Pgm-1	0.096	0.036
Mean	0.158	0.060
		N_em = 3.92

F_{IS} [1]= Inbreeding coefficient

F_{ST} [2] > 0.25 Large genetic differentiation among the subpopulations, 0.25 > F_{ST} > 0.15 Moderate, 0.15 > F_{ST} > 0.05 Small, and $F_{ST} \leq$ 0.05 Negligible.

Table 6 Linkage disequilibrium of enzymatic loci from nine populations of *Stomoxys calcitrans* (L.)

Locus pairs	Populations, *P*-value (significance level=0.05)								
	CHM	LAM	NAK	SRA	TRA	CHO	KAN	PRA	SUR
Acp-1/ *Aox*-1	ns	ns	ns	ns	ns	0.0091	ns	ns	ns
Acp-1/ *Gpd*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Acp-1/ *Got*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Acp-1/ *Got*-2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Acp-1/ *Had*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Acp-1/ *Idh*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Acp-1/ *Idh*-2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Acp-1/ *Mdh*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Acp-1/ *Mdh*-2	ns	ns	ns	ns	ns	ns	0.0108	ns	ns
Acp-1/ *Mez*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Acp-1/ *Pgd*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Acp-1/ *Pgm*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Aox-1/ *Gpd*-1	ns	ns	ns	ns	ns	ns	ns	0.0266	ns
Aox-1/ *Got*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Aox-1/ *Got*-2	0.0089	ns	ns	ns	ns	ns	ns	ns	ns

Table 6 (Continued)

Locus pairs	Populations, *P*-value (significance level=0.05)								
	CHM	LAM	NAK	SRA	TRA	CHO	KAN	PRA	SUR
Aox-1/ *Had*-1	ns	ns	ns	ns	0.0103	ns	ns	ns	ns
Aox-1/ *Idh*-1	ns	ns	ns	ns	0.0038	ns	ns	ns	ns
Aox-1/ *Idh*-2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Aox-1/ *Mdh*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Aox-1/ *Mdh*-2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Aox-1/ *Mez*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Aox-1/ *Pgd*-1	ns	ns	ns	ns	0.0105	ns	ns	ns	ns
Aox-1/ *Pgm*-1	0.0462	ns	ns	ns	ns	ns	ns	ns	ns
Gpd-1/ *Got*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Gpd-1/ *Got*-2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Gpd-1/ *Had*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Gpd-1/ *Idh*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Gpd-1/ *Idh*-2	ns	ns	ns	ns	0.0065	ns	ns	ns	ns
Gpd-1/ *Mdh*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Gpd-1/ *Mdh*-2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Gpd-1/ *Pgd*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 6 (Continued**)**

Locus pairs	Populations, *P*-value (significance level=0.05)								
	CHM	LAM	NAK	SRA	TRA	CHO	KAN	PRA	SUR
Gpd-1/ *Pgm*-1	ns	ns	ns	ns	0.0312	ns	ns	ns	ns
Got-1/ *Got*-2	ns	ns	ns	0.0235	0.0344	ns	ns	ns	ns
Got-1/ *Had*-1	0.0267	ns	ns	ns	0.0322	ns	ns	ns	0.0390
Got-1/ *Idh*-1	ns	ns	ns	ns	ns	ns	ns	ns	0.0028
Got-1/ *Idh*-2	ns	0.0421	ns	ns	0.0319	ns	ns	ns	ns
Got-1/ *Mdh*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Got-1/ *Mdh*-2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Got-1/ *Mez*-1	ns	ns	ns	ns	0.0337	ns	ns	ns	ns
Got-1/ *Pgd*-1	0.0125	ns	ns	ns	ns	ns	ns	ns	0.0113
Got-1/ *Pgm*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Got-2/ *Had*-1	ns	ns	ns	ns	0.0335	ns	ns	ns	ns
Got-2/ *Idh*-1	ns	0.0331	ns	ns	ns	ns	ns	0.0365	ns
Got-2/ *Idh*-2	ns	ns	ns	ns	0.0323	ns	ns	ns	ns
Got-2/ *Mdh*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Got-2/ *Mdh*-2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Got-2/ *Mez*-1	ns	ns	ns	ns	0.0326	ns	ns	ns	ns

Table 6 (Continued)

Locus pairs	Populations, *P*-value (significance level=0.05)								
	CHM	LAM	NAK	SRA	TRA	CHO	KAN	PRA	SUR
Got-2/ *Pgd*-1	ns	0.0328	ns	ns	ns	ns	ns	ns	ns
Got-2/ *Pgm*-1	ns	0.0334	ns	ns	ns	ns	0.0132	ns	ns
Had-1/ *Idh*-1	ns	0.0325	ns	ns	ns	ns	ns	ns	0.0415
Had-1/ *Idh*-2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Had-1/ *Mdh*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Had-1/ *Mdh*-2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Had-1/ *Mez*-1	ns	ns	ns	ns	0.0359	ns	ns	ns	ns
Had-1/ *Pgd*-1	0.0036	0.0286	ns	ns	0.0098	ns	0.0395	0.0001	0.0049
Had-1/ *Pgm*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Idh-1/ *Idh*-2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Idh-1/ *Mdh*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Idh-1/ *Mdh*-2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Idh-1/ *Mez*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Idh-1/ *Pgd*-1	0.0021	0.0326	ns	ns	0.0008	ns	ns	ns	0.0110
Idh-1/ *Pgm*-1	ns	ns	0.0393	ns	ns	ns	ns	ns	ns
Idh-2/ *Mdh*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 6 (Continued)

Locus pairs	Populations, *P*-value (significance level=0.05)								
	CHM	LAM	NAK	SRA	TRA	CHO	KAN	PRA	SUR
Idh-2/ *Mdh*-2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Idh-2/ *Mez*-1	ns	ns	ns	ns	0.0359	ns	ns	ns	ns
Idh-2/ *Pgd*-1	ns	ns	ns	ns	ns	ns	ns	ns	0.0273
Idh-2/ *Pgm*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mdh-1/ *Mdh*-2	ns	ns	ns	0.0008	ns	ns	ns	ns	ns
Mdh-1/ *Mez*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mdh-1/ *Pgd*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mdh-1/ *Pgm*-1	ns	ns	ns	ns	ns	ns	0.0273	ns	ns
Mdh-2/ *Mez*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mdh-2/ *Pgd*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mdh-2/ *Pgm*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mez-1/ *Pgd*-1	ns	ns	ns	ns	ns	ns	0.0401	ns	ns
Mez-1/ *Pgm*-1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Pgd-1/ *Pgm*-1	ns	0.0312	ns	ns	0.0209	ns	ns	ns	ns

ns = Not significant

Table 7 Above diagonal: Geographic distance (km); Below diagonal: Pairwise *F* statistics[1] and effective migration rate (N_em) of all loci at nine collection samples of *Stomoxys calcitrans* (L.)

	CHM	LAM	NAK	SAR	TRA	CHO	KAN	PRA	SUR
CHM	---	92	777	625	1,011	777	824	977	1,340
LAM	0.014 (17.61)	---	687	535	914	680	727	880	1,243
NAK	0.028 (8.68)	0.019 (12.91)	---	152	399	280	387	540	903
SAR	0.038 (6.33)	0.029 (8.37)	0.018 (13.64)	---	380	201	235	388	751
TRA	0.021 (11.65)	0.017 (14.46)	0.017 (14.46)	0.035 (6.89)	---	234	443	596	959
CHO	0.024 (10.17)	0.030 (8.08)	0.032 (7.56)	0.030 (8.08)	0.034 (7.10)	---	209	362	725
KAN	0.070 (3.32)	0.065 (3.59)	0.036 (6.69)	0.061 (3.85)	0.055 (4.29)	0.062 (3.78)	---	295	658
PRA	0.029 (8.37)	0.024 (10.17)	0.036 (6.69)	0.034 (7.10)	0.018 (13.64)	0.043 (5.56)	0.063 (3.72)	---	364
SUR	0.025 (9.75)	0.009 (27.53)	0.027 (9.00)	0.039 (6.16)	0.016 (15.38)	0.047 (5.07)	0.071 (3.27)	0.018 (13.64)	---

Coefficient of determination of isolation by distance between populations $r^2 = 0.014^{ns}$

[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

--- = Infinite

DISCUSSIONS

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 geographically expand negative economic impact and disease transmission. The temporal and spatial differences with respect to expression of enzymes that are associated with variability of vectorial capacity for disease pathogens may be influenced by the patterns of gene flow between and within populations. For example, a correlation exists 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 genetics structure of *S. calcitrans* populations in Thailand has not previously been reported. The genetic variation and gene flow between and among nine different geographical populations of *S. calcitrans* were compared using isozyme electrophoresis. The percentage of polymorphic loci and mean heterozygosities observed in this study were higher than those observed in the USA (Jones *et al*., 1991, Szalanski, 1996). Several polymorphic loci in this study were not observed by those authoers. However, our findings do correspond more closely to 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 we believe 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 (1-2 per wk) use of insecticides for controlling flies. 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 is generally quite limited and preferred breeding habitats and hosts more abundant.

Ten loci departures from the Hardy-Weinberg equilibrium occurred in the TRA as well as linkage population are expected in populations in which mating is not random, especially when there is reproductive isolation between individuals. However, other causes may have generated genetic disequilibrium, such as geographic subdivision or selection. This population was 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 inbreed 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 mechanism(s) involved, any significant reduction in adult insects from generation to generation can result in genetic bottlenecks in a localized population (Futumya, 1986). We suspect the prevailing environmental conditions at TRA have had a significant impact on development and expansion of *S. calcitrans* in the area.

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

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 8 samples. This would be expected for wild populations of insects exposed to few, if any, population control activities (Sukonthabhirom *et al*., 2005). Contrast that with the SAR population which had very low genetic variability compared to the other eight populations with a leading contributing factor being the routine use of insecticides (primarily synthetic pyrethroids) to control *Stomoxys* flies.

The high mean of F_{IS} with a value of 0.158 in all populations and F_{IS} value in each population indicated that inbreeding within the subpopulation and populations of *S. calcitrans* is occurring. More specifically, the higher positive value of F_{ST}, the less heterozygosity is in the population. The N_em value among all 9 populations was 3.92 reproductive migrants/generation which was similar with Dsouli *et al.*(2009), between Gabon-France (2.3). Although the N_em value among populations in this study lower than the N_em value obtained from Nebraska (5.85) (Szalanski, 1995) and from Reunion Island (12.25) (Gilles *et al*., 2004). As maximum flight range of *Stomoxys calcitrans* is comparatively far (several hundred kilometers) (Gersabeck and Merritt, 1985, Hogsette and Ruff, 1985, Hogsette *et al.*, 1987), so the same volume of active movement and human transportation providing passive movement of stable fly adults. The significant passive movement of immature stage appears to be the principal means of dispersal between distant localities with manure. However, Szalanski (1995) reported that a very few (<10) migrants are needed each generation to maintain genetic homogeneity.

The unbiased genetic distances obtained indicate that there is no significant genetic difference between the 9 populations examined. The resulting phenogram produced fourth closely-related clusters, Lampang and Surat Thani (LAM and SUR), the second from Nakhon Ratchasrima and Saraburi (NAK and SRA), the third from Trat and Prachuap Khiri Khun (TRA and PRA), the fourth from Chiang Mai and Chonburi (CHM and CHO). Kanchanaburi (KAN) is slightly apart from the others. KAN 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 value for the study of phylogenetic relationships for investigating speices homologies and for the studying of ecological genetic relationships.

The starch gel electrophoresis techniques allow us to explore dozens of genes on one test specimens simultaneously. In this study, starch gel electrophoresis was used to estimate the rates of gene flow between and among *Stomoxys calcitrans* populations from nine provinces six different geographic regions of Thailand. Among ten enzyme systems, 13 putative loci and 11 polymorphisms were detected. Limited genetic differentiation among the nine 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 least percent polymorphism was seen in south-central Saraburi Province.

Genetic Distance

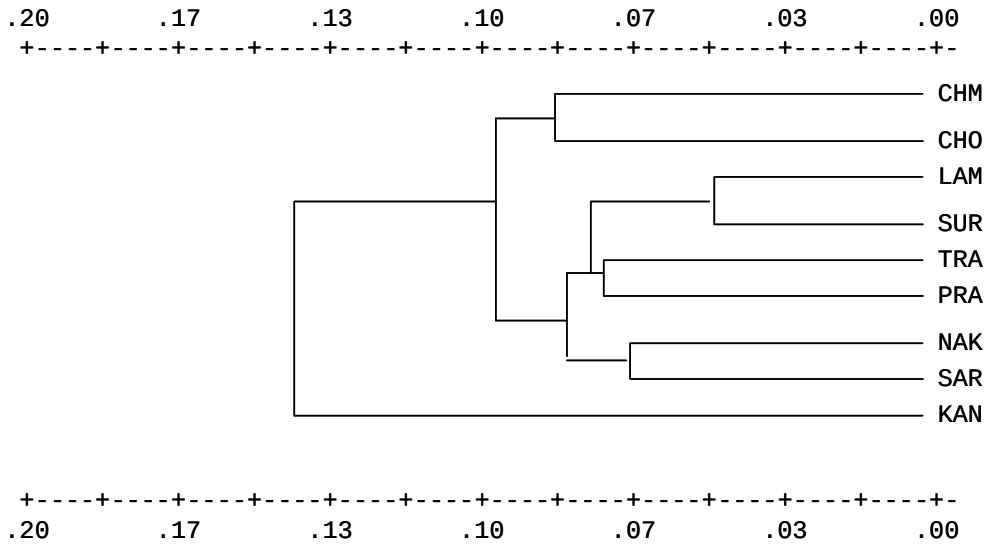

Figure 1 Unweighted pair-group method averaging phenogram from modified Roger's distance (Wright 1978) matrix among nine populations of *Stomoxys calcitrans* in Thailand (cophenetic correlation = 0.925)

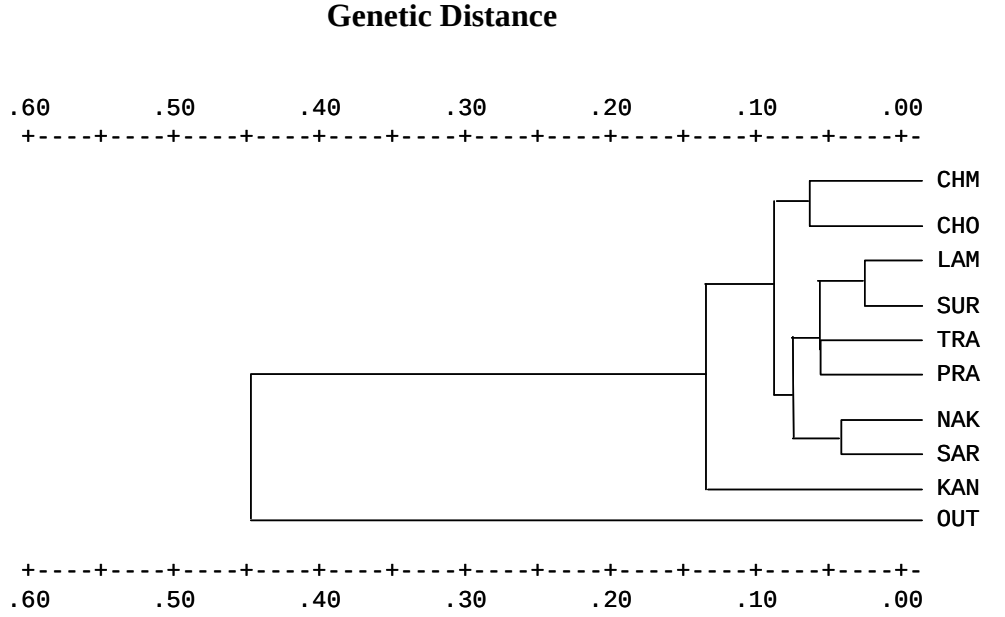

Figure 2 Unweighted pair group method averaging phenogram from modified Roger's distance (Wright 1978) matrix among all nine populations of *Stomoxys calcitrans* and one population of *Stomoxys uruma* (cophenetic correlation = 0.997)

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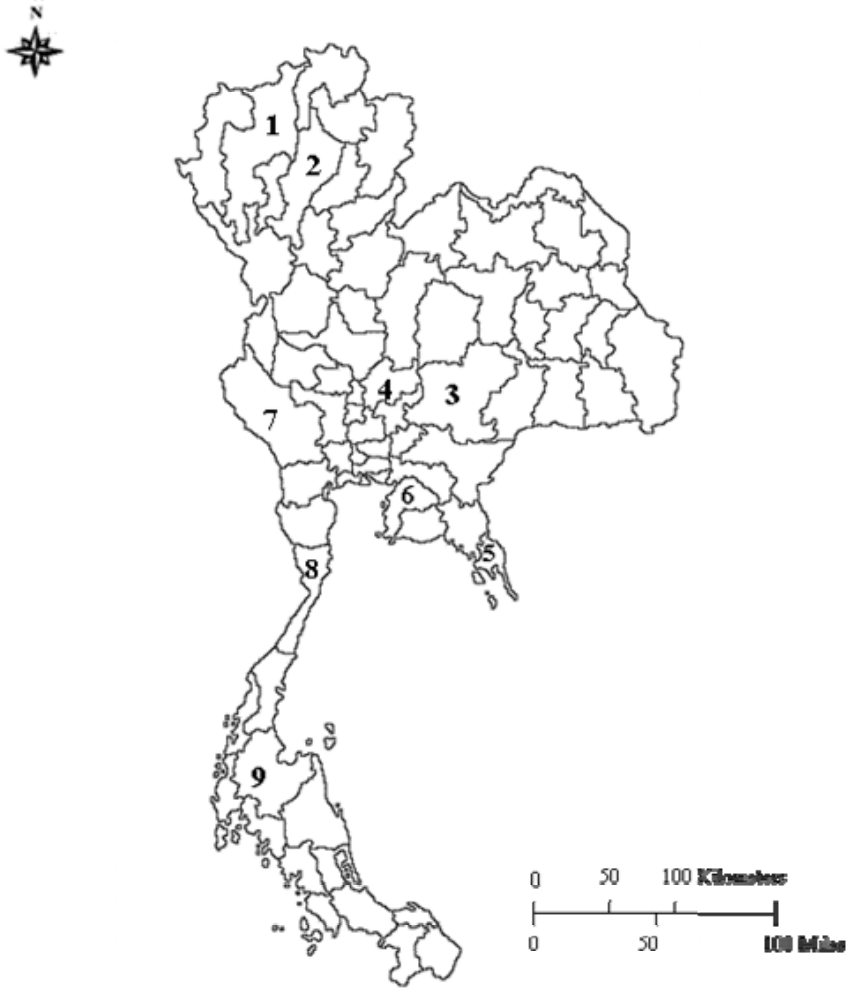
APPENDIX



Appendix Figure 1 *Stomoxys calcitrans* collection sites in Thailand

1 Chiang Mai (CHM)

2 Lampang (LAM)

3 Nakhon Ratchasima(NAK)

4 Saraburi (SAR)

5 Trat (TRA)

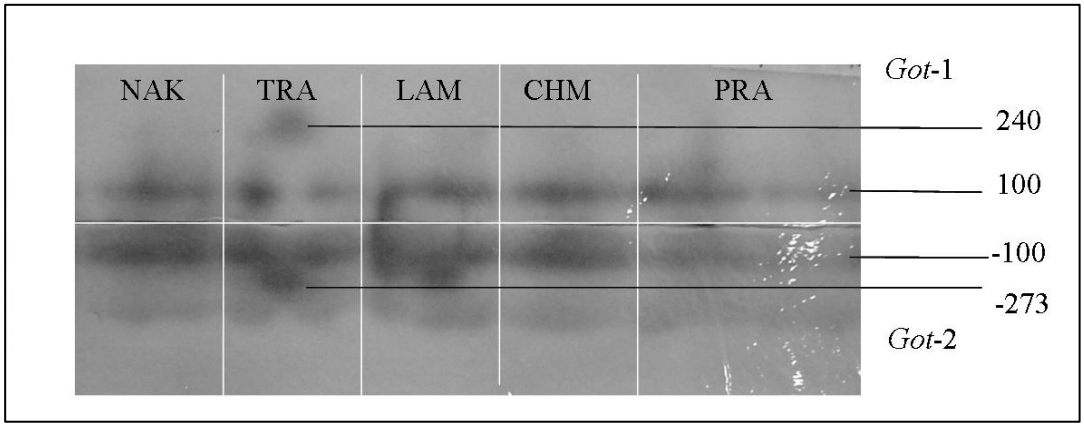
6 Chonburi (CHO)

7 Kanchanaburi (KAN)

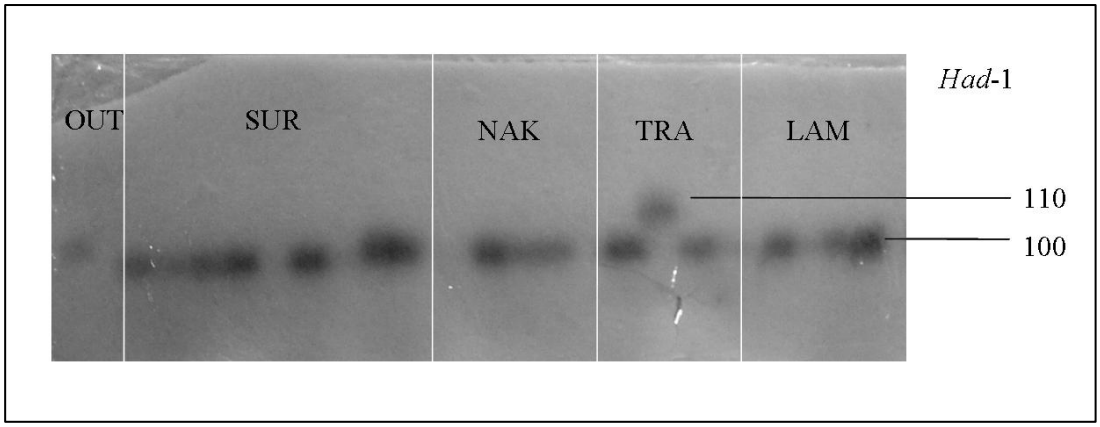
8 Prachuap Khiri Khan (PRA)

9 Surat Thani (SUR)

Appendix Figure 2 Vavoua traps



Appendix Figure 3 Electrophoretic pattern on Isocritrate dehydrogenase among five sample sites; NAK, TRA, LAM, CHM and PRA. Values on right are the relative mobilities of alleles. (above: forward, bellow: backward)



Appendix Figure 4 Electrophoretic pattern on β-Hydroxyacid dehydrogenase among five sample sites; OUT, SUR, NAK, TRA and LAM. Values on right are the relative mobilities of alleles.