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SI-MOLECULAR TECHNOLOGIES TO IMPROVE SIT

The utility of microsatellite DNA markers for the evaluation of area-wide integrated pest management using SIT for the fruit fly, *Bactrocera dorsalis* (Hendel), control programs in Thailand

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Abstract The oriental fruit fly, *Bactrocera dorsalis* (Hendel), is a key pest that causes reduction of the crop yield within the international fruit market. Fruit flies have been suppressed by two Area-Wide Integrated Pest Management programs in Thailand using Sterile Insect Technique (AW-IPM-SIT) since the late 1980s and the early 2000s. The projects' planning and evaluation usually rely on information from pest status, distribution, and fruit infestation. However, the collected data sometimes does not provide enough detail to answer management queries and public concerns, such as the long term sterilization efficacy of the released fruit fly, skepticism about insect migration or gene flow across the buffer zone, and the re-colonisation possibility of the fruit fly population within the core area. Established microsatellite DNA markers were used to generate population genetic data for the analysis of the fruit fly sampling from several control areas, and non-target areas, as well as the mass-rearing facility. The results suggested limited gene flow ($m < 0.100$) across the buffer zones between the flies in the control areas and flies captured outside. In addition, no genetic admixture was revealed from the mass-reared colony flies from the flies within the control area, which supports the effectiveness of SIT. The control pests were suppressed to low density and showed weak bottleneck footprints although they still acquired a high degree of genetic variation. Potential pest resurgence from fragmented micro-habitats in mixed fruit orchards rather than pest incursion across the buffer zone has been proposed. Therefore, a suitable pest control effort, such as the SIT program, should concentrate on the hidden refuges within the target area.

Keywords Oriental fruit fly · Invasive species · AW-IPM-SIT · Microsatellites · Gene flow · Bottleneck

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Introduction

Effective integrated pest management (IPM) comprises environmental-friendly methods that reduce insecticide problems. The modern IPM field implementation also better targets on an area-wide (AW) basis. The entire pest population area encompassing multi-owner fruit orchards, backyard gardens, wild host areas, and abandoned crop fields should be under control, otherwise the highly mobile insect pests will take refuge. Thus, the application of this AW-IPM is concerned with controlling the pests at the population level (Hendrichs et al. 2007; Lindquist 2000).

If the pests, within the entire population range, are ideally and sustainably controlled and isolated, they will not be able to re-establish their population over the economic threshold level. The AW-IPM tactic for fruit fly pests usually includes male annihilation trapping (MAT), protein baited annihilation trapping (BAT), phytosanitation of fruit orchards, wild host cutting, natural enemy augmentation, and geographical information system (GIS) assisted buffer zone establishment. In addition to those control measures, it would be desirable to have effective control agents that can penetrate micro-habitats of the entire pest population.

One of the most effective biological methods is the sterile insect technique (SIT). SIT is the most target-species specific and non-disruptive pest control technique among biologically-based methods (Enkerlin et al. 2003). The SIT technology involves a mass release of sterile insects that are supposed to win the overall mating competition among the fertile insects in the wild. This generates a means of birth control which eliminates the next generation of the target pests. SIT has already been proven to be internationally and economically viable for many economically, veterinarily, and medically important pests (Dyck et al. 2005a, b). Examples of successful AW-IPM projects on insect pests are the control of the New World screwworm and the Mediterranean fruit fly (Hendrichs et al. 2007).

One of the most destructive agricultural fruit fly pests in the Asia–Pacific areas is *Bactrocera dorsalis* (Hendel), or oriental fruit fly (OFF) (Clarke et al. 2005; White and Elson-Harris 1992). With its very broad host range, relatively wild climatic tolerance, and dispersal capacity, the OFF has the potential to establish adventive populations in various areas. In Thailand, the OFF has been recognized as a major quarantine pest by many of its trading partners, for example, New Zealand (http://www.biosecurity.govt.nz/files/ihs/mango-th.pdf; 7 October 1999) and Japan. The fruit fly significantly damages economic crops, especially a wide range of soft fruits such as mango (*Mangifera indica*). Although mangoes are cultivated in up to 300,000 hectares of farming area, most of the fruit product is currently lost due to fruit fly infestation. The consequences are a significant reduction to yields and market shares, leading to an estimated loss of millions of dollars per year (Orankanok et al. 2006, 2007). Common solutions for the fruit fly problem generally involve traditional means such as the intensive use of insecticides. It was understood that not only the fruit fly damage problem remains but also social and environmental risks increased in the region. The implementation of the AW-IPM-SIT project for fruit fly suppression has become an alternative control method for the OFF with cooperation from the International Atomic Energy Agency (IAEA) of the United Nations, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, and the Department of Agricultural Extension (DOAE) of Thailand since 1991. The first pilot campaign was launched in Ratchaburi province, in the western area of Thailand. The damage caused by fruit flies decreased from over 82% before the implementation of the AW-IPM-SIT project in 1987, to 30, 26, 21, 18, 17, and 9% in the following 6 years (from 1988 to 1993), respectively. From 1994 to 2001, the damage was further reduced to an average of less than 4%. These results suggested that the first control program was successful. In 2002, the second pilot program was implemented in Pichit province, located approximately 450 km north of Bangkok. After the control program had been carried out for only 2 years, infestation was reduced from 42.9 to 15.5% (Orankanok et al. 2006, 2007).

In order to expand the AW-IPM-SIT programs to a regional scale, an effective and sustainable control program is required for public acceptance. A few concerns are worth attention when the AW-IPM-SIT is put into action. This is due to the fact that this technology involves the release of mass-reared and radiation-sterilized flies into wild populations of the target species. The effectiveness of this approach therefore relies on the competitive mating of the released sterile flies. The consequence is the decrease of wild female reproductive potential and the subsequent reduction of the population size. Premating reproductive isolation between released sterile and wild flies becomes a big obstacle even in successful pest control when progress is slow or the release is deemed ineffective, as found in the New World screw-worm *Cochliomyia hominivorax* (Coquerel) project (Krafsur 2005).

Another concern involves the quality of isolation barriers (or buffer zoning) between fruit flies within and outside the control areas, and flies from target areas and non-target areas, respectively. Ideal conditions for a successful control program are usually found in a small area, especially an isolated island, which represents realistic geography for eradication of target pest species such as insects (Koyama et al. 2004). If the desired area is not readily isolated, a buffer zone, a boundary of intensive control areas, is needed to serve as the physical isolation mechanism for the core areas from the outside world. Nevertheless, there is still a lack of adequate information on the effectiveness of the buffer zone in preventing gene flow as well as being a technically viable and economically justifiable tool in real situations. Also, the level of sterility of the released flies is a social concern (W. Orankanok, personal communication) because no available indicator can detect accidentally released fertile flies. For these reasons, data for evaluating an effective control program should rely on the collection of baseline data, e.g., data on population ecology, genetics, dynamics and distribution of the target species in the proposed areas, and an assessment of potential immigration of the pest into the area (Dyck et al. 2005a, b; Krafsur 2005).

Population genetic analyses are indirect methods but can be complementary to direct methods, such as mark and recapture, in order to achieve mating and genetic information. These analyses might play an important role in estimating migration rates among target and non-target populations, establishing the origin of outbreaks or reintroductions, and also supporting the quality control of mass-reared flies (Krafsur 2005). To attain the population genetic data, a molecular DNA marker such as Simple Sequence Repeats (SSRs), or microsatellites is a suitable candidate. This is because microsatellites are widely distributed across all eukaryotic genomes and provide high polymorphisms (Hancock 1999; Li et al. 2002; Loxdale et al. 1996; Tautz 1989). These markers are successfully applied to an evaluation of the pest management (Abdelkrim et al. 2005a, b, 2007; Hampton et al. 2004; Robertson and Gemmell 2004; Yu et al. 2001). In recent years, microsatellite markers of *B. dorsalis* were isolated, characterized, and established (Aketarawong et al. 2006). These markers showed high polymorphism in different geographical populations (macro- and micro-geographic levels). In addition, these population genetic data were used to infer the colonization process, migration routes, and population structures (Aketarawong et al. 2007) leading to effective management of this invasive pest. Therefore, microsatellite markers could be applied in order to provide population genetic data for addressing the control program concerns.

In this study, we investigated population genetic data of nine oriental fruit fly populations collected from target and non-target areas, as well as a mass-reared colony, using seven polymorphic microsatellite DNA markers. The genetic diversity, genetic differentiation, population structure, and population demography of each fruit fly population was examined to evaluate both AW-IPM-SIT control programs in Thailand.

Materials and methods

Method of fruit fly sample collection

All wild fruit fly samples were randomly collected only in the larval stage from approximately 40–50 infested fruits from each location during the fruit season (April–May) of the year 2003. This implies that the fruit fly samples were born in the wild. The larvae in the infested fruits were brought back to the insectary and reared to the adult stage. All of the adults were identified as *B. dorsalis* sensu stricto according to Drew and Hancock (1994). The possibility of including released sterile insects was very unlikely because there was no sterile insect released practice during the fruit season and fruit flies are only released in the adult stage. Each population was composed of 18 individual fruit flies. Each of the adult samples had been preserved in 95% ethanol solution at −20°C before single fly DNA extraction was carried out according to the method of He and Haymer (1997).

Target areas

Two target areas, which had implemented Area-Wide Integrated Pest Management using Sterile Insect Technique (AW-IPM-SIT) programs, were chosen for this study. These two programs were launched in Ratchaburi and Pichit provinces in 1991 and 2002, respectively. Each target area included a core area and a buffer zone following the Area-Wide standard practice (Fig. 1). The core area is a place for growing an economic mango crop so intensive control practices were applied there while the buffer zone is a 1 km wide concentric ring around the core area (Fig. 1). The buffer zone usually serves as a physical barrier (i.e., road, rice field, and village), and has no major commercial host plantation, isolating fruit fly migration between the core area and the outside area (non-target area). The buffer zone is also an area with intensive control practices such as male annihilation trapping (MAT), protein baited annihilation trapping (BAT), and wild host cutting.

The core area population and the buffer zone population were randomly sampled from Ratchaburi province and Pichit province from all mango hosts. Only one additional population sample is also from the buffer zone in Pichit province but from rose apple hosts (Table 1).

Non-target areas

There are three different non-target areas. Two of them were nearby but outside the AW-IPM-SIT program areas, in Pichit and Ratchaburi provinces. The other non-target area is in Nakhon Pathom province, where there was no nearby (or several 100 km away from) control program (Table 1). Three different non-target population samples were collected from these three non-target areas.

Mass-rearing facility

For reducing local public skepticism of the SIT practices, a fruit fly population was randomly sampled from the mass-rearing facility in Pathum Thani province, Thailand (Table 1). This facility produced up to 40 million sterile flies weekly. The fruit flies were mass-reared in the insectary rooms using rectangular oviposition cages, 40 × 180 × 175 cm (W × L × H) constructed of stainless steel with insect screens. Quality controls were carried out in terms of egg hatch (%), pupae weight (mg), adult

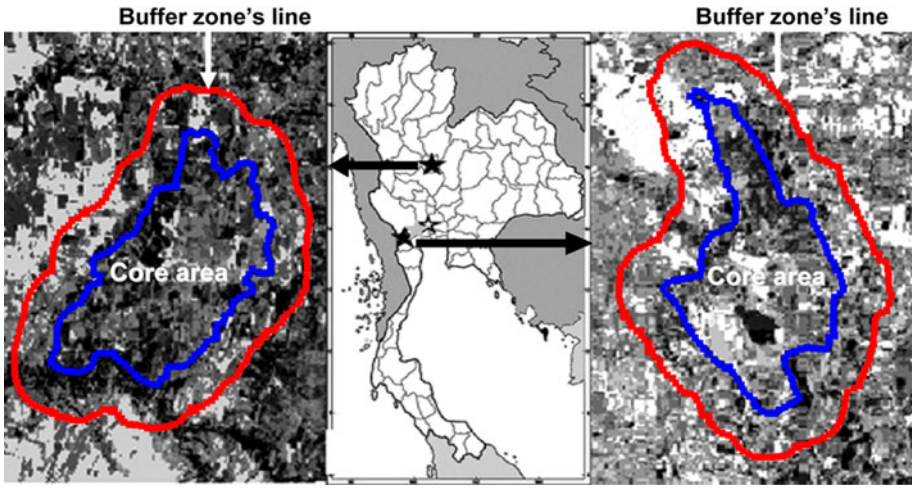

Fig. 1 Satellite maps of the pilot areas in Pichit (36 km^2) (*left*) and Ratchaburi (34 km^2) (*right*) provinces where the oriental fruit fly, *Bactrocera dorsalis*, populations were suppressed using the Area-Wide Integrated Pest Management using Sterile Insect Technique (AW-IPM-SIT) program. The buffer zone's border (*outer line*) divides the non-target area (*outside*) and target area (*inside*), whereas, the *inner line* divides the buffer zone (*outside*) and core area (*inside*). The two *black* stars, a *gray* star, and a *blank* star represent locations of the two AW-IPM-SIT programs, Nakhon Pathom province and Bangkok, respectively, in the map of Thailand (*middle*). (Modified from Orankanok et al. 2007)

Table 1 Collected population samples of *Bactrocera dorsalis* from target areas (core area or buffer zone), non-target areas (outside areas), and the mass-rearing facility in Thailand

Sample location	Sample site	Sample name	Host	Date	Coordinates	Sample size (individuals)
Pichit province	Outside area	Pichit-O	Mango	April 2003	16°43′N 100°36′E	18
	Buffer zone	Pichit-B1	Mango	April 2003		18
		Pichit-B2	Rose apple	April 2003		18
	Core area	Pichit-C	Mango	April 2003		18
Ratchaburi province	Outside area	Ratchaburi-O	Mango	April 2003	13°53′N 99°80′E	18
	Buffer zone	Ratchaburi-B	Mango	April 2003		18
	Core area	Ratchaburi-C	Mango	April 2003		18
Nakhon Pathom province	Outside area	Nakhon Pathom	Mango	May 2003	13°47′N 100°19′E	18
Mass-rearing facility	Mass-reared colony	Mass-reared colony	–	February 2003	Not applicable	18

emergence (%), flight ability, sex ratio, and sterility (%) following the International Fruit Fly Quality Control Manual (FAO/IAEA/USDA 2003). The genetic variability of the mass-reared colony was periodically enriched every 18 months by adding wild flies taken from various areas.

Microsatellite analyses

Amplifications of seven microsatellite loci (Bd1, 9, 15, 19, 39, 42, and 85B) were achieved by polymerase chain reaction (PCR) using specific primer pairs for the oriental fruit fly (Aketarawong et al. 2006). The loci were selected on the basis of reported levels of polymorphism (Aketarawong et al. 2007). Primer sequences and the methods used for DNA amplification, electrophoresis, and allele scoring with an automated ABI PRISM 310 Genetic Analyser (Applera Perkin Elmer) were described in Aketarawong et al. (2006). The individual genotype was declared as null (not amplifying at a locus) only after at least two amplification failures.

Population genetic analyses

The genetic variability of each population was examined in terms of the average number of alleles (N_a), the median variance in allele size range (V_m), the average number of effective alleles (N_e), the average number of private alleles (N_p), the average frequency of private alleles (A_p), the average observed heterozygosity (H_O), the average expected heterozygosity (H_E), and the average frequency of null alleles (A_n). These parameters were estimated using MICROSATELLITE ANALYSER (MSA; Dieringer and

Schlötterer 2003) software. GENEPOP version 3.3 (Raymond and Rousset 1995) was used to test the deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD), together with their critical levels after sequential Bonferroni correction (Rice 1989). The number of effective alleles was estimated as $1/(1 - H_E)$. The frequency of null alleles (A_n) was calculated as $[(H_E - H_O)/(H_E + 1)]$ (Brookfield 1996).

The degrees of genetic differentiation among populations were analyzed as pairwise F_{ST} values using MSA software (Dieringer and Schlötterer 2003). The statistical significance of each value was assessed by comparison of the observed value with the values obtained in 10,000 matrix permutations.

Population structure was analyzed using STRUCTURE version 2.1 (Falush et al. 2003; Pritchard et al. 2000). The Bayesian clustering algorithm in this program was applied as an exploratory analysis to determine whether the wild fruit fly populations could be subdivided into K genetically distinct groups (or clusters). STRUCTURE assumes a model in which there are K populations (where K is unknown), each of which is characterized by a set of allele frequencies at each locus. Individuals in the populations are probabilistically assigned to hypothetical original populations, or jointly to two or more populations if their genotypes indicate that they are admixed. To determine the most likely number of clusters (K) underlying our population samples, we conditioned our data on various values of K ranging from 1 to 9 (including and excluding the mass-reared colony). The program was run under the admixture model with the option of correlated gene frequencies (the "F model"). The F model allows frequencies in the different populations to be similar due to ongoing migration or shared ancestry. The additional parameters used were: different values of F_{ST} for different subpopulations, prior F_{ST} mean 0.01, standard deviation 0.05, and constant lambda valued at 1. The length of the initial burn-in period was set to 50,000 iterations followed by a run of 100,000 Markov Chain Monte Carlo (MCMC) repetitions. The value of K showing the optimal subdivision of the data (in our case $K = 5$) was selected using the formula: $[\ln P(D)_k — \ln P(D)_{k-1}]$, where $\ln P(D)$ is the estimated posterior probability of the data conditional to K (Garnier et al. 2004). In addition, the genetic admixture among the eight wild populations collected from the target (core areas and buffer zones), and non-target areas was estimated using prior population information in order to infer the impact of the AW-IPM-SIT management concept. In doing so, only the values of $K = 2$ and $K = 3$ were run under the admixture model using the previously mentioned parameters in order to estimate the posterior probability that the genetic structure may be grouped into the target and non-target populations (two groups) and/or the core area, buffer zone, and outside area populations (three groups).

The Principal Coordinate Analysis (PCoA) using GENALEX 6 (Peakall and Smouse 2006) was used to display genetic divergence among the fruit fly samples in multidimensional space using allele frequency data.

Population demographic information of each population was investigated through the estimation of migration and demographic contraction. Population assignment and an exclusion test including the estimation of origin for each individual were estimated using GENECLASS 2.0 software (Piry et al. 2004). This program can estimate the probability of each individual belonging to only its own population, the probability of being a migrant from each of the other populations, and the probability of it being a migrant to other populations. For inference of demographic contraction in each population, BOTTLENECK version 1.2.02 (Cornuet and Luikart 1996) was run. Two measures were used in this study to detect a within-population genetic signal from the bottleneck: mode-shift distortion and heterozygosity excess. The heterozygosity excess was tested under all three proposed models of microsatellite mutation: the infinite allele model (IAM), the stepwise mutation model (SMM), and the two-phased mutation model (TPM). Such a TPM model includes both 90% signal step and 10% multi-step mutation. The analysis was carried out using one-tailed Wilcoxon's signed-rank test which is suitable for a low number of loci (Piry et al. 1999).

Results

Genetic variability

Seven microsatellite loci were observed to be polymorphic in the nine populations sampled. The target populations (Pichit-B1, Pichit-B2, Pichit-C, Ratchabuti-B, and Ratchaburi-C) showed relatively high levels of genetic variability similar to the non-target populations (Pichit-O, Ratchaburi-O, and Nakhon Pathom) and the mass-reared colony (Table 2). Private alleles (N_p) were observed in all of the given populations. The Ratchaburi-O population had the highest number of private alleles (11) with a low average frequency (0.033). A core area with a long term AW-IPM-SIT program, Ratchaburi-C, presented the highest average value of private allele frequency (0.111). In the case of the mass-reared colony, it also showed quite a high number of private alleles (9) with a low average frequency (0.035).

No significant linkage disequilibrium was detected between genotypes at the different loci (data not shown). The majority of the cases of Hardy–Weinberg Equilibrium (HWE) deviations were detected in five target populations

Table 2 Genetic variability estimates from the oriental fruit fly, *Bactrocera dorsalis*, samples taken from target and non-target areas, and the mass-reared colony

Population	N_a	N_e	V_m	N_p	A_p	N_r	A_r	H_O	H_E	F_{IS}	A_n
1 Pichit-O	6.143	3.835	2.371	4	0.042	13	0.028	0.429	0.679	0.350	0.146
2 Pichit-B1	9.429	6.712	3.678	2	0.042	19	0.028	0.513	0.831	0.369	0.172
3 Pichit-B2	9.571	7.719	2.531	4	0.042	15	0.028	0.452	0.866	0.475	0.222
4 Pichit-C	7.429	4.869	2.857	3	0.056	14	0.028	0.516	0.781	0.343	0.151
5 Ratchaburi-O	8.429	5.375	2.497	11	0.033	20	0.028	0.481	0.741	0.316	0.143
6 Ratchaburi-B	9.571	6.831	3.208	4	0.064	17	0.029	0.393	0.846	0.533	0.246
7 Ratchaburi-C	10.143	7.647	3.906	6	0.111	23	0.028	0.532	0.860	0.376	0.177
8 Nakhon Pathom	8.714	5.184	2.777	4	0.035	24	0.028	0.614	0.768	0.185	0.085
9 Mass-reared colony	11.000	7.307	4.287	9	0.035	21	0.028	0.713	0.858	0.159	0.078

N_a mean number of alleles, N_e mean number of effective alleles, $1/(1 - H_E)$, V_m median variance of repeats, N_p number of private alleles, A_p mean frequency of private alleles, N_r number of rare alleles, A_r mean frequency of rare alleles, H_O mean observed heterozygosity, H_E mean expected heterozygosity, F_{IS} mean inbreeding coefficient, A_n mean frequency of null alleles, $[(H_E - H_O)/(H_E + 1)]$ (Brookfield 1996)

after the sequential Bonferroni correction (Rice 1989; $P < 0.05$) as a result of heterozygote deficiency. High values of inbreeding (F_{IS}) and frequency of null alleles were observed in the target populations, ranging from 0.343 to 0.533, and 0.151–0.246, respectively (Table 2).

Population structure

The genetic relationships among populations were quantified by pairwise F_{ST} values. Almost all of the pairwise F_{ST} values were significantly different from zero except for among the non-target populations (Phichit-O, Ratchaburi-O, Nakorn Pratom; Table 3). The highest level of genetic differentiation ($F_{ST} = 0.224$) was observed between the non-target (Pichit-O) and the target (Pichit-C) populations.

The STRUCTURE analysis showed that the overall genetic profile of the populations can be described with five different hypothetically original populations, whether the analysis excluded or included the mass-reared colony. The estimated likelihood increases from $K = 1$ to $K = 5$, and then reaches a plateau, implying that five is the smallest value of K which is the most likely number of inferred populations in the data set (data not shown). Each of the 162 individual flies from the nine populations was assigned to each of the five clusters with certain probability values. Table 4 provides the average values of ancestry probabilities (Q) of each population allocated to the five clusters. In cluster 1, the highest value of ancestry was that of Ratchaburi-B (Q(1) = 0.722) and in cluster 2, the highest value of ancestry was that of Ratchaburi-C (Q(2) = 0.729). Pichit-B1 and B2 had high probabilities of ancestry in cluster 3, Q(3) = 0.671 and 0.699, respectively. This suggested that the fruit fly population from different hosts from the same area had a common ancestor. In addition, cluster 3 shared a relatively high degree of co-ancestry with the mass-reared colony (Q(3) = 0.186). The highest proportion of individuals from the non-target areas (Pichit-O, Ratchaburi-O, and Nakhon Pathom) and the mass-reared

Table 3 Pairwise F_{ST} values (Weir and Cockerham 1984) as derived from MICROSATELLITE ANALYSER (Dieringer and Schlötterer 2003) for the nine oriental fruit fly populations analyzed

Population	1	2	3	4	5	6	7	8	9
1 Pichit-O		0.1773*	0.1787*	0.2237*	0.0216	0.1707*	0.1894*	0.0193	0.0800*
2 Pichit-B1			0.0424*	0.1057*	0.1580*	0.0865*	0.0704*	0.1460*	0.0964*
3 Pichit-B2				0.0628*	0.1489*	0.0585*	0.0640*	0.1367*	0.0803*
4 Pichit-C					0.1909*	0.0840*	0.0874*	0.1858*	0.1171*
5 Ratchaburi-O						0.1361*	0.1522*	0.0304	0.0710*
6 Ratchaburi-B							0.0858*	0.1262*	0.0812*
7 Ratchaburi-C								0.1431*	0.0867*
8 Nakhon Pathom									0.0227
9 Mass-reared colony									

The *asterisks* indicate statistical significance at $*P < 0.05$

Table 4 Average coefficients of ancestry obtained from a STRUCTURE run with $K = 5$, for the 162 individuals of *Bactrocera dorsalis* from the nine populations

Population	Cluster (Q)				
	Q = 1	Q = 2	Q = 3	Q = 4	Q = 5
1 Pichit-O	0.009	0.006	0.006	**0.962**	0.016
2 Pichit-B1	0.027	0.037	**0.671**	0.007	0.301
3 Pichit-B2	0.043	0.181	**0.699**	0.030	0.047
4 Pichit-C	0.015	0.013	0.031	0.005	**0.936**
5 Ratchaburi-O	0.048	0.032	0.022	**0.875**	0.023
6 Ratchaburi-B	**0.722**	0.047	0.070	0.037	0.124
7 Ratchabuti-C	0.036	**0.769**	0.089	0.012	0.094
8 Nakhon Pathom	0.018	0.011	0.010	**0.952**	0.010
9 Mass-reared colony	0.019	0.017	0.186	**0.646**	0.132

The highest value of co-ancestry of each population in a cluster is in bold

colony was determined in cluster 4. The Pichit-C population had high probabilities of ancestry in cluster 5, Q(5) = 0.936. The degree of co-ancestry in cluster 5 was also shared with Pichit-B1 (0.301), Ratchaburi-B (0.124), and the mass-reared colony (0.132).

Another ways to use the structure analysis was to test whether the genetic ancestry of each individual wild fly of the populations, taken from the same type of control area (according to the AW-IPM-SIT management concept), fell into the same genetic cluster group. When a K value equal two was tested against individual flies, the five target area populations tended to be clustered in the same group and likewise to all the non-target populations (data not shown). Individual flies from the core area populations were grouped together in the same way as the buffer zone population cluster and the outside area population cluster when a K value equal to three was set up (Fig. 2).

For the Principal Coordinate Analysis (PCoA), the graphical ordination of the first two axes in the plane for all of the 162 fruit fly individuals from all nine populations is shown in Fig. 3. No clear-cut separation of populations was observed along the X axis (37.41% of the total variation). Conversely, the Y axis, accounting for 15.77% of the total variation, reflected the separation of non-target and target populations. In Fig. 3, almost all of the non-target samples (the club shaped symbols) are scattered at values of more than 0.00 (mostly in the right quadrants) while the target fruit flies (the rectangular and triangle symbols) are located at values of less than 0.00 (mostly in the left quadrants). In the case of the mass-reared colony samples (the asterisk symbols), they were scattered in all of the quadrants. The PCoA seemed to provide consistent results with the STRUCTURE analysis by separating the target and non-target fruit fly samples.

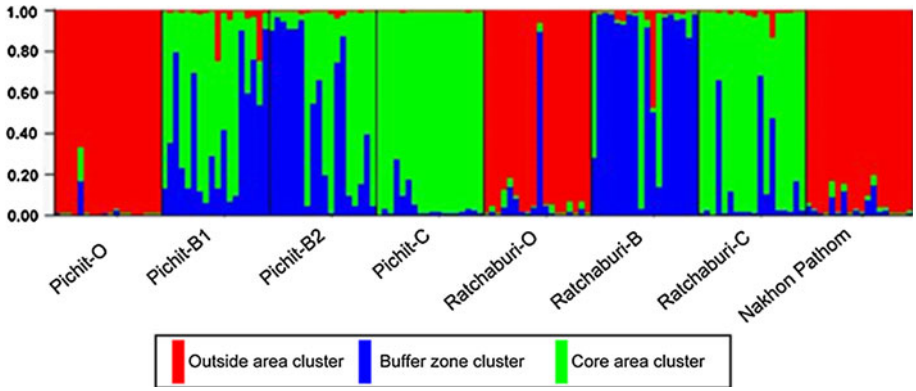

Fig. 2 The STRUCTURE analysis ($K = 3$) of 144 fruit fly samples of *B. dorsalis* from eight wild populations. Each individual represents a *vertical thin line* which is *horizontally* partitioned into *three shaded* components. Three hypothetical clusters seem to correspond to populations from (1) core areas, (2) buffer zones, and (3) outside areas

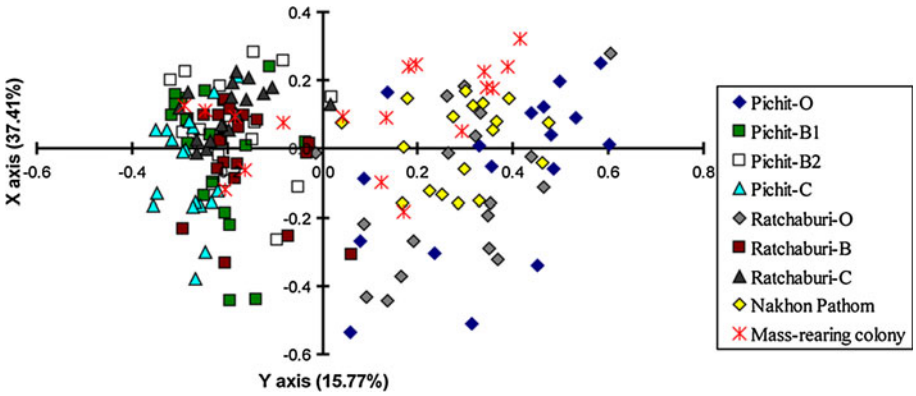

Fig. 3 Distribution of 162 individual fruit flies in the plane of the first two principal coordinates. The axes explain 37.41 and 15.77% of the total variation

Table 5 Averages of migration rate (m) into (rows) and from (columns) each population as estimated by GENECLASS 2.0 (Piry et al. 2004)

Assigned individuals	1	2	3	4	5	6	7	8	9
1 Pichit-O	*0.5296*	0.0143	0.0002	0.0000	**0.3646**	0.0067	0.0000	**0.5327**	**0.4852**
2 Pichit-B1	0.0001	*0.6650*	0.0626	0.0016	0.0012	0.0214	0.0368	0.0001	0.0477
3 Pichit-B2	0.0000	0.0458	*0.5517*	0.0025	0.0010	0.0032	0.0051	0.0001	0.0274
4 Pichit-C	0.0000	0.0159	0.0690	*0.5523*	0.0003	0.0156	0.0243	0.0000	0.0545
5 Ratchaburi-O	**0.1042**	0.0041	0.0008	0.0000	*0.5921*	0.0108	0.0001	**0.2102**	**0.2958**
6 Ratchaburi-B	0.0000	0.0177	0.0087	0.0286	0.0007	*0.6418*	0.0073	0.0005	0.0373
7 Ratchaburi-C	0.0000	0.0087	0.0087	0.0006	0.0000	0.0015	*0.5849*	0.0000	0.0099
8 Nakhon Pathom	0.0785	0.0028	0.0000	0.0000	0.0992	0.0054	0.0001	*0.6542*	**0.5079**
9 Mass-reared colony	0.0037	0.0065	0.0083	0.0001	0.0271	0.0021	0.0011	**0.1781**	*0.6883*

In rows, the populations from which the individuals probably belong to reference population as shown in columns. In italics, the proportions of individuals derived from the source population. Values of m above 0.1000 are in bold

Table 6 Tests of the demographic contraction in each population based on mode-shift distortion in allele frequency distributions and heterozygosity excess with three underlying mutation models (IAM, SMM, and TPM) performed using BOTTLENECK (Cornuet and Luikart 1996)

Population	Mode-shift[a]	Heterozygosity excess P value		
		IAM	SMM	TPM[b]
1 Pichit-O	Normal	0.0078	NS	NS
2 Pichit-B1	Normal	NS	NS	NS
3 Pichit-B2	Normal	0.0039	NS	NS
4 Pichit-C	Normal	0.0117	NS	NS
5 Ratchaburi-O	Normal	NS	NS	NS
6 Ratchaburi-B	Normal	0.0391	NS	NS
7 Ratchaburi-C	Normal	0.0039	NS	NS
8 Nakhon Pathom	Normal	NS	NS	NS
9 Mass-reared colony	Normal	NS	NS	NS

Probability values were determined using one-tailed Wilcoxon tests

NS refers to no significant value

[a] A shift in the distribution at allelic frequency classes is expected in bottleneck populations

[b] Parameters for the TPM model included 90% signal step mutation and 10% multi-step mutation

Demographic inferences

The proportions of immigrants (m) in each population estimated by GENECLASS 2.0 (Piry et al. 2004) are shown in Table 5. The values in the diagonal of the matrix were the proportions of individuals taken from the same population from which they were sampled. The highest value belongs to the mass-reared colony ($m = 0.688$). Genetic exchange among the target and non-target populations and the mass-reared colony were observed to have different migration rates, and almost all of them showed no significant migration rates. The only significant migration rates ($m > 0.1000$) were detected among the non-target populations and the mass-reared colony. The highest value of estimated migration rate was detected from the Nakorn Pathom to the Pichit-O populations ($m = 0.533$).

No bottleneck signal was detected through the mode-shift distortion approach (Table 6). To measure heterozygosity excess, for the IAM model, heterozygous excess was noted in the five populations: Pichit-O ($P = 0.008$), Pichit-B2 ($P = 0.004$), Pichit-C ($P = 0.012$), Ratchaburi-B ($P = 0.039$), and Ratchaburi-C ($P = 0.004$; Table 6). Conversely, no bottleneck signal was detected in any population analysed under the remaining mutation models. The signal of population expansion (heteozygosity deficiency) was observed in the Nakhon Pathom and mass-reared colony populations under both the SMM and the TPM models. However, the signal was observed only under the SMM model in the case of the Ratchaburi-O population (data not shown).

Discussion

This research aimed to apply the microsatellite DNA markers to generate the population genetic data of the fruit flies from a few target and non-target areas as well as the mass-rearing facility. The results were subsequently used to answer management queries and public concerns. Our results showed enough detail to argue the following key points: (1) the sterilization efficacy of the released fruit flies and quality control of SIT, (2) the possibility of insect migration or gene flow across the buffer zone, and (3) the impact of the potential risk factors from the insect control programs.

The sterilization efficacy of the released fruit flies and quality control of the SIT program

The long term sterilization efficacy of released insects was one of the highest public concerns because it was a factor

that caused failure of the control program (Macleod and Donnelly 1961; McDonagh et al. 2009). If the level of sterility is insufficient, the trapping survey and the fruit sampling would reveal the expansion of the fruit fly population size and a higher fruit damage level, respectively. The use of qualitative data such as population genetic analyses should also demonstrate the following characteristics. A significant level of gene flow between the released and target populations should be manifested and a similar pattern of genetic structure between such populations should be observed. For the studied AW-IPM-SIT programs, Orankanok and her colleges (2006, 2007) found that the population size of the fruit flies and the level of the host plant damage were reduced since the program had been started. Likewise, population genetic analyses provided data that suggested no significant gene flow ($m < 0.100$) from the mass-reared population to the target populations. Also, different patterns of genetic structure were observed over the nine populations as a result of the STRUCTURE analysis in Table 4. Two target populations have their own genetic clusters (Pichit-C, $Q(5) = 0.936$ and Ratchaburi-C, $Q(2) = 0.769$) while the mass-rearing colony is in the different cluster, $Q(4) = 0.646$. Therefore, these population genetic data support the fact that the fruit fly sterilization practice was valid.

The potential premating reproductive isolation between the sterile flies and the wild flies is also an inherent hurdle, especially when the fruit fly population suppression was slow or the SIT program gave the impression of ineffectiveness. An example of a failed program due to such an effect is the New World screwworm, *Cochliomyia hominivorax* (Coquerel) project (Krafsur 2005). The SIT program in Jamaica in 1999–2005 failed because the sterile flies originated from Mexico. This failure might be a result of mating incompatibility between the sterile and the wild flies or the existence of sexually incompatible cryptic species (McDonagh et al. 2009). Comparisons of the genetic variation within and between populations of target species should be investigated in order to infer the genetic similarity. Subsequently, maintenance of the high genetic variation in the mass-reared strain could increase the possibility of male vigor in the mating competition between the released sterile and wild flies. Our genetic data revealed that the observed genetic variation of the mass-reared strain was as high as the non-target (natural) populations (Table 2). The extent of their genetic variation was considerably large and may cover all of the genetic variations of the wild fruit fly samples, whether collected from the target or the non-target areas, as illustrated in the Principal Coordinate Analysis (PCoA; Fig. 3). Adding the fact that our control programs can suppress the fruit fly pest populations, the 18-month periodical genetic variation enrichment from the wild to the mass-reared colony seemed to be sufficient for the maintenance of the genetic variation that may be necessary for mating competitiveness of the SIT flies.

Nonetheless, the effectiveness of the competitive mating of the released sterile males was still being hindered by the release of sterile females in the control programs. This was because the mass-reared strain was a dual sex by nature. The released sterile female flies would distract the sterile male from mating with the wild females. In addition, the released females were also undesirable due to the fact they still damage the fruits by oviposition. Genetic sexing strains (GSSs) of the oriental fruit fly are needed in the future in order to separate the sterile female from the sterile male flies prior to releasing them (Wimmer 2005).

The possibility of insect migration or gene flow across the buffer zone

The Area-wide Integrated Pest Management (AW-IPM) concept was designed to suppress the total pest population covering widely isolated areas. The ideal geography for a successful Area-Wide insect control program (e.g., eradication of *Bactrocera cucurbitae* in Japan, Koyama et al. 2004) would be isolated islands. In order to imitate an isolated island geography, the combination of a natural and/or artificial buffer zone encircling a core area was set up. This terrain demarcation is analogous to the sea enclosing an island. Therefore, the fruit fly populations sampling from the effective target areas, i.e., the core areas and the buffer zones, should be genetically isolated and show no significant level of gene flow. This would be an ideal expectation on isolated islands.

Several population genetic analyses suggested that the target populations, particularly the core areas, were unlike the non-target populations. Firstly, the target populations did not show significant gene flow across the non-target populations ($m < 0.1000$; Table 5). Similarly, the genetic differentiation (F_{ST}) was significant when observed among the target and the non-target samples, as shown in Table 3. However, both patterns were not found among the non-target and the mass-reared populations. Thirdly, the nine populations were structured into five groups: two core areas, two buffer zones, and a group comprising the non-target and mass-reared colony (Table 4). When the mass-reared fly population was excluded from the STRUCTURE analysis, the other eight wild populations were separated into two (target and non-target) and/or three (core area, buffer zone, and non-target area) clusters when K values equalled 2 and 3, respectively (Fig. 2). These genetic clusters lead to a hypothesis that SIT has exactly the same sort of genetic effect in all the target populations. Since the sample size for each of the populations was modest, future studies with more samples and analyses should be carried out to test the hypothesis.

Weak bottleneck signals were also observed in most of the target populations (Table 6). This bottleneck phenomena may result from the effectiveness of SIT in recent population suppression. However, this type of bottleneck is not a direct comparison of the target versus the non-target samples. The data from population dynamic studies, such as fruit fly distribution, should be used to support such an inference.

The genetic effect of the control programs for fruit fly population suppression was also evident from the inbreeding studies. The fruit fly populations from two different buffer zones manifested a high level of inbreeding coefficient (F_{IS}) compared to their respective populations in the core areas and the non-target areas (Table 2). The analysis of the number and the frequency of the private alleles (N_p and A_p, respectively) indicated that the populations collected from core areas, particularly from the longer-term project (Ratchaburi-C), appeared to be an isolated population (Table 2). This is because one feature of an isolated population is a high frequency of private alleles (Aketarawong et al. 2007). Both the bottlenecking and inbreeding could be indirect implications that the buffer zoning practices actually isolated the core areas from the outside areas. Again, future population genetic studies should be conducted with a larger sample size and with more samples that represent different time periods to refine the genetic interpretation.

Impact of potential risk factors from the insect pest control program: success or failure?

The AW-IPM-SIT goal was to suppress, not eradicate, the oriental fruit fly population to be lower than the economic threshold level in Thailand (Orankanok et al. 2007). Therefore, the emergence of fruit flies in the target areas is a key risk factor responsible for the sustainability of the control programs. It would be advantageous to identify sources of the emergent fruit flies so that the management effort can be concentrated. This work provided different population genetic methods that make inferences, but all were based on likelihood. The following are hypothetical scenarios for the recolonization of the fruit fly: (1) resurgence from a single source, or (2) resurgence from multiple sources, or (3) incursion whether from single or multiple sources. Such a management query may be necessary for the planning, monitoring, and validation of the SIT programs (Abdelkrim et al. 2005a, b, 2007; Hampton et al. 2004; Robertson and Gemmell 2004; Hendrichs et al. 2007). In this work, the population genetic analyses were indirect but complementary tools that elucidated this aspect. For hypotheses (1) and (2), if existing fruit flies were recolonized from resurgence, i.e., a small number of individual survivals, bottleneck signals should be observed. If the populations had been re-established from a single source, they would possess less genetic variation than that of multiple sources due to the inbreeding effect. If the existing flies are newly colonized from incursions, a significant level of gene flow from the nearby non-target populations may be evident. In the case of our control programs, incursion was less likely than resurgence because there was no significant gene flow between the target and non-target areas. In addition, the presence of weak bottleneck signals of the population in the target areas also supported this view although a larger sample size in future studies may be required to confirm this statement. The proposed resurgence sources may have multiple origins based on the following data. The observed genetic variation (such as the mean rare alleles (N_r) of the Ratchaburi-C flies) of the core area populations was as high as that of the non-target populations (Table 1). A possible explanation is that the combination of fragmented micro-habitats in mixed fruit orchards may be the multiple sources of fruit flies with fast growing capabilities.

It was also evident that the two fruit fly populations (Pichit-B1 and Pichit-B2) from two different hosts, the commercial mango host and the backyard rose apple host, respectively, in the same buffer zone area had similar genetic variation (Table 2) and average coefficients of ancestry (Table 4 and Fig. 2). However, there were some significant differences found between the two populations. For example, their private alleles (Table 2), genetic differentiation (F_{ST}; Table 3) and bottleneck analysis (Table 6) were relatively different. Also, a limited gene flow was observed between the two populations (Table 5). This could imply that the Pichit-B1 and Pichit-B2 flies shared the same genetic history but now had become slightly diverse. Such a scenario, in particular among the polyphagous insects, might induce the evolution of assortative mating as a local adaptation to a new host and subsequently become genetically differentiated (Drès and Mallet 2002; Subramanian and Mohankumar 2006). Larger fruit fly sample sizes are needed to verify this type of scenario in the future. However, it is conceivable that a similar scenario happened in the core areas that had primarily commercial mangoes and plenty of secondary hosts in the backyard, wild, and/or abandoned areas, as fragmented habitats. Therefore, fruit fly populations could be subdivided into smaller groups because suitable habitats for them were not continuous (Hedrick 2011). It was reported that each micro-habitat might play a role as an important natural refuge (Subramanian and Mohankumar 2006).

The oriental fruit fly is an invasive species that has high fecundity, high dispersion ability, and the ability to establish its population in a broad host range under a wide range of climatic conditions (Aketarawong et al. 2007; Clarke

et al. 2005; White and Elson-Harris 1992). The combination of these invasive characteristics with a weak bottleneck and multiple resurgence could account for the high level of genetic diversity as found in other organisms (reviewed in Allendorf and Lundquist 2003; Eales et al. 2008; Hampton et al. 2004; Neville et al. 2007; reviewed in Sakai et al. 2001). Therefore, the current AW-IPM-SIT program management should intensify the control effort and resources toward the core area in order to reduce fragmented micro-habitat refuges of the fruit fly pests.

One simple approach to understand the effect of the bottleneck was to compare the heterozygosity in the population that was collected before the bottleneck and in the population after the event (Hedrick 2011). For the current study, no fruit flies before and after the implementation of the AW-IPM-SIT programs were collected due to the fact that the program had been launched before this research. Thus, some of our genetic inferences rely only on the extant flies in different areas under the assumption that such populations, including target and non-target populations, were originally the same populations and subsequently separated by the control program. Since then, this research has worked on only one randomly represented population of each area from a single time point so that the current information on the population genetic analysis represents merely a time-specific snapshot. More research relying on a large number of populations with a more extensive time span is still required for a better picture on the population ecology of the AW-IPM-SIT. Our number of individual fruit flies in each population was modest in size due to the effective control programs that limited the number of fruit fly samples. Nonetheless, it appeared to be sufficient for our initial evaluation which illustrated congruent results from different population genetic analyses.

Conclusion

Established microsatellite DNA markers could generate population genetic data for the evaluation of the AW-IPM-SIT programs in order to answer several management queries and public concerns. Our results suggest that the sterility procedure was effective enough to inhibit significant gene flow to the target populations. Similarly, it was inferred that buffer zone management can actually isolate the core areas from the outside areas because it can prevent significant gene flow among the target and non-target fruit flies. However, fruit fly pest resurgence, from fragmented micro-habitats in mixed fruit orchards, was suspected. Management of the AW-IPM-SIT effort, such as SIT, should concentrate on the hidden refuges within the target areas.

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