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THESIS

STRAIN EVALUATION AND PARENTAGE IDENTIFICATION OF GIANT FRESHWATER PRAWN *Macrobrachium rosenbergii* de Man BY MICROSATELLITE PROFILING

THUCHAPOL KARAKET

A Thesis Submitted in Partial Fulfillment of
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Thuchapol Karaket 2012: Strain Evaluation and Parentage Identification of Giant Freshwater Prawn *Macrobrachium rosenbergii* de Man by Microsatellite Profiling. Doctor of Philosophy (Aquaculture), Major Field: Aquaculture, Department of Aquaculture. Thesis Advisor: Associate Professor Supawadee Poompuang, Ph.D. 165 pages.

This thesis was aimed to demonstrate the usefulness of microsatellite markers for strain evaluation, parentage inference and male reproductive assessment of freshwater prawn *Macrobrachium rosenbergii*. In the first experiment, growth performance among three strains of freshwater prawn- including a commercial strain of foreign origin, a local hatchery strain and a hatchery strain of wild origin - was evaluated under separate and communal rearing conditions for 120 days. Significant differences in growth were observed among strains in both separate and communal rearing, with the commercial strain outperforming the hatchery populations. The exclusion-simulation approach was performed on different sets of one to seven microsatellite loci to determine the power of the assignment test. High accuracy of the assignment test was obtained by using seven loci, with 90% correct assignment of individuals (P<0.05). The power of the assignment test was highly dependent on the degree of population differentiation (F_{ST}). Results of this study demonstrated that an assignment score of 100% was obtained when $F_{ST} > 0.1$.

The potential use of microsatellite loci for parentage identification was assessed in a commercial strain of the freshwater prawn. Nine loci were informative, with average expected heterozygosity of 0.81 and PIC score of 0.77. The accuracy in assignment was determined in 21 full-sib and two maternal half-sib families using two contrasting methods, a pair-wise likelihood comparison approach in the CERVUS program and a full-pedigree likelihood method in the COLONY program. Use of four highly informative loci was sufficient for COLONY to resolve the genetic structure of this population, while seven loci would be required to obtain 94-99% correct assignment with CERVUS. Moreover, COLONY showed a list of full- and half-sibships, but CERVUS did not display this information. Results suggest that this set of microsatellites, used in conjunction with COLONY would be an effective tool for parentage and sibship identification in selective breeding programs of the giant freshwater prawn.

Among three male morphotypes, blue claw males were the most successful at mating under various combinations of male to female sex ratio, followed by orange claw males and small males. Morphometric traits, including condition factor, body weight, and relative claw length that were highly correlated with male type had significant effects on reproductive success of male prawn.

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Thuchapol Karaket

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LIST OF ABBREVATIONS

ANOVA	=	Analysis of variance
BC male	=	Blue claw male
BL	=	Body length
bp	=	Base pairs
BW	=	Body weight
°C	=	Degree Celsius
cm	=	Centimeter
CR (CL)	=	Carapace length
df	=	Degrees of freedom
DNA	=	Deoxyribonucleic acid
dNTPs	=	Deoxydinucletide triphosphate
FS	=	Full-sib
GLM	=	Generalized linear model
GFP	=	Giant Freshwater Prawn
g	=	Gram
HCl	=	Hydrochloric acid
HS	=	Half-sib
M	=	Molar
mg	=	Milligram (s)
$MgCl_2$	=	Magnesium chloride
ML	=	Maximum likelihood
ml	=	Milliliter (s)
mM	=	Millimolar
mm.	=	Millimeter
NaCl	=	Sodium chloride
ng	=	Nanogram (s) (10^{-9})
PCR	=	Polymerase Chain Reaction
pH	=	Logarithm of reciprocal of hydrogen (H) ion
PL	=	Post larva
pmol	=	Picomole (s) (10^{-12})

LIST OF ABBREVATIONS (Continued)

RCL	=	Relative claw length
S.D.	=	Standard deviation
Taq	=	*Thermus aquaticus*
TBE	=	Tris-borate EDTA buffer
TE	=	Tris-EDTA buffer
TL	=	Total length
TNES	=	Tris-NaCl-EDTA-sodium dodesylsulfate
Tris	=	Tris (hydroxylmethyl) methylamine
µg	=	Microgram (s) (10^{-6})
µl	=	Microliter (s)
µM	=	Micromolar
%	=	Percentage

STRAIN EVALUATION AND PARENTAGE IDENTIFICATION OF GIANT FRESHWATER PRAWN *Macrobrachium rosenbergii* de Man BY MICROSATELLITE PROFILING

INTRODUCTION

Diversity of genetic resources offers possibilities for domestication and selective breeding for sustainable development of aquaculture stocks. The applications of microsatellite markers for assessing genetic diversity of breeding populations and determining parentage to establish pedigrees have become common in selection programs of aquaculture species (Moore *et al*., 1999; Jerry *et al*., 2004). Microsatellites have proven effective tools for monitoring genetic change in selection programs, as well as for management of genetic variation in broodstock of selected populations. In addition, a successful breeding program also depends on pedigree information of broodstock for design of breeding strategies to maximize genetic responses for traits such as growth, to monitor breeding levels and to minimize the detrimental effects of inbreeding in the population (Moore *et al*., 1999).

A prerequisite for genetic gain or improvement by selection depends on the existence of genetic variation in important traits within- wild or cultured stocks. Genetic diversity within populations allows breeders to develop new traits in response to environmental change or market demand. Moreover, selection of best strains will enhance production of that species. Strain selection is a method to compare performance such as growth rate and survival, of a variety of strains under common culture conditions to identify the best strain. Strain evaluation, therefore, should be conducted prior to initiating selective breeding programs to determine the genetic basis of the suitable stocks.

Selective breeding programs for aquatic animals normally involve rearing a large number of families in separate tanks or ponds until the offspring are large enough to be marked and stocked together. However, separate rearing of families,

increases environmental differences between families which can be confounded with genetic effects (Falconer and Mackay, 1996) unless full- and half-sib or other highly interconnected designs are used, which is frequently not feasible in aquaculture. Studies indicate that the increased variation between families due to tank effects can result in high estimation of heritability for growth and reproductive traits in Pacific white shrimp *Litopenaeus vannamei* (Pérez-Rostro *et al.*, 1999; Pérez-Rostro *et al.*, 2003). Variations in pond conditions are found to have large effects, particularly on growth performance (Herbinger *et al*., 1999). Therefore, it is important to minimize the influence of confounding environmental effects by stocking tagged animals in the same tanks or ponds under communal rearing conditions.

Tagging methodologies allow mixing of families or strains in the same environment for performance testing. Generally, the animals are physically marked using external tags to identify their family or strain of origin. However, for crustaceans, external tagging is inefficient due to tag loss during molting (Menasveta *et al*., 1994). Although several types of internal tags, e.g., elastomer dyes and passive integrated transponders, are available for crustaceans, their use is limited to juvenile and adult individuals, as demonstrated in Pacific white shrimp (Godin *et al*., 1996), lobster *Homarus gammarus* (Linnane and Mercer, 1998) and crayfish *Cherax destructor* (Jerry *et al*., 2001). It is impractical to tag the animals at early life stages such as post-larvae (Arce *et al*., 2003; Jorstad *et al*., 2005).

Microsatellite DNA polymorphisms have been used as genetic markers to eliminate the problems posed by physical tagging of early life stages of animals. The popularity of microsatellites is due to their abundance, neutrality, co-dominant expression, high levels of polymorphism, and PCR-based analysis (Chistiakov *et al.*, 2006). Applications have been reported in many selective breeding programs for fish and shellfish. For example, microsatellites were used to establish the pedigrees of mixed families of rainbow trout *Onchorhynchus mykiss* (Herbinger *et al*., 1995), Atlantic salmon *Salmo salar* (Herbinger *et al*., 1999; Norris *et al*., 2000) and Humpback grouper, *Cromileptes altivelis* (Na-Nakorn *et al*., 2010), to estimate heritability in common carp *C. carpio* (Vandeputte *et al*., 2004), to assess performance

of European lobster *H. gammarus* broodstocks (Jorstad *et al*., 2005), and to assign parentage relationship in Kuruma shrimp *Penaeus japonicus* (Jerry *et al*., 2004) and tiger shrimp *P. monodon* (Jerry *et al*., 2006).

Freshwater prawn farming represents one of the important sectors in Thailand's aquaculture industry, with annual production increasing from 2,200 mt in 1997 to 28,500 mt in 2008 (FAO, 2010) and total culture area increasing from 2,200 ha in 1998 to 15,540 ha in 2007 (Department of Fisheries, 2007). Eighty percent of prawn production was from the major farming area situated in the southwest of the Chao Phraya Basin in central Thailand. Despite the expansion of culture areas, inconsistent and low levels of production have been a major concern among prawn farmers. Genetic deterioration of prawn broodstocks was suggested as a probable cause of declining yields (Chareontawee *et al*., 2007). To overcome the problem of low production, efforts have been made to upgrade the existing broodstocks and to acquire a new stock to initiate the breeding program by importing non-native prawn broodstock from India and Myanmar. In the previous study, microsatellites revealed the relatively high levels of genetic diversity among seven populations of wild and hatchery origins of freshwater prawn despite their low level of farm productions (Chareontawee *et al*., 2007).

Although the issue of association between genetic variation at neutral marker loci and variation of quantitative traits remains controversial, genetic characterization of aquaculture stocks is important because it reflects the genetic makeup and history of domestication of a particular strain. Selection of the best strains is the first priority for ensuring high production and profitability of prawn farming. However, despite the importance of strain selection, little or no research has been conducted to compare the performance of freshwater prawn strains in Thailand.

To this end, the research conducted in this thesis was aimed to demonstrate the usefulness of microsatellite markers in combination with genetic information for proper management of freshwater prawn broodstock. These microsatellites were

developed for genetic investigation of freshwater prawn in the previous study (Chareontawee *et al*., 2006).

The first experiment was designed to compare growth and survival of post-larvae and juveniles among three prawn strains which are widely cultured in the farming areas in central Thailand. Broodstock and post-larvae were obtained from private hatcheries, including the CPP strain from Petchaburi province, the KSB strain from Supunburi province, and the SKL strain from a local hatchery in Nakorn Pathom province. Strain evaluation was performed under both separate and communal rearing conditions to detect if the relative rankings of strains were the same between the two conditions. Growth and survival of three strains were compared after 120 days of rearing. Microsatellites were used to evaluate genetic diversity within strains to determine whether the association between diversity at microsatellite loci and variation at quantitative traits exists in the hatchery populations of freshwater prawn. In addition, genotype data and the exclusion assignment method (Cornuet *et al*., 1997) were used to identify strain of origin for prawn in the communal tanks, because use of physical tags was not possible for post-larvae. The amount of genetic differentiation (F_{ST}) among strains was quantified to determine the accuracy of strain assignment because high rates of assignment success would be obtained, with F_{ST} value >0.1 The assignment tests were carried out using different sets of microsatellite loci to determine the number of loci needed to optimize between genotyping cost and power of the test.

In the second experiment, the potential use of ten microsatellite markers for parentage determination of freshwater prawn was evaluated. Informative markers with high levels of polymorphic information content (PIC) were chosen for the analysis. The accuracy in parentage assignment was tested in 220 individuals from 21full-sib and two half-sib families of a commercial prawn strain. Parentage identification of individuals in 23 families was inferred using two contrasting assignment approaches, a pair-wise likelihood comparison method (Marshall *et al*., 1998; Kalinowski *et al*., 2007) and a full-pedigree likelihood method (Jones and Wang, 2010). These two

approaches are widely used for parentage inference in wild and domesticated populations of plants and animals.

The final experiment described the use of microsatellites to assess reproductive success of male morphotypes under different combinations of males and females. Males and females were genotyped at a microsatellite locus and their genotype data were used to verify genotypes of offspring obtained from each mating. Freshwater prawns are characterized by the formation of dominance hierarchy, as are some clawed decapod crustaceans, e.g., lobster and crayfish (Goessman *et al*., 2000; Herberholz *et al*., 2003). Three male morphotypes are found in sexually mature population of freshwater prawn. Blue-claw males are the slow-growing individuals with large size and long claw. Orange-claw males, the most desirable in prawn culture, are medium to large sized and have rapid growth rate. Small males are much smaller than the other two morphotypes and have a very slow growth rate. The previous study by Ra'anan and Sagi (1985) reported that BC males were the most reproductively active and successful at mating, while OC males were much less active in the presence of dominant BC males. Small males, on the other hand, due to their small size, practiced a form of sneak mating. The present study was carried out to determine if morphometric traits such as body weight, body length, claw length, and condition factor, contribute to the reproductive success of male prawn.

OBJECTIVES

1. To compare growth performance among three commercial strains of *Macrobrachium rosenbergii* under separate and communal rearing conditions.

2. To evaluate the properties of microsatellite loci for parentage identification of freshwater prawn with different family structures.

3. To examine the effects of sex ratio and morphometric traits on reproductive capacity among male broodstock of different morphotypes.

LITERATURE REVIEW

Distribution, life history, and farming of giant freshwater prawn

Macrobrachium rosenbergii de Man, commonly known as giant freshwater prawn, is a species with wide range of distribution from India, Southeast Asia to northern Australia. They are found in most inland freshwater areas, including rivers, lakes, swamps, and ponds that are directly or indirectly connected with the sea. Based on phylogenetic analysis, freshwater prawn in India and Southeast Asia, including Thailand, Myanmar, Malaysia, and Indonesian regions of Sumatra and Java, are recognized as 'western' form, while those found in the Philippines, the Indonesian regions of Sulawesi and Irian Jaya, Papua New Guinea and northern Australia are identified as 'eastern' subspecies (de Bruyn *et al*., 2004; New *et al*., 2010). In Thailand, freshwater prawn is naturally distributed in the Chao Phraya River, the Maeklong River and the Kra Buri River in the south. Following a successful breeding under hatchery conditions, freshwater prawn larvae have been released by the government fishery stations into rivers, lakes and dams throughout the country for stock enhancement.

The life cycle of giant freshwater prawn consists of four distinct phases, including egg, larva, juvenile and adult. After mating in freshwater, gravid females migrate downstream into estuaries to spawn. Larvae (free-swimming zoea) metamorphose into post-larvae within approximately 26 days under hatchery conditions. Following two to three weeks after metamorphosis, post-larvae begin to move upstream towards freshwater canals and rivers to live as adults (New *et al*., 2010).

Breeding of freshwater prawn can be practiced year-round in Thailand. Generally, local hatcheries obtain berried females from rivers, lakes as well as farm ponds during the harvest at the end of growing season. Mature females (15-20 g) can release 50,000 to 100,000 eggs during one spawning (New *et al*., 2010). However, for prawn of Thailand origins, average fecundity at first maturity was approximately

30,000 eggs (Rungsin *et al*., 2006). Hatchery survival rate of larvae to post-larval stage is about 50%.

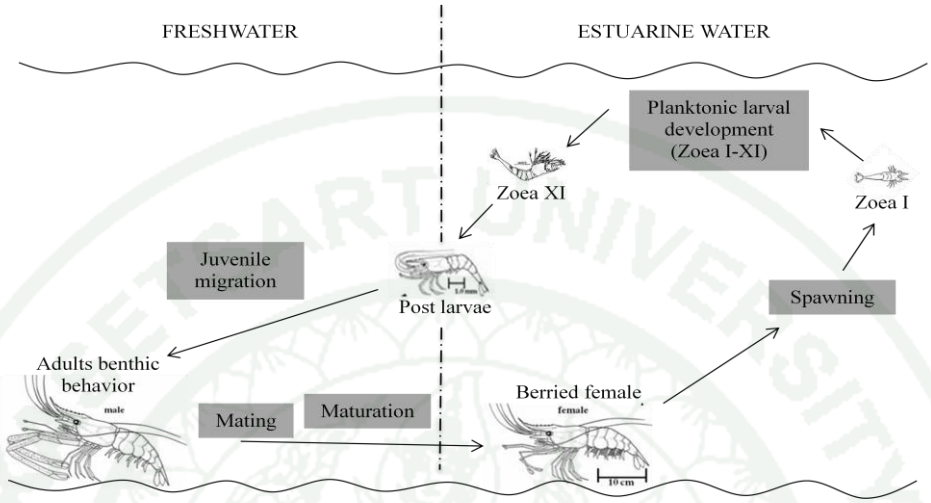


Figure 1 Life cycle of giant freshwater prawn.

Source: New *et al*. (2010)

Culture of giant freshwater prawn has been established in Thailand for nearly 40 years. In 2008, Thailand was ranked the second major producer of freshwater prawn behind China (FAO, 2010). During the past several years, rising demand for domestic consumption and export markets has led to rapid expansion of the industry, with production increasing from 2,200 tons in 1997 to 28,500 tons in 2008 (FAO, 2010) and total culture area increasing from 2,200 ha in 1998 to 15,540 ha in 2007 (Department of Fisheries, 2007). Eighty percent of prawn production was from the major farming area situated in the southwest of the ChaoPhraya Basin in central Thailand.

Prawn farmers practice three rearing strategies in Thailand. One strategy is to stock post-larvae directly into grow-out ponds. A second method is to stock post-larvae in nursing tanks or cages for two to three months and then to transfer juveniles to grow-out ponds. Several studies reported that prawn production was relatively low for the first strategy due to high mortality of post-larvae by cannibalism in the grow-

out ponds. In contrast, the use of a nursery phase increases the survival of juveniles and subsequently increases farm production (Lin and Boonyaratpalin, 1988; Schwantes *et al*., 2007). The third method is to stock 2-4 month old juveniles directly into grow-out ponds. This practice shortens the culture period and reduces production cost compared to other methods. The farmers use partial harvest for marketable size prawns after about four months of rearing, with additional harvests every 45 days. Total harvest is made at the end of the seven months of the grow-out period.

Reproductive strategies of male freshwater prawn

In giant freshwater prawn, territorial behavior and competition among males lead to a large variance in reproductive success among individual males. The social behavior results in physiological differences and different growth patterns of different morphological types. Initially, populations of post larvae (PL) are relatively homogenous and normally distributed. However, within several weeks of development, the difference in growth rate among individuals results in size variation. There are two distinct types of juveniles which can be described based on their relative growth rates: jumpers and laggards. Jumpers are very fast-growing individuals that may become up to15 times larger than the average population within 60 days (Karplus, 2005). During grow-out, most of the jumpers transform into the blue-claw (BC) and the orange-claw (OC) males, whereas the majority of the laggards become small males (SM) (Karplus, 2005).

The three morphotypes of males can be characterized based on claw color and the ratio of claw length to body length (relative claw length) (Ra'anan and Sagi, 1985; Ra'anan and Cohen, 1985). Blue-claw (BC) males are the slow-growing individuals with large size and long blue claw with relative length of 1.5-2.0. Orange-claw (OC) males, the most desirable, are medium to large sized with relative claw length of 1.0-1.5 and have rapid growth rate. Small males (SM) are much smaller than the other two morphotypes with relative claw length of 0.5-0.7 and restricted body weight between 1-10 g. The relative proportions of the three male morphotypes, SM, OC, and BC, remain nearly constant at 5:4:1, respectively, under both wide range of farming

(Ranjeet and Kurup, 2002) and environmental conditions (Cohen *et al*., 1981). When dominant males die or are removed, individual males undergo a transformation from SM to OC to BC (Karplus *et al*., 1989, 1991, 1992a, 1992b; Karplus and Hulata, 1995). This pattern of growth and metamorphosis has been termed the 'leap frogging' pattern (Ra'anan and Cohen, 1985).

Reproductive potential of male freshwater prawn has been extensively studied by Ra'anan and Sagi (1985). From their aquarium and field observations, BC males were the most reproductively active and successful at mating. A dominant BC male attracted eight to ten females and displayed a courtship behavior prior to mating. Reproductive capacity of OC males, however, was suppressed in the presence of BC males. The SM males with high mobility practiced a form of sneaking mating. Successful fertilization by SM males was observed in the absence of all other males.

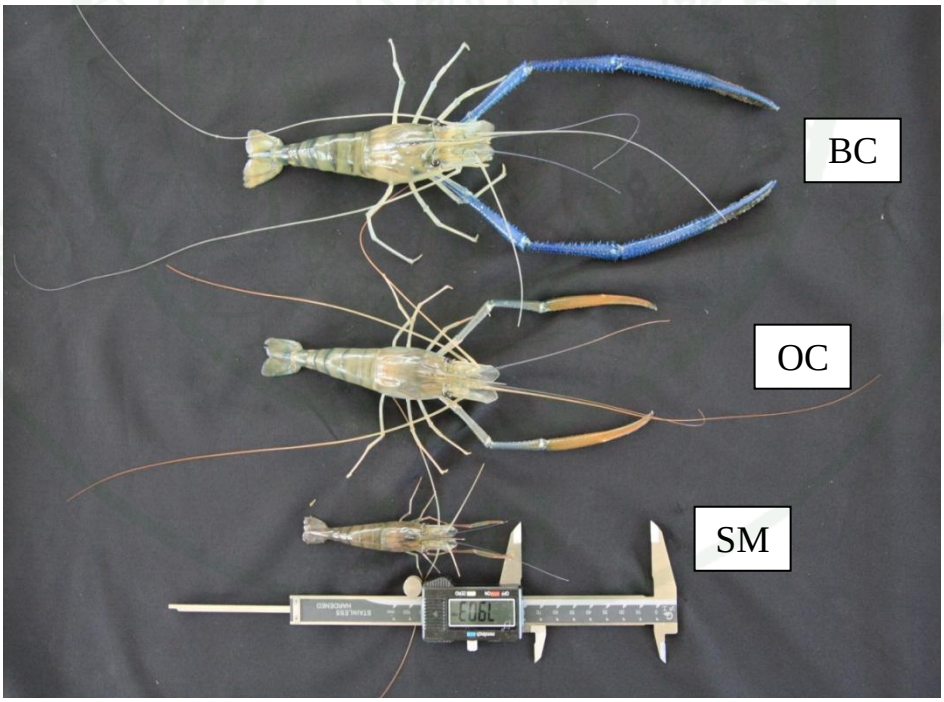


Figure 2 Three male morphotypes of the giant freshwater prawn. BC, blue claw; OC, orange claw; SM, small male.

In addition to its effect on mating behavior, morphotype was found to be the main effect on dominance ranking and resource competition for male freshwater prawn. Barki *et al*. (1992) observed the effect of morphotype between BC and OC males of similar sizes but different in claw to body length ratio. Their findings indicated that BC males dominated OC males irrespective of body size and suggested that the advantage of BC was due to their larger claws. Consequently, these BC males gained priority of access to food and shelter.

Sex hormone levels are found to be closely related to variations in reproductive system size and mating behavior of crustaceans. For example, differences in mating behavior have been attributed to varying levels of methylfarnesoate (MF), a form of juvenile hormone, in the hemolymph of male and female spider crab *Libinia emarginata* that have the largest reproductive systems, suggesting its role in reproduction (Laufer and Ahl, 1995). However, little is known about the role of MF in reproduction of freshwater prawn.

Applications of microsatellite DNA in genetic research and breeding programs

Microsatellites are simple sequence tandem repeats (SSTRs) of one to six nucleotides with the number of repeats ranges from 8 to 40 copies (Goldstein and Schlotterer, 1999). They are evenly distributed throughout the nuclear genome of most eukaryotes, approximately 10^6 copies, within coding regions, introns, and in the non-gene sequences (Liu *et al*., 2004). Most of microsatellites (30-70%) found in genome of vertebrates are di-nucleotide repeats such as $(AC)_n$, $(AT)_n$, and $(CG)_n$. Microsatellites with tri-, tetra-, and penta-nucleotide repeats are found at lower frequencies than the di-nucleotide repeats (Chistiakov *et al*., 2006). Different number of repeat units results in microsatellite diversity among individuals in species or populations. The DNA replication slippage is thought to be the predominant mutation mechanism generating microsatellite variability. This mutation process occurs at the repetitive sequences when the new strand mispairs with the template strand, altering the repeat number of microsatellites (Schlötterer, 2000). The mechanism of unequal crossing-over between homologous chromosomes can result in the loss or gain of large

number of repeats. The formation of a hairpin during synapsis causes the unequal exchange of the chromosome fragments. One chromosome will receive a larger fragment, while the homologous chromosome will receive a fragment with smaller number of repeats (Oliviera *et al*., 2006).

Large number of alleles at a microsatellite locus can be detected by the polymerase chain reaction (PCR), given that flanking sequences are known. The primers for PCR are designed from these unique flanking regions. A locus-specific microsatellite region can be amplified using a forward and a reverse primer on each side of the microsatellite. Microsatellites have been the most widely used as marker of choice in various fields of genetic research due to their abundance, high levels of polymorphism, and co-dominance mode of expression. They become a powerful tool for assessing genetic diversity of wild and cultured stocks of aquatic species as well as monitoring of genetic change in selection program (Liu and Cordes, 2004).

Microsatellite loci have been isolated for freshwater prawn of the eastern form (Chand *et al*., 2005) and the western subspecies (Charoentawee *et al*., 2006). But these microsatellites were not conserved between the two subspecies, none of the eastern form amplified samples of the western form (Chand *et al*., 2005).

Properties of microsatellites for genetic analyses

Genetic investigations which incorporate the use microsatellite markers are based on the Medelian inheritance of alleles and the population genetics assumptions of Hardy-Weinberg expectation (HWE) and independent segregation of loci. Violations of these assumptions increase the probability of biased and false conclusions. Therefore, it is important to determine the properties of a single microsatellite locus and between loci to maximize the power of the tests.

There are several factors that affect the power of molecular genetic techniques, including usefulness of markers, presence of null alleles, allele dropout, as well as and genotyping errors (Marshall *et al*., 1998). A marker's usefulness depends on the

number and frequency of alleles where highly polymorphic markers are required for genetic investigations, including population assignment and parentage determination. Heterozygosity and polymorphic information content (PIC) are widely used measures of the degree of polymorphism of a genetic marker in natural and experimental populations. Heterozygosity is defined as the probability that a chosen individual from the population is heterozygous at a locus. PIC is commonly used in linkage studies and is defined as the probability that a given marker genotype of an offspring would allow of the parental genotype at the marker locus (Botstein *et al*., 1980). PIC is always smaller than heterozygosity because some mating patterns between heterozygous individuals are not informative. The two measures tend to increase as the number of alleles increases and are maximal when allele frequencies are equal.

Presence of null alleles is commonly found at microsatellite loci across species. Microsatellite null alleles are non-amplifiable in PCR-amplification due to mutations at the priming sites and can be detected as a significant departure from HWE. Similar to the effects of null alleles, allele dropout results in heterozygote deficiencies, violating the assumption of Hardy-Weinberg proportions. The cause of allelic dropout is due to very low concentrations of DNA templates that, the one allele may be amplified more that the other.

When segregation of alleles at two or more loci is considered simultaneously, linkage disequilibrium can be detected (Hedrick, 2005). It refers to non-random associations of alleles which results in the difference between the expected and the observed genotypic frequencies. Various factors can cause linkage disequilibrium including genetic drift, mutation, inbreeding and mixing between two populations.

Population assignment

The aim of population identification is to use genetic approach for assigning population (strain) membership of individual or group of individuals based on multilocus genotype data of a particular population (Manel *et al.*, 2002). For natural populations, the assignment techniques are useful in determining the geographic origin

of a population sample, addressing relationships at the individual level and detecting immigrant individuals (Piry *et al*., 2004). Further, the assignment methods have been applied to differentiate between breeds of livestock (Maudet *et al*., 2002), to identify admixture populations of brown trout (Hansen *et al*., 2006), to distinguish between species of abalone (Sekino and Hara, 2006), and to determine hatchery origin of Atlantic salmon (Glover *et al*., 2009). The original genetic assignment method or the direct frequency method developed by Paetkau *et al*. (1995) uses the estimated allele frequencies in different populations to calculate the probability of an individual belonging to each of the possible source populations. The individual is assigned to the population of origin in which it has the highest likelihood of belonging compared to the likelihood that it is assigned to other populations. For each individual being assigned, the log-likelihood of belonging to a particular population is the product over all loci as follows:

$$Log\left(\prod_{l=1}^{n} p_{ij}^{2}\right) \text{ for } i = j \text{, and } 2p_i p_j \text{ for } i \neq j \text{,}$$

where n denotes the number of loci, i and j are two alleles at locus l, p_i and p_j are the frequencies at locus l in the population.

The drawback of this method, however, is that if one allele in an individual is absent from the possible population sample, the corresponding allele frequency is equal to zero, leading to zero likelihood in this population. Consequently, the population is excluded from being the possible origin of the individual. In some cases, the absence of this particular allele in the sample may be due to very low frequency of the allele in the population (Cornuet *et al*., 1999). Moreover, the direct method assigns the individual to the population of origin without providing the confidence levels.

The more accurate assignment approach based on simulation-exclusion method was developed (Cornuet *et al*., 1999; Piry *et al*., 2004) to provide a measure of confidence that the individual truly belongs to a given population. In this method, the probability of belonging of individuals is calculated using a Bayesian approach (Rannala and Mountain, 1997) for estimating population allele frequencies. The Bayesian approach was derived to provide the solution to the problem of null

frequencies in the sample using the direct assignment method. The simulation-exclusion approach employs Monte Carlo resampling procedure to compute the expected genotypic likelihoods of up to 10,000 simulated individuals from potential source populations. These genotype probabilities are used to generate a frequency distribution of likelihood values. The likelihood of a particular individual belonging to a particular source population is compared with the distribution of likelihoods of simulated genotypes. If the value is below a threshold level of confidence, e.g., 5% or 1%, the individual is excluded from that sample. As a result, if an individual is excluded from a sample it provides a strong indication that it does not belong to this population.

Parentage determination

Parentage identification is a method for determining the parents of an individual or group of individuals using genetic information combined with statistical methods (Manel *et al*., 2005). Two different methods are widely used to determine parentage, exclusion and likelihood-based approaches.

1. Parentage exclusion

The classical assignment method is based on the exclusion probability where all but one pair of the candidate parent can be excluded based on the multi-locus genotype of a particular offspring. Parentage analysis is generally based on Mendelian rules of inheritance and uses parent-offspring genotype combination to exclude an individual as a parent (Hedrick, 2005). For example, given that a mother and offspring have the genotypes A/A and A/B, respectively, at a single locus, the father could be either A/B or B/C, and the paternal genotype A/C can be excluded. However, when the group of candidate parents becomes large, the exclusion-based method is not possible to resolve parentage. As a result, multiple pairs of parent are not excluded (Hedrick, 2005).

2. Likelihood-based assignment

The likelihood-based assignment method (Meagher and Thompson, 1986) is more efficient for determining the most likely parent from the group of non-excluded parents (Marshall *et al*., 1998; Jones and Ardren, 2003). The likelihood approach determines the likelihood of alternative hypothesis given the observed data. The likelihood *L* of a hypothesis *H* given the data *D* is written as *L*(*H*|*D*). The likelihood of one hypothesis, for example, hypothesis 1 (H_1), relative to hypothesis 2 (H_2), is called the likelihood ratio:

$$L(H_1, H_2 \mid D) = \frac{P(D \mid H_1)}{P(D \mid H_2)},$$

where $P(D|H_i)$ is the probability of obtaining data D given hypothesis *i* (H_i). For parentage analysis, the data D are the genotypes of the offspring, mother, and alleged father at a particular locus. The hypothesis H_1 is that the alleged father is the true father and this is compared with H_2 that the alleged father is an unrelated male selected at random from the population (Marshall *et al*., 1998). When multiple loci are used, the likelihood ratios, the natural logarithm of the combined likelihood ratios can be taken to obtain the likelihood of odds (LOD) score (Hedrick, 2005). Offspring are assigned to the parent (or parental pair) with the highest LOD score. A LOD score of zero indicates that the alleged father is equally as likely to be the father of the offspring as a random male from the population. A positive LOD score indicates that the alleged male is more likely to be the father than a random male. Negative LOD scores may occur if the alleged father and offspring share a particular common set of alleles (Marshall *et al*., 1998).

The likelihood approach is used in parentage analysis under the following situations (Jones and Ardren, 2003).

(a) Identifying one parent when the other is known.

Let *C* represent the known parent and *D* the alleged parent, the LOD score for *D* being the parent of *B* is:

$$\text{LOD score } (D \text{ parent of } B) = \log_e \frac{T(g_B \mid g_C, g_D)}{T(g_B \mid g_C)},$$

where $T(g_B \mid g_C, g_D)$ is the Mendelian segregation or transition probability of g_B given g_C and g_D, and $T(g_B \mid g_C)$ is the transition probability of g_B given g_C.

(b) Identifying one parent with no information about the other parent.

In this case, no information is available concerning parentage of *B*. The single parent LOD score for *C* being the parent of *B* is:

$$\text{LOD score } (C \text{ parent of } B) = \log_e \frac{T(g_B \mid g_C)}{P(g_B)},$$

where $P(g_B)$ is the frequency of the offspring's genotype in the population.

(c) Identifying a parental pair starting with no prior information.

Parental pair allocation is an approach for identifying parent–offspring relationships by constructing genotypic triplets consisting of a proposed offspring and proposed maternal and paternal parents. This procedure involves calculating a breeding likelihood, which is defined as the likelihood of a parental pair producing the multilocus genotype found in the offspring being examined. The breeding likelihood of a given offspring on the basis of a single locus is:

$$\text{LOD score } (C, D \text{ parents of } B) = \log_e \frac{T(g_B \mid g_C, g_D)}{P(g_B)}.$$

The extension of the likelihood-based approach called full-pedigree likelihood methods was developed by Wang and Santure (2009). The methods are more efficient than the original likelihood approach which is based on relationships between pairs of individuals. The full-pedigree likelihood methods simultaneously identify parentage and infer sib-ship among individuals with likelihood considered over the entire pedigree configuration of a population (Fig. 3). Offspring O1 and O3 do are linked through offspring O2, although they do not share a parent.

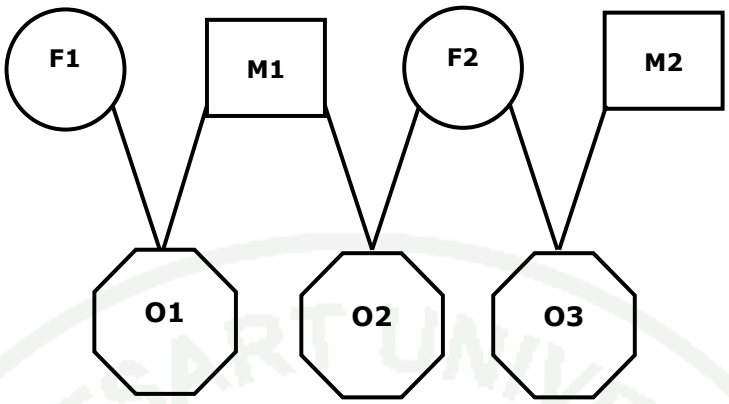


Figure 3 A full-pedigree likelihood analysis, showing relationships among individuals. F, female parent; M, male parent; O, offspring.

Source: Jones and Wang (2010)

The methods can determine accurate parentage and relationships among individuals in populations with more complex mating systems, i.e., populations comprise full-sib, paternal half-sib, and maternal half-sib. Knowledge of multiple relationships among three or more individuals increases statistical power of assignment tests (Wang and Santure, 2009; Jones and Wang, 2010). The drawback of the pairwise likelihood method is that it is difficult to assemble relationships between two individuals into a relationship structure when three or more individuals are considered. For instance, in pair-wise sibship analysis of three individuals, A, B and C, the pairs A–B and A–C might be inferred as full-sibs, while the pair B–C might be inferred as half- or non-sib. Similarly, in a parentage analysis it is possible that A–C and B–C could be inferred as father–offspring and mother–offspring respectively, but, when considered jointly, this relationship structure might be rejected (Wang and Santure, 2009).

Pedigree and parentage determination in breeding populations

Pedigree information permits the design of breeding program to maximize genetic responses, while minimizing the effects of inbreeding which can reduce fitness and production in the cultured populations. Microsatellite data have been used to

establish pedigree from a particular experimental design and to infer parentage in selective breeding programs of fish. For example, Herbinger *et al.* (1995) established pedigree of mixed families from complete factorial crosses between ten males and ten females of rainbow trout using four to five microsatellite loci. In Atlantic salmon, Norris *et al.* (2000) demonstrated that in the absence of pedigree information, a set of eight microsatellite markers was efficient for determining parentage and relatedness in mixed families. Further, simulations suggested that this set of microsatellites was suitable for discriminating between related and unrelated fish. In a selective breeding program of common carp, Vandeputte *et al*. (2004) estimated heritabilities for growth traits in juvenile common carp (8-weeks of age). Eight microsatellites were used to assign parentage in a full factorial cross of 10 females x 24 males and 550 offspring.

The applications of microsatellite markers for parentage inference have been reported in a number of selective breeding programs for commercial crustacean species. Jerry *et al*. (2004) examined the potential of microsatellite loci for parentage assignment for *P. japonicus* using genotype data from computer simulations and controlled breeding. Results showed that at least five loci were needed to assign progeny to their correct maternal parent. However, assignment success of progeny was only 47% compared with 92% from the simulations. Null alleles and allele dropout were indicated as possible causes for low assignment success rate. In their related study, Jerry *et al*. (2006) used eight microsatellite loci to determine relative growth rates of Kuruma shrimp from 22 families communally reared in commercial ponds. After six months of rearing, parentage analysis indicated male G x E interactions in some families. To identify parentage of captive black tiger shrimp *P. monodon*, Jerry *et al.* (2006) demonstrated that seven microsatellite loci was required for inferring parentage of 13 families. Further, simulations indicated that this set of seven loci was highly informative for parentage assignment up to 20 families. In cultured populations of Chinese shrimp (*Fenneropenaeus chinensis*), Dong *et al*. (2006) reported that five microsatellite loci were required for assigning 97% of 215 progeny to parent pairs in mixed family groups. The authors suggested that discrepancies between simulations and real data set were due to scoring errors at microsatellite loci.

Assessing reproductive capacity of broodstock, in particular, those of group spawning become possible with the use of microsatellite markers. The contribution of male parents in group breeding of tropical abalone (*Haliotis asinina*) was assessed using microsatellites in crosses with different combinations of males and females (Selvamani *et al*., 2001). The number of males used in each cross ranged from 2 to 4 with one or two females. They reported that five loci were appropriate for parental assignment. Analysis of microsatellite data from all crosses indicated different reproductive success of male parents, with most of the offspring were produced from a single male. In this case, induced spawning of multiple abalones resulted in reduced genetic diversity in the offspring generation. The authors suggested a more controlled breeding practice would be required to maintain genetic diversity of cultured stocks. In other study, Lucas *et al*. (2006) used five microsatellite loci for parentage assignment in 500 offspring from 84 families of a full factorial design of 14 males x 6 females, of which, 465 were successfully assigned to their parents. The pedigree information was incorporated in the linear model to estimate heritability for growth related traits in this abalone species.

Genetic characterization and evaluation of aquaculture stocks

Genetic diversity which refers to the variety of genes is the fundamental component of a population. Genetic diversity can be defined by the amount and distribution of genetic variation within and among populations as determined by the effects of mutation, natural selection, genetic drift and gene flow. Genetic variation within populations determines the ability of a species to survive in changing environment. Loss of genetic diversity reduces the potential for that species to adapt to new conditions in the long term, and results in inbreeding depression in the short term, particularly, for small populations both in the wild and in captivity (DeWoody, J. and Avise, 2000). The amount of genetic variation within populations can be measured by the parameters such as allelic diversity or allelic richness, effective number of alleles and the observed and expected heterozygosities. Information on genetic diversity that can be used to measure levels of inbreeding, mixing of populations and population differentiation within and among populations.

Assessing the genetic diversity of wild and culture stocks has become an important part in selective breeding of both livestock and aquaculture species despite the controversy about the relationship between variation at molecular measures and variation at quantitative traits. Reed and Frankham (2001) conducted a meta-analysis of 71 datasets of molecular data and quantitative traits from plant and animal species. They found weak correlation between variation at isozymes and quantitative genetic variation, as well as non-significant relationships between the two measures for heritability of fitness traits. Conversely, a meta-analysis of empirical data from 20 species by Merilä and Crnokrak (2001) suggested a strong correlation between quantitative trait variation and microsatellite marker diversity.

In the previous study, microsatellites revealed the relatively high levels of genetic diversity in terms of the average allele per locus and the observed heterozygosities among seven populations of wild and hatchery origins of freshwater prawn despite their low level of farm productions (Chareontawee *et al*., 2007).

Strain selection is a method to compare performance, in particular, growth rate and survival, of a variety of strains under common culture conditions to identify the best strain. Generally, strain selection, should be practiced prior to initiating selective breeding programs and broodstock domestication. In addition, the interaction variance component between the genotype and the environment (G x E) can be exploited by growing the best strain following performance tests of various strains. Strain evaluations have been practiced for a number of fish species, including, channel catfish (Dunham *et al*., 1986), rainbow trout (Ayles *et al*., 1983; Thompson, 1985), Atlantic salmon (Skaala *et al*., 2004; Rengmark *et al*., 2006), common carp (Vandeputte *et al*., 2008), and tilapia (Danting *et al*., 1995; Romana-Eguia *et al*., 2004; Eknath *et al*. 2007), but there have been no reports of strain selection of freshwater prawn and marine shrimp.

Evaluation of strains should be conducted under the identical environmental conditions to eliminate confounding effects of genetic and environments. The accuracy of strain evaluation depends on the variation of environmental factors,

particularly on tank or pond conditions, which can have large effects on the expression of growth traits (Herbinger *et al*., 1999). Therefore, it is important to minimize the influence of confounding environmental effects by stocking animals in the same tanks or ponds. Wohlfarth and Moav (1985) introduced communal rearing as a technique to provide a common environment for strain evaluation of common carp. In classical communal rearing, the animals will be physically marked using external or internal tags to identify their strain or family of origin. Communal rearing can reduce the cost of replicate ponds and was found to enhance expression of phenotypic differences among strains of common carp and other species such as channel catfish, and tilapia (Tave, 1993), and in European lobster (*Homarus gammarus*) (Jorstad *et al*., 2005).

MATERIALS AND METHODS

PART I

1. Stock evaluation and strain identification by microsatellite profiling

1.1. Sample collection

Prawn broodstock samples were obtained from three private hatcheries, including the CPP strain (N=95) from Petchaburi province, the KSB strain (N=59) from Supunburi province, and the SKL strain (N=66) from a local hatchery in Nakorn Pathom province (Fig 4). The CPP strain was a commercial strain derived from broodstock of Indian origin, while the KSB strain was developed from the local strain of the ChaoPhrya River origin. The SKL strain was developed from broodstock collected from Songkla Lake, Songkhla province. These samples were used to determine the levels of genetic variation within stocks and to assess genetic differentiation among stocks.

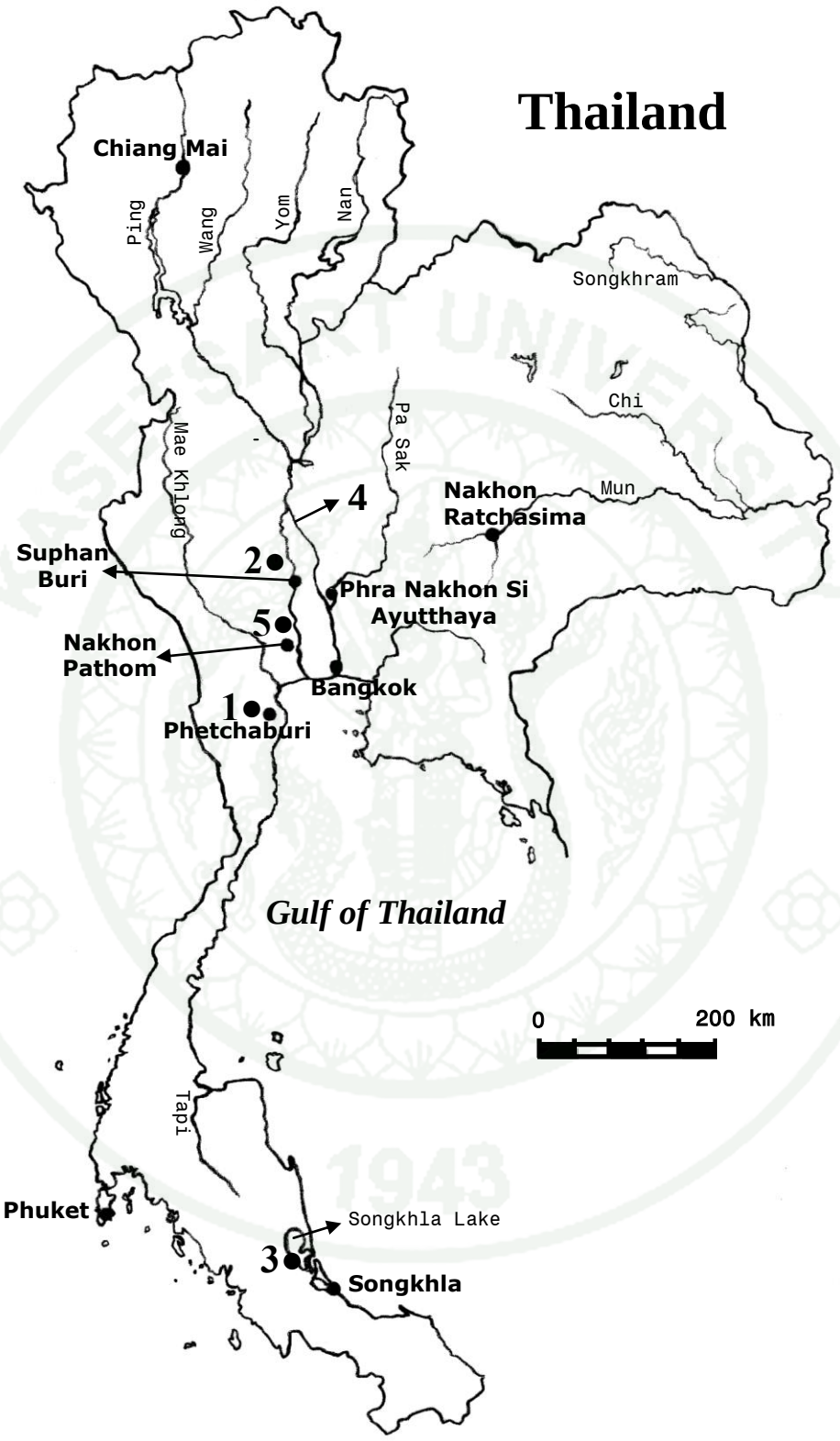


Figure 4 A map showing the three hatchery locations: 1, CPP; 2, KSB; 3, SKL, the ChaoPhrya River (4) and Khampaengsean Fisheries Research station (5).

1.2. Nursing of prawn larvae

Post-larval prawn (PL30) of similar age and size of three prawn strains were obtained from private hatcheries, including the CPP strain from Petchaburi province, the KSB strain from Supunburi province, and the SKL strain from a local hatchery in Nakorn Pathom province. The average body mass was 0.0087, 0.0077, and 0.0076 g for the CPP, KSB, and SKL strains, respectively. Prior to stocking in experimental tanks, post-larvae were nursed separately for 30 days in 3,000-l fiber glass tanks. Thereafter, 100 post-larvae from each strain were measured to determine average total length, carapace length and body weight. During the experiment, post-larvae were fed fresh *Artemia* nauplii and an artificial diet twice a day.

1.3. Experimental design

After 30 days of nursing, post-larvae of each stock were randomly separated into two groups for communal and separate rearing experiments and stocked at a density of 100/m^2 in twelve concrete tanks (3 x 2 x 0.6 m). A plastic mosquito mesh was fixed vertically at the middle of each tank to increase holding capacity. For communal rearing, 200 post-larvae from each strain were stocked together in each of three concrete tanks. For separate rearing, 600 post-larvae from each strain were stocked in three single-strain tanks. Fifty percent of the water in the tanks was changed daily. Post-larvae and juveniles in the single-strain tanks were sampled every 4 weeks to determine growth and survival rates. In the communal-rearing tanks, growth was measured at the completion of the study (after 120 days). All remaining juveniles were collected and stored in individual tubes containing 99% ethanol for DNA extraction and microsatellite genotyping. Survival rates of each strain were calculated after strain identification. Both communal and separate stocking experiments were conducted in the same greenhouse at Kamphaengsaen Fishery Research Station, Kasetsart University, Nakorn Pathom Province (Fig 4).

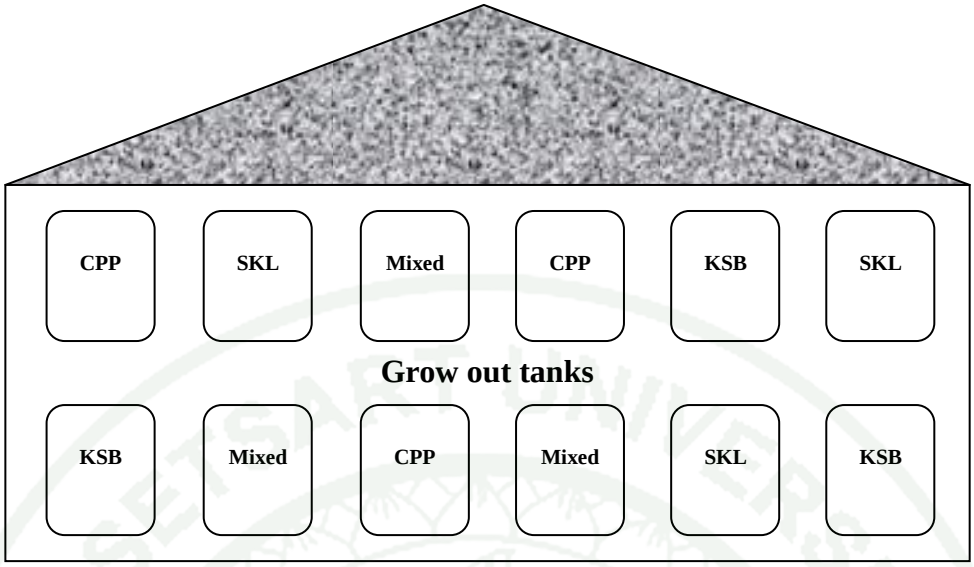


Figure 5 The diagram showing tank arrangement for separate (CPP, KSB, and SKL) and communal (mixed) rearing experiments.

1.4. Statistical analysis

A two-factorial design was used to evaluate the results for the communal stocking. The statistical model is as follows:

$$Y_{ijk} = \mu + T_i + S_j + TS_{ij} + e_{ijk} \quad (1),$$

where Y_{ijk}, μ, T_i, S_j, and e_{ijk} are the record for the k^{th} progeny, a common mean, the effect of the i^{th} experimental tank, the effect of the j^{th} strain, the effect of the ij^{th} tank-strain interaction and the uncontrolled environmental and genetic deviation, respectively.

A nested design was used to evaluate the results for separate stocking, with strain treated as fixed effect and tank nested within strain as random. The statistical model is as follows:

$$Y_{ijk} = \mu + S_i + T_{(i)j} + e_{ijk} \quad (2),$$

where Y_{ij}, μ, S_i, $T_{(i)j}$ and e_{ij} are the record of the j^{th} progeny, a common mean, the effect of the i^{th} strain, the effect of the j^{th} tank which nested in the i^{th} strain and the uncontrolled environmental and genetic deviation, respectively.

The mean and standard error were estimated for all parameters (survival rate, body weight, body length and carapace length) for each strain and subjected to one-way analysis of variance using the software package SAS (SAS Institute, 2003) to determine significant differences ($P < 0.05$) among strain means. Multiple comparison by Duncan's new multiple range test ($\alpha = 0.05$) was used to compare means of survival and growth traits between pairs of strains.

1.5. Microsatellite DNA genotyping

Swimmeret tissues of prawn broodstock and the surviving 4-month old juvenile prawns in the communal rearing experiment were used for DNA extraction using a standard phenol-chloroform extraction procedure (Taggart *et al*., 1992). Samples of prawn broodstock were genotyped at seven microsatellite loci (*Mbr-1*, *Mbr-2*, *Mbr-3*, *Mbr-5*, *Mbr-7*, *Mbr-8*, and *Mbr-10* with GenBank accession numbers DQ019863, DQ019864, DQ019865, DQ019867, DQ019869, DQ019870 and DQ019872, respectively) using the PCR conditions described by Chareontawee *et al.* (2006) (Table 1). PCR reactions were performed in 15-µl reactions which contained 10 ng template DNA, 0.3 µM forward and reverse primers, 0.2 mM each dNTP, 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, and 1 unit of *Taq* DNA polymerase (Promega). The PCR profile was initial denaturation at 94˚C for 3 min; 35 cycles of: 94˚C for 30 sec, annealing temperature for 45 sec, and 72˚C for 1 min; and 1 cycle of 72˚C for 7 min. Following amplification, PCR products were mixed with 2.5 µl of sequencing dye and heated for 5 min at 95˚C. The reaction mixtures were subjected to electrophoresis on a 5.5% denaturing polyacrylamide gel at 80 W for 3 hr. The gel was denatured at 100˚C for 30 min before electrophoresis. Bands in the gel were visualized by silver staining. Allele sizes were estimated by comparison to an M13 sequence ladder.

Table 1 Locus name, accession number, primers sequences and annealing temperature of microsatellites for freshwater prawn.

Locus	GenBank Accession Number	Primer sequences (5'-3')	T_a (°C)	Repeat sequence	Size Range (bp)
Mbr-1	DQ019863	F: CCC ACC ATC AAT TCT CAC TTA CC R: TCC TTT TCA CAT CGT TTC CAG TC	60	$(GA)_{24}$	272-320
Mbr-2	DQ019864	F: TTC CCG ACC AAT TTC TCT TTC TC R: GGC AAA AAT GAT CTT GGA TTC AC	60	$(GT)_{22}$	298-336
Mbr-3	DQ019865	F: CAA CTC TAT GTT TCG GCA TTT GG R: GGG GAA TTT TAC CGA TGT TTC TG	62	$(AG)_{14}$	232-284
Mbr-4	DQ019866	F: CCA CCT ACC GTA CAT TCC CAA AC R: CGG GGC GAC TTT TAG TAT CGA C	62	$(GT)_{29}$	205-310
Mbr-5	DQ019867	F: CAA GGC TCG TGT CTC TTG TTT C R: GCT TGT ACT TGT TCA GCT TTT GC	62	$(AG)_{25}$	286-328
Mbr-7	DQ019869	F: ATA AAA GAG TCG CCA AAT GAG CA R: ATT GGG AAT TGT TGA CCT CCA AG	60	$(TGC)_{16}$	274-286
Mbr-8	DQ019870	F: AAC CAG CCG ACT TAG ACT GTG C R: CGC CAT TTG CGT CTA TCT CTT AC	62	$(AGC)_6(AG)_5$ AA$(AG)_4$	256-266
Mbr-9	DQ019871	F: ATG ACG ATG ATG AGG AAT GAA GC R: TTT CAG GCT ATA TCA AGC AAC AG	60	$(TG)_5(AG)_{17}$	235-258
Mbr-10	DQ019872	F: ATG ACG ATG ATG AGG AAT GAA GC R: TTT CAG GCT ATA TCA AGC AAC AG	60	$(ATG)_3A$ $(ATG)_4$	241-265
Mbr-11	DQ019873	F: TTG TTT GCT TGT TTA GTG TCA AGG R: CTC CAA AAC CGA AAA ATC CTC AC	60	$(AG)_{31}$	238-274

Source: Chareontawee *et al*. (2006)

1.6. Genetic data analysis

1.6.1. The Micro-checker version 2.2.3 (Oosterhout *et al*., 2004) was used to detect the presence of null alleles at all loci.

1.6.2. Genetic variation within each of three populations including mean number of alleles per locus (*A*), allelic richness, A_r observed *(*H_o) and expected (H_e) heterozygosities were calculated using GENEPOP version 3.1c (Raymond and Rousset, 1995). The significance of differences in average values of A_r, and H_e among populations were tested by independent *t*-test of Archie (1985) using FSTAT version 2.9.3 (Goudet, 2001).

1.6.3. Hardy-Weinberg equilibrium and linkage disequilibrium were tested by the exact *P* values for tests of Hardy-Weinberg expectations and calculated by a Markov chain randomization method (Guo and Thompson, 1992) using GENEPOP version 3.1c. A sequential Bonferroni correction method was used to adjust significance levels for multiple tests (Rice, 1989).

1.6.4. Population differentiations were examined by testing for heterogeneity of allele distributions between pairs of populations were conducted using GENEPOP version 3.1c. Fisher's combined method (a chi-square test) was used to calculate *P* values of all loci for overall test.

1.6.5. The TFPGA program (Miller, 1997) was used to calculate pair-wise F_{ST} values and *P* values between all pairs of strains. Prawn samples of Myanmar origin (MYN) from the previous study by Chareontawee *et al.* (2007) was used as a reference population.

1.6.6. Assignment tests of prawn individuals based on multi-locus genotypes at seven loci were carried out on a total of 220 broodstock individuals from the three strains, which were used as the baseline populations. Two contrasting assignment methods, a direct method and a simulation-exclusion method were used in

the analysis. Both assignment algorithms calculated a likelihood probability of an individual belonging to each of the possible source populations. The direct method originally developed by Paetkau *et al*. (1995) assigns the individual to the population of origin in which it has the highest likelihood of belonging compared to the likelihood that it is assigned to other populations, without providing confidence levels. The simulation-exclusion approach developed by Cornuet *et al*. (1999) determines the proportion of correct assignment at user-defined threshold levels of significance. In this approach, up to 10,000 genotypes are generated based on the allele frequencies of the populations tested to obtain the expected distribution of genotypes and the distribution of genotype likelihood values (Piry *et al*., 2004). The likelihood of a particular genotype belonging to a particular source population is compared with the distribution of likelihoods of simulated genotypes. If the value is below a certain threshold level, the individual is excluded from that sample.

In the present study, GENECLASS2 (Piry *et al*., 2004) was used to calculate the probability of belonging of individuals based on a Bayesian approach. Both direct and simulation-exclusion methods were performed among the three prawn strains and between pairs of strains. In addition, the assignment tests were carried out using different sets of microsatellite loci, ranging from one to seven loci in order to determine the number of loci needed to optimize between genotyping cost and power of the test. Genotype data of prawn broodstock were used as the reference populations for the assignment of individuals in the communal rearing tanks. For each individual, the most likely strain of origin was determined based on the likelihood value and the score of an individual. Genetic diversity within each of the assigned strains was calculated using GENEPOP version 3.1c. The same program was used to test for deviations from Hardy-Weinberg expectations.

PART II

2. Parentage identification

2.1. Production of families

The experiment was carried out at the Kamphaengsaen Fishery Research Station, Kasetsart University, Nakorn Pathom Province. Broodstock of a commercial strain were used to produce twenty-one full-sib and two half-sib families. After mating, berried females were removed and placed in individual 100 l spawning tanks. Nauplii were obtained within 48 h and were nursed for 25 days. These families were used to verify parentage assignment with known parents and offspring genotypes.

2.2. Microsatellite amplification

Swimmeret tissues of prawn broodstock and whole body of 10 larvae from each family were collected for DNA extraction using a standard phenol-chloroform extraction procedure (Taggart *et al*., 1992). Genotyping was performed at ten microsatellite loci (*Mbr-1, Mbr-2, Mbr-3, Mbr-4, Mbr-5, Mbr-7, Mbr-8, Mbr-9, Mbr-10* and *Mbr-11*) using the PCR conditions described by Chareontawee *et al.* (2006). PCR reactions were performed in 15-µl reactions which contained 10 ng template DNA, 0.3 µM forward and reverse primers, 0.2 mM each dNTP, 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, and 1 unit of *Taq* DNA polymerase (Promega). The PCR profile was initial denaturation at 94˚C for 3 min; 35 cycles of 94˚C for 30 sec, annealing temperature for 45 sec, and 72˚C for 1 min; and 1 cycle of 72˚C for 7 min. The reaction mixtures were subjected to electrophoresis on a 5.5% denaturing polyacrylamide gel at 80 W for 3 hr. The gel was denatured at 100˚C for 30 min before electrophoresis. Bands in the gel were visualized by silver staining. Allele sizes were estimated by comparison to an M13 sequence ladder.

2.3. Simulations and parentage analysis

Estimates of genetic variation, including observed/expected heterozygosity, PIC value, the frequency of null alleles, and the average non-exclusion probability of each locus were calculated based on the allele frequencies of 45 broodstock using the CERVUS 3.0 (Marshall *et al*., 1998; Kalinowski *et al*. 2007). An estimated null allele frequency > 0.05 was considered significant. The presence of null alleles also was tested using the program MICROCHECKER 2.2.0 (Oosterhout *et al*., 2004). GENEPOP 3.1c (Raymond and Rosset 1995) was used to test for departure from Hardy-Weinberg expectations and genetic linkage disequilibrium between pairs of loci.

Two computer programs were used for parentage analysis, CERVUS 3.0 and COLONY 2 (Jones and Wang, 2010). CERVUS assigns offspring to their parent pairs based on the pair-wise likelihood comparison approach. The program generates locus-by-locus likelihood scores for each candidate parent for each offspring and assigns parentage to a candidate parent with the highest LOD score. In contrast to CERVUS, COLONY infers sibship and parentage simultaneously by generating pedigree configurations of all individuals and comparing the likelihoods of potential pedigree configurations. Parentage and sibship structure are inferred according to the maximum-likelihood configuration.

The simulation program within CERVUS was used to generate genotypes of 10,000 offspring and candidate parents from the allele frequencies of 45 parents to get significant LOD scores at 95% confidence level (Marshall *et al*., 1998). The numbers of family tested in the simulations were from 10 to 1000, with the assumption that each family consisted of one male and one female. To minimize the cost of genotyping, simulations were performed to determine the number of informative loci that would be needed to obtain a given level of assignment success. Loci with the highest PIC scores were chosen and sequentially added to the set. To determine the effects of genotyping error or mutation on parentage inference, the error rate was set at 1% and 5%.

Parentage assignment of the real offspring was performed in 21 full-sib and two half-sib families. Genotype data of 230 offspring (ten from each family) were pooled and analyzed, with parent information known to the experimenter but unspecified in the program. The accuracy in parentage identification was determined by comparing the observed assignment success rates in CERVUS or the best pedigree configuration in COLONY with known pedigree information of the hatchery population of freshwater prawn.

PART III

3. Assessment of reproductive potential among male broodstocks

3.1. Broodstock collection and genotyping

Five month old prawn broodstock consisting of three male morphotypes and mature females were purchased from a farm in Nakorn Pathom province and were kept in 3 x 2 x 0.6 m. cement tanks at the Kamphaengsaen Fishery Research Station, Kasetsart University, for acclimation. DNA samples were extracted from 150 males and 87 females for genotyping at the microsatellite locus *Mbr*-5 (total number of alleles = 25). PCR amplification conditions were as described by Chareontawee *et al.* (2006). The PCR products were subjected to capillary electrophoresis on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). Peak Scanner software v.1.0 was used to visualize the alleles.

3.2. Measurement of body traits

Fifty prawn from each male types and fifty females were measured for body weight (g), body length (cm), carapace length (cm), relative claw length (cm) and condition factor. Relative claw length was calculated as the ratio of claw length to body length.

Condition factor (*K*) was calculated as: $K = \frac{BW}{BL^b}$,

where BW is the body weight (g), BL is the body length (cm), and *b*, the exponent in the formula is the slope of the regression of $\text{Log}_{10}BW$ on $\text{Log}_{10}BL$ (Bolger and Connolly, 1989).

Correlations between body weight and length of male morphotypes were estimated using log transformation values of body weight and body length.

3.3. Experimental design

Mating capacity of BC, OC, and SM males were examined by stocking different combinations of males and females as follows:

1) 1 BC male, 1 OC male, 1 SM male and 12 females (1M:4F)
2) 1 BC male, 1 OC male, 1 SM male and 3 females (1M:1F)
3) 1 BC male, 1 OC male, 1 SM male and 1 female (3M:1F)

For each combination, males of the three morphotypes having different genotypes at the *Mbr*-5 locus were stocked for mating competition with gravid females. Seven plastic tanks (500 1) were set up for each stocking combination. After mating, all berried females were removed and placed in individual 100 l spawning tanks. Nauplii were obtained within 19-21 days and were nursed for 30 days.

DNA was extracted from whole body of ten offspring from each female for genotyping at microsatellite locus *Mbr-5* to determine parentage and the number of female sired by each male type within a single tank by comparing with genotypes of all three male types and their mother.

3.4. Statistical analysis

Male reproductive success (*RS*) was calculated as the proportion of female sired by each male type as follows:

$$RS = \frac{x_i}{n} \qquad (1),$$

where x_i is the number of female sired by each male type and n is the total number of female that were mated in a single tank.

Analysis of variance was performed using a generalized linear model (PROC GLM) implemented in SAS computer software (SAS, 2003) to evaluate the effects of

sex ratio, male type, and sex ratio-male type interaction on the reproductive success of males. The statistical model is as follows:

$$RS_{ijk} = \mu + SR_i + M_j + SRM_{ij} + e_{ijk} \quad (2),$$

where RS_{ijk} = reproductive success of males

μ = mean value of male reproductive success

SR_i = the male to female sex ratio (i = 1, 2, 3)

M_j = type of males (j = 1, 2, 3)

SRM_{ijk} = sex ratio-male type interaction

e_{ijk} = the residual error term

Multiple comparisons by Duncan's new multiple range test ($\alpha = 0.05$) were used to compare mean of reproductive success between pairs of male morphotypes.

Because morphometric traits of males, including bodyweight, length, condition factor, carapace length and relative claw length might be associated with male's reproductive success, a covariate was included in the model as follows:

$$RS_{ijk} = \mu + SR_i + M_j + SRM_{ij} + \beta\left(X_{ijk} - \overline{X}\right) + e_{ijk} \quad (3),$$

where RS_{ijk} = reproductive success of males

μ = mean value of male reproductive success

SR_i = the male to female sex ratio (i = 1, 2, 3)

M_j = type of males (j = 1, 2, 3)

SRM_{ij} = sex ratio-male type interaction

X_{ijk} = additional covariate

$\overline{X}$ = mean value of the covariate

β = regression coefficient of each additional covariate on RS_{ijk}

e_{ijk} = the residual error term

The effects of additional covariates on reproductive success of males were evaluated for one covariate at a time using analysis of covariance (ANCOVA) implemented in SAS computer software (SAS, 2003).

RESULTS

PART I

1. Stock evaluation and strain identification using microsatellite markers

1.1. Genetic variation among and within stocks

A total of 220 prawn broodstock (CPP = 95, KSB = 59, and SKL = 66) were genotyped at seven microsatellite loci. All three prawn stocks exhibited relatively high genetic variation, with average numbers of alleles per locus of 8.4, 12.6 and 14.7, and observed heterozygosities of 0.75, 0.73, and 0.79 for the CPP, KSB, and SKL strains, respectively (Table 2). Null alleles were present at loci *Mbr-1* and *Mbr-3* in the CPP. The CPP stock showed significant departures from Hardy-Weinberg at three loci after applying a sequential Bonferroni correction. The overall estimate of F_{ST} (0.076) indicated statistically significant levels of differentiation among stocks. Significant pairwise F_{ST} values were observed for all strain comparisons (P<0.05, Table 3). With the exception of the MYN stock (the out-group), the highest genetic differentiation was observed between the CPP and the SKL stocks (F_{ST} = 0.12). Genetic parameters were also calculated using genotype data at four loci (*Mbr-1*, *Mbr-2*, *Mbr-3*, and *Mbr-5*) which displayed higher levels of polymorphism (Table 4). The average numbers of alleles per locus were 11.7, 17.2 and 19.7 for the CPP, KSB, and SKL strains. Similarly, higher observed heterozygosities were observed for the three populations, with the values ranged from 0.83 to 0.91. Moreover, an overall F_{ST} value increased from 0.076 to 0.081 with the use of the four loci.

Table 2 Genetic variability at seven microsatellite loci in three prawn strains, including sample size (N), total number of alleles (A), allelic richness (A_r), observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index (F_{is}), and P value for test of Hardy-Weinberg expectations (HW). Bonferroni correction: P<0.0071 (0.05/7).

Popn (N)	Locus							Average across loci
	Mbr-1	*Mbr-2*	*Mbr-3*	*Mbr-5*	*Mbr-7*	*Mbr-8*	*Mbr-10*	
CPP (95)								
A	10	12	10	15	3	5	4	8.43 ± 4.5
A_r	9.40	9.96	9.12	11.74	2.99	5.00	3.99	7.46 ± 3.4
H_o	0.74	0.79	0.75	0.86	0.48	0.98	0.68	0.75 ± 0.16
H_e	0.81	0.78	0.81	0.81	0.45	0.74	0.69	0.71 ± 0.13
F_{is}	0.120	-0.008	0.104	-0.025	-0.079	-0.250	0.007	-0.017
HW	0.000	0.017	0.000	0.008	0.031	0.000	0.007	Highly sig.
KSB (59)								
A	18	12	19	20	6	5	8	12.57 ± 6.4
A_r	18.00	11.41	18.04	17.48	5.28	4.94	7.53	11.81 ± 6.0
H_o	0.85	0.74	0.81	0.88	0.42	0.60	0.81	0.73 ± 0.16
H_e	0.91	0.78	0.89	0.90	0.45	0.62	0.79	0.76 ± 0.17
F_{is}	0.091	0.091	0.112	0.022	0.139	0.107	0.010	0.077
HW	0.002	0.151	0.038	0.028	0.356	0.390	0.582	0.0003
SKL (66)								
A	16	17	26	20	6	7	11	14.71 ± 6.67
A_r	15.12	13.91	22.90	16.82	5.34	5.94	8.86	12.70 ± 6.30
H_o	0.98	0.75	0.96	0.95	0.70	0.50	0.75	0.79 ± 0.16
H_e	0.92	0.83	0.95	0.92	0.64	0.51	0.81	0.80 ± 0.15
F_{is}	-0.056	0.101	-0.006	-0.037	-0.094	0.038	0.065	0.001
HW	0.064	0.001	0.362	0.811	0.074	0.393	0.099	0.003

Table 3 Genetic distance (below diagonal) and pair-wise F_{ST} (above diagonal) at seven microsatellite loci with *P* values among three prawn populations. The data of MYN population from the previous study (Chareontawee *et al*., 2007) is used as a reference population. Asterisk indicates significant differentiation ($P<0.05$).

Population	CPP	KSB	SKL	MYN
CPP	-	0.093*	0.116*	0.154*
KSB	0.065	-	0.027*	0.155*
SKL	0.073	0.024	-	0.129*
MYN	0.122	0.125	0.110	-

Table 4 Genetic variability at four microsatellite loci in three prawn strains, including sample size (*N*), total number of alleles (*A*), allelic richness (A_r), observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index (F_{is}), and *P* values for test of Hardy-Weinberg expectations (HW). Bonferroni correction: $P<0.0125$ (0.05/4).

Popn (*N*)	Locus				Average across loci
	Mbr-1	*Mbr-2*	*Mbr-3*	*Mbr-5*	
CPP (95)					
A	10	12	10	15	11.75 ± 2.36
A_r	9.28	9.67	9.09	11.31	9.84 ± 1.01
H_o	0.75	0.79	0.78	0.86	0.79 ± 0.05
H_e	0.84	0.78	0.84	0.84	0.83 ± 0.03
F_{is}	0.107	-0.008	0.075	-0.025	0.038
HW	0.000	0.077	0.000	0.007	Highly sig.
KSB (59)					
A	18	12	19	20	17.25 ± 3.59
A_r	18	11.03	17.87	16.65	15.89 ± 3.30
H_o	0.97	0.72	0.97	0.88	0.89 ± 0.12
H_e	0.92	0.82	0.92	0.90	0.89 ± 0.05
F_{is}	-0.047	0.116	-0.062	0.022	0.004
HW	0.009	0.068	0.303	0.008	0.0008
SKL (66)					
A	16	17	26	20	19.75 ± 4.50
A_r	15.22	14.10	23.16	16.99	17.37 ± 4.04
H_o	0.98	0.75	0.96	0.95	0.91 ± 0.11
H_e	0.92	0.83	0.96	0.92	0.91 ± 0.05
F_{is}	-0.057	0.101	-0.006	-0.037	-0.002
HW	0.043	0.009	0.265	0.735	0.0146

1.2. Assignment test results

Assignment test results are shown in Table 5. The Paetkau *et al*. (1995) direct method assigned 98 to 100% of the individual prawn to their correct strain of origin. Figure 6 shows the plots of the log-likelihoods between pairs of strains for direct assignment. For example, all 95 individuals of CPP had higher values of log-likelihood when they were assigned to the CPP in comparison with the log-likelihoods that they were assigned to the KSB. Similarly, between the CPP and SKL, 100% of the CPP individuals were assigned to their correct origin. While between the KSB and SKL, the direct method assigned 98.4% of the prawn to the KSB strain. The likelihood of one out of 59 KSB individuals being SKL was higher than that of being KSB. When the confidence levels were defined using the Cornuet *et al*. (1999) exclusion method, the overall proportions of correct individual assignment decreased from 90 to 88% at *P*-values <0.05, 0.01, and 0.001. The accuracy of individual assignment using a *P*-value of 0.05 was highest for the CPP strain (96.8%), followed by the SKL (91%) and KSB (76.3%) strains. Because PCR products were not amplified for some of the KSB samples, approximately 9.4% of the KSB genotypes were treated as missing data. The adjustment of allele frequencies due to missing data reduced the power of the assignment test for this strain.

Assignment tests between pairs of strains revealed that using the exclusion method at a *P*-value of 0.05, 100% correct assignment was obtained between CPP and KSB, followed by 98.7% between CPP and SKL, and 84.8% between KSB and SKL (Table 5). The assignment accuracy for each pair of strains varied slightly when using *P*-values of 0.01 and 0.001.

To optimize cost of genotyping and assignment power, simulations were performed for 127 sets of marker combinations. Assignment test results indicated that using a set of four loci (*Mbr-1*, *Mbr-2*, *Mbr-3*, and *Mbr-5*), the accuracy of the test was comparable to that using seven loci (Table 5). Therefore, this set of loci was used for genotyping prawn individuals in the communal rearing experiment. Of 1,366 surviving juvenile prawns, 428 were identified as the CPP strain, while 446 and 492 individuals

were assigned to KSB and SKL strains respectively. Each individual was assigned to the most likely strain of origin, with the highest values of likelihood and score for that strain. For example, an unknown individual was assigned to the CPP strain with the likelihood value of -4.9 and the score of 95.9% in comparison with the log-likelihoods of -6.3 and -7.5 and the scores of 3.8% and 0.3% when it was assigned to the KSB and SKL strains respectively.

Table 5 Strain assignment test results, showing the proportions of correct assignment of individuals using the Paetkau *et al.* (1995) direct (real assigned) method or the Cornuet *et al*. (1999) exclusion-simulation method with the probability of belonging to a population, based on seven (four) loci. The overall F_{ST} values are 0.07 for seven loci and 0.08 for four loci.

Strain (*N*)	Direct (real assigned)	Correctly assigned (simulated)		
		P<0.05	*P*<0.01	*P*<0.001
Assignment for each strain				
CPP (95)	100 (100)	96.8 (97.9)	95.8 (97.9)	95.8 (97.9)
KSB (59)	98.3 (98.3)	76.3 (83.1)	74.6 (81.4)	74.6 (81.4)
SKL (66)	98.5 (92.4)	90.9 (83.3)	90.9 (83.3)	89.4 (83.3)
Total	99.2 (97.3)	89.5 (89.6)	88.6 (89.1)	88.2 (89.1)
Assignment between two strains				
CPP-KSB	100 (100)	100 (98.7)	98.7 (98.7)	98.7 (98.7)
CPP-SKL	100 (100)	98.7 (100)	98.7 (100)	98.7 (100)
KSB-SKL	98.4 (95.2)	84.8 (83.2)	84.0 (82.4)	83.2 (81.6)

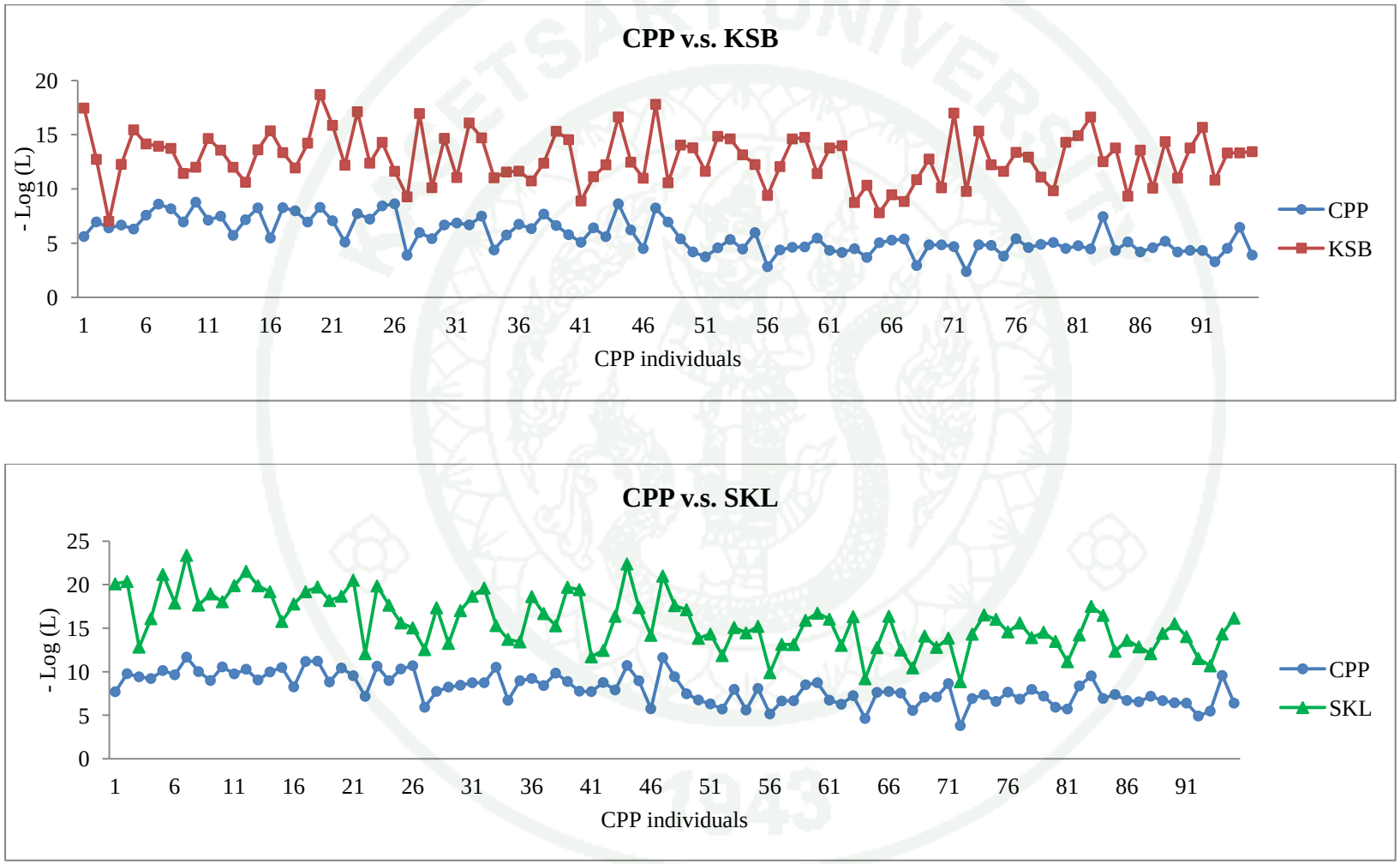
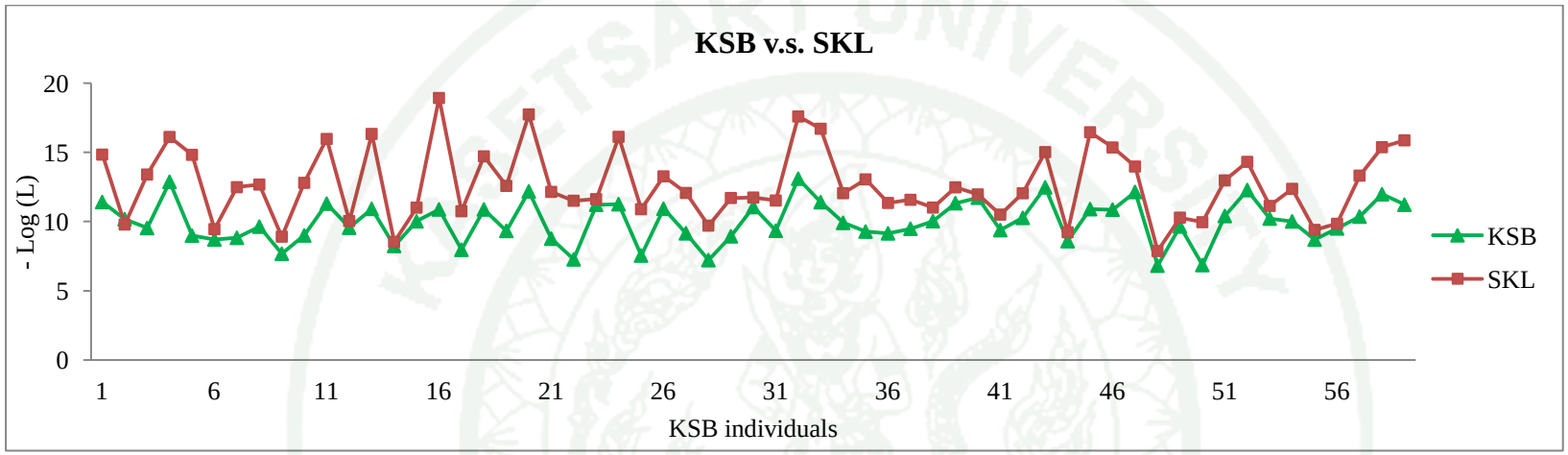


Figure 6 Individual log likelihood, -Log (L) for the direct assignment based on seven loci: between two strains.



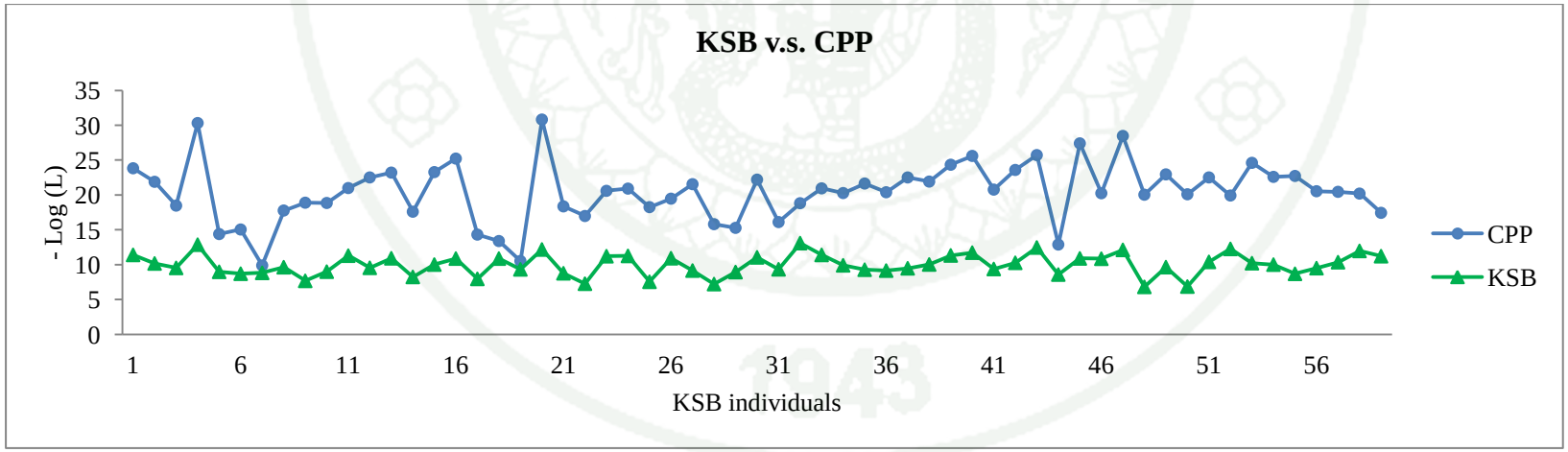


Figure 6 (Continued)

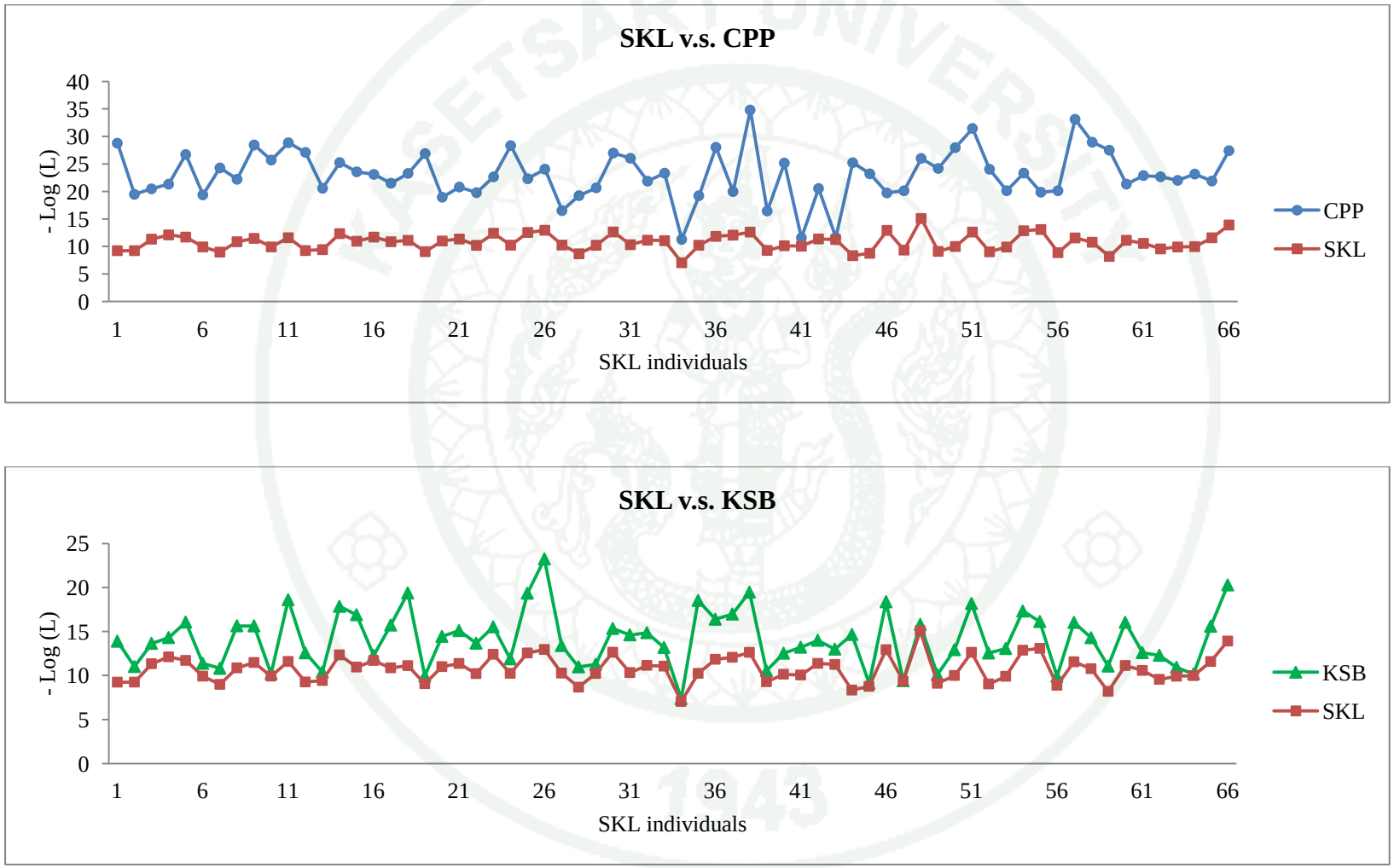


Figure 6 (Continued)

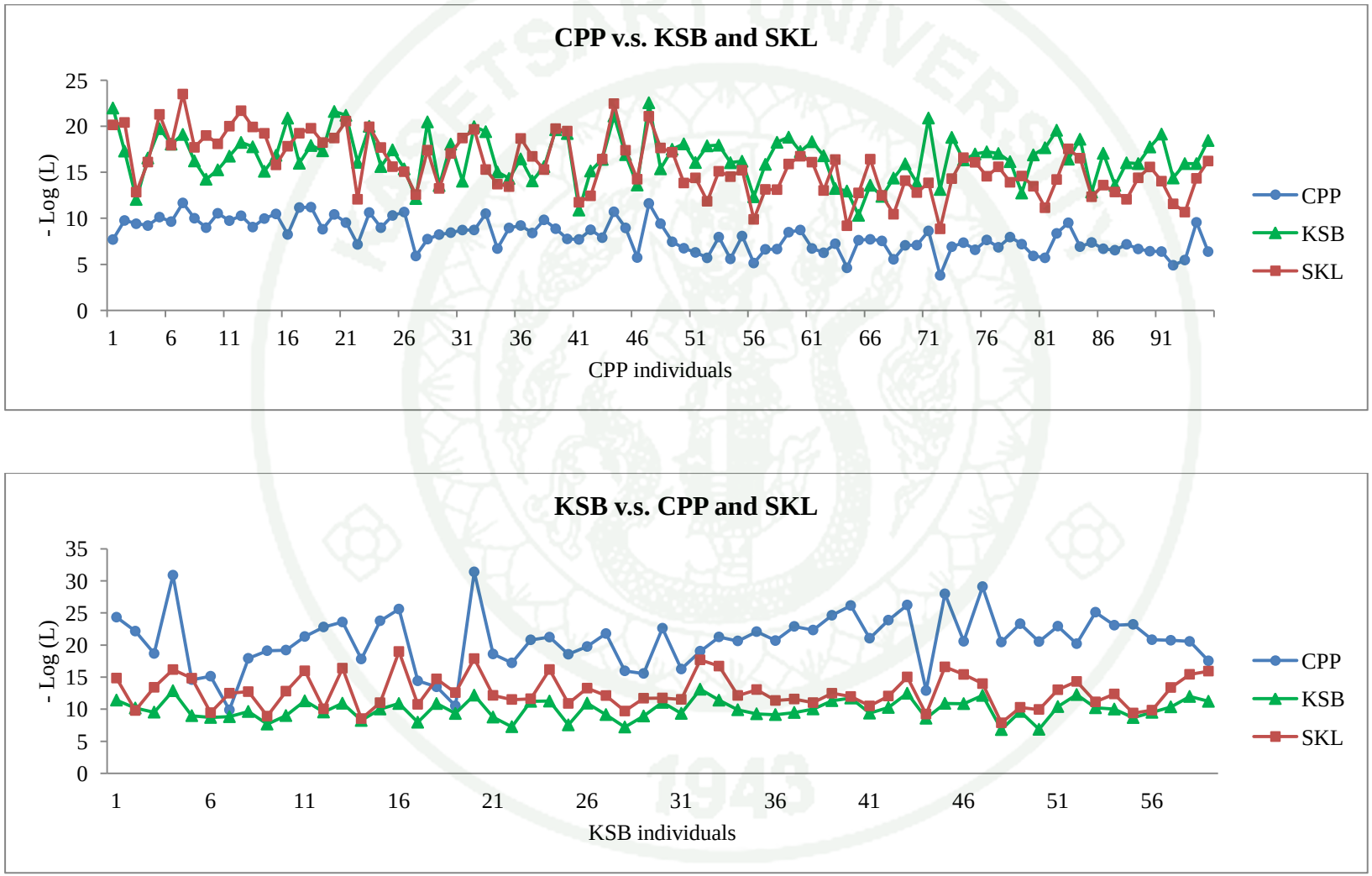


Figure 7 Individual log likelihood, -Log (L) for the direct assignment based on seven loci: between three strains.

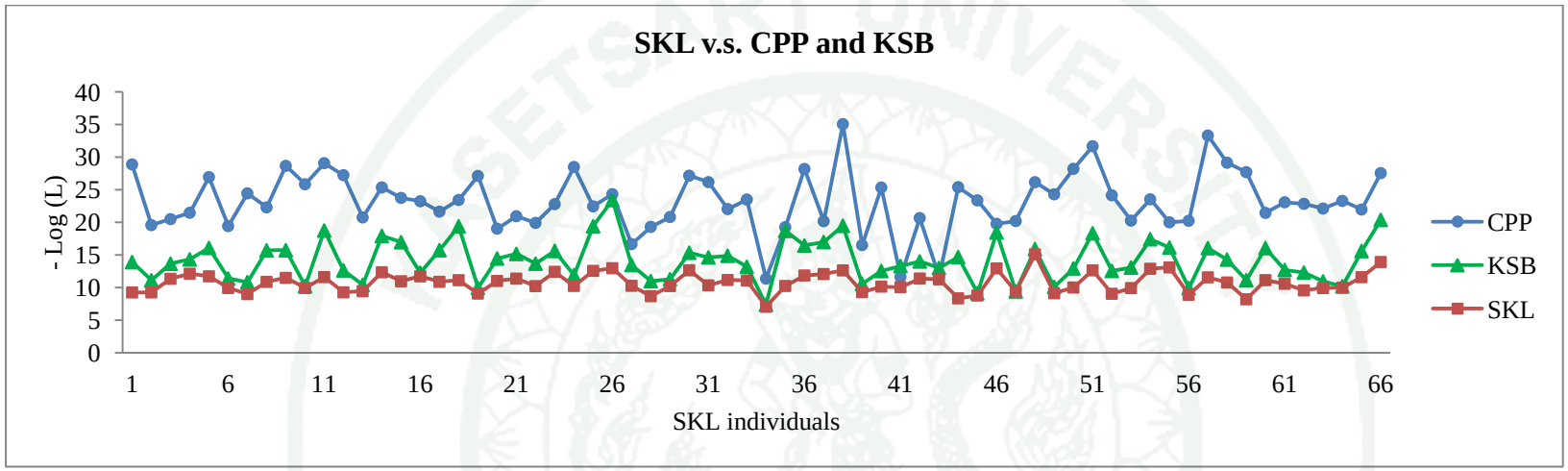


Figure 7 (Continued)

Genetic variation at four loci of the three assigned strains from the communal rearing experiment is presented in Table 6. All three prawn stocks exhibited relatively high genetic variation, with average numbers of alleles per locus of 11.25, 15.0 and 18.5, and observed heterozygosities of 0.90, 0.93, and 0.96 for the CPP, KSB, and SKL strains, respectively.

1.3. Growth and survival of juveniles

In separate testing, the average daily growth (ADG), body weight (BW), carapace length (CL) and total length (TL) of 4-month old juveniles differed significantly among strains ($P<0.05$), with the CPP strain displaying the highest values for all characters (Table 7, Fig. 8). For example, body weights of CPP, KSB, and SKL at 120 days were 6.75±2.93, 5.43±3.19, and 4.13±3.10 g, respectively. Tank effects were not significant in separate rearing. Survival rates were considered low to moderate for the three strains, at 71, 50.6, and 76%, respectively, for CPP, KSB, and SKL strains. However, testing for strain and tank effects on survival among strains was not possible in the nested design.

Table 6 Genetic variability at four microsatellite loci in three prawn strains from the communal rearing experiment, including sample size (*N*), total number of alleles (*A*), allelic richness (A_r), observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index (F_{is}), and *P* value for test of Hardy Weinberg expectations (HW). Bonferroni correction: $P<0.0125$ (0.05/4).

Popn (*N*)	Locus				Average across loci
	Mbr-1	*Mbr-2*	*Mbr-3*	*Mbr-5*	
CPP (428)					
A	10	10	10	15	11.25 ± 2.50
A_r	10.00	9.16	9.50	12.73	10.35 ± 1.63
H_o	0.80	0.98	0.99	0.80	0.90 ± 0.11
H_e	0.87	0.76	0.85	0.84	0.83 ± 0.05
F_{is}	0.077	-0.281	-0.176	0.047	-0.077
HW	0.000	0.000	0.000	0.007	Highly sig.
KSB (446)					
A	10	11	18	21	15.00 ± 5.35
A_r	10.00	10.62	16.76	16.49	13.47 ± 3.66
H_o	0.98	0.99	0.93	0.84	0.93 ± 0.07
H_e	0.88	0.84	0.92	0.89	0.88 ± 0.03
F_{is}	-0.12	-0.186	-0.012	0.048	-0.066
HW	0.000	0.000	0.000	0.000	Highly sig.
SKL (492)					
A	13	15	26	20	18.50 ± 5.80
A_r	12.88	13.88	22.33	18.51	16.90 ± 4.37
H_o	0.98	0.99	0.98	0.87	0.96 ± 0.06
H_e	0.88	0.88	0.94	0.93	0.91 ± 0.03
F_{is}	-0.12	-0.128	-0.048	0.068	-0.055
HW	0.000	0.000	0.000	0.000	Highly sig.

Table 7 Mean body weight (BW), mean total length (TL), and mean carapace length (CL) of three prawn strains after 120 days of separate rearing with ANOVA results showing *F*-values for differences among strains and tanks. Asterisk indicates significant difference ($P<0.05$).

Strain	Mean BW ± SD (g)	Mean TL ± SD (mm)	Mean CL ± SD (mm)
CPP	6.75 ± 2.93[a]	85.01 ± 11.05[a]	42.12 ± 5.76[a]
KSB	5.43 ± 3.91[b]	80.23 ± 14.11[b]	39.19 ± 7.73[b]
SKL	4.13 ± 3.10[c]	72.91 ± 14.44[c]	35.07 ± 8.06[c]
ANOVA			
F-value (strain)	26.41*	64.03*	63.07*
P-value	0.0011	<0.0001	<0.0001
F-value (tank)	2.08	0.99	1.13
P-value	0.05	0.43	0.34

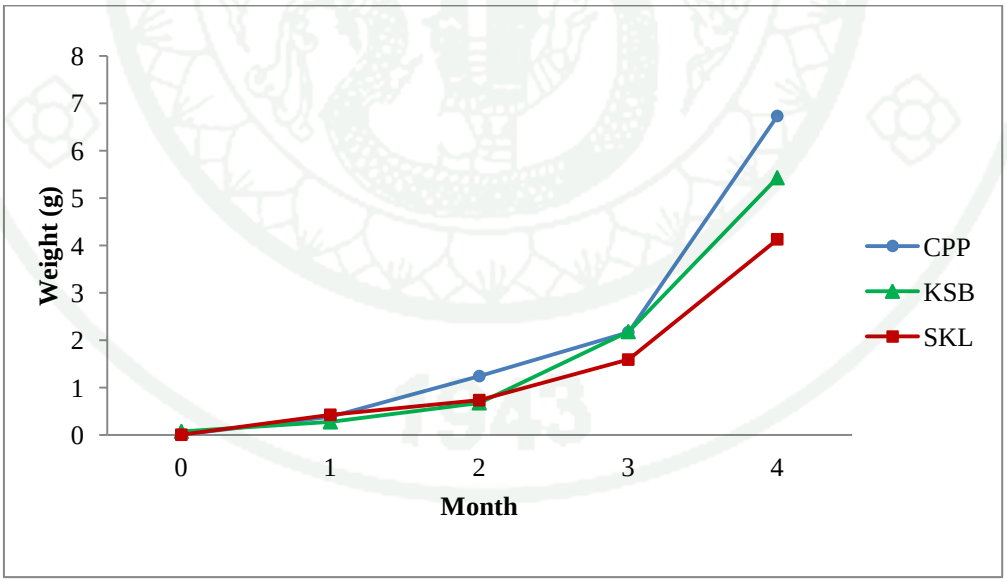


Figure 8 Average daily weight gain (g) among three prawn strains reared in separate tanks for 120 days.

In communal testing, a total of 1,366 surviving juvenile prawns, including 428 CPP, 446 KSB and 492 SKL individuals were measured. Similar to the results of the

separate rearing experiment, the CPP stock exhibited the highest values for growth parameters (Table 8). For example, mean body weights of CPP, KSB, and SKL individuals at 120 days were 7.05±4.95, 5.10±3.58, and 4.85±6.79 g, respectively. Although differences in growth were observed within strains of juvenile prawns between the two tests, the ranking of strains was the same for separate and communal rearing. Tank effects were significant in total length and carapace length tests but significant difference was not observed in body weight test. Strain-tank interactions were not significant in communal stocking. Survival rates of the CPP strain (71.3%) in the communal tanks did not differ from that in separate tanks, but survival rates of the KSB (74.3%), and SKL (82%) strains in communal tanks were higher than those in separate rearing.

Table 8 Mean body weight (BW), mean total length (TL) and mean carapace length (CL) of juveniles of three prawn strains after 120 days of communal rearing with ANOVA results showing *F*-values among tanks, strains and tank-strain interaction. Asterisk indicates significant difference ($P<0.05$).

Strain	Mean BW ± SD (g)	Mean TL ± SD (mm)	Mean CL ± SD (mm)
CPP	7.05 ± 4.95[a]	83.99 ± 18.58[a]	40.60 ± 9.73[a]
KSB	5.10 ± 3.58[b]	78.16 ± 26.28[b]	37.64 ± 8.79[b]
SKL	4.85 ± 6.79[b]	75.04 ± 32.70[b]	35.81 ± 9.03[b]
ANOVA			
F-value (tank)	2.14	3.61*	4.92*
P-value	0.12	0.03	0.01
F-value (strain)	18.87*	12.72*	30.76*
P-value	<0.0001	<0.0001	<0.0001
F-value (tank-strain)	0.75	0.38	0.45
P-value	0.56	0.82	0.77

PART II

2. Parentage identification of freshwater prawn broodstock

2.1. Properties of microsatellite markers

The parental stock exhibited high genetic variation relative to wild and hatchery populations of *M. rosenbergii* (Chareontawee *et al*., 2007), with the number of alleles per locus ranging from 5 to 22, expected heterozygosities ranging from 0.437 to 0.958, and PIC ranging from 0.384 to 0.937 (Table 9). The average non-exclusion probabilities over ten loci were close to zero for one candidate parent (0.0003), for one candidate parent given the genotype of known parent of the opposite sex, as well as for a candidate parent pair. The average non-exclusion probability is the probability of not excluding a single unrelated candidate parent or parent pair from parentage of a given offspring at one locus (Kalinowski *et al*., 2007). None of the loci displayed the presence of null alleles when analyzed by the program MICROCHECKER. Although CERVUS indicated the presence of low null allele frequencies at the ten loci, the values were not significant. Genotype frequencies at all loci conformed to Hardy-Weinberg expectations. Linkage disequilibrium was detected between *Mbr-9* and *Mbr-10* (P<0.000), therefore, locus *Mbr-10* was not included in parentage analysis.

Table 9 Characteristics of microsatellite loci used for parentage assessment of 45 freshwater prawn broodstock, including number of alleles, H_o: observed heterozygosity, H_e: expected heterozygosity, HWE: Hardy-Weinberg expectation (*P*-value), polymorphism information content (PIC), and frequency of null alleles F (Null).

Locus	No. alleles	H_o	H_e	HWE	PIC	F (Null)	NE-1P[a]	NE-2P[b]	NE-PP[c]
Mbr-1	21	0.893	0.958	0.251	0.937	+0.024	0.215	0.120	0.025
Mbr-2	13	0.644	0.739	0.299	0.695	+0.005	0.656	0.478	0.280
Mbr-3	22	0.917	0.941	0.056	0.917	-0.002	0.267	0.155	0.038
Mbr-4	20	0.822	0.916	0.052	0.899	+0.038	0.316	0.188	0.056
Mbr-5	16	0.889	0.907	0.447	0.889	+0.006	0.343	0.206	0.067
Mbr-7	5	0.400	0.437	0.635	0.384	+0.035	0.904	0.779	0.643
Mbr-8	5	0.778	0.731	0.600	0.687	-0.037	0.680	0.497	0.305
Mbr-9	6	0.733	0.801	0.061	0.760	+0.038	0.591	0.412	0.231
Mbr-10	6	0.711	0.799	0.052	0.758	+0.043	0.594	0.415	0.234
Mbr-11	14	0.964	0.893	0.065	0.866	-0.042	0.390	0.241	0.086
Average	12.80	0.775	0.812	0.0122	0.779		3.43×10^{-4}	5.50×10^{-6}	9.19×10^{-10}

[a] Average non-exclusion probability for one candidate parent.

[b] Average non-exclusion probability for one candidate parent given the genotype of a known parent of the opposite sex.

[c] Average non-exclusion probability for a candidate parent pair.

2.2. Simulations of parentage inference

The number of loci that would be required to obtain high rate of assignment were chosen based on the highest PIC scores and were sequentially added to the set. For example, loci *Mbr-1* (PIC = 0.937) and *Mbr-3* (PIC = 0.913) were used in the initial analysis, followed by *Mbr-4* (PIC = 0.899), *Mbr-5* (PIC = 0.889), *Mbr-11* (PIC = 0.866) and the remaining loci with lower PIC scores.

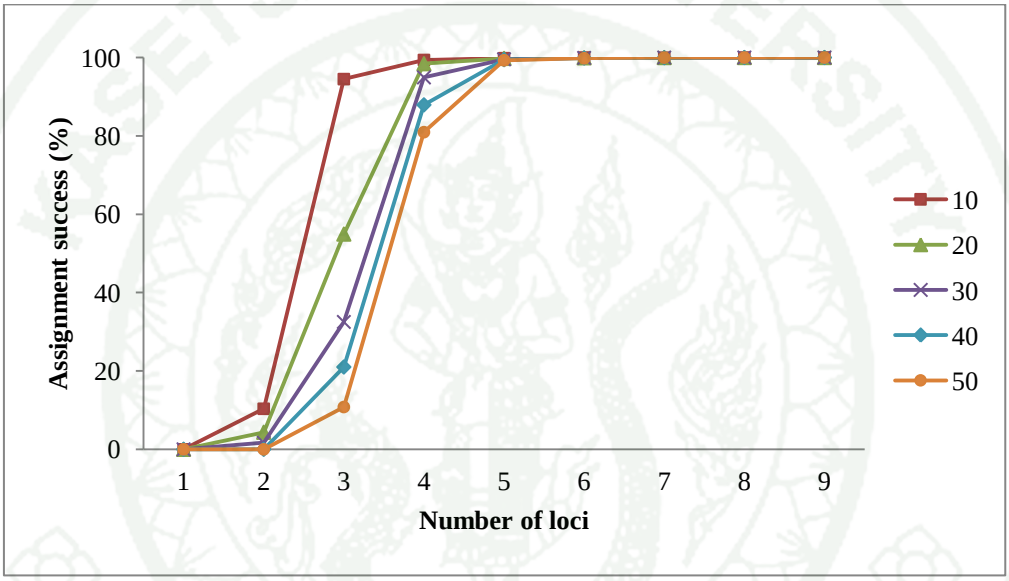


Figure 9 Simulation of assignment success for parent pairs from 10 to 50 families.

Assignment was not success when using one locus (*Mbr-1*), whereas 10% of the offspring was assigned to parent pairs from 10 families with the use of two loci (*Mbr-1*and *Mbr-3*) (Fig 9). The number of offspring assigned to parent pairs increased with additional loci. Five loci (*Mbr-1*, *Mbr-3*, *Mbr-4*, *Mbr-5*, and *Mbr-11*) were needed to obtain 99% predicted assignment for 50 families. Simulations predicted that 100% assignment of parent pair would be obtained for up to 1,000 families based on nine loci (Fig. 10). However, only 300 families were correctly assigned based on father or mother information and the assignment rates dropped to 65% for 1,000 families.

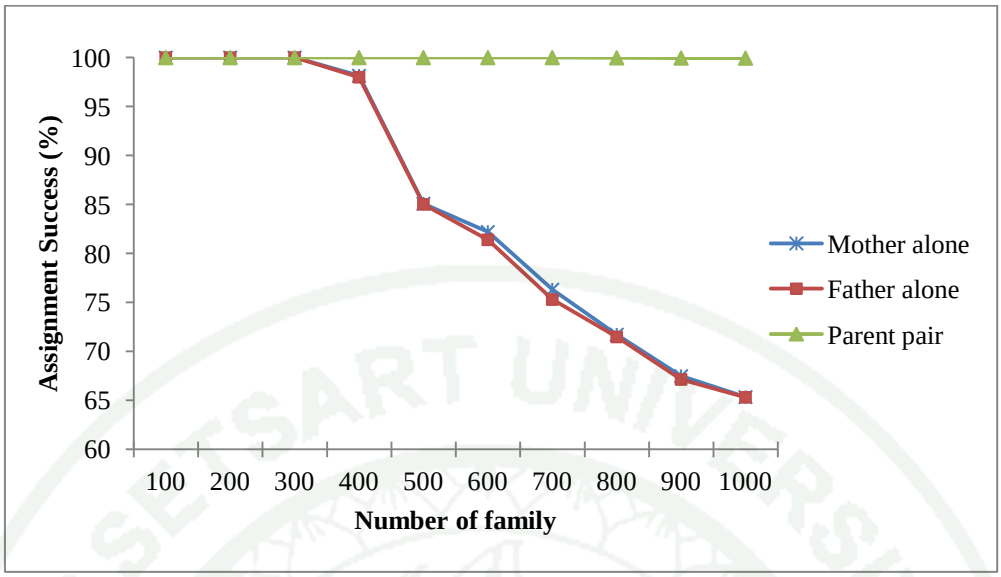


Figure 10 Simulation of assignment success based on nine microsatellite loci.

Figures 11 and 12 display the plots of the log-likelihoods of parent and parent pair of a single offspring. Genotype data of 22 males, 23 females and 506 (22×23) pairs of parent were used to calculate log-likelihoods. Parentage was assigned to the candidate sire or dam having the highest value of log-likelihood of assignment. For example, the tested offspring was assigned to dam #21 (LOD 9), sire #20 (LOD 7.5) and parent pair #460 (LOD 13). When using real data from 23 families to verify parentage assignment, results indicated that all offspring were unambiguously assigned to a pair of parents using from six to nine microsatellite loci.

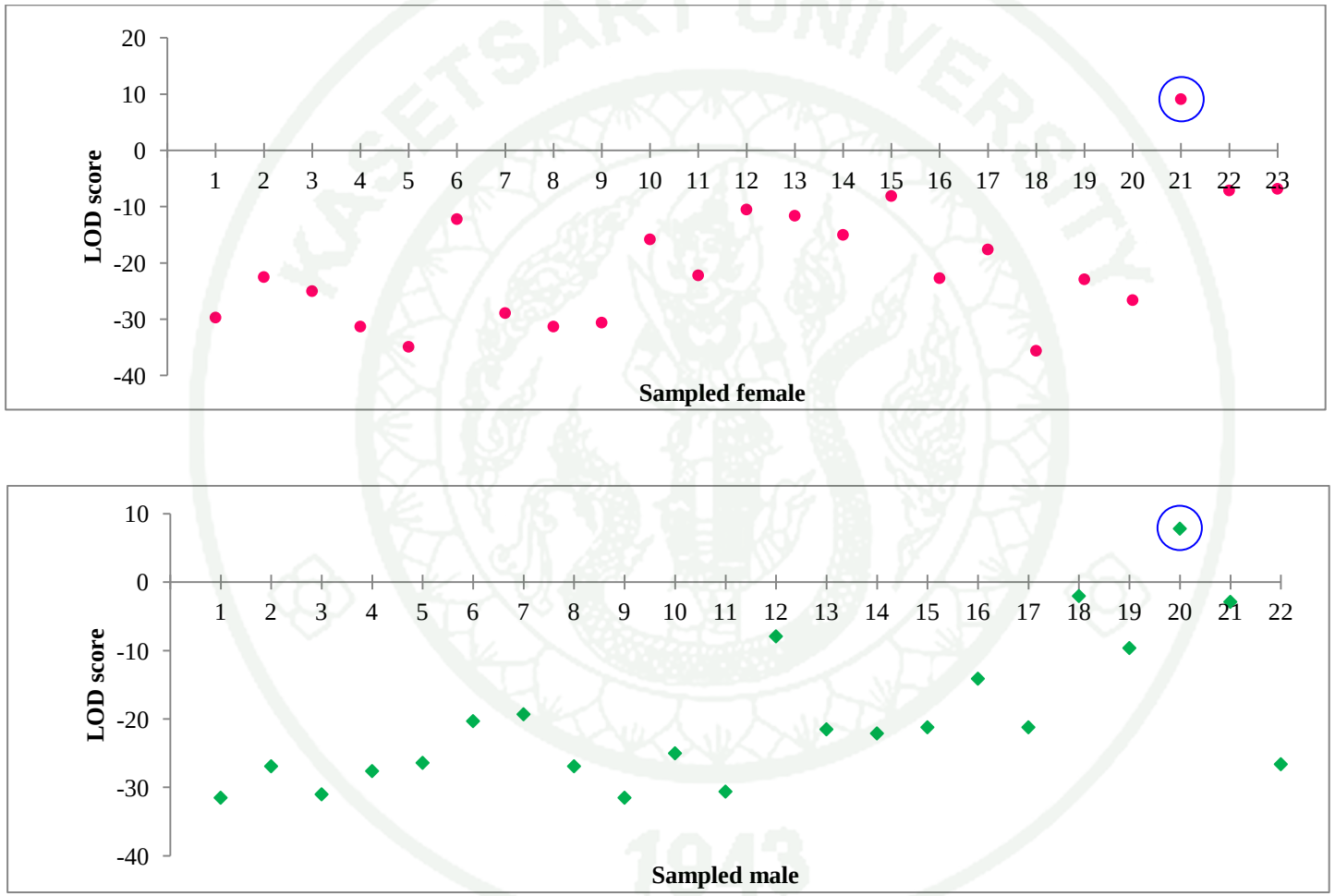


Figure 11 LOD scores for 23 females (top) and 22 males (bottom) of freshwater prawn broodstock. Female #21 (LOD 9) and male #20 (LOD 7.5) were the most likely dam and sire of the offspring.

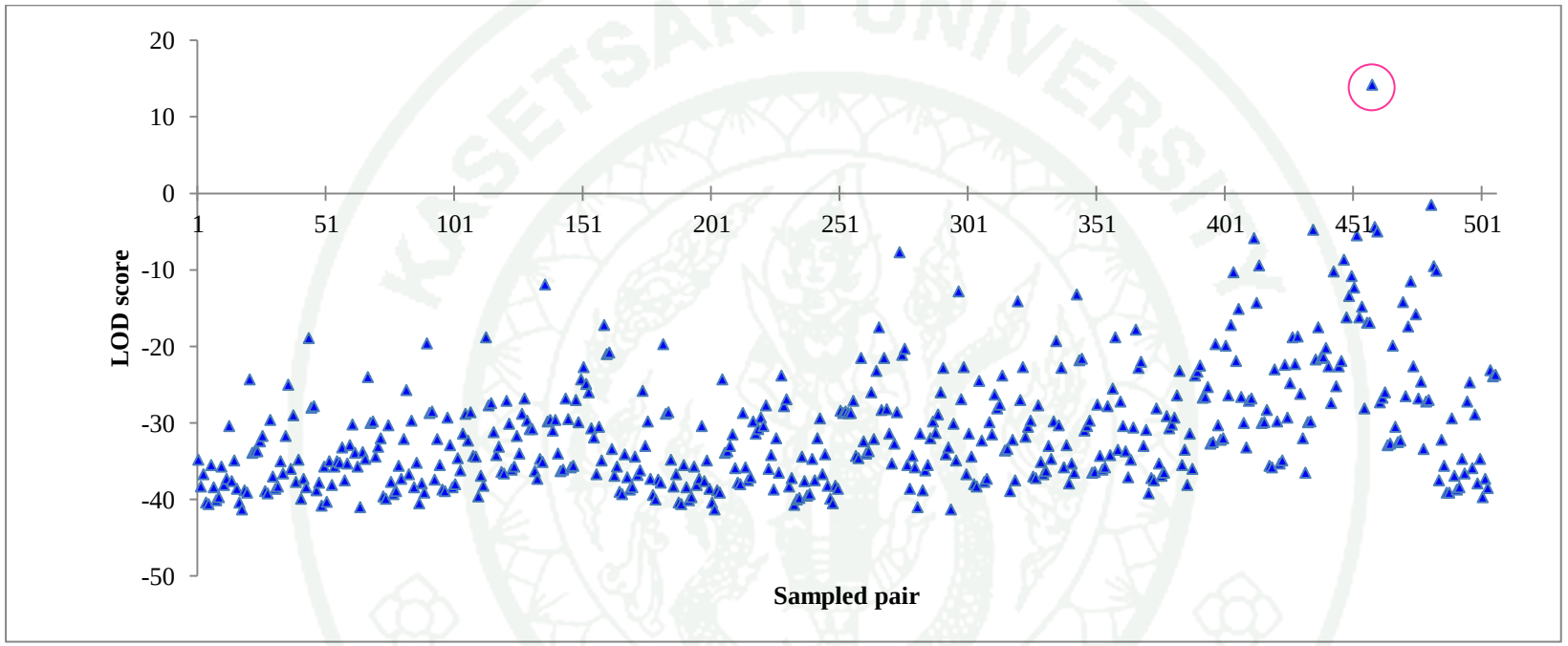


Figure 12 LOD scores for 506 parent pairs of freshwater prawn. Pair #460 (LOD 13) was the most likely parents of the offspring.

Results of assignment success rate for simulations and actual (observed) assignment using CERVUS are presented in Table 10. Genotyping errors were 3% and 5% for parental and offspring genotypes, respectively, due to non-amplified PCR products. When typing error rate was set for 1% with the use of two loci, the success rates of parentage assignment were very low for simulations (1.2-2.5%) and actual assignments (3.5-6.9%). The assignment rates increased to 100 % when six loci were included. Similarly, the assignment rates at typing error of 5% were very low when 2-3 loci were used and 100% success rates were obtained for seven loci.

Table 10 Results of assignment success rate for simulating (real) assignment with known parents for 23 families at genotyping errors = 0.01 and 0.05, and number of offspring = 10,000 using CERVUS.

No. loci	Genotyping error = 0.01			Genotyping error = 0.05		
	Father	Mother	Parent pair	Father	Mother	Parent pair
2	1.2(6.9)	2.5(4.3)	2.4(3.5)	0.4(0.8)	0.0(0.0)	2.9(7.4)
3	35.9(36.1)	28.3(27.4)	61.3(45.6)	21.4(27.8)	20.7(25.6)	38.5(36.9)
4	96.1(94.4)	94.2(83.5)	98.8(98.7)	59.7(66.9)	57.3(61.3)	77.2(88.7)
5	99.9(99.5)	99.8(99.6)	99.7(99.6)	86.3(91.7)	83.6(82.6)	97.2(98.2)
6	99.9(100)	99.9(100)	99.9(100)	96.1(95.6)	94.5(95.6)	99.8(100)
7	100(100)	100(100)	100(100)	98.9(99.1)	98.9(99.6)	99.9(100)
8	100(100)	100(100)	100(100)	100(100)	100(100)	100(100)
9	100(100)	100(100)	100(100)	100(100)	100(100)	100(100)

A comparison of assignment accuracy (with typing error rates of 1% and 5%) for actual data from 23 families between CERVUS and COLONY showed that COLONY correctly assigned paternity, maternity and parent pair to all 230 offspring using four loci, whereas CERVUS assigned parentage to 84-92% of the offspring (Table 11). Results indicated that use of the four highly informative loci was sufficient for COLONY to resolve genetic structure of freshwater prawn, while seven loci would be required to obtain 94-99% correct assignment with CERVUS. Additionally, COLONY accurately inferred relationships of full- and half-sibs of 230 individuals. With the inclusion of the five less informative loci in COLONY, the accuracy of maternal assignment did not changed, but the assignment accuracy of father and parent pair decreased from 100 to 96%.

Table 11 Parentage assignment test results, showing the percent of correct assignment of individuals for 23 families using CERVUS version 3.0 and COLONY 2 based on genotyping errors at 0.01 (0.05).

No. loci	CERVUS			COLONY		
	Father	Mother	Parent pair	Father	Mother	Parent pair
2	70.8(72.2)	66.5(69.6)	55.6(55.6)	80.8(65.22)	77.4(63.1)	71.7(44.3)
3	85.6(84.3)	87.8(86.9)	76.1(76.1)	96.1(96.1)	95.6(95.6)	94.3(93.5)
4	92.2(87.4)	87.4(92.6)	84.7(85.2)	100(100)	100(99.6)	100(99.6)
5	93.5(88.7)	96.9(93.1)	91.7(83.9)	94.3(94.3)	100(100)	94.3(94.3)
6	97.7(95.6)	99.1(99.1)	94.3(95.2)	95.6(94.7)	100(100)	95.6(94.7)
7	94.7(94.7)	99.6(99.6)	94.7(94.7)	95.6(94.7)	100(100)	95.6(94.7)
8	95.2(95.2)	99.6(99.6)	95.2(95.2)	95.6(94.7)	100(100)	95.6(94.7)
9	95.2(95.2)	99.1(99.1)	95.2(95.2)	95.6(94.7)	100(100)	95.6(94.7)

PART III

3. Assessment of reproductive potential among male broodstocks

3.1. Genotyping of prawn broodstock and their progeny

A total of 63 males and 87 females were genotyped at *Mbr-5* locus. A total number of allele at this locus was 25. Genotype data for parents and offspring for each mating is shown in Appendix Table A3. Genotypes of offspring from each female were used to identify their male parent. Electrophoregrams of three individuals are shown in Appendix Figure C1.

3.2. Measurement of morphometric traits

The average body weight of males were 76.91 ± 17.94, 64.09 ± 14.25, 14.51 ± 5.26, and 25.68 ± 4.63 g, respectively for BC, OC, SM males and females (Table 12). Mean body lengths and mean carapace lengths were similar between blue claw and orange claw males and were greater than those of small males and females. Average relative claw lengths were 1.45, 1.11 and 0.85 for BC, OC and SM males, respectively. Females were larger than SM males, with greater body weight, body length and carapace length.

Table 12 Mean body weight, body length, carapace length, and relative claw length and standard deviation of females, BC, OC, and SM males.

Male type /female	Mean body weight ± SD (g)	Mean body length ± SD (cm)	Mean carapace length ± SD (cm)	Mean relative claw length ± SD (cm)
BC	76.91±17.94	13.40±0.90	8.85±0.86	1.45±0.24
OC	64.09±14.25	13.05±0.76	8.87±0.54	1.11±0.12
SM	14.51±5.26	8.28±0.91	5.42±0.52	0.85±0.10
Female	25.68±4.63	10.11±0.63	6.64±0.45	-

The length-weight relationships (LWR) obtained from three male morphotypes of freshwater prawn, estimating from log transformation values of length and weight, are shown in Table 13 and Fig. 13. The LWR values often referred to as *b* values were 3.29, 3.71 and 3.19 for BC, OC and SM males, respectively. Condition factors (*K*) were significantly different among the three male types ($P < 0.0001$), with the values of 0.0165 ± 0.0019, 0.0148 ± 0.0012 and 0.0046 ± 0.0003, for SM, BC, and OC males respectively.

Table 13 Length-weight relationship and condition factor of male morphotypes. Values in the same column with different letters differ significantly.

Male morphotypes	*a*	*b*	*r*	r^2	Mean of *K*
BC	-1.828	3.29	0.94	0.88	0.0148 ± 0.0012^{b}
OC	-2.336	3.71	0.96	0.93	0.0046 ± 0.0003^{c}
SM	-1.789	3.19	0.96	0.92	0.0165 ± 0.0019^{a}

a, *b* = regression coefficients; *r*, r^2 = correlation coefficients

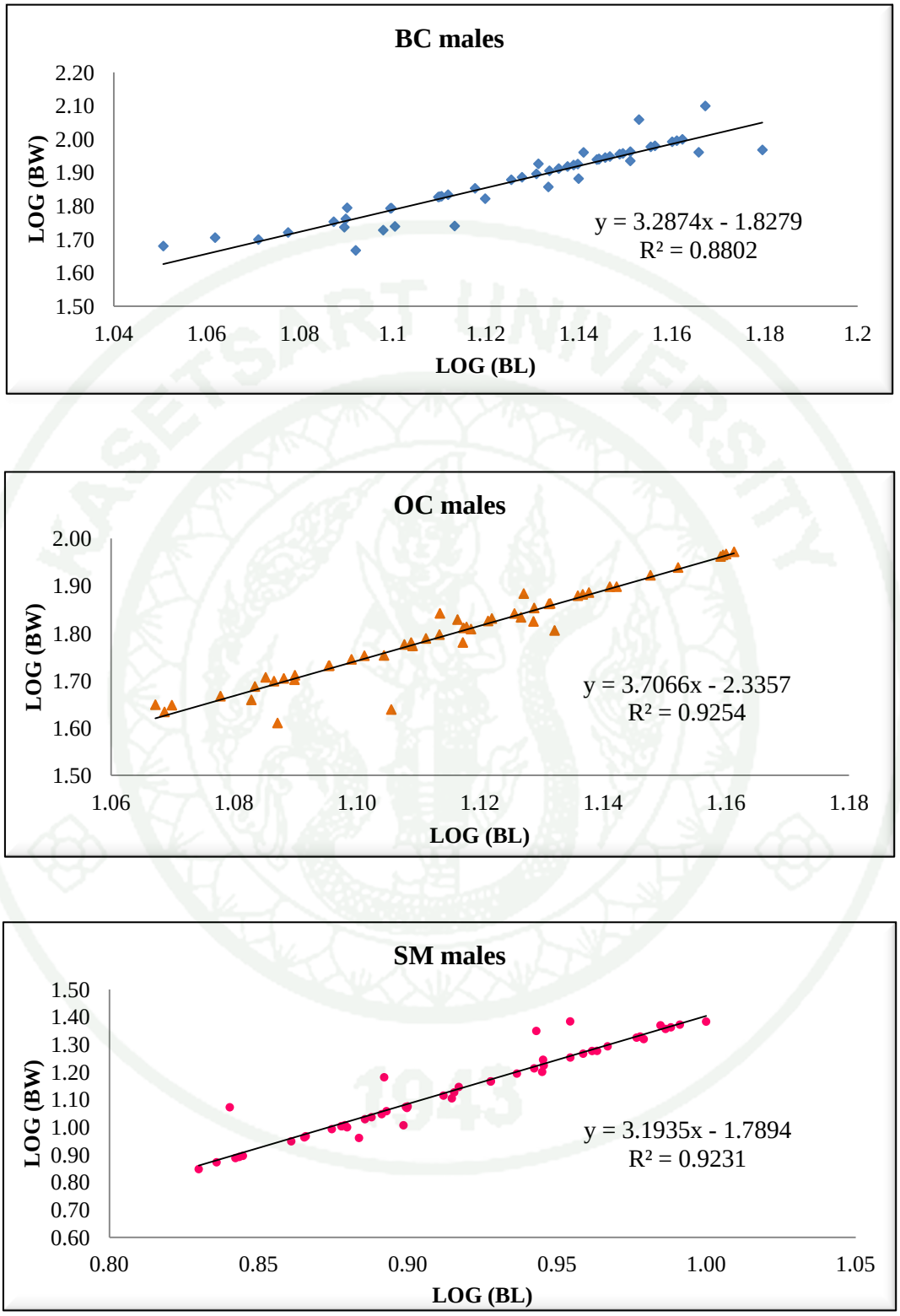


Figure 13 Log length-weight relationship for three male morphotypes.

3.3. Mating success of male morphotypes

Reproductive success of different male types when using different combinations of males and females is shown in Table 14. In mating type I (sex ratio of 1 male: 4 females) BC males had the highest reproductive success scores, ranging from 0.5 to 0.9 from seven tanks, followed by OC males (0.09 to 0.33) and SM males (0.1 to 0.28). The number of females sired in each tank ranged from 5 to 12.

Table 14 Reproductive success of male types from three mating designs.

Mating type/replication	Number of females sired by males (*n*)	Reproductive success of male morphotypes (RS=x_i/n)		
		BC	OC	SM
1 male: 4 females (1BC:1OC:1SM:12F)				
R1	6	0.500	0.333	0.167
R2	9	0.556	0.333	0.111
R3	9	0.889	0.000	0.111
R4	12	0.917	0.083	0.000
R5	7	0.714	0.000	0.286
R6	5	0.800	0.200	0.000
R7	11	0.909	0.091	0.000

Table 14 (Continued)

Mating type/replication	Number of females sired by males (*n*)	Reproductive success of male morphotypes (RS=x_i/n)		
		BC	OC	SM
1 male: 1 female (1BC:1OC:1SM:3F)				
R1	3	0.333	0.000	0.667
R2	3	1.000	0.000	0.000
R3	3	0.333	0.667	0.000
R4	3	1.000	0.000	0.000
R5	3	1.000	0.000	0.000
R6	3	0.667	0.333	0.000
R7	3	0.667	0.333	0.000
3 males: 1 female (1BC:1OC:1SM:1F)				
R1	1	1	0	0
R2	1	1	0	0
R3	1	0	1	0
R4	1	0	1	0
R5	1	1	0	0
R6	1	1	0	0
R7	1	1	0	0

When the ratio of male: female was 1:1, a single BC male in each tank successfully mated with at least one female in all seven tanks and the reproductive success scores ranged from 0.33 to 1. All three females were sired by a single BC male in three occasions (tanks # 2, 4, and 5). In tank # 1, two females were sired by a small male and the other female by a BC male. In tank # 3, two females were sired by an orange claw male and the other female by a BC male. In tanks # 6 and 7, two females were sired by a BC male and the other female by an OC male. When the ratio of male:

female was 3:1, a single female was sired by a BC male in five occasions (tanks # 1, 2, 5, 6, and 7) and by an OC male in two occasions (tanks # 3 and 4), while the SM male never had a chance to mate.

3.4. Factors affecting reproductive success of males

The least squares mean± S.D. of male reproductive success scores for different sex-ratios and male types were 0.73±0.33, 0.21±0.32 and 0.06±0.16 respectively for BC, OC and SM males. Results of ANOVA indicated significant effects of sex ratio, male type as well as sex ratio-male type interaction on reproductive success of males (P<0.0001) (Table 15). When covariates were included, ANCOVA results revealed significant effects of condition factor (β = 394.6, P<0.0005), body weight (β = 0.03, P<0.0039) and relative claw length (β = 2.17, P<0.029) on reproductive success of males, while the effects of body length and carapace length were not significant.

Table 15 The effects of sex ratio, male morphotype, sex ratio-male morphotype interaction, condition factor, body weight, standard length, carapace length, and relative claw length on male reproductive success, the regression coefficient (β) of covariates, *F*-values and *P*-values.

Effect	*df*	Parameter estimate (β)	*F*- value	*P*-value
Sex ratio (SR)	2	-	22.47	<0.0001**
Male morphotype (M)	2	-	32.24	<0.0001**
SR-M interaction	4	-	10.77	<0.0001**
Condition factor (*K*)	1	394.6153 ± 105.53	13.98	0.0005**
Body weight (BW)	1	0.0301 ± 0.01	9.10	0.0039**
Standard length (SL)	1	0.1823 ± 0.20	0.80	0.3747^{ns}
Carapace length (CR)	1	0.2898 ± 0.27	1.11	0.2961^{ns}
Relative claw length (RCL)	1	2.1768 ± 0.97	5.00	0.0296*

DISCUSSIONS

PART I

1. Stock evaluation and strain identification by microsatellite profiling

1.1. Genetic diversity of freshwater prawn

Assessing the genetic diversity of wild and cultured stocks and strain evaluation have become common practices in selective breeding of aquaculture species. Genetic characterization is important because it reflects the genetic makeup and a history of domestication of a particular strain. Although the issue of association between genetic variation at neutral marker loci and variation of quantitative traits remains controversial (Reed and Frankham, 2001), analyzed data for 20 species – including plants, invertebrates and vertebrates – indicated positive correlation of quantitative trait variation and neutral marker divergence (Merila and Crnokrak, 2001). Genetic improvement of prawn strains is still at an early stage compared to that for domesticated fish species (Amrit and Yen, 2003; Nhan *et al*., 2009; Thanh *et al*., 2010). Only a few genetically improved strains of prawn have been developed for commercial use (New, 2005).

Prawn strains in this study – including CPP, KSB, and SKL – are widely used in the major farming areas in central Thailand. The original CPP broodstock was derived from prawns of India origin and has undergone selective breeding for a number of generations (Nithid Patarakulchai, pers. comm.). The other two strains were developed from wild populations native to Thailand. KSB originated from the ChaoPhraya River, but the record of domestication for this strain is not known. The SKL strain was brought recently from Songkla Lake in south Thailand. The CPP strain was believed to have better growth rate than the other two strains. However, differences in other traits such as survival rate and disease resistance among the three strains were not known. Analysis of population genetic data indicated that the three stocks exhibited relatively high genetic variation in terms of average numbers of

alleles (8.3-14.7) and observed heterozygosities (0.76-0.79). Among them, the SKL strain displayed the highest number of alleles and percent heterozygosity, followed by KSB and CPP. Overall, the observed genetic diversity of the three strains was comparable to that of hatchery and wild population samples of freshwater prawn in the previous study (Chareontawee *et al*., 2007). Significant departures from HWE in the CPP strain could have resulted from the presences of null alleles at *Mbr-1* and *Mbr-2*, while heterozygote excess at *Mbr-8* may indicate mixing of populations. The F_{ST} value of 0.076 showed high levels of genetic differentiation among prawn strains and was in agreement with their different origins.

1.2. Assignment tests

Microsatellite DNA markers are an effective tool for strain or population identification studies. A number of computer programs have been developed to perform genetic assignment tests based on different algorithms (Cornuet *et al*., 1999; Hansen *et al*., 2001; Piry *et al*., 2004; Manel *et al*., 2005). These assignment methods use genotype data and statistical tests to assign individuals to their most likely stock of origin under different assumptions regarding the data. For instance, assumptions of HWE and linkage equilibrium between loci are embodied in the original assignment test developed by Paetkau *et al*. (1995). The test was shown to be effective in a study of the genetic population structure of polar bears *Ursus arcticus* and in other investigations (Paetkau *et al*., 1997).

In the present study, however, the exclusion-simulation method implemented in the program GENECLASS2 was used specifically because it does not assume HWE and linkage equilibrium among loci. Application of this method is more appropriate for aquaculture stocks, where departure of HWE is a common phenomenon. The performance of the exclusion-simulation approach is quantified as the proportion of correctly assigned individuals at a threshold probability of belonging to a population (Cornuet *et al*., 1999; Piry *et al*., 2004). The exclusion method performed well in this study, showing high accuracy, with 90% correct assignment of individuals among the three strains at $P<0.05$. Moreover, the assignment scores (88%) changed only slightly

when the confidence level was decreased to $P<0.01$ and $P<0.001$. The utility of the exclusion-simulation method for individual assignment has been assessed in other studies, with varying results. For instance, in their study on cattle breed assignment, Maudet *et al*. (2002) indicated that 67% correct assignment was obtained among seven breeds at a significance level of $P<0.05$. The accuracy of breed assignment, however, decreased to 54 and 33% at $P<0.01$ and $P<0.001$, respectively. In other studies, the exclusion method failed to distinguish between hybrids and wild individuals in admixed populations of brown trout *Salmo trutta* (Hansen *et al*., 2006), but proved very effective in discrimination among three species of Pacific abalone *Haliotis spp*. (Sekino and Hara, 2007).

There are several factors that determine the efficacy of assignment methods, including the amount of genetic differentiation among stocks, the number and sample sizes of populations and the number of loci studied (Cornuet *et al*., 1999; Hansen *et al*., 2001; Piry *et al*., 2004; Manel *et al*., 2005). The power of the exclusion method was determined in a simulation study by Cornuet *et al*. (1999); for example, using 10 simulated populations, an assignment score of nearly 100% was obtained when ten loci and a sample size of 30 individuals were used with an F_{ST} of 0.1. The performance of the exclusion method was further evaluated using empirical microsatellite data sets from 10 species, including bear, cattle, fish, bees, and *Drosophila* (Manel *et al*., 2002). Similarly, analysis of the empirical data sets indicated that nearly all individuals were correctly assigned when populations were highly differentiated ($F_{ST} > 0.1$). The effect of genetic differentiation on power of the assignment test also was demonstrated in this study, where a score of 100% correct assignment was obtained between the CPP and SKL strains with an F_{ST} of 0.12. In contrast, only 81-83% of individuals were correctly assigned between the least differentiated populations, KSB and SKL ($F_{ST} = 0.02$). Cornuet *et al*. (1999) suggested that if the amount of genetic differentiation existing among populations is low, the power of assignment test can be increased by using larger population samples and larger numbers of loci. It should be noted, however, that the accuracy of the test decreases with an increase in number of populations due to increased numbers of individuals wrongly assigned to each population (Hansen *et al*., 2001).

Although microsatellites are the most suitable genetic markers for individual assignment, genotyping at a large number of loci can be very expensive and may reduce the benefit of assignment tests. Therefore, it would be useful to determine the number of markers that optimizes assignment success and the cost of genotyping. Simulation results from the three baseline populations showed that high assignment scores were obtained with the use of several subsets of markers comprising four to seven loci. Of these, a subset of four loci (*Mbr-1*, *Mbr-2*, *Mbr-3*, and *Mbr-5*) showing 89% of correct assignment among the three strains at $P<0.05$, 0.01 and 0.001 was chosen for genotyping of surviving prawn in the communal rearing experiment. The explanation for the high rate of assignment at the four chosen loci is that removal of three loci (*Mbr-5*, *Mbr-7*, and *Mbr-10*) which were less informative resulted in increased population differentiation from $F_{ST} = 0.07$ (seven loci) to 0.08 (four loci). By using the subset of four loci, the number of genotypes from the communal tanks was reduced considerably, from 9,562 (1,366 individuals x 7 loci) to 5,464 (1,366 individuals x 4 loci), with up to 45% reduction of genotyping cost.

1.3. Comparison of growth performance

Communal rearing has proven an effective strategy for testing different genetic groups under the same environmental conditions. However, the presence of competition among different strains may invalidate application of communal testing for certain species (Wohlfarth and Moav, 1985; Wohlfarth and Moav, 1991). Competitive advantage is indicated if the ranking of strains is different between communal and separate testing. The results of the present study did not suggest the presence of competition among prawn strains; i.e., the same ranking of strains for growth traits was observed between separate and communal testing, with the CPP strain ranked first, followed by KSB and SKL. Without competition between different strains, the growth of juveniles in communal rearing may be used as a predictor for their performance in separate rearing (Wohlfarth and Moav, 1991). However, the application of communal stocking for comparison of survival for juvenile prawn may be restricted because the results varied between separate and communal tests. While the survival of the CPP strain (71%) was consistent in separate and communal culture,

differences in survival were observed for the KSB and SKL strains. The variation in survival rates at the end of the experiment were not unexpected and likely were due to the cannibalistic nature of prawn larvae and juveniles.

Growth and survival of juveniles are the important traits that determine prawn production. Prawn farmers practice two rearing strategies in Thailand. One strategy is to stock post-larvae (PLs) directly into grow-out ponds. A second method is to stock post-larvae in nursing tanks or cages for 2-3 months and then juveniles are transferred to grow-out ponds. Several studies reported that prawn production was relatively low for the first strategy due to high mortality of PLs by cannibalism in the grow-out ponds. In contrast, the use of a nursery phase increases the survival of juveniles and subsequently increases farm production (Lin and Boonyaratpalin, 1988; Schwantes *et al*., 2007). According to our recent survey, most prawn farmers in the areas have adopted a new strategy by stocking 2-4 months old juveniles directly into grow-out ponds. This practice shortens the culture period and reduces production cost. As a result, demand for juvenile prawns is increasing while demand for post-larvae tends to decrease.

PART II

2. Parentage identification of freshwater prawn broodstock

2.1. Parentage assignment

The performance of the likelihood-based assignment methods were shown to be effective in determining genetic relationship in natural populations of red deer *Cervus elaphus* (Marshall *et al*., 1998), cheetah (Wang and Santure, 2009) as well as in cultured stocks of crustaceans (Jerry *et al*., 2006; Dong *et al*., 2006). The power of these techniques is dependent on several factors, including usefulness of markers, presence of null alleles, independent segregation of loci, as well as mutation and genotyping errors (Marshall *et al*., 1998; Wang and Santure, 2009). Of ten microsatellite loci analyzed in this study, all but one locus (*Mbr-7*) were highly informative, with average expected heterozygosity of 0.81 and PIC score of 0.77. In addition to the measure of polymorphism, CERVUS also calculates the non-exclusion probability for each locus. Results indicated that the probability for a particular locus was low to moderate, but the overall non-exclusion probability for the set of 10 loci was very close to 0 due to high levels of polymorphism of the loci. Therefore, high assignment success based on the exclusion method would be expected. With genotyping errors and missing genotypes, however, the probability of non-exclusion can be higher and can decrease the power of the test. Nevertheless, these probabilities have little effects on the likelihood-based assignments, but may be useful for comparison with similar work on the same species (Kalinowski *et al*., 2007).

Presence of null alleles, deviation from HWE and linkage disequilibrium can bias the results of parentage analyses. Microsatellite null alleles are non-amplifiable in PCR-amplification due to mutations at the priming sites and can be detected as a significant departure from HWE. In this study, the presence of null alleles was not detected when using MICROCHECKER, but CERVUS detected low frequencies of null alleles (<0.05). Including loci with low null allele frequencies in the parentage analysis, however, should not cause any problems in likelihood calculations (Marshall

et al., 1998). Moreover, the exact test revealed that all loci conformed to HWE (P>0.05). The assignment tests in CERVUS and COLONY assume that all pairs of loci are in linkage equilibrium. The locus *Mbr-10* was not included in the parentage analysis due to significant linkage disequilibrium with *Mbr-9* (P<0.000). Use of linked loci decreases the accuracy of parentage assignment because nonrandom associations between loci reduce the amount of genetic variation for discriminating parentage in the population (Jones and Ardren, 2003).

Although microsatellites are the most suitable genetic markers for parentage studies, problems of mutation, null alleles and typing errors can reduce the accuracy of the test by causing mismatch between parent and offspring. In their study, Jerry *et al*. (2004) showed that a large discrepancy in assignment success between simulations (92%) and real data (47%) for *P. japonicus* was due to null alleles and typing errors. However, mutation rate at microsatellite loci is very low, i.e., 10^{-4} per locus and generation for turbot *Scophthalmus maximus* (Borrell *et al*., 2004). In such case, one of 10,000 offspring would not be assigned to any candidates parents if the mutation produces a new allele. Although mutation was expected to occur at higher rate in crustaceans due to their high fecundity (Jerry *et al*., 2004), the presence of new alleles was not observed in the freshwater prawn population in this study. The classical exclusion approach is very sensitive that a mismatch at a single locus can result in false exclusions (Hedrick, 2005). Because the probability of typing errors tends to increase when additional loci are used, the computer programs are designed to accommodate genotyping errors to increase success in parentage assignment (Kalinowski *et al*., 2007; Wang and Santure, 2009; Jones and Wang, 2010). It is likely that the inclusion of less informative loci in the prawn population may introduce more noise, resulting in decreased accuracy for assignment of paternity and parent pair obtained in COLONY.

Knowledge of full-pedigree relationships is required to increase accuracy in estimating heritability and genetic correlations. Experimental designs for genetic parameter estimations and performance tests in aquaculture usually involve mixing of full- and half-sib families to eliminate confounding environmental effects. Genetic

relatedness among candidate parents and, in particular, the family structure for polygamous population, may have confounding effects on parentage analysis (Marshall *et al*., 1998; Jones and Arden, 2003). Wang and Santure (2009) demonstrated that the full-pedigree likelihood method was not affected by the mating system in the population. In the present study, the advantage of using COLONY was that the program performed inference of both parentage and sibship structure for freshwater prawn. COLONY showed a list of full- and half-sibships, but CERVUS did not display this information.

PART III

3. Assessment of reproductive potential among male broodstocks

3.1. Morphometric traits of males

The morphometric traits were used to characterize adult male prawn into three distinctive morphological types (Ra'anan and Sagi, 1985; Ra'anan and Cohen, 1985). The relative claw lengths (RCL) were 1.5-2, 1-1.5 and 0.5-0.7, the body weights (BW) were 40-50, 30-40 and 5-10 g for BC, OC and SM males, respectively (Ra'anan *et al*., 1985). For male prawn in present study, the RCLs were similar but the BWs were higher. This might be due to the use of older prawn (5-6 months) and/or genetic improved prawn from good cultured farm.

The length-weight relationship is commonly used to describe growth in fish for stock assessment ((Abohweyere and Williams, 2008)). Growth is considered isomeric (fish grows without changing shape) when the value of the exponent (*b*) is 3. If *b* value is different from 3, growth is said to be allometric (fish changes shape as it grows larger) (Wooton, 1992). For male prawn in this study, their growth was allometric, because *b* values varied among morphotypes and were 3.29, 3.71, and 3.19 for BC, OC, and SM males respectively. High correlations between length and weight were observed with $r > 0.9$. When compared with growth ($b = 2.95$) of freshwater prawn *M. macrobrachion* in the Lagos-Lekki Lagoon System, Nigeria (Abohweyere and Williams, 2008), prawns in this study appeared to grow faster. This may be due to the effects of larger amount of feeds that enhance growth of cultured prawn used in this study.

3.2. Factors affecting reproductive success of male

Reproductive success in several species is dependent on male-male interaction and female mate choice (Johnstone and Earn, 1999). An investigation by Ra'anan and Saki (1985) suggested that blue claw males were the most successful at

mating, followed by the orange claw and small males. The dominant BC males are territorial, sexually active and often associated with eight to ten females. The OC males are sub-dominant, non-territorial, occasionally mated with females. The SM males are submissive to all other males, non-territorial, and sexually active than the OC males (Ra'anan and Saki 1985; Saki *et al*., 1988). Overall, results in the present study were consistent with most of the previous investigations.

In addition to sex ratio, and male type, the effects of morphometric traits of males on their reproductive success were evaluated in this study. The analysis of covariance (ANCOVA) was used because there were good correlations between body traits and male type. The approach is more powerful than the corresponding one-way or two-way ANOVA since including a covariate in the model help reduce residual variation (SAS, 2003). ANCOVA results revealed that condition factor (β = 394.6, P<0.0005), body weight (β = 0.03, P<0.004) and relative claw length (β = 2.17, P<0.03) had significant effects on reproductive success of males.

The condition factor is normally used as determination of period and duration of gonadal maturation, depending on age of fish, sex, season, and maturity stages (Anyanwu *et al*., 2007). Rakitin *et al.* (1999) suggested that sire condition factor was a critical factor for determining sperm fertilization potency in Atlantic cod *Gadus morhua*. Among mature males of freshwater prawn, Sagi *et al*. (1988) revealed that the relative weight of the testes, measured by the gonado-somatic index (GSI) from small males was significantly greater than in the other morphotypes. Orange claw males had lower GSI values (0.09) when compared to small males (0.24) and blue claw males (0.14). Histological observations of testes of small males showed a large amount of mature sperm, and were active in spermatogenesis, while those of orange claw males contained mainly spermatocytes and almost only mature sperm was observed in testes of blue claw males. These stages of male gonadal development might be correlated with the corresponding male condition factor reported in this study. Condition factor for small males (0.0165) was significantly greater than those of blue claw males (0.0148) and orange claw males (0.0046). However, more experiments are needed to determine the GSI-condition factor correlation.

The effects of body weight and relative claw length on male reproductive success were suggested in the present study. These findings were in agreement with the previous study by Ra'anan and Saki (1985). They reported that dominant BC males used their claws to attract eight to ten females and displayed a courtship behavior. The advantage of bigger males in reproductive competition was also suggested in tilapia *Oreochromis niloticus* (Fessehaye *et al.* 2009). In the present study, mating success of BC males indicates the importance of size in reproductive competition of giant freshwater prawn. Use of BC males for post-larval production has become common in most of prawn hatcheries. Despite higher mating capacity of dominant BC male, studies report that not all gravid females were fertilized, and as a result, the production of prawn post-larvae were low. Furthermore, high stocking density, low fecundity of females and especially female mate choice, contribute to low productivity of post-larvae. Although, almost females are thought to get benefit by mating with dominant males, evidence indicates that females do not always prefer dominant males. For example, females showed a preference for unmated males with sufficient sperm reserves over sperm-depleted males in stone crab *Hapalogaster dentata* (Sato and Goshima, 2007). In BC males, stored sperm were ejaculated when they were used to mate frequently with more females (Sagi *et al*., 1985). It is likely that, due to the depletion or absence of BC male sperm, gravid females were mated by OC or SM males under laboratory conditions in the present study. These findings also suggest that sperm limitation might occur in prawn male populations. However, more studies are required to investigate the reproductive system and mating ability of each male morphotypes.

Male of giant freshwater prawn is polygamous in nature and captivity. As a result, it is more profitable in post-larval production to use more than one female with a single male (Celada *et al.*, 2005). The sex ratio of 1:4 for BC male and female mating scheme has been suggested for commercial production of prawn post-larvae (Sureshkumar and Kurup, 1998; Chantaganond *et al.*, 2004). Although, the sex ratio of 1:4 has gained popularity for farming but it does not control the rate of inbreeding in a closed breeding population (Gjerde, 2005). It is widely accepted that improper mating design is the major cause of accumulated inbreeding in the population. For both nested

and factorial mating designs, the rate of inbreeding increases with increasingly skewed sex ratio (Gjerde, 2005). For example, inbreeding rates of 0.38, 0.50 and 0.63% per generation can occur when the nested mating ratio (sire per dam) at 1:2, 1:3 and 1:4 are used in fish breeding programs. In contrast, the lowest rate of inbreeding (0.25% per generation) is obtained with the paired (1:1) mating design. It is worthwhile to consider the sex ratio for a sustainable genetic improvement program of freshwater prawn.

CONCLUSIONS AND RECOMMENDATIONS

Microsatellite DNA polymorphisms have become the most widely used molecular markers for genetic management of aquaculture stocks. In particular, they have proven effective tools in assessing genetic diversity, identifying strains of origin, and establishing pedigrees in breeding programs of various species of fish and shellfish. In this thesis, a set of polymorphic microsatellites previously developed from freshwater prawn of the Thai origin by Chareontawee *et al.* (2006) has been successfully used to compare growth performance and identify parentage for hatchery strains of freshwater prawn.

The first experiment demonstrated that seven microsatellite loci were useful for assessing genetic diversity within prawn strains. All three hatchery strains exhibited similar and relatively high levels of genetic diversity in terms of average numbers of alleles (8.3-14.7) and observed heterozygosities (0.76-0.79), with high levels of genetic differentiation (F_{ST} = 0.076) among strains. Genotype data at seven loci were used for strain identification by the exclusion-simulation method implemented in the program GENECLASS2. This particular program does not assume HWE and linkage equilibrium among loci and is more appropriate for aquaculture stocks, where departure of HWE is a common phenomenon. The exclusion method performed well in this study, showing high accuracy, with 90% correct assignment of individuals among the three strains (P<0.05).

The power of assignment methods depends on the amount of genetic differentiation among stocks, and the number and sample sizes of populations. When populations were highly differentiated (F_{ST} > 0.1), 100% assignment would be expected as demonstrated in this study. Consequently, a score of 100% correct assignment was obtained between two prawn strains (F_{ST} = 0.12). The power of the tests increases with sample sizes of the populations, but decreases with increased numbers of the populations used in the analysis, i.e., the accuracy of assignment tests is higher between two populations than that among three or more populations. Simulations included in GENECLASS2 are useful for determining the number of

markers that optimizes assignment success and the cost of genotyping. Use of four markers was sufficient for strain identification among the three strains and greatly reduced the cost of genotyping.

Communal rearing has proven an effective strategy for comparing growth performance of different prawn strains in this study. It should be noted that competition among different strains may invalidate the application of communal rearing techniques. The presence of competition among strains can be detected by comparing performance of the same strains between separate and communal rearing conditions. Competitive advantage is indicated if the ranking of strains is different between the two rearing systems. Without competition between different strains, the growth of juveniles in communal rearing may be used as a predictor for their performance in separate rearing. The application of communal stocking for comparison of survival for juvenile prawn, however, may not be valid because the results varied between separate and communal tests. Communal rearing proved an effective technique for comparison of growth performance of different prawn strains because inter-strain competition was not observed in this study. However, the communal rearing approach was not appropriate for comparing survival due to different rankings of strains between separate and communal rearing.

The second experiment described the potential of ten microsatellite loci for parentage determination of freshwater prawn. Of ten microsatellite loci analyzed, all but one locus (*Mbr-7*) were highly informative, with average expected heterozygosity of 0.81 and PIC score of 0.77. Two computer programs, CERVUS 3.0 (Marshall et al., 1998; Kalinowski et al. 2007) and the recently developed COLONY 2 (Jones and Wang 2010), were used for parentage analysis. The two programs are designed to accommodate common problems of genotyping errors and mutation to increase success in parentage assignment. Both programs performed well at inferring pedigree relationships in this hatchery population of freshwater prawn, with COLONY showing a clear advantage in accuracy and economical use of markers. Use of four highly informative loci was sufficient for COLONY to resolve the genetic structure of this

population, while seven loci would be required to obtain 94-99% correct assignment with CERVUS.

Results in the third experiment suggested that blue claw males were the most successful at mating, followed by the orange claw and small males. Sex ratio, male type, and morphometric traits, including condition factor, body weight and relative claw length of males had significant effects on male reproductive success. Stages of male gonadal development might be correlated with the corresponding male condition factor.

Finally, the findings in this thesis have provided recommendations for the effective use of microsatellite markers for strain identification and parentage assignment in freshwater prawn stocks as follows:

1. The properties of microsatellite loci should be examined for presence of non-amplifying alleles at a single locus and the non-random association among loci by testing for HWE and linkage disequilibrium. Violations of the assumptions of Hardy-Weinberg expectation and linkage equilibrium between loci may lead to false conclusions.

2. It is essential to quantify the amount of genetic differentiation (F_{ST}) because knowledge of the F_{ST} value for a set of populations provides a useful prediction of the performance of assignment methods. The accuracy of assignment test for strain identification is maximized when $F_{ST} \geq 0.1$.

3. If a large number of microsatellite loci (>10) are available, use of loci with high value of polymorphic information content (PIC ≥ 0.7) will maximize the power of assignment tests and optimize the cost of genotyping. Inclusion of less informative markers not only reduces the accuracy of assignment but also increases time and genotyping costs.

4. For strain evaluation by communal testing, it is essential to determine if inter-strain competition occurs within the particular species. This can be accomplished by set up the separate rearing experiment parallel to the communal rearing.

5. The COLONY program is more powerful for exploring possible pedigree relationships in a domesticated population of freshwater prawn than CERVUS. In particular, COLONY showed a list of full- and half-sibships, but CERVUS did not display this information.

6. For parentage determination in a large number of breeding families (>100), multiplexing of microsatellite markers can reduce time and genotyping costs.

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APPENDICES

Appendix A

Genetic raw data for evaluations

Appendix Table A1 Allele frequency of three prawn strains for population genetic analysis.

Locus	Allele size (bp)	CPF	KSB	SKL	Locus	Allele size (bp)	CPF	KSB	SKL
Mbr-1	246	0.000	0.000	0.008	*Mbr-3*	262	0.000	0.000	0.038
	252	0.000	0.009	0.000		264	0.000	0.000	0.015
	254	0.000	0.026	0.015		266	0.000	0.042	0.038
	260	0.005	0.035	0.000		268	0.000	0.000	0.015
	262	0.043	0.026	0.000		270	0.000	0.034	0.008
	264	0.037	0.219	0.054		272	0.022	0.000	0.015
	268	0.000	0.000	0.062		282	0.000	0.000	0.038
	272	0.000	0.053	0.085		284	0.000	0.017	0.000
	274	0.000	0.070	0.000		286	0.000	0.000	0.008
	276	0.000	0.044	0.038		288	0.000	0.008	0.000
	278	0.027	0.018	0.069					
	280	0.000	0.114	0.162	*Mbr-5*	276	0.000	0.010	0.000
	282	0.271	0.026	0.054		278	0.000	0.000	0.031
	286	0.239	0.000	0.038		280	0.000	0.020	0.039
	288	0.000	0.149	0.100		282	0.082	0.000	0.000
	290	0.176	0.044	0.038		284	0.000	0.020	0.031
	292	0.128	0.009	0.000		286	0.005	0.039	0.008
	294	0.000	0.009	0.000		288	0.011	0.000	0.000
	296	0.032	0.044	0.085		290	0.000	0.020	0.031
	298	0.000	0.026	0.085		292	0.000	0.010	0.016
	300	0.043	0.079	0.092		294	0.110	0.020	0.000
	304	0.000	0.000	0.015		296	0.044	0.049	0.023
						298	0.005	0.069	0.000

Appendix Table A1 (Continued)

Locus	Allele size (bp)	CPF	KSB	SKL	Locus	Allele size (bp)	CPF	KSB	SKL
Mbr-2	293	0.074	0.000	0.000		300	0.000	0.000	0.047
	296	0.000	0.412	0.344		302	0.000	0.010	0.031
	298	0.330	0.219	0.313		304	0.022	0.147	0.125
	300	0.309	0.000	0.023		306	0.143	0.147	0.148
	302	0.000	0.000	0.023		308	0.005	0.137	0.148
	304	0.000	0.000	0.023		310	0.022	0.029	0.094
	306	0.000	0.053	0.016		312	0.044	0.020	0.008
	308	0.000	0.000	0.008		314	0.000	0.010	0.055
	310	0.000	0.044	0.031		316	0.313	0.176	0.070
	313	0.000	0.053	0.055		318	0.000	0.000	0.016
	315	0.011	0.000	0.023		320	0.011	0.029	0.023
	317	0.011	0.018	0.031		322	0.000	0.029	0.000
	318	0.053	0.009	0.008		326	0.000	0.000	0.008
	320	0.043	0.079	0.063		328	0.159	0.010	0.047
	323	0.011	0.000	0.000		332	0.022	0.000	0.000
	326	0.000	0.018	0.008					
	328	0.069	0.018	0.000	*Mbr-7*	268	0.000	0.076	0.265
	330	0.005	0.000	0.000		271	0.226	0.186	0.098
	334	0.000	0.061	0.008		274	0.000	0.017	0.015
	337	0.053	0.000	0.016		277	0.705	0.686	0.530
	339	0.032	0.000	0.000		279	0.000	0.025	0.015
	347	0.000	0.000	0.008		282	0.068	0.008	0.076
	355	0.000	0.018	0.000					

Appendix Table A1 (Continued)

Locus	Allele size (bp)	CPF	KSB	SKL	Locus	Allele size (bp)	CPF	KSB	SKL
Mbr-3	222	0.000	0.000	0.098	*Mbr-8*	254	0.147	0.127	0.023
	224	0.000	0.042	0.015		257	0.165	0.027	0.008
	228	0.006	0.110	0.015		260	0.135	0.136	0.200
	230	0.133	0.000	0.023		263	0.282	0.200	0.046
	232	0.206	0.093	0.098		265	0.271	0.509	0.669
	236	0.000	0.000	0.038		269	0.000	0.000	0.031
	238	0.000	0.008	0.045		272	0.000	0.000	0.023
	240	0.000	0.051	0.023					
	242	0.044	0.017	0.015	*Mbr-10*	222	0.000	0.052	0.031
	244	0.028	0.203	0.167		231	0.000	0.000	0.008
	246	0.000	0.025	0.045		233	0.000	0.000	0.008
	248	0.261	0.034	0.015		234	0.000	0.009	0.008
	250	0.167	0.051	0.023		243	0.000	0.190	0.138
	252	0.000	0.025	0.015		246	0.068	0.259	0.354
	254	0.000	0.042	0.045		249	0.274	0.034	0.077
	256	0.056	0.110	0.030		251	0.000	0.112	0.062
	258	0.078	0.034	0.091		254	0.232	0.241	0.154
	260	0.000	0.051	0.023		257	0.426	0.103	0.146
						260	0.000	0.000	0.015

Appendix Table A2 Genotypes of 23 families for parentage identification.

ID	*Mbr1a*	*Mbr1b*	*Mbr2a*	*Mbr2b*	*Mbr3a*	*Mbr3b*	*Mbr4a*	*Mbr4b*	*Mbr5a*	*Mbr5b*
F101M*	280	302	298	298	254	254	238	248	298	310
F101F**	266	266	298	335	272	272	205	258	300	306
F101P1***	266	280	298	298	254	254	248	258	298	300
F101P2	266	302	298	335	254	254	248	258	300	310
F101P3	266	280	298	298	254	272	238	258	298	306
F101P4	266	302	298	335	254	272	205	248	300	310
F101P5	266	280	298	335	254	254	238	258	300	310
F101P6	266	302	298	298	254	254	205	238	306	310
F101P7	266	280	298	298	254	272	248	258	300	310
F101P8	266	302	298	298	254	272	248	258	298	300
F101P9	266	280	298	335	254	254	205	238	298	306
F101P10	266	302	298	335	254	272	205	248	306	310
F104M	280	282	298	300	240	246	216	310	306	312
F104F	264	280	298	310	240	242	238	252	300	318
F104P1	264	280	298	300	240	242	238	310	312	318
F104P2	264	280	298	298	240	242	252	252	300	306
F104P3	280	280	298	310	240	240	216	252	300	306
F104P4	280	282	298	298	240	242	252	310	300	306
F104P5	280	282	298	298	240	240	238	238	306	318
F104P6	264	282	298	300	246	246	216	238	306	318
F104P7	264	282	298	298	240	240	216	238	300	306
F104P8	264	282	298	298	240	242	238	252	312	318
F104P9	280	280	298	310	246	246	238	310	300	312
F104P10	280	280	298	300	240	242	?****	?	312	318
F107M	274	302	296	317	232	256	238	248	298	304

Appendix Table A2 (Continued)

ID	*Mbr1a*	*Mbr1b*	*Mbr2a*	*Mbr2b*	*Mbr3a*	*Mbr3b*	*Mbr4a*	*Mbr4b*	*Mbr5a*	*Mbr5b*
F107F	242	268	328	335	268	268	238	238	290	316
F107P1	242	302	317	328	232	232	238	238	304	316
F107P2	242	302	317	328	256	256	238	238	304	316
F107P3	268	274	296	335	256	268	238	238	304	316
F107P4	242	274	317	328	256	256	238	238	304	316
F107P5	268	302	296	335	256	256	238	248	290	304
F107P6	268	302	296	328	232	232	238	238	290	298
F107P7	268	274	296	328	256	256	238	248	298	316
F107P8	242	274	317	328	232	232	238	248	290	304
F107P9	242	274	296	335	256	256	238	238	290	298
F107P10	268	302	317	328	256	256	238	248	290	304
F108M	298	298	296	298	222	252	228	248	300	308
F108F	274	302	328	328	244	244	205	218	294	308
F108P1	298	302	296	328	244	252	218	228	300	308
F108P2	274	298	296	296	222	244	205	228	294	300
F108P3	274	298	298	328	244	252	218	248	294	300
F108P4	274	298	298	298	244	252	205	228	294	308
F108P5	274	298	296	296	244	252	218	248	294	300
F108P6	298	302	296	296	244	252	205	228	300	308
F108P7	298	302	296	296	244	252	218	228	300	308
F108P8	298	302	296	328	244	252	205	228	300	308
F108P9	298	302	296	328	222	244	205	228	294	308
F108P10	274	298	296	328	244	252	205	248	294	300
F201M	?	?	298	298	238	246	216	216	304	306
F201F	242	242	313	326	244	272	205	234	312	316

Appendix Table A2 (Continued)

ID	*Mbr1a*	*Mbr1b*	*Mbr2a*	*Mbr2b*	*Mbr3a*	*Mbr3b*	*Mbr4a*	*Mbr4b*	*Mbr5a*	*Mbr5b*
F201P1	242	242	298	313	238	244	216	234	306	312
F201P2	242	242	298	326	244	246	216	234	304	312
F201P3	242	242	298	313	244	246	205	216	304	316
F201P4	?	?	298	313	238	272	205	216	306	316
F201P5	?	?	298	313	244	246	216	234	306	312
F201P6	242	242	298	326	238	244	205	216	304	312
F201P7	242	242	298	313	238	272	205	216	306	316
F201P8	242	242	298	313	238	272	216	234	306	316
F201P9	242	242	298	326	244	246	205	216	306	316
F201P10	242	242	298	313	244	246	205	216	304	312
F203M	242	242	298	298	266	266	216	248	294	306
F203F	276	286	296	315	244	244	228	246	308	316
F203P1	242	276	296	298	244	266	228	248	294	316
F203P2	242	276	298	315	244	244	246	248	294	308
F203P3	242	286	296	298	244	266	216	228	294	308
F203P4	242	286	298	315	244	266	246	248	306	308
F203P5	242	276	296	298	244	266	216	246	294	308
F203P6	242	276	296	298	244	266	246	248	306	316
F203P7	242	286	296	298	244	266	216	246	306	308
F203P8	242	286	296	298	244	266	216	246	306	316
F203P9	242	276	296	298	244	266	228	248	306	316
F203P10	242	286	298	315	244	266	228	248	306	316
F207M	282	294	298	335	260	272	240	274	298	298
F207F	262	294	298	313	268	268	240	240	310	328
F207P1	294	294	298	298	260	260	240	240	298	310

Appendix Table A2 (Continued)

ID	*Mbr1a*	*Mbr1b*	*Mbr2a*	*Mbr2b*	*Mbr3a*	*Mbr3b*	*Mbr4a*	*Mbr4b*	*Mbr5a*	*Mbr5b*
F207P2	282	294	298	313	260	260	240	274	298	310
F207P3	262	282	298	298	268	272	240	274	298	328
F207P4	262	282	298	298	268	272	240	274	298	310
F207P5	282	294	298	313	260	268	240	240	298	328
F207P6	262	294	298	298	268	272	240	274	298	328
F207P7	294	294	298	313	260	268	240	274	298	310
F207P8	262	294	313	335	268	272	240	274	298	310
F207P9	262	282	298	335	260	260	240	240	298	328
F207P10	262	282	313	335	268	272	240	240	298	328
F210M	294	294	300	300	244	244	234	234	276	304
F210F	288	288	298	300	244	244	228	266	316	316
F210P1	288	294	300	300	244	244	234	266	304	316
F210P2	288	294	298	300	244	244	234	266	276	316
F210P3	288	294	298	300	244	244	228	234	276	316
F210P4	288	294	298	300	244	244	234	266	276	316
F210P5	288	294	300	300	244	244	266	266	276	316
F210P6	288	294	300	300	244	244	228	234	276	316
F210P7	288	294	300	300	244	244	228	234	304	316
F210P8	288	294	298	300	244	244	228	234	276	316
F210P9	288	294	298	300	244	244	234	266	276	316
F210P10	288	294	298	300	244	244	234	266	276	316
F501M	?	?	298	298	250	274	228	240	292	316
F501F	264	302	298	328	232	232	228	228	292	320
F501P1	264	264	298	298	250	250	228	228	292	292
F501P2	264	264	298	298	?	?	228	240	292	316

Appendix Table A2 (Continued)

ID	*Mbr1a*	*Mbr1b*	*Mbr2a*	*Mbr2b*	*Mbr3a*	*Mbr3b*	*Mbr4a*	*Mbr4b*	*Mbr5a*	*Mbr5b*
F501P3	302	302	298	328	232	250	228	228	292	292
F501P4	302	302	298	328	232	274	228	240	292	292
F501P5	264	264	298	328	274	274	228	228	292	316
F501P6	?	?	298	298	232	274	228	240	292	320
F501P7	?	?	298	298	250	250	228	228	316	320
F501P8	302	302	298	328	274	274	228	228	292	292
F501P9	264	264	298	328	232	274	228	240	316	320
F501P10	302	302	298	298	274	274	228	240	292	292
F503M	?	?	298	328	232	232	234	296	316	320
F503F	?	?	296	335	232	232	234	296	296	298
F503P1	?	?	296	298	232	250	240	296	296	320
F503P2	?	?	328	335	246	246	234	234	298	316
F503P3	?	?	296	335	232	250	256	296	298	320
F503P4	?	?	296	335	232	246	234	256	298	298
F503P5	?	?	328	335	232	246	256	296	296	296
F503P6	?	?	296	335	246	246	234	240	298	320
F503P7	?	?	296	335	232	250	234	240	298	298
F503P8	?	?	296	298	232	250	?	?	296	320
F503P9	?	?	296	298	232	250	234	240	298	320
F503P10	?	?	296	298	250	250	256	296	298	316
F505M	266	288	296	296	228	228	238	256	308	320
F505F	?	?	298	298	244	248	228	228	298	306
F505P1	266	266	296	298	228	244	228	238	298	308
F505P2	288	288	296	298	248	248	228	238	306	320
F505P3	266	266	296	298	228	244	228	238	306	308

Appendix Table A2 (Continued)

ID	*Mbr1a*	*Mbr1b*	*Mbr2a*	*Mbr2b*	*Mbr3a*	*Mbr3b*	*Mbr4a*	*Mbr4b*	*Mbr5a*	*Mbr5b*
F505P4	288	288	296	298	228	244	228	256	298	308
F505P5	?	?	296	298	228	244	228	256	298	320
F505P6	288	288	296	298	228	248	228	238	298	320
F505P7	266	266	296	298	228	244	228	256	306	308
F505P8	288	288	296	298	244	244	228	256	298	308
F505P9	266	266	296	298	228	244	228	238	298	308
F505P10	?	?	296	298	?	?	228	256	306	308
F506M	286	300	292	298	248	250	228	234	308	318
F506F	292	300	298	298	230	244	228	234	298	306
F506P1	286	300	292	298	230	250	234	234	298	308
F506P2	292	300	298	298	244	248	228	234	298	308
F506P3	286	292	292	298	244	248	228	228	298	318
F506P4	300	300	292	298	230	250	228	228	306	308
F506P5	292	300	298	298	230	250	234	234	306	318
F506P6	286	300	298	298	230	250	228	234	298	318
F506P7	286	292	?	?	244	250	228	228	298	308
F506P8	300	300	298	298	230	250	234	234	298	318
F506P9	292	300	292	298	244	250	228	228	298	318
F506P10	286	300	298	298	244	248	228	228	298	308
F507M	264	292	296	298	244	248	240	248	316	320
F507F	276	276	296	303	244	244	248	256	306	310
F507P1	276	292	298	303	244	248	240	248	306	316
F507P2	276	292	296	303	?	?	240	248	306	320
F507P3	276	292	296	303	244	244	248	256	306	320
F507P4	264	276	298	303	244	248	248	256	310	320

Appendix Table A2 (Continued)

ID	*Mbr1a*	*Mbr1b*	*Mbr2a*	*Mbr2b*	*Mbr3a*	*Mbr3b*	*Mbr4a*	*Mbr4b*	*Mbr5a*	*Mbr5b*
F507P5	264	276	296	298	244	248	248	248	310	320
F507P6	264	276	296	303	244	244	248	248	310	320
F507P7	264	276	296	303	244	244	248	248	310	316
F507P8	276	292	296	296	244	244	240	256	310	316
F507P9	276	292	296	303	244	248	240	256	310	320
F507P10	276	292	298	303	244	244	240	256	310	320
F509M	?	?	296	335	230	260	234	240	298	320
F509F	260	286	298	298	244	248	234	240	316	320
F509P1	260	260	296	298	230	248	240	240	320	320
F509P2	260	260	296	298	248	260	234	234	316	320
F509P3	260	260	298	335	230	248	234	240	298	320
F509P4	?	?	296	298	230	244	234	240	298	316
F509P5	260	260	298	335	244	260	234	240	298	320
F509P6	260	260	296	298	244	260	234	240	298	316
F509P7	286	286	298	335	244	260	240	240	316	320
F509P8	286	286	298	335	230	248	234	240	?	?
F509P9	?	?	298	335	244	260	234	234	316	320
F509P10	286	286	296	298	248	260	234	240	298	316
F511M	264	300	298	298	244	244	228	240	310	316
F511F	286	296	296	315	248	248	228	234	296	316
F511P1	286	300	296	298	244	248	228	240	296	316
F511P2	264	286	298	315	244	248	234	240	310	316
F511P3	296	300	?	?	244	248	228	234	296	310
F511P4	264	296	296	296	248	248	234	240	296	310
F511P5	286	300	315	315	244	248	228	240	296	316

Appendix Table A2 (Continued)

ID	*Mbr1a*	*Mbr1b*	*Mbr2a*	*Mbr2b*	*Mbr3a*	*Mbr3b*	*Mbr4a*	*Mbr4b*	*Mbr5a*	*Mbr5b*
F511P6	264	286	296	298	248	248	234	240	316	316
F511P7	296	300	315	315	244	248	228	234	296	316
F511P8	264	296	315	315	244	248	228	228	316	316
F511P9	286	300	296	298	244	248	228	240	296	310
F511P10	264	286	296	296	244	248	234	240	316	316
F512M	?	?	298	298	248	260	234	238	316	316
F512-1F	254	284	296	298	244	244	228	246	306	318
F512-1P1	254	254	298	298	244	260	228	238	316	318
F512-1P2	?	?	298	298	244	248	?	?	306	316
F512-1P3	284	284	298	298	244	248	234	246	316	318
F512-1P4	254	254	298	298	244	248	234	246	306	316
F512-1P5	284	284	298	298	244	248	234	246	306	316
F512-1P6	284	284	296	298	244	260	228	234	306	316
F512-1P7	254	254	298	298	244	248	228	234	316	318
F512-1P8	254	254	296	298	244	260	234	246	316	318
F512-1P9	?	?	298	298	244	260	228	238	316	318
F512-1P10	284	284	296	298	244	260	238	246	316	318
F513M	262	300	298	298	232	232	230	236	292	308
F513F	?	?	296	335	244	244	240	258	292	308
F513P1	300	300	298	335	232	244	236	240	292	292
F513P2	262	262	296	298	244	244	230	258	308	308
F513P3	262	262	296	298	244	244	230	258	292	292
F513P4	262	262	296	298	232	244	236	240	292	308
F513P5	300	300	296	298	232	244	236	240	292	308
F513P6	?	?	296	298	232	232	230	258	292	308

Appendix Table A2 (Continued)

ID	*Mbr1a*	*Mbr1b*	*Mbr2a*	*Mbr2b*	*Mbr3a*	*Mbr3b*	*Mbr4a*	*Mbr4b*	*Mbr5a*	*Mbr5b*
F513P7	?	?	296	298	232	232	230	240	292	292
F513P8	300	300	298	335	232	244	230	240	292	292
F513P9	300	300	298	335	?	?	236	240	292	308
F513P10	300	300	296	298	244	244	236	258	292	308
F514M	?	?	296	298	244	244	230	236	316	320
F514F	284	300	296	298	232	244	230	240	298	320
F514P1	284	284	298	298	244	244	?	?	320	320
F514P2	284	284	296	298	244	244	236	240	298	320
F514P3	284	284	298	298	244	244	230	230	298	316
F514P4	300	300	296	298	244	244	230	240	316	320
F514P5	300	300	296	298	244	244	230	230	298	316
F514P6	300	300	296	298	244	244	230	240	316	320
F514P7	?	?	298	298	244	244	236	240	316	320
F514P8	?	?	296	298	232	232	230	240	298	320
F514P9	284	284	296	298	232	232	230	230	298	316
F514P10	284	284	298	298	232	244	236	240	?	?
F515M	280	296	296	296	240	244	238	248	306	320
F515F	260	300	296	298	232	244	234	242	308	316
F515P1	280	300	296	298	244	244	234	248	306	308
F515P2	280	300	296	298	232	244	242	248	316	320
F515P3	260	296	296	298	232	244	242	248	316	320
F515P4	260	280	296	296	232	244	234	238	316	320
F515P5	296	300	296	296	240	244	?	?	306	316
F515P6	260	296	296	296	232	244	234	248	306	316
F515P7	296	300	296	296	232	244	234	238	316	320

Appendix Table A2 (Continued)

ID	*Mbr1a*	*Mbr1b*	*Mbr2a*	*Mbr2b*	*Mbr3a*	*Mbr3b*	*Mbr4a*	*Mbr4b*	*Mbr5a*	*Mbr5b*
F515P8	260	280	296	296	232	240	?	?	308	320
F515P9	296	300	296	296	244	244	234	238	306	316
F515P10	280	300	296	298	?	?	234	238	306	308
F512M	?	?	298	298	248	260	234	238	316	316
F512-2F	270	270	296	296	244	254	248	248	276	310
F512-2P1	270	270	296	298	244	260	234	238	276	316
F512-2P2	270	270	296	298	244	260	234	234	310	316
F512-2P3	270	270	296	298	254	260	238	248	310	316
F512-2P4	?	?	296	298	254	260	238	248	276	316
F512-2P5	?	?	296	298	248	254	234	234	276	316
F512-2P6	270	270	296	298	248	254	?	?	276	316
F512-2P7	270	270	296	298	244	260	238	248	276	316
F512-2P8	270	270	296	298	254	260	238	248	276	316
F512-2P9	270	270	296	298	248	254	238	238	310	316
F512-2P10	270	270	296	298	244	248	?	?	276	316
F109M	276	282	296	301	230	262	216	248	298	310
F109F	294	294	296	328	256	256	234	310	310	316
F109P1	276	294	301	328	230	256	234	248	298	316
F109P2	276	294	301	328	230	256	234	248	298	316
F109P3	282	294	296	296	256	262	216	234	298	310
F109P4	282	294	296	328	256	262	216	310	298	310
F109P5	282	294	296	296	230	256	216	234	310	316
F109P6	282	294	296	301	230	256	216	310	310	316
F109P7	282	294	296	328	230	256	216	310	310	316
F109P8	282	294	296	301	230	256	216	234	310	310

Appendix Table A2 (Continued)

ID	*Mbr1a*	*Mbr1b*	*Mbr2a*	*Mbr2b*	*Mbr3a*	*Mbr3b*	*Mbr4a*	*Mbr4b*	*Mbr5a*	*Mbr5b*
F109P9	276	294	296	296	256	262	248	310	298	316
F109P10	276	294	296	328	256	262	216	310	310	316
F11M	264	264	296	298	256	264	222	234	292	310
F11F	282	282	296	298	250	258	216	228	292	310
F11P1	264	282	296	296	250	264	216	234	310	310
F11P2	264	282	296	298	250	256	228	234	310	310
F11P3	264	282	296	298	250	256	216	234	310	310
F11P4	264	282	296	296	250	256	222	228	310	310
F11P5	264	282	296	298	?	?	216	222	310	310
F11P6	264	282	296	296	250	264	228	234	?	?
F11P7	264	282	298	298	250	264	216	234	?	?
F11P8	264	282	296	298	256	258	228	234	310	310
F11P9	264	282	296	298	250	264	216	222	292	310
F11P10	264	282	296	296	250	264	222	228	292	310
F12M	268	282	298	335	244	244	252	296	306	306
F12F	260	284	296	296	256	256	252	252	306	306
F12P1	282	284	296	335	244	256	252	296	306	306
F12P2	260	282	296	298	244	256	252	252	306	306
F12P3	268	284	296	335	244	256	252	296	306	306
F12P4	260	268	296	335	244	244	252	296	306	306
F12P5	260	282	296	298	244	244	252	252	306	306
F12P6	282	284	296	335	244	244	252	296	306	306
F12P7	282	284	296	335	244	256	252	296	306	306
F12P8	260	282	296	298	244	256	252	252	306	306
F12P9	260	282	296	298	244	256	252	296	306	306

Appendix Table A2 (Continued)

ID	*Mbr1a*	*Mbr1b*	*Mbr2a*	*Mbr2b*	*Mbr3a*	*Mbr3b*	*Mbr4a*	*Mbr4b*	*Mbr5a*	*Mbr5b*
F12P10	268	284	296	335	244	256	252	296	306	306
F101M	271	277	260	260	247	258	246	257	252	262
F101F	277	277	254	265	247	255	246	254	242	242
F101P1	271	277	254	260	247	255	246	254	242	262
F101P2	271	277	254	260	255	258	254	257	262	262
F101P3	271	277	260	265	?	?	246	254	262	262
F101P4	271	277	254	260	247	258	246	257	252	252
F101P5	271	277	254	260	247	258	254	257	262	262
F101P6	277	277	254	260	247	255	246	257	252	262
F101P7	271	277	260	265	247	258	254	257	262	262
F101P8	271	277	254	260	247	258	246	246	242	252
F101P9	271	277	254	260	?	?	246	257	242	262
F101P10	271	277	254	260	247	258	246	257	242	252
ID	*Mbr7a*	*Mbr7b*	*Mbr8a*	*Mbr8b*	*Mbr9a*	*Mbr9b*	*Mbr10a*	*Mbr10b*	*Mbr11a*	*Mbr11b*
F101M	271	277	260	260	247	258	246	257	252	262
F101F	277	277	254	265	247	255	246	254	242	242
F101P1	271	277	254	260	247	255	246	254	242	262
F101P2	271	277	254	260	255	258	254	257	262	262
F101P3	271	277	260	265	?	?	246	254	262	262
F101P4	271	277	254	260	247	258	246	257	252	252
F101P5	271	277	254	260	247	258	254	257	262	262
F101P6	277	277	254	260	247	255	246	257	252	262
F101P7	271	277	260	265	247	258	254	257	262	262
F101P8	271	277	254	260	247	258	246	246	242	252
F101P9	271	277	254	260	?	?	246	257	242	262

Appendix Table A2 (Continued)

ID	*Mbr7a*	*Mbr7b*	*Mbr8a*	*Mbr8b*	*Mbr9a*	*Mbr9b*	*Mbr10a*	*Mbr10b*	*Mbr11a*	*Mbr11b*
F101P10	271	277	254	260	247	258	246	257	242	252
F104M	277	277	260	263	244	255	243	254	242	262
F104F	277	277	254	265	247	252	246	251	254	256
F104P1	277	277	263	265	244	252	243	251	254	262
F104P2	277	277	263	265	244	247	246	254	254	262
F104P3	277	277	254	260	247	255	246	254	242	256
F104P4	277	277	260	265	244	252	246	254	254	262
F104P5	277	277	263	265	247	255	243	246	242	242
F104P6	277	277	260	265	247	255	246	254	242	242
F104P7	277	277	260	265	252	255	251	254	256	262
F104P8	277	277	263	265	252	255	243	251	242	256
F104P9	277	277	254	263	247	255	243	251	242	256
F104P10	277	277	260	265	252	255	251	254	254	262
F107M	274	277	265	265	235	247	233	246	242	242
F107F	271	282	254	265	252	255	251	254	248	248
F107P1	274	282	254	265	235	252	233	254	242	242
F107P2	277	282	254	265	247	252	233	251	242	248
F107P3	277	282	254	265	235	252	246	251	242	248
F107P4	271	274	265	265	235	255	233	254	242	248
F107P5	274	282	254	265	247	255	246	251	242	248
F107P6	271	277	254	265	247	255	246	254	242	242
F107P7	277	282	265	265	247	252	246	251	?	?
F107P8	277	282	265	265	235	255	246	251	242	248
F107P9	277	282	254	265	235	255	246	254	?	?
F107P10	271	277	254	265	247	252	233	254	242	242

Appendix Table A2 (Continued)

ID	*Mbr7a*	*Mbr7b*	*Mbr8a*	*Mbr8b*	*Mbr9a*	*Mbr9b*	*Mbr10a*	*Mbr10b*	*Mbr11a*	*Mbr11b*
F108M	271	277	265	265	244	247	243	246	242	254
F108F	271	277	265	265	235	258	233	257	254	266
F108P1	271	277	265	265	247	258	246	257	?	?
F108P2	271	277	265	265	235	244	233	243	254	254
F108P3	277	277	265	265	235	244	243	257	254	266
F108P4	271	277	265	265	247	258	246	257	242	242
F108P5	271	271	265	265	247	258	233	243	254	266
F108P6	271	277	265	265	244	258	246	257	254	254
F108P7	271	277	265	265	?	?	246	257	254	266
F108P8	271	277	265	265	235	244	233	243	242	242
F108P9	271	277	265	265	247	258	246	257	254	254
F108P10	271	277	265	265	247	258	233	243	254	266
F201M	277	277	260	260	247	247	246	246	242	256
F201F	277	277	263	265	247	255	246	254	248	256
F201P1	277	277	260	263	247	247	246	246	256	256
F201P2	277	277	260	265	247	247	246	246	242	248
F201P3	277	277	260	265	247	247	246	254	242	248
F201P4	277	277	260	265	247	247	246	254	?	?
F201P5	277	277	260	265	247	247	246	246	248	256
F201P6	277	277	260	263	247	255	246	246	256	256
F201P7	277	277	260	263	247	247	246	254	248	256
F201P8	277	277	260	265	247	255	246	254	248	256
F201P9	277	277	260	263	247	255	246	246	256	256
F201P10	277	277	260	263	247	247	246	246	242	248
F203M	277	282	263	265	255	255	254	254	246	274

Appendix Table A2 (Continued)

ID	*Mbr7a*	*Mbr7b*	*Mbr8a*	*Mbr8b*	*Mbr9a*	*Mbr9b*	*Mbr10a*	*Mbr10b*	*Mbr11a*	*Mbr11b*
F203F	277	277	263	265	247	247	246	246	242	256
F203P1	277	282	265	265	247	255	246	254	?	?
F203P2	277	277	263	263	247	255	246	254	256	274
F203P3	277	277	265	265	247	255	246	254	242	246
F203P4	277	277	263	265	247	255	246	254	256	274
F203P5	277	282	263	265	247	255	246	254	246	256
F203P6	277	277	265	265	247	255	246	254	246	256
F203P7	277	277	265	265	247	255	246	254	242	246
F203P8	277	277	263	265	247	255	246	254	256	274
F203P9	277	277	263	265	247	255	246	254	256	274
F203P10	277	282	263	265	247	255	246	254	246	256
F207M	277	277	254	263	255	255	254	254	258	258
F207F	277	277	257	265	244	244	243	243	248	248
F207P1	277	277	257	263	244	255	243	254	248	248
F207P2	277	277	257	263	244	255	243	254	248	258
F207P3	277	277	263	265	244	255	243	254	248	258
F207P4	277	277	257	263	244	255	243	254	248	248
F207P5	277	277	263	265	244	255	243	254	248	248
F207P6	277	277	254	257	244	255	243	254	248	248
F207P7	277	277	254	265	244	255	243	254	248	258
F207P8	277	277	254	257	244	255	243	254	248	258
F207P9	277	277	254	257	244	255	243	254	248	258
F207P10	277	277	254	257	244	255	243	254	248	258
F210M	277	277	254	263	258	258	257	257	254	268
F210F	277	277	254	257	255	258	254	257	254	256
F210P1	277	277	254	263	255	258	254	257	254	254

Appendix Table A2 (Continued)

ID	*Mbr7a*	*Mbr7b*	*Mbr8a*	*Mbr8b*	*Mbr9a*	*Mbr9b*	*Mbr10a*	*Mbr10b*	*Mbr11a*	*Mbr11b*
F210P2	277	277	254	254	?	?	257	257	254	254
F210P3	277	277	254	257	258	258	257	257	268	268
F210P4	277	277	254	263	?	?	257	257	254	254
F210P5	277	277	257	263	?	?	254	257	268	268
F210P6	277	277	254	254	258	258	254	257	256	268
F210P7	277	277	257	263	255	258	257	257	254	256
F210P8	277	277	254	257	?	?	254	257	254	254
F210P9	277	277	254	263	258	258	257	257	256	268
F210P10	277	277	257	263	255	258	254	257	254	256
F501M	271	277	265	265	252	255	251	254	248	256
F501F	271	271	257	265	252	255	251	254	256	256
F501P1	271	271	265	265	252	252	251	251	256	256
F501P2	271	271	257	265	252	255	?	?	248	248
F501P3	271	271	257	265	252	255	254	254	256	256
F501P4	271	271	257	265	252	255	254	254	256	256
F501P5	271	277	265	265	252	252	251	254	256	256
F501P6	271	277	257	265	252	255	251	254	248	248
F501P7	271	271	257	265	252	255	251	254	248	256
F501P8	271	271	257	265	252	255	251	254	256	256
F501P9	271	277	265	265	252	255	251	251	248	256
F501P10	271	277	257	265	252	255	251	254	248	256
F503M	271	271	257	265	255	255	254	254	248	256
F503F	277	277	265	265	252	255	251	254	258	268
F503P1	271	277	265	265	252	252	251	254	256	258
F503P2	271	277	265	265	252	252	251	254	256	258
F503P3	271	277	?	?	255	258	?	?	256	258

Appendix Table A2 (Continued)

ID	*Mbr7a*	*Mbr7b*	*Mbr8a*	*Mbr8b*	*Mbr9a*	*Mbr9b*	*Mbr10a*	*Mbr10b*	*Mbr11a*	*Mbr11b*
F503P4	271	277	257	265	252	255	251	251	256	258
F503P5	271	277	257	265	255	258	254	254	256	258
F503P6	271	277	265	265	255	258	254	254	256	258
F503P7	271	277	265	265	252	252	251	251	256	258
F503P8	271	277	?	?	255	258	251	251	268	268
F503P9	271	277	257	265	252	252	254	254	256	258
F503P10	271	277	257	265	252	252	?	?	256	268
F505M	271	277	257	265	244	247	243	246	?	?
F505F	271	277	257	265	255	255	254	254	?	?
F505P1	271	271	265	265	244	255	243	254	?	?
F505P2	271	277	257	265	247	255	246	254	?	?
F505P3	271	277	265	265	247	255	246	254	?	?
F505P4	271	277	257	265	247	255	243	254	?	?
F505P5	271	277	265	265	247	255	246	254	?	?
F505P6	271	277	257	265	247	255	246	254	?	?
F505P7	277	277	265	265	247	255	243	254	?	?
F505P8	271	277	265	265	247	255	246	254	?	?
F505P9	271	277	265	265	247	255	243	254	?	?
F505P10	277	277	257	265	247	255	243	254	?	?
F506M	277	277	263	265	244	247	243	246	238	248
F506F	271	277	257	265	252	255	251	254	248	256
F506P1	271	277	?	?	244	255	243	254	238	248
F506P2	271	277	?	?	247	255	243	254	248	248
F506P3	277	277	265	265	247	252	246	254	248	256

Appendix Table A2 (Continued)

ID	*Mbr7a*	*Mbr7b*	*Mbr8a*	*Mbr8b*	*Mbr9a*	*Mbr9b*	*Mbr10a*	*Mbr10b*	*Mbr11a*	*Mbr11b*
F506P4	277	277	?	?	244	255	?	?	248	248
F506P5	277	277	?	?	244	255	246	251	238	248
F506P6	271	277	?	?	244	255	246	254	238	256
F506P7	271	277	257	263	244	255	?	?	238	248
F506P8	277	277	?	?	244	252	246	254	238	248
F506P9	277	277	257	265	244	252	243	254	238	256
F506P10	271	277	257	265	244	255	243	251	238	256
F507M	277	277	265	265	252	258	251	257	256	258
F507F	268	277	260	265	244	247	243	246	?	?
F507P1	268	277	265	265	247	252	246	251	256	258
F507P2	268	277	265	265	247	252	246	257	256	258
F507P3	277	277	260	265	244	258	246	257	?	?
F507P4	268	277	265	265	244	258	243	251	?	?
F507P5	277	277	260	265	244	252	243	257	256	258
F507P6	268	277	265	265	247	252	246	251	256	258
F507P7	277	277	265	265	247	258	243	251	256	258
F507P8	268	277	260	265	244	258	246	251	256	258
F507P9	277	277	?	?	244	258	243	251	?	?
F507P10	277	277	265	265	244	252	243	257	256	258
F509M	277	277	263	265	255	258	254	257	256	258
F509F	271	271	263	265	252	258	251	257	256	256
F509P1	271	277	?	?	258	258	257	257	256	258
F509P2	271	277	263	263	252	255	257	257	256	256
F509P3	271	277	265	265	252	255	254	257	256	258

Appendix Table A2 (Continued)

ID	*Mbr7a*	*Mbr7b*	*Mbr8a*	*Mbr8b*	*Mbr9a*	*Mbr9b*	*Mbr10a*	*Mbr10b*	*Mbr11a*	*Mbr11b*
F509P4	271	277	265	265	258	258	251	254	256	256
F509P5	271	277	265	265	252	255	251	254	256	258
F509P6	271	277	265	265	252	255	251	254	256	258
F509P7	271	277	263	263	255	258	251	254	256	258
F509P8	271	277	263	265	252	255	251	254	256	256
F509P9	271	277	263	265	252	255	251	254	256	256
F509P10	271	277	265	265	255	258	251	254	256	258
F511M	277	277	257	265	252	258	251	257	256	256
F511F	271	277	265	265	255	255	254	254	238	256
F511P1	277	277	257	265	252	255	251	254	256	256
F511P2	271	277	257	265	252	255	251	254	256	256
F511P3	277	277	257	265	252	255	251	254	238	256
F511P4	277	277	265	265	252	255	254	257	256	256
F511P5	271	277	257	265	252	255	251	254	238	256
F511P6	277	277	257	265	252	255	251	254	256	256
F511P7	271	277	265	265	252	255	251	254	256	256
F511P8	277	277	257	265	252	255	251	254	256	256
F511P9	277	277	257	265	252	255	251	254	238	238
F511P10	277	277	257	265	252	255	251	254	238	238
F512M	271	277	257	265	252	258	251	257	256	258
F512-1F	274	277	257	260	247	247	246	246	248	268
F512-1P1	274	277	257	265	247	252	246	251	256	256
F512-1P2	271	277	257	265	247	252	246	251	248	268
F512-1P3	271	277	257	257	247	258	246	251	248	268

Appendix Table A2 (Continued)

ID	*Mbr7a*	*Mbr7b*	*Mbr8a*	*Mbr8b*	*Mbr9a*	*Mbr9b*	*Mbr10a*	*Mbr10b*	*Mbr11a*	*Mbr11b*
F512-1P4	271	277	260	265	247	252	246	251	256	268
F512-1P5	277	277	257	265	247	252	246	257	256	256
F512-1P6	274	277	257	257	247	258	246	257	256	256
F512-1P7	277	277	257	265	247	252	246	251	256	258
F512-1P8	271	277	260	265	247	252	246	251	?	?
F512-1P9	271	274	257	260	247	252	246	251	256	258
F512-1P10	271	277	257	265	247	252	246	251	248	268
F513M	277	277	254	257	252	252	251	251	258	258
F513F	271	277	257	260	252	255	251	254	250	250
F513P1	277	277	257	257	244	252	251	251	250	250
F513P2	277	277	254	260	244	252	251	251	250	250
F513P3	271	277	257	260	244	258	251	251	258	258
F513P4	271	277	254	257	244	258	251	251	?	?
F513P5	277	277	?	?	244	258	251	251	250	258
F513P6	277	277	257	257	?	?	?	?	250	258
F513P7	277	277	257	257	244	252	251	254	250	258
F513P8	271	277	257	260	?	?	251	254	250	258
F513P9	277	277	257	260	?	?	251	254	250	250
F513P10	277	277	254	260	244	252	?	?	250	258
F514M	277	277	257	263	252	255	251	254	258	258
F514F	277	277	260	263	252	255	251	254	258	258
F514P1	277	277	263	263	252	252	251	251	258	258
F514P2	277	277	257	260	252	255	251	251	258	258
F514P3	277	277	260	263	252	252	?	?	258	258

Appendix Table A2 (Continued)

ID	*Mbr7a*	*Mbr7b*	*Mbr8a*	*Mbr8b*	*Mbr9a*	*Mbr9b*	*Mbr10a*	*Mbr10b*	*Mbr11a*	*Mbr11b*
F514P4	277	277	257	263	?	?	251	254	258	258
F514P5	277	277	260	263	252	255	251	251	258	258
F514P6	277	277	260	263	252	255	251	251	258	258
F514P7	277	277	263	263	?	?	?	?	258	258
F514P8	277	277	257	263	252	255	254	254	258	258
F514P9	277	277	260	263	252	252	254	254	258	258
F514P10	277	277	257	260	252	255	254	254	258	258
F515M	277	277	263	265	244	258	243	257	252	260
F515F	277	277	257	260	252	255	251	254	252	256
F515P1	277	277	257	263	?	?	251	257	252	256
F515P2	277	277	?	?	?	?	254	257	256	256
F515P3	277	277	?	?	?	?	243	254	252	256
F515P4	277	277	260	263	252	252	?	?	?	?
F515P5	277	277	260	263	255	255	251	257	?	?
F515P6	277	277	257	265	252	255	?	?	256	256
F515P7	277	277	260	263	252	252	243	254	252	256
F515P8	277	277	260	265	255	255	251	257	?	?
F515P9	277	277	257	263	252	255	251	257	252	256
F515P10	277	277	?	?	252	255	?	?	252	256
F512M	271	277	257	265	252	258	251	257	?	?
F512-2F	271	277	260	265	244	244	243	243	256	258
F512-2P1	271	277	?	?	?	?	243	251	?	?
F512-2P2	277	277	257	265	252	258	243	257	256	258
F512-2P3	271	277	257	260	?	?	243	251	?	?

Appendix Table A2 (Continued)

ID	*Mbr7a*	*Mbr7b*	*Mbr8a*	*Mbr8b*	*Mbr9a*	*Mbr9b*	*Mbr10a*	*Mbr10b*	*Mbr11a*	*Mbr11b*
F512-2P4	277	277	257	260	255	258	243	251	256	256
F512-2P5	271	277	?	?	?	?	243	257	?	?
F512-2P6	271	277	257	265	252	255	243	257	256	258
F512-2P7	277	277	257	265	?	?	243	257	256	258
F512-2P8	271	271	260	265	?	?	?	?	?	?
F512-2P9	271	271	?	?	244	255	?	?	?	?
F512-2P10	271	277	257	260	244	255	243	254	256	256
F109M	277	277	265	265	244	247	246	246	?	?
F109F	277	277	254	265	247	258	246	257	268	268
F109P1	277	277	254	265	247	247	246	246	268	268
F109P2	277	277	265	265	244	247	246	246	268	268
F109P3	277	277	254	265	247	258	246	246	?	?
F109P4	277	277	265	265	244	247	246	257	242	250
F109P5	277	277	265	265	247	247	246	246	242	242
F109P6	277	277	265	265	244	258	246	257	?	?
F109P7	277	277	265	265	247	258	246	257	256	268
F109P8	277	277	254	265	247	258	246	246	256	268
F109P9	277	277	265	265	244	247	246	246	268	268
F109P10	277	277	254	265	?	?	246	257	?	?
F11M	268	277	260	265	247	258	246	257	242	250
F11F	271	277	257	263	247	252	246	251	248	248
F11P1	271	277	263	265	247	247	246	246	250	268
F11P2	268	271	257	260	247	252	246	251	242	248
F11P3	277	277	260	263	252	258	251	257	242	248

Appendix Table A2 (Continued)

ID	*Mbr7a*	*Mbr7b*	*Mbr8a*	*Mbr8b*	*Mbr9a*	*Mbr9b*	*Mbr10a*	*Mbr10b*	*Mbr11a*	*Mbr11b*
F11P4	268	277	263	265	247	252	251	257	242	248
F11P5	271	277	257	265	252	258	251	257	242	248
F11P6	268	271	263	265	247	258	246	257	250	268
F11P7	268	271	263	265	252	258	246	246	242	248
F11P8	277	277	257	265	247	247	246	251	250	268
F11P9	277	277	257	265	247	247	251	257	250	268
F11P10	268	271	257	260	252	258	246	246	242	248
F12M	277	277	254	265	255	258	246	254	250	268
F12F	277	277	263	265	247	255	246	257	250	268
F12P1	277	277	263	265	247	258	246	257	250	250
F12P2	277	277	265	265	247	258	246	254	250	250
F12P3	277	277	263	265	247	255	246	254	250	250
F12P4	277	277	254	265	255	258	254	257	250	250
F12P5	277	277	265	265	247	255	254	257	250	268
F12P6	277	277	265	265	247	255	246	246	250	268
F12P7	277	277	254	263	247	258	246	246	250	268
F12P8	277	277	263	265	247	255	246	254	250	250
F12P9	277	277	254	265	247	255	246	254	250	250
F12P10	277	277	254	265	247	258	246	257	250	250

F___M* = Sire ID, F___F** = Dam ID, F___P___*** = Progeny ID, ?**** = Missing data, *a* and *b* indicate first and second allele in each genotypes, respectively.

Appendix Table A3 Genotypes of parents and their progeny (87 families) at *Mbr-5* locus for assessment of reproductive success among male broodstock.

Family	Sire	Dam	Progeny									
T1R1f1	295/317	305/317	295/305	295/317	305/317	295/305	295/317	295/305	295/317	305/317	295/305	295/317
T1R1f2	295/317	281/307	281/295	307/317	?*	281/317	281/317	281/317	307/317	?	281/317	281/317
T1R1f3	317/329	317/317	317/317	317/317	317/317	317/317	317/329	317/329	317/317	317/317	317/317	317/329
T1R1f4	295/317	295/319	317/319	317/319	317/319	295/317	295/295	317/319	317/319	317/319	295/317	295/295
T1R1f5	305/305	299/311	299/305	305/311	299/305	299/305	305/311	?	305/311	299/305	299/305	305/311
T1R1f6	317/329	297/299	297/329	297/317	299/317	297/317	299/299	297/317	297/317	299/317	297/317	299/299
T1R2f1	281/295	297/317	295/317	295/317	281/297	295/297	281/297	295/317	295/317	281/297	295/297	281/297
T1R2f2	281/295	317/317	317/329	317/329	291/317	317/329	317/329	291/317	317/329	291/317	317/329	317/329
T1R2f3	297/317	311/329	297/311	297/311	317/329	297/329	297/311	297/329	297/311	317/329	297/329	297/311
T1R2f4	281/295	291/317	295/317	295/317	295/317	291/295	291/295	281/291	295/317	295/317	291/295	291/295
T1R2f5	281/295	297/305	295/305	281/297	295/297	295/305	281/305	281/297	281/297	295/297	295/305	281/305
T1R2f6	291/329	311/311	291/311	311/329	291/311	311/329	291/311	291/311	311/329	291/311	311/329	291/311
T1R2f7	291/329	311/317	?	291/317	291/317	291/317	311/329	311/329	291/317	291/317	291/317	311/329
T1R2f8	291/329	299/329	291/329	?	291/299	?	291/299	291/299	?	291/299	?	291/299
T1R2f9	297/317	281/297	297/317	297/317	291/297	281/317	291/297	291/297	297/317	291/297	281/317	291/297
T1R3f1	317/317	297/313	313/317	295/317	?	313/317	295/317	295/317	295/317	?	313/317	295/317
T1R3f2	317/317	317/329	?	317/329	317/329	317/317	317/317	317/329	317/329	317/329	317/317	317/317

Appendix Table A3 (Continued)

Family	Sire	Dam	Progeny									
T1R3f3	317/317	295/319	295/317	295/317	317/319	317/319	317/319	295/317	295/317	317/319	317/319	317/319
T1R3f4	317/317	281/305	305/317	305/317	305/317	281/317	281/317	305/317	305/317	305/317	281/317	281/317
T1R3f5	317/317	297/317	317/317	297/317	317/317	297/317	297/317	317/317	297/317	317/317	297/317	297/317
T1R3f6	317/317	305/317	305/317	317/317	317/317	317/317	305/317	317/317	317/317	317/317	317/317	305/317
T1R3f7	297/311	307/309	307/311	297/307	297/307	297/307	297/309	?	297/307	297/307	297/307	297/309
T1R3f8	317/317	303/319	317/317	317/319	303/317	317/319	303/317	317/319	317/319	303/317	317/319	303/317
T1R3f9	317/317	317/317	317/317	317/317	317/317	317/317	317/317	317/317	317/317	317/317	317/317	317/317
T1R4f1	317/317	293/317	311/317	311/317	311/317	293/317	311/317	311/317	311/317	311/317	293/317	311/317
T1R4f2	317/317	309/317	317/317	309/317	317/317	309/317	309/317	309/317	309/317	317/317	309/317	309/317
T1R4f3	317/317	305/319	317/319	305/317	305/317	317/319	317/319	317/319	305/317	305/317	317/319	317/319
T1R4f4	317/317	291/317	291/317	317/317	317/317	307/317	291/317	317/317	317/317	317/317	307/317	291/317
T1R4f5	317/317	305/313	305/317	313/317	305/317	313/317	313/317	313/317	313/317	305/317	313/317	313/317
T1R4f6	317/321	297/315	297/321	297/317	297/321	297/321	297/317	315/321	297/317	297/321	297/321	297/317
T1R4f7	317/317	309/309	309/317	309/317	309/317	309/317	309/317	309/317	309/317	309/317	309/317	309/317
T1R4f8	317/317	297/313	297/317	313/317	313/317	313/317	297/317	297/317	313/317	313/317	313/317	297/317
T1R4f9	317/317	317/317	317/317	317/317	317/317	317/317	317/317	317/317	317/317	317/317	317/317	317/317
T1R4f10	317/317	305/309	309/317	305/317	305/317	309/317	309/317	309/317	305/317	305/317	309/317	309/317
T1R4f11	317/317	305/327	317/327	305/317	305/317	305/317	317/327	?	305/317	305/317	305/317	317/327
T1R4f12	317/317	297/321	297/317	297/317	297/317	317/321	317/321	297/317	297/317	297/317	317/321	317/321
T1R5f1	297/321	317/317	297/317	297/317	297/317	297/317	317/321	317/321	297/317	297/317	297/317	317/321
T1R5f2	297/317	305/309	297/309	305/317	309/317	309/317	297/305	305/317	305/317	309/317	309/317	297/305
T1R5f3	297/321	297/321	321/321	321/321	297/297	321/321	297/297	297/297	321/321	297/297	321/321	297/297
T1R5f4	297/321	305/317	317/321	317/321	305/321	297/305	297/317	297/317	317/321	305/321	297/305	297/317
T1R5f5	297/321	317/317	297/317	297/317	297/317	317/321	317/321	317/321	297/317	297/317	317/321	317/321

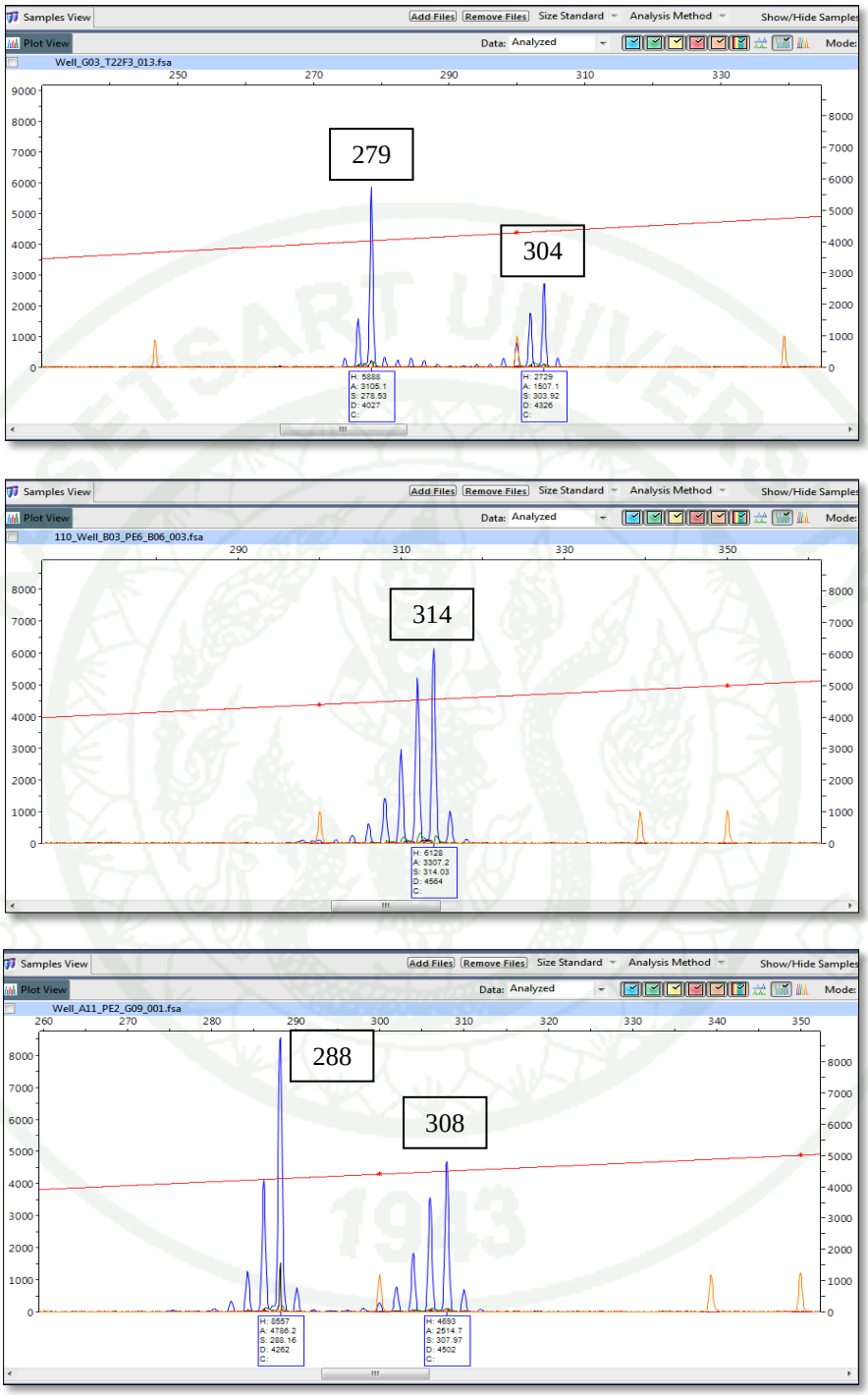
Appendix Table A3 (Continued)

Family	Sire	Dam	Progeny									
T1R5f6	297/321	291/307	309/321	297/309	291/321	309/321	309/321	309/321	297/309	291/321	309/321	309/321
T1R5f7	297/317	291/313	297/315	315/317	315/317	291/317	291/317	291/317	315/317	315/317	291/317	291/317
T1R6f1	291/321	305/327	291/327	291/305	291/327	305/321	321/327	291/305	291/305	291/327	305/321	321/327
T1R6f2	291/321	293/311	311/321	291/293	293/321	291/311	291/311	291/293	291/293	293/321	291/311	291/311
T1R6f3	291/321	299/309	299/321	309/321	309/321	299/321	291/309	309/321	309/321	309/321	299/321	291/309
T1R6f4	297/309	291/321	291/297	291/297	291/309	297/321	309/321	309/321	291/297	291/309	297/321	309/321
T1R6f5	291/321	309/309	291/317	317/321	291/309	317/321	309/321	309/321	317/321	291/309	317/321	309/321
T1R7f1	317/321	317/317	317/317	317/321	?	317/317	317/317	317/317	317/321	?	317/317	317/317
T1R7f2	317/317	305/309	305/317	305/317	309/317	309/317	305/317	305/317	305/317	309/317	309/317	305/317
T1R7f3	317/317	305/317	317/317	317/317	305/317	305/317	305/317	305/317	317/317	305/317	305/317	305/317
T1R7f4	317/317	307/309	307/317	307/317	307/317	309/317	307/317	307/317	307/317	307/317	309/317	307/317
T1R7f5	317/317	297/305	297/317	297/317	305/317	305/317	297/317	297/317	297/317	305/317	305/317	297/317
T1R7f6	317/317	313/317	317/317	317/317	317/317	317/317	317/317	313/317	317/317	317/317	317/317	317/317
T1R7f7	317/317	283/327	317/327	283/317	317/327	317/327	283/317	317/327	283/317	317/327	317/327	283/317
T1R7f8	317/317	305/309	309/317	309/317	309/317	305/317	309/317	309/317	309/317	309/317	305/317	309/317
T1R7f9	317/317	301/317	301/317	317/317	307/317	301/317	317/317	301/317	317/317	307/317	301/317	317/317
T1R7f10	317/317	291/299	299/317	291/317	299/317	291/317	291/317	299/317	291/317	299/317	291/317	291/317
T1R7f11	317/317	299/309	309/317	309/317	309/317	299/317	309/317	299/317	309/317	309/317	299/317	309/317
T2R1f1	305/307	303/317	307/317	303/305	305/317	305/317	307/317	303/305	303/305	305/317	305/317	307/317
T2R1f2	309/309	307/313	309/313	307/313	307/309	307/309	307/313	307/313	307/313	307/309	307/309	307/313
T2R1f3	305/307	281/305	281/307	281/305	281/305	305/307	281/307	305/307	281/305	281/305	305/307	281/307
T2R2f1	317/317	281/317	317/317	281/317	317/317	317/317	317/317	317/317	281/317	317/317	317/317	317/317
T2R2f2	317/317	305/305	305/317	305/317	305/317	305/317	305/317	305/317	305/317	305/317	305/317	305/317
T2R2f3	317/317	281/307	307/317	307/317	307/317	281/317	281/317	307/317	307/317	307/317	281/317	281/317

Appendix Table A3 (Continued)

Family	Sire	Dam	Progeny									
T2R3f1	297/317	307/317	317/317	317/317	297/307	307/317	297/317	297/307	317/317	297/307	307/317	297/317
T2R3f2	307/313	297/303	303/307	297/305	297/307	303/307	303/307	303/307	297/305	297/307	303/307	303/307
T2R3f3	307/313	297/321	297/305	297/307	305/321	297/307	297/305	305/321	297/307	305/321	297/307	297/305
T2R4f1	291/321	285/319	285/291	319/321	285/291	319/321	319/321	291/319	319/321	285/291	319/321	319/321
T2R4f2	291/321	295/319	319/321	291/295	295/321	319/321	291/319	319/321	291/295	295/321	319/321	291/319
T2R4f3	291/321	307/309	307/321	291/309	307/321	307/321	309/321	307/321	291/309	307/321	307/321	309/321
T2R5f1	291/321	281/327	281/291	281/321	321/327	281/291	281/321	321/327	281/321	321/327	281/291	281/321
T2R5f2	291/321	305/319	291/319	305/321	?	305/321	291/319	305/321	305/321	?	305/321	291/319
T2R5f3	291/321	297/309	297/321	291/309	291/309	291/297	291/309	291/297	291/309	291/309	291/297	291/309
T2R6f1	305/317	299/327	317/327	299/317	299/305	317/327	305/327	317/327	299/317	299/305	317/327	305/327
T2R6f2	291/307	317/317	307/317	291/317	307/317	307/317	307/317	291/317	291/317	307/317	307/317	307/317
T2R6f3	291/307	295/319	295/307	307/319	291/295	295/307	291/295	291/295	307/319	291/295	295/307	291/295
T2R7f1	291/307	317/317	307/317	291/317	307/317	307/317	307/317	307/317	291/317	307/317	307/317	307/317
T2R7f2	291/307	281/311	281/307	291/311	291/311	281/291	281/307	307/311	291/311	291/311	281/291	281/307
T2R7f3	305/317	297/321	305/319	305/319	315/319	297/315	297/305	297/315	305/319	315/319	297/315	297/305
T3R1f1	313/319	281/317	317/319	317/319	317/319	281/313	281/313	317/319	317/319	317/319	281/313	281/313
T3R2f1	299/329	281/307	281/299	305/317	281/329	281/299	281/329	299/307	305/317	281/329	281/299	281/329
T3R3f1	305/313	297/317	?	313/317	297/313	305/317	313/317	305/317	313/317	297/313	305/317	313/317
T3R4f1	309/317	297/309	297/317	309/317	309/317	?	297/317	297/317	309/317	309/317	?	297/317
T3R5f1	291/317	317/321	291/321	317/321	317/321	317/321	317/317	317/321	317/321	317/321	317/321	317/317
T3R6f1	291/321	297/305	291/305	305/321	297/321	291/305	?	291/297	305/321	297/321	291/305	?
T3R7f1	297/307	279/309	279/307	279/307	279/297	307/309	307/309	297/309	279/307	279/297	307/309	307/309

* ? indicates missing data.



Appendix Figure A1 Electrophoregrams of three individuals at *Mbr-5* locus using the ABI PRISM 3130 Genetic Analyzer (Applied Biosystems) and the Peak Scanner 1.0 software.

Appendix B

Statistical analysis results

1. ANOVA results of three prawn strains after 120 days of separate rearing.

1.1. Carapace length (CL)

Sourse	df	Sum of squares	Mean square	*F*-value	*P*-value
Strain	2	7,515.3361	3,757.6680	71.51	<0.0001
Tank(Strain)	6	357.4662	59.5776	1.13	0.3405
Error	891	46,819.7828	52.5475		
Total	899	54,692.5851			

Duncan's Multiple Range Test for CL

Means with the same letter are not significantly different.

Duncan grouping	Mean	N	Strain
A	42.1184	300	CPP
B	39.1901	300	KSB
C	35.0734	300	SKL

1.2. Total length (TL)

Sourse	df	Sum of squares	Mean square	*F*-value	*P*-value
Strain	2	22,281.3252	11,140.6626	63.13	<0.0001
Tank(Strain)	6	1,043.9920	173.9987	0.99	0.4334
Error	891	157,238.0814	176.4737		
Total	899	180,563.3987			

Duncan's Multiple Range Test for TL

Means with the same letter are not significantly different.

Duncan grouping	Mean	N	Strain
A	85.013	300	CPP
B	80.229	300	KSB
C	72.913	300	SKL

1.3. Body weight (BW)

Sourse	df	Sum of squares	Mean square	*F*-value	*P*-value
Strain	2	1,032.5228	516.2614	54.93	<0.0001
Tank(Strain)	6	117.2880	19.5480	2.08	0.0532
Error	891	8,374.5902	9.3991		
Total	899	9,524.4009			

Duncan's Multiple Range Test for BW

Means with the same letter are not significantly different.

Duncan grouping	Mean	N	Strain
A	6.7552	300	CPP
B	5.4309	300	KSB
C	4.1316	300	SKL

2. GLM (Type III error) results of three prawn strains after 120 days of communal rearing.

2.1. Carapace length (CL)

Sourse	df	Sum of squares	Mean square	*F*-value	*P*-value
Model	8	6,508.4174	813.5522	9.56	<0.0001
Tank	2	836.9402	418.4701	4.92	0.0075
Strain	2	5,233.0921	2,616.5460	30.76	<0.0001
Tank*Strain	4	152.0272	38.0068	0.45	0.7748
Error	1110	94,412.8476	85.0566		
Total	1118	100,921.2649			

Least Squares Means

Adjustment for Multiple Comparisons: Bonferroni

Strain	CL LSMEAN	LSMEAN Numer
CPP	40.6032	1
KSB	37.6376	2
SKL	35.8149	3

Least Squares Means for effect strain

Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: CL

i/j	1	2	3
1	-	0.0006	<0.0001
2	0.0006	-	0.0584
3	<0.0001	0.0584	-

2.2. Total length (TL)

Sourse	df	Sum of squares	Mean square	*F*-value	*P*-value
Model	8	24,925.5182	3,115.6898	4.31	<0.0001
Tank	2	5,223.8459	2,611.9229	3.61	0.0272
Strain	2	18,388.4943	9,194.2472	12.72	<0.0001
Tank*Strain	4	1108.7778	277.1944	0.38	0.8205
Error	1110	802,118.6210	722.6294		
Total	1118	827,044.1392			

Least Squares Means

Adjustment for Multiple Comparisons: Bonferroni

Strain	TL LSMEAN	LSMEAN Numer
CPP	83.9950	1
KSB	78.1577	2
SKL	75.0394	3

Least Squares Means for effect strain

Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: TL

i/j	1	2	3
1	-	0.0361	<0.0001
2	0.0361	-	0.5098
3	<0.0001	0.5098	-

2.3. Body weight (BW)

Sourse	df	Sum of squares	Mean square	*F*-value	*P*-value
Model	8	1,474.3128	184.2891	5.79	<0.0001
Tank	2	136.4880	68.2440	2.14	0.1178
Strain	2	1,201.6305	600.8153	18.87	<0.0001
Tank*Strain	4	95.3309	23.8327	0.75	0.5591
Error	1110	46,819.7828	31.8452		
Total	1118	54,692.5851			

Least Squares Means

Adjustment for Multiple Comparisons: Bonferroni

Strain	BW LSMEAN	LSMEAN Numer
CPP	7.0531	1
KSB	5.0953	2
SKL	4.8479	3

Least Squares Means for effect strain

Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: BW

i/j	1	2	3
1	-	0.0002	<0.0001
2	0.0002	-	1.0000
3	<0.0001	1.0000	-

3. ANOVA of condition factor (*K*) of male morphotypes.

Sourse	df	Sum of squares	Mean square	*F*-value	*P*-value
Male	2	0.00413553	0.00206776	1197.89	<0.0001
Error	147	0.00025375	0.00000173		
Total	149	0.00438927			

Duncan's Multiple Range Test for *K*

Means with the same letter are not significantly different.

Duncan grouping	Mean	N	Male
A	0.0164509	50	BC
B	0.0148156	50	OC
C	0.0045852	50	SM

4. GLM (Type III error) of reproductive success of male morphotypes.

Sourse	df	Sum of squares	Mean square	*F*-value	*P*-value
Model	8	234	29.25	19.06	<0.0001
Sex ratio	2	68.9524	34.4762	22.47	<0.0001
Male	2	98.9524	49.4762	32.24	<0.0001
SR*Male	4	66.0952	16.5238	10.77	<0.0001
Error	54	82.8571	1.5344		
Total	62	316.8571			

Least Squares Means

Adjustment for Multiple Comparisons: Bonferroni

Male	RS LSMEAN	LSMEAN Numer
BC	0.7328	1
OC	0.2115	2
SM	0.0667	3

Least Squares Means for effect Male

Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: RS

i/j	1	2	3
1	-	<0.0001	<0.0001
2	<0.0001	-	0.3871
3	<0.0001	0.3871	-

5. GLM (Type III error) and co-variables estimation of condition factor (*K*), body weight (BW), body length (BL), carapace length (CR) and relative claw length (RCL) on reproductive success of male morphotypes.

5.1. Condition factor (*K*)

Sourse	df	Sum of squares	Mean square	*F*-value	*P*-value
Model	9	251.2958	27.9218	22.57	<0.0001
Sex ratio	2	61.1248	30.5624	24.71	<0.0001
Male	2	97.1619	48.5809	39.27	<0.0001
SR*Male	4	45.6328	11.4082	9.22	<0.0001
K	1	17.2958	17.2958	13.98	0.0005
Error	53	65.5614	1.2370		
Total	62	316.8571			

Parameter	Estimate	*t*-value	*P*-value
K	394.6153	3.74	0.0005

5.2. Body weight (BW)

Sourse	df	Sum of squares	Mean square	*F*-value	*P*-value
Model	9	246.1386	27.3487	20.50	<0.0001
Sex ratio	2	45.5608	22.7804	17.07	<0.0001
Male	2	46.5190	23.2595	17.43	<0.0001
SR*Male	4	44.6191	11.1548	8.36	<0.0001
BW	1	12.1386	12.1386	9.10	0.0039
Error	53	70.7185	1.3343		
Total	62	316.8571			

Parameter	Estimate	*t*-value	*P*-value
BW	0.0301	3.02	0.0039

5.3. Body length (BL)

Sourse	df	Sum of squares	Mean square	*F*-value	*P*-value
Model	9	235.2344	26.1372	16.97	<0.0001
Sex ratio	2	63.2699	31.6350	20.54	<0.0001
Male	2	62.5137	31.2569	20.30	<0.0001
SR*Male	4	59.0838	14.7710	9.59	<0.0001
BL	1	1.2344	1.2344	0.80	0.3747
Error	53	81.6227	1.5401		
Total	62	316.8571			

Parameter	Estimate	*t*-value	*P*-value
BL	0.1823	0.90	0.3747

5.4. Carapace length (CR)

Sourse	df	Sum of squares	Mean square	*F*-value	*P*-value
Model	9	235.7054	26.1895	17.10	<0.0001
Sex ratio	2	61.8266	30.9133	20.19	<0.0001
Male	2	63.8054	31.9027	20.84	<0.0001
SR*Male	4	59.8885	14.9721	9.78	<0.0001
CR	1	1.7054	1.7054	1.11	0.2961
Error	53	81.1518	1.5312		
Total	62	316.8571			

Parameter	Estimate	*t*-value	*P*-value
CR	0.2898	1.06	0.2961

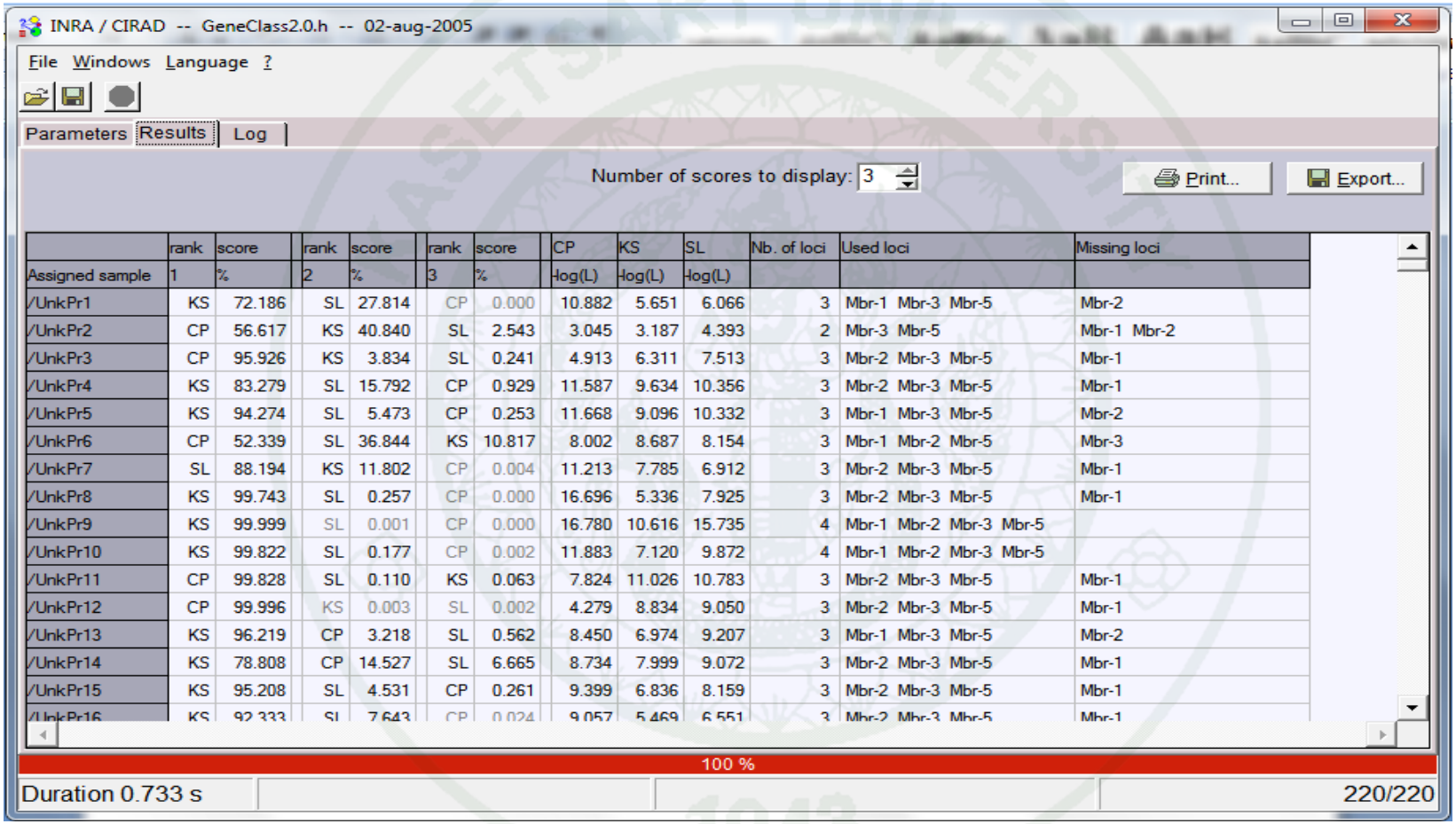
5.5. Relative claw length (RCL)

Sourse	df	Sum of squares	Mean square	*F*-value	*P*-value
Model	9	241.1445	26.7938	18.76	<0.0001
Sex ratio	2	55.0069	27.5035	19.25	<0.0001
Male	2	19.3305	9.6652	6.77	0.0024
SR*Male	4	61.8327	15.4582	10.82	<0.0001
RCL	1	7.1445	7.1445	2.24	0.0296
Error	53	75.7127	1.4285		
Total	62	316.8571			

Parameter	Estimate	*t*-value	*P*-value
RCL	2.1768	2.24	0.0296

Appendix C

Strain and parentage identification outputs from 3 computer programs

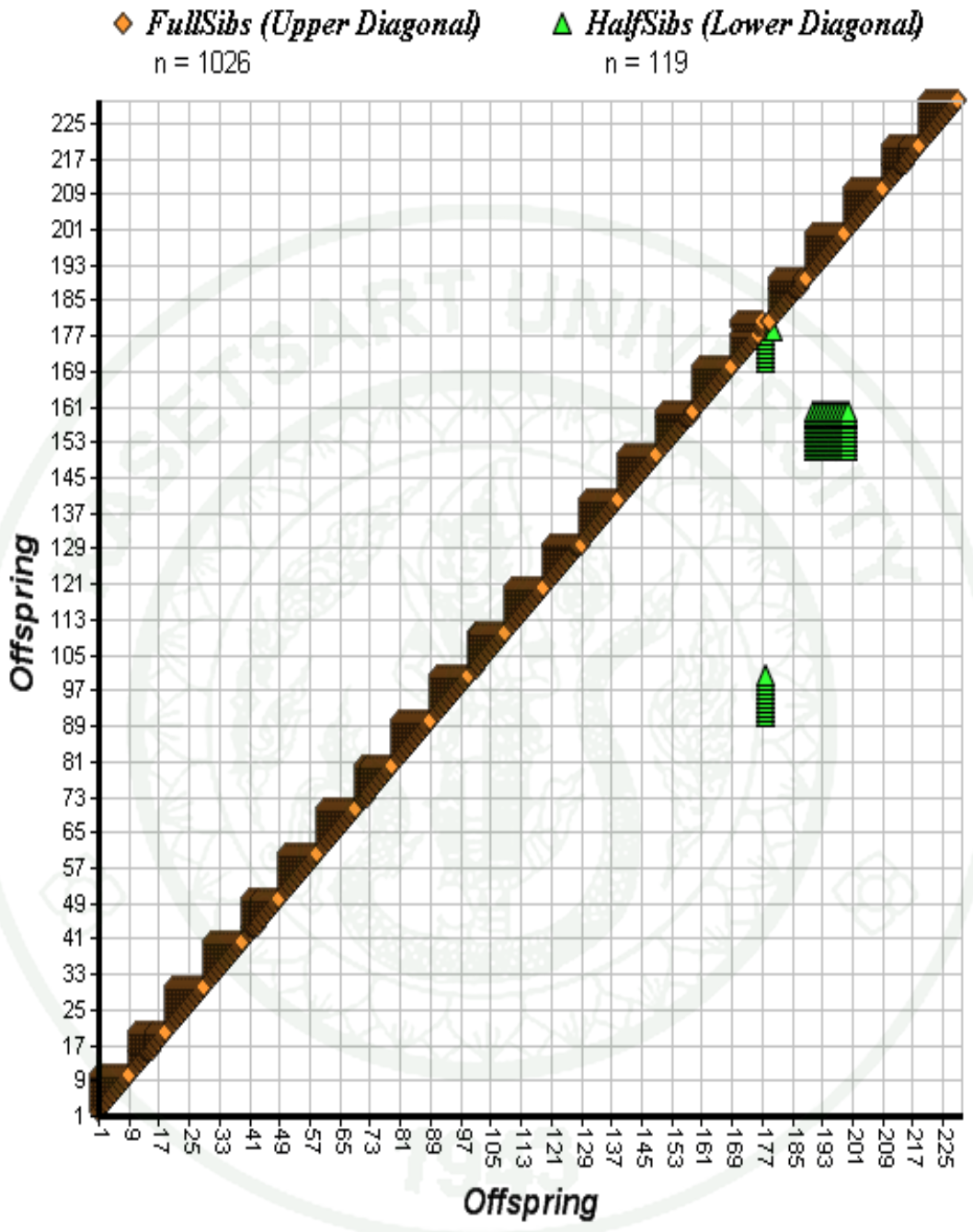


	rank	score	rank	score	rank	score	CP	KS	SL	Nb. of loci	Used loci	Missing loci
Assigned sample	1	%	2	%	3	%	log(L)	log(L)	log(L)			
/UnkPr1	KS	72.186	SL	27.814	CP	0.000	10.882	5.651	6.066	3	Mbr-1 Mbr-3 Mbr-5	Mbr-2
/UnkPr2	CP	56.617	KS	40.840	SL	2.543	3.045	3.187	4.393	2	Mbr-3 Mbr-5	Mbr-1 Mbr-2
/UnkPr3	CP	95.926	KS	3.834	SL	0.241	4.913	6.311	7.513	3	Mbr-2 Mbr-3 Mbr-5	Mbr-1
/UnkPr4	KS	83.279	SL	15.792	CP	0.929	11.587	9.634	10.356	3	Mbr-2 Mbr-3 Mbr-5	Mbr-1
/UnkPr5	KS	94.274	SL	5.473	CP	0.253	11.668	9.096	10.332	3	Mbr-1 Mbr-3 Mbr-5	Mbr-2
/UnkPr6	CP	52.339	SL	36.844	KS	10.817	8.002	8.687	8.154	3	Mbr-1 Mbr-2 Mbr-5	Mbr-3
/UnkPr7	SL	88.194	KS	11.802	CP	0.004	11.213	7.785	6.912	3	Mbr-2 Mbr-3 Mbr-5	Mbr-1
/UnkPr8	KS	99.743	SL	0.257	CP	0.000	16.696	5.336	7.925	3	Mbr-2 Mbr-3 Mbr-5	Mbr-1
/UnkPr9	KS	99.999	SL	0.001	CP	0.000	16.780	10.616	15.735	4	Mbr-1 Mbr-2 Mbr-3 Mbr-5	
/UnkPr10	KS	99.822	SL	0.177	CP	0.002	11.883	7.120	9.872	4	Mbr-1 Mbr-2 Mbr-3 Mbr-5	
/UnkPr11	CP	99.828	SL	0.110	KS	0.063	7.824	11.026	10.783	3	Mbr-2 Mbr-3 Mbr-5	Mbr-1
/UnkPr12	CP	99.996	KS	0.003	SL	0.002	4.279	8.834	9.050	3	Mbr-2 Mbr-3 Mbr-5	Mbr-1
/UnkPr13	KS	96.219	CP	3.218	SL	0.562	8.450	6.974	9.207	3	Mbr-1 Mbr-3 Mbr-5	Mbr-2
/UnkPr14	KS	78.808	CP	14.527	SL	6.665	8.734	7.999	9.072	3	Mbr-2 Mbr-3 Mbr-5	Mbr-1
/UnkPr15	KS	95.208	SL	4.531	CP	0.261	9.399	6.836	8.159	3	Mbr-2 Mbr-3 Mbr-5	Mbr-1
/UnkPr16	KS	92.333	SL	7.643	CP	0.024	9.057	5.469	6.551	3	Mbr-2 Mbr-3 Mbr-5	Mbr-1

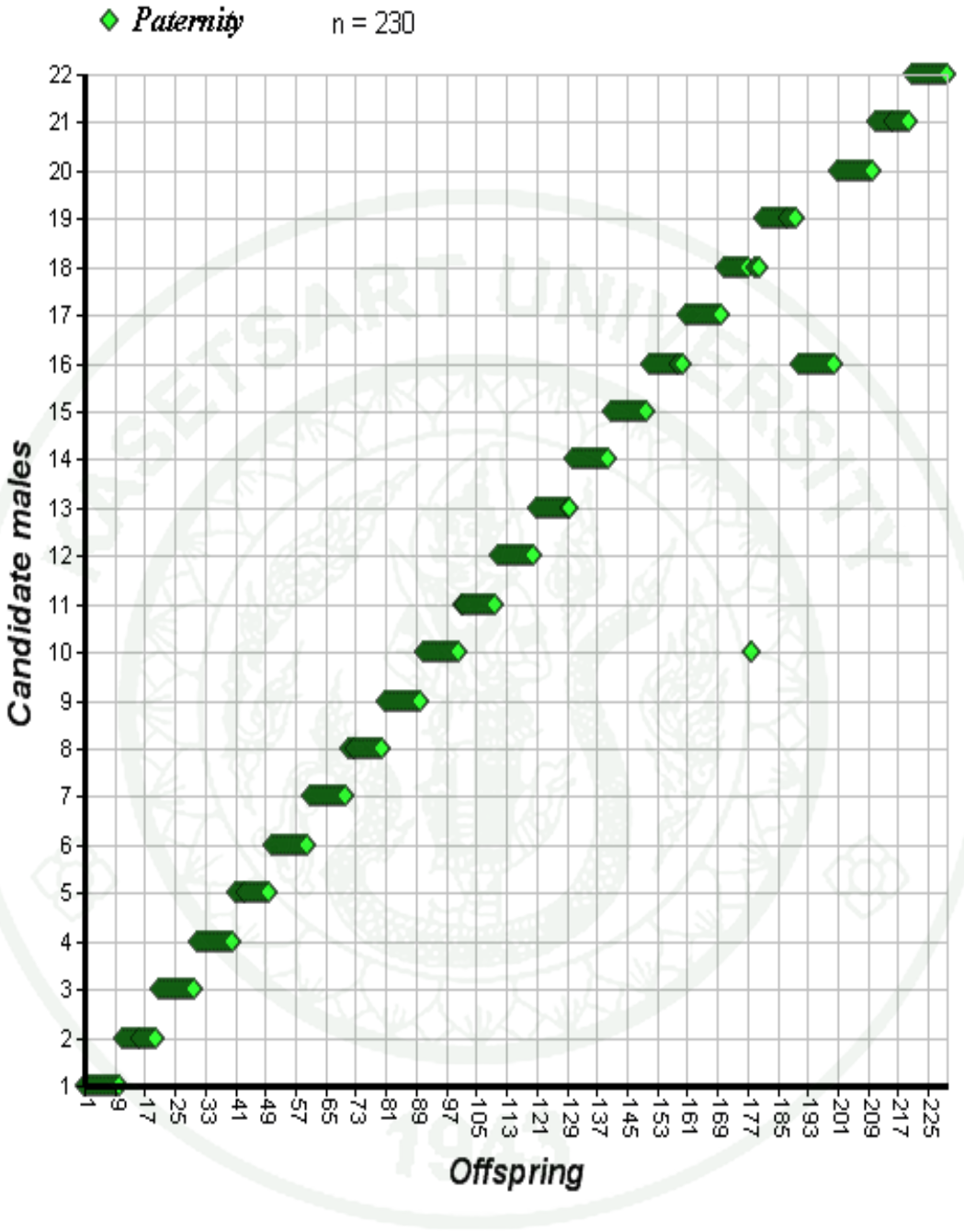
Appendix Figure C1 Sample results from GeneClass2 program.

Appendix Table C1 Summary statistics of parentage assignment simulation from CERVUS program using 9 microsatellite loci.

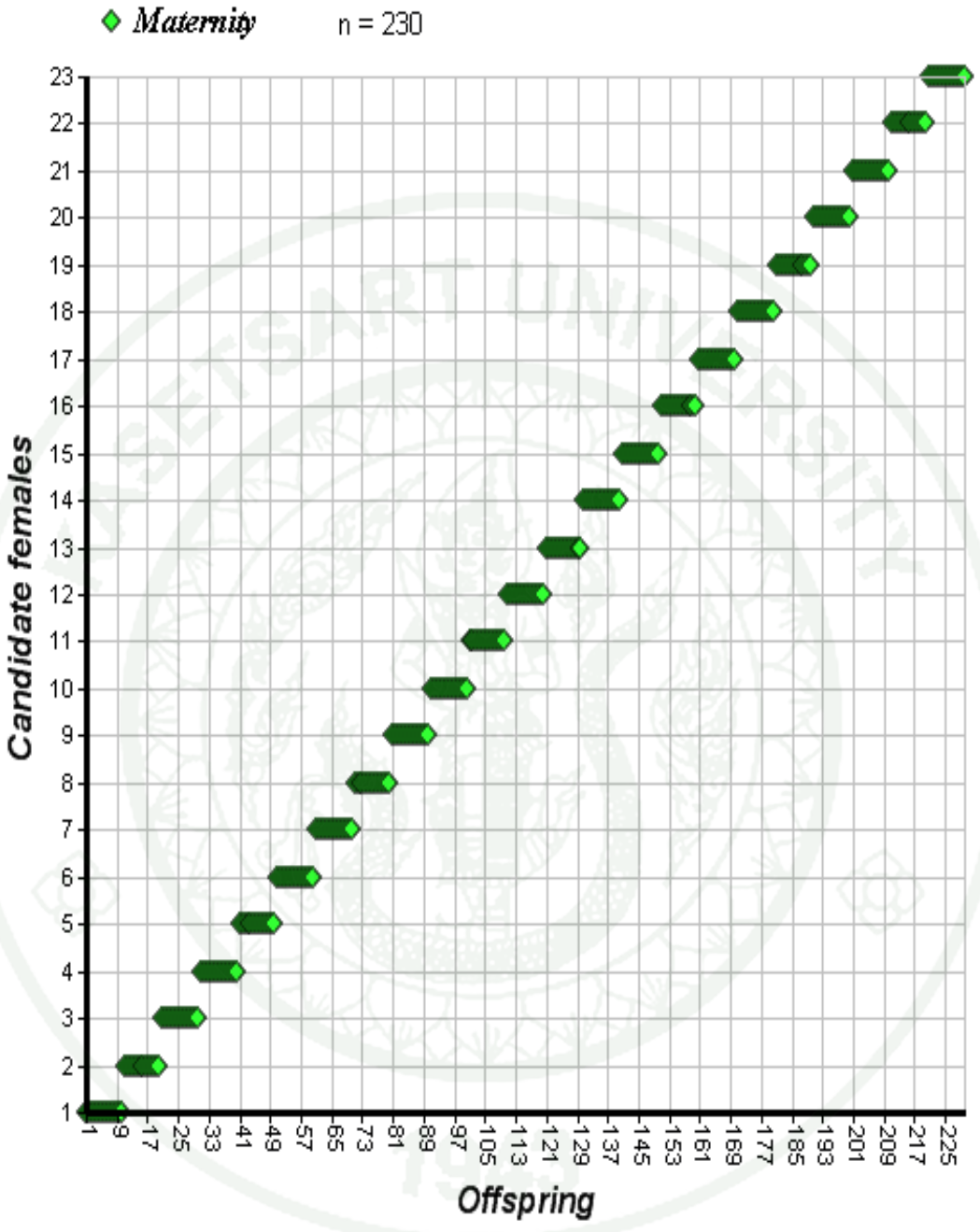
Level	Confidence (%)	Critical LOD	Assignments	Assignment Rate
Mother alone:				
Strict	95	-999	10000	100%
Relaxed	80	-999	10000	100%
Unassigned			0	0%
Total			10000	100%
Father alone:				
Strict	95	-999	10000	100%
Relaxed	80	-999	10000	100%
Unassigned			0	0%
Total			10000	100%
Parent pair (sexes known):				
Strict	95	-999	10000	100%
Relaxed	80	-999	10000	100%
Unassigned			0	0%
Total			10000	100%



Appendix Figure C2 Best (ML) sibship assignment plot from COLONY program.



Appendix Figure C3 Best (ML) paternity assignment plot from COLONY program.



Appendix Figure C4 Best (ML) maternity assignment plot from COLONY program.

Appendix D

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DNA microsatellite-based evaluation of early growth performance among strains of freshwater prawn *Macrobrachium rosenbergii* de Man

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Strain evaluation

ABSTRACT

Microsatellite DNA markers were used to assess early growth performance among different strains of freshwater prawn under separate and communal rearing conditions. Two-hundred-and-twenty broodstock individuals from three prawn strains were collected and used as baseline populations for assignment tests. The exclusion–simulation approach was performed on different sets of one to seven microsatellite loci to determine the power of the assignment test. High accuracy of the test was obtained by using seven loci, with 90% correct assignment of individuals ($P<0.05$). Simulations suggested that the use of four loci would substantially reduce the number of genotypes needed and the associated cost of genotyping while maintaining high assignment scores (88%, $P<0.05$). The power of the assignment test was highly dependent on the degree of population differentiation (F_{ST}). Results of this study demonstrated that an assignment score of 100% was obtained when $F_{ST}>0.1$. Post-larvae of the three baseline populations – including a commercial strain of foreign origin, a local hatchery strain and a wild population – were stocked in separate and communal tanks and cultured for 120 days. Significant differences in growth were observed among strains in both separate and communal rearing, with the commercial strain outperforming the hatchery populations. While communal rearing was proven an effective technique for comparing the growth of juvenile fishes, the approach may not be applicable for comparing the survival of juvenile prawns due to their cannibalistic nature.

1. Introduction

The culture of freshwater prawn *Macrobrachium rosenbergii* has been established in Thailand for nearly 40 years. In 2008, Thailand was ranked the second major producer of freshwater prawn behind China (FAO, 2010). During the past several years, the rising demand for domestic consumption and export markets has led to rapid expansion of the industry, with production increasing from 2200 t in 1997 to 28,500 t in 2008 (FAO, 2010) and the total culture area increasing from 2200 ha in 1998 to 15,540 ha in 2007 (Department of Fisheries, 2007). Eighty percent of the prawn production was from the major farming area situated in the southwest of the ChaoPhraya Basin in central Thailand. Despite the expansion of the culture areas, inconsistent and low levels of production have been a major concern among prawn farmers. The genetic deterioration of prawn broodstocks was suggested as a probable cause of the declining yields (Chareontawee et al., 2007). To overcome the problem of low production, efforts have been made to upgrade the existing broodstocks and to acquire a new stock to initiate the breeding program. Selection of the best strains is the first priority for ensuring high production and profitability of prawn farming. However, despite the importance of the strain selection, little or no research has been conducted to compare the performance of freshwater prawn strains in Thailand.

Strain evaluations are conducted to assess the performance of different genetic groups raised under common conditions and to determine the effects of genotype-by-environment (G×E) interactions (Falconer and Mackay, 1996). Strain selection has become an integral part of the broodstock domestication and selective breeding programs. Many studies indicate that the accuracy of the strain evaluation depends on the variation of environmental factors, particularly on tank or pond conditions, which can have large effects on the expression of growth traits (Herbinger et al., 1999). Therefore, it is important to minimize the influence of confounding environmental effects by stocking animals in the same tanks or ponds. Wohlfarth and Moav (1985) introduced communal rearing as a technique to provide a common environment for strain evaluation of the common carp *Cyprinus carpio*. Communal rearing can reduce the

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cost of replicate ponds and was found to enhance the expression of phenotypic differences among strains of the common carp and other species such as channel catfish and tilapia (Tave, 2003).

In classical communal rearing, the animals are physically marked using external or internal tags to identify their strain or family of origin. However, for crustaceans, external tagging is inefficient due to tag loss during molting (Menasveta et al., 1994). Although several types of internal tags, e.g., elastomer dyes and passive integrated transponders, are available for crustaceans, their use is limited to juvenile and adult individuals, as demonstrated in Pacific white shrimp *Litopenaeus vannamei* (Godin et al., 1996), lobster *Homarus gammarus* (Linnane and Mercer, 1998) and crayfish *Cherax destructor* (Jerry et al., 2001). It is impractical to tag the animals at early life stages such as post-larvae (Arce et al., 2003; Jørstad et al., 2005). Microsatellite DNA polymorphisms have been used as genetic markers to eliminate the problems posed by physical tagging of early life stages of animals. Applications have been reported in many selective breeding programs for fish and shellfish. For example, microsatellites were used to establish the pedigrees of mixed families of rainbow trout *Onchorhynchus mykiss* (Herbinger et al., 1995) and Atlantic salmon *Salmo salar* (Herbinger et al., 1999; Norris et al., 2000), to estimate the heritability in the common carp *C. carpio* (Vandeputte et al., 2004), to assess the performance of the European lobster *H. gammarus* broodstocks (Jørstad et al., 2005), and to assign parentage relationship in the Kuruma shrimp *Penaeus japonicus* (Jerry et al., 2004) and tiger shrimp *P. monodon* (Jerry et al., 2006).

This study was designed to compare the growth and survival of post-larvae and juveniles among the three prawn strains that are widely cultured in the farming areas in central Thailand. The strain evaluation was performed under both separate and communal rearing conditions. Moreover, we examined the potential use of the microsatellite markers for the strain identification of prawn individuals in communal tanks. These microsatellites were developed for the genetic investigation of freshwater prawn in our previous study (Chareontawee et al., 2006). The results of this study will provide useful information on the expression of performance traits, which are critical for the nursery period of prawn farming.

2. Materials and methods

2.1. Nursing of prawn larvae

Post-larval prawn (PL30) of similar ages and sizes of the three strains were obtained from private hatcheries, including the CPP strain from Petchaburi province, the KSB strain from Supanburi province, and the SKL strain from a local hatchery in Nakorn Prathom province. The average body mass was 0.0087, 0.0077, and 0.0076 g for individuals of the CPP, KSB, and SKL strains, respectively. Prior to stocking in experimental tanks, the post-larvae were nursed separately for 30 days in 3000-l fiber glass tanks. Thereafter, 100 post-larvae from each strain were measured to determine average total length, carapace length and body weight. During the experiment, post-larvae were fed fresh *Artemia* nauplii and an artificial diet twice a day.

2.2. Experimental design

After 30 days of nursing, the post-larvae of each stock were randomly separated into two groups for communal and separate rearing experiments and stocked at a density of 100/m^2 in twelve concrete tanks (3×2×0.6 m). A plastic mosquito mesh was fixed vertically at the middle of each tank to increase the holding capacity. For communal rearing, 200 post-larvae from each strain were stocked together in each of the three concrete tanks. For separate rearing, 600 post-larvae from each strain were stocked in three single-strain tanks. Fifty percent of the water in the tanks was changed daily. Post-larvae and juveniles in the single-strain tanks were sampled every four weeks to determine the growth and survival rates. In the communal-rearing tanks, growth was measured at the completion of the study (after 120 days). All remaining juveniles were collected and stored in individual tubes containing 99% ethanol for subsequent DNA extraction and microsatellite genotyping. The survival rates for each strain were calculated after the strain identification. Both communal and separate stocking experiments were conducted in the same greenhouse at Kamphaengsaen Fishery Research Station, Kasetsart University, Nakorn Pathom Province.

2.3. Statistical analysis

A two-factorial design was used to evaluate the results for the communal stocking experiment. The statistical model is as follows:

$$Y_{ijk} = \mu + T_i + S_j + TS_{ij} + e_{ijk}, \quad (1)$$

where Y_{ijk}, μ, T_i, S_j, and e_{ijk} are the record for the kth progeny, a common mean, the effect of the ith experimental tank, the effect of the jth strain, the effect of the ijth tank–strain interaction and the uncontrolled environmental and genetic deviation, respectively.

A nested design was used to evaluate the results for separate stocking, with the strain treated as a fixed effect and the tank nested within the strain as a random effect. The statistical model is as follows:

$$Y_{ijk} = \mu + S_i + T_{(i)j} + e_{ijk}, \quad (2)$$

where Y_{ij}, μ, S_i, $T_{(i)j}$ and e_{ij} are the record of the jth progeny, a common mean, the effect of the ith strain, the effect of the jth tank which was nested in the ith strain and the uncontrolled environmental and genetic deviation, respectively.

The mean and standard error were estimated for all parameters (survival rate, body weight, body length and carapace length) for each strain and subjected to the one-way analysis of variance using the software package SAS (SAS Institute, 2003) to determine significant differences ($P<0.05$) among strain means. The multiple comparison by Duncan's new multiple range test ($\alpha = 0.05$) was used to compare the means of survival and growth traits between the pairs of strains.

2.4. Microsatellite DNA genotyping

Swimmeret tissues of the prawn broodstock and the surviving 4-month old juvenile prawns in the communal rearing experiment were utilized for DNA extraction using a standard phenol-chloroform extraction procedure (Taggart et al., 1992). Samples of the prawn broodstock were genotyped at seven microsatellite loci (*Mbr*-1, *Mbr*-2, *Mbr*-3, *Mbr*-5, *Mbr*-7, *Mbr*-8, and *Mbr*-10 with GenBank accession numbers DQ019863, DQ019864, DQ019865, DQ019867, DQ019869, DQ019870 and DQ019871, respectively) using the PCR conditions described by Chareontawee et al. (2006). The PCR reactions were performed in 15-μl reactions which contained 10 ng template DNA, 0.3 μM forward and reverse primers, 0.2 mM each dNTP, 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris–HCl (pH 9.0), 0.1% Triton X-100, and 1 unit of *Taq* DNA polymerase (Promega). The PCR profile was the initial denaturation at 94 °C for 3 min; 35 cycles of: 94 °C for 30 s, annealing temperature for 45 s, and 72 °C for 1 min; and 1 cycle of 72 °C for 7 min. Following the amplification, PCR products were mixed with 2.5 μl of sequencing dye and heated for 5 min at 95 °C. The reaction mixtures were subjected to electrophoresis on a 5.5% denaturing polyacrylamide gel at 80 W for 3 h. The gel was denatured at 100 °C for 30 min before electrophoresis. Bands in the gel were visualized by silver staining. Allele sizes were estimated by comparison to an M13 sequence ladder.

Table 1
Genetic variability at seven microsatellite loci in three prawn strains, including sample size (N), total number of alleles (A), allelic richness (A_r), observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index (F_{is}), and P value for test of Hardy–Weinberg expectations (HW). Bonferroni correction: $P<0.0071$ (0.05/7).

Population (N)	Locus							Average across loci
	Mbr-1	*Mbr*-2	*Mbr*-3	*Mbr*-5	*Mbr*-7	*Mbr*-8	*Mbr*-10	
CPP (95)								
A	10	12	10	15	3	5	4	8.43 ± 4.5
A_r	9.4	9.96	9.12	11.74	2.99	5.00	3.99	7.46 ± 3.4
H_o	0.74	0.79	0.75	0.86	0.48	0.98	0.68	0.75 ± 0.16
H_e	0.81	0.78	0.81	0.81	0.45	0.74	0.69	0.71 ± 0.13
F_{is}	0.12	−0.008	0.104	−0.025	−0.079	−0.25	0.007	−0.017
HW	0.000	0.017	0.000	0.008	0.031	0.000	0.007	Highly sig.
KSB (59)								
A	18	12	19	20	6	5	8	12.57 ± 6.4
A_r	18.00	11.41	18.04	17.48	5.28	4.94	7.53	11.81 ± 6.0
H_o	0.85	0.74	0.81	0.88	0.42	0.60	0.81	0.73 ± 0.16
H_e	0.91	0.78	0.89	0.90	0.45	0.62	0.79	0.76 ± 0.17
F_{is}	0.091	0.091	0.112	0.022	0.139	0.107	0.010	0.077
HW	0.002	0.151	0.038	0.028	0.356	0.390	0.582	0.0003
SKL (66)								
A	16	17	26	20	6	7	11	14.71 ± 6.67
A_r	15.12	13.91	22.90	16.82	5.34	5.94	8.86	12.7 ± 6.3
H_o	0.98	0.75	0.96	0.95	0.70	0.50	0.75	0.79 ± 0.16
H_e	0.92	0.83	0.95	0.92	0.64	0.51	0.81	0.80 ± 0.15
F_{is}	−0.056	0.101	−0.006	−0.037	−0.094	0.038	0.065	0.001
HW	0.064	0.001	0.362	0.811	0.074	0.393	0.099	0.003

2.5. Genetic data analysis

The program MICRO-CHECKER version 2.2.0 (van Oosterhout et al., 2004) was used to test for the presence of the null alleles. Metrics of genetic variation within each of three strains – including mean number of alleles per locus (A), allelic richness (A_r), and observed (H_o) and expected (H_e) heterozygosities – were calculated using GENEPOP version 3.1c (Raymond and Rousset, 1995). Hardy–Weinberg expectations were tested by the exact P values and calculated by a Markov chain randomization method (Guo and Thompson, 1992) using GENEPOP version 3.1c. The TFPGA program (Miller, 1997) was used to calculate pair-wise F_{ST} values and p-values between all pairs of strains. The prawn samples of Myanmar origin (MYN) from the previous study by Chareontawee et al. (2007) was used as a reference population.

The assignment tests of the prawn individuals based on the multilocus genotypes at seven loci were carried out on a total of 220 broodstock individuals from the three strains, which were used as the baseline populations. We utilized two contrasting assignment methods, a direct method and a simulation–exclusion method. Both assignment algorithms calculated a likelihood probability of an individual belonging to each of the possible source populations. The direct method originally developed by Paetkau et al. (1995) assigns the individual to the population of origin in which it has the highest likelihood of belonging compared to the likelihood that it is assigned to other populations, without providing confidence levels. The simulation–exclusion approach developed by Cornuet et al. (1999) determines the proportion of the correct assignment at user-defined threshold levels of significance. In this approach, up to 10,000 genotypes are generated based on the allele frequencies of the populations tested to obtain the expected distribution of the genotypes and the distribution of genotype likelihood values (Piry et al., 2004). The likelihood of a particular genotype belonging to a particular source population is compared with the distribution of likelihoods of the simulated genotypes. If the value is below a certain threshold level, the individual is excluded from that sample. In the present study, GENECLASS2 (Piry et al., 2004) was used to calculate the probability of belonging of individuals based on a Bayesian approach. Both direct and simulation–exclusion methods were performed among the three freshwater prawn strains and between the pairs of strains. In addition, the assignment tests were carried out using different sets of microsatellite loci, ranging from one to seven loci in order to determine the number of loci needed to optimize between the genotyping cost and the power of the test. The genotype data from the prawn broodstock were used as the reference populations for the assignment of individual progeny in the communal rearing tanks. For each individual, the most likely strain of origin was determined based on the likelihood value and the score of an individual. The genetic diversity within each of the assigned strains was calculated using GENEPOP version 3.1c. The same program was used to test for deviations from Hardy–Weinberg expectations.

3. Results

3.1. Genetic variation among and within stocks

A total of 220 prawn broodstock (CPP = 95, KSB = 59, and SKL = 66) were genotyped at seven microsatellite loci (Table 1). All three prawn stocks exhibited relatively high genetic variation, with the average numbers of alleles per locus of 8.4, 12.6 and 14.7, and observed heterozygosities of 0.75, 0.73, and 0.79 for the CPP, KSB, and SKL strains, respectively. Null alleles were present at loci *Mbr*-1 and *Mbr*-3 in the CPP. The CPP stock showed significant departures from Hardy–Weinberg at three loci after applying a sequential Bonferroni correction. The overall estimate of F_{ST} (0.076) indicated statistically significant levels of differentiation among stocks. Significant pairwise F_{ST} values were observed for all strain comparisons ($P<0.05$, Table 2). With the exception of the MYN stock (the out-group), the highest

Table 2
Genetic distance (below diagonal) and pair-wise F_{ST} (above diagonal) with P values among three prawn populations (CPP, KSB, and SKL). MYN is used as a reference population. Asterisk indicates significant differentiation ($P<0.05$).

Population	CPP	KSB	SKL	MYN
CPP	–	0.093*	0.116*	0.154*
KSB	0.065	–	0.027*	0.155*
SKL	0.073	0.024	–	0.129*
MYN	0.122	0.125	0.110	–

Table 3
Strain assignment test results, showing the proportion of correct assignment of individuals using the Paetkau et al. (1995) direct (real assigned) method or the Cornuet et al. (1999) exclusion–simulation method with the probability of belonging to a population, based on seven (four) microsatellite loci. The overall F_{ST} values are 0.07 for seven loci and 0.08 for four loci.

Strain (*N*)	Direct (real assigned)	Correctly assigned (simulated)		
		$P<0.05$	$P<0.01$	$P<0.001$
Assignment for each strain				
CPP (95)	100 (100)	96.8 (97.9)	95.8 (97.9)	95.8 (97.9)
KSB (59)	98.3 (98.3)	76.3 (83.1)	74.6 (81.4)	74.6 (81.4)
SKL (66)	98.5 (92.4)	90.9 (83.3)	90.9 (83.3)	89.4 (83.3)
Total	99.2 (97.3)	89.5 (89.6)	88.6 (89.1)	88.2 (89.1)
Assignment between two strains				
CPP-KSB	100 (100)	100 (98.7)	98.7 (98.7)	98.7 (98.7)
CPP-SKL	100 (100)	98.7 (100)	98.7 (100)	98.7 (100)
KSB-SKL	98.4 (95.2)	84.8 (83.2)	84.0 (82.4)	83.2 (81.6)

genetic differentiation was observed between the CPP and the SKL stocks ($F_{ST}=0.12$). When using the genotype data at four loci (*Mbr*-1, *Mbr*-2, *Mbr*-3, and *Mbr*-5), an overall F_{ST} value of 0.081 was obtained.

3.2. Assignment test results

The assignment test results are shown in Table 3. The Paetkau et al. (1995) direct method assigned 98 to 100% of the individual prawns to their correct strain of origin. Fig. 1 shows the plots of the log-likelihoods between the pairs of strains for direct assignment. For example, all 95 individuals of the CPP had higher values of the log-likelihood of assignment to the CPP than to the KSB. Similarly, between the CPP and SKL, 100% of the CPP individuals were assigned to their correct strain of origin, while between the KSB and SKL strains, the direct method assigned 98.4% of the prawn to the KSB strain. One of the 59 KSB individuals exhibited higher log-likelihood of assignment to the SKL than to the KSB. When the confidence levels were defined using the Cornuet et al. (1999) exclusion method, the overall proportions of correct individual assignment decreased from 90 to 88% at *P*-values <0.05, 0.01, and 0.001. The accuracy of the individual assignment using a *P*-value of 0.05 was highest for the CPP (96.8%), followed by the SKL (91%) and KSB (76.3%) strains. Because the PCR products were not amplified for some of the KSB samples, approximately 9.4% of the KSB genotypes were treated as missing data. The adjustment of allele frequencies due to the missing data reduced the power of the assignment test for this strain (data not shown). In addition, to detect genotyping errors due to slippage during PCR-

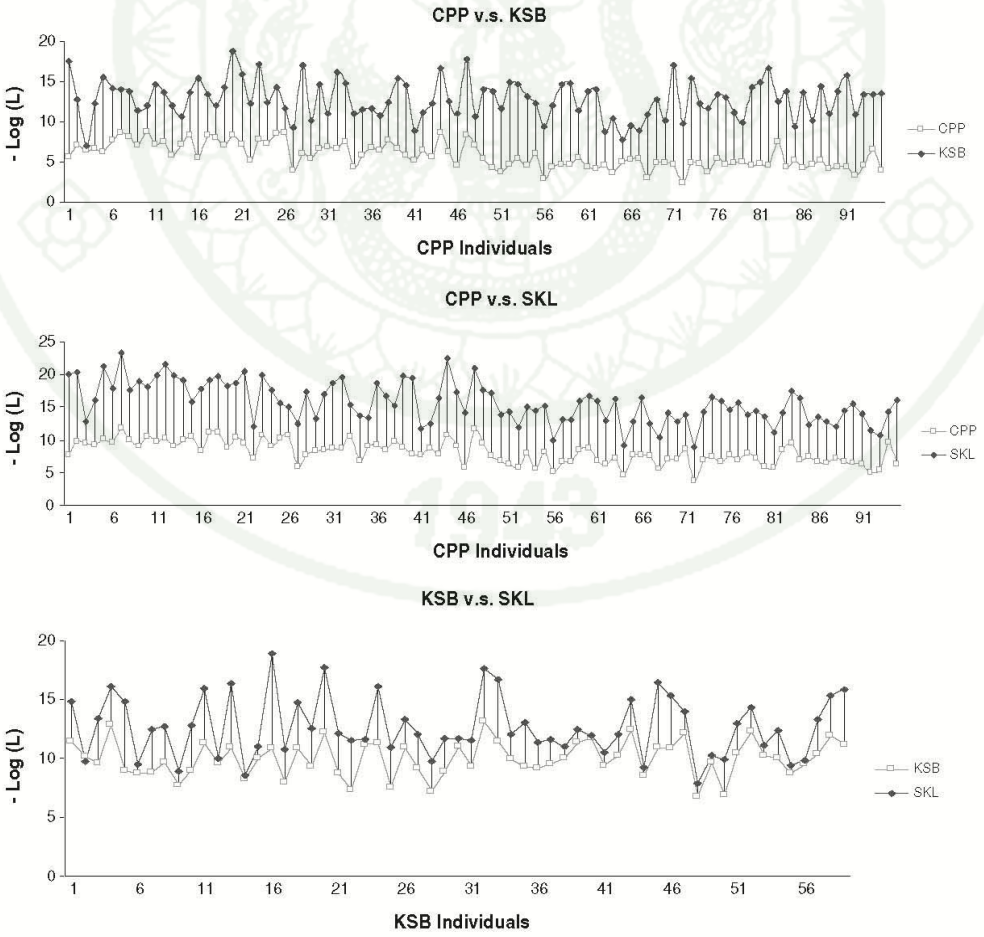

Fig. 1. Individual log likelihood, −Log (L) for the direct assignment based on seven loci: between CPP and KSB, between CPP and SKL, and between KSB and SKL strains of giant freshwater prawn.

Table 4
Genetic variability at four microsatellite loci in three prawn strains from a communal rearing experiment, including sample size (N), total number of alleles (A), allelic richness (A_r), observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index (F_{is}), and P value for test of Hardy–Weinberg expectations (HW). Bonferroni correction: $P<0.0125$ (0.05/4).

Population (N)	Locus				Average across loci
	Mbr-1	*Mbr*-2	*Mbr*-3	*Mbr*-5	
CPP (428)					
A	10	10	10	15	11.25 ± 2.5
A_r	9.28	9.67	9.09	11.3	9.84 ± 1.01
H_o	0.75	0.79	0.78	0.86	0.79 ± 0.05
H_e	0.84	0.78	0.84	0.84	0.83 ± 0.03
F_{is}	0.107	−0.008	0.075	−0.025	0.038
HW	0	0.07	0	0.007	Highly sig.
KSB (446)					
A	10	11	18	20	15.25 ± 3.59
A_r	9.52	10.62	16.76	16.65	13.47 ± 3.66
H_o	0.93	0.92	0.93	0.88	0.92 ± 0.07
H_e	0.88	0.88	0.92	0.90	0.89 ± 0.05
F_{is}	−0.02	−0.016	−0.012	0.022	−0.004
HW	0.01	0.02	0.3025	0.008	0.008
SKL (492)					
A	13	15	26	20	18.50 ± 5.8
A_r	12.81	13.88	22.33	18.51	16.90 ± 4.37
H_o	0.96	0.92	0.91	0.87	0.93 ± 0.06
H_e	0.92	0.88	0.94	0.92	0.91 ± 0.03
F_{is}	−0.057	−0.101	0.006	0.007	−0.02
HW	0.042	0.009	0.265	0.03	0.0146

amplification, repeated genotyping of some individuals was performed on the automated DNA sequencer (data not shown).

The assignment tests between the pairs of strains revealed that using the exclusion method with a P-value of 0.05, 100% correct assignment was obtained between CPP and KSB, followed by 98.7% between CPP and SKL, and 84.8% between KSB and SKL strains (Table 3). The assignment accuracy for each pair of strains varied slightly when using P-values of 0.01 and 0.001.

To optimize the trade-off between the cost of genotyping and the assignment power, simulations were performed for 127 sets of marker combinations. The assignment test results indicated that using a set of four loci (*Mbr*-1, *Mbr*-2, *Mbr*-3, and *Mbr*-5), the accuracy of the test was comparable to that using seven loci (Table 3). Therefore, this set of four loci was used for the genotyping prawn individuals in the communal rearing experiment. Of 1383 surviving juvenile prawns, 17 were unidentified due to low DNA concentrations, while 428, 446 and 492 individuals were assigned to the CPP, KSB and SKL strains, respectively. Each individual was assigned to the most likely strain of origin, with the highest values of the likelihood and score for that strain (data available upon request). For example, an unknown individual was assigned to the CPP strain with a likelihood value of −4.9 and a score of 95.9%, in comparison with the log-likelihoods of −6.3 and −7.5 and scores of 3.8% and 0.3% for the assignment to the KSB and SKL strains, respectively.

Table 5
Mean body weight (BW), mean total length (TL), and mean carapace length (CL) of three prawn strains after 120 days of separate rearing, with ANOVA results showing F-values for differences among strains and tanks. Asterisk indicates significant difference ($P<0.05$).

Strain	Mean BW ± SD (g)	Mean TL ± SD (mm)	Mean CL ± SD (mm)
CPP	6.75 ± 2.93[a]	85.01 ± 11.05[a]	42.12 ± 5.76[a]
KSB	5.43 ± 3.91[b]	80.23 ± 14.11[b]	39.19 ± 7.73[b]
SKL	4.13 ± 3.10[c]	72.91 ± 14.44[c]	35.07 ± 8.06[c]
ANOVA			
F-value (strain)	26.41*	64.03*	63.07*
P-value	0.0011	<0.0001	<0.0001
F-value (tank)	2.08	0.99	1.13
P-value	0.05	0.43	0.34

Table 6
Mean body weight (BW), mean total length (TL) and mean carapace length (CL) of juveniles of three prawn strains after 120 days of communal rearing, with ANOVA results showing F-values among tanks, strains and tank–strain interaction. Asterisk indicates significant difference ($P<0.05$).

Strain	Mean BW ± SD (g)	Mean TL ± SD (mm)	Mean CL ± SD (mm)
CPP	7.66 ± 5.16[a]	86.66 ± 18.03[a]	41.86 ± 9.71[a]
KSB	5.27 ± 3.29[b]	78.33 ± 26.28[b]	37.71 ± 8.97[b]
SKL	5.10 ± 3.58[b]	76.77 ± 39.93[b]	36.47 ± 9.53[b]
ANOVA			
F-value (tank)	0.17	0.76	0.45
P-value	0.84	0.47	0.64
F-value (strain)	14.59*	9.25*	26.55*
P-value	<0.0001	0.0001	<0.0001
F-value (tank-strain)	1.78	0.7	1.88
P-value	0.13	0.59	0.11

The genetic variation at four loci of the three assigned strains from the communal rearing experiment is shown in Table 4. All three prawn stocks exhibited relatively high genetic variation, with average numbers of alleles per locus of 11.2, 15.2 and 18.5, and observed heterozygosities of 0.79, 0.92, and 0.93 for the CPP, KSB, and SKL strains, respectively.

3.3. Growth and survival of juveniles

In a separate testing, the average daily growth (ADG), body weight (BW), carapace length (CL) and total length (TL) of 4-month old juveniles differed significantly among strains ($P<0.05$), with the CPP strain displaying the highest values for all characters (Table 5, Fig. 1). For example, body weights of CPP, KSB, and SKL individuals at 120 days were 6.75 ± 2.93, 5.43 ± 3.19, and 4.13 ± 3.10 g, respectively. Tank effects were not significant in the separate rearing experiment. Survival rates were considered low to moderate for the three strains, at 71, 50.6, and 76%, respectively, for the CPP, KSB, and SKL strains. However, testing for strain and tank effects on survival among strains was not possible in the nested design. In communal testing, a total of 1366 surviving juvenile prawns, including 428 CPP, 446 KSB and 492 SKL individuals, were measured. Similar to the results of the separate rearing experiment, the CPP stock exhibited the highest values for growth parameters (Table 6). For example, the mean body weights of CPP, KSB, and SKL individuals at 120 days were 7.66 ± 5.16, 5.27 ± 3.29, and 5.10 ± 3.58 g, respectively. Although differences in growth were observed within the strains of juvenile prawns between the two tests, the ranking of strains was the same for separate and communal rearing. Tank effects and strain–tank interaction were not significant in communal stocking. The survival rates of the CPP strain (71.3%) in the communal tanks did not differ from those in separate tanks, but the survival rates of the KSB (74.3%), and SKL (82%) strains in the communal tanks were higher than those in the separate rearing (Fig. 2).

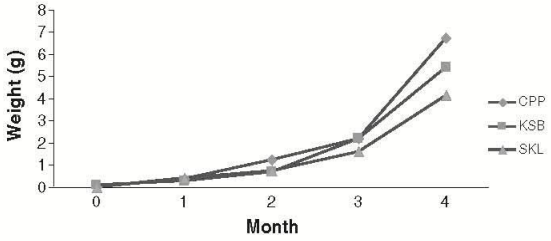

Fig. 2. Average daily growth rate among three prawn strains reared in separate tanks for 120 days.

4. Discussion

4.1. Genetic diversity of freshwater prawn

Assessing the genetic diversity of wild and cultured stocks and strain evaluation have become common practices in the selective breeding of aquaculture species. The genetic characterization is important because it reflects the genetic makeup and history of domestication of a particular strain. Although the issue of association between the genetic variation at neutral marker loci and the variation of quantitative traits remains controversial (Reed and Frankham, 2001), the analyzed data for 20 species – including plants, invertebrates and vertebrates – indicated positive correlation of the quantitative trait variation and the neutral marker divergence (Merila and Crnokrak, 2001). The genetic improvement of the prawn strains is still at an early stage compared to that for the domesticated fish species (Bart and Yen, 2003; Nhan et al., 2009; Kitcharoen et al., 2010; Thanh et al., 2010). Only a few genetically improved strains of prawn have been developed for commercial use (New, 2005). The prawn strains in this study – including CPP, KSB, and SKL – are widely used in the major farming areas in central Thailand. The original CPP broodstock was derived from the prawns of Indian origin and has undergone selective breeding for a number of generations (Nithid Patarakulchai, KasetSomboon Farm, Supunburi province, pers. comm.). The other two strains were developed from wild populations native to Thailand. KSB originated from the ChaoPhraya River, but the record of domestication for this strain is not known. The SKL strain was brought recently from Songkla Lake in south Thailand. The CPP strain was believed to have more rapid growth rate than the other two strains. However, differences in other traits, such as survival rate and disease resistance among the three strains, were not known. The analysis of the population genetic data indicated that the three stocks exhibited relatively high genetic variation in terms of average numbers of alleles (8.3–14.7) and observed heterozygosities (0.76–0.79). Among them, the SKL strain displayed the highest number of alleles and percent heterozygosity, followed by KSB and CPP. Overall, the observed genetic diversity of the three strains was comparable to that of the hatchery and wild population samples of freshwater prawns in our previous study (Chareontawee et al., 2007). Significant departures from HWE in the CPP strain could have resulted from the presence of null alleles at *Mbr*-1 and *Mbr*-3 or from the inbreeding, while the heterozygote excess at *Mbr*-8 may indicate the mixing of populations. The F_{ST} value of 0.076 showed high levels of genetic differentiation among the prawn strains and was in agreement with their different origins. The analysis of the genetic data at the four loci for the prawn strains in the communal tanks also exhibited relatively high genetic variation, with the average number of alleles, varying from 11.25 (CPP) to 18.5 (SKL) and observed heterozygosities varying from 0.79 (CPP) to 0.93 (SKL). The number of alleles detected at the four loci for prawn juveniles for the CPP and SKL strains were similar to those of their broodstock. However, for the KSB strain, the numbers of alleles at the three loci (*Mbr*-1, *Mbr*-2, and *Mbr*-3) were less than the number of alleles existing in their parental stock, suggesting that the KSB juveniles were produced from a small number of broodstock.

4.2. Assignment tests

Microsatellite DNA markers are an effective tool for the strain or population identification studies. A number of computer programs have been developed to perform genetic assignment tests based on different algorithms (Cornuet et al., 1999; Hansen et al., 2001; Piry et al., 2004; Manel et al., 2005). These assignment methods use the genotype data and statistical tests to assign individuals to their most likely stock of origin under different assumptions regarding the data. For instance, the assumptions of HWE and linkage equilibrium between loci are embodied in the original assignment test developed by Paetkau et al. (1995). The test was shown to be effective in a study of the genetic population structure of polar bears *Ursus arcticus* and in other investigations (Paetkau et al., 1997). In the present study, however, the exclusion–simulation method implemented in the program GENECLASS2 was used specifically because it does not assume HWE and linkage equilibrium among the loci. The application of this method is more appropriate for the aquaculture stocks, where the departure of HWE is a common phenomenon. The performance of the exclusion–simulation approach is quantified as the proportion of the correctly assigned individuals at a threshold probability of belonging to a population (Cornuet et al., 1999; Piry et al., 2004). The exclusion method performed well in this study, showing high accuracy, with 90% correct assignment of individuals among the three strains at $P<0.05$. Moreover, the assignment scores (88%) changed only slightly when the confidence level was decreased to $P<0.01$ and $P<0.001$. The utility of the exclusion–simulation method for individual assignment has been assessed in other studies, with varying results. For instance, in their study on the cattle breed assignment, Maudet et al. (2002) indicated that 67% correct assignment was obtained among seven breeds at a significance level of $P<0.05$. The accuracy of the breed assignment, however, decreased to 54 and 33% at $P<0.01$ and $P<0.001$, respectively. In other studies, the exclusion method failed to distinguish between the hybrids and wild individuals in admixed populations of the brown trout *Salmo trutta* (Hansen et al., 2006), but proved very effective in the discrimination among the three species of the Pacific abalone *Haliotis* spp. (Sekino and Hara, 2007).

There are several factors that determine the efficacy of the assignment methods, including the amount of genetic differentiation among stocks, the number and sample sizes of populations and the number of the loci studied (Cornuet et al., 1999; Hansen et al., 2001; Piry et al., 2004; Manel et al., 2005). The power of the exclusion method was estimated in a simulation study by Cornuet et al. (1999); for example, using 10 simulated populations, an assignment score of nearly 100% was obtained when ten loci and a sample size of 30 individuals were used with an F_{ST} of 0.1. The performance of the exclusion method was further evaluated using the empirical microsatellite data sets from 10 species, including bear, cattle, fish, bees, and *Drosophila* (Manel et al., 2002). Similarly, the analysis of the empirical data sets indicated that nearly all individuals were correctly assigned when populations were highly differentiated ($F_{ST}>0.1$). The effect of genetic differentiation on power of the assignment test also was observed in this study, where a score of 100% correct assignment was obtained between the CPP and SKL strains with an F_{ST} of 0.12. In contrast, only 81–83% of individuals were correctly assigned between the least differentiated populations, KSB and SKL ($F_{ST}=0.02$). Cornuet et al. (1999) suggested that if the degree of the genetic differentiation among populations is low, the power of the assignment test can be increased by using larger population samples and larger numbers of loci. It should be noted, however, that the accuracy of the test decreases with an increase in the number of populations due to the increased numbers of individuals wrongly assigned to each population (Hansen et al., 2001). For microsatellites, the genotyping errors caused by large allele dropout and scoring of the stuttering alleles on a sequencing gel may reduce the accuracy of the assignment. In such cases, the genotyping of individuals should be repeated by an automated DNA sequencer.

Although microsatellites are the most suitable genetic markers for the individual assignment, the genotyping at a large number of loci can be expensive and may reduce the benefit of the assignment tests. Therefore, it was our interest to determine the number of markers that optimizes the trade-off between assignment success and the cost of genotyping. The simulation results from the three baseline populations showed that high assignment scores were obtained with the use of several subsets of markers comprising four to seven loci. Of these, a subset of four loci (*Mbr*-1, *Mbr*-2, *Mbr*-3, and *Mbr*-5)

showing 89% of the correct assignment among the three strains at $P<0.05$, 0.01 and 0.001 was chosen for the genotyping of the surviving prawn in the communal rearing experiment. The explanation for the high rate of assignment at the four chosen loci is that the removal of the three loci (*Mbr*-7, *Mbr*-8, and *Mbr*-10) which was less informative resulted in the increased population differentiation from $F_{ST}=0.07$ (seven loci) to 0.08 (four loci). By using the subset of the four loci, the number of genotypes from the communal tanks was reduced considerably, from 9562 (1366 individuals × 7 loci) to 5464 (1366 individuals × 4 loci), with up to 43% reduction of the genotyping cost. However, the multiplexing of the microsatellite markers also can reduce the genotyping costs.

4.3. Comparison of growth performance

Communal rearing has proven an effective strategy for testing different genetic groups under the same environmental conditions. However, the presence of competition among different strains may invalidate the application of communal testing for certain species (Wohlfarth and Moav, 1985, 1991). Competitive advantage is indicated if the ranking of strains is different between the communal and separate testings. The results of the present study did not suggest the presence of competition among the prawn strains; i.e., the same ranking of strains for growth traits was observed between the separate and communal testings, with the CPP strain ranked first, followed by KSB and SKL. Without competition between different strains, the growth of juveniles in a communal rearing may be used as a predictor for their performance in a separate rearing (Wohlfarth and Moav, 1991). However, the application of communal stocking for comparison of survival for juvenile prawns may be restricted because the results varied between the separate and communal tests. While the survival of the CPP strain (71%) was consistent in the separate and communal cultures, the differences in survival were observed for the KSB and SKL strains. The variation in the survival rates at the end of the experiment were not unexpected and likely were due to the cannibalistic nature of the prawn larvae and juveniles.

The growth and survival of the juveniles are the important traits that determine prawn production. Prawn farmers practice two rearing strategies in Thailand. One strategy is to stock post-larvae (PLs) directly into the grow-out ponds. A second method is to stock post-larvae in nursing tanks or cages for 2–3 months and then to transfer juveniles to grow-out ponds. Several studies reported that the prawn production was relatively low for the first strategy due to the high mortality of PLs by cannibalism in the grow-out ponds. In contrast, the use of a nursery phase increases the survival of juveniles and subsequently increases farm production (Lin and Boonyaratpalin, 1988; Schwantes et al., 2007). According to our recent survey, most prawn farmers in the areas have adopted a new strategy by stocking 2–4 month old juveniles directly into the grow-out ponds. This practice shortens the culture period and reduces the production cost. As a result, the demand for juvenile prawns is increasing while the demand for the post-larvae is decreasing. It should be noticed, however, that significant differences in the performance of prawn strains may vary in the environments different from those utilized in this study.

5. Conclusion

Characterizing the genetic structure and assessing the performance of the freshwater prawn strains will assist in the selection of the best strains for increasing farm production and initiation of future breeding programs. This study demonstrated that individual prawns of different origins can be identified using four to seven microsatellite markers. The use of four markers optimized the power of assignment tests and the cost of genotyping. Communal rearing proved an effective technique for the comparison of the growth performances of the different prawn strains because the inter-strain competition was not observed in this study. However, the communal rearing approach was not appropriate for comparing survival due to the different rankings of strains between separate and communal rearing.

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Short communication

CERVUS vs. COLONY for successful parentage and sibship determinations in freshwater prawn *Macrobrachium rosenbergii* de Man

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ABSTRACT

This study assesses the properties of 10 microsatellite loci and two popular computer programs for likelihood-based parentage identification in commercial strains of the giant freshwater prawn *Macrobrachium rosenbergii*. It was our interest to determine the number of markers needed to optimize between genotyping cost and power of the test. Nine loci were informative, with average expected heterozygosity of 0.80 and PIC score of 0.76. Low and non-significant frequencies of null alleles (<0.05) were detected at the nine loci. Simulations implemented in the CERVUS program were performed to determine the number of informative loci needed to maximize assignment success. The accuracy in assignment was determined in 21 full-sib and two paternal half-sib families using two contrasting methods, a pair-wise likelihood comparison approach in CERVUS and a full-pedigree likelihood method in the COLONY program. When genotyping error was set at 0.05, use of four highly informative loci was sufficient for COLONY to resolve the genetic structure of this population, while seven loci would be required to obtain 94–99% correct assignment with CERVUS. As typing rate of error increased to 0.2, COLONY required only four loci to assign parentage to 85–90% of the offspring, while CERVUS required five to seven loci to obtain the similar proportions of assignment. Results suggest that this set of microsatellites, used in conjunction with COLONY would be an effective tool for parentage and sibship identification, testing performance of families and estimating genetic parameters in selective breeding programs of the giant freshwater prawn.

1. Introduction

Selective breeding programs for aquatic animals normally involve rearing a large number of families in separate tanks or ponds until the offspring are large enough to be marked and stocked together. However, separate rearing of families, increases environmental differences between families which can be confounded with genetic effects (Falconer and Mackay, 1996) unless full- and half-sib or other highly interconnected designs are used, which is frequently not feasible in aquaculture. Microsatellite DNA technology offers the tool to identify animals stocked in the same tanks or ponds. Applications have been reported in many selective breeding programs for fish and shellfish.

Parentage analysis is a method for determining the parents of an individual or group of individuals using genetic information combined with statistical methods (Manel et al., 2005). The classical assignment method is based on the exclusion probability where all but one pair of the candidate parent can be excluded based on the multi-locus genotype of a particular offspring. However, the weakness of the exclusion approach is that genotyping errors and null alleles can lead to false exclusions (Jones and Ardren, 2003). In some cases, the exclusion-based method is not possible to resolve parentage when multiple pairs of parent are not excluded (Hedrick, 2005). Nevertheless, some exclusion procedures can handle genotyping errors and mutations, and use distances between genotypes of individuals to assign parentage in the case of multiple paternities (Carvajal-Rodríguez, 2007). The pair-wise likelihood-based method was developed to assign offspring to non-excluded parent or parent pair with the highest log-likelihood ratio or LOD score (Marshall et al., 1998). Alternatively, Wang and Santure (2009) utilized full-pedigree likelihood methods to simultaneously infer sibship and parentage among individuals, with complex mating systems.

Giant freshwater prawn *Macrobrachium rosenbergii* (de Man) is one of the most important aquaculture species in India, China, and Southeast Asia. In 2008, Thailand was ranked the second major producer of freshwater prawn behind China with production of 28,500 tons (FAO, 2010). Despite increasing demand for freshwater prawn products worldwide, the potential growth of the industry has been limited by low level of production (Chareontawee et al., 2007). Genetic improvement of prawn strains is still at an early stage compared to that for domesticated fish species (Kitcharoen et al., 2010; Thanh et al., 2010a, 2010b). Recently, Thanh et al. (2010a, 2010b) documented the potential of selection and crossbreeding to obtain increased growth of giant

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freshwater prawn in Vietnam. Only a few genetically improved strains of prawn have been developed for commercial use in Thailand (Chareontawee et al., 2007; Karaket et al., 2011).

In this paper, we assessed the genetic properties of 10 microsatellite loci in a commercial strain of giant freshwater prawn for parentage identification. These microsatellites were developed previously for freshwater prawn (Chareontawee et al., 2006). The accuracy in assignment was determined in 21 full-sib and two half-sib families using two contrasting assignment approaches, a pair-wise likelihood comparison method (Kalinowski et al., 2007; Marshall et al., 1998) and a full-pedigree likelihood method (Jones and Wang, 2010; Wang and Santure, 2009). It was our interest to determine the number of markers that optimizes the trade-off between cost of genotyping and the efficacy of the assignment methods.

2. Materials and methods

2.1. Production of families

The experiment was carried out at the Kamphaengsaen Fishery Research Station, Kasetsart University, Nakorn Pathom Province. Broodstock of a commercial strain (CPF) were used to produce twenty-one full-sib and two half-sib families. After mating, berried females were removed and placed in individual 100 l spawning tanks. Nauplii were obtained within 48 h and were nursed for 25 days. These families were used to verify parentage assignment with known parents and offspring genotypes.

2.2. Microsatellite amplification

Swimmeret tissues of prawn broodstock and whole body of 10 larvae (PL-5) from each family were collected for DNA extraction using a standard phenol-chloroform extraction procedure (Taggart et al., 1992). Genotyping was performed at ten microsatellite loci (*Mbr-1*, *Mbr-2*, *Mbr-3*, *Mbr-4*, *Mbr-5*, *Mbr-7*, *Mbr-8*, *Mbr-9*, *Mbr-10* and *Mbr-11* with GenBank accession numbers DQ019863-67, and DQ019869-73 respectively) using the PCR conditions described by Chareontawee et al. (2006). PCR reactions were performed in 15-μl reactions which contained 10 ng template DNA, 0.3 μM forward and reverse primers, 0.2 mM each dNTP, 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris–HCl (pH 9.0), 0.1% Triton X-100, and 1 unit of *Taq* DNA polymerase (Promega). The PCR profile was initial denaturation at 94 °C for 3 min; 35 cycles of 94 °C for 30 s, annealing temperature for 45 s, and 72 °C for 1 min; and 1 cycle of 72 °C for 7 min. The reaction mixtures were subjected to electrophoresis on a 5.5% denaturing polyacrylamide gel at 80 W for 3 h. The gel was denatured at 100 °C for 30 min before electrophoresis. Bands in the gel were visualized by silver staining. Allele sizes were estimated by comparison to an M13 sequence ladder.

2.3. Simulations and parentage analysis

Estimates of genetic variation, including observed/expected heterozygosity, polymorphism information content (PIC value), the frequency of null alleles, and the average non-exclusion probability of each locus were calculated based on the allele frequencies of 45 broodstock and 230 offspring using the computer program CERVUS 3.0 (Kalinowski et al., 2007; Marshall et al., 1998). An estimated null allele frequency > 0.05 was considered significant. GENEPOP 4.0 (Raymond and Rousset, 1995) was used to test for departure from Hardy–Weinberg expectations and genetic linkage disequilibrium between pairs of loci.

We used two computer programs for parentage analysis, CERVUS 3.0 and the recently developed COLONY 2 (Jones and Wang, 2010). CERVUS assigns offspring to their parent pairs based on the pair-wise likelihood comparison approach. The program generates locus-by-locus likelihood scores for each candidate parent for each offspring and assigns parentage to a candidate parent with the highest LOD score. In contrast to CERVUS, COLONY infers sibship and parentage among individuals using their multilocus genotypes and a full-pedigree likelihood method. The program divides a sample of individuals into three subsamples: the offspring sample, a candidate father sample and a candidate mother sample, and assigns individuals in the three subsamples to family clusters. Individuals within a cluster are assumed to be either full-sibs or half-sibs, while individuals between clusters are unrelated. As a result a very large number of potential clusters can be generated. The program's algorithm calculates the likelihood of pedigree relationship in an initial cluster and compares with likelihoods of the new pedigrees to search for the best cluster with maximum likelihood (Jones and Wang, 2010; Wang, 2004; Wang and Santure, 2009).

The simulation program within CERVUS was used to generate genotypes of 10,000 offspring and candidate parents from the allele frequencies of 45 parents and to get significant LOD scores at 95% confidence level (Marshall et al., 1998). The numbers of family tested in the simulations were from 10 to 1000, with the assumption that each family consisted of one male and one female. To minimize the cost of genotyping, simulations were performed to determine the number of informative loci that would be needed to obtain a given level of assignment success. Loci with the highest PIC scores were chosen and sequentially added to the set. To determine the effects of genotyping error or mutation on parentage inference, the error rate was set at 1% and 5%.

Parentage assignment of the actual offspring was performed in 21 full-sib and two half-sib families. Genotype data of 230 offspring (ten from each family) were pooled and analyzed, with parent information known to the experimenters but unspecified in the program. The accuracy in parentage identification was determined by comparing the observed assignment success rates in CERVUS or the best pedigree relationship in COLONY with known pedigree information of the hatchery population of freshwater prawn. To further compare accuracy and robustness of the two approaches when typing error occurs at a high rate, we introduced deliberate genotyping errors of 0.1, 0.15 and 0.20 at each locus to marker data of 23 families and compared assignment accuracy of individuals.

3. Results

3.1. Properties of microsatellite markers

The parental stock exhibited high genetic variation relative to wild and hatchery populations of *M. rosenbergii* (Chareontawee et al., 2007; Karaket et al., 2011), with the number of alleles per locus ranging from 5 to 22, expected heterozygosities ranging from 0.425 to 0.942, and PIC ranging from 0.374 to 0.936 (Table 1). The average non-exclusion probabilities over nine loci were one over 10,000 animals for one candidate parent, two over 100,000 animals for one candidate parent given the genotype of known parent of the opposite sex, and one over 10,000,000 animals for a candidate parent pair, respectively. The average non-exclusion probability is the probability of not excluding a single unrelated candidate parent or parent pair from parentage of a given offspring at one locus (Kalinowski et al., 2007). CERVUS indicated the presence of low null allele frequencies at the eight loci and significant level of null alleles at locus *Mbr-11*. Significant deviations from Hardy–Weinberg expectations were observed at two loci (*Mbr-9* and *Mbr-11*) after Bonferroni correction, suggesting the presence of null alleles at these loci. Linkage disequilibrium was detected between *Mbr-9* and *Mbr-10* ($P<0.000$), therefore, locus *Mbr-10* was not included in parentage analysis.

3.2. Simulations and real parentage assignments

The number of loci that would be required to obtain high rate of assignment were chosen based on the highest PIC scores and were sequentially added to the set. Loci *Mbr-1* (PIC = 0.936) and *Mbr-4*

Table 1
Characteristics of microsatellite loci used for parentage assessment of 45 freshwater prawn broodstock and 230 progeny, including number of alleles, H_o: observed heterozygosity, H_e: expected heterozygosity, HWE: Hardy–Weinberg expectation, polymorphism information content (PIC), and frequency of null alleles F (Null). Asterisks denote significant deviations from HWE, $P<0.005$, NS = $P>0.005$.

Locus	No. alleles	H_o	H_e	HWE	PIC	F (Null)	NE-1P[a]	NE-2P[b]	NE-PP[c]
Mbr-1	21	0.975	0.942	NS	0.936	−0.0201	0.220	0.124	0.026
Mbr-2	13	0.704	0.713	NS	0.672	−0.0015	0.682	0.506	0.309
Mbr-3	22	0.817	0.867	NS	0.857	0.0259	0.398	0.247	0.081
Mbr-4	20	0.947	0.914	NS	0.906	−0.0204	0.299	0.176	0.050
Mbr-5	16	0.849	0.896	NS	0.886	0.0243	0.348	0.210	0.069
Mbr-7	5	0.420	0.425	NS	0.374	0.0087	0.908	0.787	0.658
Mbr-8	5	0.710	0.715	NS	0.677	0.0103	0.689	0.507	0.314
Mbr-9	6	0.831	0.791	*	0.756	−0.0285	0.596	0.418	0.237
Mbr-11	14	1.000	0.866	*	0.851	−0.0794	0.422	0.266	0.103
Average	13.56	0.806	0.792	–	0.768	–	1.07×10^{-3}	2.54×10^{-5}	1.0×10^{-8}

[a] Average non-exclusion probability for one candidate parent.
[b] Average non-exclusion probability for one candidate parent given the genotype of a known parent of the opposite sex.
[c] Average non-exclusion probability for a candidate parent pair.

(PIC = 0.906) were used in the initial analysis, followed by *Mbr-5* (PIC = 0.886), *Mbr-3* (PIC = 0.857), *Mbr-11* (PIC = 0.851) and the remaining loci with lower PIC scores.

Results of assignment success rate for simulations and actual (observed) assignment using CERVUS are presented in Table 2. Genotyping errors were 4%, 2% and 5% for father, mother and offspring genotypes, respectively, due to non-amplified PCR products. When typing error rate was set for 1% with the use of two loci, the success rates of parentage assignment were very low for simulations (1.2–2.5%) and actual assignments (3.5–6.9%). The assignment rates increased to 100% when six loci were included. Similarly, the assignment rates at typing error of 5% were very low when 2–3 loci were used and 100% success rates were obtained for seven loci. Simulations predicted that 100% assignment of parent pair would be obtained for up to 1000 families based on nine loci (Fig. 1). However, only 300 families were correctly assigned based on father or mother information and the assignment rates dropped to 65% for 1000 families. A comparison of assignment accuracy (with typing error rates of 1% and 5%) for actual data from 23 families between CERVUS and COLONY showed that COLONY correctly assigned paternity, maternity and parent pair to all 230 offspring using four loci, whereas CERVUS assigned parentage to 84–92% of the offspring (Table 3). Results indicated that use of the four highly informative loci was sufficient for COLONY to resolve genetic structure of freshwater prawn in this study, while seven loci would be required to obtain 94–99% correct assignment with CERVUS. With the inclusion of the five less informative loci in COLONY, the accuracy of maternal assignment did not changed, but the assignment accuracy of father and parent pair decreased from 100 to 96%.

When higher genotyping error rates were generated and taken into account, assignment rates were lower for CERVUS using from two to four highly polymorphic loci (Table 4). For example, at typing error of 0.10 and the use of three loci, approximately 71, 72 and 61% assignment rates were obtained for father, mother and parent pair with CERVUS, whereas higher assignment rates of 92, 93 and 90% for father, mother and parent pair were obtained with COLONY. When typing error increased to 0.2, COLONY required only four loci to assign parentage to 85–90% of the offspring, while five to seven loci would be required to obtain the similar proportions of correct assignment with CERVUS.

4. Discussion

The power of microsatellite-based assignment techniques is dependent on several factors, including usefulness of markers, presence of null alleles, independent segregation of loci, as well as mutation and genotyping errors and the logic underlying the test procedure itself (Marshall et al., 1998; Wang and Santure, 2009). Of ten microsatellite loci analyzed in this study, all but one locus (*Mbr-7*) were highly informative, with average expected heterozygosity of 0.79 and PIC score of 0.77. In addition to the measure of polymorphism, CERVUS also calculates the non-exclusion probability for each locus. Results indicated that the probability for a particular locus was low to moderate, but the overall non-exclusion probabilities over nine loci were close to zero due to high levels of polymorphism of the loci. Therefore, high assignment success based on the exclusion method would be expected. With genotyping errors and missing genotypes, however, the probability of non-exclusion can be higher and can decrease the power of the test. These probabilities have little effects on the likelihood-based assignments, but may be useful for comparison with similar work on the same species (Kalinowski et al., 2007).

Although microsatellites are the most suitable genetic markers for parentage studies, problems of mutation, null alleles and typing errors can reduce the accuracy of the test by causing mismatch between parent and offspring. It is suggested that loci with null allele frequencies (>0.05) should be excluded from parentage analysis. However, including loci *Mbr-9* and *Mbr-11* in the parentage analysis increased the accuracy of assignment in CERVUS. Mutation was expected to occur at higher rate in crustaceans due to their high fecundity (Jerry et al., 2004), but the presence of new alleles was not observed in this study,

Table 2
Results of assignment success rate for simulating (actual) assignment using CERVUS with genotyping errors = 0.01 and 0.05. Simulations were performed based on 10,000 offspring, and actual assignments were based on pooled data of offspring in 23 families.

No. loci	Genotyping error = 0.01			Genotyping error = 0.05		
	Father	Mother	Parent pair	Father	Mother	Parent pair
2	1.2(6.9)	2.5(4.3)	2.4(3.5)	0.4(0.8)	0.0(0.0)	2.9(7.4)
3	35.9(36.1)	28.3(27.4)	61.3(45.6)	21.4(27.8)	20.7(25.6)	38.5(36.9)
4	96.1(94.4)	94.2(83.5)	98.8(98.7)	59.7(66.9)	57.3(61.3)	77.2(88.7)
5	99.9(99.5)	99.8(99.6)	99.7(99.6)	86.3(91.7)	83.6(82.6)	97.2(98.2)
6	99.9(100)	99.9(100)	99.9(100)	96.1(95.6)	94.5(95.6)	99.8(100)
7	100(100)	100(100)	100(100)	98.9(99.1)	98.9(99.6)	99.9(100)
8	100(100)	100(100)	100(100)	100(100)	100(100)	100(100)
9	100(100)	100(100)	100(100)	100(100)	100(100)	100(100)

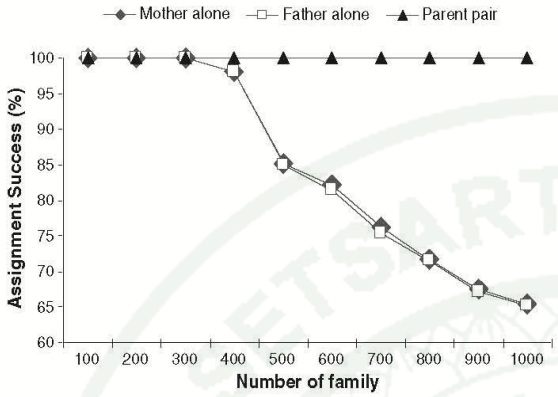

Fig. 1. Simulation of assignment success based on genotypes of 10,000 offspring at nine microsatellite loci using CERVUS. The numbers of family tested in the simulations were from 10 to 1000 at 95% confidence level.

probably due to small number of offspring used in the analysis. The classical exclusion approach is so very sensitive that a mismatch at a single locus can result in false exclusions (Hedrick, 2005). Because the probability of typing errors tends to increase when additional loci are used, the computer programs are designed to accommodate genotyping errors to increase success in parentage assignment (Jones and Wang, 2010; Kalinowski et al., 2007; Wang and Santure, 2009). In the present study, when genotyping error rates increased from 0.1 to 0.2 and were accounted for, assignment accuracy decreased slightly for both CERVUS and COLONY. Our results indicated that the accuracy of assignment decreased if genotyping errors were ignored (data not shown). It is likely that the inclusion of less informative loci (e.g., *Mbr-2*, *Mbr-7*, and *Mbr-8*) in the prawn population may introduce more noise, resulting in the slightly decreased accuracy for assignment of paternity and parent pair obtained in COLONY when the number of loci was increased from 5 to 9.

Experimental designs for genetic parameter estimations and performance tests in aquaculture usually involve mixing full- and half-sib families to eliminate confounding environmental effects. Genetic relatedness among candidate parents and in particular, the family structure for polygamous population, may have confounding effects on parentage analysis (Marshall et al., 1998). Increased relatedness between parents can result in multiple assignments of parentage to the individuals thus, reducing the confidence of assignment (Jones and Ardren, 2003). However, Wang and Santure (2009) demonstrated that the full-pedigree likelihood method was only slightly affected by relatedness and the mating system in the population. It is well understood that domesticated populations, including freshwater prawn are assumed to have significantly connected pedigree. However, the degrees of relatedness in such populations are usually not known. COLONY, therefore, would be appropriate for analysis of parentage and sibship structure for freshwater prawn because the whole field of pedigree relationship can be explored. Knowledge of full-pedigree relationships is useful for estimating genetic parameters and assessing performance of families in the same tanks (Jerry et al., 2006).

In the present study, the only drawback of COLONY is that it requires up to 3 days for analyzing our data with typing error rates of 0.1, 0.15 and 0.2 on a Notebook SONY with a 1.86-GHz Intel Pentium Dual-Core processer and 1 GB RAM. In contrast, CERVUS requires approximately 30 min for simulations and parentage analysis when genotyping error is set at 0.1. Moreover, simulations in CERVUS are very useful to provide the quality control of allele information from real marker data.

Despite the potential for parentage assignment of the nine microsatellite markers, it should be noted that there are few limitations in the present study, including the size of the test population and the number of candidate parents. For example, only 230 progeny were used in the analysis. While in the real situation, a parentage test could be required for a much larger population size. Potential parents consisted of only 45 real parents and there were no other non-parental genotypes included as candidates. In practice, the number of candidate parents to be tested could far exceed real number of parents. Consequently, this will affect the robustness of nine markers utilized in this study.

In conclusion, the set of microsatellites previously developed for *M. rosenbergii* has proven an effective tool for parentage analysis in breeding populations of freshwater prawn. This study demonstrated that both CERVUS and COLONY do well at inferring pedigree relationships in this domesticated population of freshwater prawn, with COLONY showing a clear advantage in accuracy and economical use of markers.

Table 3
Parentage assignment test results of the actual offspring, showing the percent of correct assignment of individuals for 23 families (22 fathers, 23 mothers, and 506 parent pairs) using CERVUS version 3.0 and COLONY 2 based on genotyping errors at 0.01 (0.05).

No. loci	CERVUS			COLONY		
	Father	Mother	Parent pair	Father	Mother	Parent pair
2	70.8(72.2)	66.5(69.6)	55.6(55.6)	80.8(65.22)	77.4(63.1)	71.7(44.3)
3	85.6(84.3)	87.8(86.9)	76.1(76.1)	96.1(96.1)	95.6(95.6)	94.3(93.5)
4	92.2(87.4)	87.4(92.6)	84.7(85.2)	100(100)	100(99.6)	100(99.6)
5	93.5(88.7)	96.9(93.1)	91.7(83.9)	94.3(94.3)	100(100)	94.3(94.3)
6	97.7(95.6)	99.1(99.1)	94.3(95.2)	95.6(94.7)	100(100)	95.6(94.7)
7	94.7(94.7)	99.6(99.6)	94.7(94.7)	95.6(94.7)	100(100)	95.6(94.7)
8	95.2(95.2)	99.6(99.6)	95.2(95.2)	95.6(94.7)	100(100)	95.6(94.7)
9	95.2(95.2)	99.1(99.1)	95.2(95.2)	95.6(94.7)	100(100)	95.6(94.7)

Table 4
Parentage assignment test results of the actual offspring, showing the percent of correct assignment of individuals for 23 families (22 fathers, 23 mothers, and 506 parent pairs) using CERVUS version 3.0 and COLONY 2 when genotyping error rates were generated at 0.10, 0.15, and 0.20.

Error	No. loci	CERVUS			COLONY		
		Father	Mother	Parent pair	Father	Mother	Parent pair
0.10	2	50.8	56.5	40.4	64.8	72.2	53.5
	3	71.7	72.6	61.3	92.6	93.0	90.0
	4	89.5	94.3	86.5	96.5	98.7	96.5
	5	92.2	93.5	88.7	92.6	98.7	92.6
	6	92.6	96.0	90.4	92.6	98.7	93.0
	7	89.6	91.3	87.3	92.2	97.8	92.2
	8	94.7	97.8	93.9	94.3	99.6	94.3
	9	94.7	96.6	93.9	93.0	99.6	93.4
0.15	2	34.3	42.6	26.9	54.7	70.4	45.2
	3	35.6	43.7	27.5	87.4	86.1	82.6
	4	88.0	90.6	84.3	93.5	94.7	92.2
	5	91.7	91.7	86.9	89.1	91.8	89.1
	6	91.3	93.9	89.1	90.0	88.3	85.6
	7	89.4	85.6	88.7	83.4	89.4	80.4
	8	93.9	95.8	92.0	82.6	86.9	75.6
	9	94.3	95.5	92.6	81.3	85.6	71.7
0.20	2	35.7	41.7	22.3	50.8	60.4	39.1
	3	34.8	42.6	23.0	69.1	74.8	60.7
	4	87.4	87.0	81.3	90.8	89.8	85.8
	5	88.7	90.0	84.3	83.4	85.6	79.6
	6	90.8	90.4	86.5	74.7	84.3	78.7
	7	84.7	86.1	87.4	72.6	83.9	65.6
	8	91.4	92.6	90.7	72.1	82.9	69.6
	9	91.3	92.6	92.6	77.8	83.9	72.2

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