DEVELOPMENT OF MICROSATELLITE MARKERS AND GENETIC DIVERSITY ASSESSMENT OF KEMPAS (KOOMPASSIA MALACCENSIS) IN PENINSULAR MALAYSIA

LEE CHAI TING
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DEVELOPMENT OF MICROSATELLITE MARKERS AND GENETIC DIVERSITY ASSESSMENT OF KEMPAS (KOOMPASSIA MALACCENSIS) IN PENINSULAR MALAYSIA

By

LEE CHAI TING

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

December 2009
Specially dedicated to my beloved husband and family members,
in loving memory of my late grandmother and uncle
A total of 24 novel microsatellite markers have been successfully isolated and characterised in an important tropical timber species of the family Leguminosae, Koompassia malaccensis, locally known as kempas. The microsatellite primers were designed from a genomic library enriched for dinucleotide (CT) repeats and subsequently screened on 24 samples from a natural population. In general, these microsatellite markers are highly polymorphic (mean number of alleles per locus, $A_a = 6.84$; average gene diversity, $H_e = 0.692$), with two loci found to deviate significantly from Hardy-Weinberg equilibrium ($p < 0.05$). The utility of these microsatellite markers were tested across 13 leguminous timber tree species and the highest transferability was found with K. excelsa, the only species of the same genus tested, followed by Dialium platysepulam of the same subtribe, Dialiinae. The amplification success appeared to be inversely associated with the phylogenetic distance, in particular up to the subtribal levels. Four of the microsatellite loci were used to study the mating system of K. malaccensis, based on a fruiting season at the
Semangkok Forest Reserve in year 2005. Single- and multilocus population outcrossing estimates ($t_s$ and $t_m$) were determined using the program MLTR ver 3.0. The results showed that *K. malaccensis* is predominantly outcrossing ($t_m = 0.890$), with low tendency of mating between relatives [$t_m - t_s = 0.027$]. In addition, the level of genetic diversity of *K. malaccensis* in 34 natural populations throughout Peninsular Malaysia was assessed and its distribution described. Omitting four loci due to suspected presence of null alleles and linkage disequilibrium, 20 microsatellite loci were analysed for 974 individuals. Overall, all the populations showed high levels of genetic diversity, with gene diversity ($H_e$) ranging from 0.577 (Kuala Langat Selatan) to 0.787 (LenggorB) and mean $A_a$ being 9.0. The levels of genetic diversity for the two peat swamp (PS) populations (Kuala Langat Selatan and Pekan) were significantly lower than for the non-PS populations. The estimated coefficients of population differentiation ($F_{ST}$ and $R_{ST}$) revealed that the majority of the genetic diversity resides within populations and less among populations ($F_{ST}$: 0.077; $R_{ST}$: 0.102). Results from the analysis of molecular variance (AMOVA), cluster analysis, principal component analysis (PCA) and *STRUCTURE* analysis consistently demonstrated that *K. malaccensis* originating from the two contrasting habitats (PS vs non-PS) were genetically distinct, supporting the ecotype hypothesis. Excluding the PS populations, the among-population component of genetic diversity was even smaller ($F_{ST}$: 0.028; $R_{ST}$: 0.023), but statistically significant. Pairwise $F_{ST}$ values among the non-PS populations were positively correlated to geographical distance (Mantel test; $r^2 = 0.0936$, $p < 0.01$), indicating weak but significant isolation-by-distance. Pangkor Selatan and LenggorB were found to be relatively more divergent among the non-PS populations investigated, presumably due to genetic drift and the inclusion of freshwater swamp habitat, respectively. Significant but weaker
population genetic structure was detected among the rest of the non-PS populations surveyed, which corresponded to the topography of Peninsular Malaysia, reflecting the role of mountain ranges as geographical barriers to gene flow. The implications of the findings from this study for the genetic conservation of *K. malaccensis* are discussed and conservation strategies (both *in situ* and *ex situ*) proposed to ensure sustainable utilisation of this important timber species in Malaysia.
PEMBANGUNAN PENANDA MIKROSATELIT DNA DAN PENILAIAN KEPELBAGAIAN GENETIK BAGI KEMPAS (*KOOMPASSIA MALACCENSIS*) DI SEMENANJUNG MALAYSIA

Oleh

LEE CHAI TING

Disember 2009

Pengerusi: Faridah Qamaruz Zaman, PhD

Institut: Biosains

Sejumlah 24 penanda mikrosatelit baru telah berjaya dipencil serta dicirikan untuk satu spesies balak tropika yang penting daripada famili Leguminosae, iaitu *Koompassia malaccensis*, yang dikenali dengan nama tempatan kempas. Pencetus mikrosatelit tersebut telah direka daripada perpustakaan genomik yang diperkayakan dengan ulangan dinukleotida (CT) dan kemudiannya disaring menggunakan 24 sampel dari satu populasi semulajadi. Secara amnya, penanda-penanda mikrosatelit yang diperolehi adalah berpolimorfik tinggi (min bilangan alel setiap lokus, $A_a = 6.84$; min kepelbagaian gen, $H_e = 0.692$), dengan dua lokus didapati menyimpang daripada keseimbangan Hardy-Weinberg ($p < 0.05$). Kegunaan penanda-penanda molekul tersebut diuji ke atas 13 spesies balak legum dan kadar pemindahan tertinggi didapati pada *K. excelsa*, satu-satunya spesies daripada genus yang sama yang telah diuji, diikuti dengan *Dialium platysepalum* daripada subtrib yang sama, Dialiinae. Kejayaan amplifikasi didapati mempunyai perhubungan songsang dengan jarak filogenetik, terutamanya sehingga ke peringkat subtrib. Empat daripada lokus
mikrosatelit tersebut telah digunakan untuk kajian sistem kacukan *K. malaccensis*, berdasarkan suatu musim buah di Hutan Simpan Semangkok pada tahun 2005. Anggaran kadar kacukan luar populasi berdasarkan lokus tunggal serta gabungan lokus (*t_s* and *t_m*) telah ditentukan dengan menggunakan program MLTR ver 3.0. Keputusan menunjukkan bahawa *K. malaccensis* mengamalkan kacukan luar dengan kadar yang tinggi (*t_m = 0.890*) dan mempunyai kadar kacukan sesama saudara yang rendah [(*t_m – t_s*) = 0.027]. Di samping itu, tahap kepelbagaian genetik *K. malaccensis* bagi 34 populasi semulajadi di Semenanjung Malaysia telah dinilai dan taburannya diterangkan. Setelah mengasingkan empat lokus yang disyaki mempunyai alel-nul serta ketakseimbangan rangkaian, 20 lokus mikrosatelit telah dianalisis untuk 974 individu. Secara keseluruhannya, kesemua populasi menunjukkan tahap kepelbagaian genetik yang tinggi, dengan kepelbagaian gen (*H_e*) menjulat daripada 0.577 (Kuala Langat Selatan) ke 0.787 (LenggorB) dan min *A_s* sebanyak 9.0. Tahap kepelbagaian genetik untuk kedua-dua populasi paya gambut (Kuala Langat Selatan dan Pekan) adalah lebih rendah secara statistik berbanding dengan populasi bukan-paya-gambut yang lain. Anggaran koefisien pembezaan populasi (*F_{ST} dan R_{ST}*) menunjukkan bahawa majoriti kepelbagaian genetik dibahagikan di dalam populasi dan kurang di kalangan populasi (*F_{ST}: 0.077; R_{ST}: 0.102*). Keputusan daripada analisis varians molekular (AMOVA), analisis kelompok, analisis komponen prinsipal (PCA) serta analisis STRUCTURE secara konsisten menunjukkan bahawa *K. malaccensis* yang berasal daripada dua habitat yang berlainan (paya gambut dan bukan-paya-gambut) adalah berbeza secara genetik, iaitu menyokong hipotesis ekotip. Dengan pengecualian populasi paya gambut, komponen kepelbagaian genetik di kalangan populasi adalah jauh lebih kecil (*F_{ST}: 0.028; R_{ST}: 0.023*), tetapi signifikan secara statistik. Nilai *F_{ST} secara berpasangan di kalangan populasi bukan-
paya-gambut berhubungan secara langsung dengan jarak geografi masing-masing
(Mantel test; \( r^2 = 0.0936, \ p < 0.01 \)), menggambarkan wujudnya pengasingan-oleh-
jarak yang lemah tetapi signifikan. Pangkor Selatan dan LenggorB didapati lebih
mencapai secara relatif di antara populasi bukan-paya-gambut yang dikaji, kemungkinan disebabkan akibat hanyutan genetik serta perangkuman habitat paya-
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pergunungan sebagai halangan geografi terhadap aliran gen. Implikasi penemuan
kajian ini terhadap pemuliharaan genetik telah dibincang dan strategi pemuliharaan
(secara in situ dan ex situ) disyorkan demi memastikan penggunaan mampan spesies
balak yang penting ini di Malaysia.
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I certify that a Thesis Examination Committee has met on 16 December 2009 to conduct the final examination of Lee Chai Ting on her thesis entitled "Development of Microsatellite Markers and Genetic Diversity Assessment of Kempas (Koompassia malaccensis) in Peninsular Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Examination Committee were as follows:

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Date: 17 Mac 2010
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

LEE CHAI TING

Date: 17 January 2010
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4.12C Principal coordinate analysis (PCA 3) of the 22 non-peat swamp *Koompassia malaccensis* populations investigated; with the exclusion of the nine populations from the Subregion NW (Northwest). The populations from the Subregions ES (East & South), NC (North & Central) and SW (Southwest) were colour coded as blue, green and orange, respectively. Population codes correspond to Table 3.2.

4.13 Bayesian clustering analysis of all the populations investigated to determine the optimal $K$. (A) Mean $L(K)$ ($\pm$ S.D.) over five runs for each $K$. The graph exhibits the optimal number of clusters at $K = 2$, after which $L(K)$ at larger $K$s almost plateaus with only slight increases. (B) Rate of change of the likelihood distribution, $L'(K)$.

4.14 Bayesian clustering analysis of the non-peat swamp populations to determine the optimal $K$. (A) Mean $L(K)$ ($\pm$ S.D.) over five runs for each $K$. (B) $\Delta K$ calculated as $\Delta K = m(|L''(K)|) / s[L(K)]$. The modal value is $K = 8$, inferring eight clusters.

4.15 *STRUCTURE* analysis of *Koompassia malaccensis* in Peninsular Malaysia based on microsatellite data. The width of each segment corresponds to the sample size of each population. Population codes correspond to Table 3.2. (A) All populations surveyed; the highest likelihood was found for $K = 2$, populations of peat swamp ecotype (red; Population 12, KLSelatan and Population 28, Pekan) are distinctive from those of non-peat swamp; with Population 8 (SKarang) showing admixture genotypes; (B) Non-peat swamp populations investigated in more detail, i.e., excluding Populations 8, 12 and 28; $K = 2$. Geographical subregions of the populations are given on top of the figure (NW = Northwest; SW = Southwest; NC = North & Central; ES = East & South).

4.16 Contribution of each population of *Koompassia malaccensis* to (A) total diversity, CT, and (B) allelic richness, CTR. Population codes correspond to Table 3.2.

5.1 (A) A *Koompassia malaccensis* tree from a peat swamp forest (Kuala Langat Selatan Forest Reserve, Selangor), exhibiting its steep, sinuous plank buttress; in comparison to *K. malaccensis* trees from the non peat swamp forests: (B) Endau Rompin State Park, Pahang; (C) Sungai Udang Forest Reserve, Melaka.

5.2 Topography map of Peninsular Malaysia superimposed with arbitrary boundaries of the four subregions of non-peat swamp
*Koompassia malaccensis* (Northwest, NS; Southwest, SW; North & Central, NC; East & South, ES) as identified from the cluster analysis (see Figure 4.10).
LIST OF ABBREVIATIONS

AMOVA  Analysis of molecular variance
bp    Base pair
CAPS  Cleaved amplified polymorphic sequences
AFLP  Amplified fragment length polymorphism
CITES Convention on International Trade in Endangered Species
CpDNA Chloroplast DNA
CsCl  Caesium chloride
CTAB  Hexadecyltrimethyl-ammonium bromide
DAF   DNA amplification fingerprint
dbh   Diameter at breast height
DNA   Deoxyribonucleic acid
dNTP  2’-deoxynucleoside 5’-triphosphate
EDTA  Diaminoethanetetra-acetic acid
EMBL  European Molecular Biology Laboratory
EST   Expressed sequence tag
ESUs  Evolutionarily significant units
FAO   Food and Agriculture Organization of the United Nations
FIASCO Fast isolation by AFLP sequences containing repeats
FR    Forest Reserve
FRIM  Forest Research Institute Malaysia
GSM   Generalized stepwise model
IAM   Infinite alleles model
IBD   Identical-by-descent