UNIVERSITI PUTRA MALAYSIA

GENETIC STRUCTURE OF GIANT HONEYBEE (Apis dorsata Fabricius) POPULATION IN PENINSULAR MALAYSIA

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By

NAJMEH SAHEBZADEH

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

December 2012

Specially dedicated to

Beloved Dad

- Words cannot express my gratitude for his love and support that has sustained me during my life and study-

… and

In loving memory of Mom…

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in partial fulfilment of the requirement for the Degree of Doctor of Philosophy

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The knowledge on the genetic structure of *Apis dorsata* is needed for effective management and conservation strategies of this species in Malaysia, especially to prevent over-harvesting of honey. The main objective of this research was to estimate genetic structure and relatedness of Malaysian *A.dorsata* populations (2009-2010) from Terengganu and Kedah using 30 DNA microsatellites. The results showed that all the loci and sample size were informative for measuring the genetic variation in *A.dorsata* aggregations.

The results of genetic structure revealed that there was no significant deviation Hardy-Weinberg equilibrium and linkage disequilibrium ($P>0.05$) within and

between aggregations. Population differentiation coefficient (F_{ST}) indicated a great level (F_{ST}>0.15) within five aggregations and a moderate level (F_{ST}=0.095) of genetic differentiation within an aggregation of Terengganu. A moderate level (F_{ST}>0.07) of F_{ST} was detected within Pedu Lake aggregations. The values of F_{ST} between Terengganu 2009 aggregations showed no genetic differentiation ($P>0.21$), whereas genetic differentiation was detected ($P<0.001$) between Terengganu 2010. Pair-wise of F_{ST} values between the aggregations, before (2009) and after (2010) migration, showed that two of three aggregations from Terengganu returned to their nest sites. A same result was obtained by Pedu Lake aggregations.

Analysis of molecular variance (AMOVA) indicated that the nests within each aggregation were genetically and statistically distinct, as a result of the restricted drone congregation areas (DCA) of each aggregation. Amount of misclassification at assignment tests was in line with the results of F_{ST} and AMOVA, indicating two of three aggregations returned to the natal site afetr the migration (2010).

The intra-colony pedigree relatedness values within aggregations showed a low level of genetic relatedness (0.29). Furthermore, genetic relatedness analyses confirmed that the queens within the aggregations were genetically different. However, presence of full and half siblings between same aggregations site before and after migration supported a probability that a particular aggregation returns to its natal site after the migration if there are available empty tree timbers. The finding of an appreciable level of aggregation fidelity indicated that a comprehensive study with a large number of aggregations throughout the region would be necessary to provide concrete proof for this idea.

Finally, the genetic structure and relatedness among the solitary nests from Terengganu, 2010 showed only one nest pair were headed away by same queens. However, the finding of an appreciable level of intercolonial relatedness between the nests of *A.dorsata* sheds light on a larger sample size of solitary nests which were found to provide concrete proof for that particular hypothesis. This finding suggests that the formidable anthropogenic pressures may change the genetic structure of *A.dorsata* population in Malaysia, which still needs to be researched on in the future.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai
memenuhi keperluan untuk ijazah Doktor Falsafah

STRUKTUR GENETIK POPULASI LEBAH MADU GERGASI (*APIS DORSATA* FABRICIUS) DI SEMENANJUNG MALAYSIA

Oleh

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Ilmu berkenaan struktur genetik *Apis dorsata* adalah penting untuk strategi pengurusan dan pemuliharaan yang efektif terhadap spesis ini di Malaysia, terutamanya untuk mengelakkan penuaian madu yang berlebihan. Objektif utama kajian ini adalah untuk menganggarkan struktur dan keberkaitan genetik populasi *A.dorsata* dari Terengganu dan Kedah menggunakan 30 microsatelit DNA. Keputusan telah menunjukkan bahawa kesemua lokus dan saiz sampel adalah cukup untuk menganggarkan variasi genetik pada aggregasi *A.dorsata.*

Keputusan daripada stuktur genetik telah mendedahkan tiada sisihan yang nyata ($P>0.05$) daripada keseimbangan Hardy-Weinberg dan ketidakseimbangan rangkaian di dalam dan di antara aggregasi. Pekali pembezaan populasi (F_{ST}) menunjukkan nilai pembezaan genetic yang besar ($F_{ST}>0.15$) dalam lima aggregasi dan nilai pembezaan genetik yang sederhana ($F_{ST}=0.095$) dalam satu aggregasi daripada Terengganu. Aggregasi daripada Tasik Pedu telah menunjukkan nilai F_{ST} yang sederhana ($F_{ST} >0.07$). Nilai F_{ST} di antara aggregasi 2009 daripada Terengganu tidak menunjukkan pembezaan genetik ($P>0.21$) manakala pembezaan genetik telah dikesan di antara aggregasi Terengganu 2010. Perbandingan nilai F_{ST} di antara aggregasi yang bermigrasi sebelum (2009) dan selepas (2010) telah menunjukkan bahawa dua daripada tiga aggregasi daripada Terengganu kembali kepada tapak sarang mereka. Keputusan yang sama telah diperoleh untuk aggregasi Tasik Pedu.

Analisa variasi molekul (AMOVA) menunjukkan bahawa sarang di dalam dan di antara setiap aggregasi adalah berbeza secara genetik dan statistic, disebabkan oleh ‘drone congregation areas (DCA) yang terhad dalam setiap aggregasi. Jumlah kesalahan klasifikasi pada assignment test adalah selari dengan keputusan F_{ST} dan AMOVA, menunjukkan bahawa dua daripada tiga aggregasi kembali kepada tapak kelahiran mereka selepas migrasi (2010). Nilai keberkaitan keturunan intra-koloni dalam aggregasi menunjukkan nilai keberkaitan genetik yang rendah (0.29). Di samping itu, analisa keberkaitan genetik mengesahkan bahawa ratu dalam aggregasi adalah berbeza secara genetic. Walau bagaimanapun, kehadiran adik-beradik penuh dan separa di antara tapak aggregasi yang sama sebelum dan selepas migrasi menyokong kebarangkalian bahawa sesetengah aggregasi kembali kepada tapak kelahiran mereka selepas migrasi jika terdapat balak kayu yang kosong. Kemunculan paras kesetiaan aggregasi yang ketara menunjukkan bahawa satu kajian yang menyeluruh dengan bilangan aggregasi yang lebih besar di sepanjang rantau ini adalah diperlukan untuk memberikan bukti yang kukuh terhadap idea ini.

Akhirnya, struktur genetik dan keberkaitan di antara sarang berasingan daripada Terengganu, 2010 menunjukkan bahawa hanya satu pasangan sarang yang diketuai oleh ratu yang sama. Walau bagaimanapun, pencarian tentang paras keberkaitan yang ketara di antara koloni di antara sarang *A.dorsata* menyediakan bukti yang kukuh bahawa saiz sampel sarang yang berasingan telah menyokong hipotesis tersebut. Keputusan ini menunjukkan bahawa kemusnahan yang dibawa oleh manusia adalah berkemampuan untuk mengubah struktur genetik populasi *A.dorsata* di Malaysia, dan lebih banyak kajian adalah perlu dijalankan pasa masa depan.

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I certify that an Examination Committee has set on 27 December 2012 to conduct the final examination of Najmeh Sahebzadeh on her Doctor of Philosophy thesis entitle "**GENETIC STRUCTURE OF GIANT HONEYBEE (*APIS DORSATA* FABRICIUS) POPULATION IN PENINSULAR MALAYSIA**" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The committee recommends that the candidate be awarded the Doctor of Philosophy.

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DECLARATION

I declare that the thesis is 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.

NAJMEH SAHEBZADEH

Date: 27 December 2012

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