

# 台灣黑眶蟾蜍之親緣地理研究

魏勢璋、賴伯琦

E-mail: 319614@mail.dyu.edu.tw

## 摘要

黑眶蟾蜍 (*Duttaphrynus melanostictus*) 現今廣泛分布於中國南部及東南亞，在台灣主要分布於五百公尺以下的闊葉林或開墾地。本研究於台灣採集到的地點分別來自不同縣市的 23 個族群，並利用粒線體 DNA 中 cytochrome b 和 control region 之間片段，長度為 982 bp。由 90 個定序樣本 所得的序列發現有 23 個變異位置 (variable sites)，18 種單倍型 (haplotypes)，其中僅單倍型 Hp01 廣泛分布於台灣以及蘭嶼、綠島，並佔了所有序列的 46.7%。透過 Bayesian skyline plot 顯示台灣黑眶蟾蜍在末次冰期後族群量顯著地增加，推測是因氣候穩定所致。而基因多樣性與族群變動分析則顯示台灣僅有西部族群發生過族群擴張現象，推測是由少數之族群快速發展而來。經計算 Kimura-2-parameter 基因距離後再以 neighbor-joining 及 maximum-parsimony 方法重建單倍型間之樹狀 圖後發現所取樣的黑眶蟾蜍序列與採集地點間沒有明顯的親緣分化現象，但在每個地區或鄰近地區 (除了雲林以外) 都發現當地特有之單倍型。經由分析親緣網狀圖推測黑眶蟾蜍進入台灣地區可能有兩條播遷路徑：1. 由台灣中部地區進入，並向東散播，隨後才向南拓殖，最後才往北擴散 2. 從台灣南部地區進入，並先後向北與向南散播，其次才向東擴散，最後又由宜蘭播遷回大陸。由於目前所得之研究資料僅得福建地區部分族群，若欲進一步推斷台灣之黑眶蟾蜍應是否至少有兩次遷入現象，尚須大陸其他沿海地區樣本以進行更深入的探討。

關鍵詞：黑眶蟾蜍、親緣地理、中心散播假說、同宗效應假說、粒線體 DNA 分析

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## 參考文獻

- 參考文獻 1. 王健勳，2001，“以粒線體之 12S 核糖體 RNA 探討海鰱首目魚類的親緣關係”，國立中山大學碩士論文。 2. 王子元，2007，“從全球氣候變遷談台灣兩種間吸鯀魚類的遷徙過程”，自然保育季刊第58期，行政院農業委員會特有生物保育中心，pp. 54-60。 3. 呂光洋，1985，“由島嶼生態談到自然保育”，科學月刊 16，pp. 408-412。 4. 李逸鴻、林仲平，2008，“淺談基因交流下的種化現象與研究法”，生物科學 51，pp. 10-20。 5. 林弘都，2008，“台灣與中國大陸地區鯉科魚類之親緣地理研究”，國立成功大學博士論文。 6. 林朝棨，1957，“台灣地形”，台灣省文獻委員會出版，台北。 7. 林朝棨，1966，“概說台灣第四紀的地史並討論其自然史和文化史的關係”，國立台灣大學考古人類學刊 28，pp. 7-44。 8. 林彥博、張廖年鴻、周文豪，2005，“盤谷蟾蜍之遺傳多樣性初探”，台灣野生生物遺傳多樣性與保育遺傳研究研討會，行政院農業委員會，pp. 169-181。 9. 周以正、鍾郁涵、張學偉、蔡奇立、郭金泉，2006，“論台灣鮭魚身世之謎和正名”，自然保育季刊第56期，行政院農業委員會特有生物保育中心，pp. 51-58。 10. 洪東奇、黃獻文、梁佑全、歐柏榮，2003，“台灣長鬃山羊族群具有顯著遺傳變異之兩個分群以粒線體 DNA 之 D-loop 序列比對”，特有生物研究第5卷第1期，行政院農業委員會特有生物保育中心，pp. 15-25。 11. 韋昕林、侯平君，2004，“溫度耐受限制黑眶蟾蜍的海拔分布”，中國生物學會與中華民國溪流環境協會聯合年會暨2004生物、溪流、行為與生態聯合學術年會。 12. 許桂菁、蔣鎮宇、王建平、麥愛堂，1999，“台灣石蟾的親緣地理及保育”，自然保育季刊第27期，行政院農業委員會特有生物保育中心，pp. 41-45。 13. 張廖年鴻，2007，“台灣兩種赤蛙的親緣地理結構比較”，國立中興大學博士論文。 14. 楊懿如，2002，“賞蛙圖鑑”，鄉宇文化出版社，pp. 38-95。 15. 葉文珊，1997，“莫氏樹蛙族群地理親緣關係之研究”，國立台灣大學碩士論文。 16. 賴俊祥、呂光洋，2007，“阿里山地區阿里山山椒魚的分布與族群監測”，生物學報 42

, pp. 105-117。 17. 陳志雄、黃敦友、陳文山、王源 , 1991 , "台灣東部海岸山脈泰源地區之生物地層" , 地質 11 , pp. 133-144。 18. 陳文山 , 2005 , "末次冰期以來台灣海岸平原區的海岸線變遷" , 國立台灣大學考古人類學刊 66 , pp. 40-55。 19. Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, E., Reeb, C.A., Saunders, N.C., 1987, "Intraspecific phylogeography the mitochondrial DNA bridge between population genetics and systematics", Annual Review of Ecology and Systematics 18, pp. 489-522. 20. Avise, J.C., Bowen, B.W., Lamb, T., Meylan, A.B., Bermingham, E., 1992, "Mitochondrial DNA evolution at a turtle's pace: evidence for low genetic variability and reduced microevolutionary rate in the testudines ", Molecular Biology and Evolution 9, pp. 457-473. 21. Avise, J.C., Walker, D., 1998, "Pleistocene phylogeographic effects on avian populations and the speciation process", Proceedings of the Royal Society of London. Series B-Biological Sciences 265, pp. 457-463. 22. Avise, J.C., 2000, "Phylogeography: the history and formation of species", Harvard University Press, Cambridge, MA. 23. Bermingham, E., Martin, A.P., 1998, "Comparative mtDNA phylogeography of neotropical freshwater fishes: testing shared history to infer the evolutionary landscape of Lower Central America", Molecular Ecology 7, pp. 499-517. 24. Bossart, J.L., Prowell, D.P., 1998, "Genetic estimates of population structure and gene flow: limitations, lessons and new directions", Trends in Ecology and Evolution 13, pp. 202-206. 25. Breton, S., Dufresne, F., Desrosiers, G., Blier, P.U., 2003, "Population structure of two northern hemisphere polychaetes, *Neanthes virens* and *Hediste diversicolor* (Nereididae), with different life-history traits", Marine Biology 142, pp. 707-715. 26. Brito, P.H., 2005, "The influence of Pleistocene glacial refugia on tawny owl genetic diversity and phylogeography in western Europe", Molecular Ecology 14, pp. 3077-3094. 27. Cao, S.Y., Wu, X.B., Yan, P., Hu, Y.L., Su, X., Jiang, Z.G., 2006, "Complete nucleotide sequences and gene organization of mitochondrial genome of *Bufo gargarizans*", Mitochondrion 6, pp. 186-193. 28. Chao, L., Carr, D.E., 1993, "The molecular clock and the relationship between population size and generation time", Evolution 47, pp. 688-690. 29. Chen, S.F., 2004, "Phylogeography of *Fatsia* (Araliaceae): an endemic genus in East Asia", Master's Thesis, Department of Biology, Cheng Kung University, Tainan, Taiwan. 30. Chen, X.L., Chiang, T.Y., Lin, H.D., Zheng, H.S., Shao, K.T., Zhang, Q., Hsu, K.C., 2007, "Mitochondrial DNA phylogeography of *Glyptothorax fokiensis* and *Glyptothorax hainanensis* in Asia", Journal of Fish Biology 70, pp. 75-93. 31. Cheng, H.L., Huang, S., Lee, S.C., 2005, "Phylogeography of the endemic goby, *Rhinogobius maculafasciatus* (Pisces: Gobiidae), in Taiwan", Zoological Studies 44, pp. 329-336. 32. Chiang, Y.C., Hung, K.H., Schaal, B.A., Ge, X.J., Hsu, T.W., Chiang, T.Y., 2006, "Contrasting phylogeographical patterns between mainland and island taxa of the *Pinus luchuensis* complex", Molecular Ecology 15, pp. 765-779. 33. Chu, J.H., Lin, Y.S., Wu, H.Y., 2007, "Evolution and dispersal of three closely related macaque species, *Macaca mulatta*, *M. cyclopis*, and *M. fuscata*, in the eastern Asia", Molecular Phylogenetics and Evolution 43, pp. 418-429. 34. Clegg, M.T., Gaut, B.S., Learn Jr., G.H., Morton, B.R., 1994, "Rates and patterns of chloroplast DNA evolution", Proceedings of the National Academy of Sciences USA 91, pp. 6795-6801. 35. Clement, M., Posada, D., Crandall, K.A., 2000, "TCS: a computer program to estimate gene genealogies", Molecular Ecology, pp. 1657-1659. 36. Creer, S., Malhotra, A., Thorpe, R.S., Chou, W.H., 2001, "Multiple causation of phylogeographical pattern as revealed by nested clade analysis of the bamboo viper (*Trimeresurus stejnegeri*) within Taiwan", Molecular Ecology 10, pp. 1967-1981. 37. Drummond, A.J., Rambaut, A., Shapiro, B., Pybus, O.G., 2005, "Bayesian coalescent inference of past population dynamics from molecular sequences", Molecular Biology and Evolution 22, pp. 1185-1192. 38. Drummond, A.J., Rambaut, A., 2007, "BEAST: Bayesian evolutionary analysis by sampling trees", BMC Evolutionary Biology 7, pp. 214. 39. Excoffier, L., Laval, G., Schneider, S., 2005, "Arlequin ver.3.1: an integrated software package for population genetics data analysis", Evolutionary Bioinformatics Online 1, pp. 47-50. 40. Frost, D.R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C?lio F.B., De S?, R.O., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., Blotto, B.L., Moler, P., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M., Wheeler, W.C., 2006, "The amphibian tree of life", Bulletin American Museum Natural History 297, pp. 1-370. 41. Fu, J.Z., Weadick, C.J., Zeng, X.M., Wang, Y.Z., Liu, Z.J., Zheng, Y.C., Li, C., Hu, Y., 2005, "Phylogeographic analysis of the *Bufo gargarizans* species complex: A revisit", Molecular Phylogenetics and Evolution 37, pp. 202-213. 42. Gibbard, P., van Kolfschoten, T., 2004, "The Pleistocene and Holocene Epochs", In Gradstein F.M., Ogg J.G., Smith A.G. (editor), A Geologic Time Scale 2004, Cambridge University Press. 43. Grant, W.S., Bowen, B.W., 1998, "Shallow population histories in deep evolutionary lineage of marine fish: insights from sardine and anchovies and lessons for conservation", Journal of Heredity 89, pp. 415-426. 44. Grassle, J.P., Grassle, J.F., 1976, "Sibling species in the marine pollution indicator *Capitella* (Polychaeta)", Science 192, pp. 567-569. 45. Graybeal, A., 1997, "Phylogenetic relationships of bufonid frogs and tests of alternate macroevolutionary hypotheses characterizing their radiation", Zoological Journal of the Linnean Society 119, pp. 297-338. 46. Heuertz, M., Fineschi, S., Anzidei, M., Pastorelli, R., Salvini, D., Paule, L., Frascaria-Lacoste, N., Hardy, O.J., Vekemans, X., Vendramin, G.G., 2004, "Chloroplast DNA variation and postglacial recolonization of common ash (*Fraxinus excelsior* L.) in Europe", Molecular Ecology 13, pp. 3437-3452. 47. Hewitt, G.M., 2001, "Speciation, hybrid zones and phylogeography or seeing genes in space and time", Molecular Ecology 10, 537-549 48. Hwang, U.W., Kim, W., 1999, "General properties and phylogenetic utilities of nuclear ribosomal DNA and mitochondrial DNA commonly used in molecular systematics", Korean Journal of Parasitology 37, pp. 215-228. 49. Igawa T., Kurabayashi A., Nishioka, M., Sumida, M., 2006, "Molecular phylogenetic relationship of toads distributed in the far east and Europe inferred from the nucleotide sequences of mitochondrial DNA genes", Molecular Phylogenetics and Evolution 38, pp. 250-260. 50. Inger, R.F., 1972. "Bufo of Eurasia", In Blair W.F. (editor), Evolution in the Genus *Bufo*, University of Texas Press, Austin, pp. 102-118. 51. Jaeger, J.R., Riddle, B.R., Bradford, D.F., 2005, "Cryptic neogene vicariance and Quaternary dispersals of the red-spotted toad (*Bufo punctatus*): insights on the evolution of North American warm desert biotas", Molecular Ecology 14, pp. 3033-3048. 52. Jang-Liaw, N.H., Lee, T.H., Chou, W.H., 2008, "Phylogeography of *Sylvirana latouchii* (Anura, Ranidae) in Taiwan", Zoological Science 25, pp. 68-79. 53. Johns, G.C., Avise, J.C., 1998, "A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome b gene", Molecular Biology and Evolution 15, pp.

1481-1490. 54. Kadoorie, F., Botanic, G., 2001, "Report of rapid biodiversity assessments at Jianfengling Nature Reserve, southwest Hainan, 1998 and 2001", South China Forest Biodiversity Survey Report Series 3, pp. 1-26. 55. Kimura, M., 1980, "a simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotides sequence", Journal of Molecular Evolution 16, pp. 111-120. 56. Klicka, J., Zink, R.M., 1997, "The importance of recent Ice Ages in speciation: a failed paradigm", Science 277, pp. 1666-1669. 57. Knowlton, N., 1993, "Sibling species in the sea", Annual Review of Ecology and Systematics 24, pp. 189-216. 58. Lee, M.S.Y., Anderson, J.S., 2006, "Molecular clocks and the origin(s) of modern amphibians", Molecular Phylogenetics and Evolution 40, pp. 635-639. 59. Li, W.H., Tanimura, M., Sharp, P.M., 1987, "An evaluation of the molecular clock hypothesis using mammalian DNA sequences", Journal of Molecular Evolution 25, pp. 330-342. 60. Librado, P., Rozas, J., 2009, "DnaSP v5: a software for comprehensive analysis of DNA polymorphism data", Bioinformatics 25, pp. 1451-1452. 61. Lin, S.M., Chen, C.A., Lue, K.Y., 2002, "Molecular phylogeny and biogeography of the grass lizards genus *Takydromus* (reptilian: lacertidae) of east asia", Molecular Phylogenetics and Evolution 22, pp. 276-288. 62. Liu, W.Z., Lathrop, A.M., Fu, J.Z., Yang, D.T., Murphy, R.W., 2000, "Phylogeny of east asian bufonids inferred from mitochondrial DNA sequences (anura: amphibia)", Molecular Phylogenetics and Evolution 14, pp. 423-435. 63. Lougheed, S.C., Gascon, C., Jones, D.A., Bogart, J.P., Boag, P.T., 1999, "Ridges and rivers: a test of competing hypotheses of Amazonian diversification using a dart-poison frog (*Epipedobates femoralis*)", Proceedings of the Royal Society of London. Series B-Biological Sciences 266, pp. 1829-1835. 64. Macey, J.R., Schulte, J.A., II, Larson, A., Fang, Z., Wang, Y., Tuniyev, B.S., Papenfuss, T.J., 1998, "Phylogenetic relationships of toads in the *Bufo bufo* species group from the eastern escarpment of the Tibetan Plateau: A case of vicariance and dispersal", Molecular Phylogenetics and Evolution 9, pp. 80-87. 65. Matsui, M., 1986, "Geographic variation in toads the *bufo bufo* complex from the Far East, with a description of a new subspecies. Copeia 1986, pp. 561-579. 66. Noonan, B.P., Gaucher, P., 2005, "Phylogeography and demography of Guianan harlequin toads (*Atelopus*): diversification within a refuge", Molecular Ecology 14, pp. 3017-3031. 67. Panchal, M., 2007, "The automation of nested clade phylogeographic analysis", Bioinformatics 23, pp. 509-510. 68. Pauly, G.B., Hillis, D.M., Cannatella, D.C., 2004, "The history of a Nearctic colonization: molecular phylogenetics and biogeography of the Nearctic toads (*Bufo*)", Evolution 58, pp. 2517-2535. 69. Phillipson, I.C., Metcalf, A.E., 2009, "Phylogeography of a stream-dwelling frog (*Pseudacris cadaverina*) in southern California", Molecular Phylogenetics and Evolution 53, pp. 152-170. 70. Pramuk, J.B., Robertson, T., Sites Jr, J.W., Noonan, B.P., 2007, "Around the world in 10 million years: biogeography of the nearly cosmopolitan true toads (Anura: Bufonidae)", Global Ecology and Biogeography 17, pp. 72-83. 71. Ramos, A.C.S., Lemos-Filho, J.P., Ribeiro, R.A., Santos, F.R., Lovato, M.B., 2007, "Phylogeography of the tree *Hymenaea stigonocarpa* (Fabaceae: Caesalpinoideae) and the influence of Quaternary climate changes in the Brazilian Cerrado", Annals of Botany 100, pp. 1219-1228. 72. Rogers, A.R., Harpending, H., 1992, "Population growth makes waves in the distribution of pairwise genetic differences", Molecular Biology and Evolution 9, pp. 552-569. 73. Rowe, G., Harris, D.J., Beebee, T.J.C., 2006, "Lusitania revisited: A phylogeographic analysis of the natterjack toad *Bufo calamita* across its entire biogeographical range", Molecular Phylogenetics and Evolution 39, pp. 335-346. 74. Rubinoff, D., Holland, S.H., 2005, "Between two extremes: mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference", Systematic Biology 54, pp. 952-961. 75. Savage, J.M., 1973, "The geographic distribution of frog: patterns and predictions". In: evolutionary biology of the anurans: contemporary research on major problem, Univ. of Missouri Press, Columbia, Mo, pp. 351-445. 76. Schaal, B.A., Olsen, K.M., 2000, "Gene genealogies and population variation in plants", Proceedings of the National Academy of Sciences USA 97, pp. 7024-7029. 77. Shih, H.T., Hung, H.C., Schubart, C.D., Chen, C.A., Chang, H.W., 2006, "Intraspecific genetic diversity of the endemic freshwater crab *Candidiopotamon rathbunae* (Decapoda, Brachyura, Potamidae) reflects five million years of the geological history of Taiwan", Journal of Biogeography 33, pp. 980-989. 78. Smith, H.M., Chiszar, D., 2006, "Dilemma of name-recognition: why and when to use new combinations of scientific names", Herpetological Conservation and Biology 1, pp. 6-8. 79. Sumida, M., Kanamori, Y., Kaneda, H., Kato, Y., 2001, "Complete nucleotide sequence and gene rearrangement of the mitochondrial genome of the Japanese pond frog *Rana nigromaculata*", Genes and Genetic systems 76, pp. 311-325. 80. Tajima, F., 1989, "Statistical method for testing the neutral mutation hypothesis by DNA polymorphism", Genetics 123, pp. 585-595. 81. Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007, "MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0", Molecular Biology and Evolution 24, pp. 1596-1599. 82. Templeton, A.R., 1998, "Nested clade analysis of phylogeographic data: testing hypotheses about gene flow and population history", Molecular Ecology 7, pp. 381-397. 83. Templeton, A.R., 2004, "Statistical phylogeography: methods of evaluating and minimizing inference errors", Molecular Ecology 13, pp. 789-809. 84. Toda, M., Matsui, M., Nishida, M., Ota, H., 1998, "Genetic divergence among southeast and east Asia population of *Rana limnocharis* (amphibian: anura), with special reference to sympatric cryptic species in Java", Zoological Science 15, pp. 607-613. 85. Turelli, M., Barton, N.H., Coyne, J.A., 2001, "Theory and speciation", Trends in Ecology and Evolution 16, pp. 330-343. 86. Truett, G.E., Walker, J.A., Baker, D.G., 2000, "Eradication of infections with *Helicobacter* spp. by use neonatal transfer", Comparative Medicine 50, pp. 444-451. 87. Tzeng, C.S., 1986, "Distribution of freshwater fishes of Taiwan", Journal of Taiwan Museum 39, pp. 127-146. 88. Wang, J.P., Lin, H.D., Huang, S., Pan, C.H., Chen, X.L., Chiang, T.Y., 2004, "Phylogeography of *Varicorhinus barbatulus* (Cyprinidae) in Taiwan based on nucleotide variation of mtDNA and allozymes", Molecular Phylogenetics and Evolution 31, pp. 1143-1156. 89. Wright, S., 1931, "Evolution in Mendelian population", Genetics 16, pp. 97-159. 90. Wright, S., 1965, "The interpretation of population structure by F-statistics with special regard to systems of mating", Evolution 19, pp. 395-420. 91. Zhang, Y., Ryder, O.A., 1995, "Different rates of mitochondrial DNA sequence evolution in Kirk's Dik-dik (*Madoqua kirkii*) populations", Molecular Phylogenetics and Evolution 4, pp. 291-297. 92. Zhang, P., Zhou, H., Chen, Y.Q., Liu, Y.F., Qu, L.H., 2005, "Mitogenomic perspectives on the origin and phylogeny of living amphibians", Systematic Biology 54, pp. 391-400. 93. Zhang, Y.J., Stock, M., Zhang, P., Wang, X.L., Zhou, H., Qu, L.H., 2008, "Phylogeography

of a widespread terrestrial vertebrate in a barelystudied Palearctic region: green toads (*Bufo viridis* subgroup) indicate glacial refugia in Eastern Central Asia", *Genetica* 134, pp. 353-365. 94. Zhong, J., Liu, Z.Q., Wang, Y.Q., 2008, "Phylogeography of the rice frog, *Fejervarya multistriata* (Anura: Ranidae), from China based on mtDNA D-loop sequences", *Zoological Science* 25, pp. 811-820. 95. Zhou, R., Zeng, K., Wu, W., Chen, X., Yang, Z., Shi, S., Wu, C., 2007, "Population genetics of speciation in nonmodel organisms: I. ancestral polymorphism in mangroves", *Molecular Phylogenetics and Evolution* 24, pp. 2746-2754. 96. Zink, R.M., Slowinski, J.B., 1995, "Evidence from molecular systematics for decreased avian diversification in the Pleistocene Epoch", *Proceedings of the National Academy of Sciences USA* 92, pp. 5832-5835.