黑猩猩親緣關係之基因鑑定

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摘要：截至民國 87 年 10 月，台北市立動物園內共有 21 隻黑猩猩樣本，根據動物園之現場記錄卡結果顯示，可分爲酋長和小強兩大族系。爲了進一步確定這些黑猩猩的親緣關係，本研究於 87 年 3 月至 88 年 3 月之間以分子生物學的方法來進行黑猩猩親緣關係之基因鑑定。首先收集黑猩猩所脫落的毛髮，然後抽取分離髮根細胞內的 DNA ，接著再利用聚合連鎖反應 (polymerase chain reaction; 簡稱 PCR) ，以設計好的特殊引子 (primer) 來分析黑猩猩特定位置的 DNA 重複序列，亦即微衛星基因座 (microsatellite loci) 的 DNA 序列，以求能簡單迅速準確的分析出黑猩猩親源的遠近。本實驗先後利用 12 組引子，包括 PTGT211, Mfd23, FABP, D16S407, D18S61, D8S200, RENA4, Mfd18, Mfd121, Pla2a1, Mfd32, 及 Mfd3 來進行實驗分析，其結果顯示其中 10 組可以成功地建立這些黑猩猩的基因型資料庫，進而確立他們彼此間的親緣關係。實驗結果顯示在 12 隻第二代黑猩猩中，有 7 隻已排除爲小強的後代，而不排除爲酋長的後代，有 4 隻。其中包括了在動物園之現場記錄卡中無法確定父親的美蘭、莉忠和莉孝，則已排除爲酋長的後代，而不排除爲小強的後代。另外一隻莎莉春目前無法用已分析之 12 種基因型分辨其爲酋長或小強的後代。將這些結果與動物園之現場記錄卡比對後，發現已成功分析的 11 隻黑猩猩中，除了曼莉春之外，其他均互相吻合。曼莉春經由 Mfd23 及 D18S61 引子分析所得基因型分別爲 100/96 和 169/169 ，而酋長的爲 106/104 和 175/173 ，小強的則爲 100/96 和 169/169 ，此基因型分析結果顯示曼莉春應爲小強的後代，而非現場記錄卡所記錄的爲酋長的後代。

關鍵字：聚合連鎖反應，黑猩猩，引子，微衛星基因座，基因型

前言

黑猩猩是行政院農委會與華盛頓公約組織公告爲瀕臨絕種之野生動物。本研究的主要目的係利用基因的遺傳工程技術，來建立黑猩猩的基因型資料庫，以確定動物園內 21 隻黑猩猩間的血緣關係。先以不傷害黑猩猩的方法，由黑猩猩的毛髮分離出脫氧核糖核酸 (deoxyribonucleic acid; 簡稱 DNA) ，再利用聚合連鎖反應的技術，以設計好的特殊引子，將黑猩猩 DNA 中特定的目標基因進行增幅和分析，以求能簡單迅速準確的分析出黑猩猩親源的遠近。而所選定的目標基因座爲微衛星基因座 (microsatellite loci) ，它又被稱爲簡單重複序列基因座 (simple sequence repeat loci; 簡稱 SSR loci) (Litt and Luty, 1989; Weber and May, 1989) ，是屬於一種小片段的變異的串聯重複序列 (variable tandem repeat sequence) ，其重複單位長度約爲 2 - 6 個鹼基對 (Beckman and Weber, 1992) 。由於微衛星基因座與其它類似的變異的串聯重複序列，如 VNTR 基因座 (variable number tandem repeats loci) 或迷你衛星基因座 (minisatellite loci) 在不同個體間其 DNA 重複序列的數目會有多形性 (polymorphism) ，而且遵循孟德爾遺傳定律 (Mendel's laws) ，經由父母遺傳給下一代 (Edwards wt al., 1992) ，因此均

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Paternity determination of chimpanzees at Taipei Zoo

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Abstract: Until October 1998, 21 chimpanzees were reared in Taipei Zoo. The relationships recorded in the studbook showed that two dominant males, Chiu-Chang and Hsiao-Chiang, organized them into two chimpanzee groups. To confirm the pedigree between these chimpanzees present in Taipei Zoo, we isolated DNA from hair follicle samples. The extracted DNA were applied in polymerase chain reaction (PCR) to amplify DNA segments of microsatellite or simple sequence repeat loci. From March 1998 to March 1999, we have applied 12 primer-sets including PTGT211, Mfd23, FABP, RENA4, D16S407, D18S61, D8S200, Mfd18, Mfd121, Pla2a, Mfd32, and Mfd3 in PCR. Data derived from 10 of 12 primer-sets in PCR successfully differentiated the genotype of these chimpanzees at the corresponding microsatellite loci. The results suggest : (1) 7 of the 12 offspring were excluded as the offsprings of Hsiao-Chiang and were not excluded as offspring of Chiu-Chang; (2) 4 of the 12 offspring, including 3 chimpanzees, Mei-Lan, Li-Chung, and Li-Hsiao, with uncertain familial relationships recorded in the studbook, were excluded as the offspring of Chiu-Chang and were not excluded as offspring of Hsiao-Chiang; and (3) the paternity of one of the 12 offsprings, Sha-Li-Chun, was unable to be determined by the 11 inferred genotypes. Microsatellite typing of 12 offspring, except Man-Li-Chun, was consistent with known relationships recorded in the studbook. The inferred genotype of Man-Li-Chun, Chiu-Chang, and Hsiao-Chiang at Mfd23 and D18S61 loci were 100/96 and 169/169, 106/104 and 175/173, and 100/96 and 169/169, respectively. This indicated that Man-Li-Chun was the offspring of Hsiao-Chiang. This result was not consistent with relationship recorded in the studbook in that Man-Li-Chun was the offspring of Chiu-Chang.

Key word: chimpanzee, polymerase chain reaction, microsatellite DNA, and genotype.

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