臺灣黑熊(Ursus thibetanus formosanus)之保育遺傳研究

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論文摘要

臺灣黑熊(Ursus thibetanus formosanus)是亞洲黑熊(U. thibetanus)分布於臺灣的特有亞種。臺灣黑熊的族群數量與分布範圍因為棲地的縮減、破碎化及獵捕而持續減少，分布於其他地理區域的亞洲黑熊也遭遇同樣的生存威脅。為了建立適當的保育策略，我們必須優先了解這個物種在族群內與族群間的遺傳多樣性與親緣關係。由於不同地理區的亞洲黑熊亞種間外表型態的差異並不明顯，難以利用表型的差異來區分，這樣的研究資料更顯得重要。

本研究主要的目的是釐清臺灣黑熊與其他地區亞洲黑熊的遺傳變異程度與親緣關係，以了解臺灣黑熊族群的遺傳定位與分化狀況。論文有四項主要的工作，首先是篩選適當的亞洲黑熊微衛星體基因座分子標記；其次是量化評估黑熊毛髮與排遺樣本在不同採樣時間與保存方法下 DNA 萃取的成功率，以建立黑熊樣本採集與保存的標準作業流程；接著利用粒線體 DNA 控制區域及篩選出的微衛星體分子標記進行臺灣黑熊與其他地區亞洲黑熊的遺傳分析；最後也利用同樣遺傳方法釐清臺灣地區圈養黑熊的遺傳狀況。

論文首先自 33 個具有微衛星體基因座的臺灣黑熊 DNA 序列中，篩選出 10 個具有專一性及多型性的 4 重複序列微衛星體分子標記。量化評估黑熊毛髮與排遺採樣與保存方法的結果，顯示了在亞熱帶環境下，DNA 增幅的成功率隨樣本放置於野外的時間及增幅標的 DNA 的長度增加而下降；但採集排遺內外不同位置並不影響排遺樣本中 DNA 增幅的成功率。另外，浸泡酒精的排遺樣本於採集後有否進行冷凍保存處理，對一週內樣本小片段 DNA 的增幅沒有影響，但將影響長片段 DNA 的增幅效果。粒線體 DNA 控制區域部分序列的遺傳分析結果顯示，日本黑熊與臺灣黑熊形成兩個單系群；東北黑熊在與西南黑熊混雜的支序中自成一群；而東南亞的西藏黑熊樹型複雜、並未形成單系群。微衛星體分子標記的遺傳結構分析顯示，臺灣黑熊、西南黑熊、東北黑熊與東南亞的西藏黑熊這 4 個黑熊亞種各自分群。最後，針對圈養黑熊的遺傳分析顯示，7 隻圈養黑熊具有臺灣黑熊獨特的粒線體單型，其中 3 個體的微衛星體分析確認其臺灣黑熊的亞種分類。

本研究的結果提供了亞洲黑熊亞種鑑別與擬定保育管理單位的明確基礎，並可做為臺灣黑熊保育與經營管理重要的參考資料。

論文外文摘要

The Formosan black bear (Ursus thibetanus formosanus) is an endemic subspecies of the Asiatic black bear (U. thibetanus) inhabiting Taiwan. Habitat degradation and fragmentation, as well as poaching have caused a decrease in its population and distribution. Similar threats to populations of Asian black bears have taken place elsewhere in their range. To establish proper conservation strategies for the species, a priority research is to reconstruct its evolutionary history and examine genetic diversity within and among its populations, especially when identification of Asiatic black bear subspecies by morphological characters is vague and controversial. The objectives of my study were to apply molecular techniques to delineate the phylogenetic relationships of Formosan black bears and other subspecies, and to assess genetic status of the Formosan black bears. My dissertation included 4 major aspects. The first part was to select appropriate microsatellite genetic markers for genetic analyses of Asiatic black bears. The second part was to quantitatively evaluate the effects of sample age and storage techniques on success rates of DNA extraction from various types of samples, i.e. bear hair and feces. Such results would facilitate the development of standard operation procedures for collection and storage of these samples before analysis. Thirdly, I applied the mitochondrial DNA control region and microsatellite markers developed in this study as genetic markers to delineate the phylogenetic relationship and genetic status of Formosan black bears and Asiatic black bears from other areas. Lastly, the same genetic analyses were conducted in captive bears to reveal the genetic ancestry of captive Asiatic black bears in Taiwan.

In my study, ten polymorphic microsatellite markers were developed for the Formosan black bear from a partial genomic library enriched for GAAA repeat and were used to examine the polymorphism in bear populations. The evaluation results showed that the amplification success rates decreased with sample age and amplicon size in both hair and faecal DNA, but did not show differences among different sampling locations of faeces in subtropical Taiwan. The immediate freezing of ethanol-soaked faecal samples in the field were not so critical in affecting DNA quality of short fragments from samples collected within a week but the effect of immediate freezing was significant for longer mtDNA fragments. The mitochondrial DNA analyses indicated that the Japanese black bears (U. thibetanus japonicus) and the Formosan black bears (U. thibetanus formosanus) formed two distinct clades. The northeastern Asia population (U. thibetanus ussuricus) formed a group within the clade containing a mixture of bears from southwestern China (U. thibetanus mupinensis). And the bears from southeastern Asia were not monophyletic. In addition, the population structure analysis of tetramicrosatellite loci showed a clear subdivision scenario of U. thibetanus formosanus, U. thibetanus mupinensis, U.

thibetanus ussuricus, and U. thibetanus thibetanus. Finally, in the results of captive bear analyses, seven captive bears of unknown origin showed the unique mtDNA haplotypes of the Formosan black bear. And three of them were verified as the Formosan black bear subspecies according to microsatellite data.

The results of this study have provided an explicit basis for subspecies identification for Asiatic black bears and important information for conservation and management of Formosan black bears.