沉默不脆弱　守護與重生

Silent Guardian Brings New Life

談建立物種DNA資料庫

Establishment of the Species DNA Database

採訪撰文 Interview & Text ／李坤能 Kun-neng Li、吳靜昀 Jing-yun Wu　特別感謝 Special thanks to / 成功大學生命科學系蔣鎮宇教授 Prof. Tzen-Yu Chiang, Department of Life Sciences, National Cheng Kung University、國立清華大學生命科學院生物資訊結構生物研究所暨生命科學系曾晴賢教授 Prof. Chyng-shyan Tzeng, Institute of Bioinformatic and Structural Biology and Department of Life Sciences, National Tsing Hua University.　圖片提供 Photo provided by ／蔣鎮宇 Tzen-yu Chiang 、曾晴賢 Chyng-shyan Tzeng　翻譯 Translator ／張詩臼 James Chang

雪霸國家公園將已建好的防砂壩拆除，讓鮭魚個體可以自由來去。經後續追蹤，此一措施對於改善台灣櫻花鉤吻鮭基因劣化的問題成效極佳／曾晴賢提供
Shei-Pa National Park removed the built dam so that the salmon could swim freely. Subsequent tracing had proved that this measure was highly conducive to improving the worsening genes of *Oncorhynchus masou formosanus*. / Photo provided by Chyng-shyan Tzeng

當紅影集〈CSI 犯罪現場〉中，鑑識專家可以利用遺留在犯罪現場的一滴血、一根毛髮或一小片組織殘片還原真相、找出兇手，同樣的分子科技，到了生物學家手中，又能解開哪些生物之謎呢？就由專研於植物學的成功大學生命科學系蔣鎮宇教授，以及魚類研究專家清華大學曾晴賢教授以不同層面的專業學術，帶領我們一同窺探這些物種間的生命密碼。

突破困境的新技術

位在亞熱帶又多崇山峻嶺的臺灣，本來是生物多樣性極豐富的地區，這點從日治時代的野外調查紀錄可見一斑。當臺灣本土物種的存續受到威脅，以保育為目的的國家公園便因應而生。國家公園剛成立時，只希望能減少人類對物種原生地區的開發與利用，減少遊客對當地環境的影響與破壞，以及人類對於特定物種的捕獵。但光是這樣並無法達成永續經營的目的，因而才漸漸演變成現在的管理模式。生物多樣性強調的是永續利用，要怎麼呈現生物多樣性，才是現今的課題。成功大學生命科學系蔣鎮宇教授說：「以學者的角度來看，以前是調查，弄一個漂亮報告出來，但這種作法已經不能滿足現況。」

以國寶魚台灣櫻花鉤吻鮭為例：目前國家公園所進行的台灣櫻花鉤吻鮭復育計畫，每年將一千多條復育出的魚放回原生溪流，但存活的數量總是只剩幾條，這是因為該物種本身對於環境的抵禦能力不足。物種對於環境的抵禦能力，追根究底可以歸結到牠們基因的多樣化程度。然而基因的多樣性，若是只有靠野外調查和形態分類是無法判斷的，唯有利用分子生物技術，才能得知一個族群中的基因是否具有多樣性。

In *CSI*, the popular TV series, a forensic specialist can recover the truth and find out the killer by a drop of blood, a hair, or a tiny tissue residue left on the crime scene. With the same molecular technology, can a biologist unveil the myth of living things? Let the two experts, Professors Prof. Tzen-yuh Chiang and Prof. Chyng-shyan Tzeng, lead us in the exploration of the life codes for different species.

New Breakthrough Technology

Tropical and mountainous, Taiwan is known for its biodiversity. Field investigations during the Japanese colonization are the best proof. To sustain local species, national parks were created. However, as time went by, the original purposes of minimizing development and cultivation of primitive lands, destruction to the local environment, and hunting of certain species are no longer sufficient. Since sustainability is the basis for biodiversity, what matters is how to present biodiversity. Life Science Professor Chiang Department of Life Science, from National Cheng Kung University says: “The old practice can no longer satisfy current needs.”

In the case of Formosan landlocked salmon (*Oncorhynchus masou formosanus*), the national treasure fish of Taiwan, each year, more than 1,000 of bred fish are set free in their original habitats but few managed to survive because of their lack of defense in a natural setting and the ability has to do with their genes. However, one cannot tell the genetic diversity through field investigations and morphological classification. It has to be identified with molecular biology.

分子生物技術的範圍很廣，應用在物種親緣關係遠近的分類上是所謂的分子指紋（fingerprinting）技術，這種技術是利用了微衛星 DNA（microsatellite DNA）或粒線體 DNA 等一小段的特定 DNA 序列。而這樣的小段 DNA 序列變異，已足以區別各種生物的差異，更進一步，甚至可以分析整個生物個體的 DNA 序列，建立所謂的遺傳資料庫。

台灣櫻花鉤吻鮭的危機

臺灣純淡水性的魚類種類並不多，若加上洄游性的魚類，共約有二百多種。目前由於人類對河川環境的影響與破壞，導致魚類種類愈來愈少，實地進行野外調查時，一條河川只能發現三、四十種的魚類。這些淡水性的魚類當中，大家在保育上最重視也最具有指標意義的物種就是台灣櫻花鉤吻鮭。

目前台灣櫻花鉤吻鮭分布於大甲溪上游之七家灣溪的數個支流，因為地理上的區隔和障礙，各支流間的鮭魚無法自由的穿越，造成一個大族群被劃分為許多獨立的小族群。但小族群的各自獨立會導致近親繁殖，造成基因的劣化，使遺傳的多樣性降低。清華大學生命科學系的曾晴賢教授告訴我們，藉由研究鮭魚族群的基因差異，確實發現目前台灣櫻花鉤吻鮭已經面臨基因劣化的危機。

根據曾教授的研究指出，區隔台灣櫻花鉤吻鮭族群的元凶之一是七家灣溪的防砂壩，台灣櫻花鉤吻鮭雖然強健，卻也無法越過高高的防砂壩。幾年前，雪霸國家公園進行了在國際上的一個創舉——將 4 個已經建好的防砂壩拆除，讓鮭魚個體可以自由來去。經過後續的追蹤研究，此一措施對於改善台灣櫻花鉤吻鮭基因劣化的問題成效極佳。另外，國家公園也將人工繁殖復育的魚群加以放流，只要放流的台灣櫻花鉤吻鮭族群數量夠多，我們可以期待十幾年後，鮭魚族群的遺傳基因的多樣性將會增高許多。

曾教授還說：「雖然物種親緣關係間的研究和遺傳資料庫的建立，並非保育的核心工作。然而，一旦物種的存續面臨關鍵時刻，若無相應的研究，人們將會措手不及。」簡單幾句話，清楚的點出了相關研究工作的重要性。

Molecular biology is widely applied. In species kinship classification, there is the fingerprinting technology that uses segments of microsatellite DNA or mitochondrial DNA sequences whose variance can indicate the difference of each living thing and be used in the analysis of the entire DNA sequence of the living thing to establish the so-called genetic data.

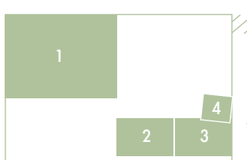
Formosan Landlocked Salmon in Crisis

There are few freshwater fishes in Taiwan. With migratory ones included, there are only around 200 of them in total, and the damage people do to rivers makes them fewer and fewer. A field investigation will only find 30 to 40 fishes in one river. Among them, the most valued and indicative fish is Formosan landlocked salmon.

The salmon lives in several branches of Cijiawan River(creek), an upper reach of the Dachia River. The geographical separation blocks them from traveling freely, resulting in several independent subgroups close in biological relationship which will propagate and lead to genetic degradation, reducing genetic diversity. Prof. Tzeng from Department of Life Sciences, National Tzeng Hua University tells us that the salmon is endangered by this crisis now.

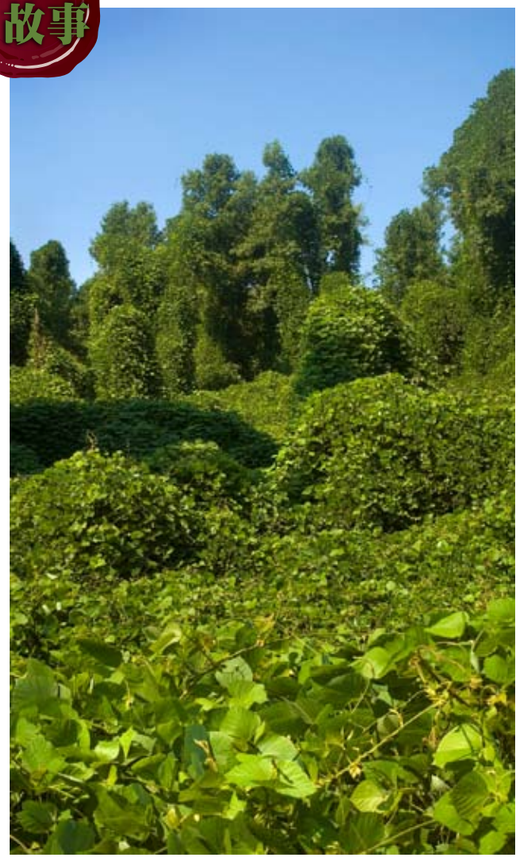
Tzeng's study indicates that the culprit for the separation of the salmon is the dam in Cijiawan River (creek) which prevents it from passing. Several years ago, Shei-Pa National Park removed four built dams so that the salmon could travel freely and the result was successful. Another measure is that national parks set free artificially bred Formosan landlocked salmon. As long as the number of the fish set free is sufficient, we can expect that in a decade, the genetic diversity of the species will develop and improve.

Tzeng also says, “Although phylogenetic relationship studies and the genetic database are not at the core of conservation, they will get us prepared for any unexpected that happens in the future.” This clearly says the importance of related researches.



1-3. 台灣櫻花鉤吻鮭是台灣淡水魚類中最具有保育指標意義的保育類物種 / 曾晴賢提供
Formosan landlocked salmon (*Oncorhynchus masou formosanus*) is the most indicative conserved species among freshwater fishes in Taiwan. / Photo provided by Chyng-shyan Tzeng

4. 埔里中華爬岩鰍主要分佈在台灣中部河川的中游地區，以湍急水域為棲地。以胸、腹鰭平貼在石頭上，並以附著性藻類為食，是保育類野生動物，同時有很明顯的河川內洄游習性 / 曾晴賢提供
Sinogastromyzon puliensis primarily primarily lives in the middle stream of rivers in central Taiwan, with waterways with rapid currents as their habitats. They lean against rocks with their pectoral fins and pelvic fins and feed on benthic algae. They are protected wildlife with obvious migratory habits. / Photo provided by Chyng-shyan Tzeng



從最根本來研究外來物種入侵的問題

根據蔣教授的說明我們知道，很多物種在原生地不會造成威脅，但漂洋過海後就變成可怕的環境殺手。美國最嚴重的入侵物種——山葛藤，會盤踞一整片山頭，原有的植物全部不見了，攀附並纏滿了一棵棵參天大樹。這種在台灣不曾造成威脅的豆科植物，為什麼會變得這麼可怕？想知道原因，微衛星 DNA 的研究，就可以從最根本，讓我們得知外來種入侵問題的答案。

微衛星 DNA 技術目前已廣泛應用在國家公園的物種研究上，蔣鎮宇教授曾接受玉山國家公園委託，分析玉山國家公園代表性植物玉山杜鵑之遺傳結構及分化程度，至玉山、南橫等高山地區採集玉山杜鵑及森氏杜鵑，藉由分子生物技術得到重大發現，玉山杜鵑和森氏杜鵑應為同種，兩物種的特徵常受到外部環境影響，經以葉綠體 DNA 片段分析，證明玉山杜鵑和森氏杜鵑為同種，外在特徵不一應是玉山山脈及中央山脈不同地區高度遺傳分化的結果，其中，南橫關山嶺山的玉山杜鵑保有最最高的基因岐異度，顯示此族群冰河時代可能即已存在。

Study Root Causes for Invasion by Foreign Species

Chiang explains that many species in their original habitats are not threats but when they relocate across ocean, they can be. For example, *Pueraria montanus (Lour) Merr* is a harmless fava plant in Taiwan, its home, but is causing problems and killing local species in the U.S. Why? Microsatellite DNA studies explain it all from the very basics and let us know answers to invasion by foreign species.

The technique of microsatellite DNA has been widely applied in researching species in Taiwan's national parks. Commissioned by Yushan National Park (YSNP), Chiang had analyzed the genetic structure and the degree of variation *of Rhododendron pseudochrysanthum Hayata*, a representative plant in YSNP. Through the molecular technology that contained an analysis of chloroplast DNA segments, Chiang discovered and proved that Rhododendron *pseudochrysanthum Hayata* and *Rhododendron morii Hayata*, which he collected in high mountains in Yushan and South Cross-Island Hwy, are actually the same species. The difference in their appearances should be the result of strong variation in their genes taking place in Jade Mountains and Central Mountains respectively. Among them, *the Rhododendron pseudochrysanthum Hayata* in Mt. Guanshanling has the highest genetic diversity, indicating the species has probably existed since the glacial age.

蔣鎮宇教授還告訴我們：經過微衛星 DNA 研究分析山葛藤的樣本後，發現這種植物到了美國後，就發生了生殖方式的改變，從有性生殖變成無性生殖，無性生殖的特色就是可以大量而快速的增加個體數目，在野外恣意生長。相似的例子就是困擾台灣的小花蔓澤蘭。在原產地墨西哥，並不會像臺灣這樣生長得漫山遍野，造成著名的綠癌問題。植物的行為改變，從基因上去看，裡面有兩個極端：山葛藤和小花蔓澤蘭的基因多樣性度低，導致物種可以快速繁衍。但歧異度高也造成浩劫，如美國的另一大為害物種檉柳，這種植物引近美國後，個體比原產地大了好幾倍，而檉柳是一種會大量吸收水分的植物，將濕地、河流吸乾，它的巨大樹根穿透至地下水層，則會讓地下水枯竭。檉柳植株變得巨大的原因就是檉柳在東亞有兩種，本來老死不相往來，因為好事者讓兩種雜交而改變了它的形態。這些原本都被劃分為生態的問題，卻可以用分子生物技術的研究而揭開謎底，在生物學的領域，沒有任何一門學問是完全獨立的，就像分子生物的研究結果可以提供作為生態的證據，而研究的成功與否取決於是否採用了適當的技術工具。

Chiang also says that analysis of *Pueraria montanus* in microsatellite DNA studies shows that the plant changed its reproductive method after it arrived in the U.S. from sexual reproduction to asexual reproduction, which features rapid multiplication in large quantities. The same problem happens to Taiwan with *Mikania micrantha* H. B. K., whose place of origin is Mexico. The behavioral changes of plants are caused by two extremities of genetics. The low genetic diversity of the two plants enables rapid propagation. However, high diversity is also catastrophic. Tamarisk, another hazardous plant in the U.S., is many times bigger than its counterparts in the place of origin and the big size makes its ability to absorb large quantity of water. It can dry a wetland, a river or even ground water. It all happened because someone hybridized the two types of tamarisk that are always separate from each other in Southeast Asia and changed its morphology. All of these ecological questions are answered in studies with molecular biology because all sciences are interrelated.

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1. 蔓生的山葛藤在台灣是平凡無奇的植物，但飄洋過海到美國，卻成為令人頭痛的環境殺手 / Vasility Koval 攝
Climbing Plant Kudzu (*Pueraria montanus (Lour.) Merr*) is very common and normal plant in Taiwan, yet across the Pacific Ocean, it becomes troublesome invasive plant. / by Vasility Koval

2-3. 危害環境甚鉅的銀合歡 (上圖為果莢、下圖為花朵)。蔓延速度快，已經是保育人士的頭號敵人，清除銀合歡亦可以用分子生物技術的技術來處理 / Vasility Koval 攝
Picture of White Leadtree (*Leucaena leucocephala (Lam.) de Wit) (Up: Seedpod, Down: Flowers)*.It causes harm to environment and has the risk of spread and invasion, the devastating enemy for conservation. The molecular biology can help to remove it / by Vasility Koval

4. 常常在市場上看到許多魚目混珠的鰻魚，要不是可以利用分子的技術加以鑑定區別，我們很可能就會被騙了！ / 曾晴賢提供
There are a variety of eels, good and bad, on the market. If it were not for the molecular technology, we may be easily fooled! / Photo provided by Chyng-shyan Tzeng

5. 鱸鰻是棲息在沙泥底部的肉食性魚類，白天全身隱藏在泥地中，只有露出一個頭部呼吸，到了夜晚即游出覓食，以其它魚類、蝦、蟹為食。 / 曾晴賢提供
The swamp eel (*Anguilla marmorata*), or marbled eel, is carnivorous and demersal, living in the bottom of the silt of a river. It hides in the silt during day time with only its head shown for breathing, and preys for food at night, feeding on shrimps, crabs, and other fish. / Photo provided by Chyng-shyan Tzeng

解析隱藏物種的真實身分

目前利用分子生物技術分析基因序列的研究工作愈來愈多，各國學者定序的研究成果則會匯入 NCBI 的基因序列資料庫（GenBank）中，成為國際性共享的資料。建立這樣的遺傳資料庫，它的主要意義為何呢？除了純粹的研究目的之外，曾教授告訴我們一個有趣的例子。

對臺灣的鰻魚養殖業來說，有時鰻苗的價格會高如黃金。然而，一些不肖商人會從各國進口許多便宜的鰻苗來販賣充數，其中多數是經濟價值不高的種類。但鰻苗的體型小，外觀形態又相似，難以辨識其種類。一旦被騙，等到養殖成魚時才發現種類不對，可能就已經浪費了大筆金錢。但利用分子生物技術加以鑑定的話，只需要一小片組織，就可以加以辨別，可說是非常實用。曾教授在利用分子生物技術幫養鰻人家鑑定一隻長得像過去被歸類為保育類野生動物鱸鰻的鰻魚時，卻發現樣本與遺傳資料庫中的世界上現有18種鰻魚都不相同，該個體竟然是未被發表過的新種。曾教授欣喜的表示，該新種鰻魚應該在今年內就會正式發表，並且會用在20年前第一個引進分子生物技術，應用在動物分類研究的中研院黃秉乾院士的名字來命名。

了解，才知道要趕快保護

在曾教授的採訪過程中我們得知，過去臺灣的原生種香魚還可以長到50公分長。時至今日，原生香魚已經從臺灣絕跡，徒留記錄。香魚分布的區域包含了中國大陸、臺灣與日本等地，雖然外型相似，但利用分子生物技術鑑定後，卻發現臺灣跟日本香魚外形雖像，卻跟大陸香魚的親緣關係較為接近。但無論如何，因為不了解、不重視造成的原生種基因流失，卻再也救不回來！

Unveil Hidden Species

Molecular biology is used in more and more research to analyze genetic sequences. Research results are compiled in the NCBI genetic sequence database (GenBank) and shared internationally. What is the main purpose of the genetic database? Tzeng answers with a fun example.

Eel fry in the eel cultivation industry of Taiwan can be as expensive as gold sometimes. To make more money, ill-natured businessmen will mix them with cheap eel fry from other countries and of course ordinary buyers cannot tell the difference. By the day the eels are big enough for them to tell the difference, it is already too late because lots of money has been invested. But only a tiny piece of tissue is required for the molecular biology to distinguish the difference. Tzeng even found a new species besides the 18 species collected in the genetic database when authenticating the eel. He cheerfully indicates that the new species will be officially made known to the public this year and named after Pien-Chien Huang, who introduced the biology into Taiwan 20 years ago.

Understanding Drives Protection

During the interview, we know that some indigenous ayu fish *Plecoglossus altivelis* can grow to 50 cm but the species is extinct now with only written records. The fish could be found in China, Taiwan, and Japan. Despite similar appearance, the molecular biology found that the ones in Taiwan look more like those in Japan but are closer in blood to those in China. This loss cause by lack of knowledge about genes could never be made up!

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1-2. 圖為實驗室經基因改造之阿拉伯芥幼苗 / Vasility Koval 攝
Picture of Thale Cress (*Arabidopsis thaliana*) in the laboratory after genetic transformation / by Vasility Koval

3. 瀕台灣鱒雖然是以台灣為名的特有屬魚類，但是最近的研究發現，它並非台灣的特有種。主要分布於濁水溪以北的溪流中，是急流中的代表性魚類 / 曾晴賢提供
Formosania lacustre, though named after Taiwan, has recently been found not exclusively available in Taiwan. The fish mainly lives in rivers north to Jhuoshuei River and is representative of fishes in rapid currents. / Photo provided by Chyng-shyan Tzeng

我們也得知，分子生物技術的研究及遺傳資料庫的建立屬於基礎研究的一環，表面上看起來是屬於純學術性的研究，難以實際應用在保育工作上。其實不然，基礎研究對於保育工作的應用是非常重要的。舉例來說：也許有些人認為台灣櫻花鉤吻鮭既然分布的涵蓋了日本及臺灣，日本的族群數量又大，臺灣的台灣櫻花鉤吻鮭若是絕滅，大可以從日本購買來臺灣放流。但是對於學者專家而言，經過地理區隔的亞種之間，其基因的差異是絕對存在的，臺灣的種類既然經過千萬年的適應，與臺灣土地本身早已密不可分。

從兩位教授的訪談的過程中我們深深體認到，如果我們輕率無知的將外來種放入原生種的棲地，會發生何種後果是無法預測的。也許外來種會因為不適應本土環境而自然消亡，但也有可能強勢的佔據了原生種的棲地資源。只要我們能好好保育本土的種類，讓物種族群量充足，基因的多樣性，經過物種的代代相傳以及後天環境的篩選，自然的提升，又何須引進外來的物種？

過去分子鑑定技術是昂貴而又耗時的工作，但隨著科技進步，DNA 定序等工作已經變的迅速而平價。應用在分類上，是一個嶄新的工具，能夠鑑定出族群的差異，分析出物種存活在地球上的時間，用來了解生物親緣關係的全貌及推演生物演化的歷史。建立遺傳資料庫的工作，只是基礎研究，實際上要如何應用在保育、保種，如何解讀分子證據給予的資訊，利用這些資訊規劃未來的保育方向，才是現今科學家應該重視、發展的課題。

We also know that studies of the molecular biology and establishment of the genetic database are only part of fundamental research. Although they seem purely academic and hard to be applied in actual conservation, fundamental research is important to conservation. For example, some may think Formosan landlocked salmon can be found in Taiwan and Japan so if they disappear in Taiwan, we can get some from Japan. However, genetic difference is unbeatable. The species spent tens of millions of years adapting to the environment in Taiwan and hence it is irreplaceable.

The interviews with the two professors make us realize that we should not misplace species as we wish because the result may be detrimental. Foreign species may die or occupy habitats of indigenous species. We must protect local species to ensure that they are of a sufficient population. Gene diversity will evolve from generation to generation. Why bother to introduce foreign species?

Molecular authentication used to be costly and time-consuming but now it is fast with a reasonable price. A brand new tool in classification, it can identify different populations of species and analyze their survival on the earth to help recover the panorama of biological kinship and history of evolution. Establishment of the genetic database only requires fundamental research, but what's important is how to use such database in the conservation and protection of species, interpretation of information provided by molecular evidence, and future conservation.

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Professor at Institute of Bioinformatics and Structural Biology and chairman of Department of Life Sciences, National Tsing Hua University; specializing in freshwater fishes; a frequent speaker actively promoting environmental conservation with close interaction with aboriginals and river protection squads in many places to whom he explains the correct ecological conservation concepts and shares his knowledge of fishes and ways to improve fish ladders and protect rivers for the ultimate goal of improving river ecology.