# A Tale of Two Pandas

Case Study

## Background

For over a century, scientists debated whether the giant panda was more closely related to bears or the red panda, an animal that is more closely related to the raccoon family.

This documents shows multiple pieces of evidence that have been collected over the years–evidence from anatomy, behavior, DNA, and fossils.



# 1. Studies of Anatomy and Behavior

#### Diet

Both the giant panda and the red panda live in the bamboo forests of China. The giant panda eats only bamboo. The red panda eats mostly bamboo, though it sometimes eats other plants, fruit, and insects. The bamboo diet of the two pandas is quite different from those of most bears and members of the raccoon family–which tend to eat a variety of plants, fruits, and small animals.



## **Skull Anatomy**

Both the giant panda and the red panda have strong jaw muscles, which help them chew tough bamboo stalks. Their skulls are extra-thick, and the places where the jaw muscles attach (the ridge at the top of skull and the upward-curving part of the lower jaw) are enlarged. (*Raccoon, black bear, and giant panda images modified from O'Brien, p. 105.*)



Raccoon



Red panda



Black bear



Giant panda

#### **Tooth Anatomy**

The drawings below show the chewing surfaces of the teeth, along with a visual summary of their surface area, which affects how well they can grind tough food. (*Images modified from Davis, p. 129.*)



#### Front Paw Anatomy

Both the giant panda and the red panda have an opposable "thumb" (highlighted). In both cases, the thumb is actually a greatly enlarged and modified wrist bone. A smaller version of this bone is present in bears, raccoons, and other carnivores. It helps the pandas handle bamboo as they eat it. (Black bear and giant panda images modified from Davis, p. 30. Red panda based on Abella 2006, Figure 1.)



# 2. A Detailed Study of Anatomy

In 1964, an biologist named D. Dwight Davis published a detailed anatomy study of the giant panda and some of its relatives. Working from zoo animals that had died naturally, Davis and his team took measurements and made drawings of the bones, muscles, joints, blood vessels, nerves, brain, glands, organs, and more–enough to fill a 340-page book. Some of the findings are summarized below.

1. The giant panda stands and moves similarly to bears, though the panda moves less efficiently. (*Drawing modified from Davis, p. 25.*)



2. The overall appearance of the giant panda is similar to that of bears. The two share body proportions that are different from those of other living carnivores (a larger group to which they belong). (*Drawings modified from Davis, p. 33.*)



- **3.** The giant panda is very similar to bears in the characteristics of its skeleton, joints, muscles, and organs. Most of the differences are related to specializations for a bamboo diet, including extra-large and strong jaw muscles, opposable "thumbs," and differences in its digestive organs.
- **4.** The lungs of the giant panda are similar to those of bears, including an extra lobe in the left lung, a characteristic that red pandas and raccoons lack. *Below left: Image from Davis, p. 236)*
- **5.** The overall structure of the brain is very similar between the giant panda and black bear. (*Below right: Image from Davis, p. 236 & 295*)





# 3. DNA Hybridization Data

Tools for molecular biology during the mid-1980s were fairly simple compared to today's tools. Researchers did not have access to very many DNA sequences, so it was difficult to compare them directly. But they did have other ways of estimating similarity. One estimate relied the fact that single-stranded DNA molecules will "hybridize" with each other through complementary base pairing, as long as their DNA sequences are similar enough.



The data below summarize the results of a DNA hybridization experiment. Researchers mixed radioactive single-stranded DNA from one species (left column) with DNA from another species (top row), and then they measured their ability to hybridize with one another. The numbers show "stability units": **a lower number means that the DNA sequences were more similar** (the DNA hybridized at a higher rate) (*data from O'Brien et al, 1985*).

	Brown bear	Giant panda	Red panda	Raccoon
Brown bear		4.3	14.0	14.4
Giant panda	4.8	_	14.4	14.2
Red panda	13.9	14.1	_	14.3
Raccoon	14.4	14.7	13.9	_
Dog (control)	18.7	18.3	18.9	18.5

# 4. Chromosome Banding Patterns

## What Are Chromosomes?

A genome contains all of the information for making an organism. In mammals, the genome is made up of billions of letters of DNA code.

To keep DNA organized, untangled, and accessible, the genome is divided into chunks called chromosomes. Each chromosome is a single DNA molecule with attached proteins called histones. Different species have different numbers of chromosomes.

Chromosomes are normally loosely spread out. But when a cell is getting ready to divide, its chromosomes condense so that, with the aid of a microscope, individual chromosomes are visible.

## **Chromosome Number**

The table shows the number of chromosomes in several species.

## Chromosome Shape

Organism	Number of chromosomes	
Raccoon	19 pairs	
Red panda	22 pairs	
Giant panda	21 pairs	
American black bear	37 pairs	
Sun bear	37 pairs	
Polar bear	37 pairs	
Spectacled bear	26 pairs	

Giant panda chromosomes are relatively large, and most of them have a centromere (the skinny, pinched-in area) near the center. Most bear chromosomes, on the other hand, are on the smaller side with a centromere close to one end. (*Images based on O'Brien et al, 1998, Figure 4; and Nash & O'Brien, 1987, Figure 4.*)

#### **Chromosome Banding Patterns**





Brown bear chromosomes

When chromosomes are dyed to make them more visible, a pattern of bands appears. Banding patterns are the same for all members of a species and similar between related species.

Several bear chromosomes are lined up below (images from Nash & O'Brien, 1987, Figure 1; permission pending):

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Chromosomes from polar bear, black bear, Asian black bear, sloth bear, and brown bear.

When scientists compared the banding patterns of bear and giant panda chromosomes, they saw some surprising patterns (*images based on O'Brien, 1998; and Nash, 1987, Figure 4; permission pending*):



chromosomes lined up together

Giant panda chromosomes do not line up this way with chromosomes from the red panda or members of the raccoon family.

# 5. Hemoglobin Sequences

## What is Hemoglobin?

Hemoglobin is a protein that is found in red blood cells. It picks up oxygen from the lungs or gills and delivers it to all the tissues in the body. Each molecule of hemoglobin is made up of 4 protein subunits: two alpha hemoglobin subunits, and two beta hemoglobin subunits. Alpha and beta hemoglobin subunits are encoded by separate genes.

Nearly every vertebrate animal makes hemoglobin proteins. Though the proteins do the same job, there are slight variations in their amino acid sequences and in the DNA sequences of the genes that code for them.



Hemoglobin protein Image from Shuchismita Dutta, David Goodsell. doi:10.2210/rcsb\_pdb/mom\_2003\_5

#### **Comparing Hemoglobin Sequences**

In 1986, a group of researchers published an analysis of beta hemoglobin amino acid sequences. They made pair-wise comparisons of the amino acids sequences of hemoglobin proteins from a variety of species. The table shows the number of amino acid differences out of 287 amino acids total (*data from Tagle et al, 1986*).

Species	Number of amino acid differences	
Giant panda vs. red panda	6	
Giant panda vs. polar bear	11	
Red panda vs. raccoon	18	

# 6. The Fossil Record of the Giant Panda

## **Vegetarian Carnivores**

Both the giant panda and the red panda live in high-altitude bamboo forests in China, and bamboo makes up all (giant panda) or nearly all (red panda) of their diet. Though they are vegetarians, both pandas belong to the carnivore lineage—a group of primarily meat-eating animals that descended from a cat-like creature that lived about 55 million years ago. The carnivore lineage includes not only raccoons and bears, but also dogs, cats, seals, badgers, and many other animals.

To help them cope with their tough bamboo diet, both pandas have some traits that are unusual for carnivores, including extra-large molars and overgrown jaw muscles to help them chew, as well as extra-strong skulls to withstand the force of all of that chewing.

Fossils can provide clues about what the ancestors to pandas looked like, and whether any of them had traits that were intermediate between those of any living species, such as giant and red pandas, or giant pandas and bears.



Do the two pandas share traits because they inherited them from a common bambooeating ancestor (left), or because they developed the traits independently in response to their shared diet and environment (right)?

## Ailuropoda baconi

This animal, which lived as recently as 750,000 years ago, looked almost identical to the modern giant panda, though it was much larger. Its fossilized remains have been found as far away as Mayunmar, suggesting that giant pandas once had a much larger range than they do today.



#### Ailuropoda microta: the Pygmy Giant Panda

The "pygmy giant panda," about half the size of a modern giant panda, lived in China about 2 million years ago. It looked much like modern giant pandas, but its skull (the only part identified so far) was less specialized for eating bamboo:

- Though its skull was similar to the giant panda's, it had a narrower brain case, a longer snout, and smaller muscle attachments-features that made it slightly more bear-like.
- Though the teeth were worn in a way that suggests that it ate bamboo or other tough plants, they had a smaller grinding surface than the living giant panda's. The graph below shows the teeth dimensions of *A. microta* and *A. baconi* compared with the giant panda and living bears. (*Graph based on Figueirido, 2011, Fig. 5. Teeth adapted from D. Davis, 1964.*)



#### Ailurarctos: the Lefung Panda

The "Lefung panda," lived in China about 7-8 million years ago. Known through teeth and pieces of skulls, it had some traits that clearly connect it to giant pandas, but it was even more bear-like, showing only precursors of the giant panda's specializations for eating bamboo.

#### Kretzoiarctos beatrix

This relatively recent fossil find (published in 2012) is from a bear that lived about 12 million years ago. It is known mainly from two lower jaws and several teeth that were found in a cave in Spain.

The teeth of K. beatrix were rather bear-like. Like modern bears, this animal most likely ate both animals and plants. However, the jaws and teeth also have some specialized traits that make them similar to those of the giant panda. For example, the shape and wear patterns on the teeth suggest that, like the giant panda, its diet included tough plants.

## Still Looking

As of February 2018, scientists have found no fossils with traits that are intermediate between the giant and red pandas.

# 7. The Red Panda's Fossil Thumb

## The Origin of the Pandas' "Thumbs"

Both the giant panda and the red panda have an enlarged wrist bone that serves as an opposable "thumb" that helps the paws hold and manipulate bamboo.

Over the years, some scientists have argued that this enlarged wrist bone must have been present in a bamboo-eating ancestor to both pandas, and others have argued that it could have arisen later and separately in both pandas (*Image adapted from Abella, Figure 8*).



The grasping action of the front paws from a giant panda (left) and red panda (right), highlighting the radial sesamoid, which acts like an opposable "thumb."

#### Simocyon batalleri

Simocyon lived between about 5 and 12 million years ago. A 2006 publication describes two nearly complete fossils from a cave in Spain. Among the fossilized remains were forelimbs, which had an enlarged wrist bone (the radial sesamoid) (*Graphic: Salesa et al, Figure 1*).



Simocyon shared many traits with modern red pandas, and the two clearly belong to the same lineage. One important difference, though, is in the jaws and teeth. Simocyon does not have the large, flattened molars or extra-strong jaw muscles of the red panda. Instead, its teeth look more like those of carnivores, suggesting that this ancestor did not eat bamboo or any other tough plants.

So if Simocyon had a "thumb" but didn't use it for handling bamboo, then what was it for? Researchers think it helped the animal hold onto thin tree branches as it walked along them. (Below: Artistic renderings of Simocyon from Anton)



#### The Radial Sesamoid is Not Unique to Pandas

In a 2015 study, researchers measured the radial sesamoid bones in several carnivores, both fossilized and living. They learned that this bone is enlarged in many carnivores, especially those that spend time in trees. The table below shows the size of the radial sesamoid bone relative to the size of the animals' paws (*table modified from Abella, 2015*):

Species	Relative size of radial sesamoid	Spends time in trees?
Giant panda	0.84	0
Wolverine	0.51	$\bigcirc$
Simocyon batalleri	0.51	$\bigcirc$
Fisher	0.50	
Pine marten	0.46	$\bigcirc$
Red panda	0.45	
Asian bearcat	0.44	
Indarctos arctoides (an ancestral bear)	0.40	0

#### **References and Image Sources**

- Abella, J., Alba, D.M., Robles, J.M., Valenciano, A., Rotgers, C., Carmona, R., Montoya, P. & Morales, J. (2012). Kretzoiarctos gen. nov., the oldest member of the giant panda clade. *PLOS One* 7, 11, e48985. doi:
- Abella, J., Perez-Ramos, A., Valenciano, A., Alba, D.M., Ercoli, M.D. & Hontecillas, D. (2015). Tracing the origin of the panda's thumb. *The Science of Nature 102*, 35
- Antón, M., Salesa, M.J., Pastor, J.F., Peigné, S. & Morales, J. (2006). Implications of the functional anatomy of the hand and forearm of *Ailurus fulgens* (Carnivora, Ailuridae) for the evolution of the 'false-thumb' in pandas. *Journal of Anatomy, 209*, 6, 757-764. doi: 10.1111/j.1469-7580.2006.00649.x
- Bininda-Edmonds, O.R.P. (2004). Phylogenetic position of the giant panda: historical consensus through supertree analysis. Chapter in *Giant Pandas: Biology and Conservation*. Lindburg, D.G. & Baragona, K., eds. Berkeley, CA: University of California Press.
- Davis, D.D. (1964). *The Giant Panda: A morphological study of evolutionary mechanisms*. Chicago, USA: Chicago Natural History Museum. Accessed online 1 September 2016, through the Biodiversity Heritage Library, http://www.biodiversitylibrary.org/bibliography/5133#/summary
- Figueirido, B., Palmqvist, P., Perez-Claros, J.A. & Dong, W. (2011). Cranial shape transformation in the evolution of the giant panda (*Ailuropoda melanoleuca*). *Naturwissenchaften 98*, 107-117. doi: 10.1007/s00114-0100-0748-x
- Hashimoto, T., Otaka, E., Adachi, J., Mizuta, K. & Hasegawa, M. (1993). The giant panda is closer to a bear, judged by α- and β-hemoglobin sequences. *Journal of Molecular Evolution* 36, 282-289.
- Jin, C., Ciochon, R.L., Dong, W., Hunt, R.M., Liu, J., Jaeger, M. & Zhu, Q. (2007). The first skull of the earliest giant panda. *Proceedings of the National Academy of Sciences 104*, 26, 10932-10937. doi: 10.1073/ pnas.0704198104
- Mayer, E. (1986). Uncertainty in science: is the giant panda a bear or a raccoon? Commentary in *Nature 323*, 769-771. doi:10.1038/323769a0
- Nash. W.G. & O'Brien, S.J. (1987). A comparative chromosome banding analysis of the *Ursidae* and their relationship to other carnivores. *Cytogenetics and Cellular Genetics* 45, 206-212. doi: 10.1159/000132455
- Nash, W.G., Wienberg, J., Ferguson-Smith, M.A., Menninger, J.C. & O'Brien, S.J. (1998). Comparative genomics: tracking chromosome evolution in the family Ursidae using reciprocal chromosome painting. *Cytogenetics and Cell Genetics* 83, 182-192.
- O'Brien, S.J. (1987). The ancestry of the giant panda. Scientific American, 1 November, 1987, 102-107.
- O'Brien, S.J., Nash, W.G., Wildt, D.E., Bush, M.E. & Benvenitse, R.E. (1985). A molecular solution to the riddle of the giant panda's phylogeny. *Nature 37*, 140-144.
- Salesa, M.J., Antón, M., Peigné, S. & Morales, J. (2006). Evidence of a false thumb in a fossil carnivore clarifies the evolution of pandas. *Proceedings of the National Academy of Sciences 103*, 2, 379-382. doi:
- Switek, B. (13 January, 2011). Bears and bamboo: the fossil record of giant pandas. *Wired*. Access 7 September, 2016, from http://www.wired.com/2011/01/bears-and-bamboo-the-fossil-record-of-giant-pandas/
- Tagle, D.A., Miyamoto, M.M., Goodman, M., Hoffmann, O., Braunitzer, G., Göltenboth, R., Jalanka, H. (1986). Hemoglobin of pandas: phylogenetic relationships of carnivores as ascertained with protein sequence data. *Naturwissenschaften* 73, 8, 512-514.