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Using footprints to identify and sex giant pandas

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ABSTRACT

Data on numbers and distribution of free-ranging giant panda are essential to the formulation of effective conservation strategies. There is still no ideal method to identify individuals and sex this species. The traditional bite-size method using bamboo fragments in their feces lacks accuracy. The modern DNA-based estimation is expensive and demands fresh samples. The lack of identifiable individual features on panda pelage and no apparent sexual dimorphism impede reliable estimation from camera trap images. Here, we propose an innovative and non-invasive technique to identify and sex this species using a footprint identification technique (FIT). It is based on a pairwise comparison of trails (unbroken series of footprints) using discriminant analysis, with a Ward's clustering method. We collected footprints from 30 captive animals to train our algorithm and used another 11 animals for model validation. The accuracy for individual identification was > 90% for individuals with more than six footprints and 89% with fewer footprints per trail. The accuracy for sex discrimination was about 84% using a single footprint and 91% using trails. This cost-effective method provides a promising future for monitoring wild panda populations and understanding their dynamics and especially useful for monitoring reintroduced animals after the detachment of GPS collars. The data collection protocol is straightforward and accessible to citizen scientists and conservation professionals alike.

1. Introduction

The giant panda (*Ailuropoda melanoleuca*) is one of the world's most iconic threatened species, with an estimated 1864 pandas surviving in the wild (State Forestry Administration, 2015). Although protected areas cover 54% of the suitable habitat (State Forestry Administration, 2015), this species still faces serious threats such as habitat loss and fragmentation (Loucks et al., 2001; Li and Pimm, 2016). The giant panda now lives in six mountain ranges and is isolated into 33 subpopulations. Of these, 22 have fewer than 30 individuals, and 18 have fewer than ten individuals and some of them are on the brink of extinction (State Forestry Administration, 2015). For their long-term survival and management, understanding giant panda population dynamics is crucial. To date, there are no ideal methods for individual and sex discrimination. Direct observation and counts are impossible because of low population densities, complex topography, and elusiveness of the species (Zhan et al., 2006). Unlike tigers or leopards, the similar appearance of individual pandas, with no identifiable features such as stripes or spots, makes them difficult to differentiate from camera trap images. Here, we suggest a practical field method to sex and identify individual pandas.

Currently, there are two primary methods to identify individual giant pandas: the bite-size technique and DNA-based approaches. The bite-size technique was originally used to differentiate age groups of pandas (Schaller, 1985) and then was extended to identify individuals (Garshelis et al., 2008). Studies of giant pandas in the wild and captivity have shown individual differences in “bite size” and “chew rates” of the bamboo stems in their droppings (Schaller, 1985; Yin et al., 2005). The bite size is usually derived from measuring 100 stem/leaf fragments in droppings (Yin et al., 2005). This method has been used for the third (1999–2003) and fourth (2011–2014) national survey of giant pandas (State Forestry Administration, 2015), but it lacks scientific rigor (Wei et al., 2002; Zhan et al., 2006). It is less reliable in denser population areas or within mating clusters because many individuals may have similar bite sizes. Moreover, some significant variation in bite sizes within individuals could result in overestimating numbers (Zhan et al., 2006). Finally, this method requires field staff to make very precise measurements to apply the threshold of 2 mm (Yin et al., 2005). Human and measurement tool errors are often unable to meet this level of precision (Zhan et al., 2006).

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The alternative is using microsatellite analysis with fecal DNA (Zhan et al., 2006). This non-invasive DNA sampling was also used in the fourth national giant panda survey (State Forestry Administration, 2015). Believed to be more accurate than the traditional bite-size estimate (Wei et al., 2015), its accuracy requires the sample to be very fresh to exclude potential degradation and contamination of DNA. The extensive survey effort required and challenges in finding sufficient samples have prevented applying this method successfully in large-scale studies. The cost of processing samples in the laboratory has impeded the use of DNA individual identification for most conservation practitioners.

There is no apparent sexual dimorphism in the giant panda. Because the external sexual organs are small and cryptic, it is difficult to identify the sex of giant pandas in the field, or even in captivity, without a DNA test. Adult males are 10–20% larger than adult females (Smith et al., 2010). There is much variation, however, and it is particularly difficult to identify the sex of a solitary, free-ranging animal, outside the breeding season. This problem is exacerbated when it comes to identifying the sex of sub-adults (Yang et al., 1999).

Reintroduction has been a crucial part of panda conservation, especially to revive the small and isolated local populations. GPS collars are only used for these reintroduced pandas and are set to drop off after two years. Reintroduction needs to be evaluated in the long term and requires novel non-invasive methods to monitor these individuals.

These challenges have motivated the development of a robust and cost-effective technique to balance the accuracy required of a population estimate with the need for a low-cost field tool. The Footprint Identification Technique (FIT) has become a promising and cost-effective tool in wildlife conservation in recent years (Pimm et al., 2015). This non-invasive technique was first developed for black rhinos (Jewell et al., 2001). More recently it has been successfully adapted and applied for cheetah (Jewell et al., 2016), white rhinos (Alibhai et al., 2008), Amur tiger (Gu et al., 2014), mountain lions (Alibhai et al., 2017; Jewell et al., 2014) and other endangered species.

Footprints have been used as signs of giant panda presence for many years (Fan et al., 2011; Wang et al., 2014; Li et al., 2015). Their footprints are characteristic of the species, and if the substrate permits, easily found.

We report the development of the giant panda FIT for individual and sex identification, a potentially powerful tool to assist with the management and conservation of this endangered species. FIT can play an important role in monitoring the demographics of giant panda populations. China now has around 375 captive giant pandas and an active re-introduction programme is underway (State Forestry Administration, 2015). Since FIT requires the initial establishment of a training database with known individuals to extract the necessary algorithms, the captive-bred population proved to be an ideal resource. The development of this technique for the giant panda could help establish an individual database of footprints for the free-ranging populations.

2. Methods

2.1. Study population

We collected footprint images from 41 captive giant pandas in the China Conservation and Research Centre for the Giant Panda (CCRCGP) in Sichuan, China. It has three major captive bases; Ya'an, Du Jiang Yan, and Wolong. The Wolong base is located in the heart of Wolong National Nature Reserve, which is one of 67 reserves designated by China's government to protect wild giant pandas (State Forestry Administration, 2015). Several enclosures are built in the forest, each with an average area of 0.33 km^2. This natural habitat provides conditions for rehabilitating animals which are to be reintroduced to the wild.

2.2. Study period

We collected images from captive animals from March 2014 to April 2016, mostly on a prepared sand substrate since snowfall was infrequent at the lower altitudes where captive pandas are held. Fresh sand was used for each animal to avoid any possible disturbance of behaviors from olfactory cues. At the same time, we collected footprints on snow from captive animals at Wolong when enough snow had accumulated in the higher-altitude enclosures.

2.3. Foot anatomy and data collection

In addition to the five digits, the giant panda has an unusual feature on the front feet – a 'sixth finger' or 'sesamoid pad'. This structure acts as an opposable digit and is an adapted and enlarged radial sesamoid bone from the wrist. This exaptation enables giant pandas to grab bamboos more efficiently and to facilitate feeding (Endo et al., 1999). Thus, a clear front footprint usually shows six distinct digit pads along with the metacarpal and carpal pads. The sesamoid bone imprints are unique to giant panda prints. For our purposes, they have the advantage of adding complexity to the footprint, thus enabling the extraction of a more effective FIT algorithm from the morphometrics (Fig. 1).

Initial trials to investigate the clarity of the prints left by each of the four feet also indicated that front foot impressions were more distinctive, detailed, and clearly outlined. This was likely due to a combination of greater weight at the front of the animal and less fur on the front feet. We arbitrarily chose the left front foot for the FIT model development. In common with bear species, pandas tend to over-step or side-step. That is, instead of registering the hind foot impression on that made by the front foot, the hind foot usually falls in front of the front foot print or to one side, leaving a clear front foot impression.

We define a *trail* to be an unbroken series of footprints from one animal. We took images of each left front footprint from directly above with a carpenter's scale in the trail according to the protocol described in Jewell et al. (2016). The form of each footprint may vary with the gait of the animal, substrate type, moisture levels, slope of the ground and weather conditions. To account for this variation within the footprint metrics of each individual, we collected multiple footprint images from each panda.

2.4. Extracting a geometric profile

In total, we collected 521 usable footprints along 76 trails from 41 individuals (see Supplementary Table 1 for individual information).

We imported each digital footprint image into a customized FIT addin in JMP software from SAS, resized and rotated for standardization (Jewell et al., 2016). Scale points 1 and 2 were placed on the ruler at an interval of 10 cm. Landmark points were then placed manually at anatomical positions on the footprint, following software prompts. In other species, the edges of the pads are more clearly defined e.g., the cheetah (Jewell et al., 2016). In the giant panda, the edges of the pads are less clearly defined due to different substrates on where footprints can be found in the field, so we used the centroids for landmark points 1 to 6 on the toe pads and sesamoid pad, and the distal end of pad for landmark point 7 (Fig. 2). Using these landmark points, JMP automatically computed a further 15 derived points and then 124 metrics consisting of lengths, angles and areas (see Supplementary Table 2 for details). The collection of these metrics allows all measurements that one anticipates might prove useful in discriminating between footprints.

3. Data analysis

3.1. Individual identification

The FIT customized model for classifying trails employs pairwise