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Analyzing the past to understand the future: Natural mating yields better reproductive rates than artificial insemination in the giant panda

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ABSTRACT

Conservation breeding programs require active management and thus selection among various management alternatives is a common practice. As in the practice of adaptive management used in ecology, it is important to reduce uncertainty about the outcomes of various management actions. Ideally this evaluation will be done using a priori hypothesis testing, but retrospective analyses can provide important insights as to which methods work better than others. The giant panda (*Ailuropoda melanoleuca*) conservation breeding program has a long history of active management and therefore is rich in potential lessons learned for panda and other endangered species breeding programs. Now self-sustaining and experiencing exponential growth, the panda population also provides sample sizes large enough to support rigorous statistical evaluation. A fundamental decision for any breeding program is whether to invest in development and application of assisted reproduction techniques or to promote natural mating. Here we analyze 21 years (1996–2016) of giant panda reproductive data from 304 insemination events to determine relative success rates of insemination methods and evaluate management strategies. The birth rate after natural mating was 60.7%, 50.6% for combined natural mating and artificial insemination techniques, and 18.5% for artificial insemination (AI). Within the combined insemination technique group, 81.8% of births could be attributed to the natural mating event with only 18.2% attributed to AI. These results suggest that while techniques for improving AI should continue to be explored and will play an important role for some conservation applications, behavioral and biological management to encourage natural mating should be the most important goal for conservation breeding of this species.

1. Introduction

The primary goal of most conservation breeding programs is to salvage threatened and endangered species or populations from extinction and to preserve the option of reintroduction and/or supplementation of wild populations (Frankham, 2008; IUCN, 2013; Seddon et al., 2007). However, obtaining a stable, self-sustaining captive population has remained an elusive goal for most programs (Bowkett, 2009). Breeding programs that have managed to reach sustainability, such as the giant panda program managed by the Chinese Conservation and Research Center for the Giant Panda (CCRCGP), could help inform protocols for other breeding programs that are still struggling to meet demographic and population goals. The giant panda conservation breeding program has one of the longest histories of any such program. Beginning in the 1960s, this program rose modestly in the 1980s and a significant increase in breeding efforts occurred from 1990 to present (Ellis et al., 2006). While early efforts met with varying reproductive success, the current system consistently produces a substantial number of cubs per year resulting in an ex situ population that is self-sustaining (Traylor-Holzer and Ballou, 2016). Indeed, the program has become so successful that it is now in an exponential growth phase producing a surplus of animals that can be used for translocation efforts to supplement the wild population (Xie, 2016; Zhang et al., 2004). Through China's extraordinary efforts and dedication to the conservation of the giant panda, the International Union for the Conservation of Nature recently 'downlisted' this iconic species from "endangered" to "vulnerable" (IUCN, 2017; Swaisgood et al., 2016; Swaisgood et al., 2017). Though the giant panda still requires continued conservation effort in order to fully recover, it is important to recognize the success of previous efforts, to review management practices that led to success, and to

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evaluate practices that will continue to help the giant panda population recover. A review of the program's strategies could inform future management of the captive population and also communicate lessons learned to similar conservation breeding programs for other species.

Five research articles have been published on the relative success of different techniques used for insemination in giant pandas (Howard et al., 2006; Huang et al., 2012a; Huang et al., 2002; Huang et al., 2012b; Zhang et al., 2004). Even though early breeding records from the giant panda program (1996–2002) show multiple successes in captive breeding techniques, the low number of reproductively available pandas limited sample sizes available for analyses. A review of these research articles suggests that artificial insemination on its own has between 25%–57.1% success rate, mixed insemination protocols 65%–66.7% (with 94.4%–100% of cubs sired from the natural mating), and natural mating alone occurred too infrequently to merit analysis. However, sample size for each of these studies was fewer than 30 insemination events, thus statistical power was low and it is difficult to generalize from these findings. Here we review 21 years of breeding data collected from two prominent Chinese breeding centers to evaluate the relative efficacy of natural mating and artificial insemination (AI) methods of breeding giant pandas. A retroactive review of success rates obtained from each insemination method will allow managers to make more informed decisions about the reliability of each protocol and will also help inform genetic management as captive panda populations are actively managed to control for inbreeding depression and loss of genetic diversity (Wildt et al., 2006a). These findings will be important as giant panda conservation programs move toward maintaining genetic diversity in captivity versus focusing on the quantity of cubs produced, a "quality over quantity" approach (Paetkau and Strobeck, 1998; Traylor-Holzer and Ballou, 2016; Woodworth et al., 2002). A principal objective of our analysis is to use findings to optimize cub production and genetic diversity, while maximizing cost-effectiveness. As with all conservation breeding programs, it is important to understand the relative efficacy of different strategies so that the most effective ones can be employed and resources saved to allocate to other conservation actions.

2. Methods

2.1. Study site and species

We conducted our study on 78 female giant pandas from 1996 to 2016 at the Chinese Conservation and Research Center for the Giant Panda. Mate pairings were conducted at Hetaoping near Gengda, China (1996–2008) and Bifengxia near Ya'an, China (2009–2016) in the Sichuan Province resulting in 304 separate insemination events. All subjects were sexually mature, with ages ranging from 5 to 24 years (Mean = 11). Subjects included in the analysis were placed with one opposite-sex individual for mating purposes. Females were often introduced to an individual male more than once in a mating season but the number of unique males introduced to a single female did not exceed five.

At Hetaoping, giant pandas were housed in concrete walled, open-air enclosures (9 m × 8 m) with an indoor den (3 m × 6 m). Pandas were housed in adjoining pens resulting in animals neighboring one or two conspecifics. All enclosures allowed opportunities for bears to interact through cage bars along the majority of the adjoining outdoor enclosure wall (7 m). Wire mesh hung between cage bars limiting direct physical contact but allowing olfactory, visual, and auditory access but limited tactile and gustatory interactions. For detailed information on animal care protocols at this institution see Swaisgood et al. (1999) and Zhang et al. (2004).

At Bifengxia giant pandas were housed in concrete walled, open-air enclosures (8 m × 25 m) that contained various forms of environmental enrichment (e.g. climbing platforms, water features, trees, etc.) and an indoor enclosure area (3 m × 8 m). All enclosures had three barred "howdy" windows and a circular barred gate located on the long sides of the enclosure (8 potential interaction windows, 4 per side). Thus, giant pandas were able to interact through cage bars with neighboring individuals in adjoining enclosures, but opportunities for physical contact were limited. For detailed information on animal care protocols at this institution see Martin-Wintle et al. (2015).

All giant pandas were exposed to natural light conditions and fed a diet of local bamboo supplemented with bamboo shoots, high-fiber biscuits, carrots, and apples. Housing and animal husbandry practices for Bifengxia are described in (Martin-Wintle et al., 2015). Animal care and use guidelines of the American Society of Mammalogists (Animal Care and Use Committee 1998; Assurance # 15-003) were followed by all facility operators.

2.2. Insemination procedures

Here we define mate pairings as the introduction of a specific male to a specific female for the purpose of breeding. All pairings were determined via genetic recommendations based on Mate Suitability Index (MSI), which combines mean kinship, inbreeding, and difference between mean kinship value of the male and female. Mating was always attempted first with the priority male according to the genetic management plan even if animals appeared indifferent or slightly aggressive toward the potential mate, but mating introductions were not attempted where excessive aggression was observed. AI was performed in the latter case or if females were recommended for insemination by a male who was not physically present (e.g. dead or at a different facility).

Prior to 2000, female estrus status was determined using either behavioral indicators and/or vaginal cytology. Intermittently between 1997 and 2002 and after 2002, female estrus was determined via behavioral indicators paired with enzyme-immunoassay for estrogen metabolites (estrone-3-glucuronide) previously validated on urine (McGeehan et al., 2002). Urine samples were collected via syringe from the enclosure floor ~3 days a week and stored at −20 °C until analysis at the CCRCGP laboratory. During the peri-ovulatory period urine samples were collected daily. Ovulation is indicated by a > 6-fold elevation of estrogen above baseline levels, followed by a return to baseline (McGeehan et al., 2002). All natural mating introductions were conducted during this peri-ovulatory period including the day before, the day of, and the day following presumed ovulation.

Males were introduced to female pens for mating between 9:00 and 17:00 h. If either animal's behavior was aggressive, animal care staff removed the male immediately to prevent injury or death. After a mating session, males were moved back to their enclosures and subsequently placed with a different female until all females had been mated to their recommended males. This method resulted in females being introduced to 1–5 males and having on average 4 (but as many as 9) mating opportunities each breeding season. As a fail-safe, female giant pandas are often artificially inseminated following natural breeding. To establish paternity, the CCRCGP used DNA obtained from hair samples and amplified utilizing the polymerase chain reaction to analyze microsatellite loci after the methods of Zhang et al. (1994). Only animals with confirmed paternity were used in these analyses.

Semen was collected via electroejaculation and spermatozoa were diluted and cooled via the protocol described in Huang et al. (2012b). Samples were stored long term in straws that were submerged in liquid nitrogen. For use during artificial insemination, samples were thawed by placing the straws in a 37 °C water bath for 30 s and kept in a warm water bath until use. AI was performed after the peak estrogen was detected from urine samples and/or behavioral signs suggested estrus. For detailed information on artificial insemination, sperm collection, sperm storage, and sperm thawing techniques see Huang et al. (2012b).