

The southernmost parakeet might be enhancing pollination of a dioecious conifer

The endangered monkey puzzle tree, *Araucaria araucana*, is a dioecious conifer that, throughout its native range in northwest Patagonia (Argentina and Chile), holds sex-specific interactions with the southernmost parrot in the world, the austral parakeet *Enicognathus ferrugineus*. This parakeet is known to be an important consumer and disperser of *A. araucana*'s seeds (Tella et al. 2016). During the pollination season, we observed parakeets feeding massively on *A. araucana*'s pollen-loaded male cones (Fig. 1a). With their bodies and feet bathed in pollen (Video S1) parakeets often flew to female trees, perching on receptive female cones, which they did not

consume at that developing stage (Fig. 1b). This feeding behavior, based on consumption of male cones releasing pollen but not of young female cones with receptive ovules, could promote parrot-mediated pollination in an otherwise wind-pollinated conifer. Parrot-mediated pollination could be potentially common across *A. araucana*'s geographic range. Beyond Díaz et al. (2012)'s first record of consumption of *A. araucana*'s pollen by parakeets in one Argentinian population, we observed parakeets feeding on pollen (groups of up to 150 individuals) and then perching on young female cones in five other Argentinian and two Chilean populations during two pollination seasons (November–December 2013 and 2015).

Biotic pollination, i.e., pollen transfer from anthers to stigmas by an animal, is one of the most fascinating and widespread plant–animal interactions in nature. More than 80% of the about 350,000 extant species of angiosperms depend to various degrees on one or several of thousands of invertebrate and hundreds of vertebrate species that act as pollinators (Ollerton et al. 2011). Unlike angiosperms, biotic pollination in extant gymnosperms is less frequent. Whereas wind is the only known pollen-transfer agent in most conifers and the relict species



FIG. 1. (a) Austral parakeets feeding on male cones in a monkey puzzle tree. While feeding destructively, parakeets contact male cones with their mouthparts but also with their feathered bodies and feet, an observation that triggered the hypothesis that pollen grains may reach ovules when (b) parakeets perch on female cones. The photographs were taken in Lanin National Park, Argentina (39°59' S, 71°41' W), during the austral spring 2015 (Photographs taken by Orlando Mastrantuoni).

Gynko biloba, insect pollination has been just reported for cycads and gnetoids (Peñalver et al. 2012). Dating back at least to the Jurassic, before the evolution of flowering plants, insect pollination may have originated from phytophagous insects feeding on reproductive tissues of ancient gymnosperms, leading accidentally to successful pollination events (Lavandeira 2010).

Although insects, and particularly bees, are the most important animal pollinators, pollen transfer can also be mediated by birds and, less frequently, by lizards and small mammals. Among birds, only 10% of species interact with flowers (as pollinators or at least as flower visitors); however, this phenomenon is phylogenetically widespread, occurring in six phylogenetically independent bird groups (Pellmyr 2002). Indeed, consumption of flower tissues, nectar, or pollen may be essential during the life cycle of some birds. Within parrots, for example, flowers have been reported to be important food resources for successful reproduction in some species, as is the case of the Australian swift parrot *Lathamus discolor*, which depends strongly on *Eucalyptus globulus*'s flower rewards during its breeding season (Hingston et al. 2004). Similarly, *Enicognathus ferrugineus* consumes massive amounts of pollen from the endemic trees *Nothofagus pumilio* and *Araucaria araucana*. Pollenivory in this parakeet has been attributed to limited food availability during late winter and early spring in these southern seasonal temperate forests (Díaz and Kitzberger 2006). Consumption of *A. araucana*'s protein-rich pollen cones, in particular, has also been suggested to be important for successful breeding of these parakeets (Díaz et al. 2012).

Avian pollination is accomplished when pollen grains remain attached to the feathers of birds capable of feeding without damaging reproductive structures (but see Pauw 1998 for a case in which pollen is successfully transported on a sunbird's tongue). Among parrots, lorises and lorikeets (Loriinae) have specialized brush-tipped tongues for pollen and nectar feeding, and are important pollinators in tropical forests from the Australasian region (Fleming and Muchhala 2008). Likewise, although stemming from granivorous ancestors, *Lathamus discolor* (Platycercinae) developed a brush tongue and morphological adaptations in the digestive tract favoring nectarivory (Gartrell et al. 2000), being an effective pollinator of *Eucalyptus globulus* (Hingston et al. 2004). On the contrary, the rest of parrot species lack specialized mouthparts to handle plant reproductive structures non-destructively. Indeed, they generally damage flower tissues when feeding on nectar or pollen, although behavioral observations suggest that some parrot species are capable of foraging non-destructively on flowers and might promote successful pollination (Cotton 2001, Blanco et al. 2015). The case of the interaction between austral parakeets and *Araucaria araucana*'s reproductive cones is unique because austral parakeets feed destructively on male reproductive cones and perch without feeding on branches with female cones bearing

receptive ovules. Given that parakeets often handle portions of male cones with their feet and bath themselves with pollen grains (Video S1) as they feed on male cones, it is very likely that a portion of pollen grains from their bodies reaches ovules contained in female cones, once they perch on these cones. Therefore, the separation of male and female functions into different structures may facilitate biotic pollination by austral parakeets.

The pollenivory interaction between austral parakeets and monkey puzzle trees is also intriguing from an evolutionary perspective because of age differences between their respective lineages. Whereas the origin of the genus *Araucaria* is thought to have occurred >100 mya, during the Mesozoic (Kranitz et al. 2014), that of the Neotropical tribe Arini (which includes the genus *Enicognathus*) probably took place <30 mya, during the Oligocene (Schweizer et al. 2011). Thus, from the viewpoint of the plant's evolutionary history, interaction with parakeets is relatively recent. Pollen wall morphology seems not to differ to a great extent between *Araucaria araucana*, the single extant species in the genus where pollenivory by vertebrates has been recorded, and a few of other extant and extinct related species studied (Del Fueyo et al. 2008), which might indicate that this is a highly conserved trait in the lineage. However, *A. araucana* has the widest male cones among all extant and probably extinct *Araucaria* (more than two-fold wider than the average across the 19 extant species; Farjon 2010), which opens the possibility that, over the last million years, they lost slenderness and some capacity of "swinging" in the wind. While the surplus pollen production associated with wide male cones could be offsetting pollen predation by parakeets, it could also be interpreted as the evolution of a reward in exchange of a pollination service.

Monkey puzzle trees, as with all other conifers, are thought to depend entirely on anemophilous pollination. Pollen flow through wind often occurs in the prevalent wind directions, thus restricting the identity of effective sires to only those favored by wind. The occurrence of pollination carried out by austral parakeets, a highly mobile species (Tella et al. 2016), could increase monkey puzzle tree's female fitness by increasing sire diversity, and thus genetic diversity within female progeny arrays (as sires would not be restricted to be only those favored by wind). Likewise, parakeet pollination might increase male fitness by increasing short- and long-distance siring opportunities. Overall, our observations on parakeets feeding behavior triggered novel unexpected questions that need to be tested. Are nutritional resources in pollen grains greater in monkey puzzle trees than in other conifers? Does pollen deposited on parakeet's feathers and feet reach receptive ovules on female trees? May parakeets act as long-distance pollinators of multiple female trees? Do male trees suffering from high consumption of their cones achieve greater siring success because of increasing pollen export? Does biotic pollination increase genetic diversity of female's

progenies over that expected solely from wind pollination? Comparative studies on pollen grains nutritional content can easily ask the first question. Live-trapping of parakeets during the pollination season would allow quantifying the amount of pollen transported on their feet and feathers and potentially transferred to female cones. Moreover, the attachment of GPS devices to parakeets would allow us to determine their daily and whole-season movements among multiple male and female trees at fine spatial scales. These field-based observations could be complemented with captive experiments conducted in large aviaries, disposing male cones as food and female cones as perches separated by a physical barrier that would exclude wind pollination, and later looking for pollen effectively transferred to female cones by parakeets. Finally, experimental manipulations conducted in monkey puzzle's natural populations (e.g. pollination exclusion treatments), combined with pollen dispersal genetic analyses would also be necessary to confirm the possibility that parakeets may effectively pollinate monkey puzzle trees, challenging the long-held thought that conifers are entirely pollinated by wind, and also that vertebrate pollination is completely absent in gymnosperms.

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