



Scanning Electron Microscopic Observation of Epizoochorys Microstructures Resembling an *origami crane* on the Seed Surface of *Desmodium paniculatum*

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Abstract

Desmodium paniculatum is a perennial Leguminosae plant that uses animal-attached seeds for dispersal. The key-shaped spines on *D. paniculatum* seeds are a recognized mechanism of attachment. However, in addition to these spines, we observed even finer attachment structures that were similarly shaped to origami cranes via scanning electron microscopy. The seeds of *D. paniculatum* are characterized by their attachment to clothing, adhering snugly similarly to a sticky seed. However, these seeds may be even more adhesive by using the large hooked spines as well as the mass of microscopic spines functioning as attachment organs.

Keywords: *electron microscopic, Desmodium paniculatum, epizoochorys, microstructures, spines.*

Introduction

Plants use various mechanisms for seed dispersal. Plant seed dispersal is classified into five disseminule types (D₁ to D₅) (Numata, 1964). The D₁ type is sprayed by the force of wind (anemochore) or water flow (hydrochore). The D₂ type is a seed carried by animals or humans. The D₃ type is spread by mechanical propulsion. The D₄ type, clitochore, have no special mechanisms for spreading and are only capable of falling due to the spreading dynamic of gravity. Finally, the D₅ type does not propagate by seed. Further, there are two major types of D₂; one is epizoochory, that is, seeds are carried by adhering to the animal's body surface with spines. Epizoochory includes seeds attached by physical structures such as hooks (oncospore), spiny (centrospore), and viscid (gloeospore). The second type is endozoochory, whereby the fleshy fruit is swallowed whole by a bird or animal and the seeds are transported through their body.

Desmodium paniculatum is a perennial Leguminosae plant native to North America (Kadota, 2013; Shimizu, *et al.*, 2003; Wulff, 1986). It was naturalized in Japan in the 1960s, and now grows wild in broad areas, for

example, the roadside (National Institute for Environmental Studies, 2022; Takahashi, 2004). *D. paniculatum* has typical epizoochory seeds with a keyed-tip spiny appearance. These seeds have been reported to attach using these spines (United States Department of Agriculture, 2022). However, using scanning electron microscopy, we determined that even finer attachment structures existed in addition to these spines.

Materials and Methods

Seeds samples of *D. paniculatum* used in this study were collected in the field of the Center for Education and Research in Field Sciences, Shizuoka University, Japan (N 34.91, E 138.27). The collected seeds were immediately observed via a scanning electron microscope (Miniscope, TM3030Plus, Hitachi Ltd., Tokyo) without any pretreatment on December 7, 2018.

Results and Discussion

For plant seeds, attachment to animal or human clothing is an effective method of dispersal over long distances. Attachment organs of animal-attached seeds have various shapes. For example, fruits, including the seeds of burdock burrs (*Arctium tomentosum*)

and oriental cocklebur (*Xanthium strumarium*) have hooked spines. Interestingly, this hook structure inspired the invention of the zipper mechanism (Bonsler and Vincent, 2007). In contrast, the seeds of pig's knee (*Achyranthes bidentata*) have clip-like attachments. The seeds of *Bidens* species such as devil's beggar ticks (*Bidens frondosa*) and hairy beggar ticks (*B. Pilosa*) possess harpoon-like spines. Further, the adhesion to animals using adhesive substances is observed in the seeds of *Sigesbeckia pubescens* and pale-seed plantain (*Plantago asiatica*).

D. paniculatum is called *Nusubito-Hagi* in Japanese and 'Nusubito' means 'thief'. The origin of this Japanese name is either because it attached to clothing to avoid being noticed or because the seeds on the clothing resemble a thief's footprints. The seed possesses hooked spines when viewed under stereo microscopes and they attach to animal and

human clothing using these spines (United States Department of Agriculture). However, we also observed hooked microscopic spines, approximately 0.1-0.2 mm in size, on the seed surface of *D. paniculatum* (Fig 1 -left) and identified a myriad of finer clumps of microscopic spines, approximately 10 μm , covering the seed's surface (Fig 1 -right). These spines had multiple curved spines originating from one place, and resembled "origami cranes" (Fig 1- top right inset). These microscopic spines may also function as an adhesive.

The seeds of *D. paniculatum* are characterized by its attachment to clothing because it lands snugly as if it were a sticky seed. However, these seeds may be more adhesive by using the large hooked spines as well as this mass of microscopic spines as their attachment organs.

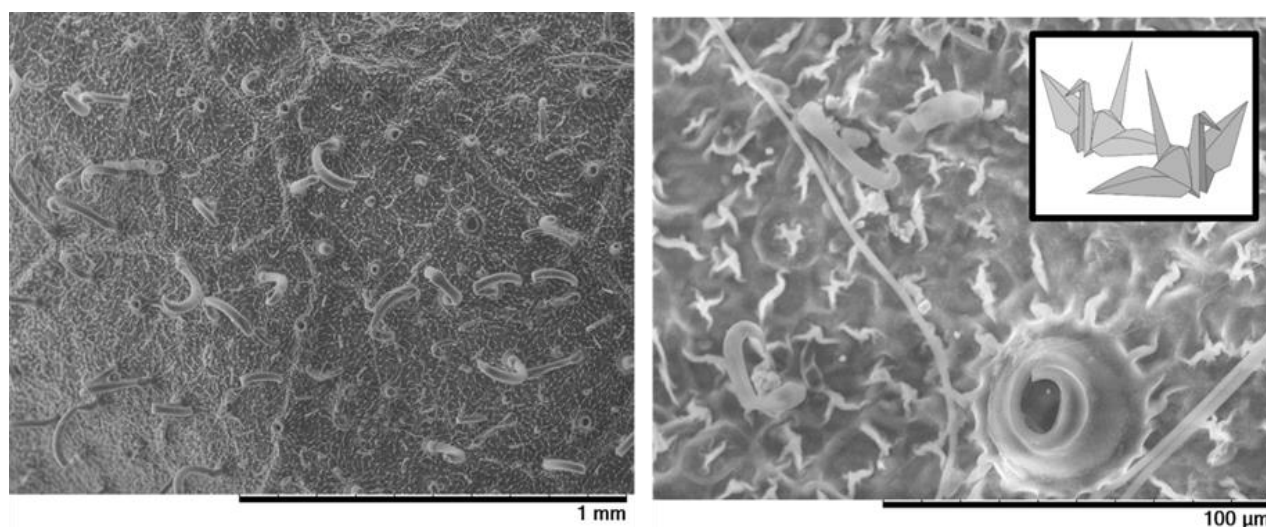


Fig 1: Electron micrograph of the seed surface of *Desmodium paniculatum*. Inset on right is an illustration of an "origami crane".

Conclusion

The seeds of *Desmodium paniculatum* are attached by key-shaped spines. However, in addition to these spines, in this study, we discovered that there are even finer attachment structures that have a similar shape to origami cranes. It is thought that the structure of the attachment of the seeds was the inspiration for the invention of the zipper mechanism. In the future, it is expected that more detailed observations of seed structures using electron microscopy will provide inform the development of novel biomimetics

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