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Diversity, evolution and taxonomic significance of fruit in *Cuscuta* (dodder, Convolvulaceae); the evolutionary advantages of indehiscence

Anna Ho, Mihai Costea*

Department of Biology, Wilfrid Laurier University, 75 University Avenue W, Waterloo, Ontario N2L 3C5, Canada

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ABSTRACT

Cuscuta (dodder) is one of the few angiosperm genera characterised by multiple fruit indehiscence/dehiscence modes. Dodder fruit is a capsule with thin pericarp and contains one to four seeds. In some species fruits open at the base (circumscissile dehiscence; DE) while in others they remain closed (indehiscent; IN). IN has evolved multiple times from DE, and is most common in the North American clades of subgenus *Grammica.* In addition, different forms of "irregular dehiscence" have been anecdotally reported. To determine fruit types and assess homology, we explored fruit development, structure and ultrastructure together with a comparative morphology and character evolution study of other fruit traits. Because it is commonly assumed that IN is associated with a loss of dispersal capability when diaspores contain several seeds, we studied the geographical distribution of species with DE and IN fruits in North America to ascertain whether the latter are less widely distributed than DE species. To determine the putative dispersal advantage(s) of IN we studied the floatability of both capsules and seeds of *C. gronovii*, a species with IN fruits. Also, in this species we examined the effects of the fruit on seed germination. Pericarp structure/ultrastructure revealed three distinct fruit types: DE, with an abscission zone (AZ); IN without an AZ, and irregularly dehiscent capsules (IrA) which lack an AZ but open because of the thin endocarp cell walls at the fruit base. A fourth functional fruit type, the irregularly dehiscent type B capsules (IrB) was noted, in which dehiscence apparently occurs because of mechanical pressures created among ripening IN fruits within the dense infructescences of the same plant. IrA fruits were posited to be an intermediate evolutionary step between DE and IN fruits; IrA and IrB fruits are not homologous. The only statistically significant fruit trait associated with the dehiscence mode is the size of the interstylar aperture which is larger in IN fruits than in DE capsules. Although not statistically significant, species with IN fruits appear to be more widely distributed in N America that species with DE capsules suggesting an enhanced dispersal capability of IN fruits. Also, species with IN fruits possess a significantly higher northern latitudinal limit in North America. Capsules of *C. gronovii* were capable of floating for at least one week longer than their seeds indicating that long-distance dispersal by water is possible under certain conditions. Seeds of *C. gronovii* not removed from their IN fruits exhibited a delayed germination pattern compared to removed seeds, which heightened germination bet hedging and may prevent sibling competition for the same host plants.

1. Introduction

The morphology of diaspores and the number of seeds they contain directly affect dispersal (e.g., Van der Pijl, 1982; Tiffney, 1984), indirectly influencing both population level evolutionary processes such as adaptive divergence and geographic isolation (e.g., Levin et al., 2003; Cousens et al., 2008), as well as species level patterns of distribution and diversification (e.g., Fernández et al., 2002; Cousens et al., 2008; Bonte and Dahirel, 2017). Diaspore characteristics also have an effect on seed dormancy and germination, thus influencing population dynamics (reviewed by Baskin and Baskin, 2014).

Cuscuta (dodder) fruit contains one to four seeds and has been considered a capsule since first mentioned in the succinct descriptions circulating in the pre-Linnaean literature (e.g., Bauhin, 1623; Tournefort, 1694). Engelmann (1842) noted first that while in some species capsules open through a line at the base (circumscissile capsules), in others the fruits remain indehiscent (which he called "baccate capsules"). Thus, dodder diaspores are either the indehiscent fruits or the seeds themselves which is a rare situation in angiosperms (e.g., *Amaranthus*, Costea et al., 2001; *Lepidium*, Mummenhoff et al., 2009). Engelmann used the dehiscence/indehiscence of capsules to circumscribe six of the nine sections within the three major infrageneric

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* Corresponding author.
E-mail address: mcostea@wlu.ca (M. Costea).

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"groups" of *Cuscuta* (Engelmann, 1859) which are currently accepted as subgenera *Monogynella*, *Cuscuta*, and *Grammica* (Costea et al., 2015). Yuncker (1921, 1932) also considered dehiscence/indehiscence a character of paramount importance for the systematics of *Cuscuta* and divided the largest subgenus *Grammica* into two sections based on their dehiscence modes: *Cleistogrammica*, with indehiscent capsules, and *Eugrammica* with dehiscent capsules. The latter author also attempted a polarity interpretation of dehiscence/indehiscence, regarding the former as derived from the latter (Yuncker, 1932). This character evolution hypothesis was, however, refuted by two recent large scale phylogenetic studies (Stefanović et al., 2007; García et al., 2014) which indicated that indehiscence is derived from dehiscence. Species of subgenera *Monogynella* and *Cuscuta* have usually circumscissile fruits (exceptions *C. capitata*, and *C. triumvirati* in subg. *Cuscuta*; Yuncker, 1932; García, 2012), while subg. *Pachystigma* has "irregularly" circumscissile or indehiscent fruits (Yuncker, 1932). Indehiscence has evolved at least nine times in subg. *Grammica*, especially in N America (Stefanović et al., 2007; García et al., 2014; Costea et al., 2015).

Unfortunately, circumscissile dehiscence and indehiscence are not always clear-cut character states in *Cuscuta*. A literature review revealed another, still unexplored dehiscence mode, referred to as "irregular" dehiscence. For example, Engelmann (1859) mentioned capsules that are "circumscissile but sometimes irregularly bursting" (e.g., *C. umbellata*); Yuncker (1932) referred to "tardily/late and irregularly circumscissile" fruits [e.g., Subsect. *Africanae* (now subg. *Pachystigma*; Costea et al., 2015)]; *C. gracillima*, *C. incurvata*, *C. hyalina* in subg. *Grammica*), while Costea et al. (2006a) noted "irregularly dehiscent" fruits (e.g., in sect. *Cleistogrammica*). It is obvious that an "irregular" attribute is shared by all these examples, but the homology of this condition is unclear.

Species level taxonomy in *Cuscuta* has relied heavily on flower morphology (Engelmann, 1859; Yuncker, 1932; reviewed by Costea et al., 2015) because as a result of the evolution to obligate parasitism the vegetative organs of dodders have undergone drastic reductions (Kuijt, 1969; Stefanović et al., 2007). Nevertheless, dehiscence/indehiscence and various fruit morphological characters have also been used in species descriptions (e.g., Engelmann, 1859; Yuncker, 1921, 1932, 1965), but the diversity and evolution of fruit characteristics (except for dehiscence/indehiscence; García et al., 2014) have remained unstudied.

Last but not least, the convergent evolution scenario of fruit indehiscence in subg. *Grammica* (García et al., 2014; Costea et al., 2015) begs questions about its evolutionary advantage(s). Fruit indehiscence, in general, is involved in seed protection and affects dispersal (Roth, 1977; Bazzaz et al., 2000; Baskin and Baskin, 2014). Since the fruit wall (pericarp) in *Cuscuta* is essentially a thin membrane (Engelmann, 1859; Yuncker, 1921; Wright et al., 2011), it is unlikely it plays a significant role in seed protection. "Dispersal syndromes" have been defined to connect morphological attributes of diaspores with dispersal vectors, such as wind, water, gravity or animals (e.g., Van der Pijl, 1982; Tiffney, 1984). From this point of view, *Cuscuta* seeds have been regarded as "unspecialized" (Kuijt, 1969; Dawson et al., 1994; Costea and Tardif, 2006), while the fruits have been anecdotally reported as being capable of floating (Verdcourt, 1948). When diaspores contain multiple seeds, indehiscence has usually been associated with a reduction of dispersal capability (e.g., Augspurger and Hogan, 1983; Snijman and Linder, 1996). However, in some cases, indehiscence may coevolve with dispersal-enhancing traits (e.g., Willis et al., 2014) or as a trade-off with life-history traits that are not directly related to dispersal. For instance, in the latter case, indehiscence can influence dormancy and germination (e.g., Hu et al., 2009; Andrade et al., 2015; Lu et al., 2015). The existence of different dehiscence modes and diaspore types in *Cuscuta* offers a unique opportunity to investigate if such fruit traits affect the dispersal ability and geographical distribution of species, and if indehiscence provides a germination advantage under certain conditions. Thus objectives of this study are: 1) Determine the ontogeny and structural/ultrastructural mechanism of dehiscence/indehiscence modes as a means to distinguish fruit "types"; 2) Survey the morphological diversity of fruits in *Cuscuta* and reconstruct ancestral character states; discuss the usefulness of capsule characters for the systematics and taxonomy of dodders; (3) Investigate possible relationships between dehiscence/indehiscence modes and other fruit traits; (4) Analyze the geographical distribution of N American species of subg. *Grammica* species in relationship to their fruit dehiscence modes. (5) Compare the floating ability of *Cuscuta* indehiscent fruits and seeds to establish if the two diaspore types have different capabilities of dispersal by water. (6) Determine if and how the fruit pericarp affects seed germination and discuss possible advantages of indehiscence at this stage.

2. Materials and methods

2.1. Structural and ultrastructural basis of dehiscence/indehiscence modes

Anatomy of pericarp was documented in 14 species selected to represent the four currently accepted subgenera of *Cuscuta* (García et al., 2014; Costea et al., 2015): *C. monogyna*, C. *japonica* (circumscissile dehiscent; subg. *Monogynella*), *C. planiflora*, *C. approximata* (dehiscent; subg. *Cuscuta*), *C. nitida, C. africana* (irregularly dehiscent; subg. *Pachystigma*), *C. campestris* and *C. gronovii* (indehiscent), *C. chilensis, C. costaricensis*, *C. chapalana*, *C. cotijana*, and *C. purpurata* (dehiscent; subg. *Grammica*); *C. umbellata* (dehiscent and irregularly dehiscent; subg. *Grammica*). Developing and mature fruits of *C. campestris*, *C. costaricensis, C. chapalana*, *C. cotijana*, and *C. gronovii* were fixed directly in the field using 3% glutaraldehyde + 2% paraformaldehyde in 0.025 M sodium phosphate buffer at pH 6.8 while *C. monogyna*, *C. chilensis* and *C. purpurata* were grown in the greenhouse from seeds collected in Israel and Chile, respectively. Herbarium vouchers of all these plants were deposited in the WLU herbarium. These species were examined both with light and transmission electron microscopy (TEM). Species for which fresh/fixed material was not available (*C. japonica*, *C. planiflora*, *C. approximata, C. nitida*, *C. africana*, C. *umbellata*) were obtained from herbarium specimens and their pericarp anatomy analyzed only with light microscopy using the protocol for rehydration developed by Wright et al. (2011). Ten fruits were examined for each species. Samples were embedded using a modified Spurr's Resin protocol (Riviere et al., 2013). For light microscopy, Spurr blocks were sectioned at 2 μm with a Sorvall MT-1 ultra-microtome and stained with toluidine blue O (pH 4.4) for 2 min. Observation and imaging was conducted on Nikon Eclipse 50i brightfield and Nikon Eclipse E600 epifluorescence microscopes using a PaxCam digital arc camera and Pax-it 7.8 software. For transmission electron microscopy (TEM), blocks were cut with a diamond ultra-knife at 80–100 nm and mounted onto formvar and carbon-coated copper grids which were then post-stained with 5% uranyl acetate for 10 min, and then stained with Reynolds lead citrate for 5 min. Observations and images were taken with a Gatan Ultrascan digital camera and 'Digital Micrograph' software on a JEOL 2011 Transmission Electron Microscope at 200 kv (Gatan Inc. 2007, Pleasanton, CA). Herbarium vouchers used for the anatomical study are marked with an "*" in the online supplemental appendix.

2.2. Comparative morphology of fruit in Cuscuta

The morphology of mature fruits was examined in 126 taxa (118 species and 8 varieties) using ca. 400 herbarium specimens (online supplemental appendix). Mature fruits were considered those containing mature seeds. Dried fruits removed from herbarium specimens were placed in 50% ethanol, heated to boiling point and allowed to rehydrate for several minutes. Ten fruits per specimen were examined with a Nikon SMZ1500 stereomicroscope and imaged with a PaxCam Arc digital camera equipped with Pax-it 12 software (MIS Inc., Villa Park, IL). To determine thickening of endocarp cells in a large number