Cuscuta, the Merchant of Proteins

Andrea Paterlini [1,*], Ykä Helariutta [1,2]

[1] The Sainsbury Laboratory, University of Cambridge, Cambridge, United Kingdom
[2] Helsinki Institute of Life Science, University of Helsinki, Helsinki, Finland

* Correspondence: Andrea Paterlini (andrea.paterlini@slcu.cam.ac.uk)

"If thou wilt lend this money, lend it not
As to thy friends, […]
But lend it rather to thine enemy,
Who, if he break, thou mayst with better face
Exact the penalty"

The merchant Antonio speaking to the moneylender Shylock

The Merchant of Venice, Act 1 Scene 3 – *William Shakespeare*

Parasitic plants, such as the obligate ones of the *Cuscuta* genus (commonly known as dodders), form intimate connections with their hosts via specialised structures called haustoria. Using these interfaces, dodders draw water and nutrients to sustain themselves (Clarke *et al.*, 2019). While the hosts might be unwilling lenders of resources (very much as the character Shylock in the play *The Merchant of Venice* by W. Shakespeare), it seems they require in exchange their own "pound of flesh" from the parasite (a role comparable to Antonio, the merchant). Examples of dodders to host transfers have indeed been reported (Shahid *et al.*, 2018; Kim *et al.*, 2014), albeit - similarly to the play - not necessarily to the advantage of the host.

The study from Liu *et al.,* (2019) shows a new facet of this process by focusing on proteins (Figure 1). While transfer was known for a few (Jiang *et al.*, 2013; Haupt *et al.*, 2001) this had not been comprehensively assessed. The authors showed that around 30% of the repertoire of proteins expressed in the stems of the hosts (*Arabidopsis thaliana* and *Glycine max*) could be detected in the parasite (*C.australis*). At the same time, remarkably, about 20% of the parasite protein repertoire could be detected in the host. This exchange of 600-1500 proteins is clearly bi-directional and sizeable (foreign proteins accounted for about 10% of the proteome in the receiving partner). The types of transferred proteins ranged broadly and while enrichments for functions were detected, it will take more work to see if such categories are truly meaningful. The foreign proteins could also be stored in the seeds of the receiver (about 5% of the seed proteomes) or be transferred across a dodder between two plant hosts (15% of the host proteomes were mobilised and represented 8% of the proteins in the receiving partner).

To determine if proteins were *bona fide* transferred, the authors analysed transcript abundances. Foreign transcripts in either direction accounted to less or around 1% of transcriptomes in receiving partners, similar to what reported in Kim *et al.*, (2014) when using *C.pentagona* and Arabidopsis or tomato hosts. More relevant for the argument of the authors, detected instances of mRNA and protein co-transfer were very low for mobilised host factors and unlikely to argue for translation in dodder tissue. However, for factors moved from dodder to host, likelihood might be higher with larger mRNA-protein overlaps (a few hundred cases). A notable difference with the Kim *et al.*, 2014 paper is the extent of different mRNAs being transferred. There, almost ten thousands unique transcripts were mobilised from Arabidopsis to *Cuscuta*, conversely, in Liu *et al.*, (2019) they are in the order of

hundreds. The use of a different parasitic plant species might be the explanation. In Thieme *et al.*, (2015) the mobile transcript number reduced to few thousands using *C.reflexa* and in Kim *et al.,* (2014) to few hundreds using tomato as a host. Combined efforts from the groups in resolving these discrepancies might be informative considered the relevance this has for *bona fide* protein movement or foreign translation.

Trying to generalise the results obtained in their specific dodder-host case, Liu *et al.*, (2019) bring good support to a non-specific model of long distance protein transport, largely defined by the mass and the abundance of the proteins. The molecular mass of most of the dodder and plant mobile proteins in Liu *et al.*, (2019) tapered off after 70kDa, highlighting that proteins with small masses are more likely to move. This cut-off was similarly detected in Paultre *et al.*, (2016). Larger mobile proteins were still detected in both studies and these might be experiencing specific mechanisms of transport (including possibly gating of plasmodesmata, the small channels connecting plant cells). From a cell-cell movement standpoint of view, this protein subset is highly interesting. At the same time, deviating from this argument that smaller proteins are on average more likely to move, Liu *et al.*, 2019 shows that mobile proteins in most instances seemed several kDa larger on average than native ones. Whether this is an effect of the 20% subset of large mobile proteins they detected or it represents a general trait of significance in mobile proteins remains to be established and represents an interesting conundrum. In Paultre *et al.*, 2016 most organelle targeting sequences (with the exception of those for ER and Golgi) were insufficient in preventing proteins from being lost to the translocation stream. The findings in Liu *et al.*, (2019) agree with this but also point out that not even ER/Golgi targeting might be sufficient (4% of detected mobile proteins). However, the percentage remains small relative to those from other compartments (about 30% for chloroplasts, 25% nucleus and 10% mitochondria). Mobile proteins seemed to be more abundant in all of their native tissue relative non-mobile proteins. This represents a nice addition to a similar conceptual result obtained by Calderwood *et al.*, (2016), when looking at the mRNA transcription level of mobile proteins in native tissue. Lastly, no obvious sequence motifs were identified in mobile proteins relative to nonmobile, arguing for a default mechanism of protein transport or at least for a non-univocal signal. There are, however, clear examples in the literature where a signal/feature driven mobility seems to be the case (latest one being in Yang *et al.*, 2019 for mRNAs).

In presence of such likely default protein stream mechanism, it will be of prime importance to see if, at least in some cases, the detected mobile proteins execute evolutionary intended biological functions in the recipient. Transfer of miRNA from dodders is for instance used to silence defences in the hosts (Shahid *et al.*, 2018). Liu *et al.*, (2019) showed that transported proteins, both of transgenic nature or native, retain function in the recipient partner. Seeds from dodders parasitizing EPSPS plants for instance acquired resistance to glyphosate during germination. Transfer of an AOS protein (related to jasmonic acid biosynthesis) from soybean, across the dodder, to the corresponding *dde2-2* Arabidopsis mutant rescued the male infertility of the latter. While this is described as a rare event, further studies might surprisingly turn dodders into potential laboratory tools to deal with some sterile genotypes.

However, these results do not really clarify if there is an intended evolutionary gain at play or these are by-products of the default protein transport hypothesis. Are the proteins imported from the host being used as N sources in the dodder? Do they compensate for gene functions missing in the parasite, which has undergone extensive gene loss (Sun *et al.*, 2018)? Alternatively, does the plant fight back by taking advantage of this stream of proteins? In the opposite direction, are proteins specifically mobile from parasite to host required for the parasitism process itself?

Overall, the paper by Liu *et al., (*2019) is a valuable addition to our knowledge of long distance transport by providing both reference datasets to study parasitic processes and by adding evidence to protein transport models. What it represents for the single host and parasite is somewhat intertwined with Shylock's destiny in the play by William Shakespeare. With the boundaries of their identities being challenged by living and exchanging in such close quarters, will we still be able to determine who's who in the partnership? Arabidopsis certainly hopes not to experience the same fate of the moneylender and have to forego its identity at the demand of the merchant and his community.

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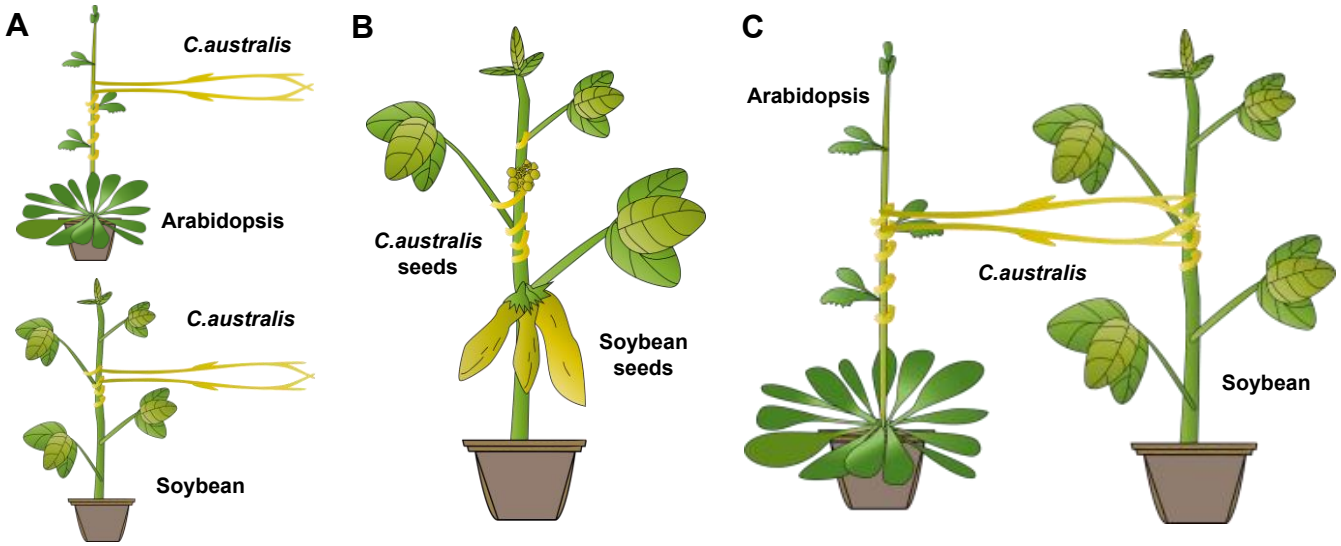
Figure legend

Figure 1: Cases of protein transfer being studied in Liu et al., 2019. A) The parasitic plant *C.australis* parasitizing Arabidopsis or Soybean plants. Bi-directional transfer of proteins occurs in the pairs. B) *C.australis* parasitizing soybean, at seed setting stage for both. Foreign proteins get stored in the seeds of either plant. C) Soybean and Arabidopsis bridged by *C.australis*. Proteins are exchanged between the two hosts via the parasite. Adapted from Figures 1, 3 and 4 from Liu *et al.*, 2019.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