

# Strong potential for endozoochory by waterfowl in a rare, ephemeral wetland plant species, *Astragalus contortuplicatus* (Fabaceae)

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## Abstract

It was proposed previously that passive dispersal by migratory aquatic birds explain the widespread distribution of many wetland organisms. Several experimental studies have shown that many widespread wetland plant species can be readily dispersed within the guts of Anatidae. However, it is unclear whether plants with a more restricted distribution are able to disperse via waterbirds. This paper addresses the dispersal ability and germination ecology of the little-known Hungarian milkvetch *Astragalus contortuplicatus*, which occurs on banks of continental rivers and has a limited and unpredictable distribution. To test whether limited capacity for endozoochory by waterfowl could explain the sporadic appearance of this species, we force-fed ten captive mallards (*Anas platyrhynchos*) with 100 milkvetch seeds each. Droppings were collected for up to 45 h after feeding. Intact and viable seeds were found in the droppings of each mallard, and altogether 24.7% of seeds fed were recovered intact. The proportion of retrieved seeds that germinated (27.0%) was significantly higher than that of untreated control seeds (0.5%), but significantly lower than that of mechanically scarified seeds (96.0%). Retrieved seeds that germinated developed into healthy mature plants. Given the average flight velocity of mallards, seeds of *A. contortuplicatus* may travel up to 1600 km inside the digestive tract of migrating individuals. Our results suggest that avian vectors may be more important for the dispersal of rare higher plants (especially those with a hard seed-coat) than hitherto considered. Moreover, they suggest that rarity does not necessarily indicate limited dispersal ability, and may instead be explained by specific habitat requirements.

**Keywords:** *Anas platyrhynchos*; germination experiment; mallard; ornithochory; seed dispersal; seed ecology; seed viability

## Introduction

Waterbirds play an important role in the dispersal of the propagules of wetland plant and animal species [1,2]. Seeds and fruits of vascular plants [3–5], eggs of aquatic invertebrates [6–9], and algal spores and cysts [10,11] can all be disseminated by birds. Such dispersion with the help of animal vectors is called zoochory, of which there are two main types. Epizoochory implies the external transportation of propagules, for instance on the feathers, beaks or feet of birds. Endozoochory, on the other hand, is considered to be much frequent [12], and implies the internal transport of propagules within the digestive tract of animals. Zoochorous seed dispersal has already been recognized by Darwin [13], but we still lack some important information on its functioning in ecosystems.

The members of family Anatidae (ducks, geese and swans) play an especially important role in the dispersal of plants lacking a fleshy fruit [2]. In a recent review [14] propagules of 223 plant species were recorded within the digestive tract and in the faeces of four dabbling duck species (*Anas* spp.) in Europe. The majority of these plant species are common with broad distributions covering a wide latitudinal range across migratory flyways [14]. The potential for zoochory of plant species having a sporadic distribution remains largely unexplored, and it is unclear to what extent a limited distribution can indicate a poor capacity for endozoochory. The Hungarian milkvetch *Astragalus contortuplicatus* is a rare and endangered plant species in Hungary [15], and its occurrence is sporadic and ephemeral there and elsewhere in its range [16].

In this study, we aim to investigate whether limited capacity for endozoochorous dispersal by waterfowl could explain the sporadic distribution of this rare plant species. We ask whether the seeds of *A. contortuplicatus* can survive passage through the guts of migratory waterfowl, whether

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gut passage changes the germination response of intact seeds, and whether the effects of gut passage on germination can be simulated by mechanical scarification.

## Material and methods

Hungarian milkvetch (*Astragalus contortuplicatus* L., Fabaceae) is an annual, ephemeral plant species, occurring sporadically in salt meadows, river banks and semideserts [16]. The Central European distribution of *A. contortuplicatus* is restricted to periodically flooded habitats like river floodplains (mainly of the River Tisza and of its tributaries; Fig. 1). It has a disjunctive distribution range across continental Eurasia (from China to Central Europe; Fig. 1a [17]), ranging from latitudes of 26.1–57.7° N and longitudes of 16.1–94.5° E. It also has a very limited distribution as an alien plant in Massachusetts, USA [18]. Between 1849 and 2013 there were only 24 years when it was recorded in Hungary, with a total number of 64 records (Tab. S1, Fig. 1b). The irregular temporal pattern of its emergence may be related to long-term persistence of its seeds and to physical dormancy caused by the hard, water-impermeable seed coat [19]. Seeds are greenish brown and have a flattened shape [dimensions (mean  $\pm$ SD,  $n = 50$ ): 1.00  $\pm$ 0.06  $\times$  0.82  $\pm$ 0.05  $\times$  0.51  $\pm$ 0.04 mm; Fig. 2a]. The mass of a thousand seeds is 0.3033 g according to Török et al. [20] and 0.331  $\pm$ 0.027 g based on our measurements (3 replicates). Seeds used in this study were collected near Tiszaroff (Hungary, N 47.4° E 20.4°) in September 2012.

For our study, we used the mallard (*Anas platyrhynchos* Linnaeus, Anatidae), a widely distributed, cosmopolitan generalist, which is the most common duck species in Europe [21]. It is not threatened and is a game species in Hungary. The migratory behavior of this species is indicated by Hungarian bird ringing data [21], with individual displacements of up to 2304 km (Russia–Hungary).

Ten adult mallard individuals (seven females and three males, 1.5-years-old) were used (Tab. 1). They were born in captivity, kept in outdoor facilities and fed with mixed grains (corn, wheat and oat) and fresh herbaceous plants

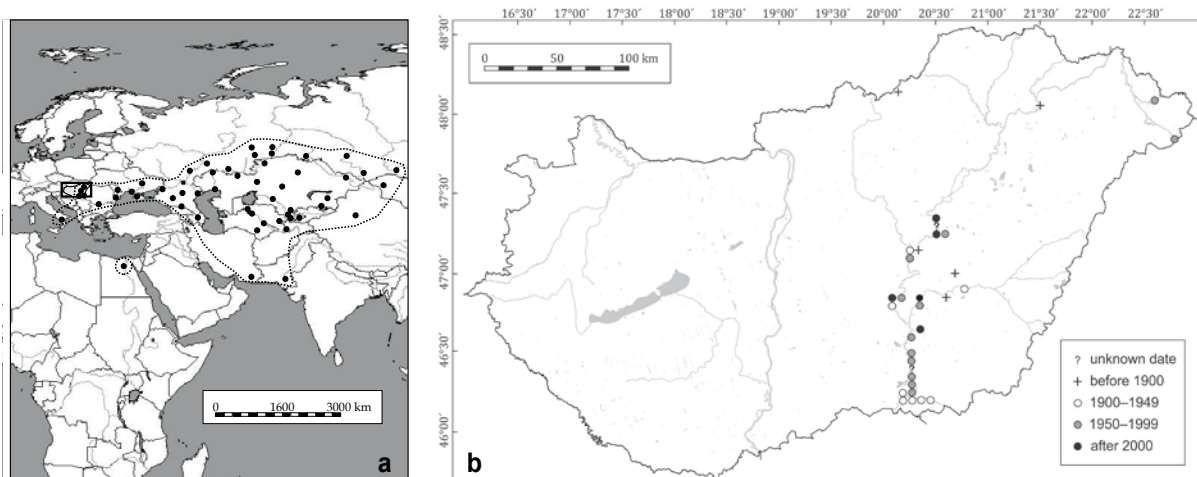
(e.g., *Stellaria media*, *Taraxacum* sect. *Ruderalia*). For the experiment (from 22nd to 24th October 2012) they were housed in separate cages of 50  $\times$  50  $\times$  50 cm. A plastic foil was placed under each cage to collect faecal samples. Food, but not water was withdrawn from each individual 24 hours prior to feeding, in order to empty their digestive tracts. Then, 100 seeds of *A. contortuplicatus* were force-fed to each bird. To simulate long distance flight periods during migration, no more food was given to them until the end of the experiment, although water was provided. Droppings were collected from the foils underneath the cages every hour for the first seven hours, as well as 21 and 45 hours after feeding. All experimental ducks remained healthy and were returned to the outdoor facilities for future experiments.

Samples were air-dried at room temperature and subsequently scanned under a light microscope. Apparently intact seeds were collected from the samples and were stored at 5°C for 70 days. Seeds used in control germination tests were stored under identical conditions. Viability of the seeds was tested with germination tests in Petri-dishes at 22  $\pm$ 2°C daytime and 18  $\pm$ 2°C night-time temperature, with a 14 h photoperiod (30  $\mu$ mol m<sup>-2</sup> sec<sup>-1</sup> light intensity). A 1% agarose gel was used as a substrate for germination-tests for a 50 day period (Fig. 2). Furthermore, two control germination tests were conducted: (i) 3  $\times$  100 seeds without any treatment, and (ii) 3  $\times$  50 seeds mechanically scarified, following the method of Patané and Gresta [22], using Bosch red, Woodeco P60 120/1305 sandpaper.

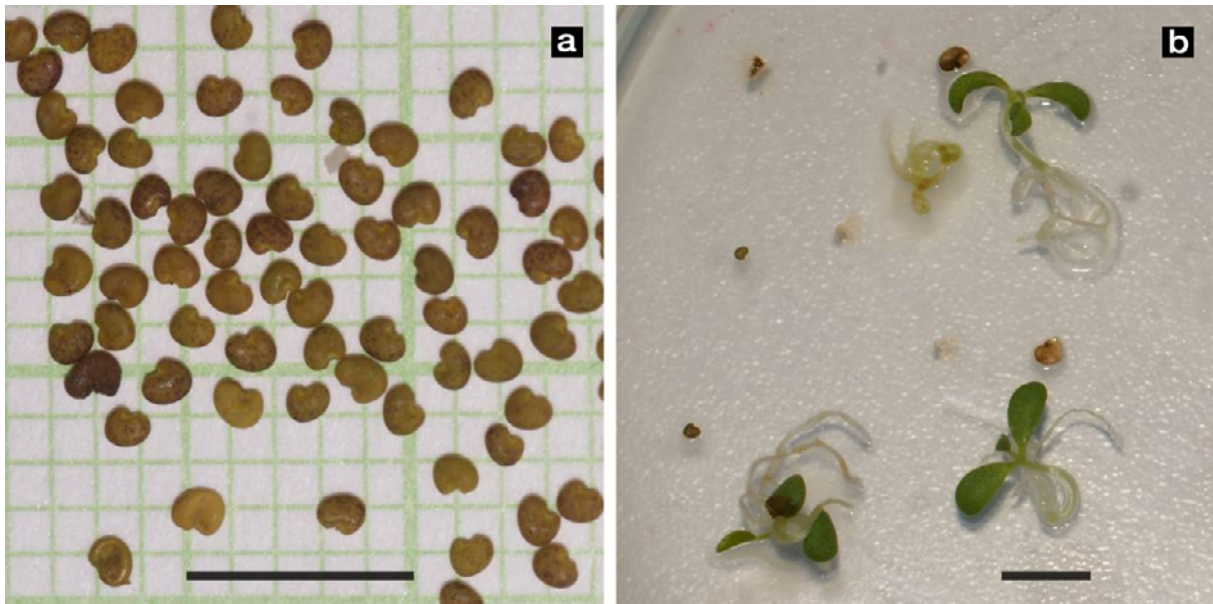
All statistical analyses were performed in R statistical programming environment, version 3.1.2 [23]. Two-sample *t*-tests were used to compare the germination of retrieved seeds with the germination of control and scarified seeds, and to compare seed passage and germination differences between sexes. All statistical tests were two-sided, with 95% confidence intervals.

## Results

Seeds of *A. contortuplicatus* were found in the faeces of each of the 10 mallards, but the number of intact seeds



**Fig. 1** a Native distribution range of *Astragalus contortuplicatus* based on dataset of Roskov et al. [17]. b Known occurrences in Hungary (see also Tab. S1).



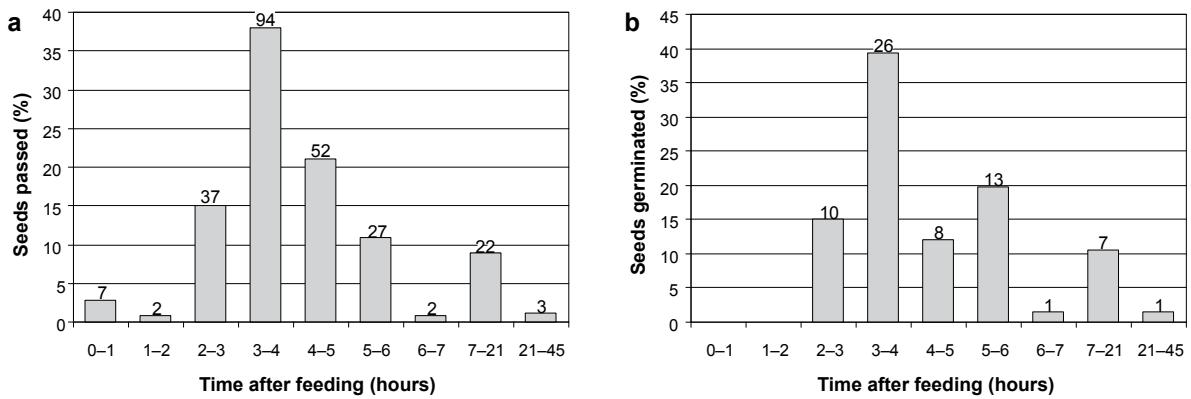
**Fig. 2** a Intact seeds of *A. contortuplicatus*. b Seedlings on 1% agarose gel developed from seeds retrieved from mallard faeces. Scale bars represent 5 mm. Images: A. Molnár V.

**Tab. 1** Temporal pattern and individual variation of seed passage and germination percent of passed seeds.

Duck No. (gender)	Number of seeds retrieved				Number of seeds germinated				Germinability
	1-7 h	7-21 h	21-45 h	Total	1-7 h	7-21 h	21-45 h	Total	
1. (♀)	7	4	1	12	3	1	0	4	33.3%
2. (♀)	4	0	0	4	1	0	0	1	25.0%
3. (♂)	14	2	0	16	3	3	0	6	37.5%
4. (♀)	47	6	0	53	6	2	0	8	15.1%
5. (♀)	42	1	0	43	12	0	0	12	27.9%
6. (♀)	35	3	0	38	20	0	0	20	52.6%
7. (♀)	44	5	2	51	6	0	1	7	13.7%
8. (♂)	6	1	0	7	2	1	0	3	42.9%
9. (♀)	15	0	0	15	3	0	0	3	20.0%
10. (♂)	8	0	0	8	2	0	0	2	25.0%
Mean	22.2	2.2	0.3	24.7	5.8	0.7	0.1	6.6	29.3%
SD	17.6	2.2	0.7	19.3	6.2	1.1	0.3	5.7	12.4%

retrieved varied highly among individuals (median  $\pm$ SD = 15.5  $\pm$ 19.3, range: 4–53). In total, 24.7% of all seeds fed (247 out of 1000) were retrieved apparently intact. Of the retrieved seeds, 89.9% passed through the digestive tracts of mallards within 7 hours of feeding (Fig. 3). In the first 7 hours, intact seeds of *A. contortuplicatus* were recorded in the faeces of all mallard individuals. Between 7 and 21 hours after feeding, seeds were found in the faeces of seven individuals, and between 21–45 hours after feeding they were found in only two individuals (Tab. 1).

From the total of 247 seeds retrieved, 66 (26.7%) germinated. Each mallard passed at least one germinable seed, but the number of germinable seeds varied highly among individuals (median  $\pm$ SD = 5.0  $\pm$ 5.7, range: 1–20; Tab. 1). The highest proportion (39.4%) of germinable seeds was found in samples collected between 3 and 4 hours after feeding (Fig. 3). The probability of germination did not vary with retention time (Fig. 3). In a linear regression, there was no significant relation between germinability and retention time ( $r = 0.1184$ ,  $P = 0.3645$ ).



**Fig. 3** a Relative frequency of seeds passed through intestinal tract of mallards. b Relative frequency of germinated seeds passed through intestinal tract of mallards. The numbers above the bars indicate the total number of seeds at each time interval.

The proportion of retrieved seeds that germinated was significantly higher ( $t = 7.20$ ,  $df = 9.49$ ,  $P < 0.0001$ ) than that of untreated control seeds (0.5%), but was significantly lower ( $t = -16.44$ ,  $df = 10.57$ ,  $P < 0.0001$ ; Tab. 2) than that of artificially scarified seeds (96.0%). There were considerable differences among mallard individuals in both the ratio of seeds passed and the germination percent of retrieved seeds. Sex of the mallard did not affect passage ratio (two-sample  $t$ -test,  $t = -1.63$ ,  $df = 3$ ,  $P = 0.2017$ ). Seeds passed through the digestive tracts of male mallards showed higher germinability than seeds passed through females, but the difference was not significant ( $t = 2.53$ ,  $df = 3$ ,  $P = 0.0855$ ). Note however, that the number of males and females was both limited and their ratio unbalanced, therefore our tests for sex differences are weak.

**Tab. 2** Germinability of control and scarified seeds.

	Number of seeds	Number of seeds germinated	Germination rate
1st control	100	2	2%
2nd control	100	0	0%
3rd control	100	0	0%
Control total	300	2	0.67%
1st scarified	50	48	96%
2nd scarified	50	48	96%
3rd scarified	50	50	100%
Scarified total	150	146	97.3%

*Astragalus contortuplicatus* seeds that were retrieved from mallards and germinated afterwards developed into healthy mature plants, which flowered richly and developed fruits. Voucher specimens of cultivated mature plants (Fig. S1) were deposited in the herbaria of University of Debrecen (Debrecen, DE) and Hungarian Natural History Museum (Budapest, BP).

## Discussion and conclusions

Our study demonstrates that the rare milkvetch, *A. contortuplicatus* has a high potential for endozoochory by Anatidae. A quarter of seeds ingested by ducks were retrieved intact, and of these more than a quarter retained their germinability. Note that we used resting animals, and due to the reduced efficiency of digestion in active birds, significantly more seeds can retain their germinability after passing through the digestive tract when the birds are active [24]. Some of the seeds were retained in the digestive system for more than 21 hours. The mallard has a maximum flight speed of around 78 km/h [25]. Thus, seeds retained for more than 21 hours in their digestive system can potentially travel as far as 1600 km inside the digestive tract of migrating birds. Our experiment ended at 45 h, and similar experiments conducted with other plant species suggest it is possible that some seeds were retained for 72 h or more [5]. Endozoochory is considered to be a major dispersal mode for the Fabaceae in Europe [26]. Species known to be ingested by dabbling ducks in the field in Europe include *Trifolium campestre*, *T. pratense* and *Lotus uliginosus* [27,28], *Medicago sativa* [29] and *M. lupulina* [14]. *Medicago polymorpha* is also dispersed by Anatidae in its introduced range in Australia [30].

According to our results, rarity of a wetland-associated plant species is not necessarily an indication of dispersal limitation. The distribution of *A. contortuplicatus* in Hungary (Fig. 1b) could easily be interpreted as evidence that the species is strongly dependent on dispersal via water (hydrochory), with a poor ability to disperse between hydrological catchments. Its air-filled, inflated ripe legumes float on the surface of water. However, our results indicate that the species has a high potential for long-distance dispersal of seeds by waterfowl between catchments, which is likely to explain its sporadic presence in different catchments. This supports Ali [31], who suggested that dispersal by migratory birds explains the “rather abnormal” distribution pattern of *A. contortuplicatus* in Pakistan.

Mechanical scarification of the seed coat is known to increase germination in hard seed-coated species, including Fabaceae species [22,32–35]. Likewise, for *A. contortuplicatus*,

artificially scarified seeds had higher germination percent than untreated seeds. A high proportion (24.2%) of *A. contortuplicatus* seeds were able to germinate following artificial mechanical scarification after 131 years of dry storage [19]. Our results suggest two physical dormancy-breaking mechanisms are likely to operate in nature. First, the hard seed coat of *A. contortuplicatus* may be degraded by abrasion caused by alluvial sediment during floods. Second, the seed coat may be damaged by the mechanical effect of grit and/or the chemical effect of digestive enzymes within the gizzard of waterfowl that ingest the seeds.

We chose not to provide mallards with additional food during the course of our experiment, and this may have changed the retention time of seeds compared to ducks provided with ad libitum food [36]. As in similar experimental studies with ducks [4,5], we observed considerable variation between individuals in rates of seed survival and germination. Factors such as variation in the amount, size

and quality of gizzard grit, body condition and the quality and quantity of food previously consumed may contribute to individual differences in both passage rate and germinability of passed seeds.

This study demonstrates that waterfowl can disperse not only widely distributed, common plants (such as most of those listed by Brochet et al. [14]), but rare plants with a limited distribution, such as *A. contortuplicatus* also has a high potential to be dispersed by them. For many such plant species, specific habitat requirements are more likely to explain rarity than dispersal limitation. A limited ecological niche for this species is also suggested by its failure to expand within North America since its introduction into Massachusetts in wool waste in the early 20th century [18]. Many studies of plant dispersal in the European flora (e.g., [37]) ignore the role of non-frugivorous birds such as ducks, and further research into the importance of these birds as seed vectors remains a priority.

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### Authors' contributions

The following declarations about authors' contributions to the research have been made: designed and performed the experiment: ÁLK, AMV; analyzed data: OV, JS; prepared figures and tables: AMV, AT; wrote the manuscript: JS, AJG, AMV, OV, ÁLK, AT. All authors reviewed the drafts of the paper.

### Competing interests

No competing interests have been declared.

### Supplementary material

The following supplementary material for this article is available online at <http://pbsociety.org.pl/journals/index.php/asbp/rt/suppFiles/asbp.2015.030/0>:

1. Tab. S1: occurrences of *Astragalus contortuplicatus* in Hungary.
2. Fig. S1: flowering and fruiting individuals of *A. contortuplicatus* cultivated from seeds passed through the digestive tract of mallards.

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