Experimental Endozoochory of *Cannabis sativa* Achenes

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**Abstract**

The mechanism by which *Cannabis sativa* dispersed from its center of origin remains an open question. The literature provides many hypotheses, which we review for the first time, but experiments are few. Darwin was interested in zoochory – the transport of plants by animals. He demonstrated endozoochory (transport of seeds via animal digestive systems) of *C. sativa* achenes (seeds) by carrier pigeons, but he did not quantify achene survival rates. We assessed mammalian endozoochory in a triplicate experiment: feeding *C. sativa* achenes into a simulated gastrointestinal system, a dog, and a human. The in vitro system subjected achenes to sequential digestive enzymes. Achenes were planted in potting soil and monitored for emergence under growroom conditions. The in vivo experiments added achenes to a normal morning meal (dog food or granola). Feces were collected for daily instillation into an outdoor garden and monitored for seedling emergence for 16 days. Control achenes were planted directly into soil without ingestion. In the in vitro study, 34.7% of the digested achenes emerged as seedlings. The in vivo emergence rates were 10.3, 1.3, and 76.0% for the dog, human, and control conditions. The three groups differed significantly ($\chi^2 = 1,264.93$, $p < 0.0001$). Achene survival was greatest under in vitro conditions, which lacked a mastication step, compared to dog (minimal chewing) and human (maximal chewing) conditions. Although *C. sativa* lacks evolutionary traits for classic endozoochory (i.e., a fleshy fruit), it seems well adapted to this manner of seed dispersal.

**Introduction**

Understanding the origin of medicinal plants and their ecological selection pressures may offer insights into their evolution of secondary metabolites. *Cannabis* and her sister genus *Humulus* diverged 27.8 million years ago [1]. *Cannabis sativa* has a center of origin in Central Asia [2], or more specifically the northeastern Tibetan plateau [3]. A meta-analysis of fossil pollen studies suggests *C. sativa* had dispersed to Europe by 1.8 million years ago [4]. The European distribution of *C. sativa* expanded and contracted with glacial cycles, like that of many plants.
ing interstadials (warmer, wetter periods, like our present time), *C. sativa* pollen was limited to “refugia” – steppe landscapes that persisted in otherwise forested Europe.

Conventional wisdom states that differences between *C. sativa* subsp. *sativa* (in Europe) and *C. sativa* subsp. *indica* (in Asia) are due to human selection, and therefore they are not “natural” segregates. However, European *C. sativa* likely went through repeated genetic bottlenecks during interstadials, when the population shrank to small numbers during range contractions. Small populations experience genetic drift, where new genotypes arise randomly. Thus, differences between European and Asian *C. sativa* began with vicariance and genetic drift, and not human selection [3].

The species’ migration velocity during glacial cycles is suggestive of rapid biological dispersal. Biological dispersal refers to the movement of individuals away from the population into which they were born. Dispersal has consequences for individual fitness, gene flow, population genetics, and species distribution. Plants rely on passive transport of diaspores (e.g., seeds), by vectors such as wind (anemochory), water (hydrochory), and animals (zoochory). Animals transport seeds via their digestive systems (endozoochory) or via seeds externally attached to their bodies (epizoochory).

Ever since Darwin [5], biologists have studied the roles of zoochory in the biological dispersal of plants. Darwin focused upon long-distance dispersal (LDD) to distant oceanic islands, vectored by birds, but LDD has been defined to include distances as little as 100 m, with roles played by terrestrial mammals [6]. Plants adapted for endozoochory classically have seeds embedded in the fleshy pulp of an edible fruit or berry. The fruit provides a nutritional reward to the disperser.

Plants with dry nuts or achenes, such as *C. sativa*, have been considered “unspecialized” because they lack classic adaptations for dispersal [7]. Nevertheless, Ridley [8] collated empirical evidence of endozoochory amongst plants with dry nuts or achenes. His examples included many agricultural weeds that passed through cattle into excreta: “we can realise at once how very many small herbaceous plants with small dry fruits and seeds in capsules are so widely spread” [8]. Seeds excreted in a germinable state explain why agricultural fields manured with cattle dung may acquire mass infestations of weed species [9].

In plants adapted for classic endozoochory, the fruit pulp often contains germination inhibitors. Animals’ digestive processes, both mechanical and chemical, strip away the pulp from the seeds. Thus, in classic endozoochory, excreted seeds often show higher percentages of germination, and/or accelerated emergence, compared to noningested seeds [9, 10]. This is not the case with dry nuts or achenes, which usually show reduced germination rates compared to noningested seeds [8–11].

**Cannabis Dispersal Mechanisms**

The dispersal mechanism of *C. sativa* and its “unspecialized” achene has generated many anecdotes and observations but few experimental studies. Moravcová et al. [12] included *Cannabis ruderalis* in an experimental study of dispersal mechanisms of 93 invasive plants. Janischevsky [13] coined *C. ruderalis* (along with the alternative rank *C. sativa var. ruderalis*) for the wild-type phenotype of *Cannabis*. Wild-type achene characteristics include a small size, a prominent abscission zone, an elongated base, a thickened pericarp, and a camouflaged and persistent perianth (achene covering).

Ridley [8] observed that the small bracts of *Humulus japonicus* and *C. sativa* are not adapted for anemochory, whereas the larger, wing-like bracts of *H. lupulus* permit some dispersal by wind. In agreement, Moravcová et al. [12] measured the terminal velocity of seeds in a wind tunnel, and *C. ruderalis* achenes dropped third fastest out of 93 species.

Hydrochory seems likely for a plant known colloquially as “ditchweed.” Basu [14] conducted the first ecological study of ruderal *C. sativa*. He emphasized achene dispersal via water, enhanced by plants colonizing riverside ditches and mounds of alluvial soil. Cappers [15] observed water transport of feral hemp achenes, and their subsequent deposition in riverside litter. Moravcová et al. [12] measured the time it takes for 100 seeds to sink in a beaker of water. *C. ruderalis* achenes took 52 h. Only 18 out of 93 species were more buoyant.

The achenes of *C. sativa* lack classic adaptations for epizoochory, such as hooks, spines, and barbs. Moravcová et al. [12] modeled epizoochory by testing the ability of seeds to attach to the fur of wild boar (*Sus scrofa*). A piece of fur was pressed against 25 seeds scattered on a sheet of paper (replicated 4 times). Surprisingly, a mean of 60% of hemp achenes stuck to the fur – the species ranked 44th out of 93. Sanchis Serra et al. [16] found *C. sativa* achenes in the nest of an Egyptian vulture (*Neophron percnopterus*). The achenes came from carcasses the vulture carried to the nest, perhaps via epizoochory.

Janischevsky [13] observed a case of epizoochory – the fire bug, *Pyrrhocoris apterus*, carried achenes of *C. ruderalis*. Janischevsky claimed the elongated base of wild-type achenes contained special cells “rich in oily inclusions,” which he characterized as an elaiosome. This term
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was coined by Sernander (1906) to describe fleshy and edible appendages of seeds dispersed by ants. *P. apterus* allegedly sucked oil out of the elaiosome, while the rest of the seed remained intact and capable of germination. In the process of feeding, the bug carried the seed “far distances.” No one else has observed *P. apterus* feeding upon hemp achenes. Small [17] dissected a number of wildtype accessions, including plants of Russian origin, and stated: “I have not been able to perceive much basal oil cell proliferation in most achenes of wild plants.”

Darwin [5] documented endozoochory vectored by carrier pigeons (*Columba livia domestica*) flying from France to England. He actually described diploendozoochory, where endozoochory spans two trophic levels: the pigeons, upon arrival in England, were preyed upon by hawks and owls. “Some hawks and owls bolt their prey whole, and after an interval of from twelve to twenty hours, disgorge pellets, including seeds capable of germination. Some seeds of the oat, wheat, millet, canary, hemp, clover, and beet germinated after having been from twelve to twenty-one hours in the stomachs of different birds of prey.”

The literature abounds with reports of birds feeding upon hemp achenes, dating back to 544 AD in China [reviewed in 18]. Henry David Thoreau observed feral hemp “sprung up” from dispersal of “bird’s seed” in 1856 [19]. A report by Harry Anslinger, Commissioner of the Federal Bureau of Narcotics, included a photograph of feral hemp growing in Philadelphia, “the result of bird-seed dissemination” [20].

Passeriformes (perching birds) use their beaks to crack open hemp achenes, killing the prospect of seed dispersal. Seed preference experiments with various Passeriformes highlight the desirability of *C. sativa* compared to other plants [21–26]. Experiments show that hemp achenes are cracked and deshelled quite efficiently [22, 23, 27]. However, songbirds occasionally swallow hemp achenes whole without cracking them [24, 28–30]. Some species temporarily hold hemp achenes in their beaks, and scatterhoard the achenes in hiding places – documented observationally [31–33] and in experimental studies [34, 35].

Columbiformes (pigeons and doves) also show preferences for hemp achenes [36, 37]. Studies of species in two genera, *Columba* and *Streptopelia*, show they swallow hemp achenes whole [38, 39]. Seed still face a formidable barrier to survival – the gizzard – but pigeons and doves temporarily store seeds in the crop, anterior to the gizzard. Wilson and Korovin [40] counted an astounding 793 hemp achenes in the crop of an Oriental turtledove, *Streptopelia orientalis*. The species is migratory and its range spans Central Asia (Russia, Kazakhstan, northern India, and China). The genera *Columba* and *Streptopelia* diverged about 30 million years ago in Central Asia [41], so they may have coevolved with *C. sativa*.

One study quantified hemp seed survival through the avian gut. Small et al. [42] cited an unpublished typescript authored by B.J. Eaton in 1972, who studied the viability of achenes excreted in bird droppings. Eaton used the tetrazolium test, a redox indicator of metabolic activity and a marker of seed viability [43]. Bobwhite quail (*Colinus virginianus*) passed 1 viable seed per 700 achenes consumed. Game bird “doves” (*Zenaida macroura*) passed 1 viable seed per 12,400 achenes consumed.

Less is known about mammalian endozoochory. Mammals that feed on feral or cultivated hemp seed include horses, cattle, goats, deer, dik-dik, rabbits, hams ters, field voles, rats, and mice [18]. Vavilov [44] noted that ruderal hemp thrived in soil manured by grazing cattle, and in gorges and ravines where the dung of wild animals accumulated. Giles [45] identified hemp achenes in the scat of raccoons (*Procyon lotor*). Gary Snyder wrote about hemp seed in horse manure, in a poem [46]. Okulova et al. [47] found achenes in the feces of two Russian voles, *Microtus arvalis* and *M. levis*. De Barba et al. [48] extracted *Cannabis* DNA from the scat of the brown bear (*Ursus arctos*) in northern Italy. Rodents, like birds, will cache seeds in hiding places, including hemp achenes [49, 50].

No studies have quantified hemp seed survival through the mammalian gut. We conducted a feeding experiment in triplicate to test this potential. Rather than using the tetrazolium test of seed viability, or germinating achenes in sheets of filter paper, we opted for a more functional test: sow-excreted achenes in soil, and using seedling emergence as our metric of successful endozoochory. We hypothesize that mastication is the survival-limiting step in endozoochory, so we tested achenes in an in vitro digestion model that lacked mastication. Then we conducted two in vivo tests with variable amounts of mastication, using a human and a dog as test subjects.

Materials and Methods

The in vitro and in vivo parts of this study were conducted in separate jurisdictions (Vermont and Ontario), using locally permitted germplasm. This necessitated the use of two different dioecious cultivars of fiber-type hemp. The in vitro study used “FINOLA,” obtained from a commercial vendor. The in vivo studies used an unnamed Polish landrace, gifted by The Vermont Hemp Company. Achenes were visually inspected; mature-and-intact achenes were separated from chaff, green (immature) achenes, and dam-
aged achenes (necrotic, cracked along their keel, or otherwise deformed). Maturity was judged by the presence of a mottled seed coat; Haney and Kutscheid [51] determined that this corresponded to the development of a viable embryo, based on germination experiments.

For the in vitro digestion model, 10 g mature seed were fed into a simulated gastrointestinal system (DRUID; Ryerson University, Toronto, ON, Canada). Achenes were subjected to sequential digestive enzymes (pancreatin, pepsin, trypsin, chymotrypsin, peptidase, α-amylase, and lipase), bile salts, and mucin at 38.5 °C for 1 + 3 h (gastric + intestinal). Achenes were planted in sterile potting soil (N-P-K: 0.03%-0.03%-0.03%), 1 seed per cell in the planting tray to facilitate counting of emerging seedlings. Growroom conditions were kept at 20–25 °C and a relative humidity of 50–60% during 16 h of light (250 W/m²). Emergent seedlings were counted as soon as identification was possible. Seedling emergence was monitored for 16 days. Percent emergence in 72 control achenes (not subject to in vitro digestion) was compared to 72 achenes subjected to full gastrointestinal digestion.

For the in vivo studies, achenes were field planted. The germination bed consisted of a fenced 200 ft² area, part of a larger 2,000 ft² raised-bed complex, in the backyard of the principle investigator. The soil was a Vergennes series clay, amended with composted manure 4 years earlier, and previously planted with Phaseolus vulgaris, which lacks allelopathic activity. For the control plot, 500 achenes were drilled 1.5 cm deep in an 8 × 8 cm grid – a slightly greater planting density than recommended by Columella [52]. Two subjects were fed 1,000 hemp achenes each, mixed into normal-sized rations of food. The principle investigator (male, 60 years old, 175 lbs) ate achenes mixed into generic granola breakfast cereal, chewed and swallowed normally. The canine subject (Chesapeake Bay retriever, female, 0.9 years old, 50 lbs) ate achenes mixed into a bowl of dog food (Orijen Puppy Large Breed Grain-Free Dry Dog Food). All feces were collected in feces bags and refrigerated at −20 °C for 10 days. Feces were then thawed and mixed into a bowl of dog food (Orijen Puppy Large Breed Grain-Free Dry Dog Food). The canine subject was fed 1,000 achenes; after 8 days, 100 seedlings emerged (10.0%), and after 16 days the total rose to 103 seedlings (10.3%). All seedlings emerged from post-feeding day 1 feces; nothing emerged from feces excreted on post-feeding days 2–4.

The human subject was fed 1,000 achenes; after 8 days, 8 seedlings emerged (0.8%), and after 16 days the total rose to 13 seedlings (1.3%). Six seedlings emerged from day 1 feces, 7 from day 2 feces, and none emerged from post-feeding days 3 and 4. Percent emergence differed significantly between the three groups (control, canine, and human, day 16; χ² = 1,264.93, p < 0.0001). Pairwise tests of independence showed significant differences between control and dog (χ² = 650.94, p < 0.0001), between control and human (χ² = 961.97, p < 0.0001), and between dog and human (χ² = 77.28, p < 0.0001).

To compare the in vitro and in vivo results, we addressed the difference in their respective control arms. We normalized the control emergence rate to 100% and the digestion emergence rate as a percentage of the control emergence rate (in vitro: 34.7% ÷ 58.3% × 100 = 59.5%; in vivo canine: 10.3% ÷ 76.0% × 100 = 13.6%; in vivo human: 1.3% ÷ 76.0% × 100 = 1.7%). Qualitatively, seedlings from canine and human feces appeared more robust than control seedlings. Four days after emergence, a thunderstorm caused ~25% of the control seedlings to lodge, whereas none of the feces-feritized seedlings fell over (Fig. 1). However, any visual differences between the test groups disappeared within 3 weeks of emergence.

Discussion

This study demonstrated C. sativa endozoochory vectored by mammals. The canine’s feces were collected up to 400 m from the feeding site, which qualifies as LDD, defined as ≥ 100 m [6]. Mastication appears to be the primary impediment to seed survival. Seedling emergence was greatest after in vitro digestion, which lacked mastication. The canine subject “wolfed” her food with minimal chewing, compared to the human subject, with a commensurate difference in seedling emergence. Digestive acids and enzymes did contribute to viability loss, however.

This study has several limitations. The in vitro and in vivo arms of the study, conducted in different jurisdictions, utilized different seed sources. The in vivo conditions were not entirely natural. Instead of counting emergence directly from fecal stools lying upon the soil sur-
face, we distributed feces in a slurry across a 0.5 m² soil area. This facilitated the counting of emergent seedlings but reduced natural seedling competition.

In canine feces, a majority of intact achenes appeared on the rind of fecal stools. The mechanism that forced achenes to the external surface is unknown. It would enhance seedling survival, however, compared to a seed germinating in the center of a hard, desiccated stool. Similarly, achenes excreted in multi-pelleted sheep feces face better conditions than achenes excreted in feces heaps produced by cattle [53].

Newly-emerged seedlings from canine and human feces appeared more robust than control seedlings (Fig. 1). This observation supports Ridley [8], who proposed that nutrients in feces benefit seedlings arising from defecated seeds. An accelerated germination time has been reported in classic endozoochory [10]. We did not observe significant differences in emergence time between the controls and the treatment arms.

The gut transit time of viable achenes was shorter in the dog (16 h) than in the human (24–48 h). A short transit time correlates with seed survival. Cosyns et al. [11] measured mean transit times of seeds consumed by rabbits (19 h), cows (49 h), horses (55 h), sheep (58 h), and donkeys (66 h). Rabbits, with the shortest transit time, had the highest germination success. However, a short transit time also correlates with decreased LDD – a short transit time means less distance traveled.

Successful LDD depends upon many factors, but two prominent considerations are (1) the distance travelled by an animal vector and (2) the quantity of surviving seeds (quantity of consumed seeds × percent survival). Avian vectors can disperse seeds over a larger area than mammalian vectors. But large mammalian vectors (the dog and the human in this study) have the capacity to consume a larger quantity of seeds. Percent survival is also greater in mammals. In two bird species studied by Eaton [reported in 42], the mean survival rate of hemp achenes was 0.075%. Our mammalian experiments showed an 18- and 140-fold greater survival rate after passage through the human and the dog, respectively.

Some researchers argue that plants with an “unspecialized” means of dispersal (sensu Grime et al. [7]) may be adapted to endozoochory [54, 55]. Are C. sativa achenes adapted to endozoochory? They are rich in lipids and protein, thereby providing the disperser with an attractive nutritional reward. However, they are enveloped by a bract expressing Δ⁹-tetrahydrocannabinol (THC), whose psychoactivity may repel potential dispersers. THC may also repel dispersers by interfering with digestion, via antibacterial effects on the gut microflora [18]. THC is not present in abscised achenes, free of bracts and lying on the ground; these are eaten by ground-feeding birds (e.g., pigeons and doves), ungulates (deer), and rodents.

Adaptations that favor endozoochory include a small seed size and a thick pericarp. Janzen [56] argued that herbivores feeding on foliage may accidently eat seeds “sufficiently small, tough, hard and inconspicuous to escape the molar mill.” Small seeds are more likely to escape

**Fig. 1.** Visual comparison of seedlings emerging from canine feces (left) versus control seedlings (right), after a thunderstorm.
mastication as well as rumination, and they have a faster gut transit time than large-seeded species. Small seeds can be ingested in larger quantities, increasing the probability of successful endozoochory. Seed size/mass inversely correlates with seed presence in mammalian feces [54, 55, 57–65], but not always [11, 66–68].

Seeds with a thick pericarp or testa are harder (imparting some protection against mastication) and more impermeable (slowing the penetration of acids, enzymes, and bacteria). Seed coat thickness correlated with seed survival in mammalian feces [54, 55, 57–65], but not always [60, 66, 68].

Achenes of wild-type plants compared to domesticated landraces and cultivars. Achenes of wild-type *C. sativa* are smaller than domesticated varieties (2.7–3.0 mm long [44]), and they weigh as little as 2.1–2.7 mg [73]. In contrast, the achenes in our in vitro study were 5.0 mm long and weighed 26.1 mg. Domesticated *C. sativa* easily escapes cultivation and reverts to a wild-type phenotype within 50 years [17]. Small and Cronquist [74] defined the wild-type phenotype as < 3.5 mm in length. The literature suggests this size is larger than optimal for mammalian endozoochory (see Table 1 [75]).

Achenes of *C. sativa* are hard, with a thick pericarp, a character that facilitates endozoochory. Janischevsky [13] commented on the “great strength of the pericarp” of *C. ruderalis*. The pericarp along the ribs and in the basal part of the seed: “Small wild fruits have walls as thick as larger domesticated fruits, and therefore in proportion to the size of the fruit, are better protected” [74]. Van der Meij and Bout [76] measured the force required to crack a domesticated hemp achene, a mean of 12.16 N, approximately the weight of a 1.24-kg mass.

In conclusion, endozoochory of *C. sativa* achenes can be vectored by mammals, with a high survival rate. Mastication, rather than digestive enzymes, is the survival-limiting step. *C. sativa* achenes express an adaption to endozoochory – a thick pericarp – but they are larger than optimal for mammalian endozoochory. The vectors used in this experiment (dog and human) evolved after the early evolutionary history of *Cannabis*. We plan to test mammals that have a longer evolutionary history in Central Asia, such as rabbits and horses. Comparing the survival rates of wild-type versus domesticated achenes will also be informative.

### Acknowledgements

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### Statement of Ethics

All procedures involving the human participant were in accordance with the ethical standards of the University of Vermont research committee, and with the 1964 Helsinki Declaration and its later amendments. For this type of study, where the human participant also designed and performed the study, informed consent is implicit. The animal research protocol was submitted to the in-

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**Table 1. Literature specifying seed size or mass in cases of successful endozoochory in other plant species with dry nuts or achenes**

<table>
<thead>
<tr>
<th>Disperser</th>
<th>Seed size (length) or mass (weight)</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cottontail rabbit</td>
<td>1.2- to 1.4-mm seeds more likely than larger ones (2.0–2.4 mm) to germinate</td>
<td>57</td>
</tr>
<tr>
<td>Brocket deer</td>
<td>5-mm Rubiaceae seeds passed intact</td>
<td>58</td>
</tr>
<tr>
<td>Sheep</td>
<td>0.3-mg seeds recovered at twice the rate of 2.0-mg seeds</td>
<td>60</td>
</tr>
<tr>
<td>Red deer</td>
<td>5-mm seeds rarely survived gut passage; most were smaller, i.e., 0.1–1.0 mm</td>
<td>61</td>
</tr>
<tr>
<td>Saki monkey</td>
<td>Intact seeds were &lt;2 mm</td>
<td>54</td>
</tr>
<tr>
<td>Cattle and sheep</td>
<td>Seeds with a mean weight of 0.25 mg showed good dispersibility, and those &gt;1.1 mg in weight showed poor to no dispersibility</td>
<td>62</td>
</tr>
<tr>
<td>White-tailed deer</td>
<td>3- to 7-mm seeds comprised 7% of the seeds germinated from dung; 81% were &lt;1 mm</td>
<td>75</td>
</tr>
<tr>
<td>Kerama deer</td>
<td>The mean size of the germinated seeds was 1.3 mm</td>
<td>71</td>
</tr>
<tr>
<td>Red deer</td>
<td>“Large” seeds (&gt;4 mm) comprised 0.6% of the seeds in dung; 90.8% were &lt;2 mm</td>
<td>65</td>
</tr>
</tbody>
</table>
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Achenes

Disclosure Statement

The authors have declared no conflict of interest.

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