The relative importance of olfactory signaling to fly-moss spore dispersal network structure of two sympatric moss species in Newfoundland, Canada

by

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A thesis submitted to the
School of Graduate Studies
in partial fulfillment of the requirements for the degree of

Master of Science
Biology
Department of Biology
Memorial University of Newfoundland

May 2019

St. John's Newfoundland and Labrador

Abstract

In Newfoundland, *Splachnum ampullaceum* (herbivore dung scent mimic) and *S. pensylvanicum* (omnivore dung scent mimic) grow in bogs on summer moose (*Alces alces* L.) dung and via olfactory and visual deception have their spores dispersed to dung by flies. In Chapter 2, the addition of carnivore and herbivore-mimicking scent increased the number of visiting flies, and the addition of carnivore scent attracted carrion flies, and this effect was greatest for *S. pensylvanicum.* In Chapter three consecutive years of fly trapping data for both species of moss were compared using Network analyses. Results showed that both mosses attract a generalized fly fauna (lower network specialization ($H_{2\ average}$) 0.171), most of which are also associated with summer moose dung (average connectance low 0.799). Also, the fly faunas of *S. ampullaceum* and *S. pensylvanicum* did not differ from each other in all three years.

Acknowledgements

I’d like to thank Dr. Paul Marino for being an excellent guide, mentor and a friend and for being there always when I needed help throughout these experiments.

Also, I am grateful to Prof. Robert Raguso and Anne Gaskett for directing me to think about these research questions, providing feedback and sharing their data with me.

I’d like to thank Dr. Tom Chapman for mentorship, encouragement, feedback and coffee and chocolates. Those meant a lot when I needed motivation.

Nick Peddle and Emily Benteau are acknowledged here for their help in the field sites, preparing insect collections and being an awesome company in the lab. I’d like to thank Thomas Rahal and Laura Dawson for their help in database preparation, data entry process and sorting insects in the lab. I am glad that I had these young research assistants around.

To my supervisory committee, Dr. Julissa Roncal and Dr. Shawn Leroux: thanks for all the feedback on my research design till the end, and I couldn’t have done it without your help.

Finally, I like to thank my loving wife, Ilesha Sandunika for encouraging me every time I am ready to give up, being a friend in need and make me feel that I have an awesome family waiting to see me succeed.

Table of Contents

List of Tables

List of Figures

List of Appendices

Chapter 1: Introduction

The transfer of pollen among conspecifics by animal pollinators is among the most common mutualisms in nature. It is a key mutualism responsible for ecosystem functioning and played a key role in the diversification of angiosperms (Cumming, Bodin, Ernstson, & Elmqvist, 2010). The diversification of plants in relation to pollinators evolved through adaptations to guilds of pollinators, commonly known as ‘pollinator syndromes’ (Van der Pijl, 1982). Since the effective pollinator abundance varies across the landscape, floral traits and their animal pollinators range from tightly coevolved interactions to those that are facultative (Daehler, 2003). The key floral traits that vary are morphology, floral rewards floral odour and visual signals (Bosch, Martín González, Rodrigo, & Navarro, 2009; Campbell, Waser, & Melendez-Ackerman, 1997; Costanzo & Monteiro, 2007).

Angiosperms are predominantly pollinated by insects (De Bruyne & Baker, 2008). Most insects rely heavily on the sense of smell for finding mates, food (Dobson, 1987) and brood sites (Dobson, 1990). The olfactory system of many insects is capable of identifying many volatile compounds in the environment, and the olfactory organs are accordingly fine-tuned to detect a limited spectrum of relevant odors (Dobson, 1987, 1990). Although olfaction is often the primary modality of plant-insect attraction, visual and tactile signals are also exploited alone or as a part of an integrated system (Campbell et al., 1997; Lord, Huggins, Little, & Tomlinson, 2013). To accomplish efficient insect attraction, plants often display flashy, colorful and fragrant flowers and are visited by insects that will primarily try to collect as much food (pollen and/or nectar) as possible, while minimizing energy and time expenditure (Costanzo & Monteiro, 2007).

The energetic cost of providing food rewards for insect pollinators, however, has also led to the evolution of plant taxa that are frauds that manipulate the behavior of insects via visual and olfactory signals, yet provide no nutritional reward to the pollinating insects (Woodcock, Larson, Kevan, Inouye, & Lunau, 2014). There are three main types of deceptive strategies: food (Jersáková, Johnson, & Jürgens, 2009), mate (Schiestl & Peakall, 2005) and brood site (Jürgens, Wee, Shuttleworth, & Johnson, 2013). Many of these deceptive plants attract insects by chemical mimicry. In this type of deception, odors often signal the presence of a mate, prey or of a brood-site (Dafni, 1984). Such plants are considered to be "mate-deceptive", "food-deceptive" and "brood site-deceptive". Deceptive strategies are found among numerous families of angiosperms, including the families Orchidaceae, Apocynaceae, Bigoniaceae and Ranunculaceae (Urru, Stensmyr, & Hansson, 2011). However, the deceptive strategy is not restricted to angiosperms as it is also found in the Phallaceae fungi (Borg-Karlson, Englund, & Unelius, 1994; Urru et al., 2011) and in the moss family Splachnaceae (Marino, Raguso, & Goffinet, 2009). In both the Phallaceae fungi and Splachnaceae mosses, insects disperse their propagules (spores).

Among the Splachnaceae mosses, nearly half of the species of this globally distributed family have evolved brood-site deception as a strategy of spore dispersal. Species of Splachnaceae exhibiting this strategy of spore dispersal share two key ecological traits: they have gametophytes that are 'coprophilous'; growing on feces and occasionally on carrion and other animal matter, and their small, sticky spores are 'myophilous,' i.e., dispersed by flies (Cameron & Troilo, 1982; Koponen & Koponen, 1977; Marino, 1991b, 2014; Marino & Raguso, n.d.; Marino et al., 2009). The sporophytes of myophilous Splachnaceae are, in most species, elongated and all entomophilous species have an often-colored (e.g., yellow, magenta, brownish red, white) inflated sterile region (hypophysis) below the sporangium. Furthermore, myophilous species produce small, thin-walled, sticky spores that are extruded, as a highly visible yellow-green mass, by a false columella as the capsule walls shrink

(Demidova & Filin, 1994). This can be comparable to the visual display of Cypripedium orchid’s flowers that present their pollen as sticky yellowish masses. However, unlike many carrion or dung mimicking flowers, sporophytes of these myophilous Splachnaceae do not entrap flies; visiting flies simply depart with the sticky spores adhering to their bodies and fly to appropriate substrates, upon which spores are dislodged and germinate into protonema (early gametophytic developmental stage).

This study focuses on two species of Splachnaceae, *Splachnum ampullaceum* Hedwig and *S. pensylvanicum* (Bridel) Grout ex H. A. Crum. Both these species coexist on the summer dung of moose (*Alces alces* L.) in peatlands on the island of Newfoundland, Canada. *S. ampullaceum* is a circumboreal species and Newfoundland, being predominantly boreal, is well within its geographic distribution. However, the distribution of *S. pensylvanicum* is more southerly, ranging from the Southeastern U.S.A. northward, east of the Appalachian mountains, to the Northeastern U.S.A. and Atlantic Canada (Marino, 1988). Newfoundland is at the extreme northern limit of its distribution. Both *S. ampullaceum* and *S. pensylvanicum* can be found growing either in single-species populations on individual droppings or in mixed-species populations on the same dropping. Summer moose dung is colonized by either or both species from late spring through summer. Dung is most attractive to flies when it is one or two days old, with rapidly decreasing visitation thereafter (Marino, 1991b), consequently, spore dispersal is directed to fresh dung. Dung is an ephemeral resource that is overgrown by the surrounding bryophyte vegetation within 1-2 years in the moist bog and fen habitats in which most boreal entomophilous *Splachnum* species grow. Because of that spore germination and subsequent gametophye growth is rapid (Marino, 1988). In the year following colonization by spores, the colonized droppings become covered with leafy gametophyte, and in the third growing season, sporophytes grow and mature, producing spores. Thereafter, in most cases, the population senesces and is overgrown by the surrounding bryophytic vegetation.

The phenology of sporophyte production in Newfoundland differs between the two species of *Splachnum.* The sporophytes of S. *ampullaceum* mature mainly from late June to late July whereas those of *S. pensylvanicum* produce two sets of mature sporophytes; the first set matures in mid-May to early June and the second set matures in late July to early August. Nevertheless, *S. ampullaceum* and *S. pensylvanicum* coexist growing on summer moose dung in the same habitat, often presenting mature sporophytes at the same time and are thus rarely, at the local population level, ever completely phenologically uncoupled.

The visual signals, as well as olfactory signals of these two species of mosses, differ considerably (Marino & Raguso, n.d.; Marino et al., 2009).When mature, *S. ampullaceum* has 5-10 cm tall sporophytes with a yellow (sometimes turning pink/red with maturity and senescence), broad and top-shaped hypophyses whereas the sporophytes of *S. pensylvanicum* is 0.5-2 cm tall, barely inflated greenish hypophysis with red, purple coloring distally (Marino, 2014). When growing together as mixed colonies on the same dropping, *S. pensylvanicum* grows under the canopy of the taller sporophytes of *S. ampullaceum*.

Figure 1.1 visual signals of study species; a single mature population of short, distally red/brown colored *S. pensylvanicum* (left) and a mature single population of tall, yellow colored *S. ampullaceum* (right).

The two species also differ in their olfactory signals (Marino & Raguso, n.d.). The olfactory signals of *S. ampullaceum* and *S. pensylvanicum*, which are associated with their hypophyses and capsules, are strong, complex and differ considerably between the two species. Mature sporophytes of *S. ampullaceum* emit a biosynthetically diverse blend of 51 volatiles including short-chain oxygenated compounds (e.g. 2-methyl butanol), unsaturated carotenoid derivatives (6-methyl-5-hepten-2-one and its associated alcohols), oxygenated aromatic compounds found in herbivore dung (acetophenone and p-cresol) and unusual cyclohexane carboxylic acids more commonly found in mammalian urine (McCuaig, Dufour, Raguso, Bhatt, & Marino, 2015). Interestingly, the mature sporophytes of *S. pensylvanicum* produce many of the same compounds emitted by sporophytes of *S. ampullaceum,* but lack the cyclohexane carboxylic acids and instead produce compounds such as dimethyl disulfide and indole. Given the categories identified by Jürgens et al., (2013) in their large-scale study of volatile chemistry emitted by brood site-deceptive angiosperms, both *Splachnum* species fall within the realm of herbivore dung mimicry, but the presence of indole and dimethyl disulfide in *S. pensylvanicum* suggest the possibility of a more generalized strategy of attracting flies that visit omnivore or carnivore dung as well (Marino, 1991a, 2014; Marino et al., 2009).

From previous experiments in Newfoundland, it is known that, when both visual and olfactory signals are present, *S. ampullaceum* and moose dung attract the same fly fauna, *S. ampullaceum* and *S. pensylvanicum* each usually attract a distinct fly fauna and *S. pensylvanicum* and moose dung each attract a distinct fly fauna. Marino and Raguso, (In prep) measured the effect of visual signals by completely blocking all visual signals of both *S. ampullaceum* and *S. pensylvanicum* and comparing visitation to non-manipulated treatments of both species and concluded that the odour signals determine the taxa of flies visiting each species of moss whereas the visual signals magnify the number of visitors. Piercey and Marino (2016) also conducted a manipulative experiment to observe changes occurring in fly fauna when only the visual signal of the bright yellow spores were eliminated by dyeing them green and also found a significant reduction in the number of fly visitors to *S. pensylvanicum* but did not influence the fly taxa visiting each species of moss or affect the number of flies visiting *S. ampullaceum* (Piercey, 2016) . Given these results, it is apparent that olfactory signaling, not visual signaling is the primary determinant of which taxa are attracted to each species of moss. However, we know that the two species of moss overlap, to some extent, in scent (Marino et al., 2009); the main difference is that *S. pensylvanicum* produces dimethyl disulfide and indole (scent of carrion) whereas *S. ampullaceum* does not. The presence of dimethyl disulfide and indole makes the scent emitted by *S. pensylvanicum* more omnivore dung like (Jürgens & Shuttleworth, 2015). Hence, in Chapter 2, a series of manipulative experiments were used to assess the role of scent in determining spore-dispersing faunal associations of *S. ampullaceum* and *S. pensylvanicum* by comparing fly faunal associations of each species of moss in non-manipulated natural populations to populations in which odor was manipulated to be either more 'herbivore dung-like' or more 'omnivore dung-like'.

In Chapter 3, the fly faunas visiting populations of *S. ampullaceum* and *S. pensylvanicum* were examined over three consecutive summers to explore the year-to-year constancy of the fly faunas associated with *S. ampullaceum* and *S. pensylvanicum* and the relative roles of generalist versus specialist insects to the dispersal of their spores. The level of the ecological generalization of the interacting partners is often measured as the number of insect visitors or diversity of spore dispersal agents (functional groups) visiting them (Blüthgen, Menzel, & Blüthgen, 2006; Vázquez, Morris, & Jordano, 2005). The level of these interactions with individual plant species is thought to be affected by the abundance of other interacting species in the community (Bascompte & Jordano, 2007; Fründ, Linsenmair, & Blüthgen, 2010; Wallace, Maynard, & Trueman, 2002). In the case of coprophilous

Splachnaceae, the efficacy of spore-dispersal is a function of the presence of dung, the type of dung present (e.g., herbivore, omnivore, carnivore) and its associated fly fauna. Network analysis was used to examine the degree of specialization between individual fly taxa and each species of moss. Connectivity and species strength analysis was done to better understand how olfactory signals contribute to the dispersal abilities of *S. ampullaceum* and *S. pensylvanicum* and consequently, to better understand the role of spore dispersal by brood-site deception in promoting their coexistence.

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Co-Authorship Statement

Both manuscripts presented here were authored with the help of Dr. Paul Marino. I was responsible for all aspects of the study design, execution, analysis, and manuscript preparation.

Chapter 2: Measuring the relative importance of olfactory signaling in a fly-moss spore dispersal network of two sympatric moss species in Newfoundland, Canada

2.1 Abstract

Nearly half the species of the globally distributed moss family Splachnaceae use brood-site deception as a strategy of spore dispersal. Brood-site deceptive species grow on feces and carrion and other animal matter. Their spores are dispersed to these substrates by flies that are attracted by visual and olfactory signals of their mature sporophytes.

I focused on two sympatric species, *Splachnum ampullaceum* and *S. pensylvanicum*, both of which are frequently found growing on summer moose dung either as mixed-species or single-species populations in peatlands on the island of Newfoundland, Canada. The sporophytes of each species have relatively distinct olfactory signals and very distinct visual signals. The odor chemistry of *S. ampullaceum* mimics herbivore/moose dung whereas that of *S. pensylvanicum* mimics omnivore dung. I manipulated the odor signals in each species of moss by adding carnivore-mimicking scent to *S. ampullaceum* and herbivore mimicking scent to *S. pensylvanicum* populations to test whether manipulating scent converged the associated fly faunas of carnivore scent manipulated *S. ampullaceum* to be more similar to the fauna associated with *S. pensylvanicum* and herbivore scent manipulated *S. pensylvanicum* to be more similar to the fauna associated with *S. ampullaceum.*

Data analysis shows that altering olfactory signals shifts the composition of the fly assemblage attracted to each species of moss such that scent manipulation converged each moss species' fly fauna closer to that associated with the other (non-manipulated) moss species. This result confirms that of an earlier study focusing on visual signals that suggested that olfactory, not visual signals promote visitor

specificity whereas visual signals primarily act to magnify the degree of attractiveness of mature moss populations to potential spore-dispersing flies.

2.2 Introduction

The energetic cost of providing food rewards for insect pollinators has led to the evolution of plant taxa that are frauds that manipulate the behaviour of insects via visual and olfactory signals, yet provide no nutritional reward to the pollinating insects (Woodcock, Larson, Kevan, Inouye, & Lunau, 2014). Three main types of deceptive strategies are identified as food (Jersáková, Johnson, & Jürgens, 2009), mate (Schiestl & Peakall, 2005) and brood site (Jürgens, Wee, Shuttleworth, & Johnson, 2013). The majority of these deceptive plants are known to use chemical mimicry (Pacini, 1992; Schaefer & Ruxton, 2009) with the odors often signaling the presence of brood sites, prey or mating partners to trigger obligate innate responses in insects to attract them (Dafni, 1984). Deceptive strategies are found among several families of angiosperms including the families Orchidaceae, Apocynaceae, Bignoniaceae and Ranunculaceae (Urru, Stensmyr, & Hansson, 2011) as well as lower plants such as family Splachnaceae and fungal family Phallaceae (Borg-Karlson, Englund, & Unelius, 1994; Urru et al., 2011). While higher plant families use deceptive mechanisms to disperse their pollen and seeds via insects, in Phallaceae fungi and Splachnaceae mosses, insects disperse their propagules (spores).

Nearly half the species of Splachnaceae mosses have evolved brood-site deception as a strategy of spore dispersal (Marino, Raguso, & Goffinet, 2009). These species have gametophytes that are ‘coprophilous’; growing on feces and occasionally on carrion and other animal matter, and their small, sticky spores are ‘myophilous’; i.e., dispersed by flies (Cameron & Troilo, 1982; Cameron & Wyatt, 1986; Koponen & Koponen, 1977; Marino, 1991a; Marino & Raguso, n.d.; Marino et al., 2009). Myophilous Splachnaceae have, in most species, elongated sporophytes. All myophilous species have a variously swollen, often-colored (e.g., yellow, magenta, brownish red, white) inflated sterile region (hypophysis) below the sporangium. Furthermore, myophilous species produce small, thin-walled, sticky spores that are extruded, as a highly visible yellow-green mass, by a false columella as the capsule walls shrink (Demidova & Filin, 1994). However, myophilous Splachnaceae do not entrap flies as is common among brood-site mimicking angiosperms. Rather, flies visiting Splachnaceae simply depart with the sticky spores adhering to their bodies which are then dispersed to dung and/or carrion upon which the spores are dislodged and germinate into protonema (early gametophytic developmental stage).

I studied two species of Splachnaceae, *Splachnum ampullaceum* Hedwig and *S. pensylvanicum* (Bridel) Grout ex H. A. Crum, found growing either in single-species populations on individual droppings or in mixed-species populations on the same summer moose dropping. Summer moose dung is colonized by either or both species from late spring through summer. Spore dispersal is directed to fresh dung as fly visitation declines substantially within 1-2 days (Marino, 1991a). Once dung is colonized by *Splachnum* spores, spore germination and subsequent gametophyte growth occurs in years 1 and 2 with sporophytes produced in year 3. Thereafter, in most cases, the population senesces and is overgrown by the surrounding bryophytic vegetation (Marino, 1988).

Phenologically, the sporophytes of S. *ampullaceum* mature mainly from late June to late July, in Newfoundland whereas those of *S. pensylvanicum* produce one set of mature sporophytes in mid-May to early June and the second set in late July to early August. Nevertheless, *S. ampullaceum* and *S. pensylvanicum* often have mature sporophytes at the same time and are thus rarely, at the local population level, ever completely phenologically uncoupled.

The visual signals, as well as olfactory signals of these two species of mosses, differ considerably (Marino & Raguso, n.d.; Marino et al., 2009). When mature, *S. ampullaceum* has 5-10 cm tall sporophytes with a yellow (sometimes turning pink/red with maturity and senescence), broad and top-shaped hypophyses whereas the sporophytes of *S. pensylvanicum* is 0.5-2 cm tall, barely inflated greenish hypophyses with red, purple colouring distally (Marino, 2014a). When growing together as mixed colonies on the same dropping, *S. pensylvanicum* grows under the canopy of the taller sporophytes of *S. ampullaceum*.

The olfactory signals of *S. ampullaceum* and *S. pensylvanicum* are primarily associated with their hypophyses and capsules and differ between the two species. Mature sporophytes of *S. ampullaceum* emit a biosynthetically diverse blend of 51 volatiles including short-chain oxygenated compounds (e.g. 2-methyl butanol), unsaturated carotenoid derivatives (6-methyl-5-hepten-2-one and its associated alcohols), oxygenated aromatic compounds found in herbivore dung (acetophenone and p-cresol) and unusual cyclohexane carboxylic acids more commonly found in mammalian urine (McCuaig, Dufour, Raguso, Bhatt, & Marino, 2015). The mature sporophytes of *S. pensylvanicum* produce many of the same compounds emitted by the sporophytes of *S. ampullaceum* but lack the cyclohexane carboxylic acids and instead produce compounds such as dimethyl disulphide and indole. Both *Splachnum* species fall within the realm of herbivore dung mimicry (Jürgens et al., 2013), but the presence of indole and dimethyl disulphide in *S. pensylvanicum* suggest the possibility of a more generalized strategy of attracting flies that visit omnivore or carnivore dung as well (Marino, 1991a, 2014a; Marino & Raguso, n.d.; Marino et al., 2009).

From previous experiments in Newfoundland, it is known that when both visual and olfactory signals are present, *S. ampullaceum* and moose dung attract the same fauna of flies, *S. ampullaceum* and *S. pensylvanicum* each attract a distinct fauna of flies and *S. pensylvanicum* and moose dung each attract a distinct fauna of flies (Marino & Raguso, n.d.; Rathnayake, 2019). Marino and Raguso (in prep), also measured the effect of visual signals by completely blocking all visual signals of both *S. ampullaceum* and *S. pensylvanicum* and comparing visitation to non-manipulated treatments of both species. In this study, Marino and Raguso concluded that the odor signals determine the taxa of flies visiting each species of moss whereas visual signals magnify the number of visitors. Piercey (2016) also conducted a manipulative experiment to observe whether the bright yellow spores that are distinctly apparent given their contrast with the dark red-brown coloration of the hypophysis is an important visual signal in *S. pensylvanicum* and found that green dyed spores compared to non-manipulated spores resulted in a significant reduction in the number of fly visitors to *S. pensylvanicum* but did not influence the fly taxa visiting each species of moss. These results suggest that olfactory signalling, not visual signalling, is the primary determinant of which taxa are attracted to a particular species of moss.

The major difference in scent between *S. ampullaceum* and *S. pensylvanicum* is that *S. pensylvanicum* produces dimethyl disulphide and indole (the scent of carrion) (Marino et al., 2009) whereas *S. ampullaceum* does not produce them. Because of that, I took a manipulative experimental approach in this study to assess the role of scent in determining spore-dispersing faunal associations of *S. ampullaceum* and *S. pensylvanicum*. Specifically, I manipulated the scent of populations of both species of moss and their moose dung substrates to be either more 'herbivore-like' and/or more 'carnivore-like,' and examined the effect of these scent manipulations on the fly faunal associations.

I predicted that the fly fauna trapped on *S. ampullaceum* + carnivore scent would converge with the fauna trapped on *S. pensylvanicum* as well as that associated with moose dung plus carnivore scent. Also, it was expected that the fly fauna trapped on *S. pensylvanicum* + herbivore scent would converge with the fly fauna trapped on *S. ampullaceum* and moose dung. The results of this study will help clarify the relative roles of scent, especially dimethyl disulphide, indole, phenol cresol, in determining the taxa of visitors associated with each species of moss and the substrate to which the spores of each species are most likely to be dispersed. The results of this study will also examine whether, despite the complexity of the volatiles being emitted from the sporophytes (69 for *S. ampullaceum* and 62 for *S. pensylvanicum*), the role of scent in deceptive mimicry amongst these mosses is essentially a function of key volatiles that constitutes ‘herbivore’ vs. ‘carnivore’ dung mimicry (Jürgens et al., 2013).

The study also was designed to identify those specific fly taxa that are specialized vs. generalized visitors with respect to scent mimicry in *S. ampullaceum* and *S. pensylvanicum*. The level of the ecological generalization of interacting partners is often measured as the number and diversity of insect visitors or, in the case of myophilous Splachnaceae, of spore-dispersal agents visiting them (Blüthgen, Menzel, & Blüthgen, 2006; Vazquez et al., 2012; D.P Vázquez & Aizen, 2014). This is important for community resilience to perturbations and selection (Blüthgen et al., 2006; Diego P. Vázquez, Morris, & Jordano, 2005). The level of these interactions with individual plant species is thought to be affected by the abundance of other interacting species in the community (Bascompte & Jordano, 2007a; Fründ, Linsenmair, & Blüthgen, 2010; Wallace, Maynard, & Trueman, 2002). In the case of coprophilous Splachnaceae, the efficacy of spore-dispersal is a function of the presence of dung or carrion, the type of dung present (e.g., herbivore, omnivore, carnivore) and its associated fly fauna. This study also uses Network analysis to examine the relationship between individual fly taxa and experimental treatments to determine taxon-specific associations and the influence of scent on the degree of specialization of fly visitors associated with each species of moss. It is expected that flies associated with *S. pensylvanicum* and *S. ampullaceum* will be more tightly nested in treatments with carnivore scent and herbivore scent, respectively. Lastly, connectivity and modularity analyses were performed to understand better how olfactory signals potentially contribute to the dispersal abilities of *S. ampullaceum* and *S. pensylvanicum* and consequently, to better understand the role of spore dispersal by brood-site deception in promoting their coexistence.

2.3 Methods

The study was conducted 2 km south of Salmonier Nature Park, Holyrood (47.25 N - 53.30 W) in eastern Newfoundland, Canada in a single *Sphagnum*-dominated bog in which both moss species have routinely been found. Fieldwork was carried out from June to August 2017. Initial fieldwork involved finding single-species populations of both species of moss in and near the study site so that they could be moved to the study site for the experimental design.

2.3.1 Study species

69 and 62 volatiles have been identified in *S. pensylvanicum* and *S. ampullaceum,* respectively, with 31 volatiles being shared (Marino & Raguso, n.d.). One of the key differences between the odor chemistry of the two species is that *S. pensylvanicum* contains dimethyl disulphide a volatile compound commonly found in rotting flesh, as well as several alkyl 2-ketones and some traces of dimethyl trisulfide, were identified in *S. pensylvanicum* but not *S. ampullaceum*. In contrast, higher amounts of 6 sesquiterpenes ($C_{15}H_{24}$) hydrocarbons, 10 compounds of cyclohexane carboxylic acid esters (McCuaig et al., 2015), Para-cresol and phenol, which are common volatile compounds found in herbivore dung, were found in higher concentrations in *S. ampullaceum* relative to *S. pensylvanicum.* Relatively, summer moose dung and *S. ampullaceum* have approximately a 1:1:1 ratio of the three key herbivore scent compounds (indole, phenol and para-cresol) and *S. pensylvanicum* has an approximately 1:1.5:1.5 ratio of DMDS and its two herbivore odors (para-cresol and indole). This odor chemistry suggests that *S. ampullaceum* is an 'herbivore dung mimic' whereas, due to the presence of dimethyl disulphide and indole, *S. pensylvanicum* is an omnivore dung mimic.

2.3.2 Experimental design

I manipulated the scent of moose dung and *S. ampullaceum* and *S. pensylvanicum* using artificial scent emitters using the aforementioned chemical ratios. Five mL of equal parts indol, para-cresol and phenol mixture was diluted in 45mL of dichloromethane (DCM) to create "herbivore scent," and 10mL of DMDS was diluted in 40mL of DCM to create "carnivore scent." The mixtures and volatiles are taken from a previous experiment (Gaskett, Marino and Raguso, unpublished) in which flies were trapped, in the same study site, on these mixtures alone (i.e., not associated with Splachnaceae mosses) and on pure populations of *S. ampullaceum* and *Tetraplodon mnioides* (Hedw. B.S.G., F. Splachnaceae); a carnivore dung specialist). From this study, we knew that the mixtures themselves (i.e., completely unassociated with Splachnaceae mosses) are attractive to flies and that the carnivore and herbivore mixtures attracted flies associated with *T. mnioides* and *S. ampullaceum*, respectively.

Flies were trapped on natural and scent-manipulated single-species populations of both species of moss, summer moose dung treatments with and without scent mixtures and on small 1ml Eppendorf tubes with herbivore and carnivore scent mixtures. The moose dung was collected fresh, frozen for storage and thawed prior to use. The treatments were: 1*) S. ampullaceum*, S. *pensylvanicum* and summer moose dung with no scent manipulation; 2) *S. ampullaceum*, S. *pensylvanicum* and summer moose dung with 'herbivore' scent (indol, phenol and cresol mixture) added; 3) *S. ampullaceum*, S. *pensylvanicum* and summer moose dung with 'carnivore' scent (Dimethyl Di sulfide mixture) added. These experimental substrates were moved to the study site and, together with the moose dung, placed in a randomized block experimental design consisting of 4 blocks (Figure 2.1). Three Eppendorf tubes (1ml) filled with dichloromethane; solvent used to dissolve scent mixtures, herbivore mixture and carnivore mixture were placed in each block as controls. Each block contained 12 treatments with one replicate of each treatment/block. Each replicate block was approximately 10m apart with treatments within blocks approximately 2 meters apart. These are not uncommon distances between which the two species of moss can be found growing at the study site. Fresh moose dung and 1ml each from fresh herbivore and carnivore mixture were added to appropriate treatments each day of fly trapping.

Flies were trapped using funnel traps constructed of clean 2-litre clear plastic soda bottles cut just below the tapered end of the bottle. The spout was then inverted and inserted into the lower half creating a funnel trap (Marino, 1991b; Marino & Raguso, n.d.). Traps were placed approximately 5-10 cm over the moss treatment using metal stakes. Nylon mesh funnels were inserted in the spout of each trap to prevent downward movement of flies to escape from the trap. Each trap was labelled with treatment and replicate number with a permanent marker.

Trapping was done for 15 days from 20 July to 5 August 2017. Fieldwork was conducted on mild dry days as the peristome teeth of the capsules close when wet, covering the spores and preventing spore dispersal. To kill trapped flies, cotton balls moistened with ethyl acetate were inserted into each trap after each 24-hour trapping period. Killed flies were placed in vials labelled with collector's name, treatment, replicate number and trapping date. All flies were pinned, identified to the family or genus when possible (McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R. & Wood, 1981). DNA barcoding (Canadian Center for DNA barcoding) was subsequently done to confirm identification and to further identify flies to species. Flies are all maintained in the personal collection of Paul Marino at the Memorial University of Newfoundland, and the digitized collection was submitted to the BOLD system (Rathnasingham & Hebert, 2007) under the project code name MKR- Diversity of Dipteran visitors on two sympatric Splachnum sp. in eastern Newfoundland.

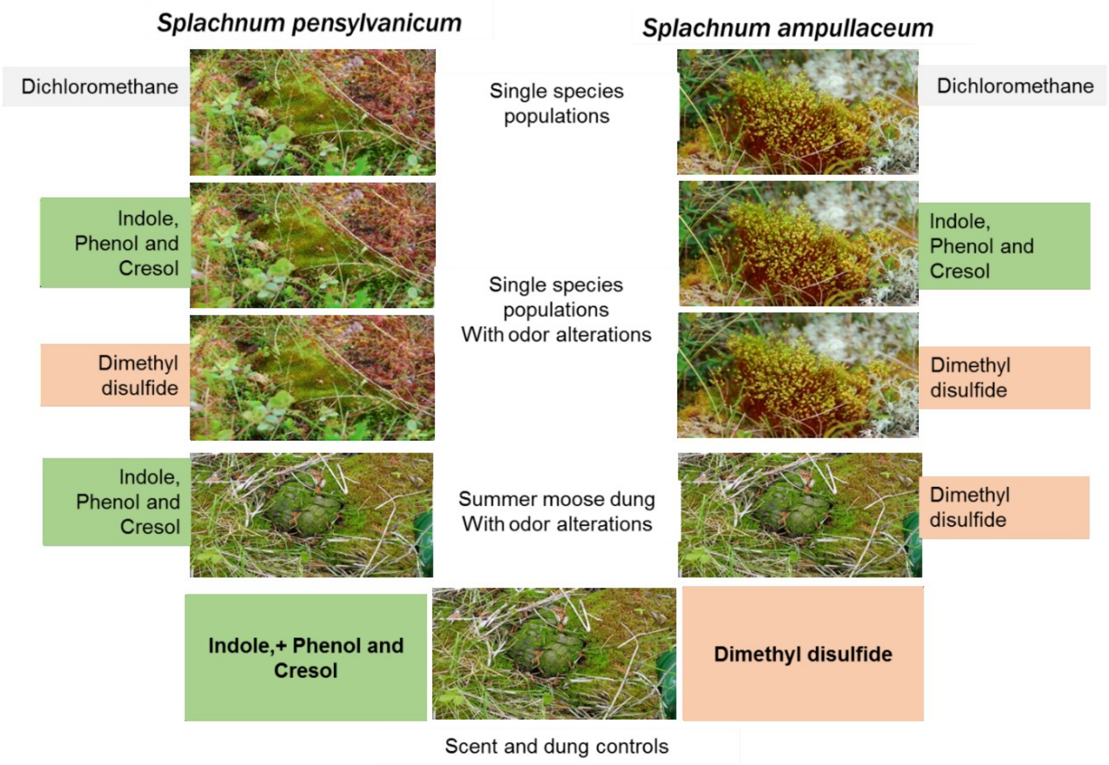

Figure 2. 1 Graphical representation of how natural and single-species populations of both species of moss, summer moose dung manipulated with scent mixtures (Dichlorometane – odorless solvent, Indol, Phenol and Cresol –Herbivore mixture, Dimethyl disulfide – Carnivore mix,) and control treatments placed in complete randomized block design

2.4 Data Analysis

2.4.1 Statistical analyses

Since previous work in a neighbouring bog suggests that flies can effectively disperse spores over distances up to 200m (Hammill, 2016), it was hypothesized that most flies foraged on a spatial scale that encompassed all four sites. Therefore, all observations were pooled in a contingency matrix in which cell values contain a number of fly species observed in each treatment and replicate. Summary statistics for this interaction matrix were calculated including the total number of flies trapped, the average number of flies trapped in each treatment, the number of unique associations between trapped flies and treatments.

Rarefaction was used to compare species richness of treatment-fly associations across treatments and blocks in which sampling effort differs due to scent manipulation. It has been shown that rarefaction is ideal for comparing different blocks as flies are non-randomly distributed among each treatment in each block (Birks & Line, 1992; Foote, 1992). Rarefaction analysis provides a minimum variance unbiased estimate of the expected number of taxa (Birks & Line, 1992) in a sample of *n* individuals taken from a collection of N individuals containing T taxa (Hammer, Harper, & Ryan, 2001). Therefore, it removes the bias in richness estimates caused by different count sizes.

2.4.2 PERMANOVA analyses

The experiment was analyzed using PERMANOVA+ add on available in PRIMER-E 7 ver. 7.0.13 (M. J. Anderson, Gorley, & Clarke, 2005, 2008). A non-parametric rank-based approach PERMANOVA (Permutational ANOVA) is ideal for analyzing multivariate ecological data as it does not make explicit assumptions on the original distribution of variables but acts on ranks (M. J. Anderson et al., 2005) of their resemblance measures such as distance, dissimilarities or similarities using permutation methods. Therefore, it is a rank-based non-parametric approach to analyze over dispersed, heavily skewed distributions as well as for data sets with many empty cells (zeros). The sum of individual fly species over the entire trapping period was assigned as the response variable and treatments as the independent variable (Marino & Raguso, n.d.) in the PRIMER-E software (K. R. Clarke & Gorley, 2015). Pairwise comparisons among treatments were also made to observe the effect of scent manipulations to mosses. The effect of scent manipulations for each moss species was analyzed using the similarities calculated between treatments using the Bray-Curtis similarity measure (M. J. Anderson et al., 2008). Fly assemblages on non-manipulated moss samples, manipulated moss samples, scent manipulated, and non-manipulated moose dung and controls were compared. Fly assemblages trapped on scent alone and vials containing dichloromethane were excluded from the analysis because scent controls were used only to test their efficacy of attracting flies. Moreover, if included in the analysis that might be obscuring a simpler result by introducing a large number of individuals from each taxa into the data matrix.

The dataset was then used to compare similarities of faunal associations between different olfactory treatment levels. (K. Clarke, Chapman, Needham, & Somerfield, 2006; K. Clarke, Somerfield, & Marine, 2006). This creates a resemblance matrix consisting of pair-wise similarity indices to each pair of treatments. After the generated resemblance matrix was partitioned into factors assigning block numbers and treatment levels, PERMANOVA was performed to test the multivariate null hypothesis of no difference among the blocks and treatments. The permutation method used in this test was of residuals under a reduced model with 9999 permutations. Subsequently, pair-wise comparisons among all treatments were obtained by performing an additional separate run of the PERMANOVA procedure in PRIMER-E (M. J. Anderson et al., 2008; Marino & Raguso, n.d.). The PERMANOVA pairwise comparison between treatments provides information on attraction levels of mosses when scents were manipulated. Also, by comparing faunal associations recorded in controls versus mosses, we can decouple the scent signal from mosses and observe the effect of added volatiles in attracting flies.

2.4.3 Interaction network analysis

The topology of the interactions observed between flies and scent manipulated treatments were explored by network statistics. All calculations were performed in RStudio (Team, 2011) using the Vegan (Oksanen et al., 2007) and Bipartite packages (Dormann, Gruber, & Fründ, 2008). The complete interaction frequency matrix (RC matrix) was divided into two matrices, one containing manipulated treatments and their interaction and a second containing non-manipulated samples. To visualize differences due to olfactory signal alteration, these matrices were analyzed individually for different network properties such as nestedness, connectance, species level and the community level specialization of species with respect to treatments in order.

Species-level specialization for flies (higher trophic level), mosses and dung (lower trophic level) were calculated to compare the effect of olfactory signal manipulation in each level. The community-wide specialization index (or connectance index) was calculated as a two dimensional Shannon entropy (H_2) (Blüthgen et al., 2006) and standardized between 0 and 1 in which 0 indicates extreme specialization and one extreme generalization. Also, network connectance was measured of each network as a measure of the stability of networks (Poisot & Gravel, 2014). Analysis of this network-level specialization was done to explain the partitioning of resources across fly assemblages found in the experiment as well as to quantify the overall effect on the fly-moss interaction network by olfactory signalling. Furthermore, the niche overlap index was calculated using the mean similarity (Hernández-Yáñez, Lara-Rodríguez, Díaz-Castelazo, Dáttilo, & Rico-Gray, 2013) in interaction patterns among treatments and species.

Nestedness analysis was used to examine the distribution pattern of species in the network as a function of their degree of specialization and generalization with respect to each treatment (Almeida-Neto, Guimarães, & Lewinsohn, 2007). Network nestedness, which is a measure of the distribution of species among treatments, tests if highly specialized species interacts with a well-defined subset of treatments with which most generalized species would also interact (Bascompte & Jordano, 2007b).

Nestedness analysis examined whether the species were distributed randomly vs. non-randomly and the stability of the observed interactions in the moss-fly network. The nestedness index (*NODF* index) and network temperature (*T*) (Atmar & Patterson,

1993) were calculated using ANINHADO (P R Guimarães & Guimarães, 2006). Both nestedness and temperature were calculated for each matrix and compared to a randomly generated null model of 1000 randomizations (Díaz-Castelazo et al., 2010; P R Guimarães & Guimarães, 2006; Hernández-Yáñez et al., 2013)

The contribution of each species of fly and treatment level in maintaining the spore dispersal network was quantified using the standardized Kullback-Leibler distance, (*d'*) as calculated following Blüthgen, 2006 (Blüthgen, Fründ, Vázquez, & Menzel, 2008; Blüthgen et al., 2006). Species-level specialization or generalization, which is described by *d',* is attributed to the partner diversity (Blüthgen et al., 2006) or the number of links a species has with their interacting counterpart. Therefore, it is believed to be a proxy to quantify the potential of spore dispersal by each species, also known as the heterogeneity in link strength (Blüthgen et al., 2006).

2.5 Results

In this study, 1693 flies comprised of 48 species and seven families were trapped (Table 2.1). Most trapped flies belonged to four families; Sepsidae (39.6%), Muscidae (30.3%), Anthomyiidae (15.3%) and Sarcophagidae (6.9%). Overall, the most attractive substrate, in terms of the number of flies trapped, was moose dung either with or without added scent. The scent alone attracted 125 flies (Table 2.1) including the majority of carrion flies (Sarcophagidae) trapped on carnivore scent and additional herbivore flies on herbivore scents supporting their efficacy as an attractant. When manipulated with carnivore and herbivore scent, dung increased in attractiveness to flies and the addition of either scent made moose dung hyper-attractive to several species of flies (Table 2.1). Sixteen species of flies were trapped on *S. ampullaceum*, eleven of which, were also trapped on summer moose dung. Fourteen fly species were trapped on *S. pensylvanicum*, of which nine were also trapped on summer moose dung. However, scent manipulation of mosses resulted in a large increase in the number of flies of several families attracted to each species of moss regardless of the scent manipulation treatment. For example, *Myospila meditabunda* is routinely captured in large numbers on moose dung, but the number of *M. meditabunda* trapped on *S. pensylvanicum* increased considerably when herbivore scent was added to the moss. Similarly, *Sepsis punctum* showed the same pattern on both the species of moss regardless of the manipulation (Table 2.1). Moreover, commonly known carrion flies such as *Fletcherimyia fletcheri* and *Sarcophaga sarraceniae* were trapped in higher numbers when the mosses are treated with carnivore scent. In general, both species of moss are visited by a wide diversity of fly species, but most are in low abundance and, the addition of scent had a little noticeable effect on species that were trapped infrequently. For those species of flies trapped in large numbers on the mosses, *M. meditabunda* increased considerably when herbivore scent was added to *S. pensylvanicum,* but the addition of herbivore scent did not affect the number trapped on *S. ampullaceum*. In contrast, *Sepsis punctum* was trapped in much greater numbers when carnivore scent was added to *S. ampullaceum* (SA = 46, SAC=86) and when either carnivore or herbivore scent was added to *S. pensylvanicum* (SP=17, SPH=99, SPC=65). Lastly, both Sarcophagid flies (*F. fletcheri* and *S. sarraceniae*), species associated with rotting flesh, increased from a very low abundance (SA=8,3 SP=5,1) on either moss to a moderate abundance (SA=16,20, SP=14,13) when carnivore scent was added to either species of moss. With respect to dung, the addition of herbivore scent resulted in the Anthomyiid flies *Hylemyza partita* (D=14 DH=35) and *Lasiomma nr. picipes* (D=12 DH=24) and the muscid fly *M. meditabunda* (D=57 DH=134) to be trapped in greater numbers whereas the addition of either herbivore or carnivore scent to dung increased the abundance of *S. punctum* (D=70 DC=132 DH=115). The addition of carnivore scent to moose dung appeared to have its greatest impact on the Sarcophagid flies *F. fletcheri* and *S. sarraceniae* by increasing their abundance.

Table 2. 1 Family, species and the total number of individuals trapped. SA (S. ampullaceum), SAH (with herbivore scent added), SAC (with carnivore scent added, SP (S. pensylvanicum), SPH(with herbivore scent added), SPC (with carnivore scent added), D (Moose dung), DH (with herbivore scent added) and DC (with carnivore scent added), CS (Carnivore mix), H (Herbivore scent)

Family	***Species***	**Treatments**											
		SA	**SAH**	**SAC**	**SP**	**SPH**	**SPC**	**D**	**DH**	**DC**	**CS**	**HS**	**Sum**
Anisopodidae	*Sylvicola punctata*	5	5	10	11	4	3	9	9	12	3	1	72
Anthomyiidae	*Hylemyza partita*	2	3	2	3	5	6	14	35	22	11	9	112
	Lasiomma nr. picipes	5	2	1	1	1	2	12	24	16	1	3	68
	Pegoplata tundrica	1	1	5	1	0	0	9	13	6	0	5	41
Calliphoridae	*Pollenia pediculata*	0	0	1	0	0	1	0	0	0	12	1	15
	Protophormia terraenovae	0	0	0	0	0	0	0	0	1	0	0	1
Dolichopodidae	*Dolichopus wheeleri*	0	0	1	1	0	0	0	0	6	0	3	11
	Dolicopus sp.	1	0	1	0	0	1	0	2	0	0	1	6
Fannidae	*Fannia atripes*	0	1	6	0	0	3	0	1	1	0	0	12
	Fannia coracina	0	0	1	0	0	0	0	0	0	1	0	2
	Fannia depressa	0	0	3	0	0	0	0	3	0	0	0	6
	Fannia fuscula	1	2	5	0	1	1	1	0	3	0	0	14
Heleomyzidae	*Neoleria inscripta*	1	1	4	0	1	1	0	0	1	0	0	9

Hybotidae	*Ocydromia glabricula*	0	0	0	0	1	0	1	1	1	0	0	4
Limoniidae	*Limoniidae sp*	0	0	0	0	0	0	0	1	1	2	0	4
Muscidae	*Graphomya minor*	0	0	2	0	0	0	1	1	4	5	3	16
	Graphomya minuta	1	0	2	0	0	0	1	3	1	1	2	11
	Graphomya transitionis or minuta	0	0	0	0	0	0	0	1	0	0	0	1
	Haematobosca alcis	0	0	0	0	0	1	0	0	0	0	0	1
	Hebecnema nigra	0	0	0	0	1	0	0	2	0	0	1	4
	Hebecnema umbratica	0	1	0	0	0	0	0	0	0	0	1	2
	Helina evecta	0	1	5	3	0	2	0	1	1	0	0	13
	Helina maculipennis	1	1	1	2	1	2	1	0	0	0	1	10
	Hydrotaea houghi	0	0	0	0	0	0	0	0	1	1	1	3
	Hydrotaea ponti	0	0	0	0	0	0	3	2	4	0	0	9
	Hydrotaea unispinosa	0	0	0	0	0	0	1	1	1	0	0	3
	Morellia micans	0	0	0	0	0	0	0	1	0	0	0	1
	Morellia podagrica	0	0	0	0	0	0	0	1	0	0	0	1
	Muscina levida	0	0	6	0	0	7	0	1	1	0	4	19
	Mydaea brevipilosa	0	0	0	0	0	0	2	4	1	0	0	7
	Mydaea obscurella	0	1	0	0	0	0	0	1	1	0	0	3
	Myospila meditabunda	6	8	1	26	96	37	57	134	64	0	7	436
	Spilogona Sp.	0	0	0	0	0	0	0	0	1	0	0	1

Mycetophilidae	*Sciophila lutea*	0	0	0	0	0	0	0	0	1	0	0	1
Sarcophagidae	*Fletcherimyia fletcheri*	8	2	16	5	4	14	2	0	9	13	1	74
	Sarcophaga sarraceniae	3	3	20	1	0	13	1	4	13	2	0	60
Scathophagidae	*Megaphthalma pallida*	0	0	0	0	0	1	0	0	0	0	0	1
	Scathophaga furcata	4	5	0	2	5	6	0	1	0	1	0	24
	Scathophagidae sp	0	0	0	0	0	1	0	0	0	0	0	1
Sciomyzoidea	*Tetanocera plebeja*	2	2	0	1	3	1	0	1	1	4	1	16
Sepsidae	*Sepsis punctum*	47	39	86	17	99	65	70	132	115	7	15	692
Stratiomyidae	*Microchrysa polita*	0	0	0	0	0	0	0	2	0	0	0	2
	Sargus decorus	0	0	1	1	0	0	0	0	6	0	0	8
Syrphidae	*Eristalis cryptarum*	0	0	0	0	0	0	1	0	1	0	0	2
	Rhingia nasica	0	0	0	0	0	0	2	5	5	0	0	12
Tachinidae	*Lixophaga sp*	0	0	1	0	0	0	0	0	0	0	0	1
	Periscepsia clesides	0	0	0	0	0	0	1	0	0	0	0	1
Ulidiidae	*Herina nigribasis*	1	0	1	0	0	0	0	0	0	0	1	3

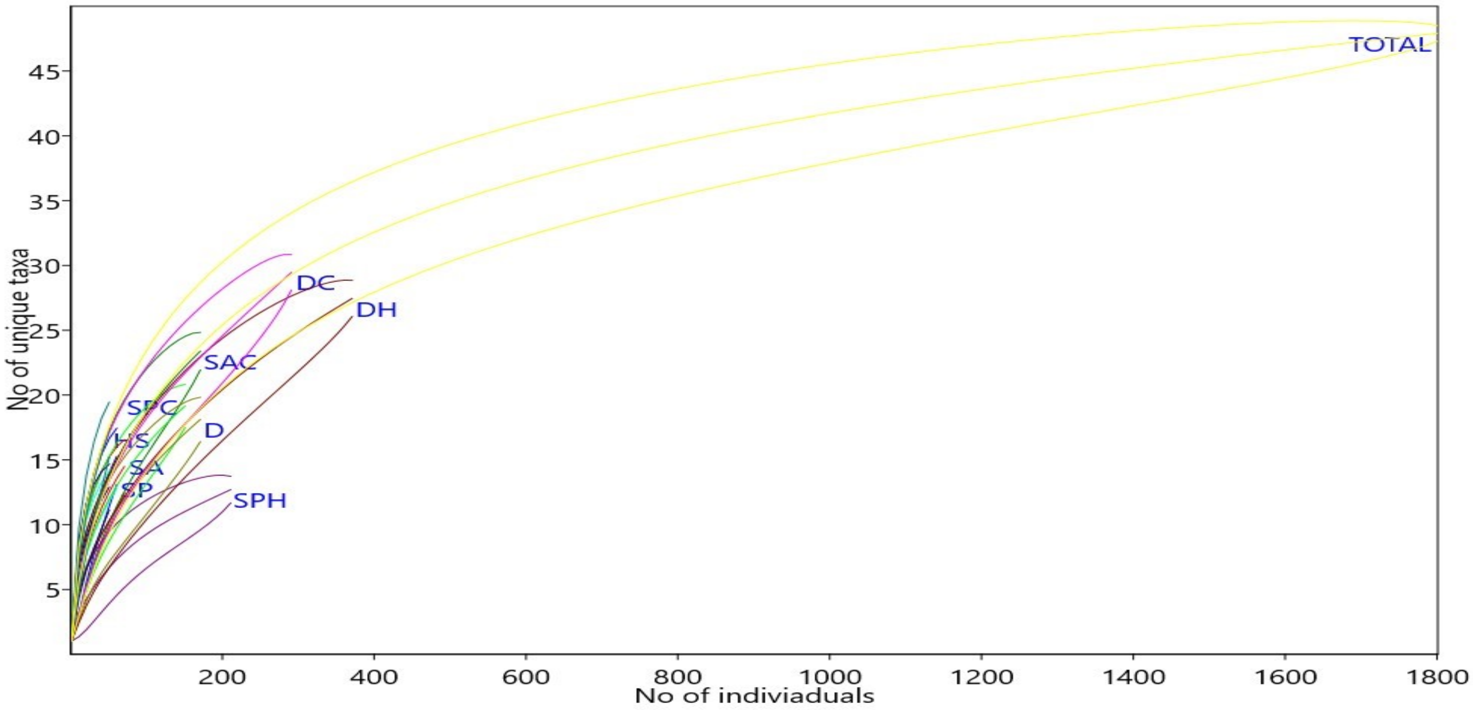

Figure 2. 2 Rarefaction curves for the number of unique fly-moss-dung and scent only sample interactions recorded versus the number of individuals observed in each treatment for the whole summer of 2017. The accumulation of links observed over the entire trapping period is significantly greater than what is observed in any single treatment, indicating the presence of unique links in the study system with scent manipulations.

2.5.1 PERMANOVA analysis

PERMANOVA results indicate that there is significant variation among the Dipteran fauna associated with the different treatments (*F* (3,8) = 2.88-, $p_{(perm)}$ <.001; Table 3). Given the high number of unique permutations which approached the number of random permutations chosen, the permutation P-value was preferred over Monte Carlo *P*-value in making inferences (M. Anderson, Gorley, & Clarke, 2008).

Table 2. 2 PERMANOVA results of blocking (BL) and treatment (TR) effects on faunal differences.

SOURCE	***DF***	***SS***	***MS***	***PSEUDO-F***	***P(PERM)***	***UNIQUE PERMS***
Bl	3	6155.6	2051.9	1.2592	0.1724	9895
tr	8	37585	4698.2	2.8831	0.0001	9849
Res	24	39109	1629.5			
Total	35	82850				

2.5.1.1 Pairwise Comparisons

Summer moose dung: Faunal associations

When manipulated with carnivore or herbivore scent, faunal associations in the dung treatments did not change significantly compared to non-manipulated dung samples (D vs. DH= t=2.018, p= 0.077/ D vs DC= t=1.041, p=0.399). *S. ampullaceum* attracted a fly fauna more strongly associated with summer moose dung whereas untreated dung attracted a significantly different fly fauna from both *S. pensylvanicum* (SA vs D = t=1.942, p= 0.056)*,* and *S. ampullaceum* + carnivore scent (D vs SAC = t= 2.200 p=0.036). Dung treated with herbivore scent attracted a significantly different fly fauna from untreated *S. pensylvanicum,* (DH vs SP= t=3.034 p=0.029) *S. pensylvanicum* + either herbivore (DH vs SPH= t=2.289, p=0.040) or carnivore scent

(DH vs SPC= t=2.530, p=0.036)*,* *S. ampullaceum* + herbivore scent (DH vs SAH= t=2.264, p=0.032) and *S. ampullaceum* + carnivore scent (DH vs SAC= t=3.081, p=0.029). Dung treated with carnivore scent had a significantly different fly fauna from untreated *S. ampullaceum* (DC vs SA= t=2.598, p=0.038) and untreated *S. pensylvanicum* (DC vs SP= t=2.333, p=0.029). Whereas dung treated with carnivore scent did not differ significantly from any of the other treatments.

Splachnum ampullaceum: faunal associations

The fly assemblage trapped on *S. ampullaceum* did not differ from those trapped on *S. pensylvanicum* (SA vs SP= t=1.611, p=0.111) or *S. ampullaceum* + either herbivore (SA vs SAH= t=0.731, p=0.755) or carnivore scent (SA vs SAC= t=1.542, p=0.103). The fly faunas trapped on populations treated with carnivore scent differed from non-manipulated populations of *S. pensylvanicum* (SAC SP= t=2.308, p=0.036). Addition of herbivore scent to *S. ampullaceum* attracted a higher number of fly taxa than when attracted with the addition of carnivore scent. Whereas, the addition of herbivore scent to *S. ampullaceum* resulted in fly assemblages that did not differ significantly between *S. pensylvanicum* + herbivore scent (SAH vs SPH= t=1.718, p=0.087) or non-manipulated summer moose dung (SAH vs D= t=1.343, p=0.204).

S. pensylvanicum: *Faunal associations*

Untreated *S. pensylvanicum* attracted a significantly different fly fauna from *S. ampullaceum* carnivore scent (SP vs SAC= t=2.368, p=0.036) and similar fly fauna to *S. ampullaceum* treated with herbivore scent (SP vs SAH= t=1.113, p=0.339). In contrast to S. *ampullaceum*, *S. pensylvanicum* had a similar fly fauna as *S. pensylvanicum* + carnivore scent (SP vs SPC= t=1.756, p=0.112). However, significantly different fly assemblages were observed when the fly assemblages of untreated *S. pensylvanicum* was compared to the fly assemblages trapped on *S. pensylvanicum* + herbivore scent (SP vs SPH=t=2.660, p=0.030) and compared to either those trapped on summer moose dung + herbivore (SP vs DH t=3.034, p=0.029) or carnivore scent (SP vs DC t=2.333, p=0.029).

Comparison of faunal communities between scents manipulated mosses

Herbivore scent treated *S. ampullaceum* attracted a fly assemblage similar to carnivore (SAH vs SPH=t=1.718, p=0.087)or herbivore scented *S. pensylvanicum* (SAH vs SPC=t=1.148, p=0.382), carnivore scent added summer moose dung (SAH vs DC=t=1.709, p=0.089) and carnivore scented *S. ampullaceum* (SAH vs SAC=t=1.260, p=0.223). Adding herbivore scent to *S. ampullaceum* attract a different fly fauna than herbivore scented summer moose dung (SAH vs DH=t=2.264, p=0.032). Similarly, when carnivore scent was added to *S. ampullaceum*, it attracted a similar fly fauna to that of carnivore scented *S. pensylvanicum* (SAC vs SPC=t=1.138, p=0.340) and carnivore scented dung (SAC vs DC=t=1.680, p=0.090). However, carnivore scented *S. ampullaceum* attracted significantly different fly taxa than herbivore scented *S. pensylvanicum* (SAC vs SPH=t=0.646, p=0.027) and summer moose dung (SAC vs DH=t=3.081, p=0.029). Regardless of the scent manipulation, *S. pensylvanicum* attracted significantly different fly fauna than herbivore scented summer moose dung (SPH vs DH=t=2.289, p=0.029/ SPC vs DH=t=2.530, p=0.040). Similarly, similar fly communities were observed on scent manipulated *S. pensylvanicum* and carnivore scented dung samples (SPH vs DC=t=1.411, p=0.145/ SPC vs DC=t=1.520, p=0.108). For all summarized results of multiple comparison see Table 2.3.

Table 2. 3. PERMANOVA paired-comparison results of all treatment combinations. Treatment abbreviations are: SA = *S. ampullaceum*, SP = *S. pensylvanicum*, D = moose dung, DC = Dung treated with carnivore scent, DH = Dung treated with herbivore scent, SAC = *S. ampullaceum* treated with carnivore scent, SAH= *S. ampullaceum* treated with herbivore scent, SPC= *S. pensylvanicum* treated with carnivore scent, SAH= *S. pensylvanicum* treated with herbivore scent.t = probability, P(*perm*) = p-value in PERMANOVA test. Significant treatment comparisons are in bold.

	Treatment	**t**	**P*(perm)***
1	D, SA	1.942	0.056
2	**D, SP**	**2.027**	**0.046**
3	D, DH	2.018	0.077
4	D, DC	1.041	0.399
5	DH, DC	1.663	0.112
6	SA, SP	1.611	0.111
7	SA, SAH	0.731	0.755
8	SA, SAC	1.542	0.103
9	SA, SPH	2.198	0.053
10	SA, SPC	1.530	0.174
11	SA, DH	1.720	0.095
12	**SA, DC**	**2.598**	**0.038**
13	**SAC, SP**	**2.368**	**0.036**
14	**SAC, D**	**2.200**	**0.039**

	Treatment	**t**	**P*(perm)***
21	SPH, D	1.408	0.149
22	SPC, D	1.503	0.109
23	SAH, SAC	1.260	0.223
24	SAH, SPH	1.718	0.087
25	SAH, SPC	1.148	0.382
26	**SAH, DH**	**2.264**	**0.032**
27	SAH, DC	1.709	0.089
28	**SAC, SPH**	**2.646**	**0.027**
29	SAC, SPC	1.138	0.340
30	**SAC, DH**	**3.081**	**0.029**
31	SAC, DC	1.680	0.090
32	SPH, SPC	1.509	0.132
33	**SPH, DH**	**2.289**	**0.040**

15	SAH, SP	1.113	0.339
16	SAH, D	1.343	0.204

17	**SP, SPH**	**2.660**	**0.030**
18	SP, SPC	1.756	0.112
19	**SP, DH**	**3.304**	**0.029**
20	**SP, DC**	**2.333**	**0.029**

34	SPH, DC	1.411	0.145

35	**SPC, DH**	**2.530**	**0.036**
36	SPC, DC	1.520	0.108

2.5.2 Network analysis

The overall network specialization (*H_2* index) for the interaction network of non-manipulated treatments and manipulated treatments were 0.1436 and 0.1519, respectively, suggesting that these networks are, as expected, very generalized. Two measures of species' distribution linked with the degree of specialization in each network were assessed by calculating network nestedness and connectance of weighted interaction matrices. The interaction network of non-manipulated samples was not significantly nested (*NODF* = 29.98 p>0.01) and had a connectance of 0.6049. Moreover, the nestedness of the interaction network was not significantly higher than randomly generated networks by null model II (T= 50.23° P>0.01). The interaction network in scent manipulated populations was significantly nested (*NODF*=46.69 P<0.01) and connectance was 0.4680. These indicate that scent manipulation had a direct effect on directing species towards enhanced chemical cues making the network more nested and arranged. But, low connectance indicates that regardless of scent manipulations, the networks are loosely arranged and are not robust to the loss of interacting partners.

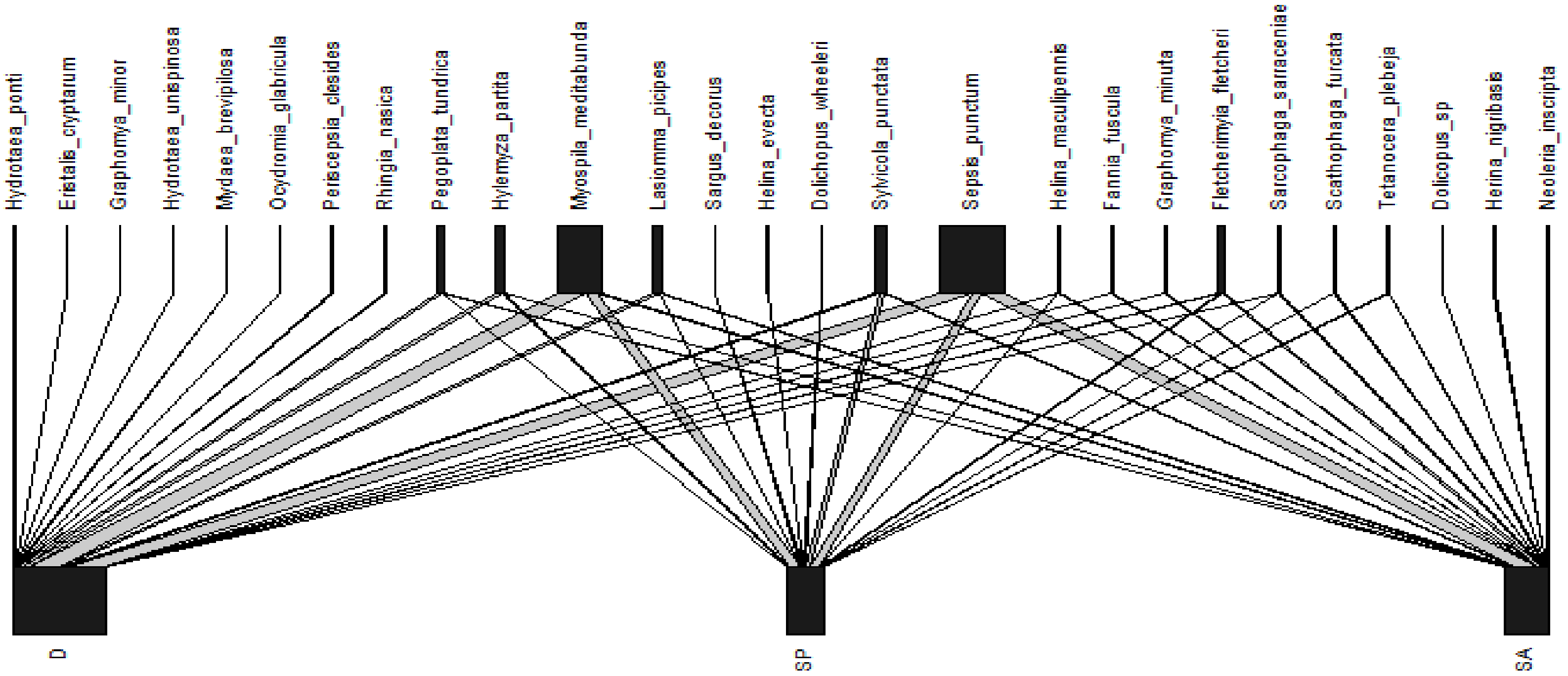

Figure 2. 3. The unconstrained functional size-based bipartite network of the non-manipulated interaction network. Upper nodes represent *S. ampullaceum* (SA), *S. pensylvanicum* (SP) and summer moose dung (D) and lower nodes represent taxa of flies captured in each treatment. Lengths of the nodes are proportional to the number of interactions of each node, and the width of edges indicates the number of individuals that have been captured on each treatment.

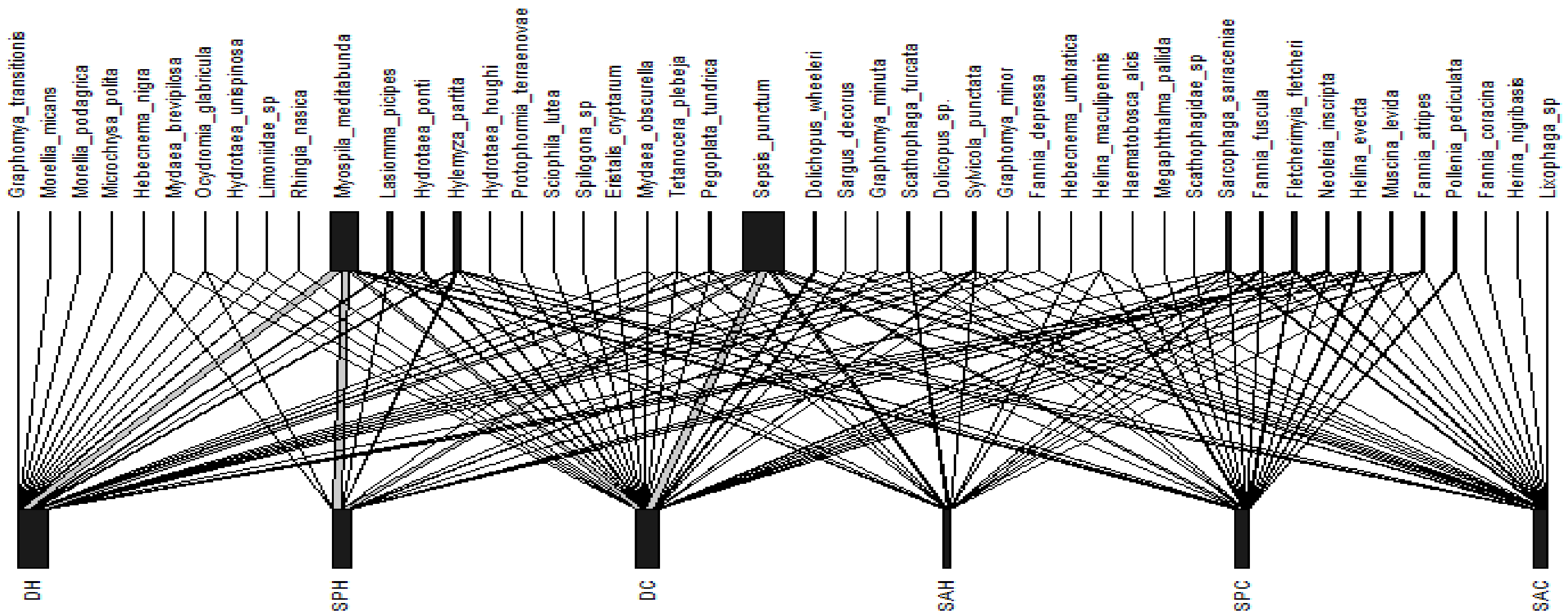

Figure 2. 4. The unconstrained functional size based bipartite network built for the scent manipulated interaction network. Upper nodes represent *S. ampullaceum* (SA), *S. pensylvanicum* (SP) and summer moose dung (D), *S. ampullaceum* treated with carnivore scent (SAC), *S. ampullaceum* treated with herbivore scent (SAH), *S. pensylvanicum* treated with carnivore scent (SPC), *S. pensylvanicum* treated with herbivore scent (SAH), Dung treated with carnivore scent (DC), Dung treated with herbivore scent (DH). Lower nodes represent taxa of flies captured in each treatment. Lengths of the nodes are proportional to the number of interactions of each node and width of edges indicates the number of individuals that have been captured on each treatment.

The structural properties of the two networks were compared using the species level specialization values (the standardized Kullback-Leibler distance; *d'*) and showed that both networks are dominated by highly generalized fly taxa (low *d'*). The non-manipulated Splachnaceae network was dominated by *Helina evecta, Hydrataea ponti* (Muscidae), *Fletcherimyia fletcheri* (Sarcophagidae), *Dolichopus sp. Dolichopus wheeleri* (Dolicophodidae), *Sargus decorus* (Stratiomyidae), *Scathophaga fructata* (Scathophagidae) and *Tetanocera plebeja* (Sciomyzoidea). These taxa were all recorded more commonly on each non-manipulated Splachnaceae population and dung (Fig. 2.2). In comparison, the scent manipulated Splachnaceae network differed from the non-manipulated network by attracting additional, but also generalized fly taxa, such as *Fannia depressa, Fannia fuscula* (Fannidae), *Graphomya minor*, *Haematobosca alcis, Hebecnema umbratica, Muscina levida, Mydaea brevipilosa* (Muscidae), *Megaphthalma pallida* (Scathophagidae), *Pollenia pediculate* (Calliphoridae), *Sarcophaga sarraceniae* (Sarcophagidae) (Fig. 2.3).

2.6 Discussion

In concordance with previous experiments examining the faunal associations of *S. ampullaceum* and *S. pensylvanicum* in Newfoundland, this manipulative experiment found that the fly faunas trapped on each species differed significantly from each other. Moreover, in all cases, there was high overlap in the taxa trapped, although the relative number of individuals trapped often differed considerably (See Chapter 3). In general, while both species of moss share a fly fauna similar to that associated with summer moose dung, *S. ampullaceum* attracts a fly fauna more strongly associated with moose dung than *S. pensylvanicum.* However, it was shown that the magnitude of this difference differs among years such that in most years the faunas associated with the two species differs significantly whereas infrequently they do not (See chapter 3). However, as expected there was low complimentary specialization between flies and mosses and a low degree of nestedness and connectance between interacting partners. Moreover, in this study, the scent chemistry of each species of moss and summer moose dung was manipulated by adding additional herbivore scent and additional carnivore scent to both species of mosses and to summer moose dung and results determined that the observed faunal differences between the two species was primarily a function of the herbivore vs. carnivore scent dichotomy. Furthermore, the study suggests that the difference in faunal associations may promote differential spore aggregation on potential habitats, thereby promoting coexistence of these two species of mosses in eastern Newfoundland.

2.6.1 Number and taxa of visitors in treatments

This study supports the expectation that key components of the olfactory signals determine the taxa of flies associated with *S. pensylvanicum* and *S. ampullaceum* and it shows that the magnitude of the olfactory signals contributes to change the number of individuals attracted to each moss species. The results of this study, therefore, support the idea that small quantities of dimethyl disulfide and indole are responsible for modest differences in fly assemblages attracted to each species of these coexisting mosses.

Myophilous Splachnaceae mosses are associated with particular types of substrates and, for those species that have been examined, their odor chemistry mimics the substrates on which they grow (Marino et al., 2009). For example, boreal species whose volatiles mimic herbivore dung (e.g., *S. ampullaceum, Splachnum luteum*, *Splachnum rubrum* and *Splachnum sphaericum*) grow on herbivore dung (e.g., moose, bison, horse) in boreal habitats. In Chilean Patagonia, *Tayloria mirabilis* mimics the scent of herbivore dung and grows on cattle dung (Goffinet, 2012; Marino et al., 2009), whereas, *Tayloria dubyi* which grows on goose droppings mimics the scent chemistry of rotting shellfish (Jofre et al., 2010) and *Tetraplodon fuegiensis*, similar to its North American counterparts *T. mnioides* and *T. angustatus* (Marino, 1991b, 1991a) mimic the scent of carnivore dung (e.g., produce DMDS and Indole) and grow on carnivore droppings (Marino et al., 2009). From all studies done on myophilous Splachnaceae mosses, we know that taxa that grow on carnivore dung produce olfactory signals mimicking decaying flesh and attract flies associated with decaying flesh. In contrast, the flies attracted to *Splachnum* spp., with the exception of *S. pensylvanicum*, that have exclusively been found growing on herbivore dung and, whose odor chemistry has been examined, do not produce volatiles associated with rotting flesh (McCuaig et al., 2015)

Splachnum pensylvanicum is the only species of *Splachnum*, whose odor chemistry has been examined that also produces, albeit in small quantities, carnivore scent (DMDS and Indole), suggesting it is an omnivore dung mimic. However, in Newfoundland, this species is primarily found growing on herbivore (moose) dung (Marino et al., 2009). However, in two instances either in our study site or a neighboring study site, very large populations of hundreds to thousands of sporophytes of *S. pensylvanicum* have also been found growing on moist soil at the edge of bogs upon which moose carcasses have rotted and decayed (Marino pers. observation). At these same sites, there were only relatively tiny populations of *S. ampullaceum* (< 10 sporophytes). Although it is unclear as to whether this difference is population size is a consequence of differential dispersal and/or growth ability on the enriched soil substrate, it does suggest that *S. pensylvanicum* does have a substrate type available to it that is relatively unavailable to *S. ampullaceum.*

This study, as well as previous trapping experiments, suggest that the olfactory signal of *S. ampullaceum* is a relatively stronger attractant than that of *S. pensylvanicum* given the relatively higher number of taxa and flies attracted to *S. ampullaceum*. Consequently, in this experiment, I expected that altering the scent would both affect the taxa of flies and that simply adding scent (irrespective of which one) would increase the number of individual flies trapped. However, in contrast to my prediction, it was found that adding carnivore or herbivore scent did not significantly increased the number of fly taxa visiting *S. ampullaceum* but did significantly increase the number of individuals of each taxon to treated moss populations. However, there were several additional fly taxa such as *Fannia atripes, F. coracina, F. depressa, Helina evecta,* and *Muscina levida* that were trapped on *S. ampulaceum* populations after being treated with carnivore scent. These taxa are generally associated with herbivore dung such as cattle dung (Blackith & Blackith, 1993; Martinez-Sanchez, Rojo, & Marcos-Garcia, 2000). However, these taxa were also trapped on the carnivore dung specialist Splachnaceae mosses *Tetraplodon angustatus* and *T. mnoides*, both of which generally grow on carnivore dung and owl pellets and produce DMDS (Marino, 1991b). These observations suggest that these taxa are associated with both types of dung and attracted to both carnivore and omnivore scents. However, well-known carnivore dung and carrion (flesh) specialists such as *Fletcherimyia fletcheri* and *Sarcophaga sarraceniae,* (Bänziger & Pape, 2004; Krawchuk & Taylor, 1999; Marino & Raguso, n.d.; Martín-Vega & Baz, 2013; Rango, 1999) were trapped in greater abundance on *S. pensylvanicum* and dung and *S. ampullaceum* treated with DMDS. In overall, recording very few numbers of known specialist taxa on *S. pensylvanicum* and carnivore scented treatments than *S. ampullaceum* suggest that the presence of DMDS create a difference in faunal attractions in mosses and manipulated treatments.

In this study it was predicted, if there is a strong effect from scent, flies attracted to *S. ampullaceum* should be similar to those trapped on dung, dung + herbivore scent *S. ampullaceum* + herbivore scent, *S. pensylvanicum* + herbivore scent but not *S. pensylvanicum*, dung + carnivore scent, *S. ampullaceum*+ carnivore scent or *S. pensylvanicum* + carnivore scent. In contrast, flies attracted to *S. pensylvanicum* should be similar to dung + carnivore scent, *S.ampullaceum* + carnivore scent, *S. pensylvanicum* + carnivore scent but not dung, dung+ herbivore scent, *S. ampullaceum*, *S. ampullaceum* + herbivore scent or *S. pensylvanicum* + herbivore scent. However, in the study, S. *pensylvanicum* attracted a fly fauna that was significantly different from summer moose dung, but *S. ampullaceum* attracted one that was similar to moose dung. Although the magnitude of these differences was very small for each species. Treating S. *pensylvanicum* with herbivore scent attracted the same fly fauna as *S. ampullaceum*. Whereas, adding carnivore scent to *S. ampullaceum* did not cause it to attract the same fly fauna as S. *pensylvanicum* except attracting a few taxa of flesh flies (e.g. *Fletcherimyia fletcheri, Fannia atripes, Sarcophaga sarraceniae*). Therefore, our predictions were met for *S. pensylvanicum* in that I could “convert” *S. pensylvanicum* into *S. ampullaceum,* but the scent manipulations did not “convert” *S. ampullaceum* to *S. pensylvanicum*. Moreover, my predictions were met on enhancing olfactory signals in that the fly fauna attracted to both mosses and dung treatments were increased with one exception, that adding herbivore scent to *S. ampullaceum* didn't increase the number of individuals attracted. The increase of taxa trapped on *S. pensylvanicum* + herbivore scent and the unchanged attraction levels of *S. ampullaceum* + herbivore scent enhanced strongly suggest that the olfactory signals of the mosses define the taxa attracted to each moss species and visual signals magnify the number of individuals of each taxa attracted.

When herbivore scent was added to *S. pensylvanicum* it attracted the same fly taxa trapped on summer moose dung. This observation suggests that the addition of herbivore scent resulted in an increased convergence between the fauna attracted to *S. pensylvanicum* and that of herbivore dung. However, the pair-wise analyses also suggest that scent manipulation did not result in all the expected convergences of the fly faunas of *S. pensylvanicum* and S. *ampullaceum.* For example, adding DMDS increased sarcophagid numbers to every treatment for both species of moss, suggesting that Indole, para-cresol and phenol plays a fundamental role in attracting more generalized herbivore dung fly fauna to both *S. pensylvanicum* and *S. ampullaceum* whereas DMDS increased visitation by more carrion/carnivore dung specialized fly taxa such as Sarcophagids and Sepsids.

This difference in the effect of scent manipulation on the two species of moss may partially be a consequence of their visual signals. Visual signals are magnifying the attractiveness of both species of mosses to flies (Marino & Raguso, n.d.). When comparing with other North American counterparts the hypophyses of Splachnaceae mosses have a wide range of colours (yellow; *S. ampullaceum*, green; *Tetraplodon angustatus* and *S. pensylvanicum,*, purple; *Tayloria dubyi*, white; *Tayloria mirabilis* and shades of red; *T. mnioides*, *S. rubrum*) in contrast to almost exclusively dull brown/reddish colors of brood site deceptive angiosperms. Among Splachnaceae, those taxa associated with carnivore dung (*T. mnioides, T. fueginiensis* and *T. angustatus*) are dull-coloured whereas among *Splachnum* there are also two dull-coloured taxa (*S. pensylvanicum* and *S. sphaericum*). However, *S. sphaericum* seems almost always to grow mixed with the very showy *S. luteum* (Marino, 1988). Dull colours in brood site deceptive angiosperms attract carrion flies for pollination (Urru et al., 2011), and Splachnaceae species having reddish-brown coloured of the hypophyses (e.g., *T. mnioides*, and *S. pensylvanicum*) are taxa relatively more attractive to carrion flies (Marino et al., 2009) and consequently their spores should be dispersed to carnivore dung or other carrion enriches substrates. Clearly visible contrasting yellow coloured sticky spore mass on top of the dull-coloured hypophyses are very suggestive of pollen to flies and stands out very distinctly (Marino & Raguso, n.d.; Piercey, 2016). These floral-like visual signals also suggest that floral mimicry is also a likely attractant, e.g., for both pollen and nectar feeders. This may be the reason why we haven't observed much escalation in numbers of fly taxa on mosses even with the enhancement of scent levels due to lack of deceiving colours that pollinating flies are attracted.

Overall, the effect of the scent manipulations was relatively minor, suggesting a highly generalized strategy of deception on the part of these two species of mosses. For the North American species, all species of Splachnum (with the exception of S. pensylvanicum) whose odour has been examed are, given their scent chemistry (Marino & Raguso, n.d.; Marino et al., 2009) herbivore dung mimics and grow on herbivore dung (Marino et al., 2009) whereas the two species of Tetraplodon are carnivore dung mimics (Marino et al., 2009) and grow on carnivore dung. To date, several North American species of Splachnaceae have been examined for their volatile compounds (*T. mnioides, T. angustatus*, *S. ampullaceum, S. luteum*, *S. pensylvanicum, S. rubrum and S. sphaericum)*. Their distributions, with the exception of *S. pensylvanicum,* which is mainly restricted to temperate forests of North America east of the Appalachian Mountains, overlap as they are mainly circumboreal (Marino, 1988; Marino & Raguso, n.d.). However, it is known (Marino, 2014b) that different species of Splachnaceae mosses are restricted to growing on different types of organic substrates. For example, *Tetraplodon* species grow on carnivore dung, and they have never been collected growing on herbivore dung, and *Splachnum* species have not been collected on carnivore dung (Marino pers. comm). The complex and diverse scent profiles of North American *Splachnum* species can, in general, have their scent chemistry divided into to two broad classes; sesquiterpenoid hydrocarbons (ubiquitous in terrestrial plants) and the octane-derived odors (Marino et al., 2009). *Splachnum* species are known to emit more than 50 volatiles from several biosynthetic classes including indole and phenol (odors in herbivore faeces), benzyl alcohol and 2-phenylethanol (flowers), and the alcohols and esters of propanoic and butanoic acids (fermenting sugar). However, each species has its own unique, pungent blend and concentrations of volatiles. Collectively, these volatiles represent a generalized strategy of targeting diverse fly taxa to disperse their spores by mimicking a broad spectrum of scent related to both brood sites and, likely, floral resources. This generalization in olfactory signaling can be suggested as the reason for the observed highly generalized dispersal networks with much faunal overlap. For example, *S. pensylvanicum* produce DMDS (Carnivore dung like scent) in addition to other volatile chemicals which are usually known as floral scents and herbivore dung scents. So, its attracted fauna overlaps broadly with *S. ampullaceum* but also attracts taxa associated with decaying flesh. This difference should provide *S. pensylvanicum* with alternative resource sites (e.g., the moose carcass enriched soil) but still result in *S. ampullaceum* and *S. pensylvanicum* growing together on moose dung as found at our study site. However, scent is not the only possible factor involved in promoting the diversity and coexistence of Boreal Splachnaceae as well as *S. pensylvanicum* since, for those species sharing the same resources in the same habitats e.g., S*plachnum* species growing on herbivore dung, the patchy ephemeral nature of dung and the potential of differential spore aggregation on fresh dung as a consequence of variable dispersal distances of fresh dung from mature populations may be a key factor promoting their coexistence (Hammill, 2016).

2.6.2 Effect of scent manipulation to the topology of the interaction network

Regardless of scent manipulation, the overall network specialization (Blüthgen, Menzel, Hovestadt, & Fiala, 2007) was low in these fly-moss interaction networks. This was expected because the *Splachnum*/fly interaction, being a propagule dispersal interaction with the absence of a reward, is not a tight complementary specialization as, for example, would be found in many plant-pollinator interactions (Bascompte & Jordano, 2007b; Blüthgen & Klein, 2011; Fenster, Armbruster, Wilson, Dudash, & Thomson, 2004; Paulo R Guimarães, Rico-Gray, Furtado Dos Reis, & Thompson, 2006; Johnson & Steiner, 2000; Waser, Chittka, Price, Williams, & Ollerton, 1996).

The majority of fly taxa were shared among both species both in unmanipulated and scent manipulated treatments, with few specialists identified (e.g. *F. fletcheri, S. sarraceniae, S. furcata*). However, for both *S. pensylvanicum* and carnivore scented treatments there was a small increase in overall network specialization primarily because of the addition of carrion flies whose abundance and frequency of visits increased.

The fly-moss interaction network is similar to seed dispersal networks in which there is also a low degree of network specialization (H_2 index for manipulated and non-manipulated *Splachnum*/fly interaction networks 0.1436 and 0.1519) (Blüthgen & Klein, 2011). This was expected as seed dispersal and deceptive dispersal do not require a high degree of specificity, unlike pollination (Gu, Goodale, & Chen, 2015; Mello et al., 2011, 2015) The complementarity of the overall network is high because most species of the network are associated with herbivore dung as herbivore dung is the most available brood site resource. Escalated attraction levels of dung and mosses with the addition of herbivore and carnivore scent mixtures resulted in more specialized flies and thus a more tightly connected network by increasing faunal fidelity to resources.

2.6.3 Scent manipulations and network nestedness

Scent manipulation influenced the affinity of fly taxa to each treatment and the number of flies attracted to each moss and dung treatment (Fig 2.4). As the number of taxa increased, the absolute number of interactions increased, which lowered the connectance (Jordano, 1987). This reduction of connectance in response to scent manipulation suggests that overall, the network gained more generalists where herbivore scent increased, whereas specialists were lost due to decreasing of both herbivore and carnivore scent levels. Thus the original non-manipulated moss-fly network may be more stable and robust (Dunne & Williams, 2002b, 2002a; Jordano, 1987) than the scent manipulated the fly-moss network. However, most taxa trapped in this study were also trapped on moose dung and, most of these taxa were trapped on both species of mosses as well. Thus there was no overall nested pattern. This observation is somewhat surprising as I expected that the interaction network would be significantly nested since most flies trapped on both species of mosses are trapped on moose dung. It is likely that this result was a consequence of the high overlap between the flies trapped on *S. ampullaceum* and those trapped on moose dung, whereas those trapped on *S. pensylvanicum* not only included the flies trapped on moose dung but in addition included flies associated with carnivore dung and/or carrion. Nonetheless, a nested pattern was found in two instances: when the scent of *S. pensylvanicum* was manipulated with herbivore scent and when *S. ampullaceum* was manipulated with carnivore scent. Both consequently attracted flies that are attracted to both herbivore and omnivore dung (dung treated with carnivore scent). Indeed, *S. ampullaceum* treated with DMDS could be treated as a mixed population of the two species of *Splachnum*; a situation that is common in the field. However, visual signals magnify visitation but have no statistical effect on faunal associations (, Marino and Raguso2016) Therefore, it is likely that some specialist flies associated with carnivore dung or carrion were less abundant with the addition of DMDS to *S. ampullaceum* as there is no *S. pensylvanicum* visual signal.

2.6.4 Implications for co-existence

The goal of this study was to better understand how Splachnacea mosses coexist. In previous experiments, it has been suggested that coexistence of these mosses has been achieved via several mechanisms. Competition-colonization trade-off (Chesson, 2000; Tilman, 1982) and aggregation mediated coexistence (Marino, 1991b) and dividing dung resources temporally and spatially (Hammill, 2016) were suggested as likely mechanisms. Additionally, Hammil (2016) suggested that the relative dispersal abilities achieved through species-specific differences may be in play promoting coexistence of mosses, but it hasn’t been tested. In this study, I set out to test whether there are any species-specific interactions occuring as a result of different olfactory signals in moss species at play resulting in differential spore dispersal to promote coexistence in Splachnaceae mosses.

This experiment re-confirms that olfactory signalling is the key factor that determines faunal associations. It also confirms that, in Newfoundland, both species attract a generalist fauna of flies that is closely associated with summer moose dung with few specialists. Although *S. ampullaceum* is more attractive to flies associated with moose dung, *S. pensylvanicum* attracts much the same fauna, although for some key taxa, fewer individuals. Since *S. pensylvanicum* produces DMDS in its hypophyses, although tiny and reddish-brown, the typical colour attractant associated with flesh flies (Jersáková, Johnson, & Kindlmann, 2006; Jürgens & Shuttleworth, 2015), it attracted more flesh flies when treated with DMDS than did *S. ampullaceum* suggesting that the combination of olfactory and visual signal is critical in determining the taxa than one sigtnal alone.

The underlying question of exploring the roles of signalling in *S. ampullaceum* and *S. pensylvanicum* is to understand better how these species coexist despite growing on the same substrate in exactly the same bog habitat. It is also know, from laboratory studies that *S. ampullaceum* is competitively superior to *S. pensylvanicum* (Hammill, 2016). Also, my study has shown that *S. ampullaceum* and *S. pensylvanicum* both attract a similar fly fauna to disperse their spores and that adding herbivore and carnivore scent to both species and to summer moose dung, does influence, although not profoundly, both the abundance and composition of flies and the taxa of flies visiting populations of each moss species of moss. The fact that *S. pensylvanicum* does attract flesh flies and, anecdotally at least, I know that *S. pensylvanicum* appears to have almost exclusive access to soils enriched with decayed carrion suggests that, perhaps, this resource provides a source population of *S. pensylvanicum* that is free of competition with *S. ampullaceum*. It is also possible that *S. pensylvanicum* is more tolerant of dry conditions than *S. ampullaceum* (Hammill, 2016). I also know that *S. ampullaceum* attracts a fly fauna more similar to that attracted to summer moose dung than does *S. pensylvanicum* and, from this and study done by Marino & Raguso, 2016that scent appears to play a key role in determining the differences in taxa and the abundance of individual taxa visiting these two species of moss. Taken together these differences may explain how *S. ampullaceum* and *S. pensylvanicum* coexist in Newfoundland habitats. Also, since there is much more herbivore than carnivore dung, i.e., there should be many more herbivore dung flies than carnivore dung/carrion flies which are specialized to *S. pensylvanicum*. Thus, the attraction levels for herbivore dung flies increased when S. *pensylvanicum* was manipulated with herbivore scent. This flexibility in signalling is important to *S. pensylvanicum* to survive on herbivore dung as well as carrion enriched substrates by spore dispersal to substrates visited by fly taxa associated with both moose dung and carrion. However, these two coexisting mosses have highly overlapping fly faunas, but they may be different enough such that spores of S. *pensylvanicum* are getting to resources to which *S. ampullaceum* is either not dispersed to and/or does not grow well on. In two instances, *S. pensylvanicum* have been found growing as a single large population on moist soil at the edge of bogs upon which moose carcasses have rotted and decayed. Only a few sporophytes of *S. ampullaceum* has been found growing with these patches in the same sites. However, it is unclear whether this difference in population size is a consequence of differential dispersal and/or growth ability on the enriched soil substrate. But it does suggest that *S. pensylvanicum* does have a refugia (decaying animal matter/flesh/enriched soil) from which is relatively unavailable to *S. ampullaceum.* Hence, this may be a contributing mechanism by which these mosses coexist. The spores of *S. pensylvanicum* that arrived in refuges less crowded with *S. ampullaceum* will be safe from competition, and thereby promote the local coexistence of these two mosses.

Considering the results from previous experiments on visual signal manipulation, growth experiments in different moisture conditions, and my study, all suggest that there can be several mechanisms in play to promote coexistence of these two mosses. However, it seems that suggesting a perfect mechanism of coexistence is difficult as well as way more complex in these mosses because similar to any other coexisting species there are many niche axes and many determinants of vital rates (Chave, 2004; Hammill, 2016).

However, appending all studies together, differential dispersal to different substrates through 1. attracting different taxa of spore dispersing flies using a combination of visual and olfactory signaling, 2. differences in maturation/phenology in the time of dispersal may likely cause spore aggregation on moose dung, can be suggested as likely mechanisms through which mosses can coexist even if they use only exactly the same resource.

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Chapter 3. Year to year variation in the spore-dispersing fly assemblages of brood site mimicking Splachnaceae mosses

3.1 Abstract

Understanding the dynamics of spore dispersal among sympatric Splachnaceae mosses is important to assess the importance of differential spore dispersal in promoting their coexistence. However, attempts to quantify temporal variation in the structure of dispersal networks and to determine the dynamics of the status of interacting species as generalists and specialists are rare. Here, I examined the deceptive fly-moss spore dispersal networks over three consecutive summers in two coexisting Splachnaceae mosses (*Splachnum ampullaceum* and *S. pensylvanicum* in Newfoundland, Canada. Both species grow primarily on summer moose dung either in single or mixed-species populations in the same bog habitat. Both species attract a subset of a larger Dipteran fauna associated with freshly deposited summer moose dung. However, the fly fauna associated with *S. ampullaceum* is generally more similar to flies associated with summer moose dung than those associated with *S. pensylvanicum* suggesting a higher dispersal advantage in *S. ampullaceum*. However, the relative abundance of taxa that appear to be reciprocally specialized were relatively constant over the three years.

Although the network structure varied among years, the assemblage of spore-dispersing flies was loosely arranged and highly generalized all three summers. Moreover, the lower degree of complementary specialization observed in this deceptive dispersal system suggests that it is different from plant-pollinator networks and more similar to seed dispersal networks. Additionally, both the moss and moose dung fly faunas changed synchronously from year to year. These results suggest that the availability of summer moose dung in the environment may cause the variation of the fly fauna and, consequently, the dispersal of spores. The presence of carrion flies, which are primarily attracted to *S. pensylvanicum* due to the presence of the scent of decaying flesh (dimethyl disulfide and indole), also likely ensures that *S. pensylvanicum* has a dispersal advantage to substrates such as carrion enriched soils

and may be a factor promoting the coexistence of *S. ampullaceum* and S. *pensylvanicum.*

3.2 Introduction

An understanding of propagule dispersal patterns is critical in understanding plant population dynamics and their geographic distribution (Nathan et al., 2002). Animal-mediated seed dispersal, for example, has been extensively studied and is among the most studied biotic interaction systems in terrestrial ecosystems (Petanidou, Kallimanis, Tzanopoulos, Sgardelis, & Pantis, 2008). During last two decades, development of plant-animal interaction network studies (Bascompte & Jordano, 2007) has further added to our understanding of animal-mediated seed dispersal (Heleno, Olesen, Nogales, Vargas, & Traveset, 2013; Mello et al., 2011). Most studies have explored seed dispersal by large frugivorous animals (Carnicer, Jordano, & Melian, 2009; Jordano, Garcia, Godoy, & Garcia-Castano, 2007; Kissling, Böhning-Gaese, & Jetz, 2009) whereas entomophilous dispersal has been relatively overlooked. However, several studies have examined "directed dispersal" by ants (Bond, 1983; Brew, O'Dowd, & Rae, 1989; Hanzawa, Beattie, & Culver, 1988; Howe & Smallwood, 1982; Miles & Longton, 1992; Nathan & Muller-Landau, 2000; Sorensen, 1986).

In several studies, properties of seed dispersal networks have been compared with pollinator networks (Bascompte, Jordano, Melián, & Olesen, 2003; Nico Blüthgen, Menzel, Hovestadt, & Fiala, 2007; Bosch, Martín González, Rodrigo, & Navarro, 2009). The network size of plant-pollinator, seed disperser and plant-ant networks are positively correlated with nestedness and the degree of asymmetry of these interaction networks (N Blüthgen, Cagnolo, Chacoff, & Va, 2009; Guimarães, Rico-Gray, Furtado Dos Reis, & Thompson, 2006; Vásquez, D.P., Aizen, 2004). However, in reality, plant-pollinator networks differ than seed dispersal networks in some properties. In general, most seed dispersal interaction networks deviate from a 1:1 ratio in the species richness of plants and animals. The deviation is not stronger than plant-pollinator systems but fluctuates around 1:2 (Nico Blüthgen et al., 2007; Guimaraes Jr et al., 2007) animal to plant ratio. Moreover, they are relatively loosely connected (Dupont, Padrón, Olesen, & Petanidou, 2009a; Smith-Ramírez, Martinez, Nuñez, González, & Armesto, 2005) with fewer specialists interacting with highly generalized species in the network (Bascompte et al., 2003). Also, Blüthgen et al. (2007) found that pollination networks are significantly more specialized than seed-disperser networks. This specialization occurs in networks because many flowers restrict visitation and accessibility to floral rewards through morphological modifications acting as barriers to some potentially interacting species whereas seeds and fruits are exposed and available to a wide spectrum of species (Stang, Klinkhamer, & Van Der Meijden, 2007). This difference in the accessibility of consumable rewards contributes to the observed difference in the specialization.

Seed plants, as is seen in most plant-pollinator and seed dispersal-disperser interactions, often use sensory signals to advertise the presence of floral rewards and seeds and fruits to their pollen and seed dispersal agents, respectively (H. Schaefer & Ruxton, 2009). In contrast, seed dispersal by passive adhesion does not actively recruit animals using sensory signals (Sorensen, 1986). Among plants, Splachnaceae mosses are, however, an exception as they use both visual and olfactory signals to attract Diptera to disperse their sticky spores thus resembling a similar mechanism present in most angiosperm seed dispersal systems. Splachnaceae species that attract flies to disperse their spores are restricted to growing on dung and other nitrogen-rich organic substrates such as old bones and owl pellets (Koponen & Koponen, 1977; Marino, 1991b; Marino, Raguso, & Goffinet, 2009). The sticky spores of Splachnaceae mosses provide no nutritional reward to insect visitors. Thus they resemble deceptive flowering plants that use both visual and olfactory signals to attract pollen dispersal agents by deception. The Splachnaceae is a globally distributed (Koponen, 1990) monophyletic lineage (Goffinet, Shaw, & Cox, 2004) with three subfamilies and 73 species (Crosby, Magill, Allen, & He, 2000; Goffinet et al., 2004). Nearly half of these species have their spores dispersed through brood-site deception (Cameron & Troilo, 1982; Marino et al., 2009).

In this study, I focused on the ecology of adhesive spore dispersal (epizoochory) of two sympatric moss species in the family Splachnaceae, *Splachnum ampullaceum*

Hedwig and *S. pensylvanicum* (Bridel) Grout ex H. A. Crum in eastern Newfoundland, Canada. *Splachnum ampullaceum* is an entirely circumboreal species, whereas the distribution of *S. pensylvanicum* is mainly restricted to North America east of the Appalachian Mountains (Marino, 1988; Marino & Raguso, n.d.). Consequently, in the study site in eastern Newfoundland, Canada, *S ampullaceum* is well within its geographic distribution, whereas *S. pensylvanicum* is at the northern limit of its geographic distribution. However, in Newfoundland, both species are found growing as single or mixed-species populations on summer moose (*Alces alces* L.) dung in bogs and fens. Summer moose dung is a patchy but widely available ephemeral substrate in Newfoundland as moose are very abundant (Hammill, 2016; Joyce & Mahoney, 2001; McLaren, Roberts, Djan-Chekar, & Lewis, 2004). At the study site from late spring through summer, fresh summer moose dung is colonized by spores that are carried by flies that are attracted to both the Splachnaceae mosses and fresh dung. The spores germinate quickly and, in general, the droppings become covered by leafy gametophytes within two summers. Sporophytes are, in general, produced the third summer (Marino, 1991b; Marino & Raguso, n.d.). Moose dung is mainly attractive to spore-dispersing flies when fresh; primarily one-two days after being deposited (Marino, 1991b). In the wet bog habitats in which these mosses grow, moss populations are generally overgrown following sporophyte production in year 3. At the study site, there is not complete overlap in sporophyte maturation between the two species of Splachnaceae. *Splachnum pensylvanicum* populations produce mature spores two times a season; initially, in mid-May early-June and the second set from late July to mid-August whereas*, S. ampullaceum* produces one set of mature spores per growing season generally from late June-mid August. Overall, however, spore production of these two coexisting mosses are never completely phenologically uncoupled, and mature spores can be seen in both mosses throughout the summer (Marino & Raguso, n.d.).

Both *S. ampullaceum* and *S. pensylvanicum* attract spore-dispersing flies using visual and olfactory signals that are associated with their specialized hypophysis; an inflated sterile region below the sporangium (Koponen, 1990; Koponen & Koponen, 1977; Marino et al., 2009; Pyysalo, Koponen, & Koponen, 1978). Sporophytes of *S.*

ampullaceum have long setae (15-65mm) relative to the short setae of *S. pensylvanicum* (2-10mm). At the apical end of the seta, distal to the sporangium both species have an inflated hypophysis. The hypophysis of *S. ampullaceum*, is initially yellow turning pink and occasionally red with senescence whereas *S. pensylvanicum* has a barely inflated green hypophysis that is dark red/brown or purplish distally (Marino, 2014). The olfactory signals of these mosses are strong but differ considerably and, in both species, are restricted to the hypophyses (Marino & Raguso, n.d.). *Splachnum ampullaceum* produces 69 volatiles including oxygenated aromatic compounds such as acetophenone, para-cresol and unusual cyclohexane carboxylic acids that are common in, for example, mammalian urine (Marino & Raguso, n.d.; McCuaig, Dufour, Raguso, Bhatt, & Marino, 2015) whereas the sporophytes of *S. pensylvanicum* also produce 62 volatile compounds includes cyclohexane carboxylic acids and dimethyl disulphide (DMDS) and indole both of which are found in carnivore dung. Consequently, the volatiles of *S. ampullaceum* mimics herbivore dung whereas the volatiles, of *S. pensylvanicum,* are more generalized due to the presence of DMDS and indole and mimic omnivore dung (Marino & Raguso, n.d.).

Marino and Raguso (unpublished) examined the relative roles of visual and olfactory signalling in *S. ampullaceum* and *S. pensylvanicum* and found that *S. ampullaceum* and *S. pensylvanicum* both attract flies associated with fresh moose dung. However, certain taxa of flies were relatively more associated with one species of moss relative to the other. Moreover, they found that the olfactory signals determined the taxa of flies associated with each species of moss, whereas the visual signals magnified the number of flies attracted to each species of moss. Consequently, each species of moss generally attracted a relatively distinct fly fauna and, regardless of whether the visual signals were masked or not, the fly fauna associated with each species did not change (Marino & Raguso, n.d.).

As the survival and coexistence of these mosses depend upon spore-dispersing flies, the moss-fly interactions of both species were examined in the context of interaction networks (Bascompte, Jordano, & Olesen, 2006; Olesen, Bascompte, Dupont, & Jordano, 2007). Exploring interaction networks can provide insights into the diversity of myophilous Splachnaceae, their geographic distributions and the demography of local associations. All recent studies exploring dispersal and coexistence in Splachnaceae mosses have considered fly-moss associations observed in one season and can be treated as ‘snapshot’ studies of faunal associations. As all myophyilous Splachnaceae that have been examined to date attract a wide diversity of flies (e.g., *S. ampullaceum* and *S. pensylvanicum* 12 and 10 families, and 25, 22 species, respectively (see chapter 2), only a multi-season approach provides a complete understanding of the degree of specificity and/or lack thereof among spore-dispersing flies. Moreover, insect populations fluctuate among years in response to various demographic factors such as local extinctions, changes in resource abundance, and the colonization of new species (Dupont et al., 2009a). External abiotic factors such as temperature (Alarcón, Waser, & Ollerton, 2008), precipitation and climate change (McMeans, McCann, Humphries, Rooney, & Fisk, 2015; Memmott, Craze, Waser, & Price, 2007; Tylianakis, Didham, Bascompte, & Wardle, 2008; Tylianakis & Morris, 2017) affect species’ abundances.

All myophilous Splachnaceae explored to date attract a relatively generalized fauna of flies of which relatively few taxa are found in large numbers, fluctuations in the abundance of key taxa may impact dispersal and potentially influence the coexistence of species that grow on the same dung type. Consequently, the role of fly spore dispersers in this deceptive spore dispersal interaction may be influenced by inter-annual fluctuations in the abundance of key taxa affecting the frequency and strength of interactions (efficiency of spore dispersal) and, ultimately, altering the topology of the network. Marino and Raguso (in prep.) have examined taxon-specific associations of both species of moss and mixed-species populations using network theory and found that the interactions were non-nested and consisted of highly generalized associations. In this study, I explore the possibility that annual variation in the abundance of local populations of flies may result in shifts in the degree of specialization and generalization between spore-dispersing flies and these brood site mimicking mosses. In this study, I examine the fly-moss interaction network in the same peatland over three consecutive summers to examine potential shifts in the network to better understand the dynamics of spore dispersal in these two species of

Splachnaceae and to gain insights into the potential role differential dispersal plays in promoting their coexistence.

3.3 Methods

This study was conducted in a roadside bog approximately 1 km from Salmonier Nature Park, Holyrood, Newfoundland (47.25 N -53.30 W). In this area, both *S. pensylvanicum* and *S. ampullaceum* are relatively common. Flies were trapped over three consecutive summers from June to August in 2007, 2008 and 2009. Flies were only trapped on dry days as the peristome teeth of Splachnaceae mosses close when wet and enclose the spores, preventing spore dispersal. Flies were trapped on mature equal-sized blocks of single-species populations of *S. pensylvanicum* and *S. ampullaceum* and fresh summer moose dung piles using funnel traps. Funnel traps were constructed using clean 2-litre plastic soda bottles and cutting just below their tapered end and inserting the inverted spout into the lower half (Marino, 1991b). To prevent trapped flies from escaping from the trap, nylon mesh funnels were inserted into the spout of each trap. Funnel traps were erected over single species moss populations and dung piles placed randomly in 5 blocks using metal stakes. Altogether, 15 treatments were placed on the study sites with five extra traps on the bog vegetation as controls.

During dry weather, the traps were set each morning for a total of 20 trapping days in 2007, 29 days in 2008 and 25 days in 2009 from July to August and trapped insects collected the following morning with the traps reset at that time. Insects were collected from traps by inserting a cotton ball dipped in ethyl acetate into the trap to kill the flies. The dead flies were placed into labelled plastic vials and taken to the lab for identification. Flies were identified to their families by using available keys (McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R. & Wood, 1981) and subsequently, DNA barcoding (Canadian Center for DNA barcoding) was done to confirm identifications and to identify flies to the species level. Collection of flies was deposited in the personal collection of Paul Marino at the Memorial University of Newfoundland, and complete digitized collection of flies was submitted to the BOLD (Rathnasingham & Hebert, 2007) system under the project name MKR.

3.3.1 Statistical analyses

Two fly-moss interaction matrices were created; a binary interaction matrix for years in which treatments (species of mosses and summer moose dung) were placed in rows and the flies trapped in each treatment were placed in columns. Interaction frequency matrices were constructed in the same way but replacing ‘1’s in columns by the number of individuals captured in each treatment. These two matrices were used in the following analyses.

Interaction frequency matrices were used to compare richness and inter-annual variation of network properties of the fly-moss-dung interaction networks. Species richness was calculated in each year, and rarefaction analysis was used to compare the richness of moss-spore dispersers. Rarefaction analysis was used to confirm that the number of unique interactions introduced to the network is accurate and not due to sampling effort. I generated the rarefaction curves with their 95% confidence intervals for three consecutive years to characterize the variation in the unique links formed between flies and treatments as a function of the total number of individuals captured in field samples, i.e. taxa plotted as a function of the accumulated number of individuals (Alarcón et al., 2008).

Interaction frequency matrices produced for three consecutive summers were analyzed to observe the concordance among matrices in each year using orthogonal least-squares Procrustes analysis performed in R (RStudio.inc, 2015), package “vegan” (Oksanen et al., 2007). Procrustes analysis is a powerful tool to compare network structure as based on matching species landmarks in each dataset. The m^2 (error) statistic was calculated as the sum of squared deviations between landmarks through translating, scaling and rotating the network configuration to match with the comparing configuration (Gower, 1971, 1975). The m^2 statistic varies from 1 to 0, with identical matrices having a value of 0. Deviations among species landmarks in the first network and superimposed comparing networks after the rotation are calculated as vector residuals. Close concordance between landmarks is indicated by small vector residuals. Vector residuals can be used to identify the fly species that exhibited greater changes over the three-year sampling period. The significance of these statistics was determined by performing 10,000 permutations (Jackson, 1995).

Over the three years of sampling, the design and treatments were kept the same, and the sampling effort was not drastically changed. However, the total number of individual flies captured, and unique moss-spore disperser associations formed in each year differed. Consequently, the possibility of observing inter-annual differences in network properties due to sample size is large (Alarcón et al., 2008). Therefore, to confirm that the analysis was not affected by sampling effort, I partitioned interaction frequency data from each summer into two subsets, respectively, from an observation made on alternating blocks, i.e. observations from the first, third and fifth blocks vs. those from second and fourth. All these six sub-matrices produced for three years were compared to each other using the Procrustes analysis similar to three full matrices to confirm that our sampling effort was sufficient to detect actual inter-annual changes in the fly-moss-dung network topology.

The binary version of the interaction matrices was used to calculate the network parameters. Network nestedness, which is a measure of the disorder of the network, was calculated for each of the three interaction networks. Nestedness (N), a measure of the degree of hierarchy in the organization of the interaction (i.e. information on whether the network consists of asymmetrical, specialized, random or compartmentalized interactions) was also calculated for each year (Bascompte et al., 2006; Reid & Armesto, 2011). N ranges from 0, in which the network is randomly organized, to 1, in which it is perfectly nested. In a perfectly nested network, the relatively more specialized species interact with a subset of all counterparts more so than do more generalized species. I used the ‘nestedrank’ function to calculate the rank of species in a matrix sorted for maximum nestedness. This gives generalists, who usually interact with all treatments, a rank closer to 1 and more specialized and rare species a higher rank. Standardized nested rankings of spore-disperser flies observed across three summers were tested for significant correlation (critical α=0.01, Spearman ranked correlation) to determine if the species appeared in the community matrix similarly over the three years (Alarcón et al., 2008).

To examine the interaction strength between fly taxa, species of moss and dung, I calculated species dependence (Bascompte et al., 2006). Species dependence is based upon the fraction of all visits by each fly taxa to a specific species of moss or moose dung as well as the standardized specialization index *d* (*d prime*) (Nico Blüthgen, Menzel, & Blüthgen, 2006) for flies for each year. The index *d* explains how specialized a given fly taxon is with respect to each species of moss and moose dung. Overall specialization, a measure of the selectiveness of bipartite networks (network specialization (H_2)), was calculated for every three bipartite matrices. When the observed interactions deviate more from the expected interactions given the species' marginal totals, H_2 becomes larger, suggesting species are more selective. All analyses were performed in RStudio 1.0.13 (RStudio.inc, 2015), and values were obtained by 1000 randomized networks generated by the null model r2dtable available in package bipartite (Dormann & Strauss, 2014; Mesquita-Neto, Blüthgen, & Schlindwein, 2018).

3.4 Results

Thirty-six species of flies from nine families were identified over the three consecutive years (2007: 31, 2008: 19 and 2009:16 species). There were a total of 2612 species links over the three sampling seasons (Table 3.1; species links/year: 2007 – 1414, 2008 – 556, 2009 - 642). Regardless of the different number of individuals in three yearly interaction networks, linkage densities were very close to each other.

Table 3. 1 Basic network parameters calculated for Splachnaceae moss-fly-summer moose dung binary networks in 2007, 2008 and 2009

Year	No of species	No of links	Connectance	Link per species	Nestedness	Linkage density	Shannon Diversity	Interaction evenness	H_2	Mean no of shared partners
2007	31	1414	0.699	1.912	24.973	4.215	2.835	0.625	0.224	16.000
2008	19	556	0.825	2.136	7.551	5.115	3.115	0.771	0.134	13.000
2009	16	642	0.875	2.211	5.687	4.538	2.933	0.758	0.136	12.667

Close to three times as many flies were captured in 2007 as were captured in either 2008 or 2009. The 2007 rarefaction curve and its 95% confidence interval fell inside the cumulative rarefaction curve, suggesting that the majority of Splachnaceae moss and dung associated flies were captured in 2007. Given that all three curves approached an asymptote (Fig. 3.1), it is unlikely that increased sampling would have identified more unique links. Moreover, compared to most diversity studies, the number of unique links between flies and mosses is relatively low (Chao, Colwell, Lin, & Gotelli, 2009). This result suggests that the sampling effort was sufficient to capture nearly the full spectrum of interacting species of flies with mosses and summer moose dung each year.

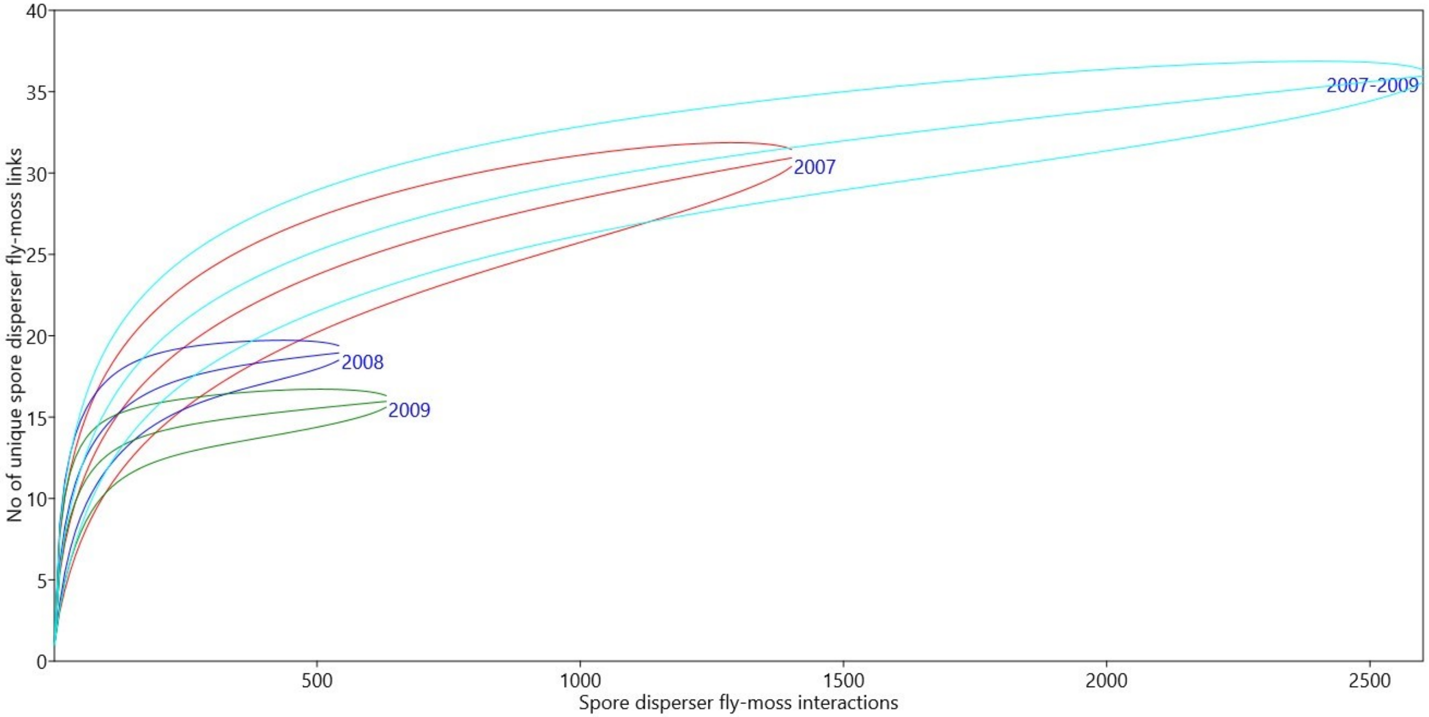

Figure 3. 1 Rarefaction curves with (95% CI) for the number of unique moss-fly-dung interactions recorded versus the number of visits observed in each summer and for the whole three years. The accumulation of links observed over the entire 3-year period is significantly greater than what is observed in a single summer, indicating significant annual variation in the identity of moss-fly-dung links.

The Procrustes analysis suggests that the topologies of three interaction networks differ. The matrices of 2007 and 2008 (m^2= 0.8613, p= 0.0125) and 2008 and 2009 (m^2= 0.7387, p= 0.0001) were concordant. However, the topologies of 2007 and 2009 were not concordant (m^2= 0.9155, p= 0.0842). However, since all Procrustes analyses approached zero, weak correspondences were present among matrices. The Procrustes analyses of the 6 sub-matrices showed a similar pattern: (2007 - 2008: m^2 = 0.8400, SE = 0.0587; 2008 - 2009 m^2 = 0.66565, SE= 0.000354). Similar to the full matrices, the four submatrices had low concordance (Mean m^2= 0.90450, SE = 0.01131). All submatrices exhibited low correspondence and non-significance ($p > 0.05$).

Residual vectors from the Procrustes analyses suggest that many fly taxa vary in their interaction levels among years (Table 2). Bipartite graphs were used to compare the differences in fly-moss-moose dung links in each year and show that some fly taxa appear on all three substrates (*S. ampullaceum, S. pensylvanicum,* and Moose dung) in one year and appear on only one or none of the substrates in the consecutive summer. Differences in links (Table 3.2) show what years each of the ten most abundantly trapped species was or were not trapped (+ vs. -). For example, the flesh fly, *Boettecheria cimbicis* was trapped on *S. ampullaceum* and *S. pensylvanicum* in 2007 but, it was not trapped on either species in 2008 or 2009 (see fig 2, 3 and 4). Similarly, the Calliphorid fly *Cynomya* sp. was trapped in 2007 and 2009 but not in 2008. These observations are unlikely a consequence of sampling errors as they are abundant on both species of mosses and moose dung when present.

Table 3. 2 Residual vectors from the Procrustes analyses for the ten most numerous fly taxa trapped that have the greatest between-year variation in fly-substrate interactions, the changes in the numbers of links to moss species and moose dung treatments with + representing the increased links while – showing the loss of unique links. The change in the number of trapped individuals is shown for each taxon and compared to the previous year.

Species	Residuals	ΔLinks	Δ Individuals
2007- 2008			
Boettcheria cimbicis	0.203	-2	4
Calliphora sp.	0.236	2	3
Cynomya sp.	0.194	-3	3
Dolichopus wheeleri	0.236	2	6
Drymeia sp.	0.194	-3	4
Hylemya sp.	0.215	1	1
Lucillia sp.	0.193	-1	1
Phervellia sp.	0.193	-1	1
Poccilagrapha sp.	0.215	1	6
Pollenia pediculata	0.219	2	2
Ravinia acerba	0.193	-1	1
2007-2009			
Boettcheria cimbicis	0.208	-2	4
Dolichopus wheeleri	0.233	1	1
Drymeia sp.	0.195	-3	4
Helina evecta	0.195	-3	19
Hydrotaea sp.	0.195	-3	17
Lucillia sp.	0.205	-1	1
Phervellia sp.	0.168	1	3
Pollenia pediculata	0.228	2	1
Ravinia acerba	0.205	-1	1
Tachytrechus sp.	0.238	2	9
2008-2009			
Calliphora sp.	0.210	-2	3
Cynomya sp.	0.252	3	24
Graphomya minuta	0.208	-2	26
Helina evecta	0.210	-2	7

Hydrotaea sp.	0.206	-2	8
Paraprosalpia sp.	0.252	3	18
Pegoplata tundrica	0.206	-2	37
Phaonia sp.	0.257	-3	1
Phervellia sp.	0.203	2	4
Tachytrechus sp.	0.203	2	9

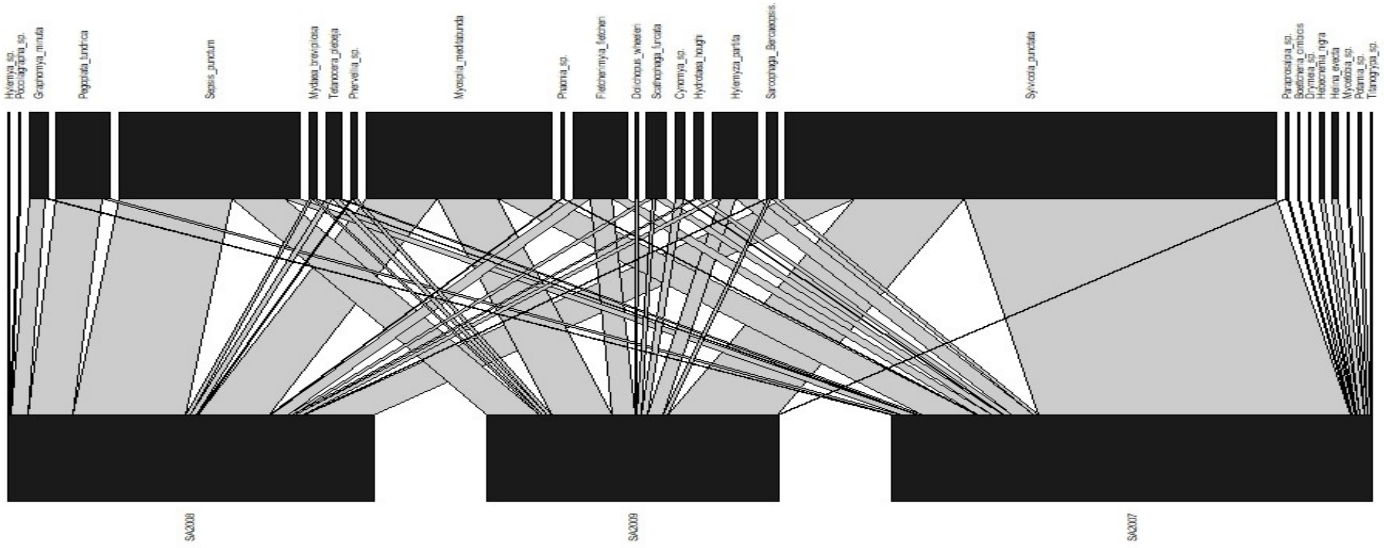

Figure 3. 2 An unconstrained functional size-based network of the moss-fly interaction network observed over three consecutive summers. Upper nodes represent *S. ampullaceum* (SA2007, SA2008, SA2009) and lower nodes represent taxa of flies captured in each treatment. Lengths of the nodes are proportional to the number of interactions of each node and width of edges indicates the number of individuals that have been captured on each treatment.

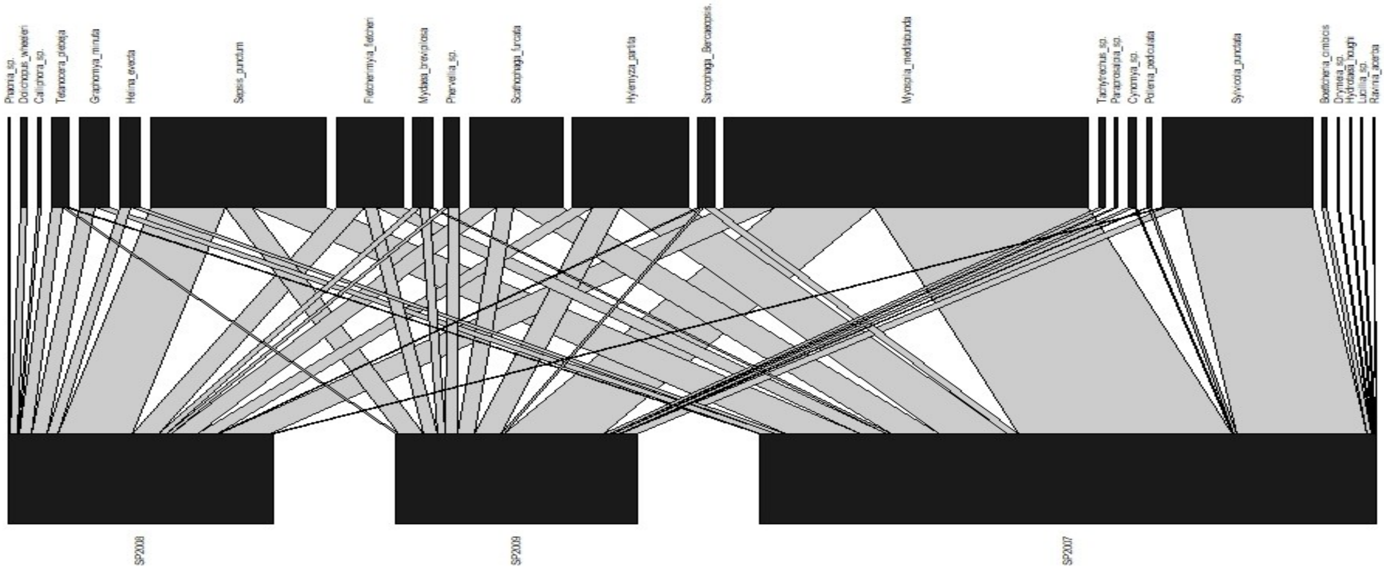

Figure 3. 3 An unconstrained functional size-based network of the moss-fly interaction network observed over three consecutive summers. Upper nodes represent *S. pensylvanicum* (SP2007, SP2008, SP2009) and lower nodes represent taxa of flies captured in each treatment. Lengths of the nodes are proportional to the number of interactions of each node and width of edges indicates the number of individuals that have been captured on each treatment.

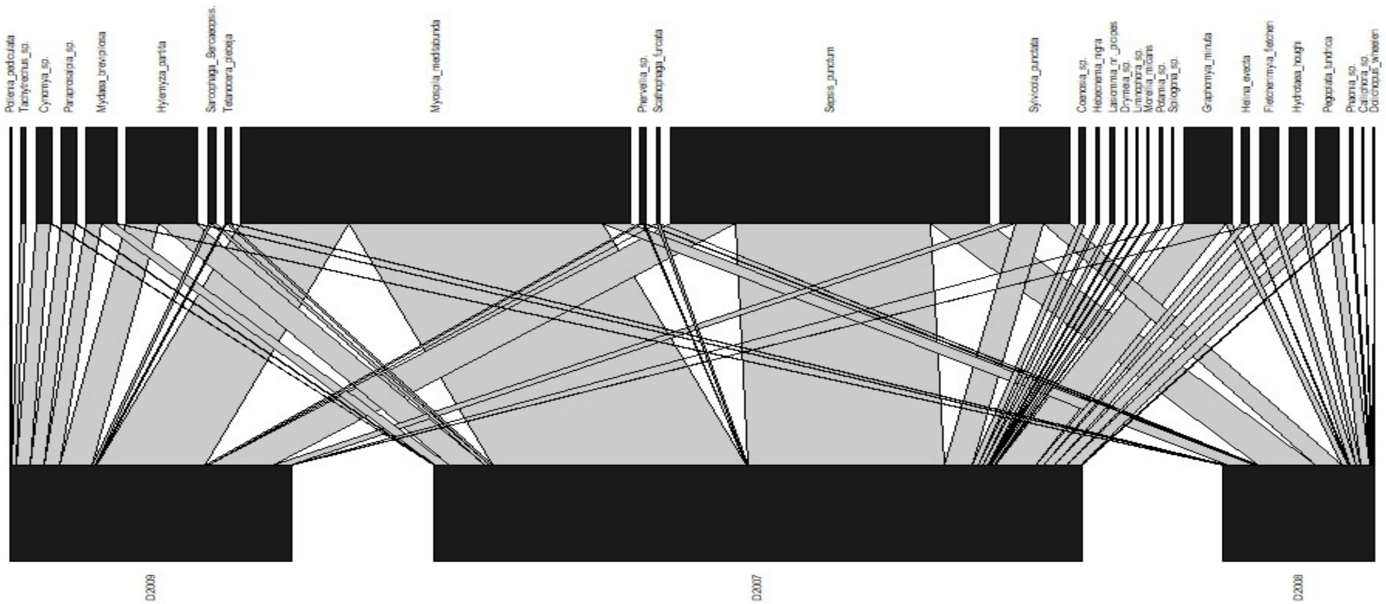

Figure 3. 4 An unconstrained functional size-based network of the summer moose dung-fly interaction network observed in three consecutive summers. Upper nodes represent dung treatments (D2007, D2008, D2009) and lower nodes represent taxa of flies captured in each treatment. Lengths of the nodes are proportional to the number of interactions of each node and width of edges indicates the number of individuals that have been captured on each treatment.

NOODF values suggest that the moss- fly-dung interaction network is very loosely arranged (2007 = 24.618, 2008 = 7.551, and 2009 = 5.687) even when pooled across the three summers (37.702). The networks also changed considerably from each other across the three years. Nestedness varied considerably (mean = 12.62, SE= 6.02) as the number of taxa and links changed over time Nestedness rankings were not correlated with the preceding years (2007 vs. 2008 rho = 0.405, p= 0.325; 2007 vs. 2009 rho= 0.024, p= 0.955; 2008 vs. 2009 rho = 0.595 p= 0.120) suggesting that, despite being present in the habitat and the links established, each fly does not interact in the same way in each summer with mosses, and moose dung and/or the abundance of many of the taxa vary considerably year to year.

Comparing the interaction networks among years showed that the identity/importance of flies varies among years and that there are low levels of specialization. Several species (*Sylvicola punctata, Sepsis punctum, Scathophaga furcata, Fletcherimyia fletcheri,* and *Pegoplata tundrica*) appeared in at least two consecutive summers and showed higher species strength and levels of specialization (Table. 3.3). Fly species that were detected over consecutive summers did not differ significantly in the level of specialization or dependence on mosses and moose dung.

Table 3. 3 Fly species captured in three consecutive summers, calculated species strengths and specialization values (*d*).

Family	Species	2007		2008		2009	
		d	Strength	*d*	Strength	*d*	Strength
Anisopodidae	*Mycetobia sp.*	0.147	0.004	0.000	0.000	0.000	0.000
	Sylvicola punctate	0.323	0.906	0.369	0.140	0.499	0.202
Anthomyiidae	*Hylemya sp.*	0.000	0.000	0.005	0.000	0.000	0.000
	Hylemyza partita	0.025	0.210	0.108	0.087	0.284	0.017
	Lasiomma picipes	0.118	0.007	0.000	0.000	0.000	0.000
	Pegoplata tundrica	0.084	0.034	0.192	0.165	0.000	0.000
Calliphoridae	*Calliphora sp.*	0.000	0.000	0.017	0.096	0.000	0.000
	Cynomya sp.	0.000	0.007	0.000	0.000	0.100	0.021
	Dolichopus wheeleri	0.000	0.000	0.033	0.148	0.006	0.109
	Lucillia sp.	0.077	0.002	0.000	0.000	0.000	0.000
Dolichopodidae	*Pollenia pediculata*	0.150	0.005	0.000	0.000	0.015	0.086
	Tachytrechus sp.	0.000	0.000	0.000	0.000	0.040	0.071
Muscidae	*Coenosia sp.*	0.125	0.008	0.000	0.000	0.000	0.000
	Drymeia sp.	0.000	0.009	0.000	0.000	0.000	0.000
	Graphomya minuta	0.074	0.090	0.146	0.004	0.003	0.000
	Hebecnema nigra	0.066	0.014	0.000	0.000	0.000	0.000
	Helina evecta	0.006	0.037	0.044	0.174	0.000	0.000
	Hydrotaea houghi	0.048	0.037	0.040	0.125	0.000	0.000
	Morellia micans	0.000	0.001	0.000	0.000	0.000	0.000
	Mydaea brevipilosa	0.042	0.032	0.043	0.043	0.097	0.057

	Myospila meditabunda	0.057	0.851	0.565	0.082	1.002	0.030
	Paraprosalpia sp.	0.036	0.005	0.000	0.000	0.067	0.077
	Phaonia sp.	0.048	0.006	0.022	0.012	0.000	0.000
	Poccilagrapha sp.	0.000	0.000	0.005	0.000	0.000	0.000
	Potamia sp.	0.067	0.013	0.000	0.000	0.000	0.000
	Spilogona sp.	0.000	0.001	0.000	0.000	0.000	0.000
Sarcophagidae	*Fletcherimyia fletcheri*	0.019	0.090	0.189	0.034	0.136	0.150
	Boettcheria cimbicis	0.125	0.011	0.000	0.000	0.000	0.000
	Ravinia acerba	0.077	0.002	0.000	0.000	0.000	0.000
	Sarcophaga sarraceniae	0.015	0.034	0.016	0.082	0.034	0.000
	Titanogrypa sp.	0.147	0.004	0.000	0.000	0.000	0.000
Sepsidae	*Sepsis punctum*	0.117	0.456	0.980	0.008	0.519	0.024
Scathophagidae	*Scathophaga furcata*	0.181	0.104	0.118	0.160	0.087	0.180
Sciomyzoidea	*Tetanocera plebeja*	0.003	0.016	0.069	0.058	0.037	0.020
	Phervellia sp.	0.000	0.001	0.039	0.028	0.076	0.060

3.5 Discussion

The analyses of the moss-fly interaction network over three consecutive summers supports my prediction that network topology and the role of spore-dispersers in the network fluctuate over time. In comparison to pollinator and seed disperser networks, this fluctuation is not atypical. But, there are few notable differences between these networks as well. For example, in general, most plant-pollinator and seed dispersal networks, both of which provide rewards, have more pollinator/disperser species than associated plant species (Dupont, Padrón, Olesen, & Petanidou, 2009b; Mello et al., 2011; Stang et al., 2007; Theodora, Athanasios, Joseph, Stefanos, & John, 2008). Moreover, most have few specialists (Atmar & Patterson, 1993; Bastolla et al., 2009; Dupont, Hansen, & Olesen, 2003; García, Martínez, Stouffer, & Tylianakis, 2014; Theodora et al., 2008; Vásquez, D.P., Aizen, 2004) interacting with core generalized plants and animals (Bascompte et al., 2006). This suggests that Splachnaceae brood site deception triggers innate behavioural responses in flies interacting with the mosses resulting in weak but sufficient interaction strength to provide spore dispersal service effectively. However, the generalized, loosely arranged networks and the lower degree of connectance observed in the fly-moss-dung deceptive spore dispersal network suggest that it is more similar to seed dispersal networks than pollinator networks. Furthermore, the study supports the idea that fluctuating network structure may be a result of the availability of multiple varying resources and thedifferential responses among functional groups of fauna towards those resources (McMeans et al., 2015) as influenced by climate, availability of breeding sites (Marino pers. communication) and synchrony of population trends between the two interaction groups (Kondoh, 2003; McCann, Rasmussen, & Umbanhowar, 2005).

Given the results of previous studies with myophilous Splachnaceae (Jofre et al., 2010; Marino, 1991a), the fauna of flies associated with *S. ampullaceum* and *S. pensylvanicum* was expected to be generalized with many taxa of flies attracted to the mosses. Moreover, I expected lower levels of faunal fidelity, specialization and loosely arranged networks given the deceptive nature of the interactions involving these mosses. This is unlike pollination interactions in which faunal fidelity and specializations are facilitated by floral morphology and floral rewards via the necessity that pollen is dispersed to the same species of plant. Thus, the high degree of variation among years in the abundance of individuals of the various taxa of flies attracted to *S. ampullaceum* and *S. pensylvanicum* was expected. Confirming the above hypothesis, results of this study suggest that these networks vary from year to year both in the number of individuals of the various taxa attracted and, to a lesser extent, the presence vs. absence of several taxa.

3.5.1 Are the moss/fly networks consistent over time or do their general patterns change in a similar manner?

This study revealed that faunal associations fluctuate among years. The three interaction matrices showed that the majority of fly taxa that were captured in large numbers on *S. pensylvanicum* and *S. ampullaceum* were also captured in large numbers on summer moose dung. Thus, these taxa can be considered key spore-dispersing species. Despite the year-to-year changes in the overall faunal composition, these taxa appeared in each network and maintained the same pattern in sharing substrates. This compartmentalization of species into varying and non-varying species may be due to the manipulation of these key species by the mosses by their unique olfactory and/or visual signaling. This manipulation or coupling can be seen as homologous to tightly connected primary consumers in food webs (McMeans et al., 2015) whereas more generalist flies appearing in the networks can be viewed as species that more regularly vary in abundance due to variation in local resource availability and/ or more flexible oviposition and/or foraging habits (McMeans et al., 2015). However, (Marino & Raguso, n.d.; Rathnayake & Marino, 2019) have shown that the differences in olfactory signals in the two mosses define the taxa of flies associated with each species and that the visual signals increase the abundance of visiting individuals. Thus, it appears that either these key species are tightly connected to the moss and dung substrates via signalling or they are simply the most abundant moose dung associated taxa in the local environment.

The network fluctuations appeared to be a function of the number of taxa whose abundances changed from year to year. This gives rise to notable differences in network structures in different summers. In 2007, 31 species were recorded, but the number of species trapped on mosses decreased from 19 to 16 in 2008 and 2009, respectively. The number of shared partners decreased due to the disappearance of highly connected taxa (for example *Boettcheria cimbicis* only appeared in 2007, *Hydrotaea houghi* appeared in 2007 and 2008 but not in 2009, and a gradual decrease in the number of individuals of *Hylemyza partita* from 2007 to 2009) and the addition of rare specialized taxa to the interaction network. For example, *Graphomya minuta* and *Pegoplata tundrica,* which were strongly associated with summer moose dung and *S. ampullaceum,* were abundant in 2007, declined in number in 2008 and disappeared in 2009. Also, rare taxa known to associate with moose dung and *S. pensylvanicum* such as *Phervellia* sp., *Tachytrechus* sp, and *Paraprosalpia* sp appeared in the 2009 network but not in the previous years. The 2007 network was especially unique and different from the other two as it had many more taxa, lower connectivity, and more shared partners. Though, these changes in species abundances and loss of tightly connected taxa can be attributed to the changes observed in network topology, the reason for these observed shifts in species guilds on mosses and dung is unclear. But, differential responses of some fly taxa to macro and microclimatic variation, flexible behaviors (McMeans et al., 2015) associated with these deceptive mosses and simply the asynchrony of life stages of mosses and flies can be suggested as possible reasons to these alterations observed in fly abundances.

3.5.2 How do these interactions compare to those in pollination and fruit dispersal networks?

The moss-fly-dung spore dispersal networks differ, as expected, compared to pollinator or seed dispersal networks. The Splachnaceae network is loose and generalized compared to pollinator networks with a mean nestedness (NODF) of 12.62, which is significantly higher than 0.72-0.98 (Alarcón et al., 2008) that have been calculated for plant-pollinator networks by Bascompte et al. (2003). Also, the specialization levels of individual fly taxa in the network are lower than what has been found among pollinator (Dupont et al., 2003; Smith-Ramírez et al., 2005) and seed dispersal (Gu, Goodale, & Chen, 2015) networks. However, because both species of mosses and summer moose dung shared the same visitors, the networks exhibited a higher connectance than has been recorded in pollination or seed dispersal interactions (mean connectance,: moss-fly-dung network = 0.799 vs. 0.0575 in literature) (Basilio, Medan, Torretta, & Bartoloni, 2006; Olesen & Jordano, 2002). With a higher level of connectance, the overall specialization of the network (*H'2*) tends to be lower than 0.3. This generalization of networks was expected as the network is based on deceptive signalling in which the flies receive no reward whereas in both pollination and seed dispersal networks there is a reward for visitors. However, the moss-fly-dung network does share some common network properties with seed dispersal networks as both have a lower degree of complementary specialization in contrast to pollination networks as both are more diffuse (Mello et al., 2011). The likely reason for this lower specialization is that there is nothing to be specialized about other than whether the flies are attracted to either species of moss as well as to dung and/or carrion. Flies are attracted to the mosses because their visual and olfactory signals both trigger obligate innate responses by olfactory and visual signals.

3.5.3 How are these differences between the Splachnaceae vs. pollination and seed dispersal networks explained by the generalized nature of the deceptive adhesive dispersal phenomenon?

The fluctuations in network properties over time may be explained by considering the taxa of flies associated with summer moose dung and the two species of coexisting mosses. Despite the different roles in the network as exhibited by the strength of the interaction and the degree of specialization between the various fly taxa and the mosses, almost all flies that were trapped on the mosses belong to the general categories of house flies, dung flies and blowflies (Koponen, 1990). *Scathophaga furcata* and *Sylvicola puctata* exhibited a tight connection with both species of moss and are also strongly associated with moose dung. These taxa are commonly associated with herbivore dung; for example, cattle dung (Lee & Wall, 2006; Webb, Beaumont, & Nager, 2007, Marino, Pers. com). Similarly, *Fletcherimyia fletcheri*; commonly known as flesh flies in family Sarcophagidae were trapped in all years and, in all years, had a higher level of affinity towards *S. pensylvanicum*. The Sarcophagids that were trapped in this study were known to use decaying materials and decaying insect residues in pitchers of pitcher plants as their breeding sites (Rango, 1999). Thus, their attraction to *S. pensylvanicum*, whose scent includes that of decaying flesh (Marino & Raguso, n.d.; Marino et al., 2009).

Despite the changes in many individuals recorded in each year, the ten most numerous fly taxa were consistently trapped in relatively large numbers each year. However, the majority were trapped on moose dung and *S. ampullaceum* with relatively fewer trapped on *S. pensylvanicum*. These high number of captures made the overall network more generalized as there were few specialized flies. Therefore, this study contributes to our ability to make inferences on the relative stability of spore dispersal to dung for *S. pensylvanicum* and *S. ampullaceum*. The study suggests that *S. ampullaceum* has a slight dispersal advantage to moose dung than does *S.*

pensylvanicum because it attracts fly taxa having higher interaction strengths to dung than those that are attracted to *S. pensylvanicum*. *Splachnum pensylvanicum,* however, because it also attracts carrion flies and has higher interaction frequency with these taxa, has several possible alternative growing substrates; moose dung, substrates enriched by decaying flesh and carnivore dung. Indeed, at the study site, we have seen two very large populations (1000's of sporophytes) of *S. pensylvanicum* growing on soil enriched with the decaying carcasses of moose whereas there were only a < 10 sporophytes of *S. ampullaceum* on this same nitrogen-enriched soils. It is unclear as to whether this is a pure dispersal phenomenon or whether *S. pensylvanicum* but not *S. ampullaceum* grows well on these enriched soils; nonetheless, *S. pensylvanicum* has an almost exclusive dispersal site on which it can grow. Laboratory experiments (Hammill, 2016) show that *S. ampullaceum* has a growth advantage over *S. pensylvanicum*, thus this dispersal difference between the two species may play an important role in promoting their coexistence (Hammill, 2016) as does the very nature of dung as a patchy ephemeral resource (Hammill, 2016; Marino, 1991b)

Apart from the effect of the difference in visual and olfactory signals of *S. ampullaceum* and *S. pensylvanicum*, the availability of breeding sites for flies in the vicinity of moss patches may be a cause for the annual variation in the network structure. Hammil, 2016 showed that the dispersal of spores to substrates depends on the distance of the nearest moss population with no evidence of differences between *S. ampullaceum* and *S. pensylvanicum*. Therefore, the absence of breeding sites may be another key factor defining the fly assemblages associated with these mosses. Curiously, moose are only recently introduced to the island of Newfoundland (Joyce & Mahoney, 2001; Mercer & Kitchen, 1968; Pitra & Lutz, 2005) and, due to the absence of predators are now considered to be hyperabundant (Mercer & Kitchen, 1968). Other than moose, the only large herbivore is caribou (*Rangifer tarandus caribou*. Gmelin), and their populations were never as large as that of moose (J. Schaefer & Mahoney, 2013), nor has either species ever been recorded collected on caribou dung (Marino, 1988). Moreover, it is completely unclear what exactly *S.*

pensylvanicum grew on, pre-European colonization of North America because most records for *S. pensylvanicum* are from cattle dung with a few records on bear dung (Marino, 1988, 2014). Because *S. pensylvanicum* produces both herbivore and carnivore scent, an association with bear dung is likely and, bears are native to Newfoundland. In conclusion, both the patchy and ephemeral nature of the substrate on which these mosses grow and their visual and olfactory signalling to mainly generalized but also several specialized taxa of flies are likely key factors promoting their coexistence.

3.6 References

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Chapter 4: General Summary

The circumboreal moss *Splachnum ampullaceum* and the primarily eastern North American moss (Frisvoll, 1978; Lüth & Goffinet, 2006; Marino, 1988) *Splachnum pensylvanicum* are restricted to growing on dung and other organic matter such as old bones and soils enriched by rotting carcasses. Both species of moss use brood-site deceptive signaling via both visual and olfactory signals to trick flies into visiting their mature sporophytes, where the sticky spores of the mosses adhere to the flies and are dispersed to fresh patches of dung and other nitrogen-enriched substrates. In Newfoundland, both *S. ampullaceum* and *S. pensylvanicum* are most often found growing either as single or mixed species populations on summer moose dung in *Sphagnum* dominated bogs and fens. The general focus of this thesis was to explore two components related to better understanding the mechanisms by which the mosses *S. ampullaceum* and *S. pensylvanicum* coexist in their peatland habitat. In the first study, olfactory signalling in both *S. ampullaceum* and *S. pensylvanicum* was manipulated to explore the extent to which the differences in odour between these two mosses influenced the fauna of flies that each species of moss attracted whereas, the second study explored year-to-year variation in the fly faunas attracted to unmanipulated populations of each species of moss to determine the degree of constancy in the general abundance and taxonomic makeup of the fauna of flies attracted to *S. ampullaceum* and *S. pensylvanicum.*

In this study, I showed that olfactory signalling is the key factor determining faunal associations in this deceptive spore dispersal system. This makes sense as the olfactory signalling in *S. ampullaceum* (McCuaig, Dufour, Raguso, Bhatt, & Marino, 2015) and *S. pensylvanicum,* although overlapping broadly, differ considerably from each other in certain volatile compounds and, as well, the strength of olfactory signalling is comparable to that of many brood site-deceptive flowers (Dafni, 1984; Dafni, Kevan, & Husband, 2005; Marino & Raguso, n.d.; Marino, Raguso, & Goffinet, 2009; Schiestl & Peakall, 2005). It has been found in a previous study that olfactory signaling is the primary determinant of the faunal difference between the flies attracted to each species of moss and that visual signaling (Marino & Raguso, n.d.), in contrast, primarily influences the number of individuals attracted. We also know, from this and other trapping experiments that the fly taxa attracted to both species of moss overlap considerably and represent a highly generalized fauna of boreal flies associated with dung and/or carrion. Therefore, the influence of the key determinants of scent (carnivore dung/carrion mimicry vs. herbivore dung mimicry) on influencing the taxa and abundance of flies trapped visiting each species of moss was experimentally tested by adding carnivore scent and herbivore scent to each species of moss and to summer moose dung. Also examined, in a separate study, was the constancy over time of this association between fly taxa and *S. ampullaceum* and *S. pensylvanicum* by comparing the fauna of flies associated with each species of moss across three consecutive years. Overall, the results suggest that both *S. ampullaceum* and *S. pensylvanicum* attract a very generalized fauna of flies associated with summer moose dung, and, although scent manipulation influenced the degree of attractiveness and the taxa of flies associated with each species of moss and summer moose dung, the effect of scent manipulation was not strong because many of the taxa are highly generalized as to what resources they use. However, the results of this study support the idea that key components of olfactory signals including dimethyl disulphide determine the taxa associated with *S. pensylvanicum*. Also, the magnitude of the signalling has a large effect on increasing the number of individuals from each attracted taxa.

The key results of the scent manipulation study are that non-manipulated *S. pensylvanicum* attracted a different fly fauna from summer moose dung and summer moose dung in which either herbivore or carnivore scent had been added. In contrast, *S ampullaceum* attracted the same fauna as summer moose dung and summer moose dung in which herbivore scent was added but differed from that attracted to summer moose dung in which carnivore scent was added. The only influence of scent manipulation to the fly fauna associated with S. *pensylvanicum* was that adding herbivore scent to *S. pensylvanicum* populations attracted a fly fauna that differed from that of summer moose dung with added herbivore scent. The addition of carnivore scent to *S. pensylvanicum* attracted more carrion flies (e.g., *Fletcherimyia fletcheri, Sarcophaga sarraceniae*). Also, adding herbivore scent to *S. pensylvanicum* attracted a different fly fauna than that attracted to non-manipulated *S. pensylvanicum*. When comparing faunal associations in scented treatments, it is noteworthy that one moss species could "convert" to another one with scent manipulation regardless of its visual signals. For *S. ampullaceum* with added carnivore scent, a different fly fauna from *S. pensylvanicum* was attracted whereas, with added herbivore scent, *S. pensylvanicum* was made to be more of an herbivore dung mimic whereas *S. ampullaceum* was made to be more of a carnivore dung mimic. This suggests that the interactions are very loose and almost no specializations are present between interacting partners in these deceptive spore dispersal interactions. Therefore, these networks can be viewed as similar to many seed dispersal interactions known to have more generalized interactions (Bastolla et al., 2009; Howe & Smallwood, 1982; Schleuning et al., 2011) vs. pollination interactions (Bosch, Martín González, Rodrigo, & Navarro, 2009; Stang, Klinkhamer, & Van Der Meijden, 2007; Vázquez & Simberloff, 2002) which are more specialized. However, a few "key species" were found in this highly generalized interaction who exhibit a relatively high degree of specialization to olfactory cues such as DMDS, the scent associated with carrion. This suggests that this deceptive network has some features common to plant-pollinator and plant-seed dispersal networks in having few core specialized species who are more tightly linked to their interacting counterparts (McMeans, McCann, Humphries, Rooney, & Fisk, 2015) whereas most of their interacting partners are relatively loosely linked.

When comparing these fly-moss interaction networks, there was a high degree of variation among years in the abundance of individuals of the various taxa of flies attracted to *S. ampullaceum* and *S. pensylvanicum*. Year to year variation in the faunal associated with each species of moss suggests that spore dispersal may also vary among years. For example, when Sepsid flies are abundant, the dispersal potential of *S. pensylvanicum* to moose dung will be enhanced because they are abundant on dung but more tightly linked to *S. pensylvanicum* than *S. ampullaceum*. However, network analyses suggest that the interaction network is highly generalized, as expected, and the generality was maintained throughout the years. The majority of flies trapped on treatments were nested in summer moose dung and tightly linked to each treatment. Thus high connectance was observed. However, the results suggest that, despite the variability of interactions among years, each year both species of moss attracted a generalized fauna with few fly taxa appearing to be specialized towards either the herbivore dung mimic S. *ampullaceum* (*Sylvicola punctate, Pegoplata tundrica*) or the omnivore dung mimic *S. pensylvanicum* (*Myospila meditabunda, Helina evecta, Fletcherimya fletcherii*). However, despite the variation among years in the number of individual flies trapped, the specialized taxa were present each year and these key taxa were responsible for the over faunal differences associated with each species of moss. These observations suggest that both species of moss, because they attract many of the same fly taxa, will both have their spores dispersed to summer moose dung but that because *S. pensylvanicum* also attracts carrion flies, its spores are more likely to be dispersed to carnivore dung and/or carrion than those of *S. ampullaceum.* This suggests that odour differences between *S. ampullaceum* and *S. pensylvanicum* may result in differential spore dispersal to different types of substrates. There is evidence suggesting that the consequence of even this small dispersal difference between the two mosses may result in resource segregation as in the local study region a very large populations of hundreds to thousands of sporophytes of *S. pensylvanicum* was found growing on moist soil at the edge of bogs upon which moose carcasses had rotted and decayed. At these same sites, there were only relatively tiny populations of *S. ampullulaceum* (< 10 sporophytes). Although it is unclear as to whether this difference is population size is a consequence of differential dispersal and/or growth ability on the enriched soil substrate, it does suggest that *S. pensylvanicum* does have a substrate type available to it that is relatively unavailable to *S. ampullaceum* .

Consequently, this segregation of resources between the two species of mosses may be a contributing means by which they coexist in eastern Newfoundland. Furthermore, these observations suggest that the varying degree of conspecific spore aggregation (Shorrocks & Atkinson, 1981) in patchy ephemeral resources is likely a major mechanism promoting their coexistence (Hammill, 2016).

Coexistence of these deceptive mosses, however, is likely to be a consequence of several mechanisms including differential dispersal to different substrates via specialist flies and/or differential growth abilities on substrates as well as simply the patchy ephemeral nature of dung as a growth substrate. Since, patchy ephemeral resources support diverse suites of organisms (Heard & Remer, 1997; Shorrocks, Atkinson, & Charlesworth, 2006) , the mechanisms by which the organisms sharing these substrates can coexist have been the subject of considerable interest (Atkinson & Shorrocks, 1984; Hammill, 2016; Hanski, 1981; Heard, 2006; Heard & Remer, 1997; Ives, 1987, 1991; Marino, 1991; Reader, Cornell, & Rohani, 2006; Shorrocks & Atkinson, 1981; Shorrocks et al., 2006). From this work the key mechanisms found to promote coexistence is the differential aggregation of propagules (e.g., eggs of flies on dung or carrion) (Atkinson & Shorrocks, 1984; Hanski, 1981; Heard & Remer, 1997; Shorrocks & Atkinson, 1981) of potentially competing species ((Fader & Juliano, 2013)) Using myophilous Splachnaceae mosses, Marino (1991) explored whether the spatial and temporal distribution of the resource itself, summer moose dung, promoted the aggregation of their spores on patches of fresh dung and thereby promoted their coexistence. Hammil (2016) followed up on this concept and showed that spore aggregation is achieved via the variable timing in the maturation of sporophytes of mosses growing on different patches and the availability of patches of fresh moose dung (flies are attracted mainly to 1-2-day old dung) locally and the proximity of fresh dung to mature populations.

Findings of this study, together with results in earlier studies exploring the dynamics of coexisting deceptive spore-dispersed mosses, can be applied more generally on similar deceptive propagule systems as well. Here, dispersal was identified as the key component of the natural histories of organisms bound to patchy ephemeral resources. Therefore, understanding the mechanics of dispersal among potentially competing species is key to understanding how species that share identical resources can coexist (Hammill, 2016; Marino, 1991). In this study, I have examined how the small differences in olfactory signaling affect the spore dispersal by flies. Moreover, I believe the next steps for consolidating our understanding of Splachnaceae ecology is to consider the effect that different substrates play in species interaction networks. As there is much more to learn regarding this dispersal network such as comparing fly communities in different microhabitats (e.g., relatively dry vs. wet bog habitat) and associated with different substrates in the local environment. For example, carrion enriched moist soil appears to be an almost exclusive substrate for *S. pensylvanicum,* but it is unclear what the relative roles of dispersal and/or growth ability play in promoting this observation. Thereby, we could integrate the effect of microhabitat and substrate on spore movement to have a more complete insight into the mimicry strategy used by these mosses.

In conclusion, the generalized nature of deceptive dispersal interactions was, as expected, relatively generalized compared to similar interactions involving a nutritional reward (e.g., pollination and seed dispersal). Also, assessing causes of varying species abundance, patterns of interactions (McMeans et al., 2015; Memmott, Craze, Waser, & Price, 2007) in these interaction webs is important assuming that the overall differences among yearly spore dispersal networks occurred mostly due to fluctuations of spore dispersing fly populations. However, the spatial distribution of resources (summer moose dung), mosses which makes consumer species to be more flexible in their visiting frequencies and to move across habitats to actively find their brood sites (McCann, Rasmussen, & Umbanhowar, 2005), coupling of generalists and decoupling of specialists (McMeans et al., 2015) on resources based on availability and population fluctuations in response to climatic variations can be suggested as most likely determinants of these changes. Moreover, these findings are in concordance with modelling studies suggesting that the spatial and temporal variability of patchy and ephemeral resources and the differential dispersal/aggregation of potentially competing species for these resources are key components that promote the coexistence of species restricted to patchy ephemeral resources (Hammill, 2016). Therefore, the patchy ephemeral nature of substrates, the variability of taxa attracted due to differences in volatiles present in each species of moss as well as the temporal variability of spore dispersing flies are likely key factors promoting the coexistence of *S. pensylvanicum* and *S. ampullaceum*.

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Appendix 1

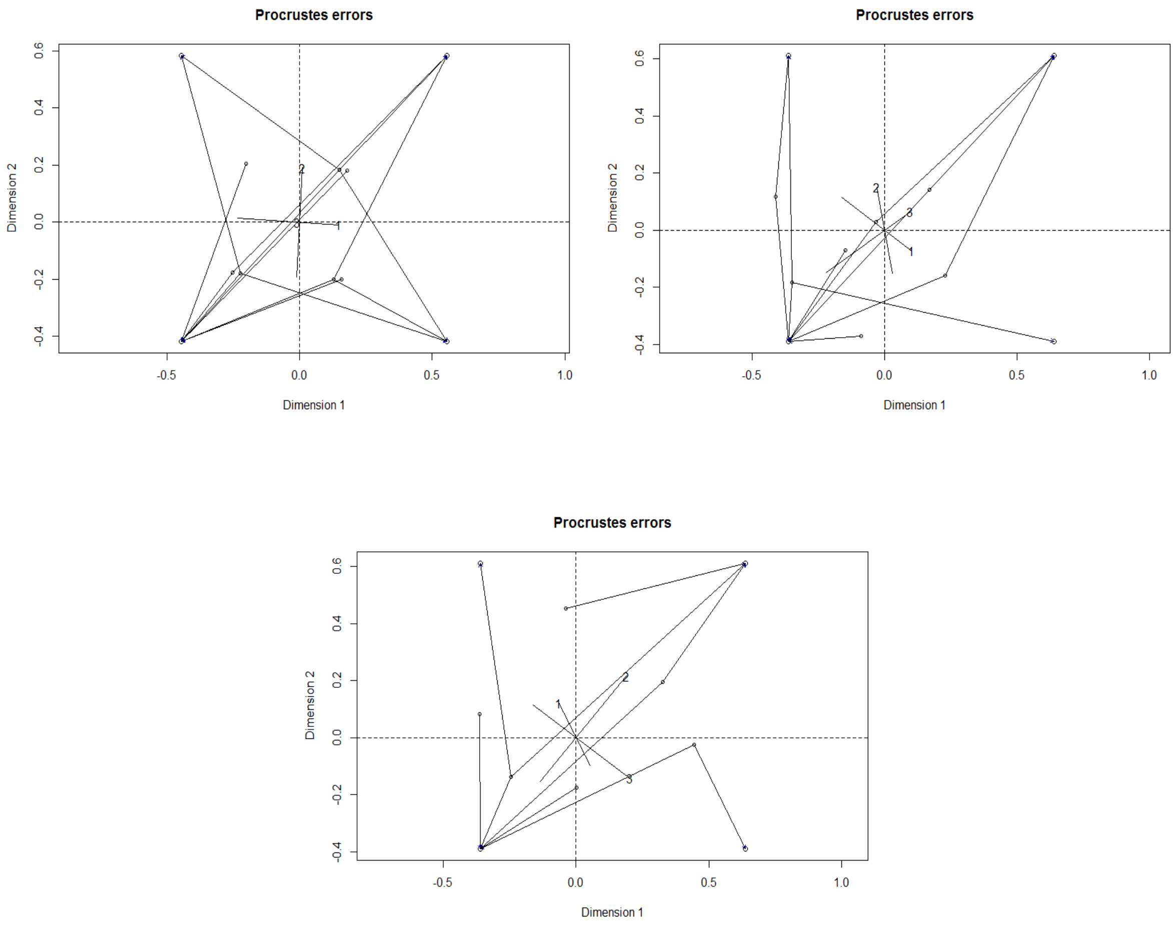
Appendix 1. 1Residual vectors from Procrustes analyses for spore disperser species between each consecutive summers.

Species	**Acronym**	**2007-2008**	**2007-2009**	**2008-2009**
Boettcheria cimbicis	**Bo**	0.203336954	0.208019522	0.081963639
Calliphora sp.	**Ca**	0.23647673	0.159480752	0.210410066
Coenosia sp.	**Co**	0.143675279	0.141685606	0.081963639
Cynomya sp.	**Cy**	0.19407729	0.056126054	0.251522478
Dolichopus wheeleri	**Do**	0.23647673	0.233471572	0.111413406
Drymeia sp.	**Dr**	0.19407729	0.194712152	0.081963639
Fletchermia fletcheri	**Fl**	0.070385499	0.056126054	0.087972483
Grphomya minuta	**Gr**	0.070385499	0.155362459	0.208468178
Hebecnema sp.	**Hb**	0.157802226	0.146366714	0.081963639
Hydrotaea sp.	**Hd**	0.133542827	0.194712152	0.20645382
Helina evecta	**He**	0.118720606	0.194712152	0.210410066
Hylemya sp.	**Hy**	0.215190638	0.159480752	0.168702521
Hylemyza partita	**Hz**	0.070385499	0.056126054	0.087972483
Lasiomma picipes	**La**	0.143675279	0.141685606	0.081963639
Limnophora sp.	**Li**	0.143675279	0.141685606	0.081963639
Lucillia sp.	**Lu**	0.192579751	0.20475282	0.081963639
Mycetobia sp.	**Mb**	0.169060536	0.163653641	0.081963639
Mydaea brevipilosa	**Md**	0.070385499	0.056126054	0.087972483
Morellia sp.	**Mo**	0.143675279	0.141685606	0.081963639
Myospila meditabunda	**My**	0.070385499	0.056126054	0.087972483
Paraprosalpia sp.	**Pa**	0.157802226	0.164852853	0.251522478
Pegoplata tundrica	**Pe**	0.082578056	0.146366714	0.20645382
Poccilagrapha sp.	**Pg**	0.215190638	0.159480752	0.168702521
Phaonia sp.	**Ph**	0.150690714	0.146366714	0.256933646
Phervellia sp.	**Po**	0.218571959	0.227989902	0.087972483
Pollenia pediculata	**Pr**	0.192579751	0.167612454	0.2032321
Potamia sp.	**Pt**	0.157802226	0.146366714	0.081963639
Ravinia acerba	**Ra**	0.192579751	0.20475282	0.081963639
Sarcophaga (Bercaeopsis)	**SB**	0.125083822	0.056126054	0.141362361
Scathophaga furcata	**Sc**	0.070385499	0.056126054	0.087972483
Sepsis punctum	**Se**	0.070385499	0.056126054	0.087972483

Spilogona sp.	**Sp**	0.143675279	0.141685606	0.081963639
Sylvicola punctata	**Sy**	0.070385499	0.056126054	0.087972483
Tachytrechus sp.	**Ta**	0.155956751	0.238420911	0.2032321
Tetanocra plebeja	**Te**	0.070385499	0.056126054	0.087972483
Titanogrypa sp.	**Ti**	0.169060536	0.163653641	0.081963639

Appendix 2

Appendix 1. 2 Rotation of network configurations of each couple of consecutive summers in Procrustes analysis. (Top left: 2007-2008, Top right: 2007-2009, Bottom: 2008-2009). Corresponding landmarks are shown in blue dots and black dots. The error (m^2) is calculated by minimizing sum of squares deviations between landmarks through rotating, translating and dilating one configuration over other till both match each other. Deviations between landmarks calculated as vectors.



Appendix 3

Appendix 1. 3 weighted fly-moss-moose dung interaction matrix recorded for three consecutive summers from 2007 to 2009. (SA= *S. ampullaceum*, SP= *S. pensylvanicum* and D= Summer moose dung)

Species	SA2007	SA2008	SA2009	SP2007	SP2008	SP2009	D2007	D2008	D2009
Boettcheria_cimbicis	1	0	0	3	0	0	0	0	0
Calliphora_sp.	0	0	0	0	2	0	0	1	0
Coenosia_sp.	0	0	0	0	0	0	6	0	0
Cynomya_sp.	1	0	4	1	0	4	1	0	16
Dolichopus_wheeleri	0	0	1	0	5	0	0	1	0
Drymeia_sp.	1	0	0	1	0	0	2	0	0
Fletcherimyia_fletcheri	8	9	13	18	19	9	13	7	1
Graphomya_minuta	1	9	0	10	11	0	45	7	1
Hebecnema_nigra	2	0	0	0	0	0	5	0	0
Helina_evecta	3	0	0	7	7	0	7	1	0
Hydrotaea_houghi	4	2	0	1	0	0	14	5	0
Hylemya_sp.	0	1	0	0	0	0	0	0	0
Hylemyza_partita	12	4	9	47	14	19	40	2	36
Lasiomma_picipes	0	0	0	0	0	0	5	0	0
Limnophora_sp.	0	0	0	0	0	0	1	0	0
Lucillia_sp.	0	0	0	1	0	0	0	0	0
Morellia_micans	0	0	0	0	0	0	1	0	0
Mycetobia_sp.	1	0	0	0	0	0	0	0	0
Mydaea_brevipilosa	1	2	1	3	5	6	15	1	17
Myospila_meditabunda	31	40	34	149	35	69	278	30	119

Paraprosalpia_sp.	1	0	1	0	0	2	1	0	15
Pegoplata_tundrica	4	26	0	0	0	0	14	11	0
Phaonia_sp.	1	1	0	0	1	0	2	2	0
Phervellia_sp.	0	1	3	0	3	8	1	3	3
Poccilagrapha_sp.	0	1	0	0	0	0	0	0	0
Pollenia_pediculata	0	0	0	2	0	2	0	0	1
Potamia_sp.	2	0	0	0	0	0	4	0	0
Ravinia_acerba	0	0	0	1	0	0	0	0	0
Sarcophaga_(Bercaeopsis)	3	1	2	7	2	2	5	0	3
Scathophaga_furcata	6	3	2	34	18	12	2	1	1
Sepsis_punctum	9	64	30	52	52	18	215	64	71
Spilogona_sp.	0	0	0	0	0	0	1	0	0
Sylvicola_punctata	177	39	62	91	2	11	31	28	18
Tachytrechus_sp.	0	0	0	0	0	4	0	0	5
Tetanocera_plebeja	2	4	3	2	8	2	3	1	2
Titanogrypa_sp.	1	0	0	0	0	0	0	0	0