Flies (Diptera) as pollinators of two dioecious plants: behaviour and implications for plant mating

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Abstract—We consider the role of generalist Diptera in the pollination of two dioecious plant species, *Clematis ligusticifolia* Nutt. (Ranunculaceae) and *Shepherdia canadensis* (L.) Nutt. (Elaeagnaceae). In particular, we assess (*i*) whether or not generalist pollinators are unable to distinguish between the sexes of dioecious species and so visit the sexes at equivalent rates, and (*ii*) the number of flowers that generalist flies visit and revisit during a foraging bout, which would affect self-pollination if plants were hermaphroditic. We determined the visitation rate to each plant species during 10 min periods and recorded the number of flowers that individual pollinators visited and revisited per foraging bout. Diptera were the main pollinators, visiting both sexes at similar rates for both plant species. The main visitors to *C. ligusticifolia* were muscoid flies (small and large), Culicidae, and halictid bees. The number of flowers visited in this species varied with pollinator group, but groups did not differ in the frequency of revisits. Visitors to *S. canadensis* were primarily Syrphidae and Empididae. Neither the number of flowers visited nor the number of revisits differed between these two pollinator groups. The results for each plant species are discussed and contrasted, particularly with other studies of the behaviour of generalist and specialist pollinators. We compare the observed pollinator behaviours, and their implications for plant mating, with the various theories of the role of pollinators in the evolution of the dioecious breeding system in plants.

Résumé—Nous étudions le rôle des diptères généralistes dans la pollinisation de deux espèces de plantes dioïques, *Clematis ligusticifolia* Nutt. (Ranunculaceae) et *Shepherdia canadensis* (L.) Nutt. (Eleagnaceae). En particulier, (*i*) nous déterminons si les pollinisateurs généralistes sont capables ou non de distinguer les sexes des plantes dioïques et, en conséquence, de visiter les deux sexes à des fréquences équivalentes et (*ii*) nous évaluons le nombre de fleurs que les mouches généralistes visitent une première fois et visitent de nouveau durant un même épisode de recherche de nourriture, ce qui affecterait l'autopollinisation si les plantes étaient hermaphrodites. Nous avons déterminé la fréquence des visites à chacune des espèces durant des périodes de 10 minutes et nous avons noté le nombre de fleurs que chaque pollinisateur visite et visite de nouveau au cours d'un même épisode d'alimentation. Les diptères constituent les pollinisateurs principaux qui visitent les deux sexes des deux espèces de plantes à des fréquences similaires. Les visiteurs principaux de *C. lusticifolia* sont des mouches muscoïdes, petites et grandes, ainsi que des Culicidae et des abeilles Halictidae. Chez cette espèce, le nombre de fleurs visitées varie d'un groupe de pollinisateurs à un autre, mais il n'y a pas de différences entre les groupes dans la fréquence des visites subséquentes. Les visiteurs de *S. canadensis* sont surtout des Syrphidae et des Empididae. Ni le nombre de fleurs visitées, ni le nombre de visites subséquentes aux mêmes fleurs ne diffèrent chez ces deux groupes de pollinisateurs. Nous comparons les résultats obtenus avec chacune des espèces de plantes et nous en discutons, particulièrement en regard d'autres études sur les comportements des pollinisateurs généralistes et spécialistes. Nous confrontons les comportements que nous avons observés chez les pollinisateurs et leurs conséquences sur

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la reproduction des plantes aux diverses théories sur le rôle des pollinisateurs dans l'évolution des systèmes dioïques de reproduction chez les plantes.

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Introduction

In contrast to most flowering plants, which are hermaphroditic, approximately 6% of flowering plant species have a dioecious breeding system, with distinct female and male individuals (Renner and Ricklefs 1995). The evolution and maintenance of dioecy in animal-pollinated species is often perplexing because female plants often seem less attractive than male plants, as they do not offer pollen and produce fewer flowers (Geber *et al.* 1999). Dioecy is commonly accompanied by pollination by small, generalist insects (Thomson and Brunet 1990; Sakai and Weller 1999), an association that is neither well explored nor understood (Charlesworth 1993; Ashman 2000). Generalist pollinators are typically considered to have few, if any, morphological or behavioural adaptations for flower feeding, and they are thought to indiscriminately visit any plants that are flowering in an area. In the absence of behavioural evidence, Charlesworth (1993) proposed that these generalist pollinators do not associate floral appearance with specific rewards (*i.e.*, pollen and nectar in males versus nectar only in females). This lack of discrimination by pollinators enables the evolution of female flowers by reducing the required resource allocation to attractive structures (Charlesworth 1993). Therefore, Charlesworth claimed that female flowers could not evolve in plants visited by discriminating pollinators because these flowers would not be visited sufficiently.

Ashman (2000) evaluated Charlesworth's proposal by studying the pollination of *Fragaria virginiana* Duchesne (Rosaceae), an herb with distinct female and hermaphroditic morphs (gynodioecious), which is pollinated by generalist Hymenoptera and Diptera. In contrast to Charlesworth's (1993) hypothesis, Ashman (2000) found that pollinators distinguish between female and hermaphroditic flowers, visiting hermaphrodites more frequently than females. These results agree with findings of previous studies of florally dimorphic plant species, which also reported that pollinators distinguish between male and female or hermaphroditic flowers (Bell *et al.* 1984; Agren *et al.* 1986; Bierzychudek 1987; Kevan *et al.* 1990; Ashman and Stanton 1991; Delph and Lively 1992; Eckhart 1992; Greco *et al.* 1996). Given these results, Ashman (2000) suggested that the association of dioecy and generalist pollinators results from the diversity of generalist pollinators, which reduces variance in pollination success by reducing dependence on any one pollinator species. Wilson and Harder (2003) came to a similar conclusion based on their theoretical finding that any process that reduces reproductive variance enhances the competitiveness of dioecy.

The role of generalist pollinators in the evolution of breeding systems and flower morphology in general remains uncertain because so few studies have explored the role of these pollinators (Kearns 1992; Kearns and Inouye 1994; Bernhardt *et al.* 2003; Goldblatt *et al.* 2004). In temperate North America, particularly at high elevations and latitudes, most generalist pollinators are opportunistic Diptera (Hocking 1968; Kevan 1973; Kevan and Baker 1983; Kearns 1992; Kevan *et al.* 1993; Larson *et al.* 2001; Vance *et al.* 2004). These adult flies use flowering plants as a source of sugars (nectar) and protein (pollen). However, most do not depend solely on flowers for nourishment, feeding also on carrion, feces, and other decaying organic matter. The role of opportunistic flies in pollination in general has received little attention, mainly because it is often thought that these flies do not depend on floral products as their primary food source and therefore visit plants in a haphazard way that does not promote effective pollination (Kearns 1992).

To date, most studies of opportunistic flies on flowers have considered their abundance (Elberling and Olesen 1999) rather than their behaviour within and between flowers and plants or their efficiency in pollen transport (but see Robertson 1992; Robertson and Lloyd 1993; Kearns and Inouye 1994; Totland 1994; Robertson and Macnair 1995). Of particular importance is the ability of flies to move pollen between plants, as opposed to either consuming pollen directly or causing self-pollination within and between flowers on the same plant (autogamy and geitonogamy, respectively).

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Self-pollination can reduce the pollen available for export to other plants (pollen discounting: *e.g.*, Harder and Barrett 1995) and can result in offspring with lower viability as a consequence of inbreeding depression (Charlesworth and Charlesworth 1987). Generalist pollinators may visit many flowers on a single plant, leading to high geitonogamy. Robertson (1992) showed that tachinid flies caused geitonogamy in about 50% of all pollinations in *Myosotis* plants (Boraginaceae) with large floral displays, but the amount of geitonogamy decreased with increasing floral display. Alternatively, generalist pollinators may visit only a few flowers on a plant and leave if they find empty flowers. This response would be particularly likely if generalists were abundant, so that individual flowers were visited frequently, and would produce little geitonogamy. Unfortunately, the pollination behaviour of generalists is not known well enough to predict whether they cause limited or extensive geitonogamy.

Plants with large floral displays are subject to extensive geitonogamy (Harder and Barrett 1995), which then may select for various self-isolation mechanisms that promote outbreeding, including dioecy (de Jong *et al.* 1999; de Jong 2000). Many studies of the evolution of dioecy have invoked a tendency of generalist pollinators to cause high levels of geitonogamy as a significant selective factor (Bawa and Opler 1975; Bawa 1980, 1994; Lloyd 1982; Charlesworth 1993; but see Renner and Feil 1993). Assessment of this claim awaits quantitative data on how generalist pollinators visit dioecious plants and their historical role in promoting geitonogamy to cause selection of dioecy over hermaphroditism in the ancestors of extant dioecious plants.

In this paper we address two main objectives. We first test Charlesworth's (1993) hypothesis that generalist pollinators, in this case flies, do not distinguish between male and female plants of dioecious species, but instead visit them at equivalent rates. Second, to assess the possibility that generalist pollinators cause extensive geitonogamy, which could select for dioecy, we consider the number of flowers that generalist flies visit per plant and the frequency of flower revisitation during a foraging bout. We will address questions of pollen removal and receipt, as well as fruit and seed set, in a subsequent paper, which will contrast reproduction of related pairs of dioecious and hermaphroditic species (see Borkent 2003).

Methods

Study species and sites

During May through July of 2001 and 2002, we studied the behaviour of insects visiting the flowers of two dioecious species, *Clematis ligusticifolia* Nutt. (Ranunculaceae) and *Shepherdia canadensis* (L.) Nutt. (Elaeagnaceae). This section describes natural-history characteristics of these species that are relevant to this study.

Shepherdia canadensis is a common dioecious shrub (Hitchcock and Cronquist 1973) of aspen (*Populus tremuloides* Michx. (Salicaceae)) and lodgepole pine (*Pinus contorta* Dougl. *ex* Loud. (Pinaceae)) forests in the Kananaskis region of Alberta, Canada. The population studied was found adjacent to the creek supplying water to the University of Calgary Kananaskis Field Station at Barrier Lake (51°02′N, 115°02′W), 500 m southeast of the station. We studied this species from 11 May to 8 June 2001 and from 25 May to 20 June 2002. Individual plants are up to 1 m tall and have many major branches splitting from the main stem near ground level, as well as further branching along these branches. This species flowers during early May before its leaves open, and often before all snow has melted. Flowers open rapidly, reaching peak flowering levels in a matter of days, which then last for 10 days (Borkent 2003). The mean maximum temperature during flowering was ~13 °C, with night temperatures frequently below 0 °C. A number of generalist Diptera species visit *S. canadensis* flowers during sunny periods when temperatures exceed 7–8 °C. No nocturnal pollinators were observed on this species, probably because of low night temperatures and a lack of nocturnal insects (as indicated by the failure of a light trap to attract insects during the flowering period). Both female and male plants produce numerous small, yellow-green flowers, with female flowers being slightly smaller than male flowers (3–4 mm *vs*. 5–6 mm diameter, respectively). Both sexes produce a thin film of nectar on the surface of swollen nectaries at the base of either the anthers or the stigma. Male plants produce 1.5 times as many flowers per inflorescence, begin flowering earlier, and display more flowers on any given day

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than females do. However, flower longevity was significantly shorter in males than in females (see Borkent 2003 for further discussion of flowering traits). Each female flower has a single uniovulate carpel and no evidence of anther development. Pollinated female flowers grow into single-seeded, fleshy, red fruits, which are dispersed during August and September by birds and large mammals (notably black bears). The male flowers typically have eight stamens (sometimes fewer) and usually no evidence of a carpel. Occasionally male plants produce a few hermaphroditic flowers, some of which are pollinated and produce fruit.

Clematis ligusticifolia is a large, dioecious (Hitchcock and Cronquist 1973), perennial vine that is supported by shrubs along prairie rivers, such as *Salix* spp. (Salicaceae) and *Shepherdia argentea* Nutt. We studied this species in Dinosaur Provincial Park, Alberta, Canada, along Little Sandhill Creek (50°46′04″N, 111°29′32″W) from 4 July to 24 July 2002, when the mean maximum temperature was 27 °C. It begins flowering during late June or early July and is pollinated by a variety of opportunistic Diptera and halictid bees. A few plants in the population had flowers that opened early (10 days before the others), with the majority of plants having flowers opening over the course of 5 days to reach peak flowering levels, which lasted for approximately 11 days (Borkent 2003). No pollinators were observed visiting plants at either dusk or dawn, suggesting that there are no nocturnal pollinators (though nocturnal pollinators were not looked for). Both sexes produce many white, open, nectar-bearing flowers and male plants produce larger flowers than female plants do (20–24 mm *vs.* 17–19 mm diameter, respectively). Male plants produce more inflorescences per plant, begin flowering earlier, and display more flowers on any given day than females do. Flower longevity did not differ between the sexes (see Borkent 2003 for further discussion of flowering traits). Male flowers lack carpels and have 10–61 stamens, which are arranged so that the anthers form a hemispherical dome, maximizing the surface exposed to pollinator contact. Female flowers have a central cone of 24–68 uniovulate carpels, which are surrounded by a ring of vestigial stamens (no anthers present). Each fertilized carpel produces a single seed and the persistent style grows into a plume that aids in dispersal by wind. Female flowers are often eaten, usually by the larva of an unidentified acalyptrate fly that consumes the ovules and then other parts of the flower. Mule deer also eat large parts of plants of both sexes, leading to a loss of flowers and fruits.

Measurement of insect behaviour

The behaviours considered included the rate of visitation (number of individuals/min), the number of flowers visited per individual, and the frequency of revisits to individual flowers. Only insects that came in contact with either an anther or a stigma were recorded as pollinators. Observers wore neither insect repellent, sunscreen, nor bright clothing to minimize effects of their presence on fly behaviour.

Fly visitation rate was measured during 10 min periods throughout the day, for both plant species, with the same number of observations for both sexes. For *C. ligusticifolia*, we observed the flowers on a random section of an individual plant, usually consisting of 2–3 branches (9–52 flowers). The number of flowers being observed was counted and the time of day noted. The day was divided into three periods (0630–1000, 1000–1600, and 1600–2100) and similar numbers of observations were made during each period to ensure that we observed the full diversity of diurnal pollinators. Visitors to the observed portion of a plant during the 10 min observation period were counted and each visitor was identified to family. The same protocol was used to determine visitation rate for *S. canadensis*, except that the number of flowers observed was not recorded owing to the large number available (>1000). Also, pollinator observations occurred from only 1000 to 1800, since no pollinators were seen before 1000 or after 1800, as it was usually too cold (<8 °C) outside of this time period for pollinators to fly. Observations were spread evenly throughout this 8 h period.

Insect behaviour on plants was measured by observing a section of a plant until a visitor arrived. The type of insect and the characteristic of each flower visit (first visit or revisit) were recorded until the insect left the plant. The total time that the visitor spent on the plant was also noted, along with time of day.

Statistical analysis

The number of visitors per 10 min period is a continuous variable and was therefore analyzed with either generalized linear models (McCullagh

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Table 1. Number and taxonomic distribution of visitors to *Clematis ligusticifolia* flowers, including number of visitors to male and female plants.

Visitor group	No. of visitors	Visitors to female plants	Visitors to male plants
Large muscoid flies (>6 mm)	89	36	53
Small muscoid flies (<6 mm)	70	31	39
Halictidae (Hymenoptera)	21	8	13
Culicidae	14	5	9
Calliphoridae	4	1	3
Mycetophilidae	1	0	1
Syrphidae	1	0	1
Large solitary bees	1	1	0
Unidentified acalyptrate Diptera visitors (with <2 visits)	12	8	4
Total	213	90	123

and Nelder 1989; genmod procedure of SAS®, release 8.2, SAS Institute Inc., Cary, North Carolina) or Kruskal–Wallis tests (Zar 1984; npar1way procedure of SAS®, release 8.2). The generalized linear model for *C. ligusticifolia* considered sex and period of day as categorical independent factors and number of flowers observed as a covariate. This analysis treated visitation rate as a Poisson-distributed variable, and so involved ln transformation (McCullagh and Nelder 1989). Statistical significance of effects was assessed with likelihood-ratio (G) tests. We compared differences in visitation rate between female and male plants of *S. canadensis* with Kruskal–Wallis tests.

We compared the numbers of flowers visited and revisited per plant with respect to a plant's sex and pollinator groups with contingency tables. Because of a high frequency of expected values <5, we used exact χ^2 tests (freq procedure of SAS®, release 8.2). The number of flowers revisited during individual pollinator visits was compared between the main pollinator groups and between the sexes. The total number of visits by each main pollinator group was also compared between the sexes.

Analyses of variance (Neter *et al.* 1996; glm procedure of SAS®, release 8.2) were used to analyze the number of different flowers visited and the total number of flower visits per pollinator. These analyses considered plant sex and pollinator type as categorical independent factors. Differences between pollinators were evaluated using Tukey–Kramer multiple comparisons (Kirk 1995).

Results

Visitors to *Clematis ligusticifolia*

A diverse fauna of flies and bees visited *C. ligusticifolia* flowers, of which nearly 90% were Diptera (Table 1). Four insect groups comprised 91% of the visitors to flowers of *C. ligusticifolia*. Large (>6 mm) and small (<6 mm) muscoid flies made up 42% and 33% of the visitors, respectively. The two other predominant visitors were Halictidae (Hymenoptera, 10%) and Culicidae (mosquitoes, 7%). We restricted analysis of behaviour on inflorescences to these four groups.

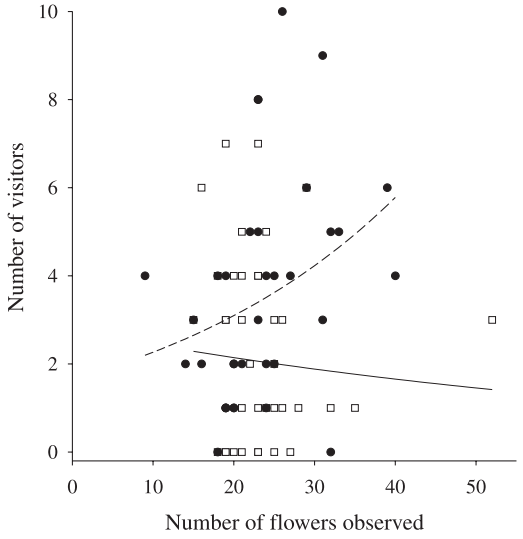
On average, about three insects visited the study portions of *C. ligusticifolia* plants during each 10 min period. The rate of visitation did not differ with a plant's sex ($G_1 = 3.10$, $P > 0.05$, $n = 72$), the period of day ($G_2 = 3.11$, $P > 0.2$), or the number of flowers observed ($G_1 = 1.35$, $P > 0.2$). However, the number of visitors increased with the number of flowers observed for male plants but not for female plants (Fig. 1, sex × flower number, $G_1 = 3.91$, $P < 0.05$).

Insects typically visited a mean of almost two *C. ligusticifolia* flowers before departing, although this number differed somewhat among pollinator groups (Fig. 2: $F_{3,59} = 4.35$, $P < 0.01$). In particular, small muscoid flies visited significantly fewer flowers than large muscoid flies (Tukey's test, $P < 0.005$), but neither group's visitation frequency differed significantly from that of halictid bees or that of mosquitoes. Insects visited the same numbers of flowers on female and male plants ($F_{1,59} = 0.30$, $P > 0.5$), regardless of their taxonomic affiliation (visitor

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Fig. 1. Relation of the number of insect visitors, at flowers on male (●, dashed regression line, $n = 35$) and female (□, solid regression line, $n = 37$) *Clematis ligusticifolia* plants, to the number of flowers observed, during 10 min periods.



type × plant sex interaction, $F_{3,59} = 1.20$, $P > 0.25$).

For the four main groups of visitors to flowers of *C. ligusticifolia*, we observed a total of 233 flower visits. The proportion of visits to female and male plants did not differ significantly among the four main pollinator groups (exact $\chi^2 = 3.32$, df = 3, $n = 67$, $P > 0.35$). Only 13 (5.6%) of the observed flower visits were revisits. The incidence of revisits did not differ between plant sexes (exact $\chi^2 = 3.80$, df = 3, $n = 75$, $P > 0.25$) or among pollinator groups (exact $\chi^2 = 10.88$, df = 9, $n = 67$, $P > 0.25$).

Insects spent much time visiting each *C. ligusticifolia* flower, with the mean duration of visits to individual flowers ranging from 20 to 142 s for different pollinators. The rate at which insects visited flowers differed primarily among taxonomic groups ($F_{3,59} = 9.62$, $P < 0.001$; Fig. 3). Small muscoid flies visited flowers at the same rate as mosquitoes (Tukey's test, $P > 0.85$) but visited significantly fewer flowers per second than large muscoid flies and halictid bees (Tukey's test, $P < 0.001$ in both cases). Mosquitoes also visited significantly fewer flowers per second than halictid bees (0.01 flower/s *vs.* 0.04 flower/s; Tukey's test, $P < 0.05$). The rate of flower visitation by large muscoid flies did not differ significantly from that of either halictid bees or mosquitoes

Fig. 2. Mean (±SE) number of *Clematis ligusticifolia* flowers visited per bout by the four main types of visitors: Halictidae, Culicidae, and small (<6 mm, □) and large (>6 mm, ▽) muscoid flies.

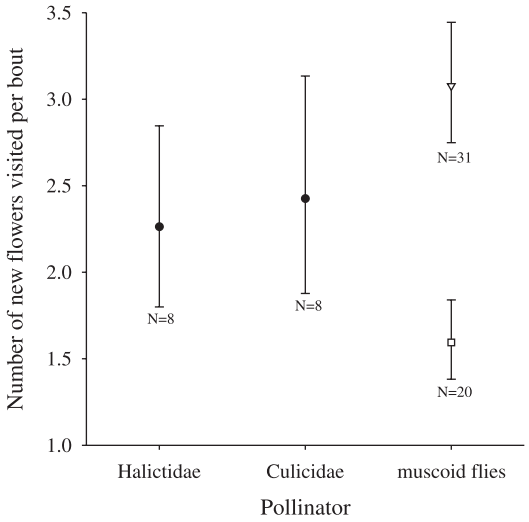
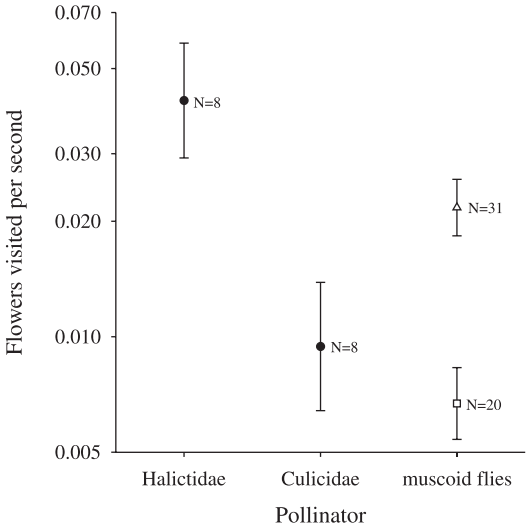


Fig. 3. Mean (±SE) rate at which individual insects visited *Clematis ligusticifolia* flowers, for the four main types of visitors: Halictidae, Culicidae, and small (< 6 mm, □) and large (>6 mm, △) muscoid flies. Note the ln scale of the ordinate.



(Fig. 3; Tukey's test, $P > 0.20$ in both cases). On average, insects visited flowers on male plants almost twice as fast as they visited flowers on female plants (0.021 *vs.* 0.012 flower/s), but this difference was not quite significant ($F_{1,59} = 3.96$, $P = 0.051$). Differences in the rate at which different pollinator groups visited

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Table 2. Number and taxonomic distribution of visitors to *Shepherdia canadensis*, including the number of visitors to male and female plants.

Visitor group	No. of visitors	Visitors to female plants	Visitors to male plants
Syrphidae	33	9	24
Empididae	30	3	27
Muscidae	4	1	3
Calliphoridae	3	0	3
Ceratopogonidae	2	2	0
Cecidomyiidae	1	1	0
Unidentified Diptera	15	4	11
Symphyta	1	1	0
Parasitic Hymenoptera	2	2	0
Total	91	23	68

Note: Not all visitors observed during measurements of visitation rate were seen when recording flower visitation patterns, owing to their infrequent visits.

flowers did not vary significantly between female and male plants (insect type × sex interaction, $F_{3,59} = 0.02$, $P > 0.9$).

Visitors to *Shepherdia canadensis*

Diptera comprised almost 97% of the insects observed on flowers of *S. canadensis*. Most dipteran visitors were either Syrphidae (36%) or Empididae (33%), with occasional visits by Muscidae (4%), Calliphoridae (3%), Ceratopogonidae (2%), and Cecidomyiidae (1%). Many flies visited too quickly to be identified. A few visiting Hymenoptera were also noted (Table 2). During the observations of flower visitation patterns, we observed more than one individual of each of Syrphidae, Calliphoridae, Empididae, Ceratopogonidae, and Cecidomyiidae.

On average, fewer than three insects visited *S. canadensis* plants per 10 min period. Even though insects visited, on average, three times more flowers on male plants (4.0 ± 1.21 visitors, $n = 22$) than on female plants (1.3 ± 0.47 visitors, $n = 20$), this difference was not statistically significant (Kruskal–Wallis test, $\chi^2 = 1.13$, df = 1, $n = 42$, $P > 0.2$).

Flies typically visited just more than 6 (SE = 1.2) flowers of *S. canadensis* before leaving, regardless of the family to which they belonged ($F_{4,17} = 1.44$, $P > 0.25$) or the sex of the plant ($F_{1,17} = 0.02$, $P > 0.85$). This pattern did not differ among Diptera families (family × plant sex interaction, $F_{4,17} = 0.95$, $P > 0.40$).

Of the 246 recorded visits to flowers of *S. canadensis*, 62 (25.2%) were revisits. Revisits were equally frequent on female and male plants (exact $\chi^2 = 8.66$, df = 7, $n = 27$, $P > 0.25$) and by the five families of visitors (exact $\chi^2 = 41.98$, df = 28, $P > 0.05$). Furthermore, the proportion of revisits on female versus male plants did not differ among the five pollinator families (exact $\chi^2 = 1.63$, df = 4, $P > 0.85$).

Visitors to *S. canadensis* spent a long time visiting the individual small, nectar-poor flowers, with means ranging between 9 and 67 s per flower for different pollinators. The rate of insect visitation to flowers varied among pollinator families (Fig. 4; $F_{4,6} = 8.05$, $P < 0.05$), with Empididae visiting fewer flowers per second (0.015) than Syrphidae (0.11; Tukey's test, $P < 0.05$). The rate of flower visitation did not differ between female and male plants ($F_{1,6} = 0.79$, $P > 0.40$), regardless of pollinator family (pollinator family × plant sex interaction, $F_{2,6} = 4.88$, $P > 0.05$).

Discussion

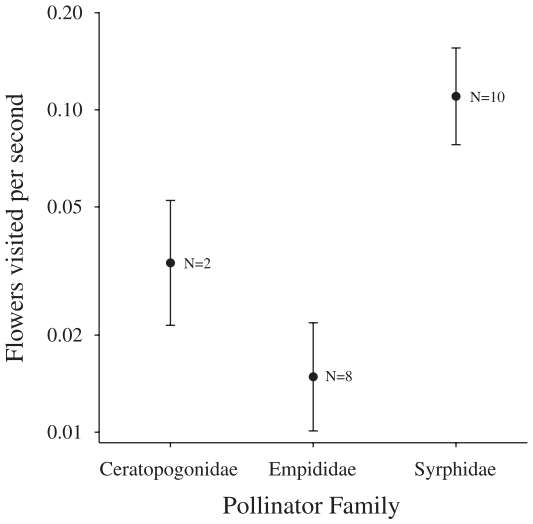
Characteristics of visits to *Clematis ligusticifolia* and *Shepherdia canadensis* flowers

Opportunistic Diptera were the main insects visiting flowers of both *C. ligusticifolia* and *S. canadensis*, but the families of Diptera differed between plant species. The dissimilar pollinator faunas likely reflect both habitat differences (prairie riparian zone *vs.* aspen and pine forest) and the emergence times of different Diptera families. Unfortunately, little is known about the emergence timing of most families of Diptera, except that they are often most abundant

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Fig. 4. Mean (±SE) rate at which individual insects visited *Shepherdia canadensis* flowers, for the three families of visitors that visited both sexes of plants: Ceratopogonidae, Empididae, and Syrphidae. Note the ln scale of the ordinate.



during spring and early summer (Kato *et al.* 1990). Mimetic Syrphidae, like those that we observed on *S. canadensis*, are known to be early-spring flower visitors (Waldbauer 1983). Adult Mycetophilidae often overwinter as adults and also emerge early in spring (Vockeroth 1981).

The rate of insect visitation to flowers was similar between the two plant species (about one visitor every 3 min) as well as between female and male plants within each plant species. Insects tended to visit male plants of *C. ligusticifolia* with many flowers more frequently than they visited female plants with any number of flowers (Fig. 1). This suggests some discrimination, although this difference may reflect plant size rather than floral characteristics; few female plants displayed many flowers. The visitation rate to *S. canadensis* varied greatly, probably because of large fluctuations in weather (see Borkent 2003). This variability may have contributed to our inability to detect a significant difference in visitation rate between female and male *S. canadensis*, despite a threefold higher average rate of visitation to flowers of males. This threefold difference is primarily due to preferential male flower visitation by both Syrphidae and Empididae. This is logical, as both of these families, though still generalist visitors in the sense of visitation behaviour, have adaptations that allow them to better exploit floral rewards (Downes and Smith 1969; Gilbert 1985).

In *C. ligusticifolia,* large muscoid flies visited more flowers per bout (3) than small muscoid flies (1.5). This difference likely reflects the lower energy requirements of small versus large flies. However, visitation rates did not differ among muscoid flies, Culicidae, and halictid bees. The similarity between the dipteran and halictid pollinators is interesting because halictids tend to visit more flowers on a plant when more open flowers are available and, as bees, they are regarded as good pollinators (Ginsberg 1984, 1985). Therefore, if Diptera are poorer pollinators than bees, then this difference must arise from qualities other than visitation rate (*i.e.*, morphology, grooming, *etc.*).

Insects visited more flowers before departing from *S. canadensis* than from *C. ligusticifolia*. This difference may simply reflect the smaller size of *S. canadensis* flowers (*i.e.*, more flowers must be visited to ingest the same amount of food) and the lack of other food sources during early spring. The observed lack of difference in the number of flower visits between pollinator families or plant sexes may reflect limited statistical power resulting from the low number of observations from *S. canadensis*, which were spread among five different insect families.

All visitors to the flowers of both species exhibited similar visitation patterns, with no difference in the number of revisits between any pollinator groups, irrespective of the sex of the plant being visited. Although insects revisited flowers much more often on *S. canadensis* than on *C. ligusticifolia*, most of the revisits (48 of 62) to *S. canadensis* flowers involved just 5 of the 31 individual flies observed. These observations involved either Cecidomyiidae or Empididae and suggest that, even though specific families did not differ statistically in number of revisits, these groups are poor pollinators that might have caused much self-pollination if the plants had been hermaphrodites. However, the large number of revisits by these two families may reflect only the low number of observations obtained on *S. canadensis*, as some Empididae are known to consume pollen and be highly specific flower visitors (Hocking 1968; Larson *et al.* 2001).

The primary behavioural differences between Diptera pollinator groups on both plant species involved the duration of flower visits. For both

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plant species, larger pollinators tended to visit flowers more quickly than smaller pollinators, possibly because of dissimilar energy requirements. Larger fly pollinators require more energy; therefore, they are often driven to remove more nectar than pollen (Gilbert 1985) and appear to remove pollen from a flower as quickly as possible so they can move on to the next resource source. These large pollinators may also have larger proboscides and stronger musculature for the uptake of nectar, thereby decreasing the time taken to collect floral resources. The tendency of larger fly or bee pollinators to visit quickly in this study is contrary to findings that show large syrphid flies take longer on flowers than small syrphids, partially because of increased proboscis and crop size (Gilbert 1985). However, the pollinators that we observed were mainly muscoid flies or halictid bees, so there may be a taxonomic difference in this characteristic. Smaller pollinators usually have lower energy requirements, but also have less musculature and smaller proboscides, which could result in a longer time required to ingest the same volume of food compared with larger fly pollinators.

That large muscoid flies and halictid bees visited flowers of *C. ligusticifolia* at equivalent rates is interesting because it suggests that these insects may have similar effects on plant mating, particularly because the number of new flowers visited per bout did not differ between them. However, individual flies may produce fewer successful pollinations than individual halictid bees if flies carry less pollen than bees do (Kearns and Inouye 1994).

Although insects spent equivalent periods visiting flowers on female and male plants, irrespective of plant species or pollinator group, it is interesting to note that pollinators of *C. ligusticifolia* tended to spend more time on female flowers than on male flowers. Such a difference seems paradoxical, as male flowers tend to be larger and present pollen. However, if male plants attract more pollinators, the actual availability of resources per flower may be lower on males than on females, possibly leading to the observed effect on number of flowers visited per second.

Generalist pollinators and the advantages of dioecy

The behaviour of generalist flies that we observed confirms the theoretical predictions of Charlesworth (1993) that pollinators of dioecious plants do not distinguish between male and female flowers, or at least do not bias visits to one sex over the other. In this study, generalist flies showed no preference for either female or male plants. This finding does not agree with that of Ashman (2000) and others (Bell *et al.* 1984; Agren *et al.* 1986; Bierzychudek 1987; Kevan *et al.* 1990; Ashman and Stanton 1991; Delph and Lively 1992; Eckhart 1992), who reported that unspecialized pollinators distinguish between female and male, or hermaphroditic (in gynodioecious species), flowers, typically choosing hermaphrodites or males over females. Obvious discrimination was not seen in either *C. ligusticifolia* or *S. canadensis*, though the mean number of visitors to males was higher than the mean number of visitors to females in both species.

Whether or not generalist pollinators, such as those observed in this study, would cause self-pollination if they visited the ancestral hermaphroditic plants, thereby favoring adaptation towards dioecy (Bawa and Opler 1975; Bawa 1980, 1994; Lloyd 1982; Charlesworth 1993), remains uncertain. On one hand, the insects that we observed on *C. ligusticifolia* and *S. canadensis* visited individual flowers slowly (>9 s/flower, Figs. 3, 4), which would tend to increase the opportunity for within-flower self-pollination in a hermaphroditic plant. Furthermore, although these insects visited few flowers per foraging bout on a plant, the frequency of revisits (*C. ligusticifolia*, 5.6%; *S. canadensis*, 25.2%) was three to ten times higher than has been observed for bumble bees (2%, Ohashi 2002) and hummingbirds (1%, Gross 2003). Such revisitation would greatly increase the opportunity for both within- and between-flower self-pollination on species with hermaphroditic flowers as compared with visits by specialized pollinators. On the other hand, visitors to these plants visited only 2 to 6 flowers on a plant per foraging bout, on average. Given the small amount of pollen that these flies carry (Kendall and Solomon 1973; Kearns 1992), this number of visits does not seem large enough to cause much geitonogamy. However, the incidence of geitonogamy depends on the amount of pollen exported: as long as flies export a large amount of collected pollen to other plants, rather than grooming it from their bodies or eating it, geitonogamy will be low.

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It is also unlikely that generalist pollinators cause unidirectional pollen movement from plants with many flowers to plants with fewer flowers, which has also been suggested as a reason for the correlation of dioecy and generalist pollinators (Bawa 1980; Beach 1981). The pollinators that we observed visited plants with few and many flowers, although many-flowered plants attracted more visits (Fig. 1). Consequently, more pollen may move from many-flowered plants to plants of all display sizes. This result conforms qualitatively with Bawa (1980) and Beach's (1981) hypothesis, but the difference in visitation to many-flowered *versus* few-flowered plants is not as strong as they predicted.

This study considered the consequences of the observed behaviour of generalist flies for the pollination of dioecious plants and supports expectations of some of the current theory about breeding system evolution. The results from these two dioecious plant species are counter to proposals that pollinators can distinguish between male and female flowers, and instead support Charlesworth's (1993) assumption of indiscriminate behaviour by generalist pollinators of dioecious species. Finally, these results also show that generalist pollinators could be an important correlate of dioecy, contrary to the prediction that reliable pollination by specialized pollinators should be more important (Renner and Feil 1993). Other generalist pollinators, such as butterflies, moths, and beetles, may discriminate between plant sexes and have different flower-visiting behaviours than those seen in Diptera, but this remains to be studied, as little is known about these groups.

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