EXPERIMENTAL EVIDENCE OF INSECT POLLINATION IN JUNCACEAE, A PRIMARILY WIND-POLLINATED FAMILY

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Premise of research.   The rush family (Juncaceae) is most often described as wind pollinated. However, flowers in the family have pollen in tetrads and numerous ovules, both unusual features for anemophilous plants. Here, we investigate evidence for wind and animal pollination in the alpine rush *Juncus allioides* in Yunnan Province, southwest China.

Methodology.   Pollen and ovule traits, insect visitors to flowers, and the relative contribution of wind, insect, and self-pollination to seed set were examined over two years in natural populations.

Pivotal results.   Unlike most Juncaceae, *J. allioides* appears to be largely insect pollinated. The pollen/ovule ratio of plants in both populations was ~700. Few pollen grains were transported by wind to sticky slides in close proximity to plants, and the seed set of bagged-and-emasculated flowers was very low. Although *J. allioides* is capable of self-fertilization, insect exclusion by bagging reduced seed set in comparison with open pollination. Field observations revealed diverse generalist insects, including flies, bees, butterflies, and beetles, visiting flowers.

Conclusions.   Our results confirm earlier, largely anecdotal reports of insect pollination in Juncaceae and suggest that insects may play a more important role in the pollination biology of some species in the family than is often assumed.

Keywords: evolutionary transition, floral traits, insect pollination, Juncaceae, *Juncus allioides*, pollen tetrads, ovule number, wind pollination.

Introduction

The transition from animal pollination to wind pollination (anemophily) involves one of the major evolutionary transformations to the reproductive biology of flowering plants. Phylogenetic evidence indicates that this transition in pollination system has occurred repeatedly in the flowering plants, with at least 65 independent origins (Linder 1998; Friedman and Barrett 2008). Wind-pollinated species frequently possess a characteristic syndrome of floral traits that distinguish them from their animal-pollinated ancestors (Faegri and van der Pijl 1979; table 1 in Friedman and Barrett 2009). These often include small, inconspicuous, unisexual, uniovulate flowers that produce large amounts of pollen. Despite the absence of showy floral displays in wind-pollinated species, insects are occasionally reported visiting flowers (Thomas 1984). This raises the question of whether this simply represents opportunistic behavior by insects feeding on pollen or involves the evolution of floral adaptations promoting animal pollination in wind-pollinated lineages.

Based on floral traits or anecdotal observations of flower visitors, transitions from wind to animal pollination have been inferred in several families, including Caryophyllaceae (Weller et al. 1998), Cyperaceae (Leppik 1955; Thomas 1984; Goetghebeur 1998; Magalhães et al. 2005), Moraceae (Datwyler and Weiblen 2004), and possibly Fagaceae (Manos et al. 2001), Salicaceae (Peeters and Totland 1999), and Joinvilleaceae and Flagellariaceae (Linder and Rudall 2005). A recent study of the South African species *Cyperus obtusiflorus* and *Cyperus sphaerocephalus* provides the first convincing experimental evidence in support of this transition in the predominantly wind-pollinated Cyperaceae (Wragg and Johnson 2011; also see Friedman 2011). Earlier observations of insect pollination in species of *Rhynchospora* section *Dichromena* implicate another independent transition (Leppik 1955), and there are several additional reports of insect visitation to other taxa of Cyperaceae (Thomas 1984), suggesting the possibility of multiple shifts from wind to insect pollination in this family.

Among the major graminoid lineages of Poales, the rush family (Juncaceae) is the most closely related to Cyperaceae (Givnish et al. 2010). Most treatments of Juncaceae describe the family as, in common with Cyperaceae, being adapted for wind pollination (Faegri and van der Pijl 1979; Proctor et al. 1996), although experimental evidence demonstrating wind pollination is sparse. The inference of wind pollination is based on (1) the observation that the vast majority of rushes have

small, inconspicuous, brown, scaly flowers with branched stigmas produced on erect, terminal inflorescences and (2) the fact that Juncaceae is closely related to other large wind-pollinated families, Cyperaceae and Poaceae. However, members of the Juncaceae also possess several traits that are unusual for wind-pollinated plants and are not typical of the related Cyperaceae or Poaceae. For example, *Juncus*, the largest genus, with ~300 species, is characterized by pollen that is produced in tetrads and by numerous ovules per flower (Michalski and Durka 2010). Michalski and Durka investigated 19 *Juncus* species and reported that pollen number ranged from 1,200 to 28,700 per flower and ovule number from 18 to 192. The resulting pollen/ovule (P/O) ratios ranged from 17 to 1,200, values significantly lower than those that are reported for most wind-pollinated species (Cruden 2000).

Several observations suggest that not all members of Juncaceae are exclusively cross-pollinated by wind. The early classic work of Buchenau (1890, 1892) warned against the assumption of exclusive wind pollination in Juncaceae and reported showy flowers and insect pollination in the species-rich *Juncus* section Stygiopsis occurring in the Himalayas and China. More recently, bumblebees were observed collecting pollen from *Juncus balticus* at a salt marsh in Vancouver Island, Canada (Pojar 1973), and *Juncus effusus* was described as wind pollinated and occasionally visited by insects in Germany (Richards and Clapham 1941). There is also evidence that self-pollination may be common in some *Juncus* species (Edgar 1964; Michalski and Durka 2007a, 2007b, 2010). Unfortunately, there are few experimental studies of the pollination ecology of Juncaceae, and experimental evidence indicating that insects play an important role in promoting cross-pollination is generally lacking.

Here, we examine the pollination system of the alpine rush *Juncus alioides* Franch. in southwest China. We were motivated to study this species because in comparison with most rushes, it possesses conspicuous white, bract-like tepals that may serve to attract pollinators (fig. 1A). Indeed, our preliminary field observations revealed frequent insect visits to flowers, raising the possibility that this species may be adapted to animal rather than wind pollination. We therefore initiated a study of the floral biology of two populations of the species, with the following main objectives: (1) to document pollen and ovule traits and determine how values compare with those for other animal- or wind-pollinated species; (2) to determine, by experimental field studies, the relative importance of wind pollination, animal pollination, and self-pollination for seed set; and (3) to document, through field observations, the diversity of insect visitors to flowers and determine which taxa are likely to be the most important pollinators.

Material and Methods

Species Description and Study Sites

Juncus alioides (fig. 1) is a perennial monocot that is common in montane meadows and wetlands of southwest China and nearby Bhutan and Sikkim (Wu and Clemants 2000; Kirschner 2002). Plants are sparsely tufted, with short creeping rhizomes. Stems are erect and ~0.5–0.8 m long, with a single terminal, cyme-like inflorescence (see Snogerup 1993 for a dis-

cussion of inflorescences in *Juncus*), which generally has 7–25 hermaphroditic flowers enclosed in three to five basal lanceolate, white bracts. Each flower has six conspicuous membranous, lanceolate, white elongated tepals and six stamens with 4–7-mm filaments and 2–4-mm anthers. The white pistil has a single ovary with a 2-mm style and three-branched stigmas up to 0.7–1 mm long. Plants generally flower from June to August, and fruits mature in July to September.

We studied two wild populations in alpine meadows in Shangri-La County, Yunnan Province, southwest China. Population A was located in a wet meadow in Shangri-La Alpine Botanical Garden (27°54′27″N, 99°38′13″E, 3200 m in altitude), and population B (fig. 1B) was located along the edge of a lake in Bitahai National Park (27°49′33″N, 99°59′38″E, 3580 m in altitude). Both populations were in open, undisturbed, herbaceous vegetation, and plants occurred at moderate to high density, with hundreds of flowering individuals.

Floral Traits

To determine whether pollen in *J. alioides* was dispersed as tetrads, we observed mature pollen from dehisced anthers under a light microscope and examined stigmas from open-pollinated flowers, using aniline blue fluorescence microscopy (Kearns and Inouye 1993). To estimate pollen and ovule production per flower, we randomly collected 20 flowers from different plants from each of our two study populations. To estimate pollen production and pollen size, we split anthers and counted pollen grains from each flower by eye under a light microscope; we then measured the pollen diameter of 60 grains from each population to 0.1 μm, using a micrometer (see methods detailed in Tang and Huang 2007). We examined whether *J. alioides* produced nectar by examining fresh flowers under a microscope and investigated the presence of nectaries by fixing flowers in FAA (formalin–acetic acid–50% alcohol at a ratio of 5 : 6 : 89 by volume) and observing them with a Hitachi S-450 scanning electron microscope. To detect nectar secretion from the nectary-like structures, we used the periodic acid–Schiff (PAS) reaction, which stains cell walls and starch in amyloplasts (Nepi 2007), and attempted to extract nectar by using microcapillary tubes and bagged inflorescences.

Role of Wind and Insect Pollination

We used two approaches to detect pollen dispersal by wind in *J. alioides*. Twenty glass slides coated with Vaseline were located in population A, where hundreds of individuals of *J. alioides* were in full flower in late July 2010 (see Kearns and Inouye 1993, p. 91–92, for method). We set out four series of five slides at 0.5-m intervals across the population. Slides were positioned at a height similar to that of the cymes of *J. alioides* (~0.6 m) during the morning of two fine days and were collected in the late afternoon. We subsequently recorded pollen tetrad deposition on the 40 slides, using a compound microscope.

To evaluate the relative contribution of insects and wind to pollination in *J. alioides*, we conducted an experiment in population A during July 2010, with three pollination treatments conducted on 20 randomly chosen plants per treatment. The three treatments were: (1) bagged intact flowers, in which 2 × 2-mm nylon mesh bags excluded insects but not wind (see

Fig. 1 *A*, Showy, cyme-like inflorescences of *Juncus allioides*, illustrating the conspicuous white, bract-like tepals. *B*, Population B of *J. allioides*, growing at the edge of a lake in Bitahai National Park, Yunnan Province, southwest China. The conspicuous white inflorescences contrast with the uniform green background of the wet alpine meadow.

Sacchi and Price 1988; Wragg and Johnson 2011); nylon bags of this type with relatively large pore sizes enable the passage of wind-borne pollen to stigmas (Bernardello et al. 1999; Neal and Anderson 2004; Duan et al. 2009); (2) bagged emasculated flowers, in which the stamens of a single flower per cyme were removed before anthesis and the remaining flowers were left intact; any pollen receipt by the emasculated flowers under insect exclusion should be dispersed by wind; and (3) open pollination, in which plants were potentially exposed to wind and insect pollination. Treated flowers were marked with white cotton thread. Three weeks later, a single fruit was collected

from each of the 20 plants per treatment, and the number of seeds and ovules was counted for each fruit.

To examine both whether seed production was limited by pollen receipt and the potential role of insect pollination in this process, we conducted a second experiment in population A in 2011, involving three treatments on 20 plants per treatment. These were (1) emasculation and open pollination; (2) supplemental hand cross-pollination; (3) open pollination, as in the preceding year. In treatment 1, we covered cymes with nylon bags before anthesis and two days later emasculated a single flower and removed the bags so that plants were exposed

Table 1

One-Way ANOVA of the Effect of Population on Pollen and Ovule Traits of *Juncus allioides* from Southwest China

	Pollen diameter (μm)	Pollen tetrads per plant	Ovules per plant	Pollen/ovule ratio
Population A, mean $\pm$ SE	36.1 $\pm$ .5	8996 $\pm$ 271.7	53.1 $\pm$ 1.3	683.1 $\pm$ 23.1
Population B, mean $\pm$ SE	36.5 $\pm$ .3	8912 $\pm$ 273.7	49.5 $\pm$ 1.7	734.9 $\pm$ 31.2
F	.52	.05	3.04	1.78
P	.47	.83	.09	.19

to insect pollination. In treatment 2, we used the same approach but hand-pollinated flowers with pollen from another individual in the population. As above, we collected a single fruit from each plant in each treatment, counted the seeds, and estimated the ovule number per flower.

In both experiments, we used ANCOVA to compare mean seed set among the three treatments conducted each year, followed by specific contrasts to evaluate differences between treatment means. For these analyses, seed set per flower was the response variable, ovule number per flower was used as a covariate, and treatment was a fixed effect. All analyses were performed in JMP (SAS Institute 2000).

Flower Visitors

Our preliminary observations indicated that diverse insects, including bees, flies, butterflies, and beetles, visited flowers of

J. allioides at our two study populations. To quantify the relative frequency of flower visitors, we observed insect visitation on seven fine days during the period July 22–August 1, 2010, at population A. On each day, we recorded insect arrivals to 40–50 randomly marked cymes for at least 2 h, and the behavior and relative frequency of visitors per cyme per hour were calculated. Insect specimens were collected and sent to the Institute of Zoology, Chinese Academy of Science, Beijing, for identification.

Results

Floral Traits

A comparison of pollen and ovule traits between the two populations of *Juncus allioides* revealed similar values (table
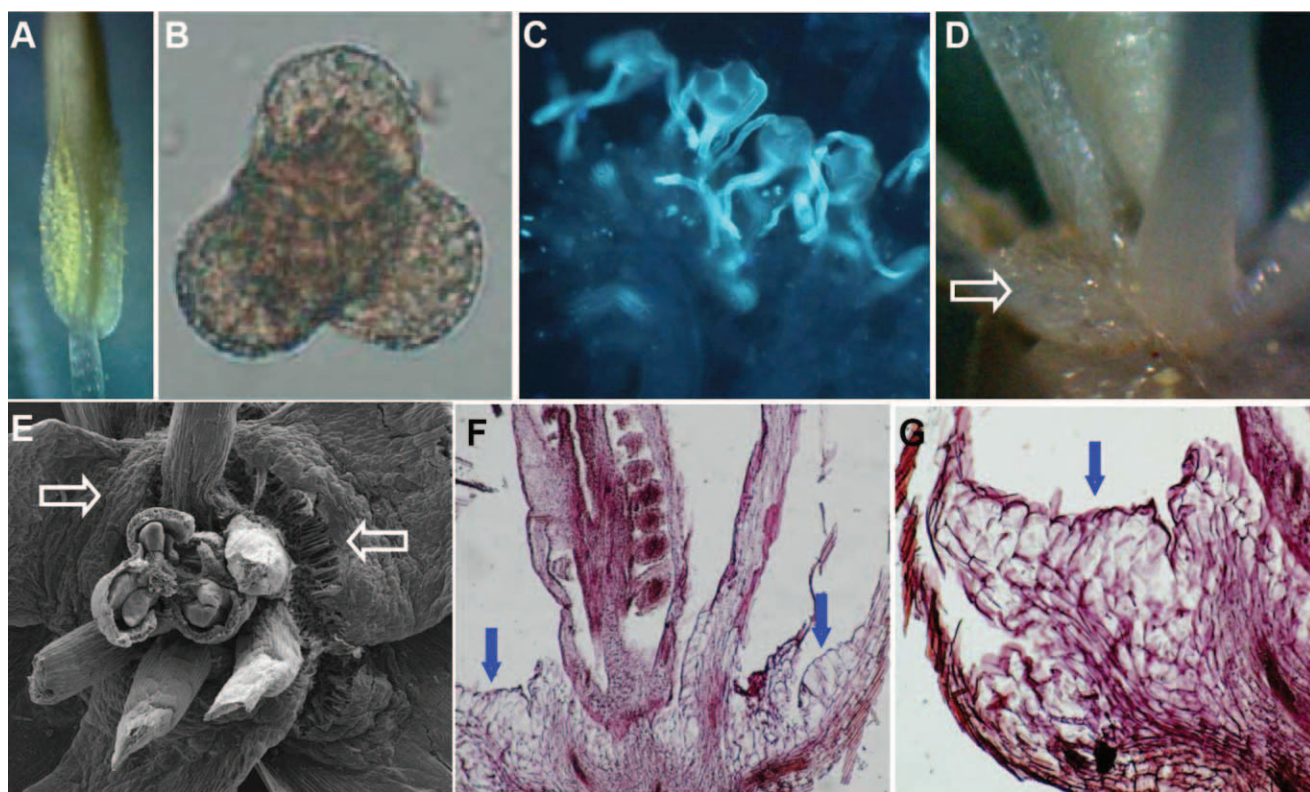
Fig. 2 Floral traits of *Juncus allioides*: *A*, anther dehiscing gradually; *B*, pollen in tetrads; *C*, numerous pollen in tetrads germinating on the stigma under fluorescence microscopy; *D*, nectary-like protuberance at the petal base; *E*, SEM image of a flower, showing numerous ovules and the petal base; *F*, flower longitudinal section stained with periodic acid–Schiff (PAS); *G*, petal base stained with PAS to detect sugar (starch) components. Arrows in *D–G* point to the nectary-like protuberance at the base of tepals.

1). Flowers produced small pollen (~36 μm in diameter) and many ovules (49–53), and P/O ratios averaged 683 and 735 in populations A and B, respectively. Anthers (fig. 2A) dehisced laterally by gradual opening, revealing numerous pollen tetrads (fig. 2B), and we also observed tetrads with numerous pollen grains germinating on open-pollinated stigmas (fig. 2C), indicating that under field conditions pollen was dispersed as tetrads. We observed a protuberance located at each petal base (fig. 2D, 2E), which appeared to function as a nectary. However, using PAS stain, we detected no starch or polysaccharides in the protuberance, as the cells at the petal base were not stained red (fig. 2F, 2G). In addition, we failed to extract nectar from bagged flowers, using microcapillary tubes.

Pollination Experiments

Only one of the 40 slides located among *J. allioides* plants captured pollen tetrads, with two tetrads observed. This indicates that airborne pollen dispersal in population A was minimal, at least on the two days of the trial, which included windy conditions.

ANCOVA indicated that in 2010, seed set per flower was significantly different among the three pollination treatments (fig. 3A; $F_{5,57} = 345.95$, $P < 0.001$). Consistent with the result from the preceding experiment with Vaseline-coated slides, the seed set of bagged-and-emasculated flowers was very low, indicating minimal wind-mediated transfer of tetrads, including any between flowers on the same plant. In contrast, both bagged and open-pollinated flowers produced abundant seed. Open-pollinated flowers produced significantly higher seed set values than bagged flowers (specific contrast: $t = 7.26$, df = 1, $P < 0.001$), probably because of the exclusion of insects from the latter treatment. However, the occurrence of seed set in bagged flowers demonstrates that *J. allioides* is self-compatible and capable of intrafloral autonomous self-pollination.

The ANCOVA results from the 2011 pollination experiment also indicated significant differences among the three treatments (fig. 3B; $F_{5,55} = 37.85$, $P < 0.001$). There was no significant difference between the seed set of open-pollinated flowers and that of flowers that received supplementary hand cross-pollination (specific contrast: $t = 0.73$, df = 1, $P = 0.47$ [not significant]), thus providing no evidence of pollen limitation of seed set. However, open-pollinated flowers that were emasculated before being exposed to pollinators set significantly less seed than those in the other two treatments (specific contrast: $t = 3.0$, df = 1, $P = 0.0041$), presumably because emasculation prevented autonomous intrafloral self-pollination and seed set resulted from insect pollination.

Insect Visitation to Flowers

We observed frequent insect visitation to flowers of *J. allioides* in both study populations (fig. 4). In a total of 30 h of observations in population A, we recorded 26 insect species visiting flowers of *J. allioides* (table 2). These were composed of 13 species of syrphid flies, 4 species of other flies, 6 species of bees, 2 species of butterflies, and one beetle. The most abundant visitors to flowers were syrphid flies, which consumed pollen (fig. 5). The most effective pollinators of *J. allioides* were bees, particularly *Bombus lucorum* (fig. 4B), *Lasioglossum* spp. (fig. 4A), and *Halictus* sp. We observed abundant

pollen adhering to their body parts, especially the abdomen and legs. *Bombus lucorum* was observed probing flowers, presumably for nectar (fig. 4B), and was particularly effective in transferring pollen between individuals, owing to its longer flight distances. Butterflies also probed flowers but rarely appeared to carry much pollen.

Discussion

This study has three major findings. (1) Pollen and ovule traits in *Juncus allioides* are not typical for wind-pollinated species but are not unusual for other members of the genus, regardless of their pollination systems. (2) In contrast to those of most rushes, flowers of *J. allioides* are visited by diverse generalist insects, which feed on pollen and possibly other floral rewards. (3) Pollination experiments indicate that wind
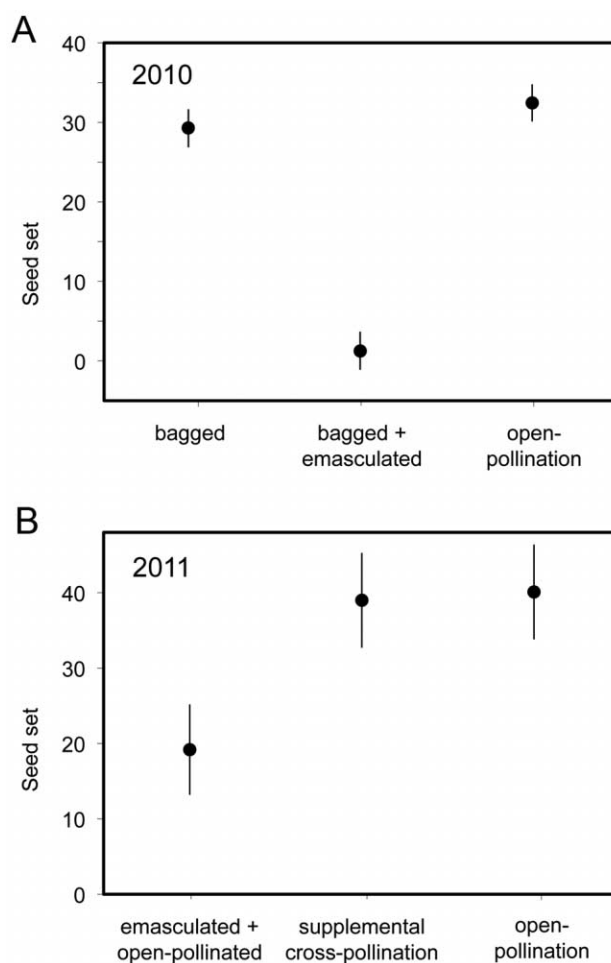
Fig. 3 Results of two pollination experiments conducted on population A of *Juncus allioides* in 2010 (A) and 2011 (B). The mean and 95% confidence interval of seed set for three treatments are compared for each year. In 2010, the treatments are bagged, bagged + emasculated, and open pollination, and in 2011, they are emasculated + open pollination, supplemental cross-pollination, and open pollination. Sample sizes for each treatment are 20 flowers sampled from 20 plants. See "Material and Methods" for further details.
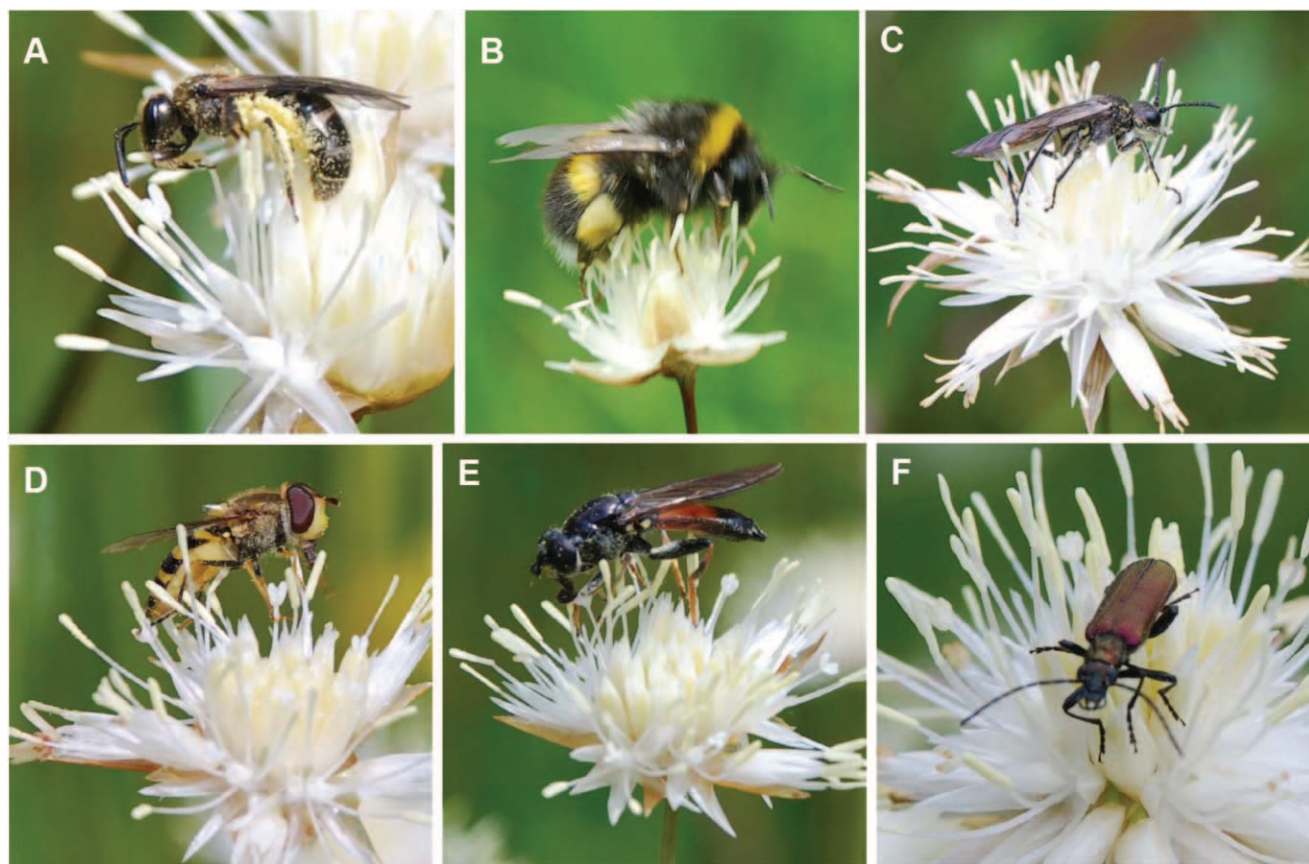
Fig. 4   Insect visitors to flowers of *Juncus allioides*: *A*, solitary bee (*Lasioglossum*), showing pollen attached to the abdomen and legs; *B*, bumblebee (*Bombus lucorum*) probing a flower; *C*, sawfly (Tenthredinidae, *Tenthredo*); *D*, syrphid fly (*Syrphus*) collecting pollen; *E*, hoverfly (*Xylota*); *F*, beetle (Cantharidae).

appears to play a minor role in pollen dispersal and that seed set largely results from a mixture of insect and self-pollination. Although we observed a nectary-like protuberance at the base of each tepal and also observed pollinators probing this area, our efforts to demonstrate the presence of nectar secretions were inconclusive. We begin by reviewing what is known about the pollination systems of Juncaceae, then discuss the main findings of our study, and finally consider the possibility that this family may show reversions from wind to insect pollination, as occurs in the related Cyperaceae.

Inferences on Pollination Systems in Juncaceae

There have been remarkably few pollination studies on members of Juncaceae, and what information is available is largely anecdotal in nature or based on inferences from floral morphology. Most authors recognize that the majority of species in the family are wind pollinated. For example, Balslev, the principal modern monographer of Juncaceae, states that "wind pollination is predominant in the Juncaceae" (Balslev 2004, p. 451; also see Balslev 1998), and other authors have come to a similar conclusion (Grant 1949; Stebbins 1970; Soderstrom and Calderón 1971; Faegri and van der Pijl 1979; Arroyo et al. 1982; Cook 1988; Smith et al. 1990; Proctor et al. 1996; Kelly et al. 2001; Michalski and Durka 2007*a*,

2007*b*, 2010; Rodríguez et al. 2007). However, the German naturalist Hermann Müller first noted insects visiting flowers of Juncaceae (see Knuth 1898), and insect visitation to flowers has been reported in *Luzula* and *Juncus* species from Europe and the Himalayas with showy yellow or white tepals, including *J. allioides* (Buchenau 1890, 1892; Knuth 1898; Balslev 2004). However, to our knowledge no experiments have been performed on these species, although both wind pollination and insect pollination have been inferred for several. On the basis of Knuth's (1898) observations, Hesse (1980) investigated pollenkitt in *Luzula* species, including at least one that was putatively insect pollinated. He found that in *Luzula nivea*, a species with showy white inflorescences, there was a small increase in pollen stickiness and pollenkitt relative to the other species. He concluded that *L. nivea* was likely to be pollinated by both wind and insects.

Floral Traits

In contrast to most members of the Juncaceae, which have small, inconspicuous brown flowers, *J. allioides* exhibits conspicuous floral displays. The large, white, bract-like tepals clustered in cyme-like inflorescences stand out against the uniform green background that characterizes the alpine wet meadows in which populations occur (fig. 1). Our investigations of pol-

Table 2

Flower Visitors to Population A of *Juncus allioides* in 2010

Diptera	Non-Diptera
Syrphidae:	Hymenoptera:
Episyrphus balteatus	Halictidae:
Allograpta sp.	*Lasioglossum kryopetrosum*
Melanostoma sp.	*Lasioglossum virideglaucum*
Cheilosia sp.	*Halictus* sp.
Melangyna sp.	Vespidae sp.
Eristalis tenax	Apidae:
Betasyrphus serarius	*Bombus lucorum*
Platycheirus sp.	Tenthredinidae
Asarkina sp.	*Tenthredo* sp.
Chalcosyrphus sp.	Lepidoptera:
Xylota sp.	Lycaenidae:
Metasyrphus nitens	*Celastrina sugitanii*
Syrphus sp.	Pierididae:
Calliphoridae sp.	*Pieris rapae*
Anthomyiidae sp.	Coleoptera:
Tachinidae sp.	Cantharidae
Muscidae:	
Helina sp.	

len and ovule traits confirmed that, in common with other members of the Juncaceae, this species produces relatively small pollen grains grouped in tetrads, produces many (∼50) ovules per flower, and has a P/O ratio (∼700) that is considerably lower than that in most anemophilous taxa (Cruden 2000). However, it is important to note that the values we obtained for pollen and ovule traits are well within the range recorded by Michalski and Durka (2010) in their investigation of wind-pollinated *Juncus* species in Europe. It is therefore possible that the relatively low P/O ratios in some *Juncus* species are more functionally related to the mating systems of populations, which may involve significant rates of self-fertilization (Michalski and Durka 2007*a*; and see below), rather than being indicators of their pollination systems.

Our observations of bumblebees and butterflies visiting flowers of *J. allioides* suggested that they were probing for nectar or some other floral reward. However, our efforts to confirm the presence of nectaries gave mixed results. Scanning electron microscopy revealed the presence of a small protuberance at the base of each of the six tepals that resembled a nectary (fig. 2*D*, 2*E*). However, using the PAS stain, we were not able to detect the presence of any nectar-like secretions, and our efforts to extract nectar from flowers with microcapillary tubes were unsuccessful. It is possible that the protuberances represent vestigial nectaries, from distant animal-pollinated ancestors, that no longer function. Buchenau (1890, 1892) reported that flowers of showy-flowered, insect-pollinated *Juncus* were nectarless but possessed swollen, sap-filled cells at the base of their flowers, which he speculated could provide nutrition for pollinators, and pseudonectaries are mentioned from several *Luzula* species in which both wind and insect pollination are reported (Knuth 1898). In *Eleocharis elegans*, a member of the Cyperaceae with showy inflorescences, floral volatiles are reported that may serve to attract insects (Magalhães et al. 2005). It is therefore conceivable that in *J. allioides*, floral scent may be produced by the swollen

protuberances. Further work is required to determine the structural and functional basis of these structures and what floral rewards, if any, probing bumblebees and butterflies obtain from flowers.

Pollen Tetrads in Juncaceae

Pollen dispersal in most flowering plants involves granular pollen produced as monads. Tetrad formation, as occurs in *J. allioides*, is restricted to 52 flowering plant families (Copenhaver 2005). It has been estimated that such aggregated pollen has evolved at least 39 times, and this condition is largely associated with animal pollination (Harder and Johnson 2008). However, tetrads are occasionally reported from wind-pollinated taxa (e.g., *Typha* [Typhaceae]: Cox 1991; *Lactoris* [Lactoridaceae]: Bernardello et al. 1999), as well as from wind-pollinated Juncaceae (Michalski and Durka 2010), so their occurrence in *J. allioides* is not altogether unexpected and may help to facilitate insect pollination.

Our observations of the open-pollinated stigmas of *J. allioides* indicate that tetrads are the principle units of pollen dispersal. However, our failure to capture significant numbers of tetrads on sticky slides placed in population A, combined with the very low seed set we obtained in bagged-and-emasculated flowers (fig. 3*A*), indicates that tetrads are not particularly effective units for dispersal by wind, at least in *J. allioides*. This is presumably because of their mass, which may pose aerodynamic constraints in comparison with the small, granular pollen that is typical of most anemophilous species. The occurrence of tetrads in wind-pollinated *Juncus* species raises important questions about their effectiveness in pollen dispersal. Investigations of the capture of airborne pollen in the Iberian Peninsula by Rodríguez and colleagues (2007) reported that the annual accumulated daily concentrations for Juncaceae were in the range of 1.8–15.8 grains/m³, substantially lower than that in any other anemophilous family they investigated. Similarly, an earlier investigation of airborne pol-
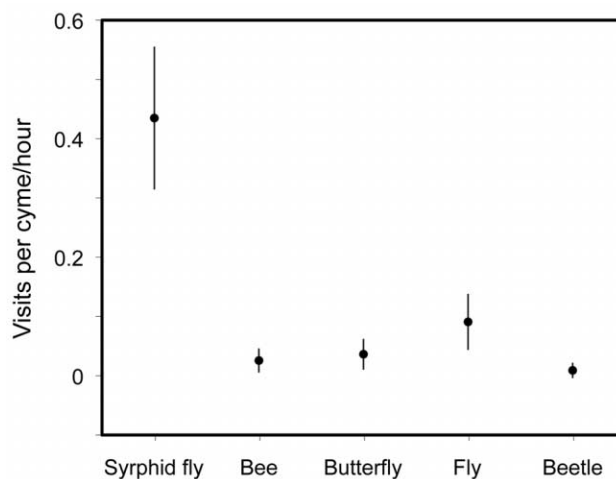

Fig. 5 Mean and 95% confidence interval of the visitation frequency per cyme per hour of five insect groups visiting *Juncus allioides*. Observations were made for ∼30 h during 2010 in population A.

len in the United Kingdom also reported low levels for Juncaceae, compared with other anemophilous groups, leading to the suggestion that pollen tetrads are associated with self-pollination (reviewed in Proctor et al. 1996, p. 267). Indeed, as discussed above, there is evidence that rushes are capable of a high degree of self-pollination (Edgar 1964; Michalski and Durka 2007a, 2010). Nevertheless, it seems improbable that tetrads are maintained in Juncaceae as an adaptation associated with self-pollination.

Pollination and Mating

A diversity of insects were observed visiting flowers of *J. allioides*, including species of flies, bees, butterflies, and beetles (table 2). A similar spectrum of generalist visitors was also reported visiting animal-pollinated *Cyperus* species (Wragg and Johnson 2011), suggesting that generalist pollination may often be involved in animal-pollinated taxa in predominantly wind-pollinated families. By far the commonest visitors to *J. allioides* were syrphids (fig. 5), with flies in general confining their foraging activity to pollen feeding, as did the small number of beetles that we observed. Given their foraging behavior, which often involved long periods on a single inflorescence, these pollinator groups probably caused a significant amount of within-inflorescence self-pollination, although the high density of some flowering patches enabled movements between plants, facilitating cross-pollination. Pollen was commonly observed on the bodies and legs of bees visiting *J. allioides* flowers, and despite their lower frequency at our study site, they are likely to be the most effective pollinators.

Our pollination experiments demonstrated that *J. allioides* is self-compatible, with the capacity for intrafloral autonomous self-pollination (fig. 3A). Although selfing is likely to be an important component of the mating system of populations, our results also indicate that insects play a role in facilitating cross-pollination. Flowers that were emasculated and exposed to open pollination set significantly less seed than those that were left intact and were open pollinated (fig. 3B). Removal of the opportunity for intrafloral autonomous self-pollination and our finding that wind does not play a role in geitonogamous pollen transport demonstrate that insects play a significant role in the pollination of *J. allioides*.

Abundant visitation by generalist pollinators and the capacity for autonomous self-pollination likely serve to maintain maternal fertility in *J. allioides*. Indeed, comparison of the seed set of open- versus supplementarily cross-pollinated flowers provided no evidence of pollen limitation of seed set. The relative importance of insect pollination versus self-pollination may be largely dependent on local environmental conditions affecting insect visitation. This flexibility in pollination system may be important in the alpine habitats that this species occupies; local environmental conditions can vary dramatically during the long flowering season, with periods often unsuitable for insect activity.

Transitions between Wind and Animal Pollination

Because of the likelihood of multiple independent origins of animal pollination from wind pollination in Cyperaceae, this family provides the most promising opportunities for detailed investigations of this shift in pollination system (Leppik 1955; Thomas 1984; Magalhães et al. 2005; Wragg and Johnson 2011). However, it is possible that similar transitions may explain the occurrence of animal pollination in Juncaceae, although the entomophilous status of other members of the family clearly requires further study. For example, several species of the otherwise anemophilous genus *Luzula*, e.g., *L. lutea*, *L. nivea*, and *L. lactea*, have showy flowers and are apparently visited by insects (Buchenau 1892; Knuth 1898). *Juncus allioides* occurs in a clade of similar showy-flowered species native to Asia that are nested among wind-pollinated species (Kirschner 2002; Roalson 2005; E. H. Roalson, personal communication). Explicit phylogenetic reconstruction of these groups would be helpful in localizing the transition(s) between wind and animal pollination and for understanding character evolution and the traits involved.

Finally, why is the evolutionary transition from wind to animal pollination so uncommon, given the frequent shifts that are evident in the opposite direction (Linder 1998; Friedman and Barrett 2009)? The answer is probably best explained by considering the severe evolutionary constraints that the anemophilous syndrome imposes on the reacquisition of floral traits required to attract, feed, and manipulate animals for effective pollen dispersal. Indeed, some have considered this constraint sufficiently severe that the transition from animal to wind pollination has been described as irreversible (Cox 1991; Dodd et al. 1999). How difficult the reacquisition of animal pollination is may depend on how "committed" taxa have become to anemophily. For example, in ambophilous taxa in which both wind and animal pollination occur, a transition to exclusive animal pollination may not be very difficult to achieve where pollinator service is reliable. However, in predominantly wind-pollinated families with fully developed anemophilous syndromes, the evolutionary remodeling of reproductive phenotype for animal pollination is likely to be more difficult. As we have shown, Juncaceae do not possess the typical suite of characters associated with the anemophilous syndrome, and this may have predisposed some members of the family to reacquire animal pollination when suitable ecological conditions prevailed, or, perhaps less likely, animal pollination may be the basal condition in the family. Future comparative studies on the functional relations between reproductive traits and pollination systems in Juncaceae would seem to be warranted.

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