



Pollen dispersal distance is determined by phenology and ancillary traits but not floral gender in an andromonoecious, fly-pollinated alpine herb

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Abstract

Pollen-mediated gene flow and spatial genetic structure have rarely been studied in alpine plants that are pollinated by dipteran insects. In particular, it is not clear how different floral traits, such as floral gender, phenology, and ancillary traits, may affect pollen dispersal distance within alpine plant populations. In this study, we conducted a paternity analysis to track pollen flow in a population of *Pulsatilla alpina*, an andromonoecious alpine herb producing male and bisexual flowers. We found that the pollen was dispersed over short distances (mean = 3.16 m), with a dispersal kernel following a Weibull distribution. Nonetheless, spatial genetic structure was weak in the population (S_p statistic = 0.013), pointing to effective seed dispersal and/or high inbreeding depression. The pollen dispersal distance was independent of the gender of the flower of origin but depended positively on floral stalk height and negatively on flowering date and tepal length. Although male siring success did not correlate with pollen dispersal distance, selection may favour traits that increase the pollen dispersal distance as a result of reduced bi-parental inbreeding. Our study not only provides new insights into the nature of pollen dispersal of alpine plants, but also reveals the effects of floral traits on a component of male reproductive success.

Keywords Sexual system · Male fitness · Sex allocation · Insect-pollinated · Intra-specific variation · Optimal foraging

Introduction

Gene flow in sessile plants is largely mediated by the dispersal of pollen and seeds, which together determine the spatial genetic structure within and among populations. The dispersal distance should depend on not only dispersal vectors, but also on plant traits that attract and manipulate the position and behaviour of pollinators. There has been substantial progress in investigating traits that affect seed dispersal (Vittoz and Engler 2007; Thomson et al. 2011; Côrtes and Uriarte 2013; Tamme et al. 2014), but we remain largely ignorant of how pollen dispersal distance varies among dispersal vectors and for different plant trait values, likely

due to the difficulties of tracking the gene flow by pollen movements.

Around 90% of flowering plants rely on a diverse group of animals for pollen dispersal (Ollerton et al. 2011). Although it is generally thought that animals with a larger body size disperse pollen further and thus reduce the genetic structure of plant populations, such conclusions are predominantly drawn from comparisons between plants pollinated by birds and bees (especially hummingbirds and bumblebees) (Krauss et al. 2017; Wessinger 2021). Importantly, we know very little about how the foraging behaviour of small insects such as flies affects pollen dispersal distances, despite the fact that they are among the most important pollinators in alpine, arctic, and agricultural ecosystems (Inouye et al. 2015; see Table 1 for a summary of studies on pollen dispersal distance in alpine plants).

A number of floral traits likely determine pollinator foraging behaviour and thus pollen dispersal distances (Waser 1983; Chittka and Thomson 2001; Ishii et al. 2008). For example, in *Delphinium virescens*, bumblebee pollinators

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Table 1 Summary of studies reporting direct estimates of pollen dispersal distances in alpine plants. References: **a.** Petré et al. (2021) **b.** Buehler et al. (2012) **c.** Scheepens et al. (2012) **d.** Aegisdóttir et al. (2009) **e.** Thomson and Thomson (1989) **f.** Pluess and Stöcklin (2004) **g.** Campbell and Waser (1989) **h.** Chen and Pannell (2022) **i.** This study **j.** Matter et al. (2013a) **k.** Matter, Kettle, Ghazoul, and Pluess (2013b)

Species	Pollination system	Mean pollen dispersal distance (m)	Methods used to estimate dispersal distance	Traits studied for within-population variation
<i>Arabis alpina</i>	Generalized (fly, bee) ^a	< 50 ^b	Paternity analysis ^b	
<i>Campanula thyrsoides</i>	Bumblebee ^c	17.4 ^c 34–62.1 ^c 4.85 ^d	Paternity analysis ^c , Pollen dyes ^{c, d}	Density ^c
<i>Erythronium grandiflorum</i>	Bumblebee ^e	0.68–16.44 ^e	Pollen dyes ^e	
<i>Geum reptans</i>	Fly ^f	< 1 ^f	Pollen dyes ^f	
<i>Ipomopsis aggregata</i>	Hummingbird ^g	1.41–2.63 ^g	Pollen dyes ^f	Phenology, floral morphology ^g
<i>Pulsatilla alpina</i>	Fly ^h	3.17 ⁱ	Paternity analysis ⁱ	Floral gender, phenology, floral morphology ⁱ
<i>Ranunculus bulbosus</i>	Generalized (fly, beetle, bee) ^j	109.5–296.5 ^{i, *} 15.9 ^k	Inferred from fitted dispersal kernel ^j , Paternity analysis ^k	
<i>Trifolium montanum</i>	Bees ^j	3490.6 ^{i, *} 10.3 ^k	Inferred from fitted dispersal kernel ^j , Paternity analysis ^k	

Note * The extremely long pollen dispersal distance reported is likely due to a non-exhausted sampling of the sires

flew longer distances after visiting flowers with a low quantity of nectar, presumably implying greater pollen dispersal distances (Waddington 1981). In hummingbird-pollinated *Ipomopsis aggregata*, mean pollen dispersal distance, estimated using pollen dyes, was found to increase with the variance but decrease with the mean stamen length, whereas it was independent of flowering date, number of flowers, and corolla size (Campbell and Waser 1989). To our knowledge, few studies have evaluated the effect of floral traits on pollen dispersal at the individual level using paternity analyses to measure realized gene flow (e.g., Tomaszewski et al. 2018; Barbot et al. 2022). This is an important lacuna in the literature, because the dispersal of pollen constitutes a critical component of male reproductive success and has important implications on spatial genetic structure (but see studies focusing on density: DiLeo et al. 2018; Diaz-Martin et al. 2023).

It is generally assumed that individuals dispersing their pollen over greater distances should enjoy higher male reproductive success in terms of the total numbers of progeny sired, largely because of a supposed increase in mate availability (Harder and Prusinkiewicz 2013) and/or because of a reduced chance of mating with relatives (Price and Waser 1979). As a result, traits that facilitate pollen dispersal should be favoured by selection via male reproductive success (Harder and Prusinkiewicz 2013). In wind-pollinated *Mercurialis annua*, for instance, taller plants were found to have a higher male siring success because they dispersed their pollen further, and the pollen dispersal distance correlated positively with male siring success

(Tonnabel et al. 2019). Surprisingly, in insect-pollinated species, although mating between distant individuals usually leads to increased performance in components of male reproductive success (Waser and Price 1991; Souto et al. 2002), few empirical studies have tested the positive correlation between pollen dispersal distance (e.g., mean and standard deviation) and male siring success.

In this study, we asked how floral traits affect pollen dispersal distance in a population of the andromonoecious alpine herb *Pulsatilla alpina*, which relies on dipteran insects as pollinators. Specifically, we selected a population comprising mostly single-flowered individuals, i.e., with a single male or bisexual flower, and used paternity analysis based on microsatellite markers to evaluate the effect of traits at the flower level on pollen dispersal distances, and we addressed the following questions. (1) What is the pollen dispersal kernel of *P. alpina*, and does it differ between male and bisexual flowers? (2) What is the spatial genetic structure of the *P. alpina* population? (3) How does pollen dispersal distance depend on floral traits, specifically floral gender, phenology, tepal length, and stalk height? And (4) to what extent does pollen dispersal distance correlate positively with male siring success?

Materials and methods

Study species and study sites

Pulsatilla alpina (L.) Delarbre (Ranunculaceae) is a perennial hemicryptophyte growing in sub-alpine to alpine habitats in central Europe (Lauber et al. 2018). Several vegetative and/or reproductive shoots emerge from the tip of a perennial underground rhizome (the species does not produce stolons and ramets) soon after the snowmelt, from early May to July. Depending on their size and age, individuals produce up to ~20 flowers with around six white tepals (the sepals and petals are not distinguishable), each on its own reproductive shoot. The species is andromonoecious. Unisexual male flowers bear only stamens, whereas protogynous bisexual flowers bear stamens and one to a few hundred uni-ovulate pistils. The sex allocation of the species is size-dependent, with larger plants (genetic individuals) allocating absolutely and proportionally more resources to their female function (Chen and Pannell 2023a). Furthermore, small individuals may produce only a single male flower and thus function as males in the respective flowering season (Chen and Pannell 2023a). Both male and bisexual flowers are predominantly visited by flies, including houseflies and syrphid flies (Chen and Pannell 2022). Ripe fruits (technically achenes) have elongated pappus hair and are dispersed by wind in early autumn (Vittoz and Engler 2007). After fruit dispersal, above-ground vegetative parts senesce, but individuals persist underground over winter.

The study was conducted during the flowering season of 2022 in a single population of *P. alpina*, located at Solalex in the pre-Alps of Vaud canton, Switzerland ('Population S1+'; latitude: 46°17'42"N, longitude: 7°09'09"E; elevation: 1758 a.s.l.). The population was located on an open slope of sub-alpine grassland and covering an area with dimensions of about 20 m x 20 m and comprising around 145 mainly small and probably young flowering individuals (following recent avalanche disturbances and/or herbivory by cattle). We set up a 10 m x 15 m temporally fenced plot within the population, enclosing 135 flowering individuals, with 22 and 82 of them producing a single male or bisexual flower, respectively (Table S1). We removed all the floral buds (i.e., around 10 buds) outside the plot at the very beginning of the flowering season to prevent nearby individuals outside the plot from siring progeny in the plot (thereby improving our ability to assign paternity).

Flowering phenology and ancillary traits

We recorded the flowering state of all individuals of *P. alpina* at the study site every three to four days for the duration of the flowering season, from late May to late June 2022, noting

the number of flowers, number of stalks, height of the tallest foliar stalk, gender, and geographic position of the flowering individuals. On each census day, we recorded the sexual stage of all flowers in terms of seven and five categories for bisexual and male flowers, respectively (see Chen and Pannell 2023b for a detailed description of the categories). The protogynous bisexual flowers are first in their female stage then their male stage, while the male flowers express only the male stage. We also photographed each flower and later counted the number of its stamens based on the photographs (see details below). The onset of the male stage (flowering date) was specified as the first date on which anthers were seen to have dehisced (M_1 stage) for both male and bisexual flowers. The height of floral stalks and the length of tepals were measured at the end of the male stage (M_2 stage), following the methods used by Chen and Pannell (2022). Around three weeks after the end of the flowering season, all flowers with developing fruits were enclosed in a paper bag until the end of the growing season (early August), at which point all fruits were collected.

Paternity analysis and estimates of pollen dispersal

To estimate pollen dispersal distance, we used variation at ten microsatellite markers to assign paternity to mature seeds (for details, see Chen and Pannell 2023b). Leaf samples of all flowering individuals were collected in July 2022 at the end of the flowering season and dried in silica gel before DNA extraction. Up to ten mature uni-ovulate achenes for all seed families ($N=104$) were arbitrarily selected for each sampled flower. Each seed comprising predominantly diploid embryo tissues (Ghimire et al. 2021) was then carefully isolated from each achene. Total DNA was extracted from both the leaves and the seeds samples using the BioSprint 96 DNA Plant Kit (Qiagen, Germany).

PCR amplification was carried out in a final volume of 10 μ l, including 5 μ l of 2 \times Multiplex PCR Master Mix (Qiagen, Germany), 2 μ l of diluted DNA, 1 μ l of distilled water, and 2 μ l of multiplex containing variable primer concentrations (Chen and Pannell 2023b). Thermal cycling was performed in a TProfessional Standard Thermocycler (Biometra GmbH, Göttingen, Germany) as follows: 95 °C for 15 min; 36 and 41 cycles for leaf and seed samples, respectively, at a temperature of 94 °C for 30 s, 60 °C for 45 s, and 72 °C for 45 s; and a final step at 72 °C for 30 min before cooling down to 4 °C. PCR products were analyzed by capillary electrophoresis on an ABI3100 Genetic Analyzer (Applied Biosystems).

Sires (fathers) were assigned from among the 135 flowering individuals to all mature seeds for which more than five loci could be genotyped, using the software Cervus v 3.0.7 and assuming a confidence level of 80% and an error rate

of 0.018 (Kalinowski et al. 2007). Pollen dispersal distance was estimated by calculating the distance between the dam (mother) and the most likely sire for each of the successfully genotyped seeds. Although *P. alpina* is self-compatible, with an average selfing rate estimated to be 0.45, the selfed progeny ultimately contributes little to the next generation due to very high (0.93) inbreeding depression (estimated by changes of inbreeding coefficients between generations in the study population, following Ritland 1990) (unpublished data). Thus, for this study, we considered pollen dispersal only for outcrossing mating events. Male outcrossing siring success was calculated following Chen and Pannell (2023b) using the paternity share and the number of mature seeds of each genotyped seed family.

Statistical analysis

We conducted the following analysis within the R statistical framework v 4.0.3 (R Core Team 2021). To quantify the pollen dispersal kernel of *P. alpina*, specifically, to compare that of bisexual and male flowers, we used the R package *dispsfit* (Proença-Ferreira et al. 2023). We fitted Weibull, geometric, 2Dt, and exponential distributions to our inferred pollen dispersal distances (i) for all the flowering individuals, (ii) for only individuals with a bisexual flower, and (iii) for only individuals with a male flower (see Table S1 for details), and we used AIC values to compare the models (for the formula of each distribution, see Proença-Ferreira et al. 2023). These four distributions are commonly assumed to describe pollen dispersal (Austerlitz et al. 2004). The best-fitted distribution was then used in subsequent analysis to evaluate the effects of traits on pollen dispersal distance.

To assess the spatial genetic structure of the population, we used the software SPAGeDi version 1.5 (Hardy and Vekemans 2002), following the procedure described by Vekemans and Hardy (2004) based on pairwise kinship coefficients between individuals. We conducted Nason's estimator of kinship coefficient ($F_{(r)}$) (Loiselle et al. 1995). The average relationship coefficients of the ten microsatellite markers per distance class were estimated and their significance per class was tested with 1000 permutations. We used S_p to evaluate the extent of spatial genetic structure, defined as $S_p = -\beta / (1 - F_{(1)})$, where β is the regression slope of $F_{(r)}$ on $\ln(\text{spatial distance})$, and $F_{(1)}$ is the mean of $F_{(r)}$ among individuals for the first distance class (Vekemans and Hardy 2004).

To evaluate how floral gender, phenology, and ancillary traits affected pollen dispersal distances, we used a generalized additive model with a Weibull distribution (*gamlss* package; Stasinopoulos et al. 2018). The pollen dispersal distance of sires with a single bisexual or male flower was set as the response variable and floral gender (i.e., bisexual

or male), flowering date, tepal length, and stalk height as explanatory variables. Here, we included only the single-flowered sires with a complete set of measurements of the traits ($N=72$; see Table S1 for details). We set the identity of the sires as a random variable because the mating events from the same flower share the same floral phenotype. We evaluated the residuals of the model using the *plot* function in the *gamlss* package (Figure S1; Stasinopoulos et al. 2018).

To investigate the relationship between pollen dispersal distance and floral male siring success, we used a generalized linear model (*glmer* function in the *lme4* package; Bates et al. 2015) with a Poisson distribution. We set the mean pollen dispersal distance and the standard deviation of each single-flowered individual as an explanatory variable in two separate models. For calculating the standard deviation in the second model, only individuals involved in more than one mating event were included. We included an observation-level random variable to account for overdispersion (Harrison 2014). We evaluated the residuals of the model using the package *DHARMA* (Hartig 2019).

Results

Pollen dispersal kernel

We identified 513 outcross mating events for 854 genotyped seeds of 104 seed families from 90 individuals. Pollen dispersal distances for outcrossing were generally short, with an average of 3.16 m separating sire and dam and 25%, 50%, and 75% of seeds sired by males < 1.0 m, < 2.15 m, and < 4.36 m away from the corresponding dam, respectively (Fig. 1A). Pollen dispersal of *P. alpina* was best fitted by a Weibull distribution for all three types of sires (Table S2; see Proença-Ferreira et al. 2023 for the formula of the Weibull distribution). The values of the parameters used in the Weibull distribution along with the mean dispersal distance, skewness, and kurtosis of the kernel are presented in Table 2. We found that the parameter b , i.e., the shape parameter, was close to 1 in all three cases, indicating a mostly fat-tailed distribution (Table 2; Fig. 1).

Spatial genetic structure

Spatial genetic structure was characterized by a significantly negative β value ($\beta = -0.013$, $P < 0.001$). We estimated $S_p = 0.013$. Mean kinship coefficients (\hat{F}_r) across all distance classes was -0.0001 . Analysis of fine-scale genetic structure indicates a significant positive autocorrelation among individuals located up to around 3 m apart (Fig. 1D).

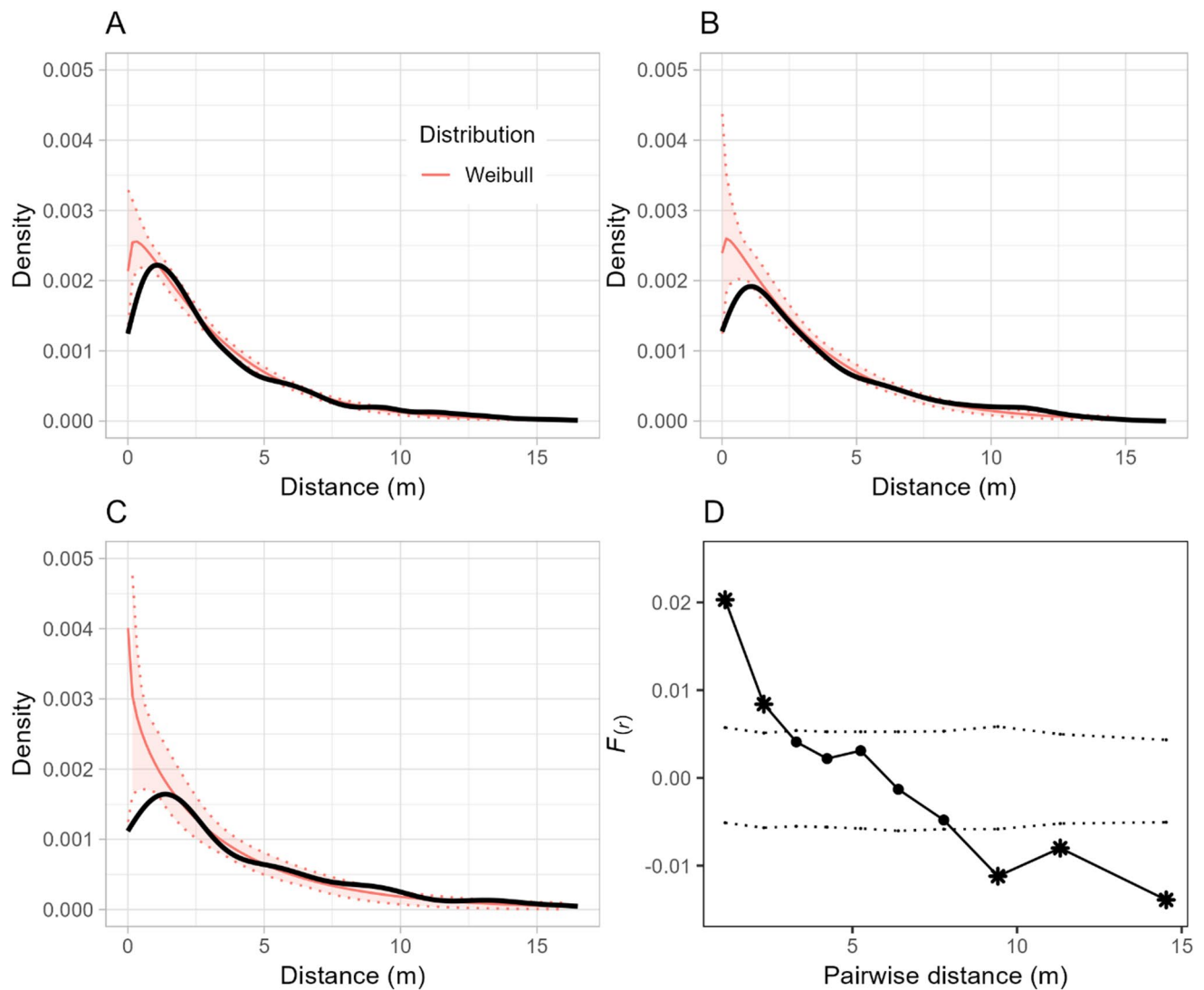


Fig. 1 Pollen dispersal kernels for all sires (A), for sires with one bisexual flower (B), and for sires with one male flower (C) in the study population of *P. alpina*. Black lines represent the density distribution of raw data. Red lines represent the best-fitted kernel with a Weibull distribution, and dotted red lines show the 95% confidence intervals of the distribution. Only outcross mating events were included in the analysis. (D) Results of a spatial autocorrelation analysis based on all

flowering individuals ($N=135$), and on the use of Nason's kinship coefficient, $F(r)$. The average kinship coefficients (marked as dots) are positioned along the x-axis at the mean pairwise distance within each of the ten distance classes. The average $F(r)$ values deviating significantly from expected for a random distribution of genotypes are marked with an asterisk. The dotted lines represent 95% confidence intervals

Effects of floral traits on pollen dispersal distance

In total, 297 outcross mating events from 72 single-flowered sires were used for the analysis. Pollen dispersal distance declined with flowering date (Fig. 2A; $P < 0.01$) and tepal length (Fig. 2B; $P < 0.001$), and increased with stalk height (Fig. 2C; $P < 0.05$), but was independent of floral gender (Fig. 1B and C; Table 3; $P > 0.05$).

Floats Kindly mention the part labels A-C in Fig.2.

Pollen dispersal distance and male siring success

Male outcross siring success did not depend on the mean ($N=72$; Fig. 3; $P > 0.05$) or standard deviation ($N=60$; Figure S2; $P > 0.05$) of the pollen dispersal distance for single-flowered individuals. The mean and standard deviation for the pollen dispersal distances were positively correlated ($N=60$; Pearson-test; $r=0.44$; $P < 0.001$). See Chen and Pannell (2023b) for how floral traits affected male siring success.

Table 2 Parameter estimates for the Weibull distribution assumed for the pollen dispersal kernel for all the sires (A), single-flowered hermaphrodites (B), and single-flowered males (C) in the study population of *P. alpina*. The parameters *a* and *b* denote the scale and shape parameters in the Weibull distribution, respectively

	a (upper CI, lower CI)	b (upper CI, lower CI)	Mean (SD) (m)	Skewness	Kurtosis
A. All sires	3.26 (2.99, 3.54)	1.07 (1, 1.15)	3.17 (2.95)	1.8	7.73
B. Sires with a bisexual flower	3.38 (2.97, 3.83)	1.04 (0.94, 1.15)	3.32 (3.19)	1.88	8.23
C. Sires with a male flower	3.62 (2.76, 4.7)	0.92 (0.76, 1.1)	3.75 (4.07)	2.26	10.83

Discussion

Short pollen dispersal distance with weak spatial genetic structure

We found that successful pollen dispersal in the fly-pollinated alpine herb *P. alpina* was largely over short distances, with 75% of mating events within 5 m. Although it has been suggested that pollen dispersal distances in herbaceous species are in general short (references in Tomaszewski et al. 2018; see also Ashley 2010; for a summary for trees and shrubs), the considerably short pollen dispersal distances found for *P. alpina* are likely a consequence of fly pollination and high population density (Levin and Kerster 1969a; van Rossum et al. 2011; Rader et al. 2011; Diaz-Martin et al. 2023). Pollination by dipteran insects (e.g., house flies and syrphid flies) is widely thought to occur over lower distances than that by hymenopteran insects (e.g., honey bees and bumble bees) as a result of their different foraging

Table 3 Partial effects of floral gender and floral traits on pollen dispersal distances in single-flowered individuals of *P. alpina* ($N=297$ mating events from 72 sires). Only outcrossing mating events were included in the analysis

	Estimate	Standard error	t-value	<i>P</i> -value
(Intercept)	1.15	0.06	20.12	***
Floral gender	-0.08	0.14	-0.58	n.s.
Tepal length	-0.19	0.05	-4.05	***
Stalk height	0.13	0.06	2	*
Flowering date	-0.21	0.07	-3.2	**

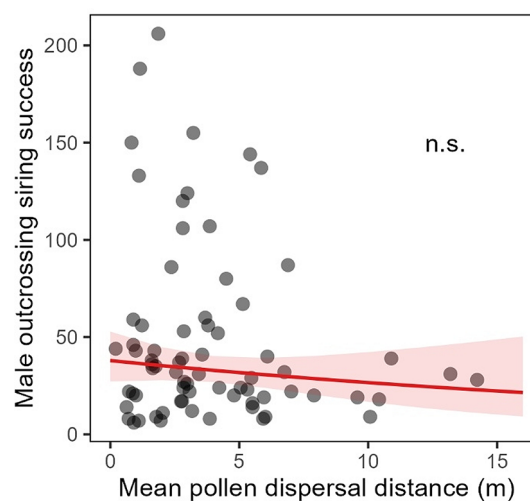


Fig. 3 Absence of a significant relationship between mean pollen dispersal distances and male outcross siring success of individuals of *P. alpina* evaluated using a generalized linear model ($N=72$; $P>0.05$). Each point represents one individual. The 95% confidence interval of the estimates is shown around the regression line

behaviour and flight distances (Inouye et al. 2015). In keeping with this view, the mean pollen dispersal distance for the congeneric bee-pollinated *P. vulgaris* was found to range between 3 and 10 m (DiLeo et al. 2018), substantially higher than our estimates for fly-pollinated *P. alpina*. Our

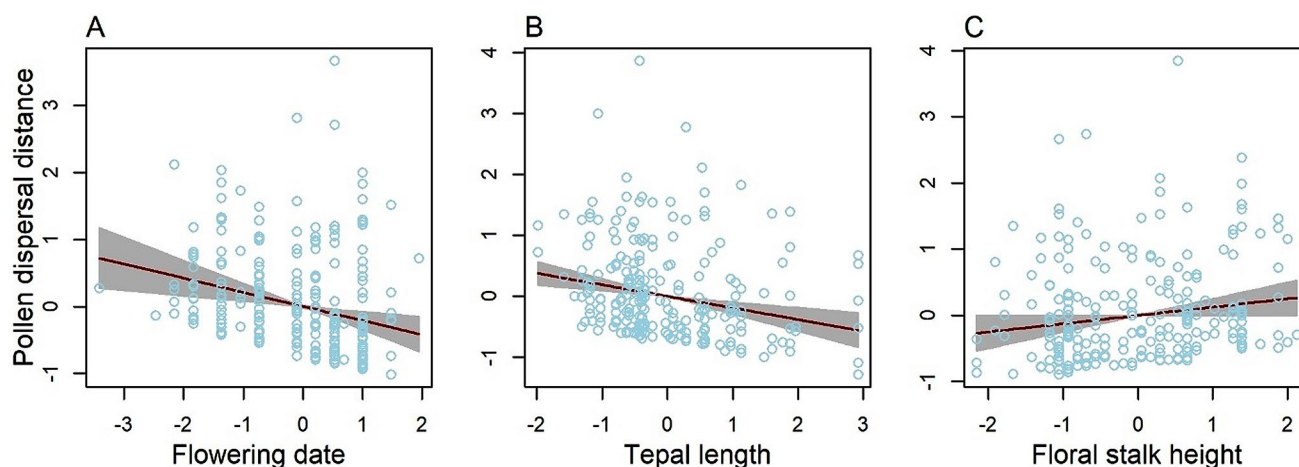


Fig. 2 Partial effects of flowering date (A), tepal length (B), and floral stalk height (C) on pollen dispersal distances of single-flowered individuals of *P. alpina* ($N=297$ outcross mating events from 72 sires).

Floral traits are all standardized. The mating events are represented by blue dots. The 95% confidence interval of the estimates is shown around the regression lines

results thus contribute to the general understanding of how pollination systems may shape within-population gene flow in flowering plants (Krauss et al. 2017).

Despite the short pollen dispersal distances and the mixed mating system and herbaceous growth form of *P. alpina*, the fine-scale spatial genetic structure of the study population was weak. Within-population spatial genetic structure in plants has been shown to depend on the mating system and pollination system of the plants concerned, with high spatial genetic structure expected for species pollinated by insects, especially those with predominant selfing (Vekemans and Hardy 2004). However, effective seed dispersal is expected to break down spatial patterns created by limited pollen dispersal (Meirmans et al. 2011). We quantified only the pollen dispersal kernel of *P. alpina*, but its seeds are dispersed by wind from elongated floral stalks (Chen and Pannell 2022), with maximum dispersal distances estimated to be of the order of 80 m (Vittoz and Engler 2007). In addition, the very high inbreeding depression estimated for *P. alpina* (i.e., 0.93) also means that progeny produced by mating among neighbouring relatives should largely be removed from the population in early life stages, further reducing any potential for the build-up of fine-scale genetic structure, as has been found for *Rhododendron brachycarpum* (Hirao 2010).

Pollen dispersal distance is independent of floral gender

We did not find any difference in the distribution of pollen dispersal distances between male and bisexual flowers of *P. alpina*, despite the fact that both types of flowers differed substantially in their sex allocation, morphology, and phenology (Chen and Pannell 2022, 2023b). First, we found that pollen dispersal kernels of male and bisexual flowers were similar in terms of their shape, skewness, and kurtosis. Second, we detected no difference in mean dispersal distance, irrespective of morphology or phenology (a supplementary analysis using a univariate *gamlss* model with floral gender as an explanatory variable showed the same results; $P > 0.05$). These results conform to those of a previous study that found that male and bisexual flowers have similar male siring success (Chen and Pannell 2023b).

Direct comparisons between male and bisexual flowers in andromonoecious species have been made for various components of male fitness (Cuevas and Polito 2004; Schlessman et al. 2004; Dai and Galloway 2012; Murakami et al. 2022), but not for pollen dispersal distance, as far as we are aware. The study on andromonoecious *Anticlea occidentalis* provided an indirect assessment of the pollen dispersal distances from bisexual versus male flowers in an experiment involving the experimental removal of anthers. That study showed that stamen removal from male flowers

increased pollen dispersal distances compared to individuals with intact flowers or with their bisexual flowers emasculated, though the observed effects largely depended on both the paternal and maternal plants (Tomaszewski et al. 2018).

Phenology and ancillary traits associated with pollen dispersal distance

The impact of flowering phenology on different aspects of female reproductive success has been extensively studied for alpine plant populations (e.g., Kudo 2006; Collin and Shykoff 2010; Kameyama and Kudo 2015; Preite et al. 2015), but we remain largely ignorant of how phenology affects the male components of reproductive success. Our results for *P. alpina* indicate that pollen dispersal distance, a likely important component of male reproduction success, depended negatively on the flowering date (onset of the male function). Pollen dispersal distance in *Ipomopsis aggregata* was found to be independent of phenology, based on an investigation using pollen dyes (Campbell and Waser 1989). In contrast, using a two-generation analysis (Smouse et al. 2001), Hirao et al. (2006) found that the number of effective pollen donors was higher in the late than the early season in *Rhododendron aureum*. So far, we can only conclude that the effect of phenology on the pollen dispersal distance in alpine plants is species-specific, and any patterns that do exist will only emerge with the study of further species and populations.

The negative correlation between pollen dispersal distance and phenology in *P. alpina* is likely a result of an increase in the flowering density of co-flowering species rather than a change in conspecific flowering density (Figure S3) or in potential mating distance throughout the flowering season (Figure S4). According to optimal foraging theory (Pyke et al. 1977), a pollinator should tend to move shorter distances between flowers in more rewarding patches, leading to shorter pollen dispersal distances (Levin and Kerster 1969b; Diaz-Martin et al. 2023). *P. alpina* is usually the sole flowering species in the early flowering season at the site we studied, but it co-flowers with other fly-pollinated species (such as *Dryas octopetala* and *Ranunculus montanus*) later in the season (KC, personal observations), such that there is a rapid increase in the density of flowers in the community as the season progresses. Although optimal foraging theory has rarely been tested for pollen-gathering dipteran insects (Inouye et al. 2015), it has been shown to predict other behaviours such as predation and oviposition (Scheirs et al. 2004; Boesi et al. 2009). If dipteran insects, as generalist pollinators (Inouye et al. 2015), follow an optimal foraging strategy by assessing floral rewards at a community level,

we would expect shorter pollen dispersal distances among late-season flowers.

In insect-pollinated *P. alpina*, pollen dispersal distances were greater from flowers on taller floral stalks. Although a positive correlation between stalk height and pollen dispersal distance is to be expected and has been found, in wind-pollinated species (Okubo and Levin 1989; Tonnabel et al. 2019; Zeng et al. 2023; but see Nakahara et al. 2018; Aljiboury and Friedman 2022), it has, to our knowledge, not been reported for species relying on animals as their pollen dispersal vector. Flowers presented on taller stalks likely attract more pollinators, and their pollen may be dispersed further. For instance, syrphid flies, the major pollinators of *P. alpina* (Chen and Pannell 2022), were found to be more likely to visit taller plants within and among species in grassland habitats (Klecka et al. 2018a, b).

It is not clear why flowers with a larger tepal length had a shorter pollen dispersal distance in *P. alpina* than those with smaller tepals. Given that tepal length showed no correlation with stamen number (Chen and Pannell 2022), it is unlikely that the short dispersal distance is a result of pollinators staying longer in a flower for greater pollen rewards, as predicted by optimal foraging theory (Pyke et al. 1977). Alternatively, dipteran pollinators may also visit the flowers for heat as a reward, which is common in arctic, temperate, and alpine environments (Hocking and Sharplin 1965; Kudo 1995; Inouye et al. 2015). Indeed, it has been shown that the actinomorphic flowers of *P. alpina* could be around 10° C warmer than the air temperature (Dietrich and Körner 2014). If larger tepals lead to warmer temperatures in the flowers, pollinators may forage shorter distances around the patch and thus cause shorter pollen dispersal distances (Pyke et al. 1977; see also the model of Rands and Whitney 2008 for how floral temperature may affect the behaviour of bees seeking nectar rewards). Although the actual mechanisms behind the observed patterns between pollen dispersal distance and different traits in *P. alpina* remain obscure, our results have nevertheless revealed how intra-specific variation in floral traits affects an important component of plant mating.

The effects of phenology and morphological traits on pollen dispersal distances in *P. alpina* imply that these traits may be under selection via male reproductive success, e.g., through enhancing the quality of offspring. It is worth noting that we detected no clear dependence between mean pollen dispersal distance and the number of seeds sired, in contrast with the common assumption of a positive correlation based on theory (Okubo and Levin 1989; Fromhage and Kokko 2010) and the confirmation of such a correlation for wind-pollinated species (Tonnabel et al. 2019; Zeng et al. 2023). In contrast with pollen dispersal in wind-pollinated species in which the mating is likely characterized by simple

mass action (Holsinger 1991), pollen flow between sires and dams in animal-pollinated plants may be much more complex (Harder 1990; Inouye et al. 2015; Krauss et al. 2017). It thus seems likely that traits that increase pollen dispersal distances in animal-pollinated species may not necessarily enhance male reproductive success in terms of numbers of progeny sired, as we have found here (for the estimates of selection gradients via siring success, see Chen and Pannell 2023b). Nonetheless, traits facilitating pollen dispersal may still be favoured as a result of the enhanced *quality* of offspring (Campbell and Waser 1989; Hirao 2010), given the somewhat elevated relatedness of *P. alpina* individuals that are less than 3 m apart and the high level of inbreeding depression estimated for the species (unpublished data).

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Data availability The datasets generated and analyzed during the current study will be available in the online repository Zenodo after acceptance.

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