



Negative correlation between dispersal investment and canopy openness among populations of the ant-dispersed sedge, *Carex lanceolata*

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Abstract Flowering plants exhibit a wide variation in the resources they invest in dispersal structures (dispersal investment), but the environmental correlates still remain unclear in many cases. Canopy openness is predicted to be negatively correlated with dispersal investment, because selective pressures on increased dispersal investment, including host-specific natural enemies and the paucity and/or ephemerality of safe sites, will be more prevalent in shady sites. Here, we tested this prediction by firstly examining the correlation between dispersal investment and canopy openness as well as abundance of the representative natural enemy (rusts, *Puccinia* spp.) through seven populations of an ant-dispersed sedge, *Carex lanceolata* (Cyperaceae). Secondly we conducted a cafeteria experiment to verify the effect of intraspecific variation in dispersal investment on diaspore preferences of seed dispersing ants. Lastly, a seedling transplant experiment was performed to clarify whether seed dispersal distances by ants are sufficient to reduce infection by the rusts. We found a negative correlation between dispersal investment and canopy openness, thus supporting the prediction. Moreover, there were more signs of rust infection caused by *Puccinia* spp. on adult plant leaves in more shady sites. The cafeteria

experiment showed that a large ant species (*Formica japonica*) with relatively long seed dispersal distances tended to prefer diaspores with greater dispersal investments, while smaller ant species with shorter dispersal distances preferred diaspores with lower dispersal investments. The seedling transplant experiment revealed that rust severity in sedge offspring was considerably reduced by the typical seed dispersal distance (ca. 4 m) afforded by the large ant species, *F. japonica*. The increased rust severity at shady sites, combined with the narrow dispersal ranges of rusts, can partially explain the negative correlation of dispersal investment with canopy openness. These results support the importance of canopy openness as a factor underlying the variations in dispersal investment seen among flowering plants.

Keywords Seed dispersal · Myrmecochory · Intraspecific variation · Canopy openness · Dispersal investment · *Carex lanceolata*

Introduction

Seed dispersal is a key facilitator of plant regeneration (Howe and Smallwood 1982; Hanzawa et al. 1988; Wenny and Levey 1998; Christian 2001; Packer and Clay 2000, 2003; Fragoso et al. 2003), therefore investments in seed dispersal structures (hereafter

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referred to as dispersal investment, see Thomson et al. 2018) such as wings or pulp can affect plant fitness and is a determinant of seed dispersal ability (Mark and Olesen 1996; Cheptou et al. 2008; Leal et al. 2014; Thomson et al. 2018). The actual relationships among dispersal investment and plant fitness appear to change in a context-dependent manner, as suggested by the tremendous diversity in dispersal investment seen among flowering plants (Hughes and Westoby 1992; Edwards 2005; Edwards et al. 2006; Thomson et al. 2018). For example, investments in wind-dispersal structures can vary among species depending on the species-specific shade tolerances, because shade intolerant species would require longer seed dispersal distances to reach sparsely distributed canopy gaps (Green 1983). Clarification of factors underlying the differentiation in dispersal investments will contribute to the better understanding of the plant demography, especially considering the important demographic consequences of seed dispersal (Hanzawa et al. 1988; Terborgh et al. 2008; Cheptou et al. 2008; Effiom et al. 2013) and potential for the rapid evolution of seed dispersal traits (Cheptou et al. 2008; Galetti et al. 2013). A few empirical studies have examined the relationships among dispersal investments and putative factors including habitat fragmentation (Cheptou et al. 2008), species-specific tolerance to negative density dependence (Takeuchi et al. 2005) and canopy openness (Venable et al. 1998). However, the number of empirical studies is still limited for each putative factor in a sharp contrast with the vegetative traits, in which a plentiful of studies are available for individual factors (Reich et al. 1998; Wright et al. 2002, 2008; Sack and Scoffoni 2013; Sendall et al. 2016).

Canopy closure following forest development can select for higher dispersal investments for at least three reasons. First, safe sites will become increasingly ephemeral as canopy closure proceeds, which reduces the benefit of staying in a former safe site while increasing the benefit of colonizing new safe sites (Venable et al. 1998). Second, negative interactions between conspecific individuals often increases as canopy openness decreases (Augspurger 1984; Comita et al. 2009), possibly due to the increased abundance of natural enemies (Augspurger 1984; Roberts and Paul 2006) and the reduction in defenses against pests and pathogens in shady areas (Roberts and Paul 2006; Norghauer et al. 2008). The high

abundance of natural enemies in areas with low canopy openness may increase the need to escape from conspecifics and hence result in the evolution of greater dispersal distances. Considering the general importance of light for forest herbs (Meekins and McCarthy 2000; Whigham 2004; Tinya et al. 2009; Westerband and Horvitz 2015), a significant proportion of the variations in dispersal investment seen among flowering plants may be explained by the among-site variation in canopy openness. Third, in the case of animal-dispersed plants, changes in the species composition of seed dispersing animals might accompany the alteration in canopy openness (Andersen 2019) and affect the selection pressure for dispersal investments via interspecific variations in the preferences for dispersal investments (e.g., Hughes and Westoby 1992; Leal et al. 2014). Despite of these multiple reasons, only one study, involving an epizoochorous herb, has examined the relationship between dispersal investment and canopy openness, in which the predicted negative correlation between canopy openness and dispersal investment was supported (Venable et al. 1998).

The perennial sedge *Carex lanceolata* (Cyperaceae) inhabits a wide range of habitats from semi-natural grasslands to forests (Koyanagi et al. 2009; Hashimoto et al. 2012). Diaspores of *C. lanceolata* possess elaiosomes as rewards and, following gravity dispersal, are dispersed by various ant species including *Formica japonica*, *Pheidole noda*, *Paratrechina flavipes*, and *Crematogaster osakensis* (Tanaka and Tokuda 2016). Of these, the large ant, *F. japonica* (body length ca. 5 mm) exhibits much greater seed dispersal distances (average and maximum dispersal distances: 4 and 15 m, respectively) than other sympatric ants (0.3 and 3 m, respectively) (Tanaka and Tokuda 2016). Furthermore, *F. japonica*, contrary to the sympatric smaller ants, clearly preferred diaspores of *C. lanceolata* with larger elaiosomes over those of *C. tristachya* with smaller elaiosomes (Tanaka and Tokuda 2016). This suggests that the higher dispersal investment into elaiosome helps to extend seed dispersal distances, resulting in the reduction in disease transmission from adults to offspring, although it remains to be determined whether *F. japonica* can discriminate the intraspecific difference in elaiosome size hence impose natural selection on the trait. As with other *Carex* spp. (Alexander et al. 2007), *C. lanceolata* is infected by

host-specific fungi, including rusts and smuts, of which rusts are much more prevalent in our focal sites (K. Tanaka: unpublished data). In wheat, majority of new lesions are formed within tens of centimeters from source lesions (Frezal et al. 2009; Farber et al. 2017). Although dispersal distances has not been reported for the rusts of *Carex*, the short dispersal distances of rusts in wheat, another graminoid, implies that the typical seed dispersal distance of *F. japonica* (4 m) is effective to separate seeds from infected adults and thereby prevent or at least delay the seedling infection. As abundance of fungal pathogens tends to be higher at shady sites than open sites (Roberts and Paul 2006), the pathogen-induced selection pressure is predicted to be stronger at shady sites as well, leading to the negative correlation between dispersal investment and canopy openness in *C. lanceolata*.

To test these predictions, we quantified the relationship between dispersal investment (as measured by the proportion of elaiosome area to diaspore area) and canopy openness across populations of *C. lanceolata*. Then, we examined if there was a greater abundance of natural enemies at sites with less canopy openness. To confirm that seed dispersal distance of *F. japonica* is sufficient to prevent or delay seedling infection by rusts, we conducted a seedling transplant experiment. We also evaluated the influence of intraspecific variation in dispersal investment on the diaspore removal by ants to confirm that ants can be a selective agent for higher dispersal investments in *C. lanceolata* when the longer dispersal distances are adaptive.

Materials and methods

Study sites

The among-population comparisons of dispersal investments and related variables were conducted at seven sites in Ibaraki and Tochigi Prefectures, central Japan (total number of plants marked: 85, Table 1). The mean annual temperature and mean annual rainfall across the study sites between 1981 and 2010 was 13.5 °C and 1289 mm, respectively (calculations based on data from the Japan Meteorological Agency, <https://www.data.jma.go.jp/obd/stats/etrn/>). Areas of study sites range from 0.4 to 12.9 ha with the

average of 4.6 ha, and vegetation in all the sites is secondary forest or forest clearance dominated by the white oak *Quercus serrata*. Understorey vegetation is composed of dwarf bamboos, tussock grasses, and evergreen shrubs, with relative abundance varies across the sites. The median distance between adjacent sites was 8.9 km (range 0.9–37.7 km). Of the seven sites, two adjacent sites (Motegi 1 and 2) were used for the cafeteria experiment.

The seedling transplanting experiment was conducted at Mt. Hinokuma, Saga Prefecture, southwestern Japan (33° 34' N, 130° 35' E, alt. 0–160 m a.s.l.). Although mean annual temperature (16.5 °C) and rainfall (1870 mm) is higher than the study sites in Ibaraki Prefecture, rust infection is similarly common as in Ibaraki.

Relationships among canopy openness, dispersal investments, and rust prevalence

In April 2019, we haphazardly marked 7–26 flowering plants of *C. lanceolata* at each site. The number of marked plants varied among the study sites due to different abundances of *C. lanceolata*. Marked plants were located at least 2 m from one another. In May 2019, the natural fruiting season of this sedge, we collected diaspores from each marked plant, placed them in plastic bags, and stored them in a refrigerator at 4 °C until use. Within 7 days of their collection, seeds were rehydrated by placing them in wet, folded filter paper in a plastic bag and storing them in a refrigerator at 4 °C for 12 h. Then, photographs at × 20 magnification of two or three diaspores per plant (depending on diaspore availability) were taken using a digital camera (WRAYCAM-EL310, WRAYMER INC., Japan) attached to a binocular microscope. The number of diaspores collected per plant was sufficient to characterize dispersal investment of each plant individual, because nested ANOVA showed that within-plant variance in dispersal investments accounted for only 4.4% of total variance. The diaspores were placed such that the maximum area of elaiosomes was visible from above. The areas of elaiosomes and whole diaspores were measured using Image J (Rasband 1997–2007). From these measurements, dispersal investment was calculated as the ratio of elaiosome area to the total area of a diaspore (i.e., elaiosome + seed body). In addition, seed body area was calculated by subtracting the elaiosome area from

Table 1 Description of the study sites

Sites (area: ha)	Coordinate	Altitude (m)	Habitat	Main tree species	No. plants sampled
Daigo (0.4)	36° 79' N, 140° 35' E	193	Secondary forest	<i>Quercus serrata</i> , <i>Prunus jamasakura</i>	9
Motegi 1 (0.8)	36° 46' N, 140° 20' E	251	Forest clearance	<i>Q. serrata</i> , <i>Rubus spp.</i>	12
Motegi 2 (11.5)	36° 47' N, 140° 21' E	290	Secondary forest	<i>Q. serrata</i> , <i>Chamaecyparis obtusa</i>	26
Kasama (5.9)	36° 29' N, 140° 25' E	249	Secondary forest	<i>P. jamasakura</i> , <i>Q. serrata</i>	10
Makabe (12.9)	36° 23' N, 140° 09' E	390	Secondary forest	<i>Q. serrata</i> , <i>Castanopsis cuspidata var. sieboldii</i>	11
Oda (0.3)	36° 16' N, 140° 13' E	272	Secondary forest	<i>Q. serrata</i> , <i>P. jamasakura</i>	10
Tsuchiura (0.3)	36° 08' N, 140° 16' E	25	Secondary forest	<i>Q. serrata</i> , <i>Q. myrsinifolia</i>	7

the diaspore area to clarify whether the variation in dispersal investment can be produced by the variation in seed sizes (due to positive allometry between elaiosome and seed size, Edwards et al. 2006). All of these diaspore traits were averaged for each plant. In August 2019, we measured the canopy openness 1 m above each marked plant using the canopy-scope method (Brown et al. 2000; Westerland and Horvitz 2015; Browne and Karubian 2016), which estimates the size of the largest visible gap on a scale of 0–25. There were 25 plants lost due to mowing or vandalism, therefore the total number of plants sampled for canopy openness was reduced to 60 plants. For each of these 60 plants, the two longest leaves were collected and taken to the laboratory. Images of the leaves were taken by a flat bed scanner, and based on the images, presence or absence of the red-colored pustules was recorded as rust symptom (confirmed by Prof. Y. Yamaoka at Tsukuba University). Then, the proportion of leaves with rust symptoms was calculated for each site, and this was used as a proxy for rust prevalence.

Effect of seed dispersal distance on rust severity in seedlings

A seedling transplant experiment was conducted to evaluate if the average seed dispersal distance by *F. japonica* (ca. 4 m) was sufficient to reduce the rust severity in offspring plants. Diaspores were collected

from at least ten fruiting plants in May 2016 and were individually sown in cell trays filled with sand. The cell trays were placed in an unheated greenhouse on Saga University campus (33° 24' 22" N; 130° 29' 15" E). Seedlings were transplanted from the trays into a field between late June and early July 2016, once they had produced two leaves. Before the seedlings were transplanted, eight adult plants with rust symptoms were haphazardly marked along a trail, each of which was at least 10 m apart from the next. Then, one seedling was transplanted at distances of 30, 100, and 400 cm from the marked adults. The three distance classes were selected to simulate seed dispersal distances by small ants (*C. osakensis* and *P. flavipes*: < 40 cm), medium ants (*P. noda*: 128 cm), and large ants (*F. japonica*: 425 cm), based on Tanaka and Tokuda (2016). Then, the rust severity on each seedling was recorded each week using the following categories: 0, no symptoms; 1, symptoms observed on less than 50% of a whole plant; 2, symptoms observed on 50% or more of a whole plant. The monitoring of seedlings was continued either until the end of the experiment (2 September 2016) or until focal seedlings died. For seedlings that died, their longevity following transplantation was recorded. The maximum value of rust severity for each seedling during the experimental period was used for the analysis.

Diaspore preferences of large and small ants

Diaspores were collected during May 2018 from more than 30 adult sedges at two sites (Motegi 1 and Motegi 2; Table 1) and refrigerated at 4 °C until use. Cafeteria experiments were conducted for *F. japonica* and small ants at Motegi 1 and Motegi 2, respectively. At Motegi 1, six nests of *F. japonica*, separated from one another by at least by 5 m, were marked. At Motegi 2, ant nest entrances were hidden under leaf litter and therefore 11 plots near flowering *C. lanceolata* plants were haphazardly chosen, with minimum distances between adjacent plots of 5 m. The minimum distance of 5 m was considered to be sufficient to ensure independent discoveries of depots by different ant colonies, because seed dispersal distances in our system rarely surpass 5 m (Tanaka and Tokuda 2016, K. Tanaka: unpublished data). The cafeteria experiments were conducted between the hours of 0930 and 1530 h. Ten diaspores were randomly selected and placed on a depot made of circular cork board (10 cm in diameter) at each of 17 experimental plot. Adjacent diaspores were separated by approximately 5 mm so that no diaspore was hidden from the ants under other seeds. Then, diaspore removal by ants was monitored for 30 min. If any diaspores were removed, both the ants and the diaspores transported by them were collected to later measure the diaspore traits. The collected ants were stored in a plastic bag during the trial to prevent them removing any further diaspores. A trial was terminated if no worker ants visited a depot within 5 min. In total, 10 and 22 trials were conducted at Motegi 1 and 2, respectively. On average, 2.4 diaspores were accidentally lost during each trial, either due to heavy wind or by the failure to retrieve diaspores removed by ants. Diaspores used in the cafeteria experiments were rehydrated by placing them in a wet, folded filter paper in a plastic bag and storing them in a refrigerator at 4 °C for 12 h. Then, the areas of elaiosomes and whole diaspores were measured using the same protocol described above.

Statistical analysis

All the following analyses were conducted using R version 3.5.1 (R Foundation for Statistical Computing 2018). Before the analyses were performed, dispersal investment, elaiosome area, seed body area, and canopy openness were averaged for each site. Because

significant spatial autocorrelation was not detected for neither variables as judged from the Moran's I test (Moran.I function from "ape" package), correlations between canopy openness and the other variables except for rust prevalence (i.e., dispersal investment, elaiosome area, seed body area) were tested using the Spearman's rank correlation test. The nonparametric correlation test was used here because inspection of the graph suggested the nonlinear relationships. The effect of canopy openness on rust prevalence was analyzed using a generalized linear model (GLM) with binomial errors its significance was assessed using a likelihood-ratio test. GLM rather than Spearman's rank correlation test (as for other variables) was used for the rust prevalence to avoid giving the same weight to the estimates based on radically different number of leaves. For example, rust prevalences in Tsuchiura and Motegi 2 were both around 60% but the former was based on just six leaves, while the latter did on 68 leaves and thus accuracy of the estimated rust prevalence was clearly higher in the latter.

For the seedling transplant experiment, the effect of seed dispersal distances on rust severity was analyzed using a generalized linear mixed model (GLMM) assuming binomial errors. The response variable in the model was the relative rust severity, which was defined as the rust severity (0–2) divided by its maximum possible value (i.e., 2). Continuous fixed effects included the distance to marked adults and seedling longevity, while the identity of marked adults was incorporated as a random effect to deal with potential spatial autocorrelation in rust severity. Longevity was incorporated to control for the potentially unequal probability of rust infection among seedlings with different longevity, because rusts would not have had sufficient time to elicit signs of disease in short-lived seedlings. The effect of distance to marked adults was tested using the likelihood-ratio test.

The results of the cafeteria experiments were analyzed separately for Motegi 1 (*F. japonica*) and 2 (small ants) using the GLM with binomial errors. In the models, the occurrence of removal was treated as the binary response variable, while dispersal investments and diaspore area were incorporated as explanatory variables. We originally included trials as a random effect but excluded it after finding that the AICs of models with a random effect are higher than

those without it. The significance of each explanatory variable was tested using the likelihood-ratio test.

Results

Relationships among canopy openness, diaspore traits, and rust severity

As expected, dispersal investments were negatively correlated with canopy openness across the seven sites (Spearman's rank correlation test; $\rho = -0.964$,

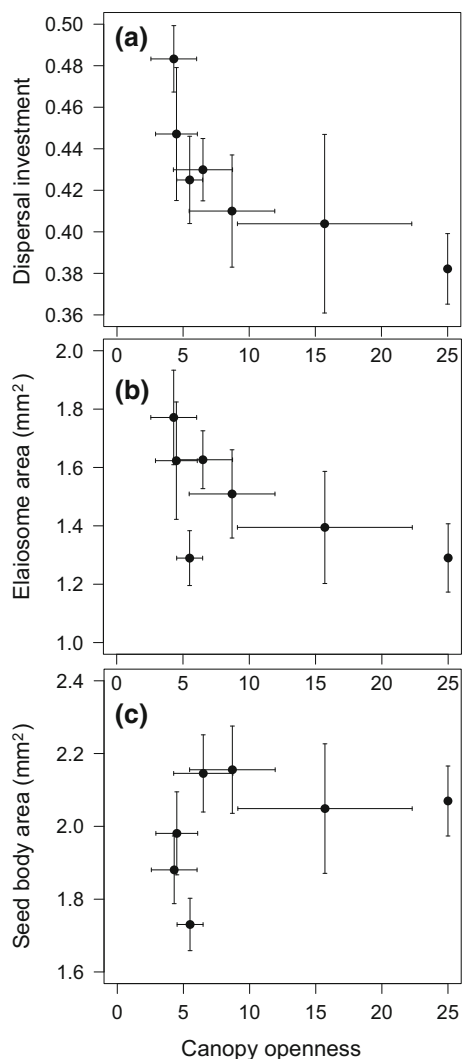


Fig. 1 Relationships between canopy openness and dispersal investment (a), elaiosome area (b), and seed body area (c) across study sites. Each point represents a site and error bars on both axes indicate 95% confidence intervals

$P = 0.003$, Fig. 1a). The correlation with canopy openness was not significant for elaiosome area ($\rho = -0.214$, $P = 0.662$, Fig. 1b) or for seed body area ($\rho = 0.07$, $P = 0.167$, Fig. 1c). Thus, the correlation between dispersal investments and canopy openness likely arose from the simultaneous and nonsignificant changes in both elaiosome area and seed body area. Dispersal investments were also correlated with rust prevalence ($\rho = 0.811$, $P = 0.027$; data not shown), but the correlation was weaker than that between dispersal investments and canopy openness ($|\rho| = 0.964$), indicating that canopy openness imposes a stronger influence on dispersal investments than rust prevalence does. The binomial GLM showed that the rust prevalence was significantly lower in sites with higher canopy openness (likelihood-ratio test: $\chi^2 = 4.653$, $P = 0.031$, Fig. 2).

Effect of seed dispersal distance on the rust severity in seedlings

Median longevity of the transplanted seedlings was 83 days and did not differ significantly between dispersal distances (Log-rank test: $P = 0.300$, data not shown). The average rust severity in the transplanted seedlings decreased to one-seventh (0.875–0.125) as the distance to marked adults increased from 30 to 400 cm (Fig. 3). Statistical significance of the trend was supported in GLMM (likelihood-ratio test: $\chi^2 = 6.456$, $P = 0.011$).

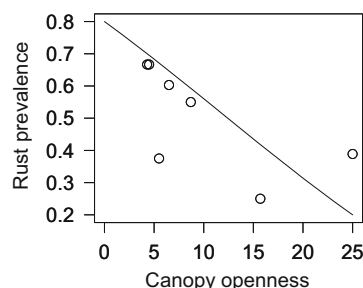


Fig. 2 Relationship between canopy openness and rust prevalence across study sites. The vertical axis denotes the proportion of leaves with rust lesions collected from the focal sites as a measure of rust prevalence. A prediction curve derived from the binomial GLM is included (see main text for details of the model)

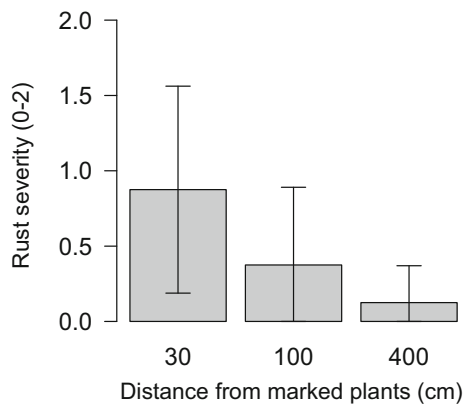


Fig. 3 Results of the seedling transplant experiment. The vertical axis shows the rust severity in a seedling measured as follows: 0, no lesions; 1, lesions observed on less than 50% of the entire plant; and 2, lesions observed on 50% or more of the entire plant. The error bars represent 95% confidence intervals

Diaspore preferences of large and small ants

Diaspore removal occurred in all trials at Motegi 1 with the exception of one case. However, diaspore removal only occurred in 36% (8/22) of trials at Motegi 2, because of both low ant abundance (50%, 11/22) and elaiosome consumption in situ (14%, 3/22). As predicted, most diaspores were removed by the large ant, *F. japonica*, at Motegi 1, while all diaspores were removed by small ants at Motegi 2 (Table 2). Among the small ants, *P. flavipes* was the dominant remover of diaspores (Table 2). At Motegi 1, *F. japonica* showed a marginally significant preference for diaspores with higher dispersal investments ($\chi^2 = 3.682$, $P = 0.055$, Fig. 4a), while the small ants preferred those with lower dispersal investment at

Table 2 Species composition of ants removing seeds in each cafeteria experiment

Site	Seed remover	No. seeds removed
Motegi 1	<i>Formica japonica</i>	20
	<i>Tetramorium tsushimae</i>	1
	<i>Lasius</i> sp.	1
Motegi 2	<i>Paratrechina flavipes</i>	9
	<i>Crematogaster osakensis</i>	1
	<i>Pheidole fervida</i>	1
	<i>Pristomyrmex punctatus</i>	1
	<i>Lasius</i> sp.	1

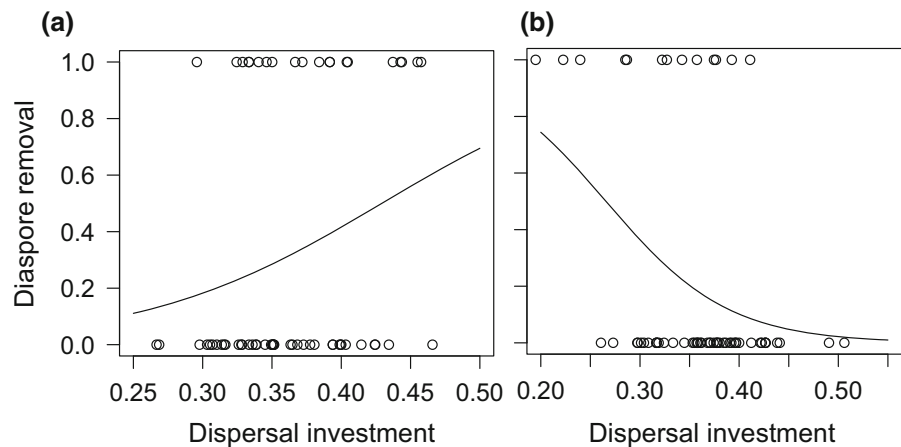
Motegi 2 ($\chi^2 = 7.250$, $P = 0.007$, Fig. 4b). Diaspore areas did not significantly affect the diaspore preference in either ant guild (*F. japonica*: $\chi^2 = 0.050$, $P = 0.823$, small ants: $\chi^2 = 2.952$, $P = 0.086$, data not shown).

Discussion

For the ant-dispersed sedge, *C. lanceolata*, dispersal investment was negatively correlated with canopy openness. Although elaiosome size is often correlated with seed size (Edwards et al. 2006), the trend in *C. lanceolata* could not be explained by among-site variations in seed size, because the seed body area was not significantly correlated with canopy openness. Instead, the trend of higher dispersal investment in shadier sites could partially be explained by the higher rust prevalence and hence the stronger selection pressure for longer seed dispersal distances in shadier sites. Furthermore, the cafeteria experiment confirmed that diaspores with greater dispersal investment were preferred by large ants with long seed dispersal distances and avoided by small ants. The stronger preference for greater rewards in large ants is consistent with findings of previous studies (Hughes and Westoby 1992; Leal et al. 2014) and suggests that the trend revealed in this study reflects among-site differences in seed dispersal strategy rather than neutral variations.

Natural enemies produce the escape benefit of seed dispersal in a wide variety of plants by causing the higher mortality around adult plants (O'Dowd and Hay 1980; Howe et al. 1985; Packer and Clay 2000, 2003; Fragoso et al. 2003; Pigot and Leather 2008) and have been proposed as the main selective pressure for the evolution of seed dispersal (Howe and Smallwood 1982; Muller-Landau et al. 2003). Rust fungi (*Puccinia* spp.) are the dominant pathogens of *C. lanceolata* at our focal sites, and although the pathogenicity of the rusts has not been determined for *C. lanceolata*, rusts in general retard photosynthesis through the formation of lesions and associated reductions in green leaf area (Paul and Ayres 1987; Springer 2002; Robert et al. 2005). Large portions of leaf area were often covered by rust lesions in heavily infected *C. lanceolata*, so this negative impact on photosynthesis also appears to be the case with *C. lanceolata*. Our seedling transplant experiment

Fig. 4 Diaspore preferences of *F. japonica* and small ants revealed by the cafeteria experiments. Prediction curves were based on the estimates from GLMs



revealed that the rust severity in seedlings was reduced by an increase in seed dispersal distances, from 30 to 400 cm, which corresponded with the shift in seed dispersers, from small to large ants (Tanaka and Tokuda 2016). The short-distance local dispersal of rusts is consistent with previous results observed with wheat rusts (Frezal et al. 2009; Farber et al. 2017), implying the generality of our result. Although all seedlings may ultimately be reached by rust spores irrespective of seed dispersal distances, any delay in spore attachment would reduce the risk of infection, owing to the increased resistance to pathogens at later developmental stages, as has been shown for many pathogens (Develey-Rivière and Galiana 2007; Farber and Mundt 2017). Therefore, the large ant, *F. japonica*, can provide plants with a much higher escape benefit than small ants can, potentially explaining the greater dispersal investment seen in sites with lower canopy openness. The higher rust prevalence at shady sites conforms to the general trend for fungal pathogens (Roberts and Paul 2006), and might be a consequence of either the susceptibility of fungi to UV-B or the enhanced defense capacity of host plants in sunny sites (Roberts and Paul 2006). Thus, the effect of canopy openness on pathogen-mediated selective pressure for dispersal investments may be a general phenomenon among flowering plants in which pathogens are their main natural enemies.

Although rust prevalence was positively correlated with dispersal investments as predicted, the correlation was weaker than that between canopy openness and dispersal investments. This implies that factors other than rust prevalence also mediate the effects of canopy openness on dispersal investment. First,

because *F. japonica* prefers open habitats (Iwata et al. 2005; Kwon et al. 2014) while smaller ants such as *P. flavipes* and *P. fervida* prefer forests (Iwata et al. 2005; Kwon et al. 2014), *F. japonica* might be more dominant at sites with increased canopy openness. The dominance of long-distance dispersers (relative to smaller ants) could have diminished the threshold dispersal investment required for attracting large ants and hence selected for lower dispersal investments to conserve energy for seed production. The effect of the dispersal easiness on the evolution of dispersal mechanisms has been supported by several theoretical models (Ezoe 1998; Encinas-Viso et al. 2014) and may have affected the selection for dispersal investments in *C. lanceolata* independently from the escape- and colonization-related selective factors. Second, the paucity and ephemerality of safe sites in shady areas might increase the colonization-related benefits of dispersal, selecting for higher dispersal abilities (Venable et al. 1998). On forest floors in temperate regions, spatial autocorrelation with light availability has been observed for distances up to 4 (von Wettberg et al. 2005) or 6 m (Washitani and Tang 1991). Moreover, in the case of the forest herb, *Impatiens capensis* (Balsaminaceae), a ranking of sites within a forest in terms of their light availability showed annual variations in relation to gap dynamics (von Wettberg et al. 2005). The large ant, *F. japonica*, can disperse seeds an average distance of 425 cm, whereas the average seed dispersal distances of smaller ants were mostly less than 100 cm (Tanaka and Tokuda 2016), therefore the fine-grained (< 4 m) spatiotemporal variation in light availability would select for higher dispersal investments to attract large ants.

Light is the main limiting resource in the forest understory (Meekins and McCarthy 2000; Whigham 2004; Tinya et al. 2009; Westerband and Horvitz 2015; Augspurger and Salk 2017) and its availability varies both spatially and temporally according to differences in successional stages and anthropogenic habitat modification (Martens et al. 2000; Breshears 2006; Lopez-Gallego and O'Neil 2014). The ability to undergo light-dependent adjustments to phenotypes is thus a key determinant of plant distributional ranges (Sultan 2001; Godoy et al. 2011; Goulart et al. 2011). Previous studies on adaptation or phenotypic plasticity to light availability have mostly focused on vegetative traits (von Wettberg et al. 2005; Gotsch et al. 2010; Godoy et al. 2011; Goulart et al. 2011; Lopez-Gallego and O'Neil 2014). For example, increases in specific leaf area and height via local adaptation and phenotypic plasticity enabled an alien herb, *Prunella vulgaris* (Lamiaceae), to colonize forests from open sites (Godoy et al. 2011). In contrast, to our knowledge, only one study has focused on seed dispersal traits (Venable et al. 1998), despite much evidence of the important demographic consequences of seed dispersal (Hanzawa et al. 1988; Packer and Clay 2000, 2003; Fragoso et al. 2003; Cheptou et al. 2008; Blake et al. 2009; Effiom et al. 2013) and the rapid evolution of seed dispersal traits (Cheptou et al. 2008; Galetti et al. 2013). In the only study which dealt with dispersal adaptation to light environments, increased epizoochorous dispersal ability of a seed dimorphic herb was observed at sites with increased canopy closure (Venable et al. 1998). We found the same trend in the ant-dispersed sedge *C. lanceolata*, implying a common response of seed dispersal traits to light environments among a wide variety of plants with distinct seed dispersal modes. It should be noted that contrary to the seed dimorphic herb examined by Venable et al. (1998), realized dispersal ability of *C. lanceolata* is not directly linked to dispersal investments, the potential dispersal ability, due to between-site differences in ant species composition. For example, despite of the lowest dispersal investments at Motegi 2 across seven sites examined, realized dispersal ability would be high at Motegi 2, because the large ant *F. japonica* was particularly abundant there. The relationship between canopy openness and realized dispersal ability remains to be investigated in future.

The relationship between canopy openness and dispersal investments found in the ant-dispersed sedge, *C. lanceolata*, offers a new model system with which to investigate the evolution of dispersal in flowering plants. Specifically, the small spatial scale of myrmecochory allows sufficient replication of field experiments across multiple sites, and enables powerful tests to be performed to explore the effect of canopy openness on the fitness consequences of greater dispersal investments. The advantage in terms of spatial replication has so far been exploited to evaluate the ecological benefits of myrmecochory such as predator avoidance (Manzaneda et al. 2005), avoidance of intraspecific competition (Leal et al. 2015), and directed dispersal (Manzaneda and Rey 2012). However, our results indicate that the advantage of myrmecochory can also be utilized for testing general hypotheses on the evolution of dispersal in flowering plants, including the effect of host-specific natural enemies on the evolution of long-distance seed dispersal (Muller-Landau et al. 2003). For *C. lanceolata*, future seedling transplant experiments at each of the current focal sites will be conducted to evaluate the validity of the hypotheses that greater seed dispersal distances are favored as canopy openness decreases.

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