

SHORT COMMUNICATION

Relationships between parasitism, bumblebee foraging behaviour, and pollination service to *Trifolium pratense* flowers

SANDRA D. GILLESPIE,¹ KAYLA CARRERO² and LYNN S. ADLER² ¹Department of Biology, Simon Fraser University, Burnaby, Canada and ²Department of Biology, University of Massachusetts, Amherst, Massachusetts, U.S.A.

Abstract. 1. Parasite effects on host behaviour frequently alter their hosts' trophic interactions. There are many compelling examples of such effects in herbivore-based trophic interactions, but less attention has been paid to how parasite effects on host behaviour can alter mutualistic interactions.

2. Pollination mutualisms depend greatly on pollinator behaviour, and many pollinators are attacked by a wide range of parasites and parasitoids.

3. To investigate whether parasites affect pollination service via changes in host behaviour, natural variation in conopid fly parasitism was used to investigate the relationship between infection and *Bombus impatiens* Cresson behaviour foraging on arrays of *Trifolium pratense* L. flowers in the laboratory. The consequences of infection for seed set and seed mass were also examined.

4. Conopid parasitism was not related to any measured behavioural response; however, flowers visited by conopid-parasitised bees set significantly heavier seeds than those visited by unparasitised bees. Larger bees were more likely to be parasitised, but the relationship between parasitism and seed set still held after accounting for body size.

5. The present results demonstrate that parasitoids may have positive impacts on per-visit pollination, but, because larger bees were more frequently parasitised, parasitism could also affect pollination by removing the largest pollinators from the population.

Key words. Behaviour, *Bombus*, conopid, parasitism, pollination, *Trifolium*.

Introduction

The effects of parasites on host populations and their trophic interactions are well documented (e.g. Sumption & Flowerdew, 1985; Matsumoto *et al.*, 2003), and there is burgeoning interest in how parasite effects on host behaviour can modify food webs (Lefevre *et al.*, 2009; Adamo, 2012; Sato *et al.*, 2012). Parasite impacts on host behaviour in mutualisms have been documented (Gegear *et al.*, 2006; Pardee & Philpott, 2011), but little is known about the indirect effects of these changes on the host's mutualistic partners. For example, bumblebees are attacked by parasitoid conopid flies (Diptera: Conopidae) while foraging (Schmid-Hempel & Durrer, 1991; Gillespie, 2010). Infection

with conopids is energetically costly and reduces host pollen collection (Schmid-Hempel & Schmid-Hempel, 1990), which could affect plant reproduction.

We examined how natural variation in conopid parasitism related to foraging behaviour and pollination service using laboratory arrays of *Trifolium pratense* L. flowers (*Trifolium* from now on). We predicted that (i) parasitism by conopids would reduce bumblebee foraging rates and (ii) that this would lead to a reduced pollination service to *Trifolium* flowers.

Methods

Our study was conducted in summer 2009 at the organic farm at Hampshire College in Amherst, Massachusetts, in an area with abundant *Trifolium*. Because conopids cannot be lab-reared, we introduced three commercial *Bombus impatiens* Cresson

Correspondence: Sandra D. Gillespie, Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, British Columbia, Canada V5A 1S6. E-mail: sgillesp@sfu.ca

colonies (Biobest, Leamington, ON, Canada) to our field site, exposing bees to natural conopid parasitism. We began trials 7 days after field placement.

Greenhouse plants

Trifolium pratense (Fabaceae; red clover) is pollinated by a small number of bee species, including bumblebees (Rao & Stephen, 2009), and bumblebee visitation is positively related to seed set (Gillespie & Adler, 2013). Flowers are composed of multiple florets arranged into a single inflorescence head ('flower' from now on). Plants were grown from seed (Greenfield Farmer's Supply, Greenfield, Massachusetts), planted in 12.7-cm-diameter pots and maintained in a screened, pollinator-free greenhouse. Owing to plant loss to powdery mildew, we also brought plants from our field site to the greenhouse and planted them using the same protocols. Only previously unopened flowers were used in trials.

Data collection

We collected bumblebee workers foraging on *Trifolium* to get experienced foragers; this limited our ability to assign colony identity to bees. Observations were conducted in a $2 \times 2 \times 1.5$ m³ enclosure containing a foraging display of 16 2.5-cm-diameter holes, spaced 12 cm apart in a 4×4 array. The array was raised so that potted plants fit underneath. Individual *Trifolium* flowers were inserted through each hole (Figure S1).

Bees were chilled in a cooler during transport and while waiting to forage to reduce stress. A single bee was placed directly on a flower and a video camera recorded behaviour. Bees were allowed to forage either until 20 floral visits occurred or 2 h had passed. Approximately 75% of bees foraged; failure to forage was not related to conopid parasitism (d.f. = 1, $\chi^2 = 0.061$, $P = 0.8$).

Each trial used 16 *Trifolium* flowers. Whenever possible, flowers were used for only a single trial; however, we reused some when there were insufficient flowers to replace the entire array between bees. Flowers that had been visited previously were not used to measure seed set. For each foraging bee, two flowers that were only visited once were marked. The flowers were returned to the greenhouse to mature, and then we counted and weighed the seeds. Each flower used to assess seed set was only visited once by a single bumblebee. Therefore, seed set was probably limited by pollen receipt rather than resources. We did not record which flowers were from a greenhouse versus field plants; however, we only used field plants later in the season. There was no pattern over time in either parasitism (logistic regression: $F_{1,42} = 0.1338$; $P = 0.7168$) or seed weight (ANOVA: $F_{1,26} = 1.012$; $P = 0.32$), suggesting that our parasitism results were not as a result of confounding effects plant origin.

Behaviour was measured from videos using behaviour quantification software (JWatcher; Blumstein *et al.*, 2006). We recorded when the bee landed and departed from each flower and the time bees spent 'out of sight' of the camera (not visible for >5 s). We then calculated the average time spent per flower and the floral interval (average time interval between flowers, excluding time out of sight).

Bees were then placed in vials covered with open mesh, provided with sugar water *ad libitum*, and maintained in the lab until death to rear conopids for identification. We measured the length of the radial cell as an estimate of bee size (Harder, 1985), and bees were dissected and inspected for the presence of conopid parasitoids. Conopid larvae reared to adulthood were identified as either *Physocephala furcillata* (Williston) or *Physocephala tibialis* (Say) (J. Gibson, pers. comm.). Bees were also examined for *Crithidia bombi* and *Nosema bombi* cells, which were not analysed because infection levels may have changed while rearing conopids. Infection by these parasites was unrelated to conopid parasitism (*Crithidia bombi*: d.f. = 1; $\chi^2 = 0.03452$, $P = 0.852$; *Nosema bombi*: $\chi^2 = 2.43$, d.f. = 1, $P = 0.119$), and inclusion in analyses did not affect our conclusions regarding conopids.

Statistical analysis

All analyses were conducted in R, version 2.15.3 (R Development Core Team, 2009). We did not include colony as a factor owing to the difficulty in assigning colony origin to workers. Of the 46 bees used in trials, 36 (17 parasitised) exhibited sequential foraging behaviour, allowing calculation of foraging metrics, and 41 (18 parasitised) visited at least one flower for seed measures. We removed one outlier bee owing to a small size (2.7 SD below the mean, with next smallest bee only 2 SD below). We used binomial logistic regression to assess whether conopid parasitism was related to days survived in the lab or radial cell length.

Floral interval and time per flower were log transformed and analysed with a Gaussian distribution and identity link function (glm). Seed set per flower and mass per seed were averaged between the two flowers collected for each bee. Because seed set was highly zero-inflated and distributed as a count variable, we examined the average seed number per flower, rounded to a whole number, using negative binomial models (log link, glm.nb). Seed mass was analysed with a Gaussian distribution and identity link function. For each response variable, we examined the main effects of conopid parasitism and radial cell length as a measure of bee size. We also included the interaction between radial cell length and conopid parasitism because previous research suggested that body size may be related to conopid parasitism (Otterstatter, 2004; Gillespie, 2010).

Results

Bees parasitised by conopids had longer wing radial cells than unparasitised bees ($F_{1,42} = 7.55$, $P = 0.008$; Fig. 1a), suggesting that larger bees were more likely to be attacked by parasitoids. Conopid-parasitised bees also survived for fewer days after their trial ($F_{1,42} = 5.2$; $P = 0.02$; uninfected bees: 10.0 ± 1.5 days, infected bees: 5.2 ± 0.7 days). Conopid parasitism did not affect any behavioural variable ($F_{1,34} < 1.0$, $P > 0.1$ for all). Total seed set increased with radial cell length ($F_{1,42} = 4.54$, $P = 0.039$; Fig. 1b) but was not related to parasitism. Seed mass increased with conopid parasitism ($F_{1,31} = 4.35$, $P = 0.046$; Fig. 1c), but was not significantly related to any other factor ($F < 1.0$, $P > 0.1$).

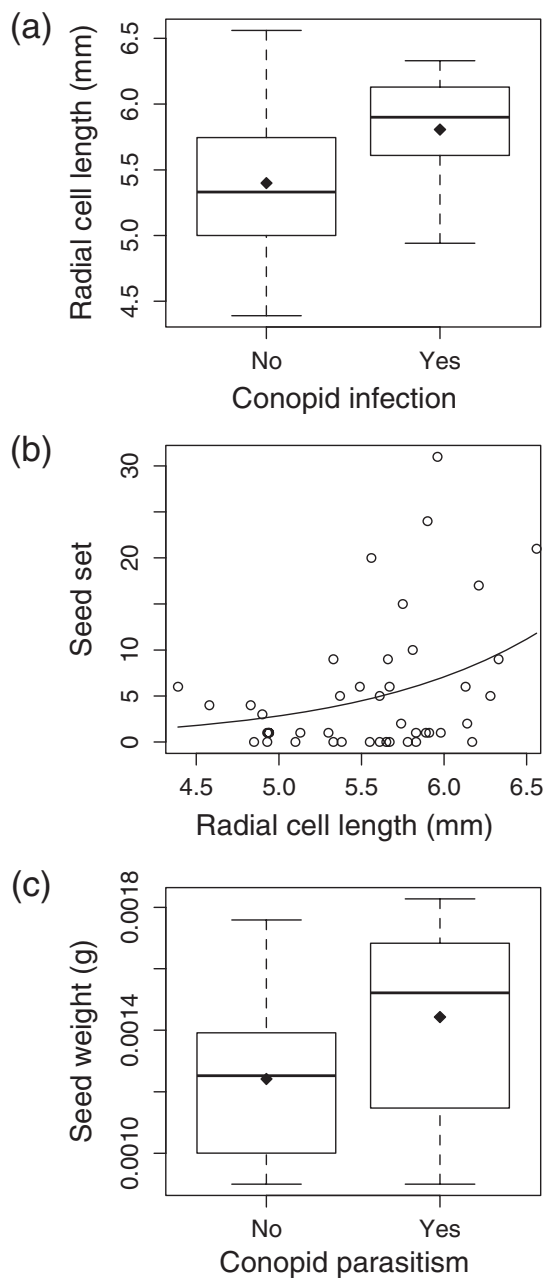


Fig. 1. (a) Body size difference between conopid infected and uninfected bees, as estimated by wing radial cell length; (b) seed set, indicated by the number of seeds produced per *Trifolium* flower head, increased with wing radial cell length, a correlate of body size. (c) Seeds from *Trifolium* flowers visited by conopid-parasitised bees were heavier than seeds produced by visits from unparasitised bees.

for all). We tested whether the positive relationship between seed mass and conopid parasitism was as a result of a confounding body-size effect, using the residuals after assessing the relationship between radial cell size and seed weight. The effect of conopids was still significant ($F_{1,31} = 4.35$, $P = 0.045$), indicating that the effect of conopid parasitism on seed mass is independent of body size.

Discussion

Our results highlight the complex relationships between parasitism and host behaviour, including how changes in behaviour can alter host interactions with mutualist partners. It is known that conopids and other parasites affect colony fitness in *Bombus* (Schmid-Hempel & Schmid-Hempel, 1988) and behaviour in the field (Schmid-Hempel & Schmid-Hempel, 1990) and laboratory (Gegear *et al.*, 2006). We document for the first time that parasitism by conopids may impact the pollination service to *Trifolium* flowers, despite no obvious behavioural differences. Determining the mechanism behind this pattern requires further investigation.

Because we relied on natural variation in parasitism, there are several potential confounding factors that should be considered. Our sample may include wild and managed bees, and we could not identify conopid parasites from all bees to species. We also could not control the extent of prior foraging experience of bees before our observations. Increased variation owing to any of these factors may obscure effects of parasitism on pollination or behaviour, but seem unlikely to create spurious results, meaning that our results are likely a conservative estimate. Parasitised bees could also respond differently to the chilling process. If this is the case, the patterns seen here may reflect an interaction between cooling and parasitism, which may also be intriguing.

Instead of the predicted negative effects, we found that parasitism could lead to positive impacts on one mutualist partner. However, larger bees were more effective pollinators of *Trifolium*, and more likely to be parasitised, possibly owing to host preference by conopids (Schmid-Hempel & Schmid-Hempel, 1996), or exposure as a result of an increased foraging by larger workers (Spaethe & Weidenmüller, 2002). In this study, parasitised bees survived on average five fewer days in the lab, suggesting conopids could remove the most effective pollinators from the population. Thus, the consequences of conopid parasitism for pollination will ultimately depend on the relative importance of pollinator density versus behaviourally mediated changes. Behaviourally-mediated effects of antagonists on prey are frequently important for multitrophic interactions (Schmitz *et al.*, 2004). Further investigation will be necessary to determine whether this is the case for mutualistic interactions.

Acknowledgements

This work was conducted while S. Gillespie was a PhD Candidate at the University of Massachusetts. We would also like to thank the managers at the Hampshire college organic farm for providing space for bumblebee cages, the Elkinton lab at UMass for space to conduct foraging trials, and three anonymous reviewers for greatly improving the manuscript. This research was supported by the National Science Foundation under Grant No. DEB-0808292 to S.D.G. and L.S.A. Further support was provided to S.D.G. from the Lotta Crabtree fellowship, and by the National Science and Engineering Research Council of Canada (NSERC). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference:
10.1111/een.12236

Figure S1. Photo of foraging apparatus.

References

- Adamo, S.A. (2012). *The strings of the puppet master: how parasites change host behavior*. In Host manipulation by parasites (ed. by D.P. Hughes, J. Brodeur & F. Thomas), pp. 36–51. Oxford University Press, Oxford, U.K.
- Blumstein, D.T., Daniel, J.C. & Evans, C.S. (2006) *J-Watcher*.
- Gegeer, R.J., Otterstatter, M.C. & Thomson, J.D. (2006) Bumblebee foragers infected by a gut parasite have an impaired ability to utilize floral information. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 1073–1078.
- Gillespie, S. (2010) Factors affecting parasite prevalence among wild bumblebees. *Ecological Entomology*, **35**, 737–747.
- Gillespie, S. & Adler, L.S. (2013) Indirect effects on mutualisms: parasitism of bumble bees and pollination service to plant. *Ecology*, **94**, 454–464.
- Harder, L.D. (1985) Morphology as a predictor of flower choice by bumble bees. *Ecology*, **66**, 198–210.
- Lefevre, T., Lebarbenchon, C., Gauthier-Clerc, M., Misse, D., Poulin, R. & Thomas, F. (2009) The ecological significance of manipulative parasites. *Trends in Ecology & Evolution*, **24**, 41–48.
- Matsumoto, T., Itioka, T. & Nishida, T. (2003) Cascading effects of a specialist parasitoid on plant biomass in a Citrus agroecosystem. *Ecological Research*, **18**, 651–659.
- Otterstatter, M.C. (2004) Patterns of parasitism among conopid flies parasitizing bumblebees. *Entomologia Experimentalis et Applicata*, **111**, 133–139.
- Pardee, G.L. & Philpott, S.M. (2011) Cascading indirect effects in a coffee agroecosystem: effects of parasitic phorid flies on ants and the coffee berry borer in a high-shade and low-shade habitat. *Environmental Entomology*, **40**, 581–588.
- Rao, S. & Stephen, W.P. (2009) Bumble bee pollinators in red clover seed production. *Crop Science*, **49**, 2207–2214.
- Sato, T., Egusa, T., Fukushima, K., Oda, T., Ohte, N., Tokuchi, N. *et al.* (2012) Nematomorph parasites indirectly alter the food web and ecosystem function of streams through behavioural manipulation of their cricket hosts. *Ecology Letters*, **15**, 786–793.
- Schmid-Hempel, P. & Durrer, S. (1991) Parasites, floral resources and reproduction in natural populations of bumblebees. *Oikos*, **62**, 342–350.
- Schmid-Hempel, P. & Schmid-Hempel, R. (1988) Parasitic flies (Conopidae, Diptera) may be important stress factors for the ergonomics of their bumblebee hosts. *Ecological Entomology*, **13**, 469–472.
- Schmid-Hempel, P. & Schmid-Hempel, R. (1990) Endoparasitic larvae of conopid flies alter pollination behavior of bumblebees. *Naturwissenschaften*, **77**, 450–452.
- Schmid-Hempel, R. & Schmid-Hempel, P. (1996) Host choice and fitness correlates for conopid flies parasitising bumblebees. *Oecologia*, **107**, 71–78.
- Schmitz, O.J., Krivan, V. & Ovadia, O. (2004) Trophic cascades: the primacy of trait-mediated indirect interactions. *Ecology Letters*, **7**, 153–163.
- Spaethe, J. & Weidenmuller, A. (2002) Size variation and foraging rate in bumblebees (*Bombus terrestris*). *Insectes Sociaux*, **49**, 142–146.
- Sumption, K.J. & Flowerdew, J.R. (1985) The ecological effects of the decline in rabbits (*Oryctolagus cuniculus* L.) due to myxomatosis. *Mammal Review*, **15**, 151–186.
- R Development Core Team (2009) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Accepted 27 May 2015

First published online 22 July 2015