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— Note on Methodology —

LONG-TERM TIME-LAPSE VIDEO PROVIDES NEAR COMPLETE RECORDS OF FLORAL VISITATION

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Abstract—Accurate records of floral visitors are critical for understanding plant pollinator interactions. However, to date, sampling methods are constrained to short sampling periods and may be subject to observer interference. Thus, complete records without sampling bias are rare. We use a portable time-lapse digital video camera to capture near-complete records of visitors to flowers over their entire blooming period. We show the broad applicability of this method by filming a wide variety of flowers of different shapes and inflorescence types. We test the importance of long-term records by studying visitors to *Cornus canadensis* (bunchberry dogwood). Visitors to *C. canadensis* filmed simultaneously at four different sites show variation (both in rates and taxa) between inflorescences, between sites, throughout the day, and throughout the season. For *C. canadensis* our films also provide a record of pollen placement (an indirect measure of male fitness) and fruit set (female fitness). This technique provides near complete records of floral visitors, is likely to capture rare events, and allows simultaneous long-term filming. These results emphasize the importance of both long-term data collection and simultaneous recording at multiple sites for pollination studies.

Keywords: pollination, time-lapse video, plant fitness, plant-pollinator interactions, Cornus canadensis, video observation

INTRODUCTION

Plant pollinator systems provide essential ecosystem services (Klein et al. 2007; Classen et al. 2009; Hein 2009), are important for understanding speciation patterns (Kay & Sargent 2009; van der Niet & Johnson 2012), and are model systems for studying mutualisms (Blüthgen et al. 2007). Recent reports of pollinator decline make understanding these systems critical for conservation (Allen-Wardell et al. 1998; Kearns, et al. 1998; Potts et al. 2010; Thomann et al. 2013). Although accurate records detailing the visitation patterns of pollinators to flowers are vital in pollination research, obtaining the data is often hampered by limited observation time and observer interference. Here we report a weatherproof digital camera system that allows for long-term uninterrupted time-lapse photography to capture near complete records of visits over the entire bloom of a flower. This system is inexpensive and portable, allowing simultaneous filming at multiple sites and use in remote field situations. We use this system to film visitors to thirty different flower species demonstrating its broad applicability to flowers with different morphologies. We then focus on bunchberry dogwood (*Cornus canadensis*, L. Cornaceae) as a case study and show that long-term videos and simultaneous filming reveal the complexity of this pollination system by documenting unexpected temporal and spatial variation in visitors among sites.

To date, both direct observations and a variety of indirect approaches are used to assess floral visitors, but to our knowledge both documenting the full visitation pattern for any flower and simultaneous observations at more than one site are rare. Common methods may also be subject to sampling bias. Pan trapping, while effective at measuring insect diversity (Taki et al. 2007; Vrdoljak & Samways 2012), does not demonstrate floral associations. Assessing the composition of pollen loads (Clements & Long 1923; Linsley & MacSwain 1958; Free 1963; Grace & Nelson 1981; Cane et al. 1985; Peterson et al. 2006; Bosch *et al.* 2009; Welsford & Johnson 2012) can broadly survey visitation patterns, but cannot measure visit efficiency or frequency. Direct observations of visitors *in situ* provide a more complete record of visitation patterns (e.g., Bennett 1883; Christy 1883; MacNaughton & Harper 1960; Mitchell et al. 2004; Olesen et al. 2008; Jacobs et al. 2010) but can be limited by short observation periods (Olesen et al. 2008; Rafferty & Ives 2011) and observer interference. Even with multiple simultaneous observers, records may be incomplete (Albrecht et al. 2012; Russell et al. 2005).

Video cameras have long been used to record floral visits (e.g. see Laverty 1980), but none, to date, have done simultaneous recordings at multiple sites or captured all the visitors to a flower over its entire bloom, both of which are important in understanding the dynamics of flower-pollinator mutualism and can be done using our time-lapse video system. Previous recording methods may be limited by

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battery power or recording space and are thus short term (1-3 hours, e.g., Manetas & Petropoulou 2000; Martén-Rodríguez & Fenster 2008; Ladd & Arroyo 2009). Other methods use time-lapse video to record over longer periods (9.5-14 hours) but the intervals between images (10-45s) are too long to ensure the complete capture of all visitors (Suetsugu & Tanaka 2013; Suetsugu & Hayamizu 2014). To extend filming periods, researchers have used external power sources such as generators (Micheneau et al. 2006) or 12-volt batteries (Steen & Aase 2011; Steen 2012; Steen & Mundal 2013). However, the noise of generators may be disruptive to visitors and the heavy 12-volt batteries reduce portability and limit replicate set-ups in field situations. Motion detection sensors that only record when there is movement also reduces the use of energy and storage space, but may result in the cameras being triggered by wind (Steen & Aase 2011) and have not yet been tested on small insects such as ants. Recently, iPod Nanos have been used to record continuous films of insect flower visitors over longer periods (Lortie et al. 2012). The iPod system has close focus and can record pollinator behaviour, but the resulting video files quickly reach the 2GB maximum file size, at which point filming must be manually restarted (iPod Nano User Guide 2009). The file size and proprietary battery also require that the camera be recharged and videos downloaded daily. A less energetically demanding system producing smaller files may be preferable in remote field situations.

Here we use time-lapse cameras to capture continuous long-term records (> three weeks) of visitors to flowers, essentially capturing all visitors to a flower over the length of its bloom. We demonstrate that the videos provide a permanent record that is clear enough to identify visitors, measure the frequency of their visits, as well as document changes in floral development from bloom times to fruit set.

MATERIALS AND METHODS

We tested this technique using Brinno TLC 200 HD and 200 Pro HDR time-lapse cameras (Brinno, Taipei City, Taiwan). These cameras come with optional weather resistant housings and can be customized to take photographs (up to 1280 × 720 resolution) at different time intervals (from less than one per second to one per 24 hours). The TLC 200 Pro HDR has the advantage of manual focus and can be used at shorter focal lengths. All of our records were captured using the 1280 × 720 resolution.

To test for use on a wide range of floral types, we filmed 30 species at three locations: Isle Royale National Park (IRNP), Keewenaw County, MI; the Sierras near Yosemite National Park, Mono County, CA; and Williamstown, Berkshire County, MA. See Appendix Tab. 1 for a list of species filmed, floral types, and their locations. Examples of camera set-ups are shown in Appendix Fig. 1.

We focused on *Cornus canadensis* because the basic ecology of bunchberry dogwood is well documented (Edwards et al. 2005; Whitaker et al. 2007) and we already had an extensive well-identified collection of insect visitors to this species, which greatly facilitates insect identification. *C. canadensis* is a sub-shrub, which forms dense patches covering the forest floor. Each flowering shoot produces a flat topped inflorescence of small self-incompatible (Barrett & Helenurm 1987) flowers that are subtended by four showy white bracts (Fig. 1 A-C). The flowers mature sequentially from the centre outwards over a period of approximately two weeks. Flowers open explosively when triggered by a visiting insect spraying the insect with pollen. If unvisited, flowers eventually explode on their own (Edwards et al. 2005).

Cornus canadensis was filmed in the relatively undisturbed boreal forest on Edwards Island, IRNP, from 17 June to 3 July 2012 and from 22 June to 15 July 2013. In 2012, we filmed four different patches of inflorescences (10 to 27) for a total of 127 hours on 12 different days. In 2013 to test for variability in visitors among sites, inflorescences, and dates, we set up four cameras to film simultaneously at four different sites (A,B,C,D). We chose sites where inflorescences were abundant and flowers had not yet opened. Sites were at most 333 meters apart. Images were captured every three seconds for a 12 hour period (0900-2100 hrs, DST) on 2 July 2013. At one site (B) we filmed a patch for the entire blooming season (0900-2100 hrs, DST) starting on 25 June and continuing through 15 July, by which time inflorescences were through flowering. Permanent tripods allowed filming the exact same inflorescences from day to day.

Videos, which are date and time stamped, were scored for pollinator visits on a frame-by-frame basis using QuickTime Player Version 10.2. For each visit, the time, inflorescence visited and taxa of visitor were recorded. Visits counted only if there was contact with the reproductive structures of the flowers. Insects were identified, often to the species level, using a reference collection compiled in previous years. We gave each new individual a unique ID number and tracked their visits within the camera frame. If an insect left the frame, and another of the same species entered, the new visitor was given a new ID number.

We timed the visit durations of insects on inflorescences during June and July 2007 to 2012. We used these direct observations of time spent on inflorescences to calculate *P(d$_s$)*, the average probability that a visit by a given species would be recorded by a given photo capture rate. We used the following expression:

(Equation 1)

$$P(d_s) = \frac{\sum_{k=1}^{n_s} f(v_{s,k})}{n_s}$$

$$f(v) = \begin{Bmatrix} \dfrac{v}{\text{int}} & if\ v < \text{int} \\ 1 & if\ \ v \geq \text{int} \end{Bmatrix}$$

Where,

P(d)s = the average probability that species *s* will be detected by the video

k = the kth individual of species *s*

n$_s$ = the total number of individuals of species *s*

int = the photo capture interval (time between successive photos)

v = the duration of an individual visit

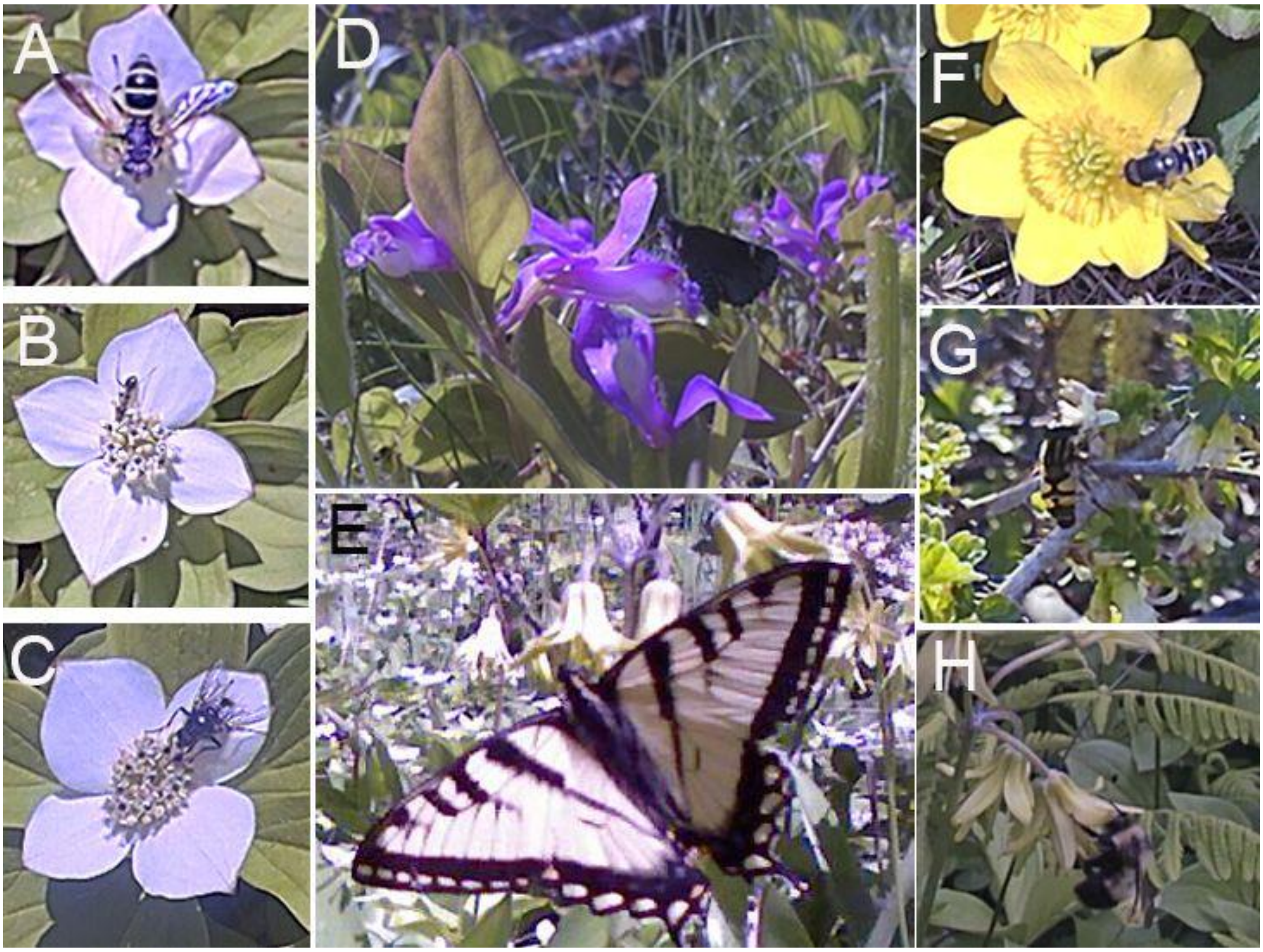

FIGURE 1. Screen captures from time-lapse videos show that images of insect visitors are clear enough for identification on a diversity of flower types: (A) *Temnostoma barberi* (Syrphidae) visiting *Cornus canadensis*; (B) ant visiting *C. canadensis*; (C) *Phaonia* sp. (Muscidae) visiting *C. canadensis*; (D) skipper (Hesperiidae) visiting the zygomorphic flowers of *Polygala paucifolia*; (E) swallowtail butterfly (*Papilio canadensis*) visiting pendant bell-shaped flowers of *Clintonia borealis*; (F) *Eristalis dimidiata* (Syrphidae) visiting *Caltha palustris*; (G) *Helophilus faciatus* (Syrphidae) visiting small tubular flowers of *Ribes oxycanthoides* and (H) *Bombus* sp. (Apidae) visiting *Clintonia borealis.*

We use the calculated values for $P(d_s)$ (from Equation 1) to estimate the overall proportion of insects captured in any one video sequence by using the following expression:

(Equation 2)

$$Pc = \frac{N}{\sum_{s=1}^{T}\left(\frac{1}{P(d_s)}\right)m_s}$$

Where,

Pc = estimate of the proportion of insect visits captured on the video

N = the total number of visitors seen on the video,

$P(d_s)$ = the probability of detection for species s,

m_s = the number of individuals of species s seen on the video, and

T = the total number of species.

Species for which there were no direct observations were assumed to be detected at a rate equal to the mean $P(d_s)$ for all species measured. See Appendix Tab. 2 for species, sample sizes, and calculated detection rates based on different capture intervals.

Statistical Analyses

Data analysis was performed using the R statistical package, version 3.0.0 (R Core Team 2013). To test how sampling effort related to achieving a measure of the species richness of the pollinator community we performed a rarefaction analysis using the Vegan Software Package (Oksanen et al. 2013). To analyze how daily and seasonal patterns of visitation varied both within and between sites, we converted the data using the Cron Package (James & Hornik 2014), which allows handling of dates and times. We then used Package 'nlme' (Pinheirl et al. 2014) to test for differences among the four sites. ANOVA generalized least squares models were used to test for differences in visit times for both ants and non-ants. An ANOVA linear mixed effects model was used to test for differences in average visit times among sites. To assess best fit curves for the visitation

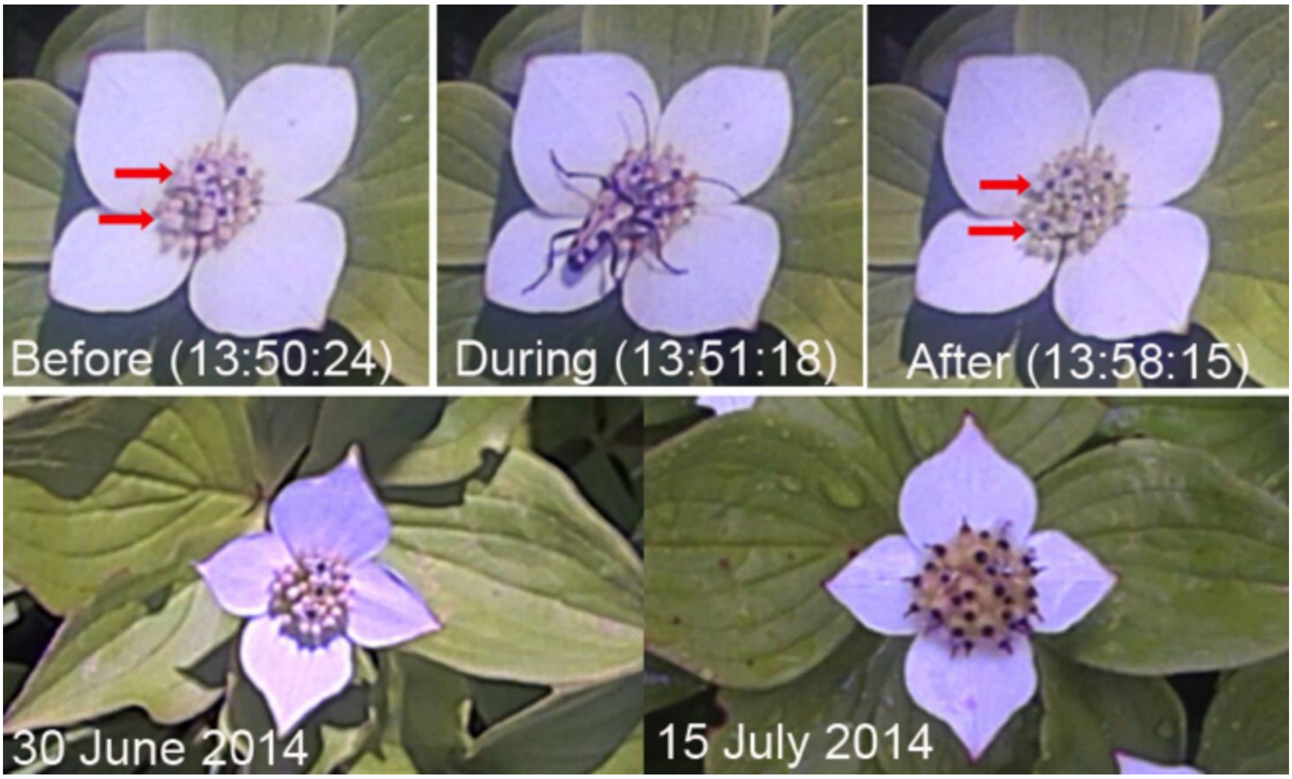

FIGURE 2. The top panel shows an inflorescence before during and after a visit by the beetle, *Evodinus monticola*. The arrows point to two flowers closed before the visit and opened after, indicating the beetle triggered flower opening and was sprayed with pollen. The bottom panel shows the same inflorescence on 30 June and again on 15 July, illustrating that fruit set can be documented by the camera images.

pattern over the flowering season we used AIC (Akaike Information Criteron).

RESULTS

Ability to film, identify and score visits

This video system captures visitation events to flowers of many different shapes and sizes and can be used to identify visitors for many floral types (Fig. 1). Dorsal views of visitors were most useful for identification. Thus, overhead views were preferable for flat open inflorescences or flowers (e.g., Fig. 1 A-C and F), whereas profile views were preferable for pendant or zygomorphic flowers (e.g., Fig. 1 D, E, G and H). Identification of visitors depends on familiarity with the insect pollinator community and a good voucher specimen collection. Insects as small as ants can be identified to group with larger more distinctive insects (e.g., many Syrphidae) identified to species. Where species could not be determined, we identified to the lowest taxon level possible.

The system is also useful for documenting changes in floral behaviour, phenology and fitness. For *Cornus canadensis*, video records are clear enough to show both when flowers were exploded open indicating pollen placement on visitors (an indirect measure of male fitness) and when flowers produced fruits (a measure of female fitness, Fig. 2). Along with recording all visitors, videos thus provide a record of fitness for this species.

The camera produces long-term uninterrupted videos that capture the majority of insect visitors. For the 21 taxa for which we have direct measurements of visit durations, the probabilities of detection, $P(d_s)$ (from Equation 1), at a 3-second capture rate varied from 0.66 in the fast visiting *Vanessa atalanta* (Red Admiral) to 1.0 in the slow moving *Evodinus monticola* (Long-horned Beetle) with an overall mean $P(d_s)$ of 0.90 (Fig. 3). For *Cornus canadensis* data from 2012, *Pc*, the estimated proportion of visits captured (Equation 2), was 93%. Shortening the photo capture interval will increase the proportion of visits captured, but will result in larger file sizes and may increase time for scoring the videos (Appendix Tab. 2). Twelve-hour videos recorded with the TLC 200 were between 1.4 and 2 GB. Twelve-hour videos recorded by the Brinno 200 Pro HDR were larger, averaging 5.43 ± 0.6 GB for each day with a range of 4.4 GB to 6.38 GB, though some videos were slightly longer than 12 hours. The four AA batteries that power the camera lasted on average 8.5 ± 1.19 days (range 4 to 13) of twelve hour filming.

In 2012 we recorded 4090 visitation events to *Cornus canadensis* and identified 39 unique taxa from 4 different insect orders. Rarefaction analysis (Fig. 4) indicates we captured most visitors over the filming period, as both the predicted rarefaction curve and observed data start to level off. Furthermore, 14 of the 39 taxa were only seen once during the film period, suggesting that shorter observation periods would either miss these rare visitors entirely or over-estimate their importance if they happened to be observed.

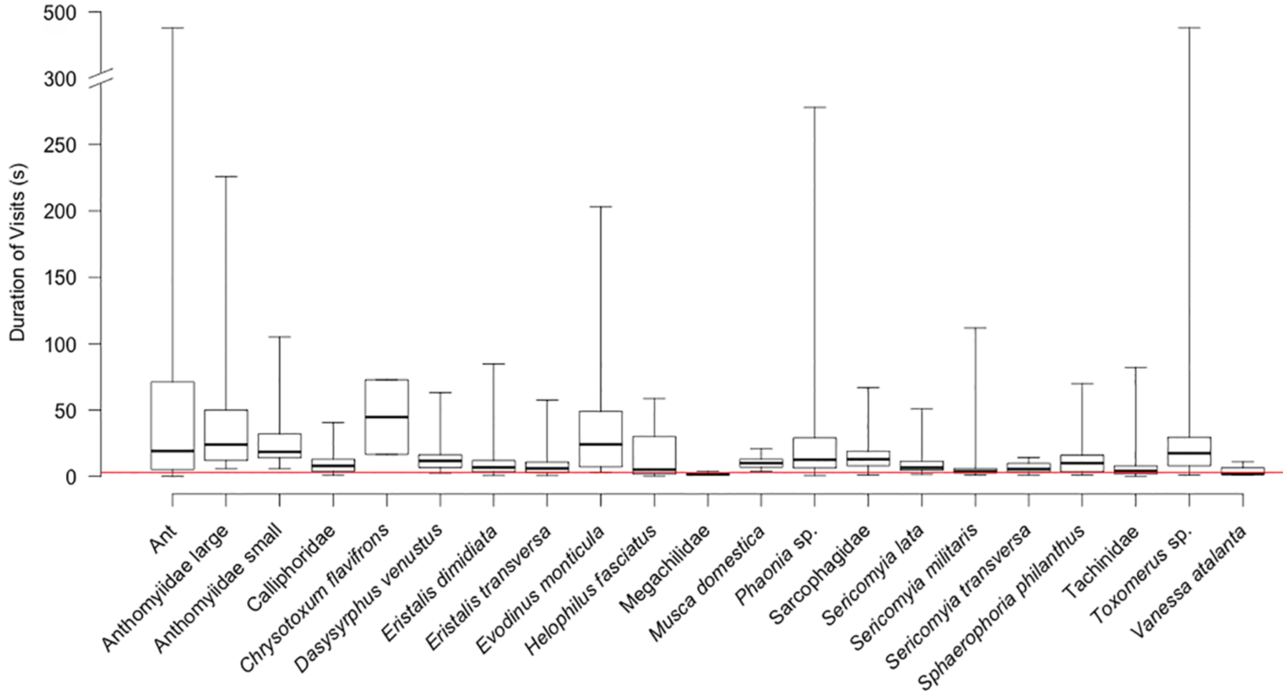

FIGURE 3: Duration of visits to *Cornus canadensis* inflorescences by common visitors shows that the majority of visits last longer than 3 seconds and will be detected by a 3 second photo capture rate. In the box plots, the heavy lines are medians; the boxes indicate the second and third quartiles, and the vertical lines the first and fourth quartiles. The horizontal red line is at 3 seconds, above which all visits are detectable by a 3-second photo capture rate.

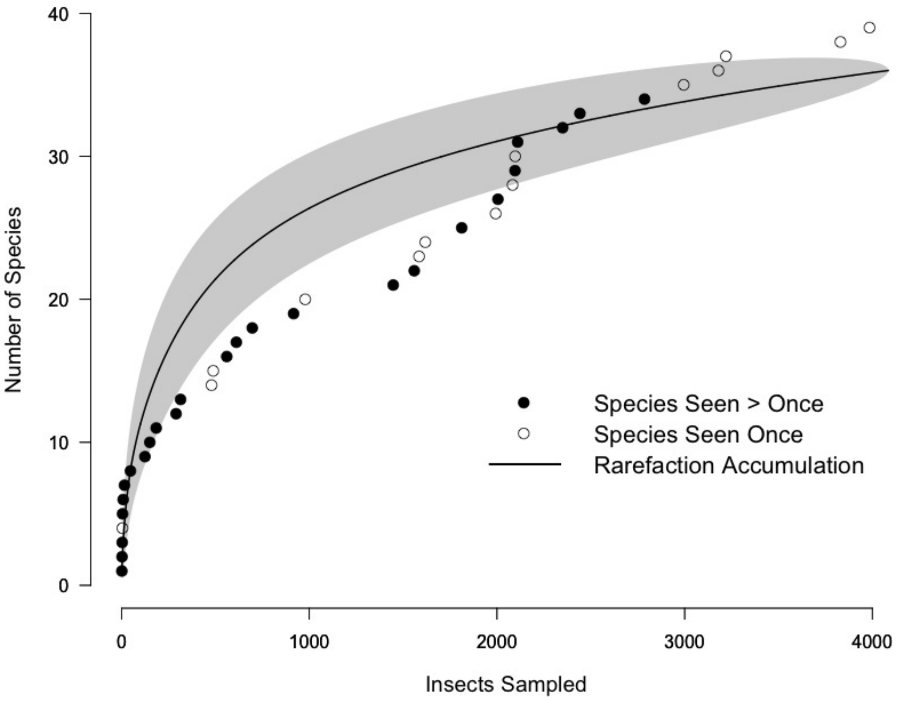

FIGURE 4: Number of novel species detected as a function of the number of insects sampled shows a high diversity (39 taxa detected) of visitors to *Cornus canadensis*, many species are rare (14 were seen only once), and the total number of species is reaching a leveling point. Points represent actual observations. The accumulation line is the species accumulation by rarefaction analysis. The gray shading represents the 95% confidence interval of the rarefaction accumulation curve. Points fall below the curve because the curve assumes random accumulation while our data show waves of species detection.

Variation in Time and Space

Our simultaneous videos of visitors to inflorescences at four different microsites on 2 July 2013 show significant differences in both the types and numbers of visitors among sites (Fig. 5, $X^2 = 797.2$, d.f. = 45, $P < .0001$). Only two taxa (ants and *Toxomerus* spp.) were present at all four sites. Six taxa were present at three of the sites, and eight taxa were only observed at one site. Although ants were the most common visitors at all sites, they were most abundant at site D, which had 1892 visits by ants on that day. Sites A, B, and C had 345, 313 and 714 visits by ants respectively. Large insects, which are more likely to open flowers and transport pollen than small visitors (Edwards et al. 2005), also

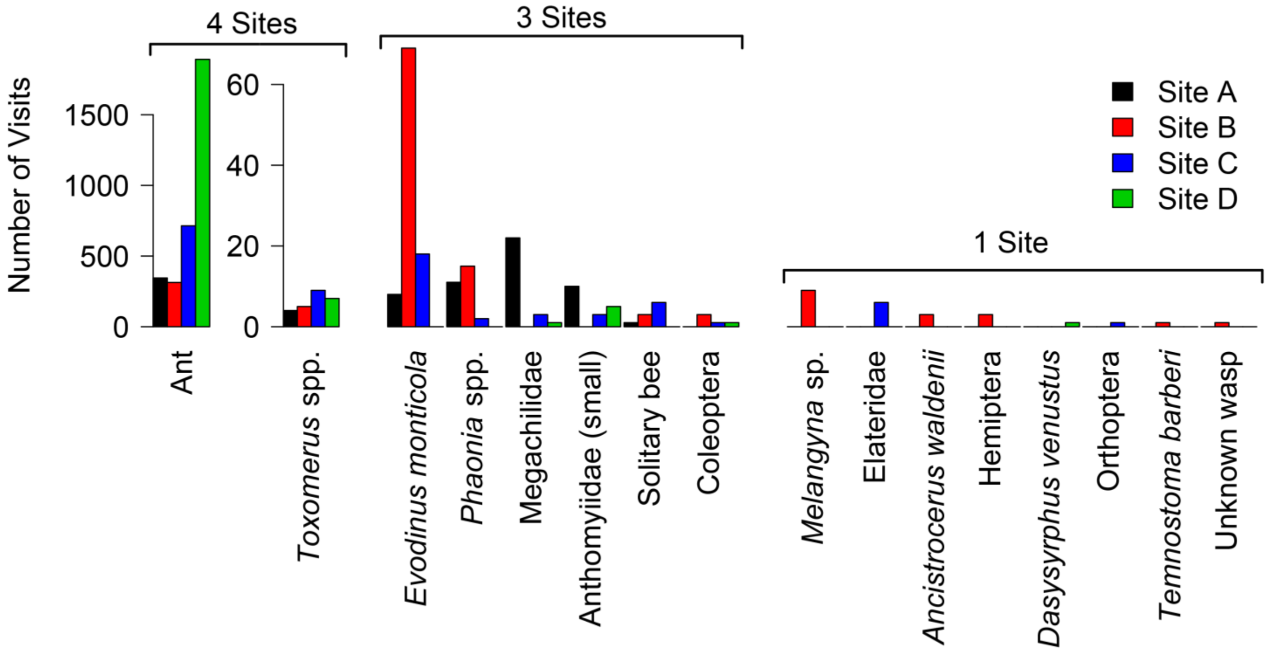

FIGURE 5. Comparisons of insect visitors to 20 inflorescences at four different sites for the same 12-hour period on 2 July 2013 show that the taxa and numbers of visitors differed significantly among sites (X^2 = 797.2, df = 45, *P*-value <0.0001). Taxa are grouped by the number of sites where they were recorded. Only two taxa (ants and *Toxomerus* spp.) were recorded at all four sites, 6 taxa were recorded at 3 sites and 8 taxa were only seen at one site.

differed in number and composition. Sites A, B, C and D had 42, 104, 37 and 3 large visitors respectively. The long-horned beetle, *Evodinus monticola,* was the most common large visitor at sites B (69 visits) and C (18 visits), while megachilid bees were the most common large visitors at site A (22 visits). Inflorescences at site D, where ants were most active, were only visited by 3 large insects.

The number of visits per inflorescence varied greatly among inflorescences even at one site (Fig. 6A). On 2 July, the number of visits ranged from 3 to 165 with an overall mean number of visits per inflorescence of 37.2 ± 4.1 (mean ± S.E.M., *N* = 80). The per inflorescence mean visitation differed among sites with mean visits of 20.1 ± 2.7, 21.3 ± 2.2, 38. 2 ± 5.3, and 56.1 ± 11.3 at sites A, B, C, and D respectively ($F_{3,52}$ = 8.15, *P* < .001, ANOVA generalized least squares model).

Furthermore, the average visitation times over the course of the day differed among sites both for ants ($F_{3,3222}$ = 93.53, *P* <.0001) and non-ant visitors ($F_{3,199}$ = 4.641, *P* = 0.0037) (ANOVA linear mixed effects models) (Fig. 6B). There was no peak visitation time that coincided at all four sites. At two sites (A and B) ant visitation peaked at 1600 hours, while at sites C and D ant visitation peaked at 1200 hours. Non-ant visitation peaks varied widely between the four sites. At site C, peak non-ant visitation occurred at 1000 hours, at site B, peak non-ant visitation occurred at 1200 hours, while at sites A and D, peak non-ant visitation occurred at 1700 hours.

Analysis of visitation patterns to inflorescences at site B over the bloom period (25 June - 15 July) show that the number of visitors differed by date with low visitation early in the blooming period, a rapid rise to a peak midway (4 July), and a gradual decline as flowers finished blooming (Fig. 6C). We fit three curves (gamma, uniform and normal) and assessed their fit using AIC (Akaike Information Criterion). The distribution of pollinator visits over time was best fit by a gamma distribution compared to either a uniform or a normal distribution (Δ AIC = -755 and -186 respectively).

DISCUSSION

Long-term time-lapse videos provide near-complete records of flower visitation that minimize sampling bias, a major concern especially in collecting data for pollination networks (Bosch et al. 2009; Sørensen et al. 2011; Rivera-Hutinel et al. 2012; Chacoff et al. 2012). Other commonly used techniques typically sample a small portion of flower-visitor interactions. The near-complete records generated by our system records rare visitors that might be missed or disproportionately weighed by shorter observations of flowers. For *Cornus canadensis* shorter direct observations would result in data that would vary based on the site, time of day, and season.

The video system is both portable and cost-effective so that multiple sites can be filmed simultaneously to document both spatial and temporal variability in floral visitors at even the most remote field situations. The camera itself is small (10.5 × 5 × 6.4 cm) and its films are stored on SD cards. Investigators without access to electricity at their sites could easily carry adequate AA batteries and SD cards to capture weeks' worth of footage. Stationary tripods allow for consistent and replicable sampling if cameras have to be removed.

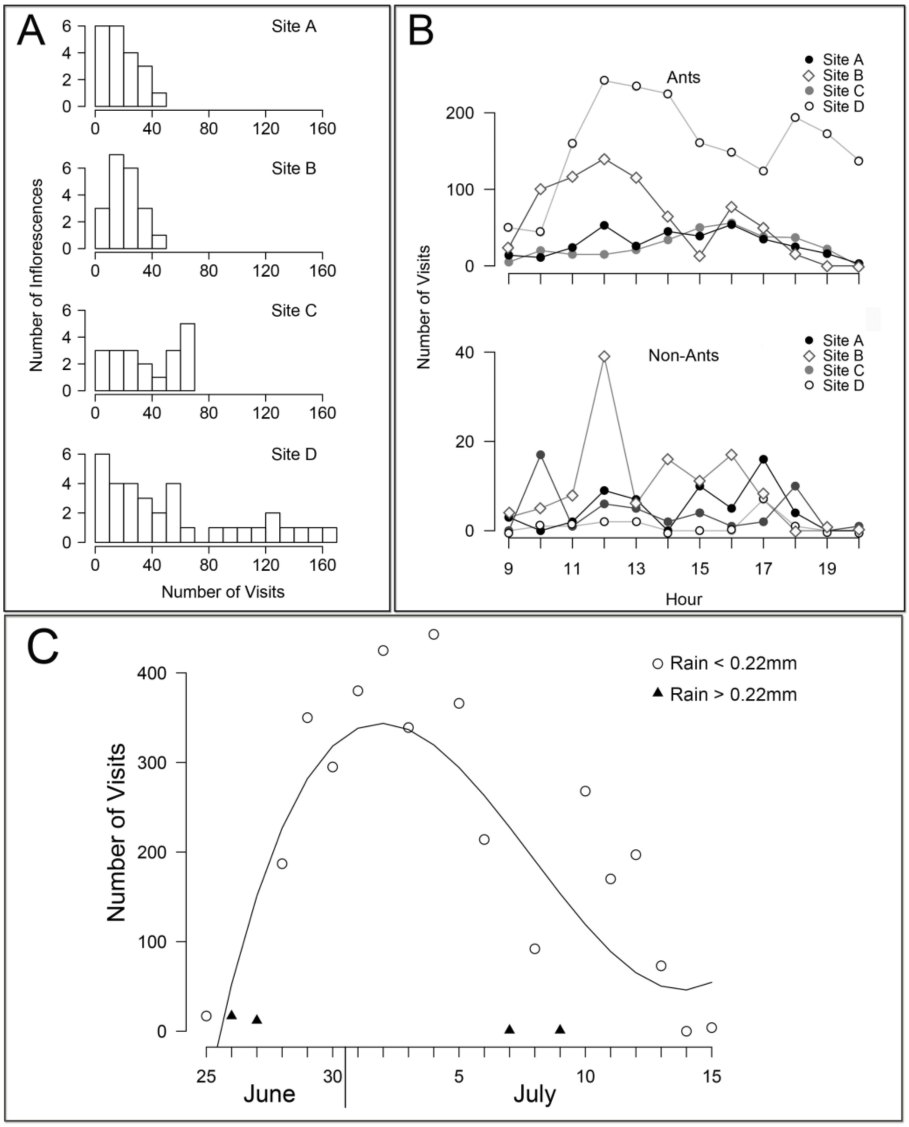

FIGURE 6. The number of visitors to flowers differs among inflorescences, during the day and over the blooming season. (A) Frequency distributions of the number visitors per inflorescence at sites A, B, C, & D on 2 July 2013 show a significant difference in the number of visitors each inflorescence received both between and within sites. (B) Visits per hour to 20 *Cornus canadensis* inflorescences for ants and non-ant visitors at four sites on 2 July 2013 show the pattern of visits change significantly hour by hour and peak visitation differs significantly among sites. (C) Number of visits to 20 inflorescences at site B throughout the season. Peak visitation occurred on 4 July 2013. Triangles indicate days where the rain exceeded .2mm. The gamma distribution, which had the lowest AIC value, is shown by the curved line.

Documenting visitation differences among sites by simultaneous filming allows investigation of differences in both visitors and floral status among different microsites. For bunchberry dogwood we found large differences in both visitor numbers and types among the four sites even though they were no more than 333 m apart. If we had viewed only one site we would have a very different record of the pollinator community. In this system, sampling more than one site is critical to pick up the full breadth of floral visitors.

The time-lapse videos filmed over the entire blooming period of flowers minimize observer effects and provide date and time stamped videos that can be carefully reviewed. Weatherproof cases and a long battery life allow the camera to record for long periods away from human activity, removing the observer interference that can occur during direct observations. When we have observed visitors directly, even small movements often startle visitors on flowers and cause them to fly away. And although the presence of observers is rarely mentioned as having an effect, early researchers of insects, wasps in these cases, reported that people can alter insect behaviour (Treat 1885; Peckham & Peckham 1905). Furthermore, the number of flowers that can be directly observed is limited. Permanent documentation allows frame-by-frame analysis and detailed scoring of each flower, which often is not possible in real time. This is especially important when visitor activity is high. In our videos we sometimes recorded over 200 visits to our twenty target infloresences in an hour. These could not be tracked through direct observation. Visits are often of short duration and can inadvertently be missed since observers may bias counts in favour of larger, more mobile insects. For example, during direct observations we often focused on large, fast flying visitors, and tended to overlook the ants. Our videos show that ant visits were more frequent than expected and have shifted our view of their potential importance. In other systems ants have been shown to effect pollination (e.g., see Gomez et al. 1996; de Vega et al. 2009). Yet in others they are detrimental reducing visitation or seed set (Galen 1983; Ness 2006; LeVan et al. 2014; Hanna et al. 2015).

The time-lapse video provides a record of how pollinators affect plant development and plant fitness. For example, in bunchberry dogwood, we can score how often different pollinators explode open flowers, get coated with pollen, and have the potential to transfer pollen to a stigma (male fitness). In addition, complete records of visitation throughout the bloom period can be used to correlate pollinator visits with seed set (female fitness).

To use this technique effectively, investigators need to know basic features of the system such as the main floral visitors, the duration of floral visits, and basic flower phenology. Once duration of floral visits is known, the appropriate time-lapse photo capture rate can be set. In addition, familiarity with behavioural cues (e.g., a syrphid fly would hover when the wasp it mimics would land) can increase the confidence of identifications. The amount of time needed to score a video varies directly with the number of visits. We scan the videos so that an hour of real time is covered in 1.75 minutes. Additional time is needed to enter data for each visit. If visitation rates are high and the pattern of visitation is known, one could devise a subsampling protocol.

This technique is an important advance in pollination biology. Long-term time-lapse video captures near complete floral visitation records for the entire bloom of the flower. It allows simultaneous data collection at multiple sites, which can document variation both over time and space. It records rare events that would be missed by shorter observation periods. In our case, the unexpected results changed our view of the bunchberry dogwood pollinator system. Complete records of this kind will allow better correlates between visitation and plant fitness and more accurate representations of floral visitation patterns that may change our understanding of these mutualisms. Although our focus is on flower-pollinator systems, the video system potentially can be used to answer a wide range of ecological questions that require careful observations over time from floral behaviour to predator-prey interactions.

APPENDICES

Additional supporting information may be found in the online version of this article:

1. Appendix Figure I shows the set-up of cameras in the field.
2. Appendix Table I provides a list of the flower species we filmed grouped by flower or inflorescence shape.
3. Appendix Table II provides the estimated camera detection rate for 21 insect taxa at three capture intervals (1, 2 or 3 *s*).
4. Appendix Video I is a sample video of insect visitors to *Cornus canadensis* at Site B filmed with a 3*s* capture interval from 12:04:34 to 12:08:31 hours on 2 July 2013. The first visitor is a muscid fly (*Phaonia* sp.) and the second is a beetle (*Evodinus monticola*).

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REFERENCES

Albrecht M, Schmid B, Hautier Y, Müller CB (2012) Diverse pollinator communities enhance plant reproductive success. Proceedings of the Royal Society B 279: 4845-4852.

Allen-Wardell G, Bernhardt P, Bitner R, Burquez A, Buchmann S, Cane J, Cox PA, Dalton V, Feinsinger P, Ingram M, Inouye D, Jones CE, Kennedy K, Kevan P, Koopowitz H, Medellin R, Medellin-Morales S, Nabhan GP, Pavlik B, Tepedino V, Torchio P, Walker S (1998) The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. Conservation Biology 12: 8-17.

Apple Inc. (2009) iPod Nano User Guide. 5th Generation.

Barrett SCH, Helenurm K (1987) The Reproductive-Biology of Boreal Forest Herbs. I. Breeding Systems and Pollination. Canadian Journal of Botany 65: 2036-2046.

Bennett AW (1883) On the constancy of insects in their visits to flowers. Zoological Journal of the Linnaean Society 17: 175-185.

Blüthgen N, Menzel F, Hovestadt T, Fiala B, Blüthgen N (2007) Specialization, constraints, and conflicting interests in mutualistic networks. Current Biology, 17: 341-346.

Bosch J, González AMM, Rodrigo A., Navarro D (2009) Plant-pollinator networks: adding the pollinator's perspective. Ecology Letters 12: 409-419.

Cane JH, Eickworth GC, Wesley, FR, Spielholz J (1985) Pollination ecology of *Vaccinium stamineum* (Ericaceae: Vaccinioideae). American Journal of Botany 72: 135-142.

Chacoff, NP., Vázquez DP, Lomáscolo SB, Stevani EL, Dorado J, and Padrón B (2012) Evaluating sampling completeness in a desert plant-pollinator network. Journal of Animal Ecology 81:190-200.

Christy RM (1883) On the methodic habits of insects when visiting flowers. Zoological Journal of the Linnaean Society 17: 186-194.

Classen A, Peters MK, Ferger SW, Helbig-Bonitz M, Schmack JM, Maassen G, Schleuning M, Kalko EKV, Böhning-Gaese K, Steffan-Dewenter I (2014) Complementary ecosystem services provided by pest predators and pollinators increase quantity and quality of coffee yields. Proceedings of the Royal Society B 281: 20133148. http://dx.doi.org/10.1098/rspb.2013.3148

Clements FE, Long FL (1923) Experimental pollination. An outline of the ecology of flowers and insects. Carnegie Institute of Washington, Washington, D.C., USA.

de Vega C, Arista M, Ortiz PL, Herrera CM, Talavera S (2009) The ant-pollination system of *Cytinus hypocistis* (Cytinaceae), a Mediterranean root holoparasite. Annals of Botany 103:1065-1075.

Edwards J, Whitaker D, Klionsky S, Laskowski MJ (2005) A record-breaking pollen catapult. Nature 435: 164.

Free, JB (1963) The flower constancy of honeybees. Journal of Animal Ecology 32: 119-131.

Galen C (1983) The effects of nectar thieving ants on seedset in floral scent morphs of *Polemonium viscosum*. Oikos 41: 245-249.

Gómez JM, Zamora R, Hódar JA, García D (1996) Experimental study of pollination by ants in Mediterranean high mountain and arid habitats. Oecologia 105: 236-242.

Grace J, Nelson M (1981) Insects and their pollen loads at a hybrid *Heracleum* site. New Phytologist 87: 413-423.

Hanna C, Naughton I, Boser C, Alarcón R, Hung KJ, Holway D (2015) Floral visitation by the Argentine ant reduces bee visitation and plant seed set. Ecology 96: 222-230.

Hein L. (2009) The economic value of the pollination service, a review across scales. The Open Ecology Journal 2:74-82.

James D, Hornik K (2014). Chronological objects which can handle dates and times. R package version 2.3-45. URL http://CRAN.R-project.org/package=chron

Jacobs JH, Clark SJ, Denholm I, Goulson D, Stoate C, Osborne JL (2010) Pollinator effectiveness and fruit set in common ivy, *Hedera helix* (Araliaceae) Arthropod-Plant Interactions 4:19-28.

Kay KM, Sargent RD (2009) The role of animal pollination in plant speciation: integrating ecology, geography and genetics. Annual Review of Ecology and Systematics 40: 637-656.

Kearns CA, Inouye DW & Waser NM (1998) Endangered mutualisms: the conservation of plant-pollinator interactions. Annual Review of Ecology and Systematics 29: 83-112.

Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T (2007) Importance of crop pollinators in changing landscapes for world crops. Proceedings of the Royal Society B 274: 303-313.

Ladd PG & Arroyo MTK (2009) Comparisons of breeding systems between two sympatric species, *Nastanthus spathulatus* (Calyceraceae) and *Rhodophiala rhodolirion* (Amaryllidaceae), in the high Andes of central Chile. Plant Species Biology 24: 2-10.

Laverty TM (1980) The flower-visiting behavior of bumble bees: floral complexity and learning. Canadian Journal of Zoology 58: 1324-1335.

LeVan KE, Hung KJ, McCan KR, Ludka JT, Holway DA. 2014. Floral visitation by the Argentine ant reduces pollinator visitation and seed set in the coast barrel cactus, *Ferocactus viridescens*. Oecologia 174: 163-171.

Linsley EG, MacSwain JW (1958) The significance of floral constancy among bees of the genus *Diadasia* (Hymenoptera, Anthophoridae). Evolution 12: 219-223.

Lortie CJ, Budden AE, Reid AM (2012) From birds to bees: applying video observation techniques to invertebrate pollinators. Journal of Pollination Ecology 6: 125-128

Manetas Y, Petropoulou Y (2000) Nectar amount, pollinator visit duration and pollination success in the Mediterranean shrub *Cistus creticus*. Annals of Botany 86: 815-820.

Martén-Rodríguez S, Fenster CB (2008) Pollination ecology and breeding systems of five *Gesneria* species from Puerto Rico. Annals of Botany 102: 23-30.

McNaughton IH, Harper JL (1960) The comparative biology of closely related species living in the same area. I. External breeding-barriers between *Papaver* species. New Phytologist 59: 15-26.

Micheneau C, Fournel J, Pailler T (2006) Bird pollination in an Angraecoid orchid on Reunion Island (Mascarene Archipelago, Indian Ocean). Annals of Botany 97: 965-974.

Mitchell RJ, Karron JD, Holmquist KG, Bell JM (2004) The influence of *Mimulus ringens* floral display size on pollinator visitation patterns. Functional Ecology 18:116-124.

Ness JH (2006) A mutualism's indirect costs: the most aggressive plant bodyguards also deter pollinators. Oikos 113: 506-514.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Henry M, Stevens HH, Wagner H (2013) Vegan: community ecology package. R package version 2.0-6. URL http://CRAN.R project.org/package=vegan

Olesen JM, Bascompte J, Elberling H, & Jordano P (2008) Temporal dynamics in a pollination network. Ecology 89: 1573-1582.

Peckham GW, Peckham EG (1905) Wasps: Social and Solitary. Constable & Company, Westminster, England.

Peterson PG, Robertson, AW, Lloyd B, & McQueen S (2006) Non-native pollen found in short-tailed bat (*Mystacina tuberculata*) guano from the central North Island. New Zealand Journal of Ecology 30: 267-272.

Pinheirl, J., Bates, D., DebRoy, S. & Sarkar, D. (2014) Linear and non-linear mixed effects models. R package version 3.1-117. URL http://CRAN.R-project.org/package=nlme

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE (2010) Global pollinator declines: trends, impacts, and drivers. Trends in Ecology and Evolution 25: 345-353.

Rivera-Hutinel A, Bustamante RO, Marín VH, Medel R. (2012). Effects of sampling completeness on the structure of plant-pollinator networks. Ecology 93: 1593-1603.

R Development Core Team (2013) *R: A language and Environment for Statistical Computing*. R version 3.0.0. R Foundation for Statistical Computing, Vienna. URL http://www.r-project.org (accessed 3 April 2013).

Rafferty NE, Ives AR (2011) Effects of experimental shifts in flowering phenology on plant-pollinator interactions. Ecology Letters 14: 69-74.

Russell KN, Ikerd H, Droege S. (2005) The potential conservation value of unmowed powerline strips for native bees. Biological Conservation 124: 133-148.

Sørensen PB, Damgaard CF, Strandberg B, Dupont YL, Pedersen MB, Carvalheiro LG, Biesmeijer JC, Olsen JM, Hagen M, Potts, SG (2011) A method for under-sampled ecological network data analysis: plant-pollination as case study. Journal of Pollination Ecology 6: 129-139.

Steen R. (2012) Pollination of *Platanthera chlorantha* (Orchidaceae): new video registration of a hawkmoth (Sphingidae). Nordic Journal of Botany 30: 623-626.

Steen R, and Aase ALTO (2011) Portable digital video surveillance system for monitoring flower-visiting bumblebees. Journal of Pollination Ecology 5: 90-94.

Steen R and Mundal D (2013) New video registration of *Autographa pulchrina* (Haworth, 1809) (Lepidoptera, Noctuidae) and *Sphinx pinastri* L., 1758 (Lepidoptera, Sphingidae) pollinating *Platanthera bifolia latiflora* (Orchidaceae) in Norway. Norwegian Journal of Entomology 60: 57-61.

Suetsugu K, Hayamizu M, (2014) Moth floral visitors of the three rewarding *Platanthera* orchids revealed by interval photography with a digital camera. Journal of Natural History 48:1103-1109.

Suetsugu K, Tanaka K (2013) Pollination of *Sedirea japonica* (Orchidaceae) by *Bombus diversus diversus* (Hymenoptera: Apidae). European Journal of Entomology. 110: 545-548.

Taki H, Kevan PG, Ascher JS (2007) Landscape effects of forest loss in a pollination system. Landscape Ecology 22: 1575-1587.

Thomann M, Imbert E, Devaux C, Cheptou P. (2013) Flowering plants under global pollinator decline. Trends in Plant Science 18: 353-359.

Treat M (1885) *Home Studies in Nature* Harper & Brothers, New York van der Niet T, Johnson SD (2012) Phylogenetic evidence for pollinator-driven diversification of angiosperms. Trends in Ecology and Evolution 27: 353-361.

Vrdoljak SM, Samways MJ (2012) Optimizing coloured pan traps to survey flower visiting insects. Journal of Insect Conservation 16:345-354.

Welsford MR, Johnson SD (2012) Solitary and social bees as pollinators of *Wahlenbergia* (Campanulaceae): single-visit effectiveness, overnight sheltering and responses to flower colour. Arthropod-Plant Interactions 6:1-14.

Whitaker DL, Webster LA, Edwards J (2007) The biomechanics of *Cornus canadensis* stamens are ideal for catapulting pollen vertically. Functional Ecology 21: 219-225.