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PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

Point and interval estimation of pollinator importance: a study using pollination data of *Silene caroliniana*

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Abstract Pollinator importance, the product of visitation rate and pollinator effectiveness, is a descriptive parameter of the ecology and evolution of plant–pollinator interactions. Naturally, sources of its variation should be investigated, but the SE of pollinator importance has never been properly reported. Here, a Monte Carlo simulation study and a result from mathematical statistics on the variance of the product of two random variables are used to estimate the mean and confidence limits of pollinator importance for three visitor species of the wildflower, *Silene caroliniana*. Both methods provided similar estimates of mean pollinator importance and its interval if the sample size of the visitation and effectiveness datasets were comparatively large. These approaches allowed us to determine that bumblebee importance was significantly greater than clearwing hawkmoth, which was significantly greater than beefly. The methods could be used to statistically quantify temporal and spatial variation in pollinator importance of particular visitor species. The approaches may be extended for estimating the variance of more than two random variables. However, unless the distribution function of the resulting statistic is known, the simulation approach is preferable for calculating the parameter's confidence limits.

Keywords Pollinator effectiveness · Pollinator visitation · Variance of product · Floral specialization · Floral generalization

Introduction

Beginning with Stebbins' (1970) assertion that floral traits evolve in response to the most effective *and* abundant pollinators, pollination ecologists have had an interest in quantifying relative pollinator importance, or the product of visitation frequency and pollinator effectiveness, and comparing it across visitor classes. The visitation component is most often measured as a proportion or percent of total visits (e.g., Larsson 2005; Wiggam and Ferguson 2005; Sahli and Conner 2007) but is also measured as a rate (Bloch et al. 2006; R. J. Reynolds, C. B. Fenster and M. R. Dudash, unpublished data), i.e., number of visits per flower, plant or inflorescence per unit time. Pollinator effectiveness may be measured as per visit pollen grain deposition (e.g., Primack and Silander 1975; Fenster 1991; R. J. Reynolds, C. B. Fenster and M. R. Dudash, unpublished data) or fruit or seed set (e.g., Schemske and Horvitz 1984; Kandori 2002; Wiggam and Ferguson 2005) or even progeny germination rates (Herrera 2000). As a product of visitation frequency and per visit pollen grain deposition, pollinator importance is a measure of a pollinator's total transfer of pollen to the stigmatic surface per unit time. Thus, pollinator importance can suggest the relative strength of the positive effects a pollinator can have on the plant partner (Thomson 2003), and as a measure of the fitness consequences of pollinator service it could indicate which pollinators are likely sources of natural selection on plant traits. For a given plant species relative pollinator importance is useful for interpreting pollination syndromes (Faegri and van der Pijl 1979) and

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may help resolve the extent of ecological specialization (Fenster et al. 2004) of a plant on a subset of a diverse pollinator assemblage (Robertson 1928; Waser et al. 1996; Ollerton 1996; Olesen and Jordano 2002).

Waser et al. (1996) inaugurated a continuing (Johnson and Steiner 2000; Fenster et al. 2004; Waser and Ollerton 2006) controversy among pollination ecologists by criticizing the pollination syndrome as the dominant theme explaining the relationship between flower forms and their visitors and determined the syndrome concept had poor predictive power. Since pollinator importance is one way to assess visitors as pollen grain vectors, it needs to be estimated efficiently and accurately to determine which of the amalgam of visitors are pollinators (Ollerton 1996). However, nearly every study conducted to date fails to present error estimates of pollinator importance. Therefore, we perceive a need to explore the inherent statistical and practical issues many researchers face when measuring the importance of a pollinator.

There are at least three statistical approaches to estimating the mean and variance of a product of random variables, some of which have been successfully applied to studies of demography (e.g., Brown et al. 1993) and mark–recapture population estimation (e.g., Hestbeck et al. 1991). First, the delta method may be used to approximate the variance of the product using the Taylor series expansion (Lynch and Walsh 1998). A simpler method of computing the variance of a product was developed by Goodman (1960) where he presents the exact formula for the variance of the product of two and three independent random variables. Furthermore, he comments on the efficiency of the product of sample means estimator under two different sampling schemes: (1) when observations are made separately (e.g., visitation and effectiveness), and (2) when the sample observations may be paired producing one dataset of products (e.g., pollinator importance). He proves that the mean of the product is more efficiently estimated (smaller variance of the mean) when the individual sample means are used to estimate the mean of the product (approach 1) rather than if the product is measured directly and the mean of the product estimated from the observations (approach 2). A third method of estimating pollinator importance is to construct its confidence interval by using computer intensive simulations from raw datasets of pollinator visitation rate and effectiveness. The main advantage of this approach is in avoiding the distributional assumptions involved with calculating confidence intervals for population parameters using estimates from the delta method or Goodman exact variance formula. For example, the simulation is preferable when the probability distribution of the estimate of mean importance is unknown and/or when the number of variables is greater than two (see Materials and methods).

The primary objective of this paper is to obtain point and interval estimates of pollinator importance using its components, visitation rate and effectiveness. Because Goodman (1960) showed that approach 1 produces an estimator with smaller variance, we use this approach to develop a computer intensive simulation method that is novel to studies of pollinator importance: bootstrap the individual visitation and effectiveness datasets, take the bootstrap means and then multiply them to get the resulting product, repeating as many times as possible. In this case, the upper and lower 95th bootstrap confidence intervals are taken from the sampling distribution of mean importance values to estimate the variation in pollinator importance. We also hand calculate the mean, variance and confidence limits of pollinator importance using Goodman's (1960) mathematical statistics result regarding the formula for the exact variance of the product of two random variables and compare these estimates with estimates from the simulations. We demonstrate the use of these methods with field-collected data of pollinator visitation rate and pollen grain deposition on stigmas for *Silene caroliniana* (Caryophyllaceae).

Materials and methods

Silene caroliniana is a protandrous herbaceous perennial wildflower of the eastern United States. At our study site near the Chesapeake & Ohio Canal National Park's Billy Goat Trail, Montgomery County, Maryland, it blooms from mid April to early May. Its corolla is tubular and variable in color, ranging from white to dark pink, but is most commonly light pink. The most common visitors are bumblebees (*Bombus* spp.), clearwing hawkmoths (*Hemaris* sp.), and beeflies (Bombyliidae), with additional infrequent to rare visits by small bees such as halictids, and lepidopterans such as cabbage whites (*Pieris rapae*) and zebra swallowtails (*Eurytides marcellus*).

Data collection

To quantify the visitation component of pollinator importance, we estimated the parameter mean visitation rate (no. visits per plant per hour), for each of the three common invertebrate visitor species during the 2006 field season, using direct observations of 46 separate patches (each patch = one experimental unit) of *S. caroliniana* individuals in a natural population. Visitation rate is defined here as the sum of visits to all the plants in a patch divided by the number of plants in the patch, and then divided by the time of observation per plant, thus number of visits per plant per hour. Observations were made of five to ten plants per patch for 20–30 min, which was appropriate given the relatively frequent visits and ease of view of a large number of plants. During each observation the count of visits to each plant and the visitor species were recorded. Every effort was made to keep the experimental units independent, by sampling across the entire phenology and observing many separate patches in a given day.

The pollinator effectiveness component of pollinator importance was estimated during the 2006 field season by measuring single-visit pollen grain deposition for each of the three most common visitor species. About 20 plants of the same population used for the visitation study were located and securely caged with fine-mesh screening prior to flowering. After the pollinator-exclusion cages were removed, female-phased flowers were identified and flowers were observed until a visit was noted. Immediately following the visit the flower was collected and its stigmas were fixed on microscope slides with fuschin glycerin jelly (Kearns and Inouye 1993). The number of pollen grains on the stigmas was counted under light microscopy at 40× power. Unvisited stigmas were collected as controls, i.e., pollen grain deposition from sources other than insects.

Data analyses (linear models)

In addition to the major focus described below of quantifying variation in pollinator importance, we also aimed to gain a greater mechanistic understanding of why different pollinators may differ in the components of pollinator importance. Thus, linear models (SAS Institute 2004) were used to determine if mean pollen grain deposition (pollinator effectiveness) and visitation rate (pollinator visitation) each vary according to visitor species. Pollen grain deposition (PROC GLM) or visitation rate (PROC GENMOD) were modeled as response variables and visitor species as the predictor variable. In the case of pollen grain deposition, an additional treatment level, no visitor (control), was used in the model. The pollen grain deposition model was run with square-root-transformed data, which made the distribution of the response variable more symmetric.

A Poisson regression model was used to model the count variable, number of visits to a patch of plants in a half hour, which ranged between 0 and 17 with a mode of 0. In this model the number of visits was the response variable, species was the predictor, the link function was log and an overdispersion parameter was used and estimated (3.7) as the Pearson χ^2 divided by its *df* (135). The model was modified by specifying an offset variable, ln[number of plants × time(hours) of observation]. The offset variable scales the count-type response data by the time of observation and the number of plants in each patch since actually mean *visitation rate* was the parameter of interest. Because visits of the three species were observed within each experimental unit, the log-linear model was further refined to account for their potential correlation (repeated statement/corr option unstructured). In using a model without this correlation or without the correction for overdispersion we would have reported all visitor species were significantly different in visitation rate (bumblebee > hawkmoth > beefly; analysis not shown). Least squares means were used to estimate the mean values of the predictor variables in both the GLM and GENMOD procedures. In both procedures a priori contrasts were used to determine if mean visitation rate differed between species or, for the case of pollen grain deposition, whether each species differed from the control (no visitor). For both models the per contrast type 1 error rate was controlled by holding the experiment-wise α-level to 0.05.

Data analyses (simulations and variance calculations)

A visual basic routine in Microsoft Excel was developed and used to simulate mean importance values and 95% bootstrap confidence limits. Simulations were done separately for each visitor species. To correct for pollen on stigmas from sources other than pollinators, the pollen deposition dataset was modified by subtracting the mean number of pollen grains on control stigmas ($n = 46$) from each observation. If the resulting observation was negative it was replaced with zero. The visitation dataset was left unmodified. For each species it consisted of 46 observations of visitation rate, one from each patch of plants.

To begin, the visitation and deposition datasets were randomly sampled to generate bootstrap samples of visitation and effectiveness each with the same number of observations as the raw datasets. Next, the sample means and variances were calculated, pollinator importance was taken as the product of the means and its variance using the formula described below. A single trial consisted of repeating the above procedure 10,000 times thus generating a distribution of 10,000 mean importance values. After a trial was complete the average of the 10,000 mean importance values and their variances were taken, the dataset was sorted in ascending order, and the 250th and 9,750th simulated observations of mean importance were taken as the estimates of the upper and lower 95% bootstrap confidence limits. In order to investigate the stability of the estimates the whole process was repeated 50 times, and the coefficients of variation (CV) of the mean and upper and lower confidence limits across the 50 trials were calculated. The final mean and upper and lower 95% bootstrap confidence limits were taken as the averages across the 50 trials.

We used the result of Goodman (1960) to make hand calculations of the mean and unbiased pollinator importance variance estimates. In general, using probability theory and the algebra of random variables the mean and variance of the product of two independent (i.e., $\mathrm{COV}_{X,\,Y} = 0$) random variables, $Z = XY$, are $E(Z) = \mu_x\mu_y$ and $\mathrm{Var}(Z) = (\mu_X)^2\sigma_Y^2 + (\mu_Y)^2\sigma_X^2 + \sigma_X^2\sigma_Y^2$ where $E(X) = \mu_x, E(Y) = \mu_y$, $\mathrm{Var}(X) = \sigma_x^2, Var(Y) = \sigma_y^2$ (Goodman 1960). Taking the random samples $\{X_1, X_2, \ldots, X_{n_x}\}$ and $\{Y_1, Y_2, \ldots, Y_{n_y}\}$, an unbiased estimate of the variance of the product of means, $\mu_x\ \mu_y$ is $V\hat{a}r(\bar{x}\bar{y}) = \bar{x}^2 s_y^2/n_y + \bar{y}^2 s_x^2/n_x - s_x^2 s_y^2/n_x n_y$ where $\bar{x}, \bar{y}, s_x^2, s_y^2, n_x, n_y$ are the respective means, unbiased variances and sample sizes of the two datasets (Goodman 1960). Note that this method does not require any model regarding the probability distribution of the sample observations or sample means. The assumptions are independent observations and there is no covariance between the random variables, which may be difficult to satisfy under field conditions.

In order to put a probability on the approximate interval containing the population mean importance using the exact variance formula we need to know the distribution of its statistic. If large random samples (e.g., >30) are taken of each variable then the means of the variables may be assumed approximately normal, regardless of the variables' underlying distribution. However, even for large samples of visitation and effectiveness where the means may be assumed normal, a confidence interval for the population mean importance value may not be the sample mean ± 1.96 times the SE. Craig (1936) published the distribution function of a product of normal random variates, and under most circumstances it is not normal. Fortunately computational methods for computing the probabilities (Cornwell et al. 1978) and statistical tables (Meeker et al. 1981) have been produced. The product of normals distribution, $g_{Z=XY}\left(z \middle| \frac{\mu_x}{\sigma_x}, \frac{\mu_Y}{\sigma_Y}, \rho_{XY}\right)$, has three parameters, the correlation, ρ_{XY}, and the ratios of the means to SDs of each variable, μ_X/σ_X and μ_Y/σ_Y (Craig 1936; Meeker et al. 1981). The tables of Meeker et al. (1981) were used to directly calculate an approximate 95% confidence interval for the population mean importance using as parameters the estimates of ratios of the sample means to SEs to find the appropriate critical values. Bivariate linear interpolation (see Meeker et al. 1981) was used to find critical values corresponding to the appropriate parameter estimates. The approximate 95% confidence interval is $P\left(\Pi_{\alpha=0.025} < \frac{\bar{x}\bar{y} - \mu_x\mu_y}{\sqrt{V\hat{a}r(\bar{x}\bar{y})}} < \Pi_{\alpha=0.975}\right) \approx 0.95$, or $(\bar{x}\bar{y} \pm \Pi_\alpha \times \sqrt{V\hat{a}r(\bar{x}\bar{y})})$ where $\Pi_{\alpha=0.025,0.975}$ are the critical values corresponding to the 0.025 and 0.975 percentiles of the product of two normals distribution, and $\sqrt{V\hat{a}r(\bar{x}\bar{y})}$ is the estimate of the SE of pollinator importance from the exact variance formula (Goodman 1960).

Comparisons were made of the simulated importance values, variances and 95% bootstrap confidence limit estimates to the mean, variance, and confidence limits of importance values calculated directly using estimates from Goodman's (1960) exact variance formula. If the point estimates differ substantially then the approximate 95% confidence limits using the mean and SE estimates from the exact variance formula may be inaccurate. Such a discrepancy may be due, for example, to violation of the methods' assumptions. For each visitor species, the relative difference of the point or interval estimates from the simulated ones was calculated as $\%\ \text{difference} = \left(\frac{\text{Est}_{\text{Sim}} - \text{Est}_{\text{Direct}}}{\text{Est}_{\text{Sim}}}\right) \times 100$.

Results

Visitation and pollen grain deposition

Overall, the linear models show bumblebees to be the most frequent and hawkmoths and bumblebees the most effective pollinators of *S. caroliniana*. The mean (±1 SE) visitation rate for bumblebees, beeflies, and hawkmoths based on the $n = 46$ observation periods was 1.1 (0.92, 1.2), 0.11 (0.086, 0.15), and 0.25 (0.18, 0.33), respectively. Thus, bumblebee least squares mean visitation rate was 4.4 times greater than hawkmoth's and 10 times greater than beefly's and these differences were statistically significant (bumblebee > hawkmoth, $\chi^2 = 16.54$, $df = 1$, $P < 0.0001$, bumblebee > beefly, $\chi^2 = 21.52$, $df = 1$, $P < 0.0001$). Visitation rate of hawkmoth pollinators was 2.3 times greater than beefly's but this difference was not significant (hawkmoth = beefly, $\chi^2 = 2.95$, $df = 1$, $P = 0.0858$).

Hawkmoths and bumblebees were the most effective pollinators. The mean (±1 SE) effectiveness for bumblebees, beeflies and hawkmoths based on the $n = 64$, $n = 9$, and $n = 29$ samples of pollen deposition were 231 (210, 253), 43.3 (25.4, 65.9), and 249 (204, 296), respectively. After adjusting the mean pollen grain deposition values by subtracting the mean pollen grain deposition from control stigmas (no visits, $n = 46$), on average hawkmoth and bumblebee pollinators deposited 9.2 times and 8.4 more pollen grains than beefly pollinators. Pairwise contrasts demonstrated that mean pollen grain deposition by hawkmoths and bumblebees was not significantly different ($F = 0.24$, $df = 1{,}144$, $P = 0.6241$). Based on the pollinator effectiveness data, beeflies were an insignificant pollinator compared to hawkmoths and bumblebees. Results from the pairwise means comparisons indicated that bumblebees ($F = 103$, $df = 1{,}144$, $P < 0.0001$) and hawkmoths ($F = 76.8$, $df = 1{,}144$, $P < 0.0001$) but not beeflies ($F = 1.31$, $df = 1{,}144$, $P = 0.2541$) deposit significantly more pollen per visit than there were pollen grains on stigmas in the absence of visitors.

Simulations and exact variance formula

The corrected effectiveness data set was used in the simulations and the Goodman exact formula estimate. The adjusted effectiveness data set of bumblebees, beeflies, and hawkmoths resulted in a mean (variance, n) pollen grain deposition of 246 (3.55×10^4, $n = 64$), 47.4 (7.79×10^3, $n = 9$), and 291 (4.92×10^4, $n = 29$). In addition, the means for the visitation rate were the same as those used for the linear models, and the variances that were used for the Goodman exact variance formula were 1.26, 0.0444, and 0.288 for bumblebees, beeflies and hawkmoths, respectively. The simulation results demonstrated that bumblebees were the most important pollinators, hawkmoths were of intermediate importance, and beeflies the least important (Fig. 1). Therefore, in the single season of 2006, a high visitation rate by bumblebees and moderate rate of pollen deposition made them more important than the less frequent but slightly more effective hawkmoths. Mean bumblebee importance (277) was greater than the mean value (127) of the 97.5th percentile of mean hawkmoth importance after 50 simulation trials. Thus, bumblebees had *significantly* higher average importance than hawkmoths (73). Although hawkmoth visitation rate was not statistically different from beefly's, the high hawkmoth effectiveness increased its pollinator importance over beefly's. Average beefly importance (5.95) was lower than the mean value of the lower 2.5th percentile of both bumblebee (190) and hawkmoth (31.2) after 50 simulation trials. The simulations exhibited remarkable stability across the 50 trials for all species. In particular the CVs for mean, lower and upper confidence limits for bumblebee importance were all less than 1%.

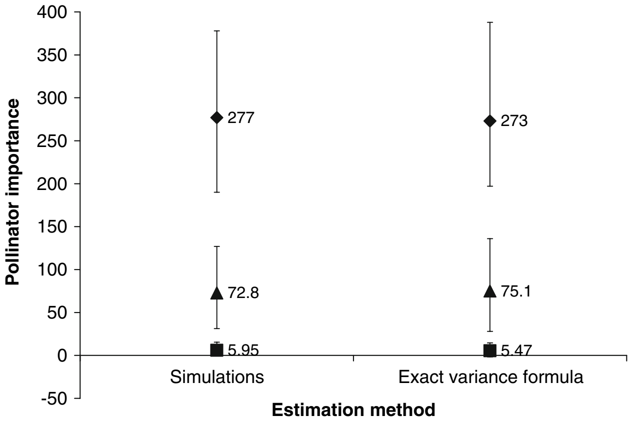

Fig. 1 Average pollinator importance of bumblebees (*diamonds*), beeflies (*squares*), and hawkmoths (*triangles*) as pollinators of *Silene caroliniana* and their 95% confidence limits estimated using two approaches, simulations and the exact variance formula. Values of means next to symbols

It appeared the precision of the estimates between the two methods was associated with the sample size of the effectiveness dataset. The simulated means, variances and confidence intervals were most similar to the estimates computed using the exact variance formula for the bumblebees ($n = 64$ observations) and most different for beeflies ($n = 9$ deposition observations). There appeared to be no pattern of either method over or underestimating the point or interval estimates of the other (Fig. 1). For example, the upper and lower bumblebee bootstrap confidence intervals were less than (−2.65%, −3.55% difference, respectively) the confidence interval from the estimates using the exact variance formula. The simulated hawkmoth upper confidence limit was less than (−7.09%) and the lower confidence limit was greater than (10.3%) the estimates using the exact variance formula.

Discussion

Here we demonstrate two methods, novel in their application to pollinator importance, of estimating the mean and variance for a product of two random samples taken separately. Both methods yielded the same conclusion: using real visitation rate and pollen grain deposition data for three visitor species to *Silene caroliniana* in the 2006 flowering season we find that bumblebee importance is significantly higher than hawkmoth's, which is significantly higher than beefly's. In fact, in no case did a pollinator's upper 95th confidence limit overlap another's lower 95th confidence limit for pollinator importance. The major advance of this paper is that the simulation method and/or the exact variance formula may be used to properly estimate the variance of pollinator importance thereby enabling pollination ecologists to test hypotheses of sources of variation in pollinator importance or any metric that involves the product of means of two random samples. First we discuss our results pertaining to the pollination system of *S. caroliniana*, and then we discuss assumptions and limitations of the methods in estimating pollinator importance and its confidence interval.

Important pollinators

The simulated point and interval estimates statistically show that pollinators are significant sources of variation in pollinator importance. The separate linear models of the visitation and effectiveness data offer suggestions as to why the importance values differ among the visitors. For example, the difference between bumblebees and hawkmoths importance was due to the quadruple visitation rate of bumblebee because the mean effectiveness was not significantly different. Hawkmoths were exceedingly more important than beeflies, more because of its very high relative effectiveness than its visitation rate, which were over twice as high as beeflies, but the difference was not statistically significant. However, the linear models of the component variables, visitation and effectiveness, do not sufficiently demonstrate pollinator importance varies among visitors because the SE of pollinator importance is a function of the mean and variance of both samples.

Pollinator importance as the product of visitation rate and pollen grain deposition can provide some biological insight into the dynamics of pollen transfer. Given that an *S. caroliniana* flower in the female phase contains about 30 ovules (R. J. Reynolds et al., unpublished data) bumblebees were delivering pollen at a rate resulting in slightly less than a 10:1 ratio of pollen grains to ovules every hour. It is likely this rate of pollinator service is sufficient to effect maximum seed set per flower since multiple studies have demonstrated seed set as a saturating function of pollen grain deposition on stigmas (Silander and Primack 1978; Mitchell 1997; Brown and Kephart 1999). With 25% the pollinator importance of bumblebees, on average, it would take the hawkmoths 4 h to achieve a similar level of pollinator service. Thus while our approaches clearly demonstrate that bumblebees were more important pollinator than hawkmoths at our study site in 2006, it is probable that both pollinators were contributing substantially to the stigmatic pollen load. Thus we suggest that bumblebees and hawkmoths were both important pollinators.

The difference in visitation rate determines the significant variation of pollinator importance between bumblebees and hawkmoths. Although components of effectiveness may be expected to differ among years (Ivey et al. 2003), yearly variation of pollinator density is an inextricable component of pollination biology (Horvitz and Schemske 1990; Fishbein and Venable 1996; Waser et al. 1996; Fenster and Dudash 2001; Ivey et al. 2003). As pollinator importance fluctuates among years so it may be expected that the dynamics of pollinator-mediated selection may also fluctuate. In the case of *S. caroliniana*, if by comparison with bumblebees, hawkmoth density increases one year such that its importance values overlap or exceed bumblebees, then in those years we would predict detection of significant selection on moth syndrome traits (e.g., tube length or tube width). In other years selection may correspond more to traits associated with bumblebee pollination (e.g., sequential anther dehiscence). Spatiotemporal variation in the densities of important pollinators that are selective agents may prevent the evolution of a strictly specialized pollination system (Aigner 2001).

Perhaps then, it is not surprising that the flowers of *S. caroliniana* exhibit traits concordant with the most common visitors. For example, the long narrow tubes, diurnal anthesis, and lack of scent and nectar guides indicate a diurnal moth syndrome (Faegri and van der Pijl 1979). However, the syndrome is not exclusively moth as we observe bumblebees readily forage for nectar located at the base of the tubes (R. Reynolds, personal observation). Sequential anther dehiscence has been noted to decrease pollen loss from bumblebee grooming behavior (Harder 1990), thus it may represent an example of a bumblebee syndrome trait for *S. caroliniana*. Since *S. caroliniana* appears to possess floral traits consistent with both bumblebee and diurnal hawkmoth syndromes, it is particularly relevant to estimate the mean *and* variance of pollinator importance in order to make comparisons between the two pollinators in a hypothesis-testing framework.

Estimation issues

The difference between the methods in their point and interval estimates appeared to be associated with sample size of the effectiveness dataset. The estimates were most in agreement for bumblebees ($n = 64$) and least in agreement for beeflies ($n = 9$), suggesting that small sample size is a serious limitation to the use of both approaches. With larger samples both approaches would yield narrower confidence intervals if the variance was constant among samples of differing size, because the variance of the mean and hence the variance of the product of means is inversely proportional to the sample size. Small sample size is problematic using the exact variance formula for possibly failing to meet the distributional assumption that the sample means of visitation and effectiveness are each normally distributed and therefore that pollinator importance has a product of normals distribution. While no distributional assumptions are required, aside from the observations being identically distributed, the bootstrap statistic's accuracy increases as the size of the samples increases because the sampling distribution then more closely resembles the population distribution (e.g., Chernick 1999). Thus, the point and interval estimates from the bootstrapping method may not represent the population with small sample size.

The interval estimates calculated using the SE of the exact variance formula are invalid if it cannot be safely assumed the mean importance statistic has a product of normals distribution, which is the case when the sample with non-normal data (e.g., visitation rate) is small. Consequently, the accuracy of the beefly importance measure may be suspect, and additional larger pollen deposition samples should be collected to confirm the very low importance estimates. The central limit theorem of mathematical statistics ensures that when large random samples (the rule of thumb being >30 observations, e.g., DeVore 2000, p. 236) are taken the sample mean becomes normally distributed regardless of the distribution of the individual observations in the sample (Hogg and Craig 1995, p. 246). If the observations are normal then the mean of the sample is normal under any sample size, and the exact variance formula may be used to estimate the SE for constructing the confidence limits. Pollinator effectiveness data may be modeled as normal if the samples have small variance and a relatively large mean (negative values are unrealistic). However, this may be an unusual case because pollen grain deposition data can have high variance. It may be more realistic to assume a Poisson distribution for the deposition data, but this probability model may not be appropriate if the data are overdispersed. One way to determine if the data are normally distributed is by examining normal quantile–quantile or probability plots (e.g., Devore 2000, p. 187), and Proc Univariate in SAS performs these analyses. Therefore, ideally large samples of both visitation and effectiveness should be taken to help satisfy the distributional assumptions required for constructing the confidence intervals.

In addition to the problems associated with estimation using small sample size, both methods assume no covariance between visitation and effectiveness. Intuitively it seems more likely that visitation and effectiveness should positively covary if pollen is limiting seed production than otherwise. In our study visitation rate was measured on 16 days, effectiveness on 10 days, and the two together for bumblebee, were measured on 8 days. The correlation between average visitation rate and pollen grain deposition for those 8 days was close to zero ($r = -0.021$, $P = 0.9641$) suggesting minimal covariance between the two pollination measures in this 1-year study. However, for the rarer pollinators, visitation and effectiveness data coincided for 4 days and thus a reliable test of the covariance assumption was not possible. Future studies of pollinator importance using the simulation method or the exact variance formula should incorporate a robust test of the no covariance assumption. If there is substantial covariance then it needs to be incorporated in the simulations and/or exact variance formula.

Since both the simulation and exact variance formula yielded similar results, and the exact variance formula is far easier and less time consuming to implement, we suggest using the SE of importance from the exact variance formula and the appropriate critical values from the distribution of two normals table to construct the confidence intervals. When estimating importance as the product of three random variables an estimate of the SE is possible using the exact variance formula, but to make a confidence interval the distribution of the statistic must be known, which is not as simple as using the published distribution tables for the product of two normals (Meeker et al.1981). Therefore, if the number of variables is greater than 2 the simulation method is preferred. Furthermore, if the sampling distribution of the mean of the two variables cannot be safely assumed to be normal then the simulation approach should be used.

Another method of modeling pollinator importance not detailed here is using the framework of hierarchical Bayesian modeling (e.g., Congdon 2003), which is gaining increasing popularity in the ecological literature (Clark 2005). These techniques have proven useful in the demographic literature where vital rates exhibit significant individual, and group level variability that present formidable modeling challenges using classical techniques (e.g., Clark 2003). Pollinators may exhibit much individual variability in visitation and pollen deposition, possibly stemming from body size variation, or nutritional status, and it is conceivable that pollinators may differ in deposition rates by grouping them based on the gender of flowers previously visited, flower plant density, and foraging time. Essentially the hierarchical framework may allow a realistic exploration of the complex relations feeding into variation in pollinator importance.

We applaud Larson (2005) and Bloch et al. (2006) for recognizing the need to add SEs to their measures of pollinator importance, which motivated this paper, but we argue that our point and/or interval estimates of importance are more accurate. The mean and variance of *both* samples of visitation and effectiveness are functions of the pollinator importance variance (Craig 1936; Haldane 1942; Goodman 1960). Accordingly, scaling each effectiveness observation by the mean (a constant:variance = 0) of the visitation data or the visitation observations by the mean of the effectiveness dataset (e.g., Larson 2005) underestimates the variance of pollinator importance. Bloch et al. (2006) incorporated a resampling procedure in which each observation of visitation was multiplied by the mean of a random subsample of the effectiveness dataset to generate a single importance dataset. However, the method needed to be repeated numerous times to generate a distribution of mean importance in order to get an estimate of population mean importance and confidence limits with the least bias as possible.

The simulations may be extended to the product of several random variables, and the statistical properties of the product of k independent random variables are known (Goodman 1962). For example one could weight the importance value by its covariance with traits, which would be indicative of its importance as a source of natural selection. Thus, if a rare pollinator that is effective exerts strong selection on a particular trait it may be more important evolutionarily than a pollinator that is frequent, effective but exerts no selection on floral traits. Therefore, the metric could measure the potential for specialization in the plant–pollinator interaction (Schemske and Horvitz 1984).

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