

# Comparison of Reproductive Efficiency between Generalist and Specialist Plant Species

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Keywords: generalist, pollination, reproductive efficiency, specialist

## Abstract

Generalist and specialist plant species vary significantly in their strategies to be pollinated (Johnson and Steiner 2000). Both strategies appear to be evolutionarily successful as there are sufficient populations of each, but they may differ in their vulnerability to habitat loss. Montane meadows in the Western Cascades of Oregon, which is where our study took place, are experiencing habitat loss due to encroachment of conifer species. Evaluation of which strategy has greater reproductive efficiency can help determine where our conservation management efforts should be directed. We expect that specialist species will exclude pollen of other species to a greater extent than generalists, thus will have greater proportions of their own pollen grains. To test this idea, we counted the relative abundance of heterospecific pollen grains on the stigmas of six flowering plants presumed to be either generalist or specialist and compared the two. We believe this proportion of relative abundance is a valuable measure of reproductive efficiency, as the plants cannot reproduce their species with another species' pollen. Counter to our expectations, the generalists had slightly higher proportions of pollen from their own species than did the specialists. As a parallel experiment, we would also need to determine the degree of specialization for each of our six species. The plant species chosen had been previously identified by one of the two pollination strategies, so we tested the validity of those designations using counts of pollinators observed interacting with a given species in our meadows over the last four years. We also tested the validity using a matrix of scores based on a plant's exclusion factors and corresponding pollinator traits. Of the six species, the three previously-identified generalists tended to have many pollinator species, and the three previously-identified specialists tended to exclude most pollinators, staying consistent with the initial designations. This is the first study to propose a comparison of reproductive efficiencies between specialists and generalists based on proportions.

## 1. Introduction

Plants have two potential types of pollination strategies that affect their reproductive efficiency: generalization or specialization. Due to the simplicity of its floral structure, a generalist plant allows for pollination by several different pollinator species. Inherently, it may also receive pollen from many other plant species, as its pollinators do not reliably return to a plant of the same species (Scopece et. al. 2009). Specialist species allow a smaller range of pollinator species, but the pollen it does receive is more than likely its own. The grains of other species cannot fertilize the ovule to reproduce its species and are therefore useless in the plant's reproduction. More "useless" pollen grains on the stigma however, may hinder the plant's

reproduction. For this reason, we hypothesize that plant species designated as specialists will receive greater proportions of their own species' pollen grains than those designated as generalists. Using proportions of appropriate pollen grains on a stigma may be a good indicator of fitness (Fang and Huang 2013) and thus reproductive efficiency. Pursuing such a study gives insight into the resiliency of the ecosystem that can be used towards ecological management. To address the question of which strategy is more reproductively efficient, we studied three species previously designated as generalists: *Boykinia major* (large boykinia), *Eriogonum nudum* (wild buckwheat), and *Hypericum perforatum* (St. John's wort), and three previously designated as specialists: *Stachys cooleyae* (giant hedge-nettle), *Orthocarpus imbricatus* (mountain owl's clover), and *Epilobium angustifolium* (fireweed) in the Western Cascades of Oregon. As a prerequisite to our comparison of reproductive efficiency, we used three methods to confirm that these previous designations are still valid, testing the hypothesis that plant species designated as specialists would be visited by a smaller number of pollinator species than species designated as generalists.

## 2, Methods

### *2.1 Hypotheses*

Six flowering species chosen for our analysis were sampled for pollen on floral stigmas in four meadows and one roadside location in the Lookout Mountain and Frissell Ridge montane meadow complexes of the HJ Andrews Experimental Forest (Figure 1) in the Western Cascades of Oregon in July of 2015. Three of the species, *B. major*, *E. nudum*, and *H. perforatum* were selected because they were previously designated as generalists, while *S. cooleyae*, *O. imbricatus*, and *E. angustifolium* were selected for their previous specialist designation (Moldenke & Luh, unpublished manuscript 2015). In order to test the hypothesis that specialists have greater proportions of their own species' pollen grains on their stigmas, we sampled the stigmas of ten individuals per species and counted the number of the species' grains against the total number of grains.

In order to test the hypothesis that specialists allow fewer species of pollinators, we used three methods:

- (1) Using plant-pollinator interaction data accumulated in our meadows from 2011-2014, we took counts of pollinator species that were observed interacting with each of our six species.
- (2) Also using the plant-pollinator dataset, we compared the proportion of pollinators that interacted with each of our species versus the total number of pollinator species observed in an interaction in that meadow during the times that each plant was present in each year of data-collection.
- (3) Using trait-interaction data based on plant exclusion factors and corresponding pollinator traits, we calculated scores that would reveal a level of specialization for our plant species.

### *2.2 Field methods*

2.2.1. Hypothesis on Strategy Designation by Interactions: From 2011 to 2014, plant-pollinator interactions were recorded within the meadow complexes of the HJ Andrews Experimental Forest. Ten plots in each meadow were watched for 15 minutes and every observed interaction was recorded.

2.2.2. Hypothesis on Strategy Designation by Traits: The data from which our trait matrix was created was based on expert knowledge from years of field sampling by Oregon State University Entomologist, Andrew Moldenke.

2.2.3. Hypothesis on Reproductive Efficiency: In testing the hypothesis that specialist plants' pollen would make up a greater portion of the pollen on their stigma, we chose Lookout Mountain and Frissell Ridge meadow complexes for sampling because they contained meadows that had a relatively high abundance of the selected flowers during the time of collection. The sampled meadows were Lookout Steep (LS), Lookout Outcrop (LO), Lookout Bog (LB), and Frissell Main (M2) (Figure 2) which were all xeric except for Lookout Bog which was mesic. Five individuals of *H. perforatum* and five of *E. nudum* were sampled in Lookout Outcrop and the other five came from Lookout Steep. All individuals of *B. major* and *S. cooleyae* were sampled in Lookout Bog and *E. angustifolium* was sampled on the side of the 1506 road near the entrance into the Lookout Mountain complex. Within each meadow a reference point was selected central to the majority of the target flowers. From this reference point, a search was made in ten randomly selected directions until an individual of the target species was encountered.

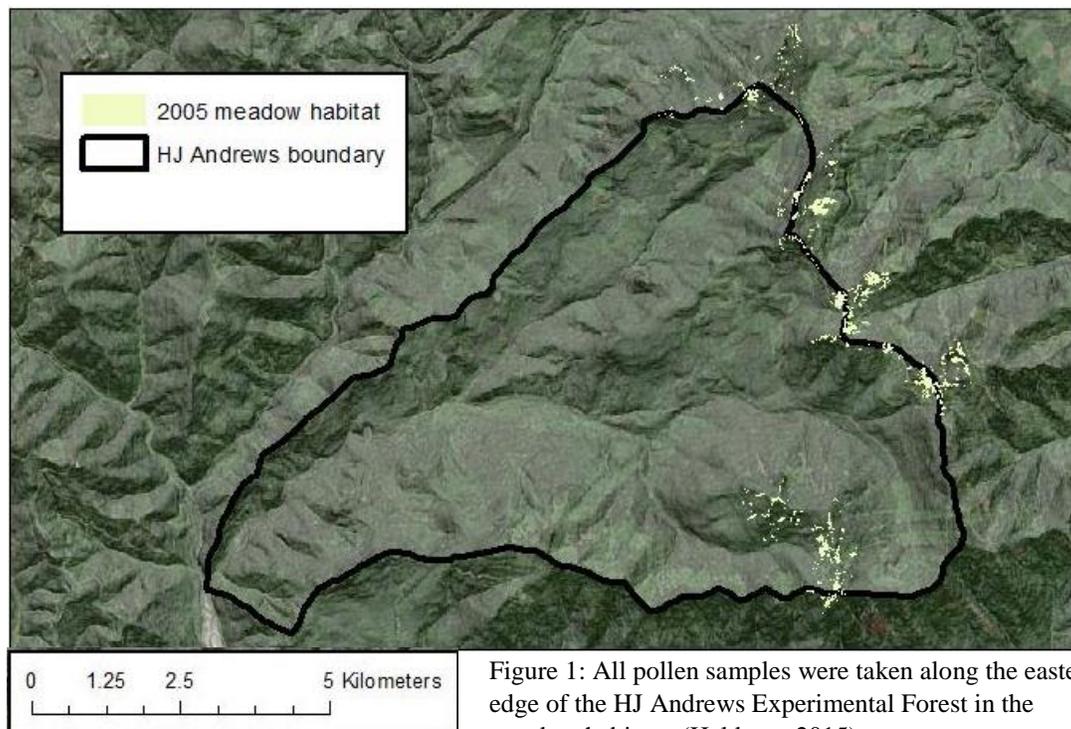


Figure 1: All pollen samples were taken along the eastern edge of the HJ Andrews Experimental Forest in the meadow habitats. (Helderop 2015)

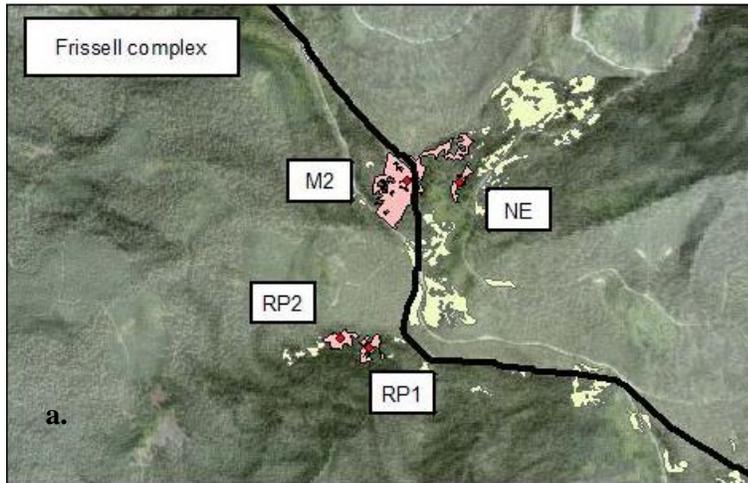
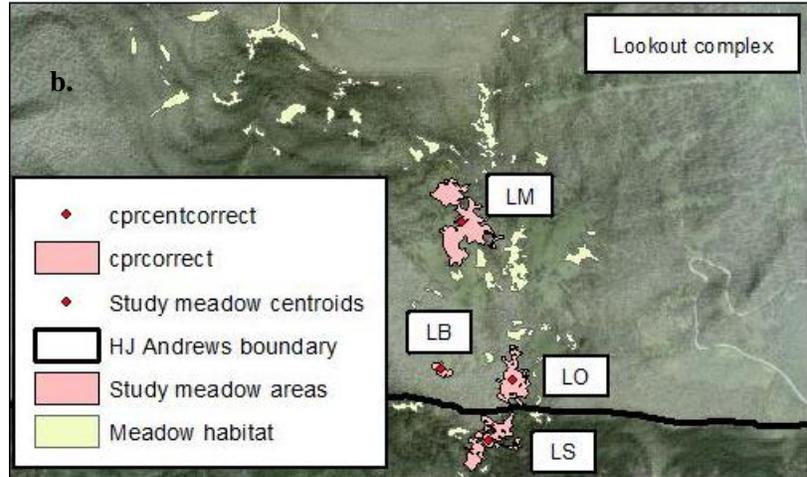


Figure 2: a. Frissell Main (M2) is a meadow within the Frissell complex where *Orthocarpus imbricatus* was sampled. b. Lookout Mountain complex was where most of our pollen samples were acquired (Helderop 2015).



When a flower of a target species was encountered, fuchsin glycerin jelly was rubbed across the stigma(s) of each flower using forceps. The gel, as a result, was embedded with pollen grains that were quickly dyed pink by the gel's fuchsin for better visibility. Then the gel was melted onto a glass slide in the field and transported to the lab at HJ Andrews Experimental Forest. Pollen grains were counted under a compound microscope at three magnifications; 40x, 100x, and 400x, for the most detailed observation.

Once the data were collected, they were input into an excel file in the following fashion. The excel file had five columns, called "Individual," "Species," "Generalist," "Pollen Grain Self," and "Pollen Grain Other." The rows each represented one sampled individual. The "Individual" column contained a number by which to refer to the sampled specimen. The "Species" column contained the name of the species of the sampled specimen. The "Generalist" column contained either the word "yes" or the word "no," indicating whether the individual was considered to be a generalist species or not. The "Pollen Grain Self" column contained the number of pollen grains from plants of the same species as the individual. The "Pollen Grain Other" column contained the number of pollen grains from plants of species other than that of the sampled individual.

### 2.3. *Data*

2.3.1 Hypothesis on Strategy Designation by Interactions: We used the interaction data collected from 2011 to 2014 to count the total number of pollinator species that visited each of our target flower species. We used these counts as a measure of how generalized or specialized each species was.

In order to fit the plant-pollinator data to a model in R Stan for a given flower species, we looked at counts of pollinator species that visited the flower species, and total counts of pollinator species that were in the same place at the same time. We sampled by meadow year, which is a unit for all plant-pollinator data from a single meadow in a single year. Note also that a meadow watch year refers to all data from a single watch in a single meadow in a single year. It became necessary to remove from the data any meadow watch years in which the flower was not recorded as present, since if the species could not be visited, a proportion of zero was not informative. The data consisted of counts sampled from the following meadow years, where for a given flower, a meadow year is actually the portion of the meadow year during which the flower was present (Table 1):

Table 1: The table displays which meadow years we used to test each plant species for the proportion of present pollinator species which visited it.

	<i>Boykinia major</i>	<i>Eriogonum nudum</i>	<i>Hypericum perforatum</i>	<i>Orthocarpus imbricatus</i>	<i>Stachys cooleyae</i>	<i>Epilobium angustifolium</i>
strategy	generalist	Generalist	Generalist	specialist	specialist	specialist
CPB		2011, 2014		2011, 2012, 2013, 2014		
CPM		2011		2011, 2012, 2013, 2014	2012, 2013, 2014	2013
CPR			2014			
CPS			2011, 2012, 2013, 2014			
M2		2011		2011, 2012, 2013, 2014		
RP1		2011, 2012, 2013, 2014	2011, 2012, 2014	2011, 2012		
RP2		2011, 2012, 2014	2013	2013, 2014		
NE				2013, 2014		
LB	2013, 2014				2013, 2014	
LM	2013, 2014	2013	2014	2011, 2012, 2013, 2014	2013	
LO		2011, 2012, 2013, 2014	2011, 2012, 2013, 2014			
LS		2012, 2013, 2014	2012, 2013, 2014			

*Epilobium angustifolium* was omitted from this analysis because it was present in only one meadow year.

2.3.2. Hypothesis on Strategy Designation by Traits: We studied a dataset compiled by Oregon State University Entomologist, Andrew Moldenke, in which there were three tables. The first was a table of nine plant exclusion factors, or plant traits that may prevent certain species from accessing their reproductive parts, for Oregon flowering plants. The second was a table of Oregon pollinators' traits that correspond to the exclusion factors. Last was a table of probabilities of an interaction occurring for every possible combination of plant exclusion factor and pollinator trait. We extracted the information about our six plant species from the first table, and using the probability table, created nine plant-pollinator interaction tables. An example of a trait-interaction table is shown in Figure 3. For every plant-pollinator interaction there would be a probability of the interaction occurring given a certain trait category. The probabilities for each interaction were multiplied to give us an ultimate probability of that interaction occurring. Then, for each plant species, the probabilities of each interaction were added. The more possibilities of

interactions and higher probabilities of them occurring would lead to greater scores, representing the versatility of generalist pollination, while the lower scores represented the precision at which specialists would be pollinated.

	A	B	C	D	E	F	G	H
1		ERIONUDU	HYPERPERF	ORTHIMBI	EPILANGU	STACCOOL	BOYKMAJO	
2	Acridid sp 1	1	1	0	1	1	1	
3	Acridid sp 2	1	1	0	1	1	1	
4	Adela trisignata	1	1	0.05	0.4	0.4	1	
5	Agapostemon texanus	1	1	0	0.01	0.01	1	
6	Agapostemon virescens	1	1	0	0.01	0.01	1	
7	Agulla sp 1	1	1	0	0.001	0.001	1	
8	Alydidae sp 1	1	1	0.01	0.1	0.1	1	
9	Alypia langtoni	1	1	0.05	0.4	0.4	1	
10	Ammophila sp 1	1	1	0.01	0.1	0.1	1	
11	Ammophila sp 3	1	1	0.01	0.1	0.1	1	
12	Ammophila sp 5	1	1	0.01	0.1	0.1	1	
13	Anaspis rufa	1	1	0	1	1	1	
14	Anastrangalia laetifica	1	1	0	0.5	0.5	1	
15	Ancistrocerus sp 1	1	1	0.01	0.1	0.1	1	
16	Ancistrocerus sp 2	1	1	0.01	0.1	0.1	1	
17	Ancistrocerus sp 3	1	1	0.01	0.1	0.1	1	
18	Ancistrocerus sp 4	1	1	0.01	0.1	0.1	1	

Figure 3: This table in Excel shows the probabilities of each plant-pollinator interaction occurring given the trait category “tube.”

2.3.3. Hypothesis on Reproductive Efficiency: Counts were taken for the number of pollen grains on each sample that belonged to the observed species and a separate count was recorded for grains that did not belong to the species observed.

## 2.4. Statistical methods

### 2.4.1. Hypotheses on Designations Based on Interactions:

We employed a probabilistic programming language called Stan, which implements Bayesian inference upon data by using the probability distributions of parameters. We used the R interface for Stan to fit the plant-pollinator interaction data available for each flower to a model and obtained predicted values for the true proportion of all present pollinators that visited the flower in question.

For a given flower species, we assumed that each pollinator species was a Bernoulli trial whose probability of visiting the flower was some  $\theta$ , and that each meadow year had its own  $\theta$  value. We assumed that each  $\theta$  was generated by a beta distribution with mean  $\lambda$ , where  $\lambda$  is the true mean proportion of pollinator species that visit the plant species in question. We also required a value representing prior pollinator species counts in the meadow

year, called kappa. In order to fit this model to the data in R we needed to use transformed parameters alpha and beta, where alpha represented prior counts of pollinator species which did interact with the flower species, and beta represented the prior counts of pollinator species which were recorded but did not interact with the flower species.

The model comprised all of the above assumptions, and we encoded it in a Stan file which we passed into the Stan function in R. We dealt with one plant species at a time. There was only one value of each of lambda, kappa, beta, and alpha. We had a different number of samples for each flower species, which was given the name J for each time a fit was obtained. The data that we passed into the Stan function consisted of the integer J and two vectors each of dimension J. One vector would contain the counts of pollinator species that visited the flower species in question, and the other would contain the total counts of pollinator species recorded in the portion of the meadow year during which the flower species was present.

With the Stan code written, we used R to take the pertinent data from the plant-pollinator data set and prepare it in the format described immediately above. A stanfit object was obtained by inputting the Stan file, the data list, a number of iterations per chain(1000), and a number of chains to run(4) into the Stan function in R. Subsequently, we examined the traceplot of the fit we obtained, and determined that the chains had not sufficiently converged. We then obtained a second fit by inputting the first fit, the same data list, a greater number of iterations per chain(100,000), a period by which to thin the data(5), and the same number of chains to run(4). We examined the traceplot of this fit, as well as the autocorrelation and partial autocorrelation plots, and determined that it converged sufficiently.

2.4.2. Hypotheses on Designations Based on Interactions: Using the trait data, we graphed nine floral traits that might influence a pollinator's access to the reproductive parts of a plant against corresponding pollinator traits. For every trait, each possible plant-pollinator interaction was assigned a probability of actually occurring, determined by years of observation (Moldenke & Luh, unpublished manuscript 2015). For each interaction, there would be nine probabilities of actually occurring, one for each trait. Those probabilities were multiplied to get ultimate probabilities of each interaction occurring. Those results were then summed to get an ultimate score. The species with the highest scores were to be considered generalists.

2.4.3. Hypotheses on Reproductive Efficiency: We made certain assumptions about the data in order to be able to identify the parameters we were seeking. We assumed that for a given individual of a flower species, there is some inherent probability theta that a single pollen grain on the stigma of the individual will be a pollen grain belonging to the species of the individual. This means that a given pollen grain represents one Bernoulli trial with p equal to theta. We assumed that this probability theta was drawn from a beta distribution with mean lambda and a value representing total prior grain counts, called kappa. Under the assumption, lambda represents the true mean of all values theta for a single species, which means for all individuals of a given species it is the true mean proportion of pollen grains which belong to the species of the plant from which it was collected. It became necessary to transform lambda and kappa into shape parameters alpha and beta since the beta distribution function in Stan expects one parameter which represents prior counts of one category, and one other parameter which

represents prior counts of a category which is mutually exclusive to the aforementioned. The shape parameters alpha and beta of the beta distribution were defined to be as follows:

$$\alpha = \lambda * \kappa$$

$$\beta = (1 - \lambda) * \kappa$$

Kappa was assumed to be drawn from a pareto distribution with scale parameter 0.1 and shape parameter 1.5. We chose the pareto distribution because we know little about kappa except that it makes somewhat more sense to us if it is small. The pareto distribution represents this well in that its highest probability density is at some minimum, positive value of the random variable, but with a scale parameter greater than one, it has no mean. Lambda, like theta, was assumed to be drawn from a beta distribution, this time with mean megalambda and a value representing average total prior grain counts called megakappa. Under the assumption, megalambda represents the true mean of all values lambda for either specialist plants or generalist plants, meaning that it is the true mean proportion of pollen grains which belong to the species of the plant from which it was collected for each of the categories generalist and specialist. The shape parameters megaalpha and megabeta of the beta distribution were defined to be as follows:

$$\text{mega}\alpha = \text{mega}\lambda * \text{mega}\kappa$$

$$\text{mega}\beta = (1 - \text{mega}\lambda) * \text{mega}\kappa$$

Megakappa was assumed to be drawn from a pareto distribution with scale parameter 0.1 and shape parameter 1.5. Megalambda was assumed to be drawn from a uniform distribution from 0 to 1.

Flower species:  $k = 1, 2, K; K = 3$

Pollen samples:  $j = 1, \dots, J; J = 10$

Pollen grains:  $g = 1, \dots, G_j$

$$\Lambda \sim \text{Uniform}(0,1)$$

$$K \sim \text{Pareto}(0.1,1.5)$$

$$\lambda_k \sim \text{Beta}(\Lambda K, (1 - \Lambda)K) \forall k$$

$$\kappa_k \sim \text{Pareto}(0.1, 1.5) \forall k$$

$$\theta_{kj} \sim \text{Beta}(\lambda_k \kappa_k, (1 - \lambda_k) \kappa_k) \forall j$$

$$x_{jkg} \sim \text{Bernoulli}(\theta_{kj})$$

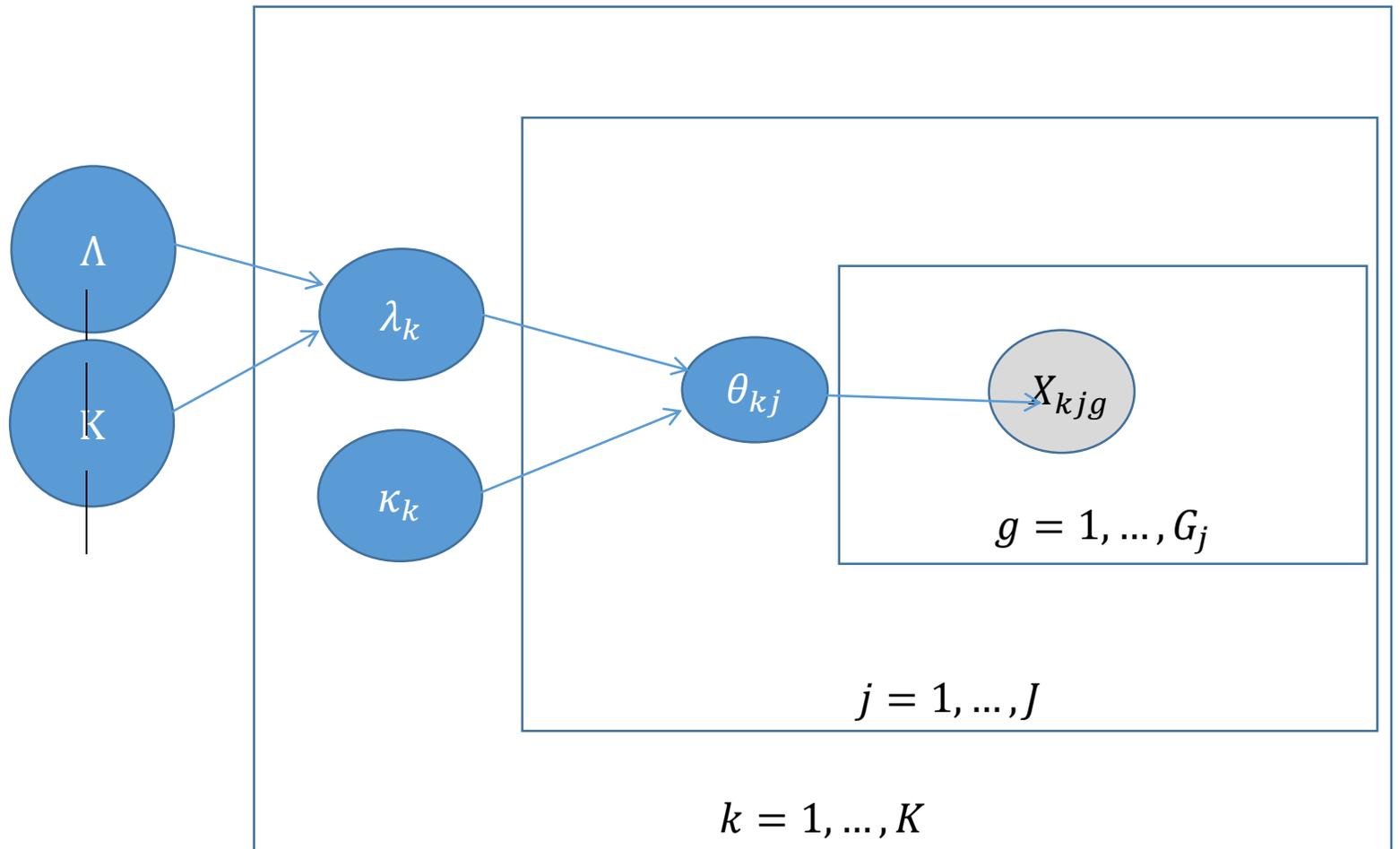


Figure 4: The above is a visual representation of the hierarchical model we chose to represent our system. Each  $x$  represents one pollen grain which belongs to the species of the individual from which it was collected with probability theta. This describes the binomially distributed level of the hierarchy. Each theta is produced from a beta distribution with mean lambda and prior total grain count kappa. Each lambda is produced from a beta distribution with mean megalambda and prior average total grain count megakappa.

The model comprised all of the above assumptions, and we encoded it in a Stan file which we passed into the Stan function in R. We dealt with one hierarchy at a time, with either specialist or generalist being the single node at the top. There was only one value of each of megalambda, megakappa, megabeta, and megaalpha. The categories “generalist” and “specialist” each contained three different species, so we had three different values each of lambda, kappa, alpha, and beta. Each species category contained ten different individuals, so we had thirty different values of theta under each branch “specialist” or “generalist.” The dimensions described above were encoded into the Stan file as K species and J individuals per species. The Stan code further specified that the Stan function in R expect the data to comprise the K dimension, the J dimension, and two matrices each having dimensions K x J. One matrix would contain the counts of pollen grains that matched the species of the sampled individual, and the other would contain the total counts of pollen grains for each sampled individual.

With the Stan code written, we used R to take the pertinent data from the excel file and prepare it in the format described immediately above. A stanfit object was obtained by inputting the Stan file, the data list, a number of iterations per chain(1000), and a number of chains to run(4) into the Stan function in R. Subsequently, we examined the traceplot of the fit we obtained, and determined that the chains had not sufficiently converged. We then obtained a second fit by inputting the first fit, the same data list, a greater number of iterations per chain(1000000), a period by which to thin the data(500), and the same number of chains to run(4). We examined the traceplot of this fit, as well as the autocorrelation and partial autocorrelation plots, and determined that it converged sufficiently.

In order to create some sense of how the megalambda values actually compared, we extracted the vectors of all 4000 megalambda values for each the specialist and generalist categories and compared the elements with matching indices in order to gain some sense of how significant our results were.

### 3. Results

#### 3.1. Hypothesis on Designations based on Interactions:

In general, the species hypothesized to behave as generalists were visited by a much greater total number of pollinator species than species hypothesized to behave as specialists (Figure 4). At the meadow year level, the overall trend was that species hypothesized to be specialists were visited by a smaller number of pollinator species in comparison to the total number of pollinator species present in the meadow year (Table 2). The estimated mean proportion across all meadow years of present pollinator species which visited *B. major* was 0.35 (Figure 5). The estimated mean proportion across all meadow years of present pollinator species which visited *E. nudum* was 0.17 (Figure 6). The estimated mean proportion across all meadow years of present pollinator species which visited *H. perforatum* was 0.29 (Figure 7). The estimated mean proportion across all meadow years of present pollinator species which visited *O. imbricatus* was 0.09(Figure 8). The estimated mean proportion across all meadow years of present pollinator species which visited *S. cooleyae* was 0.16 (Figure 9).

Figure 4. Total counts of pollinator species on each of six plant species: For the most part, species hypothesized to be generalists were visited by higher total numbers of species in the Frissell, Carpenter, and Lookout complexes in the years 2011-2014. However, *O. imbricatus* (40 pollinator species), a hypothesized specialist, was visited by a comparable number of species to *E. nudum* (43 pollinator species). Also, *B. major* (21 pollinator species) was visited by significantly fewer species than either of the other two species expected to behave as generalists. *H. perforatum* was visited by 55 pollinator species, *S. cooleyae* was visited by 4 pollinator species, and *E. angustifolium* was visited by 8 pollinator species.



Table 2. Counts of pollinator species on each plant species in each meadow year compared to total pollinator species counts in the meadow year: Overall, the trend we observed in these data was that species hypothesized to be specialists were visited by fewer pollinator species compared to the total pollinator species count in the meadow year. Note that a blank box indicates a meadow year where the corresponding plant species was not present.

	<i>O. imbricatus</i>	<i>E. angustifolium</i>	<i>S. cooleyae</i>	<i>E. nudum</i>	<i>H. perforatum</i>	<i>B. major</i>
CPB_11	3/44			0/17		
CPB_12	7/83					
CPB_13	5/45					
CPB_14	2/13			2/18		
CPM_11	5/45			0/19		
CPM_12	1/57		1/25			
CPM_13	8/75	8/42	1/35			
CPM_14	2/33		1/15			

CPR_14					0/2	
CPS_11					4/45	
CPS_12					9/37	
CPS_13					6/18	
CPS_14					5/17	
LB_13			2/17			15/40
LB_14			1/11			8/20
LM_11	2/57					
LM_12	8/55					
LM_13	13/92		1/27	0/42		
LM_14	4/39				0/6	2/23
LO_11				13/40	13/61	
LO_12				12/42	14/70	
LO_13				10/38	16/38	1/24
LO_14				1/33	17/33	
LS_12				4/29	6/29	
LS_13				7/30	10/38	
LS_14				5/31	3/15	
M2_11	9/80			1/24		
M2_12	5/78					
M2_13	10/80					
M2_14	5/46					
NE_13	10/69					
NE_14	3/63					
RP1_11	5/52			1/29	10/34	
RP1_12	2/34			4/20	6/20	
RP1_13				1/6		
RP1_14				4/19	9/19	
RP2_11				6/45		
RP2_12		0/5		6/14		
RP2_13	1/25				11/25	
RP2_14	0/26			3/35		

Figure 5. Counted proportions, estimated meadow year proportions, and estimated overall species mean proportions of pollinators which visited *Boykinia major*: The estimated mean proportion of pollinator species which visited *B.*

*major*, a hypothesized generalist, is represented by a red line, and was 0.35. Two of the individual meadow year proportions were estimated to be much lower.

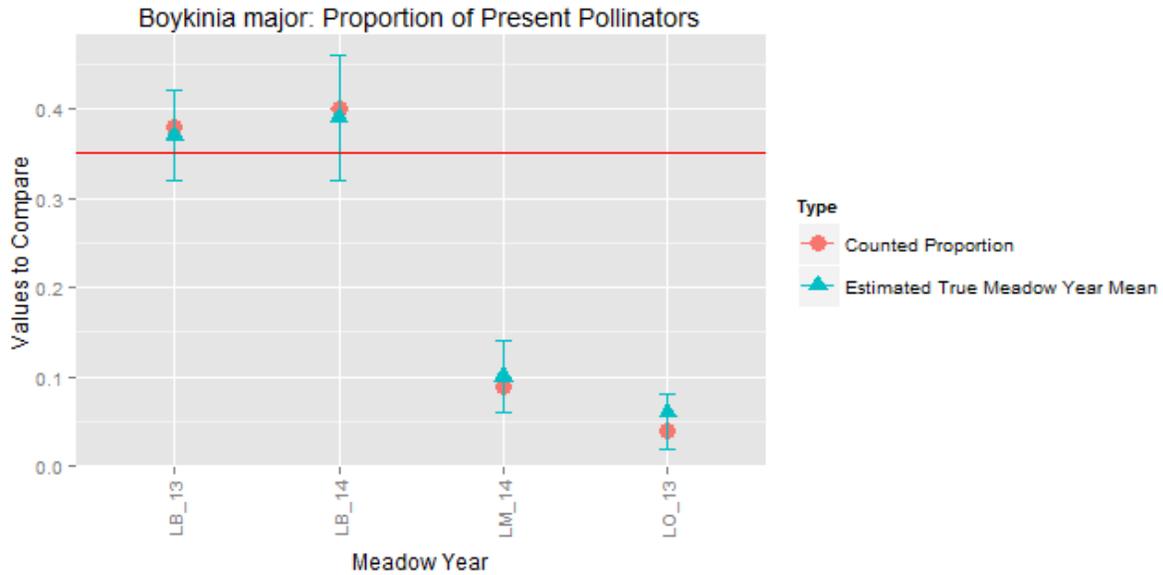


Figure 6. Counted proportions, estimated meadow year proportions, and estimated overall species mean proportions of pollinators which visited *Eriogonum nudum*: The estimated mean proportion of pollinator species which visited *E. nudum* (hypothesized generalist) was 0.17, and is represented by a red line. Neither counted proportions nor estimated proportions stayed particularly close to this estimated mean, with one counted proportion outlier of 0.43.

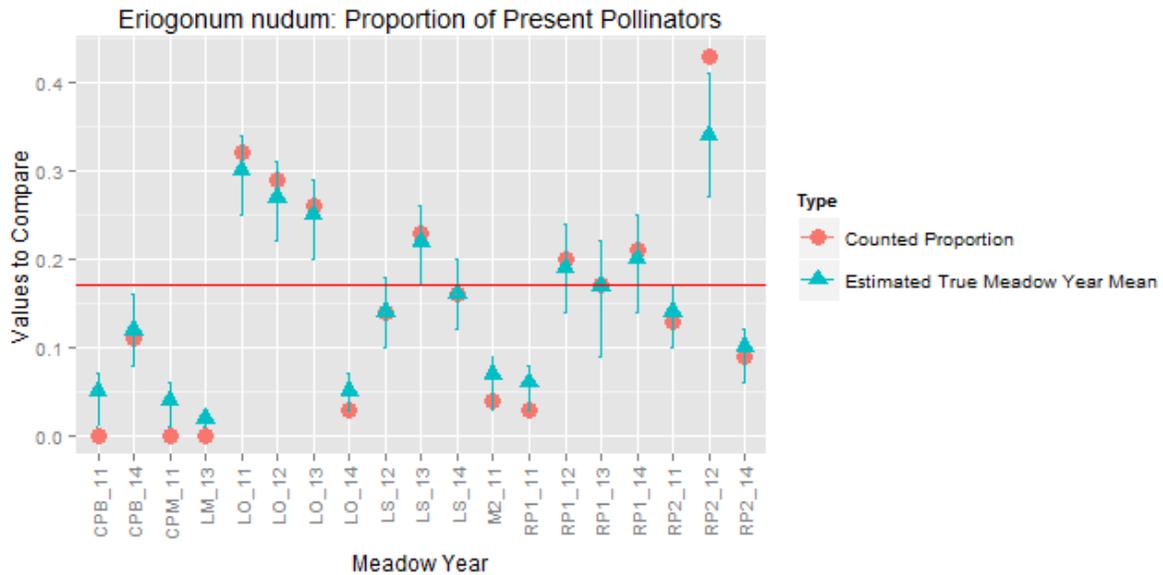


Figure 7. Counted proportions, estimated meadow year proportions, and estimated overall species mean proportions of pollinators which visited *Hypericum perforatum*: The estimated mean proportion of pollinator species which

visited *H. perforatum* (hypothesized generalist) was 0.29, and is represented by a red line. Both the counted proportions and the estimated proportions varied widely around the estimated mean.

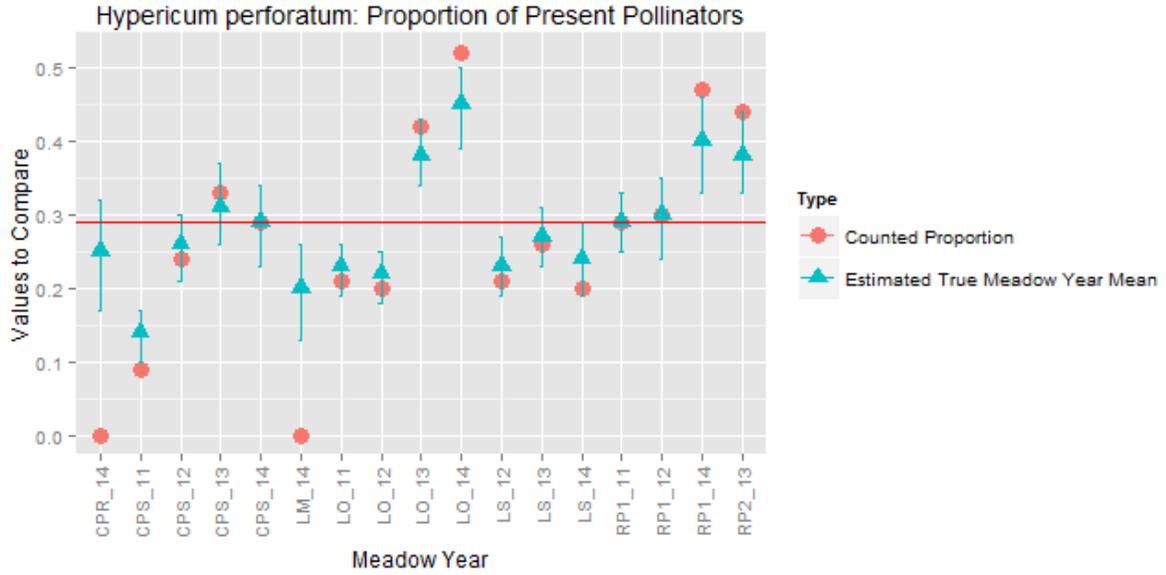


Figure 8. Counted proportions, estimated meadow year proportions, and estimated overall species mean proportions of pollinators which visited *Orthocarpus imbricatus*: The estimated mean proportion of pollinator species which visited *O. imbricatus* (hypothesized specialist) was 0.09, and is represented by a red line. Neither the counted

proportions nor the estimated meadow year proportions ever exceeded the estimated mean proportion by more than 0.06, but some are as low as 0.

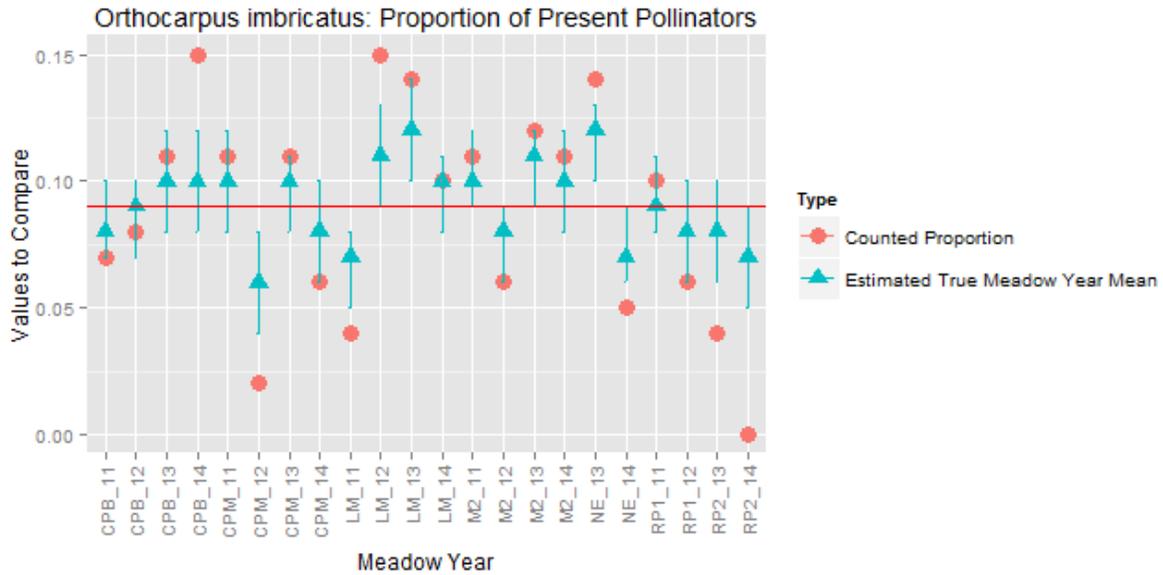
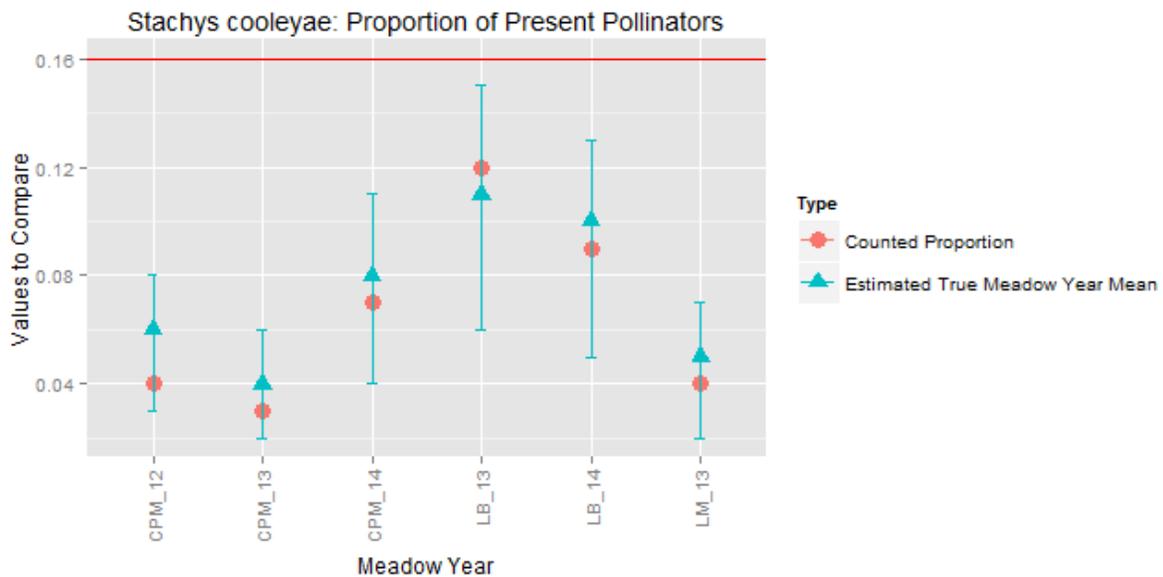


Figure 9. Counted proportions, estimated meadow year proportions, and estimated overall species mean proportions of pollinators which visited *Stachys cooleyae*: The estimated mean proportion of pollinator species which visited *S. cooleyae* (hypothesized specialist) was 0.16, and is represented by a red line. The estimated mean proportion was higher than any of the counted meadow year proportions, which all fell between 0.02 and 0.12.



### 3.2. Results of Hypothesis (2).

Overall, the heterospecific grain counts of the generalists were lower compared to the total grain counts than was the case for specialists (Table 3). The estimated average homospecific grain count for *Eriogonum nudum* was 0.33 (Figure 10). The estimated average homospecific grain count for *Hypericum perforatum* was 0.86 (Figure 11). The estimated average homospecific grain count for *Boykinia major* was 0.94 (Figure 12). The estimated average homospecific grain count for *Orthocarpus imbricatus* was 0.6 (Figure 13). The estimated average homospecific grain count for *Stachys cooleyae* was 0.46 (Figure 14). The estimated average homospecific grain count for *Epilobium angustifolium* was 0.56 (Figure 15). The generalist megalambda value was greater than that of the specialists with an estimated probability of 0.62 (Figure 16).

Table 3. Heterospecific vs. Homospecific pollen grain counts from 10 individuals from each of six species: overall, the generalist species had lower counts of heterospecific grain counts compared to their total grain counts. However, *Eriogonum nudum*, a generalist, had the lowest homospecific grain count proportions of any of the six species analyzed.

Individual	Species	Generalist	Pollen Grain Self	Pollen Grain Other
41	<i>Eriogonum nudum</i>	yes	1	5
42	<i>Eriogonum nudum</i>	yes	3	20
43	<i>Eriogonum nudum</i>	yes	1	11
44	<i>Eriogonum nudum</i>	yes	8	7
45	<i>Eriogonum nudum</i>	yes	0	10
46	<i>Eriogonum nudum</i>	yes	3	7
47	<i>Eriogonum nudum</i>	yes	2	1
48	<i>Eriogonum nudum</i>	yes	4	15
49	<i>Eriogonum nudum</i>	yes	0	4
50	<i>Eriogonum nudum</i>	yes	9	2
31	<i>Hypericum perforatum</i>	yes	308	0
32	<i>Hypericum perforatum</i>	yes	121	4
33	<i>Hypericum perforatum</i>	yes	121	26
34	<i>Hypericum perforatum</i>	yes	71	18
35	<i>Hypericum perforatum</i>	yes	108	27
36	<i>Hypericum perforatum</i>	yes	61	5
37	<i>Hypericum perforatum</i>	yes	181	5

38	Hypericum perforatum	yes	125	3
39	Hypericum perforatum	yes	347	19
40	Hypericum perforatum	yes	161	34
1	Boykinia major	yes	1574	2
2	Boykinia major	yes	73	1
3	Boykinia major	yes	717	11
4	Boykinia major	yes	17	2
5	Boykinia major	yes	14	1
6	Boykinia major	yes	97	8
7	Boykinia major	yes	64	2
8	Boykinia major	yes	63	1
9	Boykinia major	yes	456	8
10	Boykinia major	yes	152	9
51	Orthocarpus imbricatus	no	14	7
52	Orthocarpus imbricatus	no	36	3
53	Orthocarpus imbricatus	no	12	4
54	Orthocarpus imbricatus	no	58	9
55	Orthocarpus imbricatus	no	6	7
56	Orthocarpus imbricatus	no	1	9
57	Orthocarpus imbricatus	no	71	156
58	Orthocarpus imbricatus	no	17	15
59	Orthocarpus imbricatus	no	126	31
60	Orthocarpus imbricatus	no	57	9
11	Stachys cooleyae	no	20	48
12	Stachys cooleyae	no	112	83
13	Stachys cooleyae	no	6	14
14	Stachys cooleyae	no	24	27
15	Stachys cooleyae	no	5	2
16	Stachys cooleyae	no	69	75
17	Stachys cooleyae	no	31	64
18	Stachys cooleyae	no	38	104
19	Stachys cooleyae	no	44	21
20	Stachys cooleyae	no	15	16

21	Epilobium angustifolium	no	20	45
22	Epilobium angustifolium	no	17	16
23	Epilobium angustifolium	no	41	68
24	Epilobium angustifolium	no	4	8
25	Epilobium angustifolium	no	23	7
26	Epilobium angustifolium	no	316	16
27	Epilobium angustifolium	no	13	30
28	Epilobium angustifolium	no	58	59
29	Epilobium angustifolium	no	77	41
30	Epilobium angustifolium	no	70	19

Figure 10. Counted proportions, estimated individual mean proportions, and estimated species mean proportions of homospecific grain counts to the total for *Eriogonum nudum*: The estimated species mean proportion of homospecific grains (represented by a red line) for *E. nudum* (a generalist) was 0.33. The counted proportions, however, varied broadly.

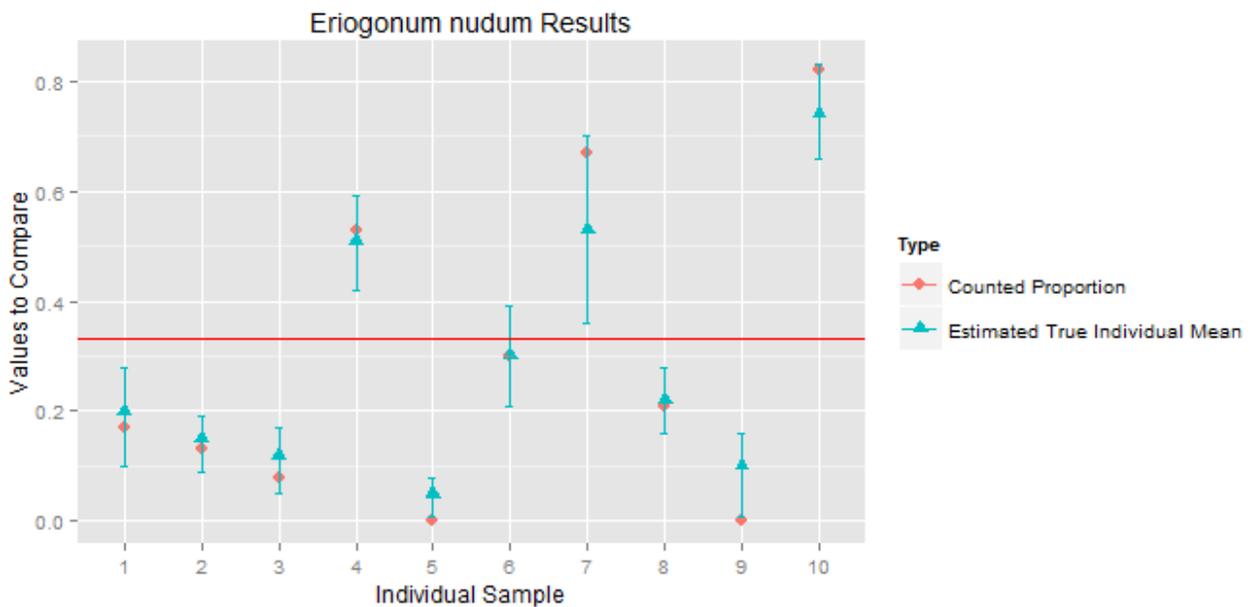


Figure 11. Counted proportions, estimated individual mean proportions, and estimated species mean proportions of homospecific grain counts to the total for *Hypericum perforatum*: The estimated species mean proportion of

homospecific grains (represented by a red line) for *H. perforatum* (a generalist) was 0.86. All of the counted proportions and estimated individual proportions were greater than 0.8.

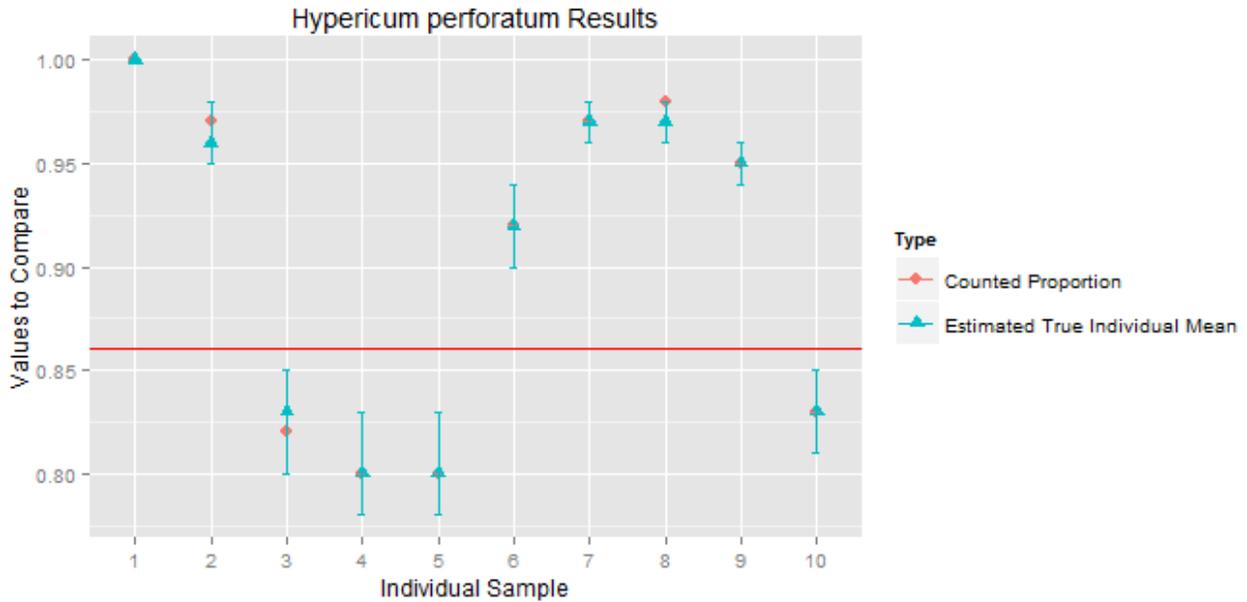


Figure 12. Counted proportions, estimated individual mean proportions, and estimated species mean proportions of homospecific grain counts to the total for *Boykinia major*: The estimated species mean proportion of homospecific grains (represented by a red line) for *B. major* (a generalist) was 0.94. No counted or estimated individual proportion was below 0.9.

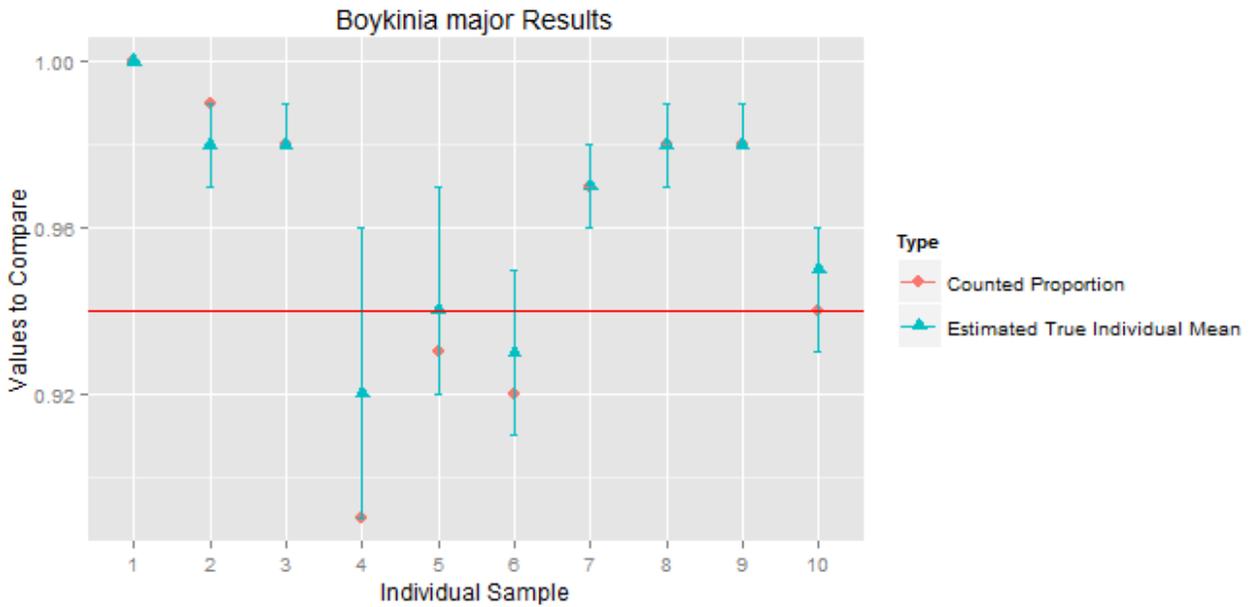


Figure 13. Counted proportions, estimated individual mean proportions, and estimated species mean proportions of homospecific grain counts to the total for *Orthocarpus imbricatus*: The estimated species mean proportion of

homospecific grains (represented by a red line) for *O. imbricatus* (a specialist) was 0.6. The counted proportions ranged broadly, between .1 and .9.

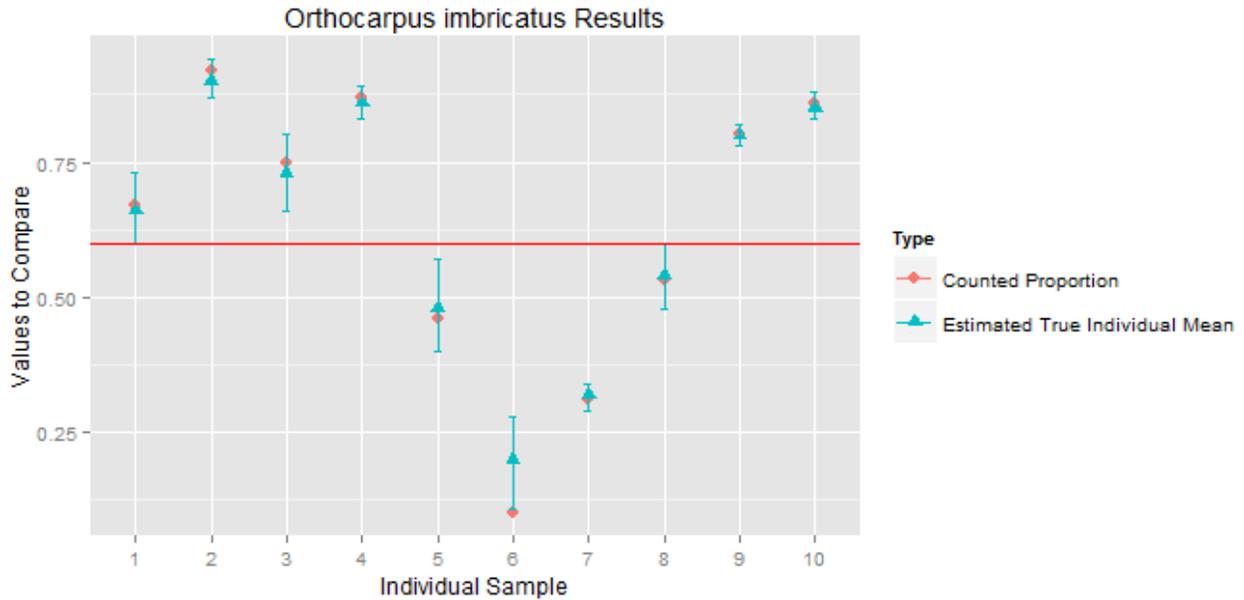


Figure 14. Counted proportions, estimated individual mean proportions, and estimated species mean proportions of homospecific grain counts to the total for *Stachys cooleyae*: The estimated species mean proportion of homospecific grains (represented by a red line) for *S. cooleyae* (a specialist) was 0.46.

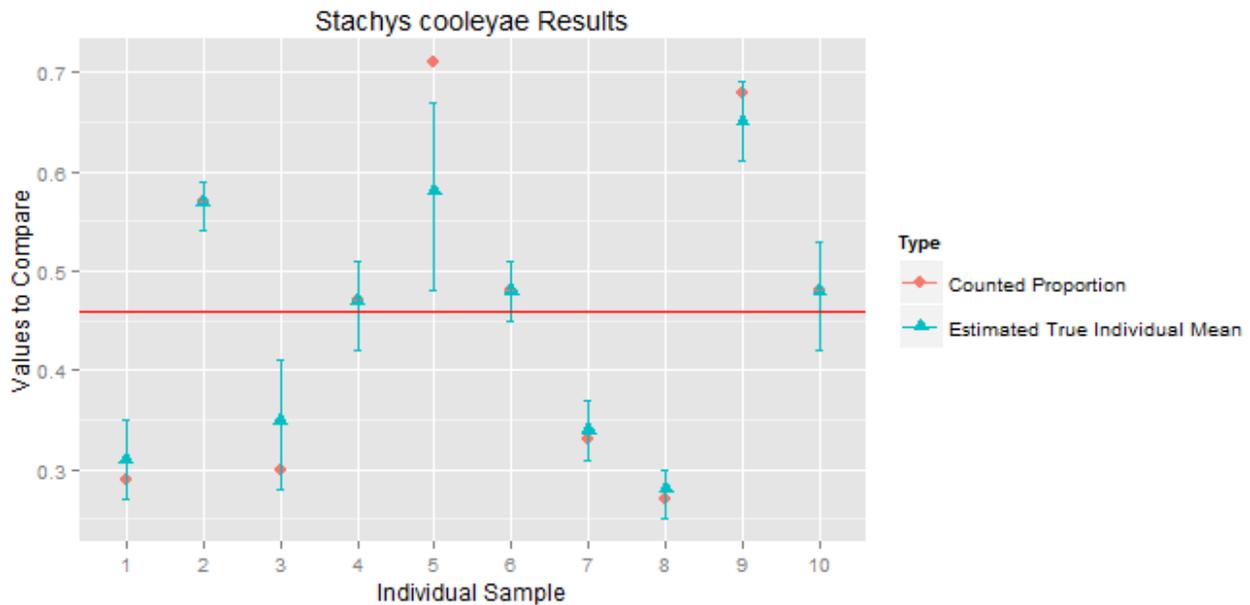


Figure 15. Counted proportions, estimated individual mean proportions, and estimated species mean proportions of homospecific grain counts to the total for *Epilobium angustifolium*: The estimated species mean proportion of

homospecific grains (represented by a red line) for *E. angustifolium* (a specialist) was 0.56. Again, the counted proportions and estimated individual means varied widely, between 0.3 and 0.95.

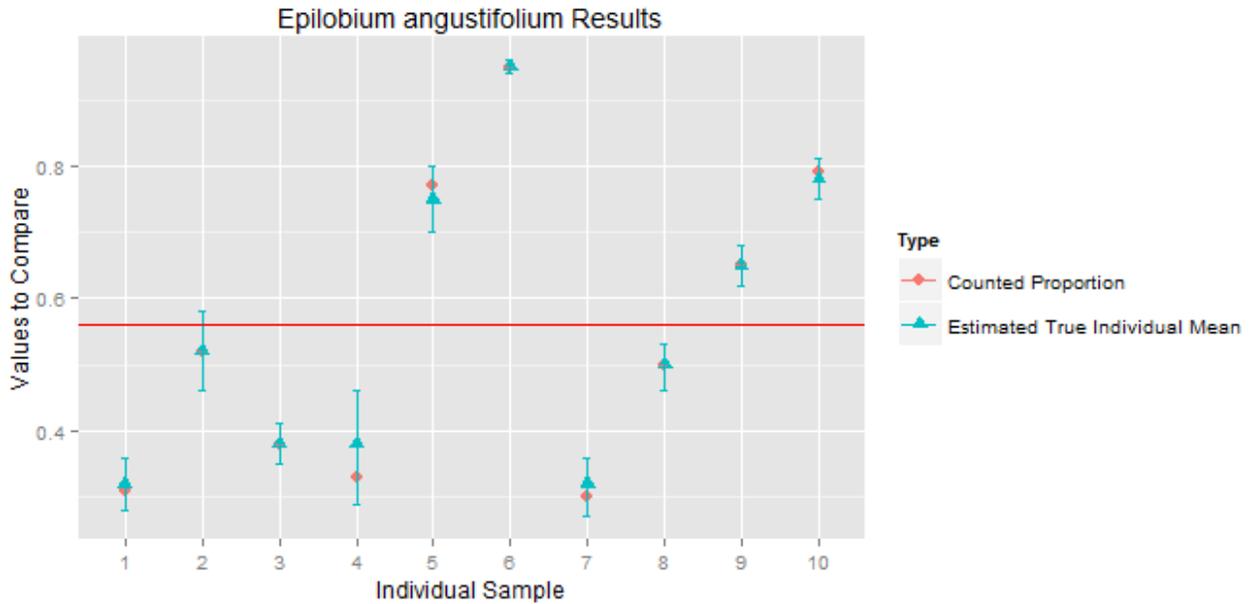
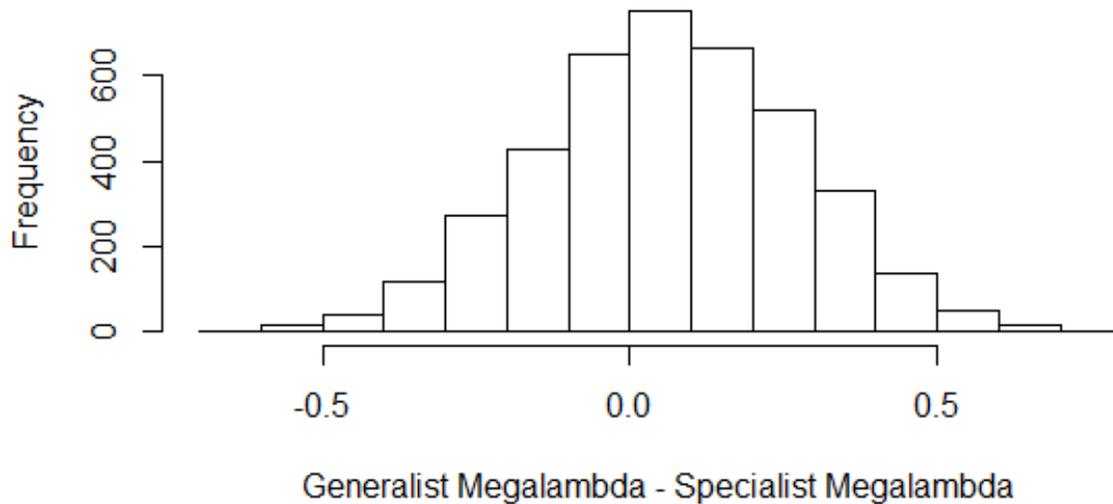


Figure 16. Histogram of the difference between estimated mean proportions of homospecific grains for generalists and specialists: In 62% of cases (out of 4000 estimates for each that were averaged to obtain the final estimate), the generalist mean homospecific grain proportion was greater than that of the specialists. The average estimate of the generalist megalambda was 0.58 while the average estimate of the specialist megalambda was 0.52.

### Difference Between Generalist and Specialist Megalambdas



3.3. Hypothesis on Designations Based on Traits: Finally, using our trait-interaction matrix to determine if plant species designated as specialists were visited by a smaller proportion of pollinator species than species designated as generalists, we acquired the following scores: *E. nudum*=9.9430875, *H. perforatum* = 9.943088, *B. major* = 10.053025, *O. imbricatus* = 0.128175, *E. angustifolium* = 0.555, and *S. cooleyae* = 0.2421.

#### 4. Discussion

##### 4.1. Hypothesis on Designations Based on Interactions:

Studying the raw counts of pollinator species that have been observed on the six plant species we were concerned with showed that two of the plants that we hypothesized were generalists (*H. perforatum* and *E. nudum*) and two of the plants we hypothesized were specialists (*S. cooleyae* and *E. angustifolium*) behaved as we expected. That is, both *H. perforatum* (55 pollinator species) and *E. nudum* (43 pollinator species) interacted with at least 35 more pollinator species than did either of *S. cooleyae* (4 pollinator species) and *E. angustifolium* (8 pollinator species). However, one hypothesized generalist, *B. major*, interacted with relatively few species (21). This could be due to the fact that it is found in relatively few places in the meadows from which the plant-pollinator data was collected compared with the other generalists in the study. We also saw an unexpected count of pollinator species that interacted with *O. imbricatus*, which we hypothesized was a specialist. It had interacted with 40 pollinator species, which is nearly double the count of *B. major*. We may gain some insight as to why this is the case by comparing the counts of pollinator species that interacted with *O. imbricatus* at a given place and time to the total number of pollinator species recorded in the same place and time, as is explained in the following paragraph.

The raw counts that we used in our Bayesian inference about pollinator species proportions indicated that, in general, the three hypothesized specialist species whose counts we obtained interacted with few pollinator species in a given meadow year compared the total number of pollinator species observed in the portion of the meadow year during which the plant species was present. The three hypothesized generalist species seemed to interact with a greater proportion of present pollinator species. *O. imbricatus*, a hypothesized specialist, was present in more meadow years than any other plant species in the study, and was present during periods of time during which the overall pollinator species count was very high. This could help to explain why in four years of data collection it interacted with comparable numbers of pollinator species to the species we hypothesized were generalists.

The results of our Bayesian inference in Stan about the proportions of all present pollinators that visited a given flower indicated that of the two hypothesized specialist species that we analyzed, both tended toward lower mean proportions than any of the three hypothesized generalist species. However, the results were not definitive in that one hypothesized generalist species, *Eriogonum nudum*, had a mean proportion that exceeded the higher of the two hypothesized specialists' (*Stachys cooleyae*) proportions by only 0.01. Additionally, it is clear from the graphic regarding *S. cooleyae* that the estimated mean proportion of pollinator species that visit the

aforementioned species(0.16) is greater than any of the sampled proportions(greatest sampled proportion 0.12). This apparent inconsistency raises two doubts; it is possible that there was a mistake in the code which we used to plot or analyze the proportions for *S. cooleyae*, although each flower species was treated with exactly the same code. It is also possible that a good estimate was hindered by the sample size, which was six meadow years in the case of *S. cooleyae*. The last concern regarding this particular method is one of model choice. For a given flower species, we chose to examine the counts of pollinator species which were present but either were or were not recorded as interacting with the flower species as a sequence of Bernoulli trials. This would be valid if the event of a pollinator species visiting the flower species in question depended solely on some inherent probability, but we have reason to believe that other influences are present. As a result, we should expect over-dispersion in our sampling distribution when compared with a binomial distribution.

#### 4.2. Hypothesis on Designations Based on Traits:

For our trait-interaction matrix, the lower-scored species are presumably more specialized than those with higher scores. Based on our calculated scores, we determined that the plant species designated as specialists were in fact visited by a smaller proportion of species of pollinators than species designated as generalists. Future studies may prove more reliable if the matrix is formed by a formal dataset where probabilities are calculated from recorded observations rather than estimations based on years of unrecorded observations.

#### 4.3. Hypothesis on Reproductive Efficiency:

The results for our reproductive efficiency hypothesis challenged the starting hypothesis that the specialist species would receive its own pollen a greater proportion of the time. Our samples indicated that generalist species have a greater overall probability of receiving pollen grains from their own species than specialist species by 0.06. However, based upon the analysis of all the megalambda values that our Markov chains produced, there is only an estimated 0.62 probability that generalists have a greater chance at all. If proportions of pollen grains captured from stigmas are in fact good indicators of reproductive efficiency, then in conservation management practices, one may decide specialists are more in need of attention than generalists.

In order for proportions to be a good indicator, we made some assumptions. First we assumed that the pollen grains are evenly distributed across a stigma; therefore the sampled proportion would represent the total proportion on the stigma. A future solution to this could be to harvest the entire stigma. Next, we assumed that none of the species could be self-pollinated. The only species for which we found information on this were *E. angustifolium* and *B. major*. *E. angustifolium* can be self-pollinated ("Index of Species Information"), but *B. major* was strongly self-incompatible (Gornall and Bohm 1983). *B. major* and any of the existing species that may be self-incompatible can only be fertilized by other individuals of the same species, but if they have been receiving their own pollen grains, then our counted proportions of viable pollen grains may be greatly overestimated. We cannot distinguish a difference between individuals of the same species. A temporary solution to this would be to only sample species that can self-

pollinate. Finally, we made the assumption that all pollen grains that could potentially fertilize the ovule were on the stigma at the time of sampling, but many of the pollen grains may have already made their way down the style toward the ovary, affecting the proportion of appropriate grains on the stigma. Flower species vary in the delay time between pollination and fertilization, but no prior information was found on the fertilization of our selected species. Species also differ on the number of ovules that need to be fertilized, thus the approximate number of pollen grains that travel down the style. Taking into account both the stage of fertilization at the time of sampling and the number of grains needed to fertilize the ovules, the estimated number of missing viable grains on the stigma should be able to be determined.

Although an accurate proportion of pollen grains on the stigma seems to make sense in measuring reproductive efficiency, we have not considered other mechanisms that might contribute to efficiency. Those species with more ovules, for example, will have will have more pollen grains traveling down the style to fertilize, and therefore may need to be weighted differently in the determination of reproductive efficiency.

Samples should be taken consistently at the stage of growth just before the style shrivels up because it will have received the season's maximum amount of pollen grains. Because all our samples were taken within a three-week period, not all of the species were in the ideal stage of growth. *E. nudum*, for example, was sampled very late in the season therefore, it is very likely that its proportions formed by small counts of pollen on the stigmas can be explained by this. Our hierarchical model accounted for the small proportions by giving more weight to the samples with the largest pollen counts. Still, collecting at a consistent stage would eliminate some margin of error. For this study to continue, it would be valuable to test other species generalists and specialists as well as collect larger samples.

### Acknowledgements

We are grateful for all the Principal Investigators and mentors who dedicated so much of their time to daily meetings, quick-feedback emails, and enthusiasm. Thanks to Dr. Julia Jones for challenging our thoughts about our observations in nature. Special thanks to Dr. Andrew Moldenke, whose lecture inspired the project, for his assistance in pollen grain identification and dataset contributions. For our many hours of statistical analysis, we would also like to thank Dr. Tom Dietterich, Dr. Alix Gitelman, and Dr. Rebecca Hutchinson. Thanks to Kate Jones for her guidance throughout this process and Eddie Helderop for his explanation of sampling protocols. Finally, thanks to Ashley Sanders, Anna Young, and Marissa Childs for their help with data collection and overall thoughts of the project.

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