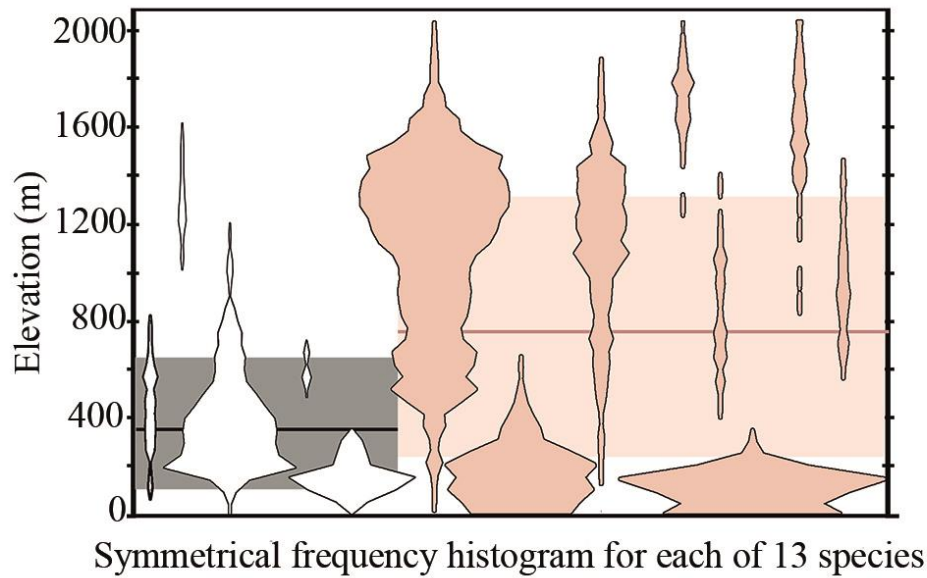# Supplemental Figure

Figure S1. Symmetrical frequency histograms of all locality records of the 13 colour monomorphic *Protea* species that are bird-pollinated and occur naturally in the CFR (6350 records from the Protea Atlas Project). The eight monomorphic pink species are coloured pink and the five monomorphic white species are coloured white. Solid horizontal lines and shading behind each group represent the average and standard deviation for all locality records. In a logistic regression model accounting for dependence of records within species, elevation is a significant predictor of the probability of being pink versus white (see Results for details).

Supplemental Tables

Table S1. *Protea* species from the Cape Floristic Region that were classified as predominately bird-pollinated, with insects contributing to the pollination of many species as well. Nectar data came from Nicholson and Van Wyk (1998). Asterisks indicate nectar chemistry mismatches expectations based on pollinator classification. When no additional references were available, pollinator classifications were based primarily on Protea Atlas data.

Species	Colour polymorphic	% sucrose in nectar (relative to hexoses)	Mean elevation (range) in meters	Additional (pollination-related) references
P. aristata	No	Low	991 (661-1474)	-
P. aurea ssp. *aurea*	Yes	Low	825 (223-1615)	Calf et al. 2003, Carlson and Holsinger 2013
P. burchellii	Yes	High*	330 (48-1212)	Calf et al. 2003
P. compacta	No	Low	188 (2-625)	Calf et al. 2003, Carlson unpub.
P. coronata	No	High*	410 (16-1185)	Wiens et al. 1983, Rebelo et al. 1984, Calf et al. 2003
P. cynaroides[a]	Yes	Low	642 (0-1742)	Rebelo et al. 1984, Wright et al. 1991, Calf et al. 2003, Steenhuisen 2012
P. eximia	No	High*	1094 (76-1981)	Calf et al. 2003
P. glabra	Yes	Low	795 (140-1463)	-
P. grandiceps	No	High*	1131 (190-1811)	Calf et al. 2003
P. holosericea	No	-	1302 (1058-1597)	-
P. inopina	No	-	593 (430-697)	-
P. lacticolor	Yes	Low	1014 (590-1444)	Rebelo et al. 1984, Calf et al. 2003
P. lanceolata	No	Low	176 (33-459)	-
P. laurifolia[a,b]	Yes	-	797 (25-1853)	Wiens et al. 1983, Wright et al. 1991, Wright 1994, Calf et al. 2003, Steenhuisen 2012
P. lepidocarpodendron	Yes	Low	316 (0-901)	-
P. longifolia	Yes	Mid and High*	293 (15-1349)	Calf et al. 2003, Carlson unpub.
P. lorea[c]	No	-	456 (101-778)	Wiens et al. 1983
P. lorifolia	Yes	Low	1068 (126-1959)	-
P. magnifica[d]	Yes	Low	1316 (259-1957)	Wiens et al. 1983, Wright et al. 1991, Steenhuisen 2012
P. mundii	Yes	Low	660 (59-1685)	Calf et al. 2003
P. neriifolia[a]	Yes	Low	626 (0-1636)	Cowling and Mitchell 1981, Rebelo et al. 1984, Wright et al. 1991, Calf et al. 2003
P. nitida[d]	Yes	Low	651 (0-1933)	Wiens et al. 1983, Rebelo et al. 1984, Wright et al. 1991, Steenhuisen 2012
P. obtusifolia[e]	Yes	Mid*	103 (0-444)	Carlson unpub.
p. punctata	Yes	High*	1438 (424-2023)	Carlson and Holsinger 2010, Steenhuisen 2012
P. repens[f]	Yes	Low	724 (0-1933)	Mostert 1980, Cowling and Mitchell 1981, Wiens et al. 1983, Coetzee and Giliomee 1985, Calf et al. 2003, Steenhuisen 2012, Carlson unpub.
P. rupicola	No	-	1605 (869-2023)	-
P. scorzonerifolia[c]	Yes	-	556 (128-1267)	Wiens et al. 1983
P. speciosa	Yes	Low	594 (0-1387)	Rebelo et al. 1984
P. stokoei	No	Low	843 (491-1381)	-
P. susannae	No	Mid*	128 (0-323)	Carlson unpublished
P. venusta[g]	No	Low and Mid*	1726 (1293-2012)	Rourke 1980, Rebelo 2013

[a] insects alone pollinated as well as birds and insects, measured in terms of seed set (Wright et al. 1991); [b] inflorescences from which birds were excluded produced lighter seeds with slower rates of germination than those that were open to both birds and insects (Wright 1994); [c] floral morphology and plant habit suggest mammal pollination is unlikely (Wiens et al. 1983); [d] insects alone did not pollinate as well as birds and insects, measured in terms of seed set (Wright et al. 1991); [e] a striped mouse and another unidentified rodent have been observed visiting inflorescences (Carlson unpublished data); [f] insects alone pollinated as well as birds and insects, measured in terms of seed set (Coetzee and Giliomee 1985); [g] morphology and scent suggest mammal pollination (Rourke 1980), but Rebelo (2013) classifies it as bird pollinated.

Table S2. *Protea* species from the Cape Floristic Region that were classified as predominately mammal pollinated, or in two species, wasp pollinated. Percent sucrose in nectar data came from Nicholson and Van Wyk (1998) for all species except *P. tenax* and *P. denticulata*, which came from Cowling and Mitchell (1981). Asterisks indicate that the percent sucrose in floral nectar mis-matches expectations based on pollinator classification. When no additional references were available, classifications were based primarily on the Protea Atlas data.

Species	Colour polymorphic	% sucrose in nectar, relative to hexoses	Additional (pollination-related) references
P. acaulos[a]	No	Mid*	Rourke and Wiens 1977, Rebelo et al. 1984
P. acuminata	No	High	-
P. amplexicaulis	No	High	Rourke and Wiens 1977, Wiens et al. 1983
P. angustata[b]	Yes	-	Rourke and Wiens 1977, Rourke 1980, Wiens et al. 1983, Rebelo unpublished
P. aspera	Yes	High	Rourke and Wiens 1977
P. caespitosa	No	-	Wiens et al. 1983
P. canaliculata	No	High	-
P. convexa	No	-	Wiens et al. 1983
P. cordata	No	-	Rourke and Wiens 1977
P. cryophila[b]	Yes	-	Wiens et al. 1983
P. decurrens	No	-	Rourke and Wiens 1977
P. denticulata[c]	No	Mid*	Cowling and Mitchell 1981, Wiens et al. 1983, Rebelo unpublished
P. effusa	Yes	-	Rourke 1980, Wiens et al. 1983
P. foliosa	Yes	-	Rourke and Wiens 1977
P. humiflora	No	High	Rourke and Wiens 1977, Cowling and Mitchell 1981, Wiens et al. 1983, Fleming and Nicholson 2002
P. intonsa	No	-	Rourke and Wiens 1977
P. laevis	Yes	-	Rourke and Wiens 1977
P. montana	Yes	Low*	Rourke and Wiens 1977
P. mucronifolia[d]	No	-	Rebelo 2001
p. nana	No	High	Wiens et al. 1983, Biccard and Midgley 2009
P. odorata[d]	No	-	Rebelo 2001
P. pendula[e]	Yes	-	Rourke 1980, Wiens et al. 1983
P. piscina	Yes	-	Wiens et al. 1983
p. pityphylla	No	High	Wiens et al. 1983, Biccard and Midgley 2009
P. pruinosa	No	High	-
p. pudens	No	High	Wiens et al. 1983
P. recondita	No	-	Rourke 1980, Wiens et al. 1983
P. restionifolia	Yes	-	Rourke and Wiens 1977
P. revoluta	No	-	Rourke and Wiens 1977
P. scabra	Yes	High	Rourke and Wiens 1977
P. scabriuscula	No	-	-
P. scolopendriifolia	Yes	-	Rourke and Wiens 1977
P. scolymacephala	Yes	-	
P. subulifolia	No	Low*	Rourke and Wiens 1977
P. sulphurea	Yes	High	Rourke and Wiens 1977, Cowling and Mitchell 1981
P. tenax	Yes	High	Rourke 1980, Cowling and Mitchell 1981, Wiens et al. 1983
P. vogtsiae	Yes	-	Rourke and Wiens 1977
p. witzenbergiana	No	-	Wiens et al. 1983, Biccard and Midgley 2009

[a] birds have been observed visiting inflorescences (Rebelo et al. 1984); [b] orange breasted sunbirds have been observed visiting inflorescences (*P. cryophila*: Wiens et al. 1983; *P. angustata*: Rebelo unpublished); [c] morphology and plant habit suggest birds are pollinators (Rebelo unpublished); [d] wasps pollinate these species (Rebelo 2001).
[e] orange breasted sunbirds have been observed visiting inflorescences (Rourke 1980).

Table S3. *Protea* species and populations used in this study. All 'focal' record sources was visited by the authors on the listed dates, with the purpose of assessing the colour class and measuring three plant traits (Flowers/head, mass of one seed and fecundity per head) and seed predation (% of heads infested per site). For four of the focal *P. repens* sites (BAN, BRD, RIV, and CDB), colour class was also assessed on separate visits by local collaborators, reserve managers or landowners. The 28 focal populations were used for the within-species analysis and included 18 *P. repens* (repens), 4 each of *P. aurea* (aur) and *P. punctata* (punc) and two of *P. lacticolor* (lacti). Different sample sizes for the focal variables within sites were the result of some plants lacking viable seeds for seed mass or being too damaged to assess flowers per head or fecundity. For RP, PK, PO2 and GB, up to 20 plants were measured only for fecundity. The 60 remaining populations are Ad hoc observation from one of three record sources: the Protea Colour Survey on ispot.org.za ('ispot'), the Protea Atlas Project on proteaatlas.org.za ('Protea atlas') or as a personal observation by Carlson ('pers. obs'). **This table is provided in a separate excel file called "table S3".**

Table S4. Results of path analysis model testing whether a population's pink floral colour morph frequency is related to its traits, seed predation intensity, or abiotic environment, and whether these variables are inter-related in four *Protea* species. Each population is classified into one of five classes of increasing pink morph frequency (see Methods). The analysis included 18 populations of *P. repens* (R) and 10 populations of *Protea* section *Exsertae* (Ex). Within *Protea* section *Exsertae*, there were 4 populations each of *P. aurea* and *P. punctata*, and 2 of *P. lacticolor*. An asterisk indicates a significant random effect of species on that particular response variable.

Response variable	Covariate	Significant transition or slope at 95% credible interval	95% credible interval	
Pink morph frequency	Elevation	R: Skew white to moderate	-8.565	-0.488
		Ex: Skew white to moderate	-10.449	-0.305
		R: Moderate to skew pink	-9.457	-1.234
	Longitude	R: White to skew white	-8.727	-0.439
		R: Skew white to moderate	-8.403	-1.592
		R: Skew pink to pink	-9.392	-1.380
	Mean annual rainfall	NS		
	Seed predation intensity	Ex: Skew white to moderate	-8.627	-0.134
	Fecundity	NS		
	Flowers per head	R: Skew white to white	1.016	10.347
	Seed mass	NS		
Seed predation intensity	Elevation	NS		
	Longitude	NS		
	Mean annual rainfall	NS		
	Flowers per head	NS		
	Fecundity	NS		
	Seed mass	NS		
Fecundity*	Elevation	R: Positive slope	0.052	0.608
	Longitude	NS		
	Mean annual rainfall	R: Positive slope	0.175	0.863
Flowers per head*	Elevation	R: Positive slope	0.048	0.728
	Longitude	NS		
	Mean annual rainfall	NS		
Seed mass*	Elevation	R: Negative slope	-0.722	-0.116
	Longitude	R: Negative slope	-0.586	-0.118
	Mean annual rainfall	NS		

Supplemental Appendices

APPENDIX S1

This study includes all members of the genus *Protea* that are both bird pollinated and naturally occur within the Cape Floristic Region of South Africa (CFR), as listed in Table S1. To determine whether a given species fit these criteria, we used a wide range of resources. According to Rourke (1980), there are 114 accepted species in the genus *Protea*, and 82 are in South Africa. Rebelo (2001) adds *Protea namaquana*, bringing the global total to 115 (Goldblatt and Manning 2000) and the South African total to 83. Other authors recognize only 110 species, but only because they designated several species as subspecies among the non-South Africa *Protea* (Valente et al. 2010). Supplemental Table S1 in Valente et al. (2010) lists the names and taxonomic treatment of the 110 species they recognize in the genus. If 110-115 species are accepted, 27-32 of these reside strictly outside of South Africa and were not considered for this study.

For the 83 South African species, we used written accounts and published distribution records (Rebelo 2001; Rebelo 2013) to exclude the 14 non-CFR species. These 14 species were *P. caffra, P. comptonii, P. curvata, P. dracomontana, P. gaguedi, P. laetans, P. namaquana, P. nubigena, P. parvula, P. roupelliae, P. rubropilosa, P. simplex, P. subvestita,* and *P. welwitschii*. Only four of these species are not restricted to South Africa; *Protea welwitschii*, *P. caffra, P. dracomontana,* and *P. gaguedi* can also be found beyond South Africa's borders. *Protea subvestita* was previously thought to have populations in and out of the CFR (Rourke 1980; Rebelo 2001), but recent a microsatellite analysis shows the CFR plants to be hybrids between *Protea* section Exsertae species (Prunier and Holsinger 2010; see Valente et al. 2010 for additional discussion).

Once we excluded the 42-47 non-CFR species, we next classified *Protea* species by pollinator types, and we dismissed all species that did not include birds among their most probable pollinators. This eliminated 36 species that are pollinated mainly by non-flying mammals (Table S3), as supported by the work of Rourke and Wiens (1977), Rourke (1980), Wiens et al. (1983), Cowling and Mitchell (1981), Nicolson and Van Wyk (1998), Fleming and Nicholson (2002), and Biccard and Midgley (2009). The two wasp pollinated species were also excluded (Rebelo 2001), and the remaining CFR species were classified as bird pollinated (Table S1). For some of these species, published studies record birds as frequent visitors to inflorescences (Mostert et al. 1980; Rebelo et al. 1984; Calf et al. 2003; Carlson and Holsinger 2013), and for the remainder, we use classifications based on expert opinion and citizen-scientist observations from the Protea Atlas database (www.proteaatlas.org.za).

Bird pollinated *Protea* species, as classified in this study, are unified by a suite of morphological and nectar differences, which differentiate them from mammal pollinated species. Bird-pollinated *Protea* usually produce more hexose-dominant nectars (i.e., a greater proportion of nectar sugars are fructose and glucose relative to sucrose), whereas mammal-pollinated *Protea* tend to produce sucrose-dominant nectars (Nicolson and Van Wyk 1998 and Cowling and Mitchell 1981; Tables S1 and S2). Bird pollinated species also have tend to have broad leaves and upright or (less frequently) sprawling habits, and/or relatively large, upward facing inflorescences lacking a strong odor. Exceptions to this include *P. lorea, P. scorzonerifolia* which have belowground stems, and these two plus *P. aristata* also have needle-like leaves. Mammal pollinated plants often have dark coloured inflorescences that are either pendulous, produced at ground level, and/or hidden within dense foliage. They also tend to produce large quantities of nectar and emit strong 'yeasty' scents (Rourke and Wiens 1977).

Insects also frequent the inflorescences of most bird-pollinated *Protea,* and in some species, they have been shown to contribute to seed set. Relevant insect visitors include bees and many species of beetles (e.g., *Trichostetha*, Scarabaeidae; *Chirodica*, Chrysomelidae; *Phloeonomus*, Staphylinidae; Mostert et al. 1980, Coetzee and Giliomee 1985, Collins and Rebelo 1987, Wright and Giliomee 1990). In the few studies comparing insect versus bird pollination, insects are equally effective pollinators for some *Protea* species but inferior pollinators for others (Coetzee and Giliomee 1985; Wright et al. 1991; Wright 1994; see footnotes in Table S1).

Recent studies outside the CFR find that several *Protea* species rely heavily on self-fertilization and beetle pollination (Steenhuisen and Johnson 2012b; Steenhuisen and Johnson 2012a). Although neither are considered central to reproduction in any CFR *Protea*, they suggest that both possibilities be re-examined within the CFR. To date, most CFR species are considered self-incompatible based on Horn (1962), but Steenhuisen and Johnson (2012a) argue that his methodology may have been flawed.

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APPENDIX S2: Assessing the impact of categorical frequency classification

From first principles it seems likely that we substantially reduce our ability to detect associations that are present by reducing a continuous variable, pink frequency, to only five categories, particularly when the middle category spans the entire range from 10-90% pink. To confirm this expectation and to explore its consequences for our analysis, we performed two simulation experiments, one that assesses our ability to detect relationships that are present and one that assesses the probability that we declare a relationship to be present when it is not.

Detection probability: In the first experiment we used regression derived from the estimated association between pink frequency and each of the covariates in our full path model to simulate individual-level observations of pink *versus* white morph status across populations with the same configuration of covariates as ours. Specifically, we assume that pink frequency in each population is given by

$$\text{logit}(p_i^{(sp)}) = \beta_0^{(sp)} + \beta_{elev}\text{elev}_i + \beta_{long}\text{long}_i + \beta_{MAP}\text{MAP}_i + \beta_{pred}\text{pred}_i + \beta_{fec}\text{fec}_i + \beta_{flno}\text{flno}_i + \beta_{seed}\text{seed}_i \quad (S2.1),$$

where $p_i^{(sp)}$ is the frequency of pink in a population of species *(sp)* at location *i*, $\beta_0^{(sp)}$ is the species random effect, and the remaining terms are the regression coefficients and covariates measured at location *i*. We calculated the regression coefficients as the average of the 4 coefficients associated with each covariate in the path analysis, with separate coefficients for *Protea repens* and members of *Protea* section *Exsertae*. We constructed a population sample using this frequency by assuming the observed number of pink morphs at a particular location was a binomial sample of size 20 from a population with the calculated frequency. We repeated this process 1000 times to produce 1000 simulated data sets.

     We then performed two sets of analyses on each of these simulated data sets: one in which we estimated the association between each of the covariates and pink frequency using an ordered categorical distribution identical to the one used in our full path analysis and one in which we estimated the associations using a logistic regression and the observed counts of pink and white morphs in each population as the dependent variable. Categories for the categorical analysis were monomorphic white (0 pink in the sample), skewed white (1 pink), moderate frequency (2 – 18 pink), skewed pink (19 pink), and monomorphic pink (20 pink). Each regression was performed using JAGS with the same priors, burnin, and sample as in our path analysis. For each regression we recorded whether or not the 95% credible interval for each coefficient included zero. In Table S2.1 we report the number of times each coefficient was detectably different from 0. Notice that with only three exceptions (fecundity in *Exsertae* and seed mass in both taxa) did the categorical analysis detect a relationship more frequently than the logistic analysis, and it detected the relationship more than 50% of the time in only one of those cases (53% for seed mass in *Protea repens*). In general, the categorical analysis had less power to detect relationships than the logistic analysis.

Table S2.1: Detecting associations in simulated data. The number in each cell is the number of replicates (out of 1000) in which the 95% credible interval for the specified coefficient did not overlap 0 in each analysis.

Coefficient	Analysis	
	Categorical	**Logistic**
Elevation (*Exsertae*)	938	995
Elevation (*repens*)	973	1000
Fecundity (*Exsertae*)	97	0
Fecundity (*repens*)	365	738
Flowers per head (*Exsertae*)	0	0
Flowers per head (*repens*)	365	738
Longitude (*Exsertae*)	965	1000
Longitude (*repens*)	965	1000
Predation intensity (*Exsertae*)	121	966
Predation intensity (*repens*)	34	328
Mean annual rainfall (*Exsertae*)	33	41
Mean annual rainfall (*repens*)	247	588
Mean seed mass (*Exsertae*)	1	0
Mean seed mass (*repens*)	530	432

False positives: In the second experiment we explored how likely each method would be to falsely declare that an association was present. We used the same simulation framework as described in the first experiments, simply setting the species random effects and all regression coefficients to 0. The results are summarized in Table S2.2. The logistic analysis had an estimated false positive rate of less than 6% except for the coefficient of longitude in *Protea repens* (6.2%). The categorical analysis had an estimated false positive rate of 0% for all coefficients except mean annual rainfall in section *Exsertae* with an upper 95% confidence bound of 0.3%. In section *Exsertae* the estimated false positive rate in the categorical analysis was 0.1%.

Table S2.2: False positives in simulated data. The number in each cell is the number of replicates (out of 1000) in which the 95% credible interval for the specified coefficient did not overlap 0 in each analysis.

Coefficient	Analysis	
	Categorical	**Logistic**
Elevation (*Exsertae*)	0	4
Elevation (*repens*)	0	51
Fecundity (*Exsertae*)	0	0
Fecundity (*repens*)	0	57
Flowers per head (*Exsertae*)	0	9
Flowers per head (*repens*)	0	58
Longitude (*Exsertae*)	0	0
Longitude (*repens*)	0	62
Predation intensity (*Exsertae*)	0	19
Predation intensity (*repens*)	0	58
Mean annual rainfall (*Exsertae*)	0	12
Mean annual rainfall (*repens*)	1	52
Mean seed mass (*Exsertae*)	0	3
Mean seed mass (*repens*)	0	55

Conclusion: The categorical analysis may fail to identify some associations that are present in the data, but it is very unlikely to suggest that an association exists when it is not present. The logistic analysis is more likely to identify associations that are present in the data, at the cost of a false positive rate that is higher than that of the categorical analysis (but still approximately consistent with expectations).

APPENDIX S3: Creation and interpretation of figure 3: cumulative probability of color morph frequency class as predicted by abiotic and biotic variables

The structure of our within-species path analysis is shown in Figure 2, and the focal results of that analysis are summarized in Figure 3a-d. Specifically, Figure 3 depicts the probability that a population falls into one of the five pink frequency classes as a function of the three abiotic predictors, three traits and seed predation intensity. Giving the complexity of these four figure panels, additional methodological and interpretation details are provided here. Using figure 3*(a)* as an example, consider the relationship between elevation and frequency of pink. For each species in that figure, we have samples from particular sites with elevations indicated by tick-marks below each panel. To plot the relationship between elevation and pink frequency for a species, we first take N vertical lines evenly spaced across the entire elevation gradient. For a given vertical line, the proportion of that line falling within each category (monomorphic white, skewed white, moderate, skewed pink, and monomorphic pink) represents the mean posterior probability of a population at that elevation belonging to that category. Low elevation populations in *P. repens*, for example, have only an undectably small posterior probability of being skewed pink or monomorphic pink. In contrast, high elevation populations are almost certain to be skewed pink or monomorphic pink. In other words, the visual impression of darker pink colours expanding from low elevations to high elevations corresponds to an increasing posterior probability of pinker categories at high elevations, which we summarize as "the frequency of pink morphs increases with elevation." Although all seven covariates could be plotted in this way, we only display the significant ones.

Because the regression relationships are highly non-linear, the predicted relationships depend not only on the regression coefficient for the covariate in question, but also on the value of other covariates and their coefficients (Gelman and Rubin 1992). Thus, we average the predictions across observed values of the other covariates. If x_i are the covariates other than elevation at site *i,* and e_n is the elevation at point *n* in the gradient, then $P_k(x_i, e_n)$ is the predicted boundary between frequency category *k* and frequency category *k+1* at elevation *n*, given the covariates at site *i*. We plot the average of $P_k(x_i, e_n)$ at each value of *n* to illustrate the influence of elevation on pink frequency. The plots for *P. aurea*, *P. lacticolor*, and *P. punctata* differ from one another even though the regression coefficients are the same because they have different intercepts and different observed covariate values.

References cited

Gelman, A. & Rubin, D.B. 1992 Inference from iterative simulation using multiple sequences. *Statistical Science* **7**, 457-472.