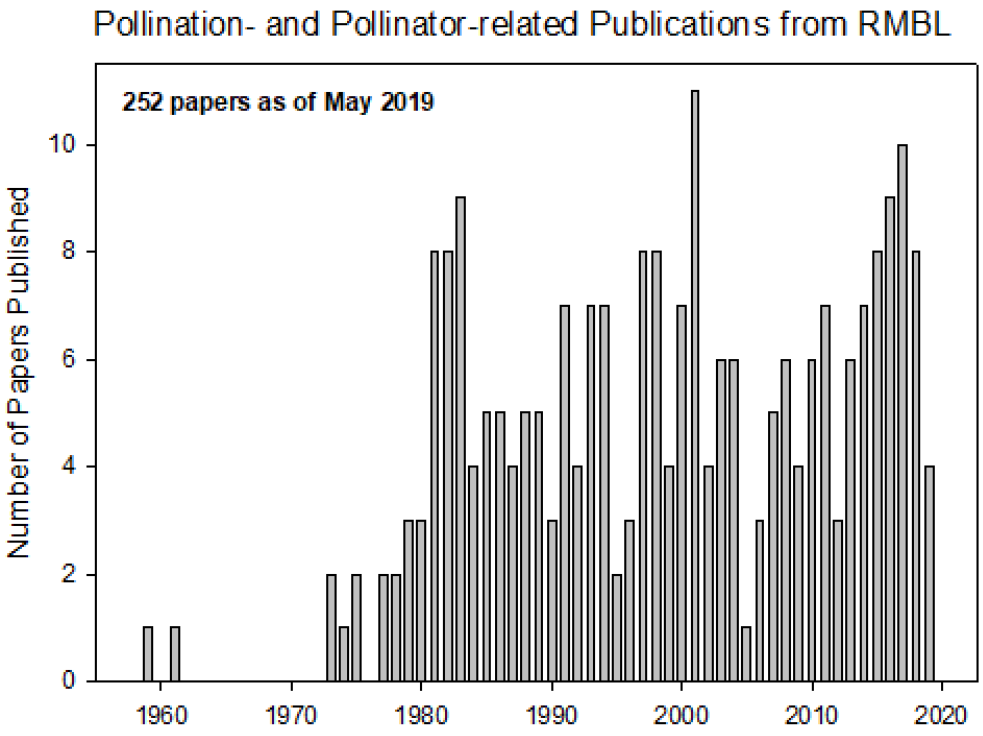
This is a list of pollination-related papers published from work at RMBL. This is exported from an EndNote file generated and maintained by David Inouye. Contact him (inouye@umd.edu) for the EndNote (or other format) file, or updates. This is current as of May 2019.



Alarcón, R. and D. R. Campbell (2000). "Absence of conspecific pollen advantage in the dynamics of an *Ipomopsis* (Polemoniaceae) hybrid zone." American Journal of Botany **87**(6): 819-824.

The frequency of hybrid formation in angiosperms depends on how often heterospecific pollen is transferred to the stigma and on the success of that heterospecific pollen at fertilizing ovules. Even if heterospecific pollen is capable of effecting fertilization it may perform poorly when conspecific pollen is also available on the stigma. We applied pollen mixtures to stigmas to determine how pollen interactions affect siring success and the frequency of hybrid formation between two species of Ipomopsis (Polemoniaceae) in Colorado. Plants of both parental species and natural hybrids were pollinated with I. aggregata and I. tenuituba pollen in ratios of 100:0, 80:20, 50:50, 20:80, and 0:100 by mass. Plants were homozygous for different alleles at an isozyme marker, allowing us to distinguish the type of pollen parent for 2166 viable seeds from 273 fruits. In contrast to studies of many other hybridizing taxa, there was no evidence of an advantage to conspecific pollen, nor did composition of the stigmatic pollen load affect seed set. Instead, the frequency of seeds sired by a given species was proportional to its representation in the pollen load. In this hybrid zone, both the frequency of first-generation hybrid formation and the relative male fitness of the two parental species should be predictable from the rates of pollen transfer to stigmas.

Aldridge, G. and D. R. Campbell (2006). "Asymmetrical pollen success in *Ipomopsis* (Polemoniaceae) contact sites." Am. J. Bot. **93**(6): 903-909.

Variation in hybridization rates among contact sites of a species pair provides an opportunity for assessing the importance of individual reproductive isolating mechanisms in limiting gene flow between species and thus promoting speciation. Conspecific pollen advantage is common in angiosperms, but its importance as a reproductive isolating mechanism is uncertain. We compared the strength of conspecific pollen advantage in two Ipomopsis aggregata-I. tenuituba (Polemoniaceae) contact sites that differ in frequency of natural

hybrids. We performed hand pollinations of single- and 1:1 mixed-species pollen loads, using donor and recipient plants from both contact sites. Paternity of offspring from mixed-species pollinations was determined using an allozyme marker. Donors from the high frequency hybrid site showed no conspecific pollen advantage; both species sired seeds in proportion to their fraction of the pollen load (0.5). In contrast, I. aggregata from the low frequency hybrid site sired 70-85% of offspring on recipients from both sites. These results suggest that pollen interactions can influence the level of natural hybridization. They also suggest the importance of geographic variation in reproductive isolation, which should be considered in studies of biological invasions and exposure of engineered crops to wild relatives.

Aldridge, G. and D. R. Campbell (2007). "Variation in pollinator preference between two *Ipomopsis* contact sites that differ in hybridization rate." Evolution **61**(1): 99-110.

Pollinator-mediated reproductive isolation is often a principal factor in determining the rate of hybridization between plant species. Pollinator preference and constancy can reduce interspecific pollen transfer between otherwise interfertile, coflowering species. The importance of this ethological isolation can be assessed by comparing the strength of preference and constancy of pollinators in contact sites that differ in the frequency of hybrid individuals. We observed visitation by hummingbirds and hawkmoths in natural single-species patches and artificial mixed-species arrays in two Ipomopsis aggregata/I. tenuituba contact sites—one with few hybrids, and one in which hybrids are abundant. Pollinator preference and constancy were stronger at the low-frequency hybrid site, especially for hawkmoths (Hyles lineata). Hawkmoths at the low-frequency hybrid site showed significant preference and constancy for I. tenuituba, while at the high-frequency site hawkmoths visited both species equally. One hypothesis that might explain these differences in hawkmoth foraging is that warmer nights at the low-frequency hybrid site allow for nocturnal foraging where the light-colored corollas of I. tenuituba have a visibility advantage. These differences in hawkmoth behavior might in turn affect hummingbirds differently at the two sites, through changes in nectar resources, leading to greater pollinator-mediated isolation at the low-frequency hybrid site. Our results suggest that differences in pollinator behaviors between sites can have both direct and indirect effects on hybridization rates between plant species.

Aldridge, G., et al. (2011). "Emergence of a mid-season period of low floral resources in a montane meadow ecosystem associated with climate change." Journal of Ecology **99**(4): 905-913.

1.Shifts in the spatial and temporal patterns of flowering could affect the resources available to pollinators, and such shifts might become more common as climate change progresses.

2.As mid-summer temperatures have warmed, we found that a montane meadow ecosystem in the southern Rocky Mountains of the United States exhibits a trend toward a bimodal distribution of flower abundance, characterized by a mid-season reduction in total flower number, instead of a broad, unimodal flowering peak lasting most of the summer season.

3.We examined the shapes of community-level flowering curves in this system and found that the typical unimodal peak results from a pattern of complementary peaks in flowering among three distinct meadow types (dry, mesic and wet) within the larger ecosystem. However, high mid-summer temperatures were associated with divergent shifts in the flowering curves of these individual meadow types. Specifically, warmer summers appeared to cause increasing bimodality in mesic habitats, and a longer interval between early and late flowering peaks in wet and dry habitats.

4.Together, these habitat-specific shifts produced a longer mid-season valley in floral abundance across the larger ecosystem in warmer years. Because of these warming-induced changes in flowering patterns, and the significant increase in summer temperatures in our study area, there has been a trend toward non-normality of flowering curves over the period 1974–2009. This trend reflects increasing bimodality in total community-wide flowering.

5.The resulting longer periods of low flowering abundance in the middle of the summer season could negatively affect pollinators that are active throughout the season, and shifts in flowering peaks within habitats might create mismatches between floral resources and demand by pollinators with limited foraging ranges.

6.Synthesis. Early-season climate conditions are getting warmer and drier in the high altitudes of the southern Rocky Mountains. We present evidence that this climate change is disrupting flowering phenology within and among different moisture habitats in a sub-alpine meadow ecosystem, causing a mid-season decline in floral resources that might negatively affect mutualists, especially pollinators. Our findings suggest that climate

change can have complex effects on phenology at small spatial scales, depending on patch-level habitat differences.

Beattie, A. J., et al. (1973). "The ecology of the pollinators and predators of *Frasera speciosa*." Ecology **54**(1): 81-91.
Frasera speciosa is a conspicuous perennial in the Rocky Mountains where it forms discrete colonies whose inflorescences are characterized by almost total absence in some years and greater abundance in others. This sporadic yet synchronous flowering was the most conspicuous feature of the reproductive biology of the species and its adaptive value was worth investigation. In a three—mile stretch of the East River Valley, Colorado, the following data were gathered over a period of three years: the distribution and abundance of colonies, the floral biology, the dispersal of insect visitors among the flowers of Frasera and its floral associates, the frequency of floral predation and seed—set. Although the percentage of plants with inflorescences was always very low and despite the occasional wide spatial separation of individuals, Frasera invariably attracted the greatest number and diversity of floral visitors and never yielded less than 52% seed—set. It was also shown that pollination was affected by a wide variety of inset visitors that maintained cross—pollination at a frequency of approximately 15%. In no colonies was floral predation nearly as heavy as in its sympatric associate Lupinus and it was concluded that seed—set Frasera was not significantly affected by predispersal herbivory. The local synchrony revealed by the occurrence of discrete colonies and the overall synchrony revealed in the almost total absence of floral colonies in some years and their abundance in others is viewed as a strategy for predator avoidance. These mechanisms, together with other aspects of the reproductive biology, also reduce pressure from sympatric species competing for pollinators. It is out that they confer reproductive advantage to a minority species which may otherwise rapidly decline to extinction in the presence of intense competition. The flowering regime of Frasera combines a predator avoidance system which yields widely dispersed colonies in space and in time with a pollination system which successfully exploits the maximum diversity of floral visitors and maintains excellent seed—set whenever and wherever the colonies appear. The systems are clearly complementary in preventing the build—up of predator populations while maintaining an attractive forage source for potential pollen vectors. The combined effect is to maintain the abundance of the species in a variety of stress environments, in turn resulting in a remarkably wide geographic success. It is considered likely that similar systems will turn out to be very common among entomophilous plants in both temperate and tropical regions.

Bischoff, M., et al. (2014). "Floral scent in natural hybrids of *Ipomopsis* (Polemoniaceae) and their parental species." Annals of Botany **113**(3): 533-544.
Background and Aims Floral traits, such as floral volatiles, can contribute to pre-zygotic reproductive isolation by promoting species-specific pollinator foraging. When hybrid zones form, floral traits could also influence post-zygotic isolation. This study examined floral volatiles in parental species and natural hybrids in order to explore potential scent mediation of pre-zygotic and post-zygotic isolation.

Methods Floral bouquets were analysed for the sister species Ipomopsis aggregata and I. tenuituba and their natural hybrids at two contact sites differing in both hybridization rate and temporal foraging pattern of hawkmoth pollinators. Floral volatiles were quantified in diurnal and nocturnal scent samples using gas chromatography–mass spectrometry.

Key Results The bouquets of parental species and hybrids showed qualitative overlap. All flowers emitted similar sets of monoterpenoid, sesquiterpenoid, aliphatic and benzenoid compounds, but separated into groups defined by multivariate analysis of quantitative emissions. The parental species differed most strikingly in the nitrogenous compound indole, which was found almost exclusively in nocturnal bouquets of I. tenuituba. Natural hybrid bouquets were highly variable, and showed emission rates of several compounds that appeared transgressive. However, indole emission rates were intermediate in the hybrids compared with rates in the parents. Volatile bouquets at the contact site with lower hybridization did not show greater species specificity in overall scent emission, but I. tenuituba presented a stronger indole signal during peak hawkmoth activity at that site.

Conclusions The two species of Ipomopsis differed in patterns of floral bouquets, with indole emitted in nocturnal I. tenuituba, but not in I. aggregata. Natural hybrid bouquets were not consistently intermediate between the

parents, although hybrids were intermediate in indole emission. The indole signal could potentially serve as a hawkmoth attractant that mediates reproductive isolation both before and after hybrid formation.

Bischoff, M., et al. (2015). "Context-dependent reproductive isolation mediated by floral scent and color." Evolution **69**(1): 1-13.

Reproductive isolation due to pollinator behavior is considered a key mode of speciation in flowering plants. Although floral scent is thought to mediate pollinator behavior, little is known about its effects on pollinator attraction and floral visitation in the wild. We used field experiments with wild hawkmoths and laboratory experiments with naïve hawkmoths to investigate attraction to and probing of flowers in response to indole, a volatile emitted by Ipomopsis tenuituba but not its close relative I. aggregata, both alone and in combination with floral color differences. We demonstrated that indole attracts wild hawkmoths to flowers, but has little effect on the rate at which those attracted moths probe flowers. In contrast, white flower color did not influence hawkmoth attraction in the field, but caused more attracted moths to probe flowers. Thus, the moths require both scent and high visual contrast, in that order, to feed at flowers at dusk. Their preference for indole-scented flowers is innate, but species-specific preference is mitigated by previous experience and plant spatial patterning. This context-dependent behavior helps explain why these Ipomopsis species show geographical variation in the extent of hybridization and may potentially explain formation of hybrid bridges in other systems of hawkmoth-pollinated plants.

Boggs, C. L. (1997). "Dynamics of reproductive allocation from juvenile and adult feeding: radiotracer studies." Ecology **78**(1): 192-202.

Nutrients used in reproduction may come from adult feeding or reserves stored from the juvenile stage. The dynamics of allocation from these sources are predicted to differ among nutrient types, depending on the relative availability of each nutrient type from adult and juvenile feeding. Using radiotracer techniques, I examined reproductive allocation of glucose and amino acids from adult and juvenile sources in two nymphalid butterflies, Euphydryas editha and Speyeria mormonia. The species used were intermediate in expected importance of adult nutrients to egg production, with abundant carbohydrates but few nitrogenous compounds available from the adult diet. As predicted, for compounds abundantly available in the adult diet, incoming nutrients were used in preference to stored nutrients. For compounds present in low amounts in the adult diet, juvenile reserves were used throughout adult life, although adult sources were used if available. Nutrients received by the female from the male at mating, although expected to be treated as stored reserves, were immediately used in egg production. Thus, restriction of adult or juvenile feeding may cause different nutrient types (e.g., carbohydrates, nitrogenous compounds) to become limiting to reproduction. These results are consistent with earlier allocation studies examining age-specific changes in body mass and reproductive effort, and the effects on fecundity of quantitative adult food reduction. The work has implications for understanding the evolution of nutrient types donated by males to females, the effects of a holometabolous lifestyle on age-specific fecundity, and the effects of using stored reserves vs. income in reproduction. The present results allow further predictions concerning effects of food supply perturbation on fecundity and, hence, population dynamics, and suggest ways in which species and individuals will differ in sensitivity to those perturbations.

Boggs, C. L. (1997). "Reproductive allocation from reserves and income in butterfly species with differing adult diets." Ecology **78**(1): 181-191.

Allocation of stored and incoming nutrients to reproduction determines an organism's age-specific fecundity curve. In holometabolous insects, differences among species in the shape of the curve are correlated with differences in the potential importance of adult food to reproduction. I examined allocation patterns underlying this association. Specific changes throughout life in body mass and reproductive effort were predicted to result from use of stored vs. incoming nutrients for reproduction and other metabolic needs at each age. Data for three nymphalid butterfly species, Euphydryas editha, Speyeria mormonia, and Heliconius charitonius, were compared with the predictions. These three species differ in adult diet and fraction of oocytes mature at adult emergence (hence, potential for adult nutrients to be used to make eggs), with E. editha showing the least potential for use of adult nutrients in egg production and H. charitonius showing the greatest potential. For all three species, body mass declined with age, although nonlinearly for E. editha. This indicated that metabolic expenditures were greater than intake at all ages, and that a constant fraction

of stored nutrients was allocated to reproduction and other metabolic uses at each age for E. editha. Reproductive effort also declined with age for all three species. The specific patterns seen suggested that incoming nutrients may be stored, to some extent, early in life and then used late in life by both S. mormonia and H. charitonius. The similarity between S. mormonia and H. charitonius is rather surprising, given the qualitative differences in adult diet and suggests either that qualitative age-specific allocation patterns for incoming vs. stored nutrients may be independent of adult diet quality, or that the observed patterns are constrained by phylogenetic relatedness of these two species.

Boggs, C. L. and D. W. Inouye (2000). "Weather-driven resource availability controls butterfly population dynamics." Abstracts of the 85th Annual Meeting, Ecological Society of America.

Boggs, C. L. and D. W. Inouye (2012). "A single climate driver has direct and indirect effects on insect population dynamics." Ecology Letters **15**(5): 502-508.

Weather drives population dynamics directly, through effects on vital rates, or indirectly, through effects on the population's competitors, predators or prey and thence on vital rates. Indirect effects may include non-additive interactions with density dependence. Detection of climate drivers is critical to predicting climate change effects, but identification of potential drivers may depend on knowing the underlying mechanisms. For the butterfly Speyeria mormonia, one climate driver, snow melt date, has multiple effects on population growth. Snow melt date in year t has density-dependent indirect effects. Through frost effects, early snow melt decreases floral resources, thence per-capita nectar availability, which determines fecundity in the lab. Snow melt date in year t + 1 has density-independent direct effects. These effects explain 84% of the variation in population growth rate. One climate parameter thus has multiple effects on the dynamics of a species with non-overlapping generations, with one effect not detectable without understanding the underlying mechanism.

Boggs, C. L. and K. Niitepold (2014). "Insights from stable isotopic tracers on reproductive allocation under stress." Integr Comp Biol **54**(5): 880-889.

Fecundity is affected by changes in the nutritional and energetic environment, as a result of changes in acquisition, assimilation, or allocation of macro-nutrients and micro-nutrients. Stable isotopes of carbon and nitrogen offer a window into the processes underlying these changes. In insects that feed on nectar as adults, carbon isotopes can be used to trace allocation of carbon to eggs from larval (capital) and adult (income) sources. If adults are fed sugar-water, there is no source of nitrogen from the adult diet. Thus, nitrogen isotopes in eggs reflect fractionation of larval nitrogen due to protein catabolism and anabolism. We subjected adult females of two butterfly species, Speyeria mormonia and Colias eurytheme, to dietary restriction (DR), larval female S. mormonia to DR, and adult female S. mormonia to extra flight. Females subjected to extra flight were previously found to eat more as adults and to have a higher resting metabolic rate. As predicted, significantly less carbon obtained by feeding as adults was incorporated into eggs in both species under DR when adult. Speyeria mormonia eggs contained significantly more carbon derived from adult feeding under DR as larvae and when subjected to extra flight as adult females. Again as predicted, eggs from females of both species subjected to DR when adults were enriched for (15)N, suggesting that increased protein catabolism or anabolism generated additional carbon compounds. Speyeria mormonia eggs from females subjected to DR when larvae or to additional flight as adults were depleted for (15)N. The result for DR of larvae suggests minimization of protein catabolism when protein reserves are relatively scarce. The results for flight were not as predicted, and deserve further exploration. In most cases, isotopic signature in eggs changed with females' age. Eggs were progressively more enriched for the carbon signature of adults, consistent with a two-compartment mixing model for the carbon sources of larvae and adults. Eggs laid across the life of a female were progressively depleted for (15)N, followed by stabilization. This could be due to high total investment in eggs early in life, as the results are consistent with those for other growing animals. Overall, these results indicate shifts in allocation of incoming and stored (capital) carbon in response to various environmental stresses. The results for nitrogen suggest hypotheses to be tested concerning nitrogen metabolism under environmental stress.

Boggs, C. L. and C. L. Ross (1993). "The effect of adult food limitation on life history traits in *Speyeria mormonia* (Lepidoptera: Nymphalidae)." Ecology **74**(2): 433-441.

Variation in food availability is likely to occur in the wild, and may affect resource allocation to various life history traits. Quantitative adult diet restriction had no effect on life—span or mean individual egg mass, but reduced fecundity in the butterfly Speyeria mormonia. The sum of fecundity plus unlaid oocytes remaining in the ovaries at death declined in direct proportion to the reduction in the adult diet. This indicates that oocytes were resorbed and resources re—allocated away from reproduction under resource stress, since the sum of laid and unlaid eggs for butterflies fed ad libitum did not differ from the number of oocytes present in the ovaries at eclosion. In this nectivorous species, then, life—span is conserved at the expense of reproduction under adult resource stress. Further, for butterflies fed ad libitum, the volume of honey—water imbibed declined with age for both sexes. Daily volume imbided by females fed ad libitum was directly correlated with daily egg production and life—span, suggesting that factors as yet unexplored may be affecting both resources intake and life history traits when resources are available ad libitum.

Bosch, M. and N. M. Waser (1999). "Effects of local density on pollination and reproduction in *Delphinium nuttallianum* and *Aconitum columbianum* (Ranunculaceae)." American Journal of Botany **86**(6): 871-879.

Plant populations vary in density both naturally and as a consequence of anthropogenic impacts. Density in turn can influence pollination by animals. For example, plants in dense populations might enjoy more frequent visitation if pollinators forage most efficiently in such populations. We explored effects of plant density on pollination and seed set in the larkspur Delphinium nuttallianum and monkshood Aconitum columbianum. At our site in the Colorado Rocky Mountains, flowers of D. nuttallianum are pollinated primarily by queen bumble bees, solitary bees, and hummingbirds, whereas those of A. columbianum are pollinated primarily by queen and worker bumble bees. We found that the quantity of pollination service to both species (pollinator visitation rate and pollen deposition) was at best weakly related to density. In contrast, seed set declined by approximately one-third in sparse populations relative to nearby dense populations. This decline may stem from the receipt of low-quality pollen, for example, inbred pollen. Alternatively, sparsity may indicate poor environmental conditions that lower seed set for reasons unrelated to pollination. Our results demonstrate the value of simultaneously exploring pollinator behavior, pollen receipt, and seed set in attempting to understand how the population context influences plant reproductive success.

Bosch, M. and N. M. Waser (2001). "Experimental manipulation of plant density and its effects on pollination and reproduction of two confamilial montane herbs." Oecologia **126**(1): 76-83.

The density of a plant population is expected to influence reproductive success through changes in the quantity and quality of pollination service, or because both density and reproduction respond to quality of the local environment. We reported previously that seed set in sparse natural populations of Delphinium nuttallianum and Aconitum columbianum was lower than in nearby dense populations, whereas quantity of pollination service was equivalent. To explore the hypotheses that environmental quality or pollination quality are lower in sparse natural populations, leading to lower seed set, we manipulated density using arrays of potted plants. In three replicate experiments with D. nuttallianum, pollinator visitation rate and seed set were indistinguishable in sparse and dense arrays, consistent with the interpretation that environmental quality contributed to the earlier result in natural populations of this species. In three replicates with A. columbianum , visitation rate tended to increase with density, and seed set increased significantly, in contrast to our earlier result. One element of pollination quality, the degree of within-plant selfing, did not vary between sparse and dense arrays. These results highlight the complexity of mechanisms by which population parameters may influence plant reproductive success, and the temporal variation that characterizes pollination service.

Briggs, H. M., et al. (2016). "Heterospecific pollen deposition in *Delphinium barbeyi*: linking stigmatic pollen loads to reproductive output in the field." Annals of Botany **117**(2): 341-347.

Background and Aims Most pollinators are generalists and therefore are likely to transfer heterospecific pollen among co-flowering plants. Most work on the impacts of heterospecific pollen deposition on plant fecundity has utilized hand-pollination experiments in greenhouse settings, and we continue to know very little about the reproductive effects of heterospecific pollen in field settings.

Methods We explored how patterns of naturally deposited heterospecific pollen relate to the reproductive output of Delphinium barbeyi, a common subalpine perennial herb in the Rocky Mountains (USA). We assessed a wide

range of naturally occurring heterospecific pollen proportions and pollen load sizes, and linked stigmatic pollen deposition directly to seed set in individual carpels in the field.

Key Results We found that heterospecific pollen deposition in D. barbeyi is common, but typically found at low levels across stigmas collected in our sites. Neither conspecific nor heterospecific pollen deposition was related to carpel abortion. By contrast, we saw a significant positive relationship between conspecific pollen amount and viable seed production, as well as a significant negative interaction between the effects of conspecific pollen and heterospecific pollen amount, whereby the effect of conspecific pollen on viable seed production became weaker with greater heterospecific deposition on stigmas.

Conclusions To our knowledge, this is the first demonstration of a relationship between heterospecific pollen and seed production in a field setting. In addition, it is the first report of an interaction between conspecific and heterospecific pollen quantities on seed production. These findings, taken with the results from other studies, suggest that greenhouse hand-pollination studies and field studies should be more tightly integrated in future work to better understand how heterospecific pollen transfer can be detrimental for plant reproduction.

Brody, A. K. (1992). "Oviposition choices by a pre-dispersal seed predator (*Hylemya* sp.). I. Correspondence with hummingbird pollinators, and the role of plant size, density and floral morphology." Oecologia **91**(1): 56-62.

Although the importance of pollinators has most often been examined in the evolution of floral characters, seed predators may also play a role in shaping floral evolution. In this study, I examined the role of interplant distance, plant size, and flower morphology on Ipomopsis aggregata's (Polemoniaceae) attractiveness to a pre-dispersal seed predator, Hylemya sp. (Anthomyiidae) and to hummingbird pollinators. The attractiveness of I. aggregata individuals to Hylemya was nonlinearly related to interplant distance in experimental arrays. Clumped and highly dispersed plants were preyed upon more frequently than those at intermediate distances. I found no relationship between interplant distance and visitation rates by hummingbird pollinators in these experimental arrays. However, in natural populations studied, clumped plants were more frequently approached by hummingbirds than those growing more widely dispersed. Display size was unrelated to visitation by Hylemya on inflorescences I clipped and maintained as "large", "small" and "control". Display size was also unrelated to the total number of visits by hummingbird pollinators to each of these experimental plants, however "large" display plants were more likely to be visited first in any given visitation sequence. Of various morphological measurements, corolla length showed the strongest positive correlation with Hylemya egg presence. To the extent that plant spacing and morphology is correlated with pollinator visits and ultimate seed set, Hylemya could be choosing flowers optimally, and playing a role in the evolution of floral traits.

Brody, A. K. (1997). "Effects of pollinators, herbivores, and seed predators on flowering phenology." Ecology **78**(6): 1624-1631.

The evolution of flowering phenology has most often been examined in light of one set of organisms, namely pollinators. However, the patterns of flowering phenology observed in nature are likely to reflect evolutionary compromises in response to a variety of selective forces. Two of the most important potentially opposing selective forces that could elicit such a compromise are pollinators and pre-dispersal seed predators. Using a case study from my own work on the pollinators and pre-dispersal seed predators of two members of the Polemoniaceae, Polemonium foliosissimum and Ipomopsis aggregata, I show that the outcome of selective pressures is not always predictable by examining one group of organisms or another. In addition, the outcome of separate and combined selective pressures is variable among years. Thus I show that only by considering all organisms that affect the fitness of a plant may we gain a complete understanding of the evolution of floral traits. I argue that we must account for both geographical and temporal variation in assessing the ecological and evolutionary importance of interacting organisms.

Brody, A. K. and R. J. Mitchell (1997). "Effects of experimental manipulation of inflorescence size on pollination and pre-dispersal seed predation in the hummingbird-pollinated plant *Ipomopsis aggregata*." Oecologia **110**(1): 86-93.

Large floral displays should theoretically provide advantages to plants through increased pollinator visitation and resulting fruit and seed set. However empirical tests of the response of pollinators to floral display size have been limited by a lack of direct experimentation, and the results of such studies have been

equivocal. In addition, other selective agents such as pre-dispersal seed predators might modulate effects of floral display on pollination. By artificially altering flower number, we examined the direct effects of floral display in the monocarpic herb, Ipomopsis aggregata (Polemoniaceae), on visitation rates by broad-tailed and rufous hummingbird pollinators, as well destruction of fruits by a pre-dispersal seed predator (Hylemya: Anthomyiidae). In addition, we quantified the ultimate effects of flower number on female reproductive success. Plants with larger floral displays were most likely to be visited first in any given foraging bout ($P < 0.01$). As expected, plants with more flowers received more total flower visits. However, we found no gain in the proportion of flowers visited for many- versus few-flowered plants, or the total number of approaches/hour. In fact, a significantly greater percentage of flowers were visited on few-flowered plants. Plants did not compensate for our reduction in flowers by increasing investment in the number or proportion of flowers that set fruit, the number of seeds/fruit, or seed weight. Pre-dispersal seed predation was greater for many- than for few-flowered plants ($P < 0.001$), but this did not offset the potential fitness gains of producing large displays. Our data support the hypothesis that large floral displays function primarily in long-distance attraction of pollinators, and enhance maternal success.

Brosi, B. J. and H. M. Briggs (2013). "Single pollinator species losses reduce floral fidelity and plant reproductive function." Proceedings of the National Academy of Sciences **110**(32): 13044-13048.

Understanding the functional impacts of pollinator species losses on plant populations is critical given ongoing pollinator declines. Simulation models of pollination networks suggest that plant communities will be resilient to losing many or even most of the pollinator species in an ecosystem. These predictions, however, have not been tested empirically and implicitly assume that pollination efficacy is unaffected by interactions with interspecific competitors. By contrast, ecological theory and data from a wide range of ecosystems show that interspecific competition can drive variation in ecological specialization over short timescales via behavioral or morphological plasticity, although the potential implications of such changes in specialization for ecosystem functioning remain unexplored. We conducted manipulative field experiments in which we temporarily removed single pollinator species from study plots in subalpine meadows, to test the hypothesis that interactions between pollinator species can shape individual species' functional roles via changes in foraging specialization. We show that loss of a single pollinator species reduces floral fidelity (short-term specialization) in the remaining pollinators, with significant implications for ecosystem functioning in terms of reduced plant reproduction, even when potentially effective pollinators remained in the system. Our results suggest that ongoing pollinator declines may have more serious negative implications for plant communities than is currently assumed. More broadly, we show that the individual functional contributions of species can be dynamic and shaped by the community of interspecific competitors, thereby documenting a distinct mechanism for how biodiversity can drive ecosystem functioning, with potential relevance to a wide range of taxa and systems.

Brosi, B. J., et al. (2017). "Experimental species removals impact the architecture of pollination networks." Biology Letters **13**(6).

Mutualistic networks are key for the creation and maintenance of biodiversity, yet are threatened by global environmental change. Most simulation models assume that network structure remains static after species losses, despite theoretical and empirical reasons to expect dynamic responses. We assessed the effects of experimental single bumblebee species removals on the structure of entire flower visitation networks. We hypothesized that network structure would change following processes linking interspecific competition with dietary niche breadth. We found that single pollinator species losses impact pollination network structure: resource complementarity decreased, while resource overlap increased. Despite marginally increased connectance, fewer plant species were visited after species removals. These changes may have negative functional impacts, as complementarity is important for maintaining biodiversity–ecological functioning relationships and visitation of rare plant species is critical for maintaining diverse plant communities.

Brunet, J. and H. R. Sweet (2006). "Impact of insect pollinator group and floral display size on outcrossing rate." Evolution **60**(2): 234-246.

Despite the strong influence of pollination ecology on the evolution of selfing, we have little information on how distinct groups of insect pollinators influence outcrossing rate. However, differences in behavior between pollinator groups could easily influence how each group affects outcrossing rate. We examined the

influence of distinct insect pollinator groups on outcrossing rate in the rocky mountain columbine, Aquilegia coerulea. The impact of population size, plant density, size of floral display, and herkogamy (spatial separation between anthers and stigmas) on outcrossing rate was also considered as these variables were previously found to affect outcrossing rate in some plant species. We quantified correlations between all independent variables and used simple and two-factor regressions to determine direct and indirect impact of each independent variable on outcrossing rate. Outcrossing rate increased significantly with hawkmoth abundance but not with the abundance of any of the other groups of floral visitors, which included bumblebees, solitary bees, syrphid flies, and muscidae. Outcrossing rate was also significantly affected by floral display size and together, hawkmoth abundance and floral display size explained 87% of the variation in outcrossing rate. None of the other independent variables directly affected the outcrossing rate. This is the first report of a significant impact of pollinator type on outcrossing rate. Hawkmoths did not visit fewer flowers per plant relative to other pollinator groups but preferred visiting female-phase flowers first on a plant. Both the behavior of pollinators and floral display size affected outcrossing rate via their impact on the level of geitonogamous (among flower) selfing. Given that geitonogamous selfing is never advantageous, the variation in outcrossing rate and maintenance of mixed mating systems in populations of A. coerulea may not require an adaptive explanation.

Buchanan, A. L. and N. Underwood (2013). "Attracting pollinators and avoiding herbivores: insects influence plant traits within and across years." Oecologia **173**(2): 473-482.

Perennial plants interact with herbivores and pollinators across multiple growing seasons, and thus may respond to herbivores and pollinators both within and across years. Joint effects of herbivores and pollinators influence plant traits, but while some of the potential interactions among herbivory, pollination, plant size, and plant reproductive traits have been well studied, others are poorly understood. This is particularly true for perennial plants where effects of herbivores and pollinators may manifest across years. Here, we describe two experiments addressing the reciprocal interactions of plant traits with herbivore damage and pollination across 2 years using the perennial plant Chamerion angustifolium. We measured (1) plant responses to manipulation of damage and pollination in the year of treatment and the subsequent season, (2) damage and pollination responses to manipulation of plant size and flowering traits in the year of treatment, and (3) plant-mediated indirect interactions between herbivores and pollinators. We found that plant traits had little effect on damage and pollination, but damage and pollination affected plant traits in both the treatment year and the subsequent year. We found evidence of indirect effects between leaf herbivores and pollinators in both directions; indirect effects of pollinators on leaf herbivores have not been previously demonstrated. Our results indicate that pollen receipt results in shorter plants with fewer stems but does not change flower number, while leaf herbivory results in taller plants with fewer flowers. Together, herbivory and pollination may contribute to intermediate plant height and plants with fewer stems and flowers in our system.

Buck, P. and E. Levetin (1985). "Airborne pollen and mold spores in a subalpine environment." Annals of Allergy **55**: 704-801.

A Rocky Mountain subalpine environment was sampled for allergenic pollen and mold spores during three growing seasons (1981-1983) using a Rotorod sampler. Pollen levels were generally low until late in the season while mold spores were present in higher levels throughout the sampling period.

Burkle, L. and R. Irwin (2009). "The effects of nutrient addition on floral characters and pollination in two subalpine plants, *Ipomopsis aggregata* and *Linum lewisii*." Plant Ecology **203**(1): 83-98.

The availability of soil and pollination resources are main determinants of fitness in many flowering plants, but the degree to which each is limiting and how they interact to affect plant fitness is unknown for many species. We performed resource (water and nutrients) and pollination (open and supplemental) treatments on two species of flowering plants, Ipomopsis aggregata and Linum lewisii , that differed in life-history, and we measured how resource addition affected floral characters, pollination, and reproduction (both male and female function). We separated the direct effects of resources versus indirect effects on female function via changes in pollination using a factorial experiment and path analysis. Resource addition affected I. aggregata and L. lewisii differently. Ipomopsis aggregata , a monocarp, responded to fertilization in the year of treatment application, increasing flower production, bloom duration, corolla width, nectar production,

aboveground biomass, and pollen receipt relative to control plants. Fertilization also increased total seed production per plant, and hand-pollination increased seeds per fruit in I. aggregata , indicating some degree of pollen limitation of seed production. In contrast, fertilization had no effect on growth or reproductive output in the year of treatment on L. lewisii , a perennial, except that fertilization lengthened bloom duration. However, delayed effects of fertilization were seen in the year following treatment, with fertilized plants having greater aboveground biomass, seeds per fruit, and seeds per plant than control plants. In both species, there were no effects of resource addition on male function, and the direct effects of fertilization on female function were relatively stronger than the indirect effects via changes in pollination. Although we studied only two plant species, our results suggest that life-history traits may play an important role in determining the reproductive responses of plants to soil nutrient and pollen additions.

Burkle, L. and R. Irwin (2009). "The importance of interannual variation and bottom–up nitrogen enrichment for plant–pollinator networks." Oikos **118**(12): 1816-1829.

Striking changes in food web structure occur with alterations in resource supply. Like predator–prey interactions, many mutualisms are also consumer–resource interactions. However, no studies have explored how the structure of plant–pollinator networks may be affected by nutrient enrichment. For three years, we enriched plots of subalpine plant communities with nitrogen and observed subsequent effects on plant–pollinator network structure. Although nitrogen enrichment affects floral abundance and rates of pollinator visitation, we found no effects of nitrogen enrichment on the core group of generalist plants and pollinators or on plant–pollinator network structure parameters, such as network topology (the identity and frequency of interactions) and the degree of nestedness. However, individual plant and pollinator taxa were packed into the nested networks differently among nitrogen treatments. In particular, pollinators visited different numbers and types of plants in the nested networks, suggesting weak, widespread effects of nitrogen addition on individual taxa. Independent of nitrogen enrichment, there were large interannual differences in network structure and interactions, due to species turnover among years and flexibility in interacting with new partners. These data suggest that the community structure of small-scale mutualistic networks may be relatively robust to short-term bottom–up changes in the resource supply, but sensitive to variation in the opportunistic behavior and turnover of plant and pollinator species among years.

Burkle, L. A. and R. E. Irwin (2010). "Beyond biomass: measuring the effects of community-level nitrogen enrichment on floral traits, pollinator visitation and plant reproduction." Journal of Ecology **98**(3): 705-717.

1. Nitrogen (N) limits primary productivity in many systems and can have dramatic effects on plant-herbivore interactions, but its effects on mutualistic interactions at the community level are not well-understood. The reproduction of many plants depends on both soil N and pollination, and N may affect floral traits, such as flower number or size, which are important for pollinator attraction to plant individuals and communities.
2. Thus, N may influence plant biomass and reproduction directly as well as indirectly via changes in pollination. The degree to which the effects of N enrichment scale from plant individuals to assemblages through emerging community-level changes in species interactions, like pollination, is relatively unknown.
3. For 4 years, we tested how N addition to subalpine plant assemblages in Colorado, USA, affected primary productivity and species diversity, floral traits and plant-pollinator interactions, and components of female and male plant reproduction.
4. At the community level, we found that high-N addition favoured the biomass and seed production of grasses, whereas low-N addition promoted forb growth, flower production and pollinator visitation. However, using a pollen supplementation experiment, we found no evidence that N addition altered patterns of pollen limitation of seed production. Pollinators distributed themselves evenly across floral resources such that per-flower visitation rate did not differ among N treatments. Thus, individual plants did not incur any extra benefit or cost from community-level changes in plant-pollinator interactions that resulted from N enrichment, and the effects of N on forb reproduction were direct.
5. Synthesis. Understanding how mutualistic and antagonistic species interactions influence individual and community responses to abiotic resources may provide insight to the dominant forces structuring communities and is especially important in the context of predicting the effects of environmental change. In this case, the direct effects of N addition on plants were stronger than the indirect effects mediated through plant-pollinator interactions, thus supporting the concept of bottom-up resource limitation controlling plant response.

Burkle, L. A., et al. (2007). "Predicting the effects of nectar robbing on plant reproduction: implications of pollen limitation and plant mating system." American Journal of Botany **94**(12): 1935-1943.

The outcome of species interactions is often difficult to predict, depending on the organisms involved and the ecological context. Nectar robbers remove nectar from flowers, often without providing pollination service, and their effects on plant reproduction vary in strength and direction. In two case studies and a meta-analysis, we tested the importance of pollen limitation and plant mating system in predicting the impacts of nectar robbing on female plant reproduction. We predicted that nectar robbing would have the strongest effects on species requiring pollinators to set seed and pollen limited for seed production. Our predictions were partially supported. In the first study, natural nectar robbing was associated with lower seed production in Delphinium nuttallianum, a self-compatible but non-autogamously selfing, pollen-limited perennial, and experimental nectar robbing reduced seed set relative to unrobbed plants. The second study involved Linaria vulgaris, a self-incompatible perennial that is generally not pollen limited. Natural levels of nectar robbing generally had little effect on estimates of female reproduction in L. vulgaris, while experimental nectar robbing reduced seed set per fruit but not percentage of fruit set. A meta-analysis revealed that nectar robbing had strong negative effects on pollen-limited and self-incompatible plants, as predicted. Our results suggest that pollination biology and plant mating system must be considered to understand and predict the ecological outcome of both mutualistic and antagonistic plantanimal interactions.

Calder, W. A. (1973). "The timing of maternal behavior of the Broad-tailed hummingbird preceding nest failure." Wilson Bulletin **85**: 283-290.

attentiveness records and other behaviors from early and late nest failures around RMBL in 1972

The temporal behavior of female Broad-tailed Hummingbirds is described for the period preceding natural abandonment of nests. Nesting failure can be considered in two classes: early and late failures. The early failures were due to infertile eggs and the death of a chick. Attentiveness persisted four days beyond normal incubation period and at least one day after the chick was dead. In the latter nest, abandonment was preceded by a lengthening of the recess periods. Of the late failures, three hens abandoned suddenly, while the other two exhibited a series of lengthened recesses. One of the latter two became hypothermic for a portion of the two nights preceding abandonment. The lengthened recesses are thought related to the declining food supply. Live chicks remained in both of those and one of the suddenly-abandoned nests.

Calder, W. A., III (1981). "Heat exchange of nesting hummingbirds in the Rocky Mountains." National Geographic Society Research Reports **13**: 145-169.

Calder, W. A., III and S. Hiebert (1982). "Some energetic aspects of behavior in a montane hummingbird nesting habitat." National Geographic Society Research Reports **14**: 89-94.

Calder, W. A., et al. (1983). "Site-fidelity, longevity, and population dynamics of Broad-tailed hummingbirds: A ten-year study." Oecologia **56**: 359-364.

In the course of other studies we have amassed a decade of records from banding, and observing the nests of, a breeding population of broad-tailed hummingbirds in Colorado, USA. In addition we have less extensive banding records for two other hummingbird species that migrate through the area but do not breed there. The rate of return of broad-tails between consecutive breeding seasons has been as high as 70% for females and 27% for males, suggesting substantial site fidelity and malebiased dispersal or mortality; our records also suggest that rufous hummingbirds are faithful to a particular migratory route. The oldest recaptured birds were at least eight years old, an age that exceeds predictions based on allometric extrapolation from other bird species; the apparent yearly survival rate of females is also unexpectedly high for birds of such small body mass. The earliest broad-tails to arrive at the start of the breeding season appear to be older, experienced individuals. Reuse of a specific nest site between consecutive years by the same female or different females seems to depend on the success of nesting efforts at that site. We have calculated the rate of change in size of the broad-tail population based on our estimates of female survivorship and fledging success. By this method the population appears to be declining, although nest counts themselves suggest

that numbers of breeding females have remained fairly constant at least over the last seven years of our study.

Campbell, D. R. (1989). "Inflorescence size: test of the male function hypothesis." American Journal of Botany **76**: 730-738.

One explanation for low fruit sets in plants with hermaphroditic flowers is that total flower production by a plant is controlled primarily by selection through male function. This male function hypothesis presupposes that success in pollen donation increases more strongly with flower number than does seed set. I tested this prediction by measuring male and female components of reproductive success as functions of flower number in natural populations of the self-incompatible, perfect flowered plant, Ipomopsis aggregata. Fruit set in this humming-bird-pollinated plant averaged 4.9 to 40.3% across the 4 years of study. Both the total amount of pollen donated and the total amount received, as estimated by movement of fluorescent powdered dyes, increased linearly with number of flowers on a plant. Total seed production, however, increased disproportionately quickly because plants with larger floral displays were more likely to set at least one fruit. An estimate of the functional femaleness of a plant, based on pollen donation and seed production, increased with flower number. These results do not support the male function hypothesis.

Campbell, D. R. (1989). "Measurements of selection in a hermaphroditic plant: variation in male and female pollination success." Evolution **43**: 318-334.

Campbell, D. R. (1991). "Comparing pollen dispersal and gene flow in a natural plant population." Evolution **45**(8): 1965-1968.

Campbell, D. R. (1991). "Effects of floral traits on sequential components of fitness in *Ipomopsis aggregata*." American Naturalist **137**: 713-737.

Campbell, D. R. (1992). "Variation in sex allocation and floral morphology in *Ipomopsis aggregata* (Polemoniaceae)." American Journal of Botany **79**(5): 516-521.

Female reproductive success (seeds/flower) showed no detectable relationship with initial allocation of biomass at time of flowering, but decreased in accelerating fashion with the proportion of final biomass including seeds that was allocated to male function.

Campbell, D. R. (1996). "Evolution of floral traits in a hermaphroditic plant: field measurements of heritabilities and genetic correlations." Evolution **50**(4): 1442-1453.

Genetic variances, heritabilities, and genetic correlations of floral traits were measured in the monocarpic perennial Ipomopsis aggregata (Polemoniaceae). A paternal half-sib design was employed to generate seeds in each of four years, and seeds were planted back in the field near the parental site. The progeny were followed for up to eight years to estimate quantitative genetic parameters subject to natural levels of environmental variation over the entire life cycle. Narrow-sense heritabilities of 0.2-0.8 were detected for the morphometric traits of corolla length, corolla width, stigma position, and anther position. The proportion of time spent by the protandrous flowers in the pistillate phase ("proportion pistillate") also exhibited detectable heritability of near 0.3. In contrast, heritability estimates for nectar reward traits were low and not significantly different from zero, due to high environmental variance between and within flowering years. The estimates of genetic parameters were combined with phenotypic selection gradients to predict evolutionary responses to selection mediated by the hummingbird pollinators. One trait, corolla width, showed the potential for a rapid response to ongoing selection through male function, as it experienced both direct selection, by influencing pollen export, and relatively high heritability. Predicted responses were lower for proportion pistillate and corolla length, even though these traits also experienced direct selection. Stigma position was expected to respond positively to indirect selection of proportion pistillate but negatively to selection of corolla length, with the net effect sensitive to variation in the selection estimates. Anther position also was not directly selected but could respond to indirect selection of genetically correlated traits.

Campbell, D. R. (1998). "Multiple paternity in fruits of *Ipomopsis aggregata* (Polemoniaceae)." American Journal of Botany **85**(7): 1022-1027.

Two different mechanisms can result in multiple paternity within fruits: deposition of a mixed pollen load due to carryover of pollen from flower to flower and multiple pollinator visits in close succession. I investigated the extent of multiple paternity within fruits of Ipompsis aggregata containing from 2 to 14 seeds. A paternity analysis based on ten polymorphic isozyme markers revealed multiple paternity in a minimum of 68% (based on simple paternity exclusion) and up to 100% (based on identification of the most likely father) of the multiseeded fruits. The estimated number of fathers increased with the number of seeds in a fruit, with an average of four sires, and up to nine sires, represented in a single fruit. To explore whether this level of multiple paternity could be explained solely by simultaneous deposition of a mixed pollen load, I constructed a computer simulation model based on previous measurements of movement patterns and pollen carryover by the hummingbird pollinators. Model predictions provided a good match to observed values for number of sires per fruit. Thus, the extensive pollen carryover in this species and consequent mixed pollen loads can explain the high levels of multiple paternity in natural populations.

Campbell, D. R. (1998). "Variation in lifetime male fitness in *Ipomopsis aggregata*: Tests of sex allocation theory." American Naturalist **152**(3): 338-353.

Sex allocation theory assumes that a shift in allocation of resources to male function both increases male fitness and decreases female fitness. Moreover, the shapes of these fitness gain functions determine whether hermaphroditism or another breeding system is evolutionarily stable. In this article, I first outline information needed to measure these functions in flowering plants. I then use paternity analysis to describe the shapes of the fitness gain functions in natural populations of the hermaphroditic herb Ipomopsis aggregata. I also explore the relationships of male fitness (number of seeds sired) and female fitness (number of seeds produced) to the number of flowers produced by a plant. Plants with greater investment of biomass in the androecium, compared to the gynoecium and seeds, showed increased success at siring seeds, assumed by the models. That sex allocation trait, however, explained only 9% of the variance in estimates of male fitness. The shapes of the fitness gain functions were consistent with theoretical expectations for a hermaphroditic plant, but the model predicted a more female-biased evolutionarily stable strategy (ESS) allocation than was observed. These results lend only partial support the classical sex allocation model.

Campbell, D. R. (2004). "Natural selection in *Ipomopsis* hybrid zones: implications for ecological speciation." New Phytologist **161**(1): 83-90.

The fitness of hybrids relative to parental species plays an important role in models of speciation. In ecological speciation, reproductive isolation evolves owing to divergent natural selection, which implies reduced fitness of hybrid phenotypes. The source of the divergent selection in flowering plants may be animal pollinators, or environmental features of habitats that lead to physiological adaptations. Reciprocal transplants, combined with examination of pollination and other fitness components, allow exploration of these mechanisms of speciation. Much of this information is available from hybrid zones between Ipomopsis aggregata and Ipomopsis tenuituba. Pollination studies reveal some disruptive selection on corolla width, but also geographical variation in pollinator preference, and hybrids do not generally suffer lower pollination. Survival of hybrids depends on both genotype and environment. One genotypic class of hybrids is as fit or more fit than the parents, while another type suffers reduced fitness in parental environments. The dynamics of this hybrid zone involve a complex mixture of selection mediated by pollinators and other sources, and this combination of selection may have contributed to the original speciation.

Campbell, D. R. (2008). "Pollinator shifts and the origin and loss of plant species." Annals of the Missouri Botanical Garden **95**(2): 264-274.

Pollinators have long been implicated in plant speciation. Peter Raven's earlier work was instrumental in integrating foraging energetics of animals into our understanding of how shifts in major pollinators influence the evolutionary diversification of floral traits. More recently, efforts by Raven and others in the area of conservation have inspired pollination biologists to consider the implications of pollinator shifts and losses due to human activities. This paper uses the shift between hummingbird and hawkmoth pollination as a model for exploring impacts of pollinator shifts on plant populations. Recent studies have quantified the degree of reproductive isolation due to such pollinators in several genera. Data from Ipomopsis Michx. further allow us to consider whether recent changes in pollinator regimes have demographic consequences for plant populations. A majority of plant populations may currently suffer from pollen limitations on seed

production, but few data exist on the demographic consequences of poor reproduction. In Ipomopsis, reduced seed production due to pollen limitation can impact the number of individuals surviving to reproduce in the next generation. Some populations of I. tenuituba (Rydb.) V. E. Grant are estimated to have finite rates of increase less than unity, which can be explained in part by current low levels of hawkmoth pollination. In the absence of an increase in hawkmoths, selection for wider corolla tubes and other floral traits could, in principle, attract enough hummingbird pollination to result in a growing population, but models show that such evolution by natural selection may leave the population vulnerable to local extinction. We need more studies of the quantitative demographic consequences of changes in pollinator regimes. Such studies should consider how evolutionary changes influence the risk of extinction.

Campbell, D. R. (2009). "Using phenotypic manipulations to study multivariate selection of floral trait associations." Ann Bot **103**(9): 1557-1566.

Background: A basic theme in the study of plant-pollinator interactions is that pollinators select not just for single floral traits, but for associations of traits. Responses of pollinators to sets of traits are inherent in the idea of pollinator syndromes. In its most extreme form, selection on a suite of traits can take the form of correlational selection, in which a response to one trait depends on the value of another, thereby favouring floral integration. Despite the importance of selection for combinations of traits in the evolution of flowers, evidence is relatively sparse and relies mostly on observational approaches.

Scope: Here, methods for measuring selection on multivariate suites of floral traits are presented, and the studies to date are reviewed. It is argued that phenotypic manipulations present a powerful, but rarely used, approach to teasing apart the separate and combined effects of particular traits. The approach is illustrated with data from studies of alpine plants in Colorado and New Zealand, and recommendations are made about several features of the design of such experiments.

Conclusions: Phenotypic manipulations of two or more traits in combination provide a direct way of testing for selection of floral trait associations. Such experiments will be particularly valuable if rooted in hypotheses about differences between types of pollinators and tied to a proposed evolutionary history.

Campbell, D. R., et al. (2003). "Reproductive isolation and hybrid pollen disadvantage in *Ipomopsis*." Journal of Evolutionary Biology **16**(3): 536-540.

One cause of reproductive isolation is gamete competition, in which conspecific pollen has an advantage over heterospecific pollen in siring seeds, thereby decreasing the formation of F1 hybrids. Analogous pollen interactions between hybrid pollen and conspecific pollen can contribute to post-zygotic isolation. The herbaceous plants Ipomopsis aggregata and I. tenuituba frequently hybridize in nature. Hand-pollination of I. aggregata with pollen from F1 or F2 hybrids produced as many seeds as hand-pollination with conspecific pollen, suggesting equal pollen viability. However, when mixed pollen loads with 50% conspecific pollen and 50% hybrid pollen were applied to I. aggregata stigmas, fewer than half of the seeds had hybrid sires. Such pollen mixtures are frequently received if plants of the two species and F1 and F2 hybrids are intermixed, suggesting that this advantage of conspecific over hybrid pollen reduces backcrossing and contributes to reproductive isolation.

Campbell, D. R. and G. Aldridge (2006). Floral biology in hybrid zones. Ecology and Evolution of Flowers. L. D. Harder and S. C. H. Barrett. Oxford, Oxford University Press**:** 326-345.

Hybridization between closely related species is relatively common in angiosperms and can create anatural hybrid zone. We review recent experimental studies of floral biology in pairs of hybridizing species, emphasizing comparisons of the floral morphology and nectar rewards of hybrid plants with tha tof their progenitors, and quantifying the influence of these floral traits on pollinator behaviour and pre-zygotic and post-zygotic reproductive isolation. Floral traits of hybrids can be intermediate or trans-gressive. Floral differences between species, which in one case are attributable to particular chromosomal regions, can have differing impacts on interspecific flights by pollinators. A simulation model of mating in a hybrid zone between Ipomopsis aggregata and Ipomopsis tenuituba shows that behavioural responses by hummingbird and hawk-moth pollinators affect pre-zygotic ethological isolation more strongly than mechanical isolation. We apply this model to compare contact sites between these species which differ greatly in the frequency of natural hybrids. Striking differences in hawk-moth behaviour between the sites generated large differences in the rate of interspecific pollen movement, potentially explaining the dis-similar frequencies of hybrids.

Although floral traits influence both the formation and fitness of hybrids primarily through effects on pollinators, impacts on plant enemies also need consideration. Recent research has revealed much about how floral traits influence pollinator visitation, but mainly for systems with hummingbird versus insect pollinators. Such studies should be extended to other pollinators. Further research is also needed on how floral traits influence pollen dispersal and other post-visitation events that impact reproductive isolation, and their genetic basis in natural populations

Campbell, D. R. and J. L. Dooley (1992). "The spatial scale of genetic differentiation in a hummingbird-pollinated plant: comparison with models of isolation-by-distance." American Naturalist **139**(4): 735-748.

FST values are 0.02-0.04, and spatial autocorrelation is significantly positive only for distances up to about 5m, based on 8 electrophoretic loci. Neighborhood areas and sizes estimated at 28-45 adults, 54m2, about double the estimates from direct pollen dispersal. Still there is less strong differentiation and on smaller scale than predicted by Wright. Discuss long-distance dispersal tail and postpollination events as contributors

Campbell, D. R., et al. (2014). "Selection of trait combinations through bee and fly visitation to flowers of *Polemonium foliosissimum*." Journal of Evolutionary Biology **27**(2): 325-336.

Pollinators are known to exert natural selection on floral traits, but the extent to which combinations of floral traits are subject to correlational selection (nonadditive effects of two traits on fitness) is not well understood. Over two years, we used phenotypic manipulations of plant traits to test for effects of flower colour, flower shape and their interaction on rates of pollinator visitation to Polemonium foliosissimum. We also tested for correlational selection based on weighting visitation by the amount of conspecific pollen delivered per visit by each category of insect visitor. Although bumblebees were the presumed pollinators, solitary bees and flies contributed substantially (42%) to pollination. In manipulations of one trait at a time, insects visited flowers presenting the natural colour and shape over flowers manipulated to present artificial mutants with either paler colour or a more open or more tubular flower. When both colour and shape were manipulated in combination, selection on both traits arose, with bumblebees responding mainly to colour and flies responding mainly to shape. Despite selection on both floral traits, in a year with many bumblebees, we saw no evidence for correlational selection of these traits. In a year when flies predominated, fly visitation showed a pattern of correlational selection, but not favouring the natural phenotype, and correlational selection was still not detected for expected pollen receipt. These results show that flower colour and shape are subject to pollinator-mediated selection and that correlational selection can be generated based on pollinator visitation alone, but provide no evidence for correlational selection specifically for the current phenotype.

Campbell, D. R. and K. J. Halama (1993). "Resource and pollen limitations to lifetime seed production in a natural plant population." Ecology **74**(4): 1043-1051.

Both hand-pollination and fertilizing during the blooming season increased total seed production, while watering alone had no effect on reproductive success. Hand-pollination boosted number of seeds per flower, with no effect on flower number. Fertilizing had its primary effect on number of flowers produced, while also increasing the number of seeds per mature fruit in hand-pollinated plants and nectar volume. These results argue against a strict dichotomy between pollen limitation and resource limitation of female reproductive success in plants.

Campbell, D. R. and J. M. Powers (2015). "Natural selection on floral morphology can be influenced by climate." Proceedings B **282**(1808).

Climate has the potential to influence evolution, but how it influences the strength or direction of natural selection is largely unknown. We quantified the strength of selection on four floral traits of the subalpine herb Ipomopsis sp. in 10 years that differed in precipitation, causing extreme temporal variation in the date of snowmelt in the Colorado Rocky Mountains. The chosen floral traits were under selection by hummingbird and hawkmoth pollinators, with hawkmoth abundance highly variable across years. Selection for flower length showed environmental sensitivity, with stronger selection in years with later snowmelt, as higher water resources can allow translation of pollination success into fitness based on seed production. Selection on corolla width also varied across years, favouring narrower corolla tubes in two unusual years with

hawkmoths, and wider corollas in another late snowmelt year. Our results illustrate how changes in climate could alter natural selection even when the primary selective agent is not directly influenced.

Campbell, D. R. and N. M. Waser (1989). "Variation in pollen flow within and among populations of *Ipomopsis aggregata*." Evolution **43**: 1444-1455.

Pollen dispersal is a major component of gene flow in plant populations. It can influence microevolution within and among populations as well as the evolution of floral characters that affect dispersal. Most previous studies have relied on point estimates to characterize dispersal distances, even though there is likely to be substantial intrapopulational and interpopulational variation. We measured variation in pollen dispersal for the hummingbird-pollinated herb Ipomopsis aggregata (Polemoniaceae), using powdered fluorescent dyes to estimate pollen movement. Analysis of 5-6 natural populations in each of three years indicated that mean and mean squared distances of pollen dispersal, measured over the reproductive lifespan of individual plants, varied more than threefold among populations and years. Dispersal distances also shifted over the season within a given population. Unlike the variation among populations, these seasonal changes were associated in part with changes in flower density. The mean distance of pollen dispersal from an individual plant was unrelated to the date of first flowering, but did reflect two floral characters. Plants with higher variance in stamen length across flowers delivered pollen farther on average, as predicted by computer simulations of pollen carryover. Plants with lower mean stamen lengths also delivered pollen farther. Such effects of plant characters on pollen dispersal are a critical prerequisite for dispersal to evolve in response to its effects on fitness.

Campbell, D. R. and N. M. Waser (2001). "Genotype-by-environment interaction and the fitness of plant hybrids in the wild." Evolution **55**(4): 669-676.

Natural hybrid zones between related species illustrate processes that contribute to genetic differentiation and species formation. A common viewpoint is that hybrids are essentially unfit, but they exist in a stable tension zone where selection against them is balanced by gene flow between the parent species. An alternative idea is that selection depends on the environment, for example, by favoring opposite traits in the two parental habitats or favoring hybrids within a bounded region. To determine whether selection of hybrids is environment dependent, we crossed plants of naturally hybridizing Ipomopsis aggregata and I. tenuituba in the Colorado Rocky Mountains and reciprocally planted the seed offspring into a suite of natural environments across the hybrid zone. All types of crosses produced similar numbers and weights of seeds. However, survival of the offspring after 5 years differed markedly among cross types. On average, the F1 hybrids had survival and growth rates as high as the average for their parents. But hybrid survival depended strongly on the direction of a cross, that is, on which species served as the maternal parent. This fitness difference between reciprocal hybrids appeared only in the parental environments, suggesting cytonuclear gene interactions that are environment specific. These results indicate that complex genotype-by-environment interactions can contribute to the evolutionary outcome of hybridization.

Campbell, D. R. and N. M. Waser (2007). "Evolutionary dynamics of an *Ipomopsis* hybrid zone: Confronting models with lifetime fitness data." The American Naturalist **169**(3): 298-310.

Interspecific hybridization is a recurring aspect of the evolution of many plant and animal groups. The temporal dynamics of hybrid zones and the evolutionary consequences of hybridization should depend on fitness of parental and hybrid individuals expressed in different environments. We measured lifetime fitness, including survival and reproduction, of plants of Ipomopsis aggregata, Ipomopsis tenuituba, and their F1 hybrids, in experimental plantings in a natural hybrid zone. Fitness, measured as the finite rate of increase (λ), depended strongly on environment. Each parental species performed well in its home locale and poorly in the locale of the other species. Hybrids performed as well as parents overall but enjoyed their highest fitness in the hybrid site. Furthermore, F1 hybrids with I. tenuituba as maternal parent survived well only at the hybrid site, suggesting a cytonuclear x environment interaction. These results support an “environmental cline” model of hybrid zone dynamics, with complexities in the fitness of hybrids consistent also with an “evolutionary novelty” model. Combined with those of earlier studies of pollination, our results suggest that both vegetative adaptation to physical environment and floral adaptation to pollinators contribute to observed patterns of phenotypic expression in this hybrid zone and to persistence of the hybrid zone.

Campbell, D. R., et al. (2008). "Lifetime fitness in two generations of *Ipomopsis* hybrids." Evolution **62**(10): 2616-2627.

Various models purporting to explain natural hybrid zones make different assumptions about the fitness of hybrids. One class of models assumes that hybrids have intrinsically low fitness due to genetic incompatibilities, whereas other models allow hybrid fitness to vary across natural environments. We used the intrinsic rate of increase to assess lifetime fitness of hybrids between two species of montane plants Ipomopsis aggregata and Ipomopsis tenuituba planted as seed into multiple field environments. Because fitness is predicted to depend upon genetic composition of the hybrids, we included F1 hybrids, F2 hybrids, and backcrosses in our field tests. The F2 hybrids had female fitness as high, or higher, than expected under an additive model of fitness. These results run counter to any model of hybrid zone dynamics that relies solely on intrinsic nuclear genetic incompatibilities. Instead, we found that selection was environmentally dependent. In this hybrid zone, cytoplasmic effects and genotype-by-environment interactions appear more important in lowering hybrid fitness than do intrinsic genomic incompatibilities between nuclear genes.

Campbell, D. R., et al. (1997). "Analyzing pollinator-mediated selection in a plant hybrid zone: hummingbird visitation patterns on three spatial scales." American Naturalist **149**(2): 295-315.

Clines across hybrid zones can be produced by several forms of selection. This paper illustrates an approach to studying pollinator selection in plant hybrid zones using Ipomopsis. Measure visitation by hawkmoths and hummingbirds at up to 3 scales: within artificial mixed plots, among plots, and in populations separated by km. On all scales hummingbirds overvisit I. aggregata relative to I. tenuituba and hybrids. One possibility is context specificity of visits. Deduce: directional selection for wider, redder flowers. Hawkmoths were seen in one year of the study; they select for narrower tubes. In that year selection was disruptive; usually however it conforms to an advancing wave model. - from Waser

Campbell, D. R., et al. (2002). "Predicting patterns of mating and potential hybridization from pollinator behavior." American Naturalist **159**(5): 438-450.

Hybridization in flowering plants is determined in part by the rate at which animal pollinators move between species and by the effectiveness of such movements in transferring pollen. Pollinator behavior can also influence hybrid fitness by determining receipt and export of pollen. We incorporated information on pollinator effectiveness and visitation behavior into a simulation model that predicts pollen transfer between Ipomopsis aggregata, Ipomopsis tenuituba, and hybrids. These predictions were compared with estimates of pollen transfer derived from movement of fluorescent dyes in experimental plant arrays. Interspecific pollen transfer was relatively uncommon in these arrays, whereas transfer between hybrids and the parental species was at least as common as conspecific transfer. Backcrossing was asymmetrical; I. aggregata flowers frequently received mixed loads of hybrid and conspecific pollen. The simulation suggests that these patterns of pollen transfer are largely explained by the visitation sequences of hummingbird and insect pollinators, with little contribution from mechanical isolation. Pollen receipt by hybrids exceeded that of both parental species in a year when pollinators preferred to visit F1 and F2 hybrids and was intermediate in another year when they preferred to visit I. aggregata. This suggests that natural variation in pollination may produce spatiotemporal variation in hybridization and hybrid fitness.

Campbell, D. R., et al. (1994). "Indirect selection of stigma position in *Ipomopsis aggregata* via a genetically correlated trait." Evolution **48**(1): 55-68.

Experimental manipulation of a trait can be used to distinguish direct selection from selection of correlated traits and to identify mechanisms of selection. Here we use experiments to investigate phenotypic selection of stigma position in angiosperm flowers. In natural populations of the subalpine herb Ipomopsis aggregata, plants with more strongly exserted stigmas receive more pollen per flower, indicating selection favoring stigma exsertion during the pollination stage of the life cycle. We pose four hypotheses for this association, two involving direct selection on stigma position and two involving indirect selection of a correlated floral trait. The first three hypotheses were tested using hand pollinations that mimicked natural hummingbird visitation, and by presenting captive hummingbirds with a series of flowers that differed in stigma and anther positions, sex ratio, and presence of anthers. In these experiments, pollen deposition either was independent of stigma exsertion or was highest on inserted stigmas, suggesting direct selection against exserted stigmas. In natural populations, however, stigma exsertion is highly correlated with time spent by the protandrous flowers in the pistillate phase. When we manipulated the latter trait in the field, pollen deposition increased

with duration of exposure to hummingbirds, indicating indirect selection for stigma exsertion. Stigma exsertion and time spent in the pistillate phase are genetically and phenotypically correlated, as shown by a quantitative genetic experiment conducted in the field with paternal half sibships. Our results suggest that the evolution of stigma position can be driven by selection of a genetically correlated trait.

Campbell, D. R., et al. (1996). "Mechanisms of hummingbird-mediated selection for flower width in *Ipomopsis aggregata*." Ecology **77**(5): 1463-1472.

The form of angiosperm flowers is thought to have evolved in part via selection that excludes ineffective pollinators and increases the efficiency of pollen transfer by effective ones. In previous studies with the montane plant Ipomopsis aggregata, we documented pollinator—mediated selection on several aspects of floral form, including flower width. This character varies continuously within natural populations of I. aggregata, and individuals with wider flowers export more pollen per flower to surrounding plants. We previously showed that this component of phenotypic selection is due primarily to the pervisit effectiveness of hummingbirds, the most important pollinator in our study populations. Here we investigate mechanisms of differential visit effectiveness in greater detail. First, counts of pollen in unvisited flowers showed that pollen production itself increases with width. In aviary experiments, hummingbirds also removed a greater proportion of available pollen as width increased. We next videotaped visits to flowers that varied in width either naturally, or by experimental treatment, and found that hummingbirds inserted their bills more deeply into wider flowers. Finally, we directly manipulated how deeply birds could insert their bills, and found that more pollen was removed after deep insertion. Thus, several mechanisms appear to underlie selection on corolla width via visit effectiveness in pollen export. One involves a phenotypic correlation with pollen production; this underscores the value of experiments for untangling indirect from direct selection. Another mechanism involves direct selection due to the depth of insertion of a hummingbird's bill; this is rare evidence for one form of selection involving the “fit” between pollinator and flower.

Campbell, D. R., et al. (1991). "Components of phenotypic selection: pollen export and flower corolla width in *Ipomopsis aggregata*." Evolution **45**: 1458-1467.

In the hummingbird-pollinated herb Ipomopsis aggregata, selection through male function during pollination favors wide corolla tubes. We explored the mechanisms behind this selection, using phenotypic selection analysis to compare effects of corolla width on two components of male pollination success, pollinator visit rate and pollen exported per visit. During single visits by captive hummingbirds, flowers with wider corollas exported more pollen, and more dye used as a pollen analogue, to stigmas of recipient flowers. Corolla width was less strongly related to visit rate in the field, and had no direct effect on visit rate after nectar production and corolla length were controlled for. Moreover, the phenotypic selection differential was 80% higher for the effect on pollen exported per visit, suggesting that this is the more important mechanism of selection.

Campbell, D. R., et al. (1998). "Pollen transfer by natural hybrids and parental species in an *Ipomopsis* hybrid zone." Evolution **52**(6): 1602-1611.

Models of hybrid zones differ in their assumptions about the relative fitnesses of hybrids and the parental species. These fitness relationships determine the form of selection across the hybrid zone and, along with gene flow, the evolutionary dynamics and eventual outcome of natural hybridization. We measured a component of fitness, export and receipt of pollen in single pollinator visits, for hybrids between the herbaceous plants Ipomopsis aggregata and I. tenuituba and for both parental species. In aviary experiments with captive hummingbirds, hybrid flowers outperformed flowers of both parental species by receiving more pollen on the stigma. Although hummingbirds were more effective at removing pollen from anthers of I. aggregata, hybrid flowers matched both parental species in the amount of pollen exported to stigmas of other flowers. These patterns of pollen transfer led to phenotypic stabilizing selection, during that stage of the life cycle, for a stigma position intermediate between that of the two species and to directional selection for exserted anthers. Pollen transfer between the species was high, with flowers of I. aggregata exporting pollen equally successfully to conspecific and I. tenuituba flowers. Although this study showed that natural hybrids enjoy the highest quality of pollinator visits, a previous study found that I. aggregata receives the highest quantity of pollinator visits. Thus, the relative fitness of hybrids changes over the life cycle. By

combining the results of both studies, pollinator-mediated selection in this hybrid zone is predicted to be strong and directional, with hybrid fitness intermediate between that of the parental species.

CaraDonna, P. J., et al. (2014). "Shifts in flowering phenology reshape a subalpine plant community." Proceedings of the National Academy of Sciences **111**(13): 4916-4921.

Phenology—the timing of biological events—is highly sensitive to climate change. However, our general understanding of how phenology responds to climate change is based almost solely on incomplete assessments of phenology (such as first date of flowering) rather than on entire phenological distributions. Using a uniquely comprehensive 39-y flowering phenology dataset from the Colorado Rocky Mountains that contains more than 2 million flower counts, we reveal a diversity of species-level phenological shifts that bring into question the accuracy of previous estimates of long-term phenological change. For 60 species, we show that first, peak, and last flowering rarely shift uniformly and instead usually shift independently of one another, resulting in a diversity of phenological changes through time. Shifts in the timing of first flowering on average overestimate the magnitude of shifts in the timing of peak flowering, fail to predict shifts in the timing of last flowering, and underrepresent the number of species changing phenology in this plant community. Ultimately, this diversity of species-level phenological shifts contributes to altered coflowering patterns within the community, a redistribution of floral abundance across the season, and an expansion of the flowering season by more than I mo during the course of our study period. These results demonstrate the substantial reshaping of ecological communities that can be attributed to shifts in phenology.

CaraDonna, P. J., et al. (2017). "Interaction rewiring and the rapid turnover of plant–pollinator networks." Ecology Letters **20**(3): 385-394.

Whether species interactions are static or change over time has wide-reaching ecological and evolutionary consequences. However, species interaction networks are typically constructed from temporally aggregated interaction data, thereby implicitly assuming that interactions are fixed. This approach has advanced our understanding of communities, but it obscures the timescale at which interactions form (or dissolve) and the drivers and consequences of such dynamics. We address this knowledge gap by quantifying the within-season turnover of plant–pollinator interactions from weekly censuses across 3 years in a subalpine ecosystem. Week-to-week turnover of interactions (1) was high, (2) followed a consistent seasonal progression in all years of study and (3) was dominated by interaction rewiring (the reassembly of interactions among species). Simulation models revealed that species' phenologies and relative abundances constrained both total interaction turnover and rewiring. Our findings reveal the diversity of species interactions that may be missed when the temporal dynamics of networks are ignored.

Cariveau, D., et al. (2004). "Direct and indirect effects of pollinators and seed predators to selection on plant and floral traits." Oikos **104**(1): 15-26.

Although flowering traits are often assumed to be under strong selection by pollinators, significant variation in such traits remains the norm for most plant species. Thus, it is likely that the interactions among plants, mutualists, and other selective agents, such as antagonists, ultimately shape the evolution of floral and flowering traits. We examined the importance of pollination vs pre-dispersal seed predation to selection on plant and floral characters via female plant-reproductive success in Castilleja linariaefolia (Scrophulariaceae). C. linariaefolia is pollinated by hummingbirds and experiences high levels of pre-dispersal seed predation by plume moth and fly larvae in the Rocky Mountains of Colorado, USA, where this work was conducted. We first examined whether female reproduction in C. linariaefolia was limited by pollination. Supplemental pollination only marginally increased components of female reproduction, likely because seed predation masked, in part, the beneficial effects of pollen addition. In unmanipulated populations, we measured calyx length, flower production, and plant height and used path analysis combined with structural equation modeling to quantify their importance to relative seed set through pathways involving pollination vs seed predation. We found that the strength of selection on calyx length, flower production, and plant height was greater for seed predation pathways than for pollination pathways, and one character, calyx length, experienced opposing selection via pollination vs seed predation. These results suggest that the remarkable intraspecific variation in plant and floral characters exhibited by some flowering plants is likely the result of selection driven, at least in part, by pollinators in concert with antagonists, such as pre-dispersal seed

predators. This work highlights the subtle but complex interactions that shape floral and vegetative design in natural ecosystems.

Caruso, C. M. (1999). "Pollination of *Ipomopsis aggregata* (Polemoniaceae): Effects of intra- vs. interspecific competition." American Journal of Botany **86**(5): 663-668.

Although plants may simultaneously experience intra- and interspecific competition for pollination, their relative strength has rarely been experimentally evaluated. Yet because intra- and interspecific competition can be caused by different mechanisms, their effect on the ecology and evolution of plants may differ. To determine the relative strength of intra- and interspecific competition for pollination, I manipulated the presence of heterospecifics and density of conspecifics using Ipomopsis aggregata as the focal species. All plots contained I. aggregata and Castilleja linariaefolia, but C. linariaefolia inflorescences were removed from half of the plots to create the heterospecifics-absent treatment. Within each plot, all I. aggregata inflorescences were removed from a 5-m radius around a focal plant to create a low conspecific density experimental unit, and a group of 12 I. aggregata plants/1 m was designated as a high conspecific density unit. Conspecific pollen deposition was reduced when C. linariaefolia was present but was not influenced by I. aggregata density. Although seed set per fruit was reduced by 17% when C. linariaefolia was present, it was not significantly influenced by either treatment. Interspecific competition for pollination is stronger than intraspecific competition in the I. aggregata–C. linariaefolia system, but neither process appears to influence plant fitness.

Caruso, C. M. (2000). "Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*." Evolution **54**(5): 1546-1557.

Although rarely tested, it is often assumed that interspecific competition results in the divergence of traits related to resource use. Using a plant-pollinator system as a model, I tested the prediction the presence of a competitor for pollination influences the strength and/or direction of pollinator-mediated selection on floral traits. I measured phenotypic selection via female fitness on five floral traits of Ipomopsis aggregata in seven populations. Four contained only conspecifics (I only) and three also contained the competitor Castilleja linariaefolia (C + I). Directional selection via fruits/plant and conspecific pollen deposited/flower on corolla length was positive and significantly stronger in C + I populations. This difference in selection was apparently driven by interpopulation variation in the degree to which reproduction of I. aggregata was pollen limited. Consistent with expectations of interspecific competition, I. aggregata plants in C + I populations received less conspecific pollen per flower and set fewer seeds per fruit and fruits per plant than those in I only populations. Ipomopsis aggregata's corollas were also significantly longer in C + I populations, suggesting that there had been a response to a similar selective regime in past generations. Phenotypic correlations between corolla length and width, which determine the variation in I. aggregata's flower shape, were significantly weaker in C + I populations. These data suggest that competition for pollination can influence the strength of selection on and patterns of correlations among floral traits of I. aggregata. If I. aggregata populations with and without competitors for pollination are linked by gene flow, then measuring selection in competitive and noncompetitive environments maybe necessary to accurately predict how floral traits will evolve.

Caruso, C. M. (2002). "Influence of plant abundance on pollination and selection on floral traits of *Ipomopsis aggregata*." Ecology **83**(1): 241-254.

Competition for pollination has been hypothesized to select for the divergence of floral traits between species and populations. A primary prediction of this hypothesis is that the strength of competition for pollination, mediated by variation in plant abundance, should directly influence the strength of selection on floral traits. To test this prediction, I examined the relationships between multiple components of plant abundance and pollination, reproductive success, and phenotypic selection via female fitness on four floral traits in artificial and natural populations of the hummingbird-pollinated Ipomopsis aggregata. In the artificial arrays, I manipulated the absolute density and interspersion of neighboring I. aggregata and a competitor for pollination (Castilleja linariaefolia). I also measured natural variation in the absolute and relative density of these two species within a 2.5-m radius of focal I. aggregata plants in three natural populations.

The strength of competition for pollination in the I. aggregata–C. linariaefolia system was only weakly influenced by local plant abundance. Both the absolute density and interspersion of plants in the arrays significantly influenced at least one component of I. aggregata's pollination and reproductive success, but the effects were not consistent across these components. For example, the treatment groups that received more conspecific pollen were not the same ones that set more seeds/fruit. Within the natural populations, variation in relative and absolute plant density influenced two components of I. aggregata's pollination and reproductive success, but only in one to two populations per component. As would be expected from these inconsistent effects of plant abundance on pollination and reproduction, the strength of selection on floral traits of I. aggregata in both the arrays and natural populations was also only weakly dependent on abundance. Previous studies have indicated (1) that self-incompatible species such as I. aggregata should experience strong, abundance-dependent effects on pollination, and (2) that variation in plant abundance at a local scale should have a stronger effect on pollination than variation at a larger spatial scale. In contrast, my results suggest that the effect of plant abundance on pollination cannot be easily predicted from simple diagnostic traits such as breeding system or spatial scale.

Citing

Castellanos, M. C., et al. (2003). "Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes." Evolution **57**: 2742-2752.

We compared pollen removal and deposition by hummingbirds and bumblebees visiting bird-syndrome Penstemon barbatus and bee-syndrome P. strictus flowers. One model for evolutionary shifts from bee pollination to bird pollination has assumed that, mostly due to grooming, pollen on bee bodies quickly becomes unavailable for transfer to stigmas, whereas pollen on hummingbirds has greater carryover. Comparing bumblebees and hummingbirds seeking nectar in P. strictus, we confirmed that bees had a steeper pollen carryover curve than birds but, surprisingly, bees and birds removed similar amounts of pollen and had similar per-visit pollen transfer efficiencies. Comparing P. barbatus and P. strictus visited by hummingbirds, the bird-syndrome flowers had more pollen removed, more pollen deposited, and a higher transfer efficiency than the bee-syndrome flowers. In addition, P. barbatus flowers have evolved such that their anthers and stigmas would not easily come into contact with bumblebees if they were to forage on them. We discuss the role that differences in pollination efficiency between bees and hummingbirds may have played in the repeated evolution of hummingbird pollination in Penstemon.

de Jong, T., et al. (1992). "Plant size, geitonogamy, and seed set in *Ipomopsis aggregata*." Oecologia **89**: 310-315.

We used powdered fluorescent dyes to estimate receipt of self vs. outcross pollen in the self-incompatible species Ipomopsis aggregata (Polemoniaceae). Flowers on small and large plants received equal amounts of outcross pollen, whereas flowers on large plants received more self pollen, so the proportion of self pollen delivered through geitonogamy increased with plant size. In natural populations emasculation of all flowers on a plant raised average seed set per flower from 5.19 to 6.99 and also raised fruit set, though not significantly. From these results one expects a negative correlation between plant size and seeds per flower. The opposite trend was observed in a sample of plants in the field, suggesting that deleterious effects of geitonogamy on female fecundity in large plants can be overruled by other factors such as size-related fruit or seed abortion. Results are discussed in relation to the evolution of gynodioecy.

Dukas, R. and N. M. Waser (1994). "Categorization of food types enhances foraging performance of bumblebees." Animal Behaviour **48**(5): 1001-1006.

The classification of food items into a selected set of categories can significantly enhance information processing by a foraging animal. The ability of bumblebees, Bombus flavifrons, to form simple categories of floral colours was tested. Bees recognized categories in a way that enhanced their ability to discriminate between rewarding and non-rewarding flowers. Bees also recognized novel members of learned categories. Both memorization and generalization based on similarity between floral types could be ruled out as the mechanisms underlying bees' behaviour. It is most likely that bees learned to attend to the stimulus that reliably identified members of a certain category; a similar mechanism may be commonly used by many other species including humans.

Forrest, J. and J. D. Thomson (2008). "Pollen limitation and cleistogamy in subalpine *Viola praemorsa*." Botany **86**: 511-519.

Early-flowering species may be especially susceptible to occasional pollen limitation and, therefore, may benefit from a mixed-mating strategy that provides reproductive assurance. We studied cleistogamous (CL) and chasmogamous (CH) fruit set of spring-flowering Viola praemorsa Dougl. ex Lindl. along an elevational gradient in the Rocky Mountains, testing whether pollen limitation or allocation to CL reproduction covaried with timing of flowering onset, within and across sites. Contrary to predictions, we found no pollen limitation of reproduction at any site, and variation among sites in the pattern of allocation to cleistogamy was not related to growing season length. Differences in reproductive strategy between early- and late-flowering plants within sites were attributable to differences in plant size, with relative allocation to cleistogamy increasing with size. This pattern has been found in some other cleistogamous species, and may indicate a cost of large CH floral displays, perhaps associated with geitonogamy or herbivory. We found no experimental evidence for resource reallocation in response to CH reproductive output, although a weak negative relationship between CH and CL fruit set across a larger sample of unmanipulated plants suggests such a trade-off. The significance of cleistogamy may be clarified by studying how pollinator visitation, self-pollination, and herbivore damage vary temporally and with floral display size.

Forrest, J. and J. D. Thomson (2010). "Consequences of variation in flowering time within and among individuals of *Mertensia fusiformis* (Boraginaceae), an early spring wildflower." Am J Bot **97**(1): 38-48.

Climate change is causing many plants to flower earlier in spring, exposing them to novel selection pressures, including-potentially-pollinator shortages. Over 2 years that contrasted in timing of flowering onset, we studied reproductive strategies, pollen limitation, and selection on flowering time in Mertensia fusiformis, a self-incompatible, spring-flowering perennial. Plants opened most of their flowers early in the flowering period, especially in 2007, the early year; but selection favored early-flowering individuals only in 2008. However, resource allocation to early vs. late seed production was flexible: In 2008, but not 2007, early flowers on a plant produced more and heavier seeds. Late flowers were capable of equal seed production if fertilization of early ovules was prevented, suggesting that late flowers serve a bet-hedging function. Evidence for pollen limitation was weak, although there was a tendency for early flowers to be pollen-limited in 2007 and for late flowers to be pollen-limited in 2008. Poor reproductive success in 2007 was likely attributable less to pollen limitation than to frost damage to flowers. We suggest that plasticity in floral longevity and resource allocation among flowers will make this species resilient to short-term pollinator deficits; whether this will help or hinder future adaptation is unclear.

Forrest, J. R., et al. (2011). "Seasonal change in a pollinator community and the maintenance of style length variation in *Mertensia fusiformis* (Boraginaceae)." Ann Bot **108**(1): 1-12.

BACKGROUND AND AIMS: In sub-alpine habitats, patchiness in snowpack produces marked, small-scale variation in flowering phenology. Plants in early- and late-melting patches are therefore likely to experience very different conditions during their flowering periods. Mertensia fusiformis is an early-flowering perennial that varies conspicuously in style length within and among populations. The hypothesis that style length represents an adaptation to local flowering time was tested. Specifically, it was hypothesized that lower air temperatures and higher frost risk would favour short-styled plants (with stigmas more shielded by corollas) in early-flowering patches, but that the pollen-collecting behaviour of flower visitors in late-flowering patches would favour long-styled plants.

METHODS: Floral morphology was measured, temperatures were monitored and pollinators were observed in several matched pairs of early and late populations. To evaluate effects of cold temperatures on plants of different style lengths, experimental pollinations were conducted during mornings (warm) and evenings (cool), and on flowers that either had or had not experienced a prior frost. The effectiveness of different pollinators was quantified as seed set following single visits to plants with relatively short or long styles.

KEY RESULTS: Late-flowering populations experienced warmer temperatures than early-flowering populations and a different suite of pollinators. Nectar-foraging bumble-bee queens and male solitary bees predominated in early populations, whereas pollen-collecting female solitary bees were more numerous in later sites. Pollinators differed significantly in their abilities to transfer pollen to stigmas at different heights, in accordance with our prediction. However, temperature and frost sensitivity did not differ between long- and

short-styled plants. Although plants in late-flowering patches tended to have longer styles than those in early patches, this difference was not consistent.

CONCLUSIONS: Seasonal change in pollinator-mediated selection on style length may help maintain variation in this trait in M. fusiformis, but adaptation to local flowering time is not apparent. The prevalence of short styles in these populations requires further explanation.

Forrest, J. R. K. and S. P. M. Chisholm (2017). "Direct benefits and indirect costs of warm temperatures for high-elevation populations of a solitary bee." Ecology **98**(2): 359-369.

Warm temperatures are required for insect flight. Consequently, warming could benefit many high-latitude and high-altitude insects by increasing opportunities for foraging or oviposition. However, warming can also alter species interactions, including interactions with natural enemies, making the net effect of rising temperatures on population growth rate difficult to predict. We investigated the temperature-dependence of nesting activity and lifetime reproductive output over 3 yr in subalpine populations of a pollen-specialist bee, Osmia iridis. Rates of nest provisioning increased with ambient temperatures and with availability of floral resources, as expected. However, warmer conditions did not increase lifetime reproductive output. Lifetime offspring production was best explained by rates of brood parasitism (by the wasp Sapyga), which increased with temperature. Direct observations of bee and parasite activity suggest that although activity of both species is favored by warmer temperatures, bees can be active at lower ambient temperatures, while wasps are active only at higher temperatures. Thus, direct benefits to the bees of warmer temperatures were nullified by indirect costs associated with increased parasite activity. To date, most studies of climate-change effects on pollinators have focused on changing interactions between pollinators and their floral host-plants (i.e., bottom-up processes). Our results suggest that natural enemies (i.e., top-down forces) can play a key role in pollinator population regulation and should not be overlooked in forecasts of pollinator responses to climate change.

Forrest, J. R. K., et al. (2019). "Two-yearbBee, or not two-year bee? How voltinism Is affected by temperature and season length in a high-elevation solitary bee." The American Naturalist **193**(4): 560-574.

Organisms must often make developmental decisions without complete information about future conditions. This uncertainty?for example, about the duration of conditions favorable for growth?can favor bet-hedging strategies. Here, we investigated the causes of life cycle variation in Osmia iridis, a bee exhibiting a possible bet-hedging strategy with co-occurring 1- and 2-year life cycles. One-year bees reach adulthood quickly but die if they fail to complete pupation before winter; 2-year bees adopt a low-risk, low-reward strategy of postponing pupation until the second summer. We reared larval bees in incubators in various experimental conditions and found that warmer?but not longer?summers and early birthdates increased the frequency of 1-year life cycles. Using in situ temperature measurements and developmental trajectories of laboratory- and field-reared bees, we estimated degree-days required to reach adulthood in a single year. Local long-term (1950-2015) climate records reveal that this heat requirement is met in only ~7% of summers, suggesting that the observed distribution of life cycles is adaptive. Warming summers will likely decrease average generation times in these populations. Nevertheless, survival of bees attempting 1-year life cycles - particularly those developing from late-laid eggs - will be <100%; consequently, we expect the life cycle polymorphism to persist.

Forrest, J. R. K. and S.-Y. Lin (2019). "The function of floral orientation in bluebells: interactions with pollinators and rain in two species of *Mertensia* (Boraginaceae)." Journal of Plant Ecology **12**(1): 113-123.

Pollinators are traditionally considered to be the primary agent of selection on floral traits. However, floral traits may also be under selection from abiotic agents (e.g. rain), which makes considering the relative importance of pollinators and abiotic selective agents on floral traits essential. The functional significance of floral orientation is often ascribed to pollinator attraction, but orientation can also protect reproductive structures from rain. Therefore, a study that incorporates both factors will enhance our understanding of the ecological roles of floral orientation in plant fitness. Mertensia brevistyla and M. fusiformis are herbaceous species that differ in their floral orientations. A series of field and laboratory experiments was used to investigate the adaptive function of floral orientation in these species, particularly with respect to pollinators and rain.

We measured and compared floral orientation and visitor assemblages between M. brevistyla and M. fusiformis populations in western Colorado, USA. We manipulated floral stems and conducted a choice experiment with floral visitors, and also compared orientations of pollinator-visited stems with those of unvisited stems in a natural setting. We examined pollinator- and rain-mediated selection on floral orientation by manipulating orientation, conducting supplemental pollinations, applying watering treatments and measuring subsequent seed set. We also experimentally tested the likelihood of rain contact with anthers, and the effect of rainwater on pollen germinability.

Mertensia brevistyla had a significantly more upright floral orientation than M. fusiformis, and seed set was highest in upright M. brevistyla and in horizontal/pendant M. fusiformis stems, supporting an adaptive function (via female fitness) of the interspecific difference in orientation. However, floral visitor assemblages did not differ significantly between the two species; visitors did not exhibit significant preference for either orientation; and pollinator-mediated selection on orientation was undetectable. Similarly, there was little effect of water on seed set in either species, regardless of floral orientation. However, pollen germinability was reduced in both species by immersion in water; and water was more likely to contact anthers in M. fusiformis than in M. brevistyla, due to interspecific differences in floral morphology. We conclude that pollinators are likely not the primary selective agent driving differences in orientation in these Mertensia species. Instead, the negative effect of rain on pollen germinability helps explain the more pendant orientation of M. fusiformis, while short anthers in more upright M. brevistyla provide an alternative adaptation to rain. The selective agent driving effects of orientation on seed set remains unclear. This study illustrates the necessity of considering male fitness and abiotic agents in interpreting the functional significance of inflorescence traits.

Forrest, J. R. K. and J. D. Thomson (2011). "An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows." Ecological Monographs **81**(3): 469-491.

One possible effect of climate change is the generation of a mismatch in the seasonal timing of interacting organisms, owing to species-specific shifts in phenology. Despite concerns that plants and pollinators might be at risk of such decoupling, there have been few attempts to test this hypothesis using detailed phenological data on insect emergence and flowering at the same localities. In particular, there are few data sets on pollinator flight seasons that are independent of flowering phenology, because pollinators are typically collected at flowers. To address this problem, we established standardized nesting habitat (trap nests) for solitary bees and wasps at sites along an elevational gradient in the Rocky Mountains, and monitored emergence during three growing seasons. We also recorded air temperatures and flowering phenology at each site. Using a reciprocal transplant experiment with nesting bees, we confirmed that local environmental conditions are the primary determinants of emergence phenology. We were then able to develop phenology models to describe timing of pollinator emergence or flowering, across all sites and years, as a function of accumulated degree-days. Although phenology of both plants and insects is well described by thermal models, the best models for insects suggest generally higher threshold temperatures for development or diapause termination than those required for plants. In addition, degree-day requirements for most species, both plants and insects, were lower in locations with longer winters, indicating either a chilling or vernalization requirement that is more completely fulfilled at colder sites, or a critical photoperiod before which degree-day accumulation does not contribute to development. Overall, these results suggest that phenology of plants and trap-nesting bees and wasps is regulated in similar ways by temperature, but that plants are more likely than insects to advance phenology in response to springtime warming. We discuss the implications of these results for plants and pollinators, and suggest that phenological decoupling alone is unlikely to threaten population persistence for most species in our study area

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

Ants of Formica neorufibarbus gelida are nectar thieves of Polemonium viscosum. Their activities lead to significant reductions in seedset. Ants interfere with seed production by severing the base of the style or chewing through the ovary during nectar foraging, rather than by lowering reward levels available to pollinators. During the summers of 1979 and 1981 flowers of over 25% of the plants of P. viscosum censused in the krummholz of the Colorado alpine were damaged by nectar thieving ants. The frequency of ant

visitation decreased at higher altitudes. Individual plants of P. viscosum have a predominant floral scent that is either sweet or skunky. I found that ants visited considerably more individuals with sweet flowers than expected from local morph abundance, and undervisited plants with skunky flowers. Laboratory preference tests supported field observations; ants showed a significant preference for sweet flowers, and this was in response to scent rather than other floral cues. In this system floral 'predators' and bumblebee pollinators show similar floral choice patterns, and their behavior may help to maintain scent variation in populations of P. viscosum. /// Муравьи Formica neorufibarbus gelida воруют нектар у Polemonium viscosum.

Gallagher, M. K. and D. R. Campbell (2017). "Shifts in water availability mediate plant–pollinator interactions." New Phytologist **215**(2): 792-802.

Altered precipitation patterns associated with anthropogenic climate change are expected to have many effects on plants and insect pollinators, but it is unknown if effects on pollination are mediated by changes in water availability. We tested the hypothesis that impacts of climate on plant–pollinator interactions operate through changes in water availability, and specifically that such effects occur through alteration of floral attractants. We manipulated water availability in two naturally occurring Mertensia ciliata (Boraginaceae) populations using water addition, water reduction and control plots and measured effects on vegetative and floral traits, pollinator visitation and seed set. While most floral trait values, including corolla size and nectar, increased linearly with increasing water availability, in this bumblebee-pollinated species, pollinator visitation peaked at intermediate water levels. Visitation also peaked at an intermediate corolla length, while its relationship to corolla width varied across sites. Seed set, however, increased linearly with water. These results demonstrate the potential for changes in water availability to impact plant–pollinator interactions through pollinator responses to differences in floral attractants, and that the effects of water on pollinator visitation can be nonlinear. Plant responses to changes in resource availability may be an important mechanism by which climate change will affect species interactions.

Gezon, Z. J., et al. (2016). "Phenological change in a spring ephemeral: implications for pollination and plant reproduction." Glob Chang Biol **22**(5): 1779-1793.

Climate change has had numerous ecological effects, including species range shifts and altered phenology. Altering flowering phenology often affects plant reproduction, but the mechanisms behind these changes are not well-understood. To investigate why altering flowering phenology affects plant reproduction, we manipulated flowering phenology of the spring herb Claytonia lanceolata (Portulacaceae) using two methods: in 2011-2013 by altering snow pack (snow-removal vs. control treatments), and in 2013 by inducing flowering in a greenhouse before placing plants in experimental outdoor arrays (early, control, and late treatments). We measured flowering phenology, pollinator visitation, plant reproduction (fruit and seed set), and pollen limitation. Flowering occurred approx. 10 days earlier in snow-removal than control plots during all years of snow manipulation. Pollinator visitation patterns and strength of pollen limitation varied with snow treatments, and among years. Plants in the snow removal treatment were more likely to experience frost damage, and frost-damaged plants suffered low reproduction despite lack of pollen limitation. Plants in the snow removal treatment that escaped frost damage had higher pollinator visitation rates and reproduction than controls. The results of the array experiment supported the results of the snow manipulations. Plants in the early and late treatments suffered very low reproduction due either to severe frost damage (early treatment) or low pollinator visitation (late treatment) relative to control plants. Thus, plants face tradeoffs with advanced flowering time. While early-flowering plants can reap the benefits of enhanced pollination services, they do so at the cost of increased susceptibility to frost damage that can overwhelm any benefit of flowering early. In contrast, delayed flowering results in dramatic reductions in plant reproduction through reduced pollination. Our results suggest that climate change may constrain the success of early-flowering plants not through plant-pollinator mismatch but through the direct impacts of extreme environmental conditions.

Gezon, Z. J., et al. (2015). "The effect of repeated, lethal sampling on wild bee abundance and diversity." Methods in Ecology and Evolution **6**(9): 1044-1054.

1.Bee pollinators provide a critical ecosystem service to wild and agricultural plants but are reported to be declining worldwide due to anthropogenic change. Long-term data on bee abundance and diversity are scarce, and the need for additional quantitative sampling using repeatable methods has been emphasized.

Recently, monitoring programs have begun using a standardized method that employs a combination of pan traps and sweep netting, resulting in lethal sampling of bees. This standardized method can remove a large number of bees from sites during each sampling day, raising concern that the sampling itself could have a negative effect on bee populations.

2.We conducted an experiment to assess whether lethal sampling for bees using pan traps and netting affected bee abundance and diversity when done every two weeks throughout a season and over multiple years. We compared bee abundance, richness, evenness and functional group composition between sites that had been sampled every two weeks from 2009 to 2012 to similar sites not previously sampled.

3.We found that the standardized method for sampling bees, with specimens from 132 morphospecies, did not affect bee communities in terms of abundance, rarefied richness, evenness, or functional group composition. Thus, our results indicate that the bee communities we sampled are robust to such sampling efforts, despite removing an average of 2,862 bees per season.

4.We discuss several explanations for why sampling did not affect bee abundance or community structure, including a density-dependent response to reduced competition for resources.

5.These results suggest that bee monitoring programs sampling once every two weeks with pan traps and netting will not affect bee community structure. We urge researchers monitoring bees to utilize standardized protocols so that results can be compared across space and time.

Gori, D. F. (1989). "Floral color change in *Lupinus argenteus* (Fabaceae): why should plants advertise the location of unrewarding flowers to pollinators?" Evolution **43**: 870-881.

I examined the adaptive significance of two floral traits in the perennial herb, Lupinus argenteus: 1) the retention of corollas on "spent" flowers, i.e., flowers containing inviable pollen, unreceptive stigmas, and negligible pollinator rewards and 2) a change in corolla color of retained "spent" flowers, which is restricted to a spot on the banner petal. At anthesis, this spot is yellow, and approximately four days later, it changes to purple. After the change, purple flowers remain on plants an additional 5-7 days before corolla abscission occurs; purple flowers were avoided by pollinators, presumably because they contained less pollen (rewards) than yellow ones. I experimentally tested the hypothesis that purple flowers contribute to the floral display of the plant by removing varying numbers of spent flowers and assessing the effect on pollination visitation. Pollinators preferentially approached and foraged on plants with greater numbers of flowers per inflorescence; they did not discriminate between yellow (rewarding) and purple (nonrewarding) flowers at interplant distances greater than 0.4 meters but would preferentially forage on plants with more total flowers, even if these individuals contained fewer rewarding flowers. Thus, spent flowers increased the overall attractiveness of plants to pollinators. In theory, color change may benefit plants in two ways. First, by directing pollinators to rewarding flowers, the change may increase pollinator foraging efficiency, with the result that pollinators visit more flowers before leaving plants (pollinator-tenure mechanism). Second, by directing pollinators to receptive flowers, the color change may prevent incoming pollen from being wasted on unreceptive stigmas and may prevent collection of inviable pollen (pollination-efficiency mechanism). I tested the pollinator-tenure mechanism experimentally by removing pollen from yellow flowers, thereby reducing the reliability of the color-reward signal. Pollinators visited fewer total flowers on experimental plants than on controls, resulting in reduced seed production in one year.

Gray, M., et al. (2018). "Consistent shifts in pollinator-relevant floral coloration along Rocky Mountain elevation gradients." Journal of Ecology **106**(5): 1910-1924.

Floral colour is a fundamental signal that shapes plant–pollinator interactions. Despite theoretical reasons why floral colours might shift in representation along biotic and abiotic gradients, few studies have examined community-level shifts in colour, and even fewer significant patterns have been detected. We examined floral colour on six replicated transects spanning 1,300 m in the Rocky Mountains of Colorado, USA. Along these transects, there is a hypothesized shift from bee-dominated to fly-dominated pollination with increasing elevation. The reflectance of flowers of 110 forb and shrub species was measured using a spectrophotometer, and was used to estimate three components of colour (hue, saturation and brightness) in relevant pollinator visual spaces. Percent cover data were collected from 67 sites and used to obtain community-weighted mean (cwm) estimates of floral colour. We found strong patterns of elevational change in floral colour. Reflectancecwm of shorter wavelengths (UVB through human blue, 300–500 nm) generally decreased linearly with elevation, while reflectancecwm of longer wavelengths (human green through red,

500–700 nm) showed hump-shaped patterns with highest reflectance at intermediate elevations. With respect to pollinators, saturationcwm increased significantly with elevation in both bee and fly visual spaces, while brightness contrastcwm showed a hump-shaped pattern in bee space and a decline with elevation in fly visual space. For hue, cover of species perceived as bee-blue declined with elevation, while cover of bee-UV-green species showed a hump-shaped pattern. In comparison, we detected no elevational shifts in floral hues as perceived by flies.

Synthesis. Hue patterns are consistent with the hypothesis that bee pollinators have shaped the geography of floral colour. The roles of fly pollinators and of abiotic drivers are more difficult to infer, although the drop in floral brightness at high elevations is consistent with predictions that low temperatures and more intense ultraviolet radiation should favour increased pigment concentrations there. Our results indicate that floral colour can be dynamic yet predictable across the landscape, a pattern that provides opportunities to tease apart the ecological and evolutionary drivers of this important plant trait.

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Groulx, A. F. and J. R. K. Forrest (2018). "Nesting aggregation as a predictor of brood parasitism in mason bees (*Osmia* spp.)." Ecological Entomology **43**(2): 182-191.

1. Parasitism can be an important source of mortality for insect populations; however, we know little about the factors influencing vulnerability of wild bees to parasites. Mason bees (genus Osmia; Hymenoptera: Megachilidae) are important pollinators of crops and wild plants and are vulnerable to attack by brood parasites. High nest densities may increase rates of brood parasitism by attracting disproportionate numbers of parasites.
2. Three years of field observations from multiple sites were analysed to assess whether mason bee brood parasitism increased with host density. Mason bees were allowed to nest in artificial nesting blocks and establish natural variation in nesting density. Nest cells constructed by bees were checked for the presence of parasite eggs.
3. Parasitism of nest cells strongly increased with the number of actively nesting bees at a nesting block. Mason bees showed no preference for nesting in blocks that were occupied or unoccupied by other mason bees. Parasitism also increased with the number of days a nest was provisioned and decreased over the course of the season. Nest cells constructed last in a nest were significantly more parasitised than inner cells, despite being sealed against invasions.

4. These findings show positively density-dependent parasitism in mason bees. They also suggest that bees terminate parasitised nests, causing parasitised cells to become outermost nest cells – a behaviour that may represent a defence against parasites. Our results have implications for the management of mason bees as agricultural pollinators, as cultivating them at high densities could reduce offspring survival.

Hainsworth, F. R., et al. (1984). "Pollination and pre-dispersal seed predation: net effects on reproduction and inflorescence characteristics in *Ipomopsis aggregata*." Oecologia **63**: 405-409.

We examined net seed production for the self-incompatible, monocarp, Ipomopsis aggregata, by monitoring pre-pollination seed parasite (Hylemya sp.) oviposition and hummingbird mediated fruit set on 21 plants of variable height. Both pollination and seed predation increased with inflorescence height. Net seed production (incorporating seed predator mortality) also was positively related to height, and this would have been the case if pollination or seed predation were doubled. Although results suggest pomopsis aggregata should be under selective pressure to maximize inflorescence height, generation time and resource limits could result in advantages for inflorescences of intermediate height.

Heiling, J. M., et al. (2018). "Why are some plant–nectar robber interactions commensalisms?" Oikos **127**(11): 1679-1689.

Many plants that bear hidden or recessed floral nectar experience nectar robbing, the removal of nectar by a floral visitor through holes pierced in the corolla. Although robbing can reduce plant reproductive success, many studies fail to find such effects. We outline three mechanistic hypotheses that can explain when interactions between plants and nectar-robbers should be commensal rather than antagonistic: the non-discrimination (pollinators do not avoid robbed flowers), visitor prevalence (robber visitation is rare relative to pollinator visitation), and pollen saturation (stigmas receive sufficient pollen to fertilize all ovules with one or very few pollinator visits) hypotheses. We then explore these mechanisms in the North American subalpine, bumble bee-pollinated and nectar-robbed plant Corydalis caseana (Fumariaceae). We first confirmed that the effects of nectar robbing on female reproductive success were neutral in C. caseana. We then tested the three mechanisms underlying these neutral effects using a combination of observational studies and experiments. We found evidence for all three mechanisms. First, consistent with the non-discrimination hypothesis, pollinators failed to discriminate against experimentally robbed flowers or inflorescences even though naturally robbed flowers offered significantly lower nectar rewards than unrobbed flowers. Second, C. caseana was more commonly visited by pollinators than by nectar robbers, in accordance with the visitor prevalence hypothesis. Third, stigmas of unvisited flowers as well as those visited once by pollinators were saturated with pollen, with all stigmas bearing pollen loads several orders of magnitude higher than the number of ovules per fruit, consistent with the pollen saturation hypothesis. Our investigation of the mechanisms driving the commensal outcome of nectar robbing in this system deepens our understanding of the ecology of nectar robbing and contributes to a more general understanding of the variation in the outcomes of interactions between species.

Hersch, E. I. and B. A. Roy (2007). "Context-dependent pollinator behavior: An explanation for patterns of hybridization among three species of Indian paintbrush." Evolution **61**(1): 111-124.

In some areas of sympatry, reproductively compatible plant species hybridize, but in other areas of sympatry, they do not and they remain reproductively isolated from one another. Explanations offered to explain patterns of hybridization that vary by population have usually focused on genetic or environmental factors. Instead, we examined whether different community contexts might change pollinator preference and constancy and thus influence the likelihood of hybridization among three Indian paintbrush species (Castilleja miniata, C. rhexifolia, and C. sulphurea). To determine whether visitation was context-dependent, we observed pollinator behavior in experimental arrays (constructed using flowering stems of the three Indian paintbrush species) in different contexts. Contexts were defined by which Castilleja species occurred in the immediate neighborhood of the arrays. Specifically, we asked, does visitation to particular species in the arrays depend on context? In general, each Castilleja species was preferred when it matched the surrounding community context, as is predicted by optimal foraging theory. More interestingly, pollinator constancy was weakened in the hybrid context (an area where the three species co-occurred with morphologically intermediate plants), which is likely to increase pollen flow among the species. Reduced pollinator constancy in hybrid zones could set up a positive feedback loop in which more flower diversity is created through

hybridization, decreasing pollinator constancy, and leading to more hybridization. This self-reinforcing mechanism could lead to "hybridization hot spots" and to a patchy distribution of hybrid populations. We expect that this mechanism may be important in other animal-pollinated plant hybrid zones.

Hodges, C. M. and R. B. Miller (1981). "Pollinator flight directionality and the assessment of pollen returns." Oecologia **50**: 376-379.

Nectar-foraging pollinators often exhibit a directional pattern of movement between plants when the energetic costs of revisiting previously utilized areas can significantly reduce foraging efficiency. However, bumblebees (Bombus spp.) foraging for pollen on flowers of Aquilegia caerulea rarely moved in a straight line among successively visited plants. Most flights from plants visited were either to closely neighboring plants or were longer and involved bypassing near neighbor plants. Bees biased their flights toward plants with relatively large numbers of flowers yet visited only a small fraction of the flowers on each plant. Such foraging tactics might result when the energetic costs of revisiting plants are minor. Alternatively we suggest that bumblebees foraging for pollen may not perceive revisitations and their associated costs because they do not assess pollen returns on a per plant basis. In this case energetic-efficiency arguments predicting the pattern of foraging movements among plants may be inappropriate. A better level of analysis would be where the bees assess net energy returns, perhaps between bouts of pollen-combing and corbiculae-packing.

Holsinger, K. E. and J. D. Thomson (1994). "Pollen discounting in *Erythronium grandiflorum*: mass-action estimates from pollen transfer dynamics." American Naturalist **144**(5): 799-812.

Pollen discounting, a reduction in success as an outcross pollen parent as a result of selfing, can reduce or eliminate the reproductive advantage commonly attributed to selfing. Previous estimates of pollen discounting have been based on segregation analysis of progeny from open-pollinated plants. Using data from E. grandiflorum, they illustrate how direct measures of pollen transfer can be used to estimate discounting rates, and discuss the relationship between absolute discounting rates measured in this way and relative discounting rates measured through segregation analysis.

Iler, A. M., et al. (2013). "Long-term trends mask variation in the direction and magnitude of short-term phenological shifts." American Journal of Botany **100**(7): 1398-1406.

• Premise of the study: Plants are flowering earlier in response to climate change. However, substantial interannual variation in phenology may make it difficult to discern and compare long-term trends. In addition to providing insight on data requirements for discerning such trends, phenological shifts within subsets of long-term records will provide insight into the mechanisms driving changes in flowering over longer time scales.

• Methods: To examine variation in flowering shifts among temporal subsets of long-term records, we used two data sets of flowering phenology from snow-dominated habitats: subalpine meadow in Gothic, Colorado, USA (38 yr), and arctic tundra in Zackenberg, Greenland (16 yr). Shifts in flowering time were calculated as 10-yr moving averages for onset, peak, and end of flowering.

• Key results: Flowering advanced over the course of the entire time series at both sites. Flowering shifts at Gothic were variable, with some 10-yr time frames showing significant delays and others significant advancements. Early-flowering species were more responsive than later-flowering species, while the opposite was true at Zackenberg. Flowering shifts at Zackenberg were less variable, with advanced flowering across all 10-yr time frames. At both sites, long-term advancement seemed to be primarily driven by strong advancements in flowering in the 1990s and early 2000s.

• Conclusions: Analysis of long-term trends can mask substantial variation in phenological shifts through time. This variation in the direction and magnitude of phenological shifts has implications for the evolution of flowering time and for interspecific interactions with flowering plants and can provide more detailed insights into the dynamics of phenological responses to climate change.

Iler, A. M., et al. (2013). "Nonlinear flowering responses to climate: are species approaching their limits of phenological change?" Philosophical Transactions of the Royal Society B: Biological Sciences **368**(1624): 20120489.

Many alpine and subalpine plant species exhibit phenological advancements in association with earlier snowmelt. While the phenology of some plant species does not advance beyond a threshold snowmelt date,

the prevalence of such threshold phenological responses within plant communities is largely unknown. We therefore examined the shape of flowering phenology responses (linear versus nonlinear) to climate using two long-term datasets from plant communities in snow-dominated environments: Gothic, CO, USA (1974–2011) and Zackenberg, Greenland (1996–2011). For a total of 64 species, we determined whether a linear or nonlinear regression model best explained interannual variation in flowering phenology in response to increasing temperatures and advancing snowmelt dates. The most common nonlinear trend was for species to flower earlier as snowmelt advanced, with either no change or a slower rate of change when snowmelt was early (average 20% of cases). By contrast, some species advanced their flowering at a faster rate over the warmest temperatures relative to cooler temperatures (average 5% of cases). Thus, some species seem to be approaching their limits of phenological change in response to snowmelt but not temperature. Such phenological thresholds could either be a result of minimum springtime photoperiod cues for flowering or a slower rate of adaptive change in flowering time relative to changing climatic conditions.

Iler, A. M. and D. W. Inouye (2013). "Effects of climate change on mast-flowering cues in a clonal montane herb, *Veratrum tenuipetalum* (Melanthiaceae)." American Journal of Botany **100**(3): 519-525.

• Premise of the study: Climate change threatens to alter the timing and magnitude of abiotic cues that synchronize mast flowering, such as temperature and precipitation. Climate change may therefore alter the frequency of masting, in turn affecting species in the community that use pulsed resources.
• Methods: We used 29-yr (1984–2012) records of climate and flowering to investigate proximate flowering cues for the clonal, mast-flowering herb Veratrum tenuipetalum. Because clonal reproduction is tied to flowering in Veratrum, we used a parallel record of ramet abundance to examine the effects of masting on long-term ramet abundance.
• Key results: Cool summer temperatures 2 years before flowering were associated with a higher percentage of flowering in Veratrum populations, consistent with its life history. Ramet abundance increased by 9.5% ± 5.6% on average following mast years compared to an average loss of 0.73% ± 1.1% in nonmast years, and ramet abundance increased over the time frame of our records.
• Conclusions: Ramet abundance has increased over the time frame of our records mainly because of clonal reproduction in masting years. If summer temperatures continue to increase at our site and Veratrum does not alter its climate thresholds, we predict that masting will become less frequent in this species, with consequent reduction in opportunities for both sexual and clonal reproduction.

Iler, A. M., et al. (2013). "Maintenance of temporal synchrony between syrphid flies and floral resources despite differential phenological responses to climate." Glob Chang Biol **19**(8): 2348-2359.

Variation in species' responses to abiotic phenological cues under climate change may cause changes in temporal overlap among interacting taxa, with potential demographic consequences. Here, we examine associations between the abiotic environment and plant-pollinator phenological synchrony using a long-term syrphid fly-flowering phenology dataset (1992-2011). Degree-days above freezing, precipitation, and timing of snow melt were investigated as predictors of phenology. Syrphids generally emerge after flowering onset and end their activity before the end of flowering. Neither flowering nor syrphid phenology has changed significantly over our 20-year record, consistent with a lack of directional change in climate variables over the same time frame. Instead we document interannual variability in the abiotic environment and phenology. Timing of snow melt was the best predictor of flowering onset and syrphid emergence. Snow melt and degree-days were the best predictors of the end of flowering, whereas degree-days and precipitation best predicted the end of the syrphid period. Flowering advanced at a faster rate than syrphids in response to both advancing snow melt and increasing temperature. Different rates of phenological advancements resulted in more days of temporal overlap between the flower-syrphid community in years of early snow melt because of extended activity periods. Phenological synchrony at the community level is therefore likely to be maintained for some time, even under advancing snow melt conditions that are evident over longer term records at our site. These results show that interacting taxa may respond to different phenological cues and to the same cues at different rates but still maintain phenological synchrony over a range of abiotic conditions. However, our results also indicate that some individual plant species may overlap with the syrphid community for fewer days under continued climate change. This highlights the role of interannual variation in these flower-syrphid interactions and shows that species-level responses can differ from community-level responses in nonintuitive ways.

Iler, A. M., et al. (2017). "Detrending phenological time series improves climate–phenology analyses and reveals evidence of plasticity." Ecology **98**(3): 647-655.

Time series have played a critical role in documenting how phenology responds to climate change. However, regressing phenological responses against climatic predictors involves the risk of finding potentially spurious climate–phenology relationships simply because both variables also change across years. Detrending by year is a way to address this issue. Additionally, detrending isolates interannual variation in phenology and climate, so that detrended climate–phenology relationships can represent statistical evidence of phenotypic plasticity. Using two flowering phenology time series from Colorado, USA and Greenland, we detrend flowering date and two climate predictors known to be important in these ecosystems: temperature and snowmelt date. In Colorado, all climate–phenology relationships persist after detrending. In Greenland, 75% of the temperature–phenology relationships disappear after detrending (three of four species). At both sites, the relationships that persist after detrending suggest that plasticity is a major component of sensitivity of flowering phenology to climate. Finally, simulations that created different strengths of correlations among year, climate, and phenology provide broader support for our two empirical case studies. This study highlights the utility of detrending to determine whether phenology is related to a climate variable in observational data sets. Applying this as a best practice will increase our understanding of phenological responses to climatic variation and change.

Inouye, D. W. (1975). "Why don't more hummingbird-pollinated flowers have dark colored pollen?" American Naturalist **109**: 377-378.

Inouye, D. W. (1977). Species structure of bumblebee communities in North America and Europe. The role of arthropods in forest ecosystems. W. J. Mattson. New York, Springer Verlag**:** 35-40.

Inouye, D. W. (1978). "Resource partitioning in bumblebee guilds: experimental studies of foraging behavior." Ecology **59**: 672-678.

A system comprising 2 species of bumblebess (Bombus appositus and Bombus flavifrons) and 2 species of flowers (Delphinium barbeyi and Aconitum columbianum) in Gothic, Colorado, USA, was manipulated to determine whether resource utilization by each bumblebee species was influenced by the presence of the other species of bumblebee. Each bumblebee species concentrated its foraging efforts on a different flower species, apparently choosing the species whose corolla tube length matched its proboscis length most closely. When each bumblebee species was temporarily removed from its preferred flower species, the remaining bumblebee species increased visitation to the other, previously less-utilized, flower species. The remaining bumblebees visited more flowers per stay in the patch, suggesting that they were finding greater amounts of nectar in the absence of other bumblebee species. These removal experiments demonstrated that the bumblebees were sampling flowers frequently enough and were flexible enough in their foraging behavior to respond rapidly to short-term changes in nectar availability. In another area, where its preferred flower species and the other bumblebee species were absent, B. flavifrons foraged actively on the flower species it rarely used in Gothic. This observation and the experiments demonstrate that resource utilization by a bumblebee species is influenced by the presence of other species and suggest that the phenomenon of competitive release can be observed in bumblebees. In this system,interspecific exploitation competition appears to be the primary mechanism involved in resource partitioning.

Inouye, D. W. (1980). "The effect of proboscis and corolla tube lengths on patterns and rates of flower visitation by bumblebees." Oecologia **45**: 197-201.

The rates at which bumblebees of different proboscis lengths forage on flowers of a series of corolla tube lengths were determined. The results indicate significant correlations between proboscis length and time spent by bees on flowers. Bumblebees of long proboscis length can forage significantly faster than bees of shorter proboscis length on flowers with long corolla tubes. There is also evidence which suggests that bumblebees of short proboscis length prefer and are more efficient on short corolla tubes. These results support the use of proboscis length as a morphological indicator of resource utilization in bumblebees.

Inouye, D. W. (1980). "The terminology of floral larceny." Ecology **61**: 1251-1253.

Inouye, D. W. (2008). "Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers." Ecology **89**(2): 353-362.

The timing of life history traits is central to lifetime fitness and nowhere is this more evident or well studied as in the phenology of flowering in governing plant reproductive success. Recent changes in the timing of environmental events attributable to climate change, such as the date of snowmelt at high altitudes, which initiates the growing season, have had important repercussions for some common perennial herbaceous wildflower species. The phenology of flowering at the Rocky Mountain Biological Laboratory (Colorado, USA) is strongly influenced by date of snowmelt, which makes this site ideal for examining phenological responses to climate change. Flower buds of Delphinium barbeyi, Erigeron speciosus, and Helianthella quinquenervis are sensitive to frost, and the earlier beginning of the growing season in recent years has exposed them to more frequent mid-June frost kills. From 1992 to 1998, on average 36.1% of Helianthella buds were frosted, but for 1999–2006 the mean is 73.9%; in only one year since 1998 have plants escaped all frost damage. For all three of these perennial species, there is a significant relationship between the date of snowmelt and the abundance of flowering that summer. Greater snowpack results in later snowmelt, later beginning of the growing season, and less frost mortality of buds. Microhabitat differences in snow accumulation, snowmelt patterns, and cold air drainage during frost events can be significant; an elevation difference of only 12 m between two plots resulted in a temperature difference of almost 2°C in 2006 and a difference of 37% in frost damage to buds. The loss of flowers and therefore seeds can reduce recruitment in these plant populations, and affect pollinators, herbivores, and seed predators that previously relied on them. Other plant species in this environment are similarly susceptible to frost damage so the negative effects for recruitment and for consumers dependent on flowers and seeds could be widespread. These findings point out the paradox of increased frost damage in the face of global warming, provide important insights into the adaptive significance of phenology, and have general implications for flowering plants throughout the region and anywhere climate change is having similar impacts.

Inouye, D. W., et al. (1991). "The effect of floral abundance on feeder censuses of hummingbird abundance." Condor **93**: 279-285.

Numbers of Broad-tailed Hummingbirds (Selasphorous platycercus) captured each summer from 1979-1989 at the Rocky Mountain Biological Laboratory were quite variable, ranging from 115 (1981) to 348 (1989), with new birds usually outnumbering returning (previously banded) birds. Capture numbers were negatively correlated with the abundance of four species of flowers they visited, Erythronium grandiflorum, Delphinium nelsonii, Ipomopsis aggregata and Delphinium barbeyi; flower numbers were also highly variable during the study period. Since most of the captures were at feeders, these data suggest that in years with high floral abundance feeders are less attractive, while in years with low floral abundance hummingbirds with nests or territories at greater distances increase their use of the feeders. This interpretation is supported by seasonal variation in use of feeders, which is highest during the beginning and end of the season when floral abundance is lowest. Estimates of hummingbird density based on activity at feeders may thus be affected by the availability of floral food resources.

Inouye, D. W. and A. D. McGuire (1991). "Effects of snowpack on timing and abundance of flowering in *Delphinium nelsonii* (Ranunculaceae): implications for climate change." American Journal of Botany **78**(7): 997-1001.

Delphinium nelsonii is an early-blooming herbaceous perennial of montane western North America, which we studied in dry subalpine meadows in the Colorado Rocky Mountains. We examined the effects of variation in annual snowfall between 1973 and 1989 on the timing and abundance of flowering. During years of lower snow accumulation, D. nelsonii plants experienced colder temperatures between the period of snowmelt and flowering. Also, flowering was delayed, floral production was lower, and flowering curves were more negatively skewed; damage during floral development probably occurred in years of low snowfall. If climate change results in decreased mean annual snowfall for the Rocky Mountains, then the seed production of D. nelsonii will probably be adversely affected. Decreased snowfall may also indirectly lower the seed production of later-blooming species by decreasing populations of bumblebees and hummingbirds that forage on D. nelsonii flowers. Decreased snowfall has the potential to reduce the number and relative proportions of species in the herbaceous flora in our study area.

Inouye, D. W., et al. (2002). "Variation in timing and abundance of flowering by *Delphinium barbeyi* Huth (Ranunculaceae): the roles of snowpack, frost, and La Niña, in the context of climate change." Oecologia **139**: 543-550.

Delphinium barbeyi is a common herbaceous wildflower in montane meadows at 2,900 m near the Rocky Mountain Biological Laboratory, and its flowers are important nectar resources for bumblebees and hummingbirds. During the period 1977-1999 flowering was highly variable in both timing (date of first flower ranged from 5 July to 6 August, mean=17 July) and abundance (maximum open flowers per 222-m plot ranged from 11.3 to 197.9, mean=82). Time and abundance of flowering are highly correlated with the previous winter's snowpack, as measured by the amount of snow remaining on the ground on 15 May (range 0-185 cm, mean=67.1). We used structural equation modeling to investigate relationships among snowpack, first date of bare ground, first date of flowering, number of inflorescences produced, and peak number of flowers, all of which are significantly correlated with each other. Snowpack depth on 15 May is a significant predictor of the first date of bare ground (R2=0.872), which in turn is a significant predictor of the first date of flowering (R2=0.858); snowpack depth is also significantly correlated with number of inflorescences produced (R2=0.713). Both the number of inflorescences and mean date of first flowering are significant predictors of flowers produced (but with no residual effect of snowpack). Part of the effect of snowpack on flowering may be mediated through an increased probability of frost damage in years with lower snowpack - the frequency of early-season "frost events" explained a significant proportion of the variance in the number of flowers per stem. There is significant reduction of flower production in La Niña episodes. The variation in number of flowers we have observed is likely to affect the pollination, mating system, and demography of the species. Through its effect on snowpack, frost events, and their interaction, climate change may influence all of these variables.

Inouye, D. W. and G. H. Pyke (1988). "Pollination biology in the Snowy Mountains of Australia: comparisons with montane Colorado, USA." Australian Journal of Ecology **13**(2): 191-210.

Various aspects of the pollination biology of the alpine flora of Kosciusko National Park, NSW, were examined from late December 1983 until the end of March 1984, including flowering phenology, corolla tube lengths, flower colour, ultraviolet reflectance patterns, visitation rates to the flowers and proboscis lengths of the flower-visiting insects. An average of 5.3 species flowered in each of 13, 2 m×2 m montane plots and 5.6 species in the 13 alpine plots. The maximum number in flower simultaneously averaged 4.1 species in the montane and 3.3 in the alpine plots; flowering peaked in mid-January, Corolla tube lengths of the flora averaged 1.73 mm.

The most common floral colour was white or predominantly white (40 species), followed by yellow (14 species). Only six of the 38 species (16%) examined had some type of reflectance pattern; the remaining species all absobed ultraviolet.

Flies appeared to be the major pollinators. The insects collected in the study area comprised 60 species of Diptera, 33 species of Hymenoptera, and several species each of Lepidoptera and Coleoptera. On average, 14.4% of flowers watched during 379 observation periods (10 min each) were visited. On average, each plant species was visited by 6.4 species of flies, 2.4 species of bees, wasps or sawflies, one species of butterfly or moth and 0.3 species of beetles. Visitation rates increased over the growing season, and were significantly affected by ambient temperature (positively), light levels (positively) and wind speed (negatively).

The maximum proboscis length for the 25 most common species of bees was 2.76 mm, but 18 of 51 species of flies had proboscis lengths longer than this. The mean proboscis length for all 25 species of bees was 1.68 mm, and for 51 species of flies was 2.31 mm. Proboscis lengths for flies were positively correlated with the average corolla length for the plant species they visited. For bees, however, the range in proboscis lengths was relatively small and did not show this pattern.

There appear to be significant differences between the plant-pollinator community of alpine Australia and other alpine areas where bumblebees are common pollinators. These differences include shorter proboscis and corolla tube lengths, and perhaps an increased diversity and significance of flies as pollinators.

Inouye, D. W., et al. (2003). "Environmental influences on the phenology and abundance of flowering by *Androsace septentrionalis* L. (Primulaceae)." American Journal of Botany **90**(6): 905-910.

We studied the timing and abundance of flowering by Androsace septentrionalis L. (Primulaceae), an indeterminate winter annual or short-lived perennial, in 2 x 2 m plots at the Rocky Mountain Biological Laboratory in Colorado, USA, from 1982 to 2000. Flowers were counted every other day for most or all of the growing season in seven plots in a rocky meadow habitat and nine plots in a wet meadow habitat. The phenology and abundance of flowering were both highly variable, with mean dates of first flowering ranging from 16 May to 12 July and maximum daily counts of flowers ranging from 1 to 1187. Snowmelt date was the primary determinant of timing of flowering. For rocky meadow plots, the previous year's summer precipitation and the current year's average minimum temperature in May had significant effects on maximum number of flowers produced, but no environmental variable we considered was significantly correlated with flower abundance in the wet meadow plots. Length of flowering in individual plots ranged from 2 to 85 d, and many plot-years had both primary (about 1 mo) and secondary (about 10-12 d) flowering periods. The predicted increase in variability of precipitation accompanying climate change will affect negatively the long-term abundance and persistence of this species at our study site.

Inouye, D. W. and F. E. Wielgolaski (2003). High altitude climates. Phenology: an Integrative Environmental Science. M. D. Schwartz. PO Box 17/3300 AA Dordrecht/Netherlands, Kluwer Academic Publ**:** 195-214.

Irwin, R. and A. Brody (2011). "Additive effects of herbivory, nectar robbing and seed predation on male and female fitness estimates of the host plant *Ipomopsis aggregata*." Oecologia **166**(3): 681-692.

Many antagonistic species attack plants and consume specific plant parts. Understanding how these antagonists affect plant fitness individually and in combination is an important research focus in ecology and evolution. We examined the individual and combined effects of herbivory, nectar robbing, and pre-dispersal seed predation on male and female estimates of fitness in the host plant Ipomopsis aggregata. By examining the effects of antagonists on plant traits, we were able to tease apart the direct consumptive effects of antagonists versus the indirect effects mediated through changes in traits important to pollination. In a three-way factorial field experiment, we manipulated herbivory, nectar robbing, and seed predation. Herbivory and seed predation reduced some male and female fitness estimates, whereas plants tolerated the effects of robbing. The effects of herbivory, robbing, and seed predation were primarily additive, and we found little evidence for non-additive effects of multiple antagonists on plant reproduction. Herbivory affected plant reproduction through both direct consumptive effects and indirectly through changes in traits important to pollination (i.e., nectar and phenological traits). Conversely, seed predators primarily had direct consumptive effects on plants. Our results suggest that the effects of multiple antagonists on estimates of plant fitness can be additive, and investigating which traits respond to damage can provide insight into how antagonists shape plant performance.

Irwin, R. E. and A. K. Brody (1998). "Nectar robbing in *Ipomopsis aggregata*: effects on pollinator behavior and plant fitness." Oecologia **116**(4): 519-527.

Hummingbirds foraging in alpine meadows of central Colorado, United States, face a heterogeneous distribution of nectar rewards.This study investigated how variability in nectar resources caused by nectar-robbing bumblebees affected the foraging behavior of hummingbird pollinators and, subsequently, the reproductive success of a host plant (Ipomopsis aggregata). We presented hummingbirds with experimental arrays of I. aggregata and measured hummingbird foraging behavior as a function of known levels of nectar robbing. Hummingbirds visited significantly fewer plants with heavy nectar robbing (over 80% of available flowers robbed) and visited fewer flowers on those plants. These changes in hummingbird foraging behavior resulted in decreased percent fruit set as well as decreased total seed set in heavily robbed plants.These results indicate that hummingbird avoidance of nectar-robbed plants and flowers reduces plant fitness components. In addition, our results suggest that the mutualisms between pollinators and host plants may be affected by other species, such as nectar robbers.

Irwin, R. E. and A. K. Brody (1999). "Nectar-robbing bumble bees reduce the fitness of *Ipomopsis aggregata* (Polemoniaceae)." Ecology **80**(5): 1703-1712.

The removal of floral nectar by nectar robbers may change the behavior of legitimate pollinators and, consequently, the pollination and fertilization success of flowering plants. We measured the effects of a nectar-robbing bumble bee, Bombus occidentalis, on male and female reproductive success of Ipomopsis aggregata (Polemoniaceae). We artificially varied nectar-robbing rates within 20 natural I. aggregata populations in Colorado. Using powdered fluorescent dyes as pollen analogs, we estimated the amount of pollen donated (male fitness component) and the amount of pollen received (female fitness component) by plants with different levels of nectar robbing. We also measured percentage fruit set per plant, seed set per fruit, and average seed mass as additional components of female fitness. Nectar robbing significantly decreased both male and female fitness components. Heavy nectar robbing (>80% of available flowers robbed) significantly decreased dye donation, pollen receipt, and average number of seeds per fruit at the whole-plant level. Furthermore, nectar robbing significantly decreased dye donation by individual flowers. Although nectar robbing has often been regarded as unimportant to plant reproductive success, we document significant effects of nectar robbing on components of both male and female fitness of I. aggregata. Our results suggest that interactions among nectar robbers, pollinators, and their plant hosts may have important consequences for plant fitness.

Irwin, R. E. and A. K. Brody (2000). "Consequences of nectar robbing for realized male function in a hummingbird-pollinated plant." Ecology **81**(9): 2637-2643.

The effects of nectar robbers on plants and their mutualistic pollinators are poorly understood due, in part, to the paucity of studies examining male reproductive success in nectar-robbed plants. Here we measured the effects of a nectar-robbing bumblebee, Bombus occidentalis, on realized male reproductive success (seeds sired) in a hummingbird-pollinated plant, Ipomopsis aggregata. To determine the effects of nectar robbing on paternity, we used a series of experimental populations of plants containing a known allozyme marker. In each population, we experimentally controlled the levels of nectar robbing on each I. aggregata plant by cutting a hole in the corolla with dissecting scissors and removing nectar with a micro-capillary tube. We measured hummingbird-pollinator foraging behavior and fruit and seed production (maternal function) for each plant. We then genotyped seeds for the allozyme marker to determine the number of seeds sired by plants with known levels of robbing. Heavy nectar robbing (> 80% of flowers robbed) significantly reduced the number of seeds sired, as well as the number of seeds produced due to pollinator avoidance of heavily robbed plants. Total plant reproduction, both male and female contributions, were reduced by 50% due to high levels of robbing. To date, no other studies have measured the effects of nectar robbing on realized male function (number of seeds sired). Ours is the first study to demonstrate that robbing can simultaneously decrease realized male reproductive success as well as female reproductive success, and that the effects are incurred indirectly through pollinator avoidance of robbed plants.

Irwin, R. E., et al. (2001). "The impact of floral larceny on individuals, populations, and communities." Oecologia **129**(2): 161-168.

Many insects and other animals that visit flowers are not mutualistic pollinators, but rather "behavioral robbers" which pierce flowers to extract nectar, and "thieves" which enter flowers in the normal way but provide little or no pollination service. Although the study of floral larceny has grown rapidly in the last two decades, the importance of larceny for individual fitness and for population- and community-level phenomena is only now becoming apparent. Here we synthesize the current understanding of floral larceny by reviewing and re-analyzing existing published data, by presenting new data of our own, and by suggesting avenues of further research. First, we perform a meta-analysis on existing studies, which shows that larceny has an overall detrimental effect on female reproductive success of plants, and that effect size depends on the types of robbers, thieves, and pollinators that interact as well as on the reproductive biology of the plant. This quantitative analysis improves upon a recently published qualitative analysis of larceny and plant fitness. Next, we discuss the possibility that larcenists and pollinators can select in different directions on floral traits, possibly contributing to the standing variation in floral phenotypes that is observed within natural populations. Larceny has the potential to affect plant population dynamics, so long as offspring recruitment and survival depend on seed production, a point we illustrate with data from the montane herb Ipomopsis aggregata. Our studies of this species also show how larcenists may influence community-level dynamics, by linking plant species that they rob or by influencing other plant species through altered

behavior of shared pollinators. Population- and community-level effects of larceny, and their possible roles in stabilizing pollination food webs, provide rich prospects for future research.

Irwin, R. E., et al. (2008). "Mechanisms of tolerance to floral larceny in two wildflower species." Ecology **89**(11): 3093-3104.

Tolerance of foliar damage is widely recognized as an effective defense against herbivores and pathogens. However, tolerance of the impacts of antagonists on pollination success is less well understood. Here, we extend the framework of tolerance to foliar damage to understand how plants mitigate the pollination and fitness costs of floral larceny (i.e., the consumption of floral nectar often without pollination). We focused on two mechanisms: high nectar rewards per flower to feed all floral visitors and high flower production to compensate for reproductive losses under reduced pollination and seed set. We compared the efficacy of these mechanisms in two plant species: Polemonium viscosum and Ipomopsis aggregata. In Polemonium, ants acting as larcenists reduce nectar accumulation but do not completely empty flowers. When nectar reserves were augmented, ant consumption increased, negating the efficacy of this putative tolerance mechanism. Similarly, in Ipomopsis, nectar addition had little effect on tolerance to larceny by bumble bees, perhaps because residual intact flowers do not have enough nectar to compensate for lost rewards. Flower production in both species mitigated some of the negative impacts of larceny on seed set. In Polemonium, flower number was not plastic in response to larceny, but large inflorescences enhanced female fitness only when larcenists were present, suggesting that "surplus" flowers in large inflorescences can function to replace reproductive losses due to larceny. In Ipomopsis, high rates of larceny induced flower production, but the fecundity benefits of making more flowers declined inversely to larcenist intensity. Overall, our results suggest (1) that tolerance to floral larceny involves "banking" extra flowers to replace lost reproduction rather than maintaining pollination of ones with larceny, and (2) that the efficacy of flower production as a tolerance mechanism varies inversely to larceny rate.

Jones, K. N. (1997). "Analysis of pollinator foraging: Tests for non-random behaviour." Functional Ecology **11**(2): 255-259.

1. A standardized protocol for analysing the behaviour of pollinators foraging on more than one plant type (species or morph) is needed.

2. A protocol is presented in which the first step is to test whether foraging trips are homogeneous in the frequency of visits to each plant type, or whether there are two or more groups of pollinators with different foraging preferences.

3. Tests for foraging preference and constancy in the sequence of plants visited then should be made separately for each homogeneous group.

4. A hypothetical example is given in which ignoring heterogeneity of preference would lead to the acceptance of a false null hypothesis of random behaviour.

5. A real example is given in which heterogeneity of preference was the only non-random aspect of pollinator behaviour (no significant positive constancy or overall preference), while transfer of powdered dye particles (pollen analogues) was assortative among floral morphs.

6. Heterogeneity of preference is probable, as many factors may cause individual pollinators to forage differently on the same patch of plants, and may be sufficient to produce non-random pollen transfer among plant morphs.

Jones, K. N., et al. (1998). "A trade-off between the frequency and duration of bumblebee visits to flowers." Oecologia **117**(1/2): 161-168.

Pollinator behavior influences plant reproduction in many ways. A traditional measure of pollination, the number of visits received, may be a poor predictor of plant reproductive success, particularly when there are trade-offs between visit quantity and components of visit quality. For example, the duration of pollinator visits may be negatively correlated with the number of visits received by a flower. We tested for a trade-off

between the number of bumblebee visits and the duration of those visits in an experimental population of snapdragons (Antirrhinummajus: Scrophulariaceae). The duration of a bumblebee visit to a flower increased significantly with the time interval since theflower had last been visited. Over the lifetime of a flower the correlation between the total number and average duration of visits received by a flower was weakly negative. However, at the whole-plant level the correlation was positive: plants whose flowers received more visits also received visits of longer duration. Factors affecting the relationship between quantity and duration of pollinator visits to flowers also were investigated. Two factors weakened the negative dependence of average visit duration on number of visits received by individual snapdragon flowers: (1) the correlation between the total number of visits to a flower and the average interval between visits was only -0.53, as visits to individual flowers were not very evenly spaced over time, and (2) newly opened flowers received fewer and shorter visits than older flowers. Comparing whole plants, nectar production per flower varied dramatically across individuals, a probable explanation for the positive correlation between visit number and average duration per flower observed at the plant level. The potential for a trade-off between these two components of pollinator service exists when visit duration depends on reward quantity; whether the trade-off is realized will depend on variation in nectar production and on whether pollinators forage systematically.

Kearns, C. A. (1992). "Anthophilous fly distribution across an elevation gradient." American Midland Naturalist **127**: 172-182.

Flower-visiting Diptera are abundant in montane and Arctic areas. This research, conducted on the western slope of the Colorado Rocky Mountains: (1) characterizes the predominant anthophilous fly taxa in comparison with other high-elevation and high-latitude sites; (2) examines changes in fly abundance with elevation, and (3) examines the potential of flies as pollinators by examining quality and quantity of pollen loads. Flies collected from 66 flowering species were identified to species to produce a data base containing taxonomic information, elevation and floral records. Malaise trap censuses were conducted in three elevational zones. Pollen carried by flies and pollen in their guts were quantified and examined to determine the number of plant species represented. Proportional representation of dipteran families changed in a similar manner in different mountain systems and the Arctic. The predominance of flies on flowers at high elevations appears to be due to a decrease in representation of other orders of insects. Most fly taxa fed at multiple plant species, but gut contents indicated that individual flies fed largely on a single pollen type. Total pollen loads of flies and solitary bees were similar although flies collected on Linum lewisii carried less pollen than bees.

Kearns, C. A. and D. W. Inouye (1993). "Pistil-packing flies." Natural History **102**: 30-37.

Kearns, C. A. and D. W. Inouye (1994). "Fly pollination of *Linum lewisii* (Linaceae)." American Journal of Botany **81**(9): 1091-1095.

This study examines the reproductive biology of Linum lewisii Pursh. (Linaceae), a polyphilic species visited by small bees and generalist flies in montane Colorado. L. lewisii plants growing at different sites experience large temporal and spatial variations in pollinator visits. Their ability to attract both dipteran and hymenopteran pollinators allows pollination under varying conditions as pollinator pool composition changes. Although L. lewisii is self-compatible, hand-pollination studies indicate that insects are required for seed production. The relative effectiveness of fly and bee pollinators is assessed in terms of per-visit pollen deposition. Insect visitation patterns are combined with per-visit effectiveness data to evaluate the relative importance of different pollinator groups. Overall, bees tend to be more effective than flies in depositing pollen. However, in many instances flies appear to be responsible for more pollen deposition due to their higher visitation rates.

Klumpers, S. G. T., et al. (2019). "Foraging efficiency and size matching in a plant–pollinator community: the importance of sugar content and tongue length." Ecology Letters **In press**.

A long-standing question in ecology is how species interactions are structured within communities. Although evolutionary theory predicts close size matching between floral nectar tube depth and pollinator proboscis length of interacting species, such size matching has seldom been shown and explained in multispecies assemblages. Here, we investigated the degree of size matching among Asteraceae and their pollinators and its relationship with foraging efficiency. The majority of pollinators, especially Hymenoptera, choose plant

species on which they had high foraging efficiencies. When proboscides were shorter than nectar tubes, foraging efficiency rapidly decreased because of increased handling time. When proboscides were longer than nectar tubes, a decreased nectar reward rather than an increased handling time made shallow flowers more inefficient to visit. Altogether, this led to close size matching. Overall, our results show the importance of nectar reward and handling time as drivers of plant–pollinator network structure.

Kohn, J. R. and N. M. Waser (1985). "The effect of *Delphinium nelsonii* pollen on seed set in *Ipomopsis aggregata*, a competitor for hummingbird pollination." American Journal of Botany **72**: 1144-1148.

Sympatric plant species can compete for pollination services in several ways. For example, pollinators may move between species and deposit heterospecific pollen on stigmas, which in turn may reduce the efficacy of conspecific pollen. We explored this possibility by determining the effect of Delphinium nelsonii pollen on seed set in Ipomopsis aggregata. These montane herbs are pollinated by hummingbirds, experience heterospecific pollen deposition in nature, and suffer reduced seed set in each other's presence. We hand-pollinated flowers of I. aggregata with either pure conspecific pollen or a mixture of pollen of the two species. Resulting pollen loads on stigmas ranged from 0-865 D. nelsonii grains and from 10-336 I. aggregata grains; mean seed set per flower was 11.3. There was no detectable effect of D. nelsonii pollen load on I. aggregata seed set. It is possible that seed set reductions seen in previous studies of competition for pollination between these species were caused by pollen wastage, pollen layering on the pollinator, or the temporal sequence of pollen arrival at the stigma.

Lambert, A. M., et al. (2010). "Changes in snowmelt date and summer precipitation affect the flowering phenology of *Erythronium grandiflorum* (glacier lily; Liliaceae)." Am. J. Bot. **97**(9): 1431-1437.

* Premise of the study: Climate change has affected species worldwide, including alterations in phenology, migration patterns, distribution, and survival. Because Erythronium grandiflorum is an early-season bloomer, alterations in its phenology may have serious implications for many North American Rocky Mountain communities, including changes in resource availability for pollinators and herbivores.
* Methods: We investigated whether changes in the snowmelt date, summer temperature, and summer precipitation have altered the timing and abundance of flowering in E. grandiflorum by collecting long-term data on floral abundance from 1975-2008 in a series of 2 x 2 m plots at the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado in the United States.
* Key results: Snowmelt date and mean summer temperature were negatively correlated. Over the 30-yr study, the snowmelt date advanced by 4.14 d/decade, and mean summer temperature increased by 0.38{degrees}C/decade. Summer precipitation was variable, showing no change. The first, peak, and last flowering dates of E. grandiflorum advanced an average of 3.2 d/decade. Furthermore, earlier snowmelt and greater summer precipitation in the previous year led to earlier flowering in E. grandiflorum. There was no change in flowering abundance in this species, indicating it may be controlled by a complex set of abiotic and biotic variables.
* Conclusions: Our study indicates that snowmelt is arriving earlier at the RMBL, which has caused earlier flowering in E. grandiflorum. Because alterations in phenology can disrupt important ecological interactions, information on potential phenological shifts in species that interact with E. grandiflorum is essential in determining the net effect of climate-driven alterations in phenology.

Lichtenberg, E. M., et al. (2018). "Costs and benefits of alternative food handling tactics help explain facultative exploitation of pollination mutualisms." Ecology **99**(8): 1815-1824.

Many mutualisms are taken advantage of by organisms that take rewards from their partners but provide no benefit in return. In the absence of traits that limit exploitation, facultative exploiters (partners that can either exploit or cooperate) are widely predicted by mutualism theory to choose an exploitative strategy, potentially threatening mutualism stability. However, it is unknown whether facultative exploiters choose to exploit, and, if so, make this choice because it is the most beneficial strategy for them. We explored these questions in a subalpine plant-insect community in which individuals of several bumble bee species visit flowers both "legitimately" (entering via the flower opening, picking up and depositing pollen, and hence behaving mutualistically) and via nectar robbing (creating holes through corollas or using an existing hole, bypassing stigmas and anthers). We applied foraging theory to (1) quantify handling costs, benefits and foraging efficiencies incurred by three bumble bee species as they visited flowers legitimately or robbed

nectar in cage experiments, and (2) determine whether these efficiencies matched the food handling tactics these bee species employed in the field. Relative efficiencies of legitimate and robbing tactics depended on the combination of bee and plant species. In some cases (Bombus mixtus visiting Corydalis caseana or Mertensia ciliata), the robbing tactic permitted more efficient nectar removal. As both mutualism and foraging theory would predict, in the field, B. mixtus visiting C. caseana were observed more frequently robbing than foraging legitimately. However, for Bombus flavifrons visiting M. ciliata, the expectation from mutualism theory did not hold: legitimate visitation was the more efficient tactic. Legitimate visitation to M. ciliata was in fact more frequently observed in free-flying B. flavifrons. Free-flying B. mixtus also frequently visited M. ciliata flowers legitimately. This may reflect lower nectar volumes in robbed than unrobbed flowers in the field. These results suggest that a foraging ecology perspective is informative to the choice of tactics facultative exploiters make. In contrast, the simple expectation that exploiters should always have an advantage, and hence could threaten mutualism persistence unless they are deterred or punished, may not be broadly applicable.

Loughnan, D., et al. (2014). "*Taraxacum officinale* pollen depresses seed set of montane wildflowers through pollen allelopathy." Journal of Pollination Ecology **13**.

Plant species that share pollinators can suffer from interspecific pollen deposition. Male reproductive success is inevitably reduced by the loss of pollen to flowers of another species. Female reproductive success can be affected by reduced stigmatic area or, more strongly, through allelopathic effects by which the admixture of some foreign pollen reduces seed or fruit set. We tested for allelopathic effects of Taraxacum officinale (Asteracaeae) pollen on the seed set of montane wildflowers Erythronium grandiflorum (Liliaceae) and Erysimum capitatum (Brassicaceae), by hand-pollinating plants with pollen mixtures. Taraxacum is a common invasive species, which produces allelopathic chemicals in its root and vegetative tissue, making it a likely candidate for pollen allelopathy. Flowers of both species produced fewer well-developed seeds when pollinated with pollen mixtures containing Taraxacum pollen. The pollen-allelopathic potential of weedy dandelion may add to its ability to disrupt communities that it invades.

Luo, E. Y., et al. (2014). "Stimulation of flower nectar replenishment by removal: A survey of eleven animal-pollinated plant species." 2014 **12**.

Understanding the interaction between reward-seeking flower feeding animals and plants requires consideration of the dynamic nature of nectar secretion. Studies on several plants suggest that nectar secretion may increase in response to its removal, but it is not clear whether the phenomenon is widespread. We determined whether 11 species of Colorado mountain wildflowers showed removal-enhanced nectar replenishment (RENR). We measured floral phenology, nectar volumes, rate of replenishment, and compared the cumulative nectar produced following five hourly removals with that accumulated after five hours. Nectar replenishment occurred rapidly, within minutes; statistically significant RENR was observed in 9 of our 11 study species, with the strongest effects in bee-pollinated species. We discuss the implications of RENR in plant species on the measurement of nectar, the adaptive advantage of RENR, and the energetic costs of RENR.

Maloof, J. E. (2001). "The effects of a bumble bee nectar robber on plant reproductive success and pollinator behavior." American Journal of Botany **88**(11): 1960-1965.

Interactions between a plant species (Corydalis caseana), a bumble bee nectar robber (Bombus occidentalis), and a bumble bee pollinator (B. appositus) were studied. There were no significant differences between naturally robbed and unrobbed flowers in fruit set or mean seed set per fruit. Plots of C. caseana plants were subjected to treatments of robbing and no robbing using commercially available colonies of B. occidentalis. Robbers did not pollinate the flowers. Pollinator behavior was observed to determine (1) the number of bees attracted to each plot, (2) the number of inflorescences visited in a plot, (3) the number of flowers visited on each inflorescence, and (4) the distance flown between inflorescences. There were no significant differences in the number of inflorescences visited per bee or the number of flowers visited per inflorescence per bee when robbed and unrobbed treatments were compared. Of the parameters measured, only distance flown between inflorescences differed in the robbed and the unrobbed treatments. Bees flew significantly further between inflorescences in the robbed plots than in the unrobbed plots. The results indicate that the nectar

robbers have no negative effect on fruit set or seed set in C. caseana and that they may cause increased pollen flow distances by changing the behavior of the pollinator.

Mayfield, M. M., et al. (2001). "Exploring the `Most Effective Pollinator Principle' with complex flowers: Bumblebees and *Ipomopsis aggregata*." Annals of Botany **88**(4): 591-596.

The `most effective pollinator principle' implies that floral characteristics often reflect adaptation to the pollinator that transfers the most pollen, through a combination of high rate of visitation to flowers and effective deposition of pollen during each visit. We looked for the expected positive correlation between quantity and quality of visits in Ipomopsis aggregata, whose red, tubular flowers are considered to be adapted to hummingbirds. Hummingbirds were indeed the most common floral visitors in 5 years of observation. However, long-tongued bumblebees deposited on average three-times as much outcross pollen per visit to virgin flowers, and elicited four-times as much seed production, as did hummingbirds. Hence visitors that are relatively infrequent, and unexpected given the `pollination syndrome' of the plant, can be surprisingly good pollinators. One interpretation of this observation is that natural selection favours a specialized floral morphology that excludes all but a single type of visitor, but that there are constraints on achieving this outcome. An alternative is that selection favours some degree of floral generalization, but that flowers can retain features that adapt them to a particular type of pollinator in spite of this generalization.

McKinney, A. M., et al. (2012). "Asynchronous changes in phenology of migrating Broad-tailed Hummingbirds and their early-season nectar resources." Ecology **93**(9): 1987-1993.

Phenological advancements driven by climate change are especially pronounced at higher latitudes, so that migrants from lower latitudes may increasingly arrive at breeding grounds after the appearance of seasonal resources. To explore this possibility we compared dates of first arrival of Broad-tailed Hummingbirds (Selasphorus platycercus) to dates of flowering of plants they visit for nectar. Near the southern limit of the breeding range, neither hummingbird arrival nor first flowering dates have changed significantly over the past few decades. At a nearby migration stopover site, first flowering of a major food plant has advanced but peak flowering has not. Near the northern limit of the breeding range, first and peak flowering of early-season food plants have shifted to earlier dates, resulting in a shorter interval between appearance of first hummingbirds and first flowers. If phenological shifts continue at current rates, hummingbirds will eventually arrive at northern breeding grounds after flowering begins, which could reduce their nesting success. These results support the prediction that migratory species may experience the greatest phenological mismatches at the poleward limits of their migration. A novel hypothesis based on these results posits that the poleward limit for some species may contract toward lower latitudes under continued warming.

Meléndez-Ackerman, E. and D. R. Campbell (1998). "Adaptive significance of flower color and inter-trait correlations in an *Ipomopsis* hybrid zone." Evolution **52**(5): 1293-1303.

Flower color is often viewed as a trait that signals rewards to pollinators, such that the relationship between flower color and plant fitness might result from its association with another trait. We used experimental manipulations of flower color and nectar reward to dissociate the natural character correlations present in a hybrid zone between Ipomopsis aggregata and Ipomopsis tenuituba. Isozyme markers were used to follow the male and female reproductive success of these engineered phenotypes. One field experiment compared fitnesses of I. aggregata plants that varied only in flower color. Plants with flowers painted red received more hummingbird visits and sired more seeds than did plants with flowers painted pink or white to match those of hybrids and I. tenuituba. Our second field experiment compared fitnesses of I. aggregata, I. tenuituba, and hybrid plants in an unmanipulated array and in a second array where all flowers were painted red. In the unmanipulated array, I. aggregata received more hummingbird visits, set more seeds per flower, and sired more seeds per flower. These fitness differences largely disappeared when the color differences were eliminated. The higher male fitness of I. aggregata was due to its very high success at siring seeds on conspecific recipients. On both I. tenuituba and hybrid recipients, hybrid plants sired the most seeds, despite showing lower pollen fertility than I. aggregata in mixed donor pollinations in the greenhouse. Ipomopsis tenuituba had a fitness of only 13% relative to I. aggregata when traits varied naturally, compared to a fitness of 36% for white relative to red flowers when other traits were held constant.

Meléndez-Ackerman, E., et al. (1997). "Hummingbird behavior and mechanisms of selection on flower color in *Ipomopsis*." Ecology **78**(8): 2532-2541.

Apparent associations between flower color and type of pollinator might be explained by correlations between color and other floral traits, rather than by narrow pollinator preferences for color. To explore this possibility, we obtained flowers varying naturally in color, nectar reward, and morphology, from a hybrid zone between Ipomopsis aggregata and I. tenuituba in western Colorado, United States. We used aviary and held experiments to study preferences of hummingbirds for unmanipulated flowers and for flowers in which we experimentally dissociated color from nectar reward and morphology. Hummingbirds preferred red flowers of I. aggregata, which contain superior nectar rewards and have relatively broad corolla tubes, relative to flowers of I. tenuituba, which are white, contain small nectar rewards, and have narrow tubes. There was no evidence of flower constancy. When presented with flowers differing only in color, birds showed a spontaneous preference for red. However, this preference could be reversed by making white flowers more rewarding than red. When plants of both parental species and their hybrids were placed in an array in the field, with all flowers painted red, hummingbirds preferred to visit the more rewarding, wider tubed I. aggregata. These results suggest that pollinator-mediated selection could act on color, shape, and nectar separately. Experimental manipulations that dissociate these traits are essential to distinguish direct from correlated selection. Also, hummingbird selection favoring red flowers may result from past experience and, thus, may depend on the ecological context.

Miller, R. B. (1978). "The pollination ecology of *Aquilegia elegantula* and *A. caerulea* (Ranunulaceae) in Colorado." American Journal of Botany **65**: 406-414.

Aquilegia elegantula Greene and A. caerulea James occur in montane and subalpine habitats in the southern Rocky Mountains of western North America The red and yellow flowers of A. elegantula are nodding, odorless, protogynous, and secrete a concentrated (44%) sucrose nectar in the floral spurs Seed set in flowers under pollinator exclosures was 12% while seed set in open-pollinated flowers was 65%. The flowers of A. elegantula are pollinated primarily by the Broad-tailed Hummingbird (Selasphorus platycercus [Swainson]) and by at least three species of pollen-foraging bumblebees, of which Bombus occidentalis Greene is the most common. The blue and white flowers of A. caerulea are erect, mildly fragrant, protandrous, and secrete a 26% sucrose nectar. Seed set in caged flowers in the field averaged 39%, in uncaged flowers 54%. The most important pollinators of A. caerulea are the crepuscular hawkmoth, Hyles (=Celerio) lineata (Fabricius) and ten species of pollen-foraging Bombus. The most abundant bumblebee species, B occidentalis, is also a frequent nectar thief. Differences in pollination systems alone probably do not constitute an effective anti-hydridization mechanism between A. elegantula and A. caerulea, but do serve to reinforce differences in habitat and flowering time that distinguish the two species.

Miller, R. B. and C. L. Willard (1983). "The pollination ecology of *Aquilegia micrantha* (Ranunculaceae) in Colorado." Southwestern Naturalist **28**: 157-164.

Aquilegia micrantha occurs in canyons of the Southwestern United States. The pinkish-yellow flowers are nodding or semi-nodding, mildly scented, protandrous, and secrete a concentrated nectar (46% total sugars). Seed set in flowers under pollinator exclosures was 25% compared with 63% for open-pollinated flowers. The most common and effective pollinators of A. micrantha in the upper Crystal River Canyon were nectar-foraging bumblebee queens, Bombus appositus and B. flavifrons, although hummingbirds and hawkmoths also visited the flowers, and hummingbirds are important pollinators at other sites. The absence of nectar-foraging Bombus queens on the red flowers of A. elegantula in the area is probably the result of the narrower spur mouths of A. elegantula that prevent the bees from inserting their heads deeply enough into the flowers to extract the nectar. This mechanical isolation may be important in restricting hybridization between the two species in the narrow elevational range of sympatry.

Miller, S. J. and D. W. Inouye (1983). "Roles of the wing whistle in the territorial behaviour of male broad-tailed hummingbirds (*Selasphorus platycercus*)." Animal Behaviour **31**(3): 689-700.

Suggested roles of avian wing sounds in territory defence have not been tested experimentally. The non-facultative, shrill wing whistle of male broad-tailed hummingbirds occurs during aerial displays used in defence of courting territories. To investigate the roles of the wing whistle in territorial behaviour, we observed males before and after being experimentally silenced, and after having wing whistles restored.

Silenced birds intruded in territories more easily than normal males, suggesting that the wing whistle is a conspicuous signal advertising the presence of adult, male birds. Birds were less active and aggressive in territory defence after silencing than they were either before silencing or after restoration of the wing whistle. Silenced birds tended to lose their territories to rival hummingbirds more readily than non-silenced birds. We propose that silenced birds defend their territories less effectively because they cannot communicate threat and because they lack sensory feedback that normally enhances a bird's aggressiveness. Results of this study suggest that the wing whistle of male broad-tailed hummingbirds is important in maintenance of courting territories and hence in reproductive success.

Miller-Rushing, A. J. and D. W. Inouye (2009). "Variation in the impact of climate change on flowering phenology and abundance: An examination of two pairs of closely related wildflower species." American Journal of Botany **96**(10): 1821-1829.

Variability in plant phenological responses to climate change is likely to lead to changes in many ecological relationships as the climate continues to change. We used a 34-yr record of flowering times and flower abundance for four species (two Delphinium [Ranunculaceae] species and two Mertensia [Boraginaceae] species) from a subalpine plant community near the Rocky Mountain Biological Laboratory to test the hypothesis that the phenologies of early-flowering species change more rapidly in response to climatological and other abiotic cues than do late-flowering species, a pattern previously found in plant communities in North America and Europe. We also explored a related hypothesis, that flower abundance of late-flowering species is more responsive to changes in climate than that of early-flowering species. The Delphinium species did not support these hypotheses, but the Mertensia species did. The difference between the peak flowering times of the early and late Mertensia species is expanding, leading to a period of diminished resources for pollinators that specialize on this genus. Mertensia ciliata populations are already severely declining in our study area, possibly as a result of earlier snowmelt. Together, these results show that the reported differences between early- and late-flowering species may be widespread, but they are not ubiquitous.

Mitchell, R. J. (1993). "Adaptive significance of *Ipomopsis aggregata* nectar production: observation and experiment in the field." Evolution **47**(1): 25-35.

Success through male function (estimated by export of fluorescent dyes) was significantly greater for plants with naturally high nectar production rates than for nearby plants with low nectar production rates, whereas success through female function (receipt of fluorescent dye) was unrelated to nectar production rate. Experimental addition of artificial nectar also produced a significant increase in male function and no increase in several estimates of female function success.

Mitchell, R. J. (1994). "Effects of floral traits, pollinator visitation, and plant size on *Ipomopsis aggregata* fruit production." American Naturalist **143**(5): 870-889.

The basic hypothesis proposes that pollinator behavior may be affected by plant traits, including floral nectar production rate, corolla size, number of open flowers, and inflorescence height, and that behavior, along with plant size, may influence reproductive success through female function. For 2 populations the basic hypothesis is significantly better as an explanation of the interactions than alternative hypotheses that ignore the effects of plant traits on pollinator behavior or plant size on reproduction.

Mitchell, R. J. and N. M. Waser (1992). "Adaptive significance of *Ipomopsis aggregata* nectar production: Pollination success of single flowers." Ecology **73**(2): 633-638.

Floral nectar rewards are expected to contribute to plant fitness by influencing several aspects of pollinator behavior. For example, when flowers have large standing crops of nectar, pollinators may take longer to probe them, thereby increasing the amounts of pollen deposited and removed. In addition, pollinators may return more often to rewarding plants and/or probe more of their flowers during each visit. We experimentally investigated how each of these behaviors influences pollination success of the hummingbird—pollinated herb Ipomopsis aggregata (scarlet gilia). Captive hummingbirds were trained to probe flowers containing known volumes of artificial nectar (25% mass/mass sucrose solution). Increasing the nectar standing crop of a flower from 1 to 5 μL significantly increased probe duration, but this had no detectable effect on pollination success through the female sexual function (pollen deposited on stigmas) or

male sexual function (pollen removed from anthers and number of fluorescent dye particles exported to other flowers). In contrast, both male and female pollination success increased with the number of times a hummingbird probed a flower. For male function, there was at best a weak tendency for pollen export to diminish with successive probes, in contrast to strongly diminishing returns reported for bee—pollinated flowers. This difference may reflect foraging mode; hummingbirds hover and remove a small fraction of available pollen with each floral probe whereas bees land and remove most pollen with the first probe. For plants such as I. aggregata whose pollination success is most sensitive to the number of probes per flower, selection on nectar production may be strongest when there are cues pollinators can use to identify rewarding plants, such as phenotypic correlations between nectar production and floral morphology.

Moldenke, A. R. and P. G. Lincoln (1979). "Pollination ecology in montane Colorado: a community analysis." Phytologia **42**: 349-379.

Montalvo, A. M. (1992). "Relative success of self and outcross pollen comparing mixed- and single-donor pollination in *Aquilegia caerulea*." Evolution **46**(4): 1181-1198.

Flowers frequently receive both self (S) and outcross (OC) pollen, but S pollen often sires proportionally fewer seeds. Failure of S pollen can reflect evolved mechanisms that promote outcrossing and/or inbreeding depression expressed during seed development. The relative importance of these two processes was investigated in Aquilegia caerulea, a self-compatible perennial herb. In the field I performed single-donor (S or OC) and mixed-donor (S plus OC) pollinations to compare the relative success of both pollen types at various stages from pollen germination to seed maturity. Single-donor S pollinations produced significantly fewer and lighter seeds (x decrease = 12% and 3%, respectively) than OC pollinations. Abortion rates differed by an average of 38% whereas fertilization rates differed by only 5%, indicating that most differences in seed number arose postzygotically. This suggests that inbreeding depression was responsible for most failure of S pollen. One prezygotic effect measured was that 10% fewer S than OC pollen tubes reached ovaries after 42 hr, suggesting S pollen might fertilize proportionately fewer ovules after mixed pollination. Using allozyme markers, I found mixed-donor pollinations produced significantly more and heavier outcrossed than selfed seeds. However, the proportion of selfed seed, fertilized ovules, and aborted seeds for mixed-donor fruits were each predictable from pollen performance in single-donor fruits, suggesting that differential paternity is best explained by inbreeding depression during seed development. Even given these similarities between mixed- and single-donor fruits in the relative performance of S and OC pollen, both individual seed weight and seed set were significantly higher in multiply-sired fruits.

Niitepold, K., et al. (2014). "Aging, life span, and energetics under adult dietary restriction in lepidoptera." Physiol Biochem Zool **87**(5): 684-694.

Stressful conditions can affect resource allocation among different life-history traits. The effect of dietary restriction (DR) on longevity and reproduction has been studied in many species, but we know little about its effects on energetics, especially in flying animals that have high energy demand. We assessed the effects of DR on metabolic rate throughout the entire adult life span in two butterfly species, Colias eurytheme and Speyeria mormonia. We cut the food intake of adult females in half and measured resting metabolic rate (RMR) and flight metabolic rate (FMR) together with body mass repeatedly throughout life. In both species, DR reduced body mass, but mass-corrected FMR was not affected, indicating that flight capacity was retained. DR lowered RMR and reduced fecundity but had no effect on life span. FMR declined with age, but the rate of senescence was not affected by DR. In contrast, aging had a strong negative effect on RMR only in control females, whereas food-restricted females had more stable RMR throughout their lives. The results suggest that flight capacity is conserved during nutritional stress but that investment in flight and survival may negatively affect other important physiological processes when resources are limited.

O'Brien, D. M., et al. (2004). "Making eggs from nectar: the role of life history and dietary carbon turnover in butterfly reproductive resource allocation." Oikos **105**(2): 279-291.

The diets of many butterflies and moths change dramatically with development: from herbivory in the larvae to nectarivory in the adults. These diets are nutritionally distinct, and thus are likely to contribute differentially to egg manufacture. We examine the use of dietary resources in egg manufacture by four butterfly species with different patterns of oviposition and lifespan; three in the Nymphalidae (Euphydryas

chalcedona, Speyeria mormonia and Heliconius charitonia), and one in the Pieridae (Colias eurytheme). Each species was fed two isotopically distinct adult diets based on sucrose, both of which differed from the larval hostplant in 13C content. Egg isotopic composition was analyzed to quantify the contribution of carbon from the larval and adult diets to egg manufacture. In all four species, egg 13C content increased to an asymptotic maximum with time, indicating that adult diet is an increasingly important source of egg carbon . The 13C increase closely resembled that of a nectar-feeding hawkmoth, and was well-described by a model of carbon flow proposed for that species. This similarity suggests that the turnover from larval to adult dietary support of egg manufacture is conserved among nectar-feeding Lepidoptera. Species varied widely in the maximum % egg carbon that derives from the adult diet, from 44% in E. chalcedona to nearly 80% in S. mormonia. These differences were related both to the extent of oocyte provisioning prior to adult emergence, and to egg composition. A species' lifetime use of larval vs adult resources in egg manufacture reflected both the carbon turnover of the eggs and the timing of oviposition. Thus, the extent to which dietary resources are important in egg manufacture in butterflies depends on development (egg provisioning in teneral adults), behavior (timing of oviposition) and nutritional physiology (nutrient synthesis and turnover).

Ogilvie, J. E. and J. R. K. Forrest (2017). "Interactions between bee foraging and floral resource phenology shape bee populations and communities." Current Opinion in Insect Science **21**: 75-82.

Flowers are ephemeral, yet bees rely on them for food throughout their lives. Floral resource phenology – which can be altered by changes in climate and land-use – is therefore key to bee fitness and community composition. Here, we discuss the interactions between floral resource phenology, bee foraging behaviour, and traits such as diet breadth, sociality, and body size. Recent research on bumble bees has examined behavioural responses to local floral turnover and effects of landscape-scale floral resource phenology on fitness, abundance, and foraging distances. Comparable studies are needed on non-social, pollen-specialist species. We also encourage greater use of information contained in museum collections on bee phenologies and floral hosts to test how phenology has shaped the evolution of bee–plant associations.

Ogilvie, J. E., et al. (2017). "Interannual bumble bee abundance is driven by indirect climate effects on floral resource phenology." Ecology Letters **20**(12): 1507-1515.

Climate change can influence consumer populations both directly, by affecting survival and reproduction, and indirectly, by altering resources. However, little is known about the relative importance of direct and indirect effects, particularly for species important to ecosystem functioning, like pollinators. We used structural equation modelling to test the importance of direct and indirect (via floral resources) climate effects on the interannual abundance of three subalpine bumble bee species. In addition, we used long-term data to examine how climate and floral resources have changed over time. Over 8 years, bee abundances were driven primarily by the indirect effects of climate on the temporal distribution of floral resources. Over 43 years, aspects of floral phenology changed in ways that indicate species-specific effects on bees. Our study suggests that climate-driven alterations in floral resource phenology can play a critical role in governing bee population responses to global change.

Ogilvie, J. E. and J. D. Thomson (2015). "Male bumble bees are important pollinators of a late-blooming plant." Arthropod-Plant Interactions **9**(2): 205-213.

Differences in the pollinator performance of flower visitor sexes are rarely considered. In bumble bees, males differ from workers in morphology and behaviour in ways that may affect their contribution to pollination. We compared the abundance, foraging behaviour, and pollen transfer ability of worker and male bumble bees on late-blooming Gentiana parryi (Gentianaceae) in subalpine meadows of the Colorado Rocky Mountains. Male bees mostly outnumbered female workers throughout blooming of the gentian. Males and workers foraged similarly, though individual males were more faithful to small foraging areas than workers. During single flower visits, males and workers caused similar levels of pollen deposition and seed production, yet female bees left fewer pollen grains in anthers to be transferred to other stigmas in the plant population. Overall, male bumble bees are common and capable pollinators of G. parryi and in some years and sites could be more important than workers. Male bumble bees may be important but unrecognized pollinators of other late-season plant species, and animal sexes may differ in their pollinator performance in other systems.

Ogilvie, J. E. and J. D. Thomson (2016). "Site fidelity by bees drives pollination facilitation in sequentially blooming plant species." Ecology **97**(6): 1442-1451.

Plant species can influence the pollination and reproductive success of coflowering neighbors that share pollinators. Because some individual pollinators habitually forage in particular areas, it is also possible that plant species could influence the pollination of neighbors that bloom later. When flowers of a preferred forage plant decline in an area, site-fidelity may cause individual flower feeders to stay in an area and switch plant species rather than search for preferred plants in a new location. A newly blooming plant species may quickly inherit a set of visitors from a prior plant species, and therefore experience higher pollination success than it would in an area where the first species never bloomed. To test this, we manipulated the placement and timing of two plant species, Delphinium barbeyi and later-blooming Gentiana parryi. We recorded the responses of individually marked bumble bee pollinators. About 63% of marked individuals returned repeatedly to the same areas to forage on Delphinium. When Delphinium was experimentally taken out of bloom, most of those site-faithful individuals (78%) stayed and switched to Gentiana. Consequently, Gentiana flowers received more visits in areas where Delphinium had previously flowered, compared to areas where Delphinium was still flowering or never occurred. Gentiana stigmas received more pollen in areas where Delphinium disappeared than where it never bloomed, indicating that Delphinium increases the pollination of Gentiana when they are separated in time. Overall, we show that individual bumble bees are often site-faithful, causing one plant species to increase the pollination of another even when separated in time, which is a novel mechanism of pollination facilitation.

Pardee, G. L., et al. (2018). "Direct and indirect effects of episodic frost on plant growth and reproduction in subalpine wildflowers." Global Change Biology **24**(2): 848-857.

Frost is an important episodic event that damages plant tissues through the formation of ice crystals at or below freezing temperatures. In montane regions, where climate change is expected to cause earlier snow melt but may not change the last frost-free day of the year, plants that bud earlier might be directly impacted by frost through damage to flower buds and reproductive structures. However, the indirect effects of frost mediated through changes in plant–pollinator interactions have rarely been explored. We examined the direct and pollinator-mediated indirect effects of frost on three wildflower species in southwestern Colorado, USA, Delphinium barbeyi (Ranunculaceae), Erigeron speciosus (Asteraceae), and Polemonium foliosissimum (Polemoniaceae), by simulating moderate (−1 to −5°C) frost events in early spring in plants in situ. Subsequently, we measured plant growth, and upon flowering measured flower morphology and phenology. Throughout the flowering season, we monitored pollinator visitation and collected seeds to measure plant reproduction. We found that frost had species-specific direct and indirect effects. Frost had direct effects on two of the three species. Frost significantly reduced flower size, total flowers produced, and seed production of Erigeron. Furthermore, frost reduced aboveground plant survival and seed production for Polemonium. However, we found no direct effects of frost on Delphinium. When we considered the indirect impacts of frost mediated through changes in pollinator visitation, one species, Erigeron, incurred indirect, negative effects of frost on plant reproduction through changes in floral traits and pollinator visitation, along with direct effects. Overall, we found that flowering plants exhibited species-specific direct and pollinator-mediated indirect responses to frost, thus suggesting that frost may play an important role in affecting plant communities under climate change.

Pelton, J. (1961). "An investigation of the ecology of *Mertensia ciliata* in Colorado." Ecology **42**: 38-52.

Pelton, J. S. (1957). "Evidence of introgressive hybridization and mutation in certain Colorado populations of *Aquilegia*." Proceedings of the Indiana Academy of Science **67**: 292-296.

Pelton, J. S. (1959). "Variation patterns in four clones of *Mertensia ciliata*." Madroño **15**(4): 123-128.

Petry, W. K., et al. (2016). "Sex-specific responses to climate change in plants alter population sex ratio and performance." Science **353**(6294): 69-71.

Males and females are ecologically distinct in many species, but whether responses to climate change are sex-specific is unknown. We document sex-specific responses to climate change in the plant Valeriana edulis (valerian) over four decades and across its 1800-meter elevation range. Increased elevation was associated

with increased water availability and female frequency, likely owing to sex-specific water use efficiency and survival. Recent aridification caused male frequency to move upslope at 175 meters per decade, a rate of trait shift outpacing reported species' range shifts by an order of magnitude. This increase in male frequency reduced pollen limitation and increased seedset. Coupled with previous studies reporting sex-specific arthropod communities, these results underscore the importance of ecological differences between the sexes in mediating biological responses to climate change.

Pleasants, J. M. (1980). "Competition for bumblebee pollinators in Rocky Mountain plant communities." Ecology **61**: 1446-1459.

Meadow plant communities at four sites in the Colorado Rocky Mountains were studied to evaluate the influence of competition for bumblebee pollinators. The niche relationships among plant species were characterized by overlap with respect to species of bumblebee visitors and times of flowering. Species in each community could be divided into two to three guilds based upon the identity of the species' major bumblebee visitor. Only the members of a guild would potentially be in competition with one another for pollinators. The competition hypothesis is that guild members have segregated blooming periods which would minimize competition. The null hypothesis is that blooming periods are dispersed randomly through time. The competition hypothesis was tested by comparing the actual temporal spacing pattern for a guild with random patterns generated by computer simulation. For 10 out of 11 guilds the sequence of blooming periods was more regular than expected (either at the statistically significant level [five cases] or near it [five cases]). I also examined the relationships among guild members using another measure of the competitive effect of one species on another which is more accurate than temporal overlap. This variable, called competitive load, includes information on the overlap, abundance, and floral attractiveness of competitors. It is an indicator of the number of visits a species loses to competitors. In general species were found to lose approximately half the number of visits they could potentially receive during the time they were in flower. All species received approximately the same number of visits despite large differences in floral abundance. Thus the pool of available pollinator visits appears to be rather evenly divided among the species in a community. This regularity in resource partitioning is further inferential evidence of competition for pollinators. Rare species avoid the potentially heavy loss of visitors to more abundant competitors by having flowers which are more attractive to pollinators. Attractiveness appears to be a function of the nectar production rate of a species' flowers. There are some exceptions to these generalizations. Species which can also reproduce vegetatively are less attractive, and lose more visits that expected. The competitive effect that on species experiences because of the presence of another may be the result of a loss of pollinator visits (exploitation) and/or a disruption of conspecific pollen flow (interference). Species may reduce the interference component of competition by spatial isolation. For the one guild that had a random flowering sequence with broad overlap among species, spatial isolation and competition between guild members were positively correlated. Temporal divergence and differential attractiveness are seen to be primarily a means of avoiding exploitation competition.

Pleasants, J. M. and N. M. Waser (1985). "Bumblebee foraging at a "hummingbird" flower: reward economics and floral choice." American Midland Naturalist **114**: 283-291.

For a brief period in 1981 Bombus appositus queens visited Ipomopsis aggregata, a hummingbird-pollinated species with floral characteristics typical of that pollination syndrome. This behavior was not observed in other years. Despite the fact that Ipomopsis lacks floral features associated with bee visitation, bumblebees foraged on Ipomopsis at a rate only somewhat slower than that for a typical bee flower. Because of the deep corolla, bumblebees are able to probe only a limited distance into Ipomopsis flowers but in 1981 nectar was more accessible due to a higher standing crop than in other years. While foraging on Ipomopsis, bees were able to obtain a net energetic profit which was similar to that obtained from their primary forage species. These observations indicate that floral choice is governed primarily by profitability - which can overcome innate preferences for bee flowers - and that the features of hummingbird flowers pose no absolute barrier to bee visitation. Bees apparently sample a wide spectrum of available flower types and will cross pollination syndrome lines if the reward economics are favorable.

Pleasants, J. M. and M. Zimmerman (1983). "The distribution of standing crop of nectar: what does it really tell us?" Oecologia **57**: 412-414.

Brink (1982) characterizes the distribution of standing crop of nectar for Delphinium nelsonii as bonanzablank, based on comparison with a Poisson. He then discusses possible effects of standing crop variability on pollinator foraging behavior. We disagree with the use of the Poisson and the resulting conclusions. The expected distribution should not be based on doling out random amounts of nectar to flowers, but based on random return times to flowers by pollinators (elapsed time=nectar accumulated). When this model is used, standing crop variance does not differ markedly from expectation. What differences do exist can be accounted for by variability in nectar production rates of individual plants. We also take issue with the use of the bonanza-blank terminology. As originally formulated this refers to nectar production differences within a plant rather than standing crop differences among plants.

Pleasants, J. M. and M. Zimmerman (1990). "The effect of inflorescence size on pollinator visitation of *Delphinium nelsonii* and *Aconitum columbianum*." Collectanea Botanica (Barcelona) **19**: 21-39.

Two factors have been suggested to play a role in determining the limit to inflorescence size within a species; energy limitation and diminishing pollination returns for larger inflorescence sizes. In an effort to assess the significance of pollination limitation we examined the effects of inflorescence size on pollinator visitation patterns for 2 species, Aconitum columbianum and Delphinium nelsonii. These species are similar in their pollination biology, and both have a racemose inflorescence, but they differ markedly in inflorescence size (A. columbianum has from 1-26 open flowers while D. nelsonnii has 1-6 open flowers). For each species the following parameters were examined as a function of inflorescence size: visits per inflorescence, flowers visited per visit, and visits per flower. For D. nelsonii all 3 parameters increased with increasing inflorescence size although for large inflorescences the rate of increase slowed slightly for flowers per visit and visits per flower. For A. columbianum all 3 parameters also generally increased with increasing inflorescence size but for the largest sizes were was no further increase in visits per inflorescence or flowers per visit and there was a decrease in visits per flower. The pattern of a smaller increase in attractiveness with progressively larger inflorescences corresponds to what would be expcted if inflorescence attractiveness were based on the concept of just noticeable difference. The observed pattern of a smaller increase in flowers per visit with increasingi inflorescence size can be described accurately by a model in which there is, on average, a fixed probability of moving from one flower to another on an inflorescence. The number of visits per flower.....

Pohl, N. B., et al. (2011). "Butterflies show flower colour preferences but not constancy in foraging at four plant species." Ecological Entomology **36**(3): 290-300.

1. The extent to which flower colour and other visual cues influence butterfly flower choice in the field is poorly understood, especially in comparison with choices by Hymenoptera.
2. Using a novel approach to studies of visitation behaviour by butterflies, flower colour of four Asteraceae species was phenotypically manipulated to decouple the influence of that trait from others (including morphology and nectar rewards) on visitation by Lycaena heteronea, Speyeria mormonia, Cercyonis oetus, and Phyciodes campestris.
3. Flower visits were recorded to experimental flower arrays in subalpine meadows to measure (i) spontaneous preference by butterflies for particular colours and other traits and (ii) flower constancy (longer than expected strings of visits made to flowers of the same species), a behaviour that can reduce interspecific gene flow in plants.
4. Over three field seasons, 3558 individual flower visits in 1386 foraging bouts were observed for free-flying butterflies. All four butterfly species responded to the phenotypic manipulations of flower colour, although in different ways. Speyeria mormonia and L. heteronea also exhibited preferences based on other flower traits. Lycaena heteronea responded to combinations of traits such that the other traits it preferred depended upon the context of flower colour.
5. None of the butterfly species exhibited flower constancy in any of the arrays employed.
6. The observed preferences show that butterflies, like some other pollinators, are potentially capable of exerting selection on colour and other floral traits. Moreover, these flower preferences can depend on the context of other flower traits. The absence of constancy contrasts with reports of high constancy in many bees.

Price, M. V., et al. (2008). "Bridging the generation gap in plants: Pollination, parental fecundity, and offspring demography." Ecology **89**(6): 1596-1604.

Despite extensive study of pollination and plant reproduction on the one hand, and of plant demography on the other, we know remarkably little about links between seed production in successive generations, and hence about long-term population consequences of variation in pollination success. We bridged this "generation gap" in Ipomopsis aggregata, a long-lived semelparous wildflower that is pollinator limited, by adding varying densities of seeds to natural populations and following resulting plants through their entire life histories. To determine whether pollen limitation of seed production constrains rate of population growth in this species, we sowed seeds into replicated plots at a density that mimics typical pollination success and spacing of flowering plants in nature, and at twice that density to mimic full pollination. Per capita offspring survival, flower production, and contribution to population increase (λ) did not decline with sowing density in this experiment, suggesting that typical I. aggregata populations freed from pollen limitation will grow over the short term. In a second experiment we addressed whether density dependence would eventually erase the growth benefits of full pollination, by sowing a 10-fold range of seed densities that falls within extremes estimated for the natural "seed rain" that reaches the soil surface. Per capita survival to flowering and age at flowering were again unaffected by sowing density, but offspring size, per capita flower production, and λ declined with density. Such density dependence complicates efforts to predict population dynamics over the longer term, because it changes components of the life history (in this case fecundity) as a population grows. A complete understanding of how constraints on seed production affect long-term population growth will hinge on following offspring fates at least through flowering of the first offspring generation, and doing so for a realistic range of population densities.

Price, M. V. and N. M. Waser (1979). "Pollen dispersal and optimal outcrossing in *Delphinium nelsoni*." Nature **277**: 294-297.

NATURAL SELECTION, in sexually reproducing plants, should often favour matings between individuals of intermediate genetic similarity. Matings between very similar individuals may lead to inbreeding depression because segregational load is revealed1,2, while matings between very dissimilar individuals may disrupt favourable gene combinations and lead to outbreeding depression3–5. Outbreeding depression in plants has been documented in crosses between species, varieties and isolated populations6–9, and reports of inbreeding depression date back at least a century10 We suggest that outbreeding depression will often occur on a much finer scale than previously recognised, especially in plants subject to restricted pollen and seed dispersal. Such plants are likely to show pronounced microgeographic genetic differentiation resulting from drift in subpopulations isolated by distance or from adaptation to local edaphic and biotic conditions11,12 Under these circumstances, a short outcrossing distance may be optimal not only because of intragenotypic effects, but also because it produces offspring sufficiently similar to the female parent to grow successfully near her, yet sufficiently genotypically diverse to maximise success of the total progeny in the face of coarse-grained temporal environmental variation13–15, frequency-dependent sibling competition16–18 or predation19–20. Here we present evidence that a short outcrossing distance is optimal for Delphinium nelsoni Greene and discuss the relationship between the optimal outcrossing distance for D. nelsoni and actual pollen dispersal by its main pollinators.

Price, M. V. and N. M. Waser (1982). "Experimental studies of pollen carryover: hummingbirds and *Ipomopsis aggregata*." Oecologia **54**: 353-358.

We present results of experiments designed to identify floral characteristics that influence patterns of pollen carryover by hummingbirds visiting Ipomopsis aggregata flowers. We used fluorescent dye powders as pollen analogues. For all four experimental treatments considered, amounts of dye deposited on recipient stigmas declined linearly as a function of flower position in a visitation sequence. The decline was significantly steeper when recipient flowers had pollen-carrying anthers than when they did not; whereas degree of stigma clogging and presence or absence of empty anthers did not influence carryover. From this we conclude that presence of pollen on recipient flowers significantly reduces the average number of subsequent flowers reached by donor pollen. We discuss mechanisms for this effect and its significance for the evolution of floral structure.

Price, M. V. and N. M. Waser (1998). "Effects of experimental warming on plant reproductive phenology in a subalpine meadow." Ecology **79**(4): 1261-1271.

Increasing "greenhouse" gases are predicted to warm the earth by several degrees Celsius during the coming century. At high elevations one likely result is a longer snow-free season, which will affect plant growth and reproduction. We studied flowering and fruiting of 10 angiosperm species in a subalpine meadow over 4 yr, focusing on plant responses to warming by overhead heaters. The 10 species reproduced in a predictable sequence during 3-4 mo between spring snowmelt and fall frosts. Experimental warming advanced the date of snowmelt by almost 1 wk on average, relative to controls, and similarly advanced the mean timing of plant reproduction. This phenological shift was entirely explained by earlier snowmelt in the case of six plant species that flowered early in the season, whereas four later-flowering species apparently responded to other cues. Experimental warming had no detectable effect on the duration of flowering and fruiting, even though natural conditions of early snowmelt were associated with longer duration and greater overlap of reproduction of sequentially flowering species. Fruit set was greater in warmed plots for most species, but this effect was not significant for any species individually. We conclude that global warming will cause immediate phenological shifts in plant communities at high elevations, mediated largely through changes in timing of snowmelt. Shifts on longer time scales are also likely as plant fitnesses, population dynamics, and community structure respond to altered phenology of species relative to one another and to animal mutualists and enemies. However, the small spatial scale of experiments such as ours and the inability to perfectly mimic all elements of climate change limit our ability to predict these longer term changes. A promising future direction is to combine experiments with study of natural phenological variation on landscape and larger scales.

Price, M. V., et al. (2005). "Temporal and spatial variation in pollination of a montane herb: A seven-year study." Ecology **86**(8): 2106-2116.

Pollination by animals is critical to sexual reproduction of most angiosperms. However, little is known about variation in pollination service to single plant species. We report results of a long-term study of Ipomopsis aggregata, a semelparous montane herb whose flowers are visited by hummingbird and insect pollinators as well as “floral larcenists.” We censused flower visitors over seven summers at permanent study sites separated by several hundred meters, and counted pollen delivered to flowers on a subset of plants observed for visitation. The species composition of the community of visitors varied significantly across years and within the flowering season; sites varied significantly only in the magnitude of parallel annual changes in the visitor community. Rates of flower visitation fluctuated over an order of magnitude or more. Variation in mean stigma pollen load among plants flowering in the same site and year was explained by a causal path model in which visitation rates by pollinators and larcenists had linear positive and negative effects, respectively. A simplified model including only pollinators explained almost as much variance as did the full model. However, qualitatively different parameter estimates were produced by an analogous causal model based on population means across site–year combinations. Discrepant results from within- and between-population levels of analysis suggest that pollen receipt is influenced by environmental factors that vary among sites and years, as well as by pollinator visit rates. We present a heuristic causal model that includes such factors, and we note its implications for ecological and evolutionary studies of pollination.

Primack, R. B. and D. W. Inouye (1993). "Factors affecting pollinator visitation rates: A biogeographic comparison." Current Science **65**(3): 257-262.

Careful observations of flower visitors can provide estimates of pollinator visitation rates. Biological communities in varying biogeographic regions were compared using the same techniques. Bees were the predominant pollinators at low elevation sites, whereas flies were the most important pollinators at high elevations. Flower visitation rates varied by a factor of 10 across biological communities, suggesting that pollination activity may be limiting seed production in some places. Visitation rates were highly variable within communities, with temperature, wind speeed, and time of the season explaining some of the variation. A picture emerges of relatively unspecialized relationships amont the plants and animals involved in pollination, influenced by a variety of environmental and ecological factors.

Pulliam, H. R. and N. M. Waser (2010). Ecological invariance and the search for generality. The Ecology of Place: Contributions of Place-Based Research to Ecological Understanding. I. Billick and M. V. Price. Chicago, University of Chicago Press**:** 69-92.

Pyke, G. H. (1978). "Optimal foraging: movement patterns of bumblebees between inflorescences." Theoretical Population Biology **13**: 72-98.

Nectar-collecting bumblebees are hypothesized to employ rules of movement which result in the maximum net rate of energy gain (i.e., are optimal). The optimal movement rules are derived from a mathematical model and are used to generate predicted patterns of movement. The predicted patterns are compared with field observations. These observations support the hypothesis. An important component of the mathematical model is the memory of the foraging animal. The field data have implications concerning the memory capabilities of the bumblebees.

Pyke, G. H. (1981). "Hummingbird foraging on artificial inflorescences." Behaviour Analysis Letters **1**: 11-15.

Hummingbirds were observed foraging for nectar on 3 kinds of artificial inflorescences—linear, spiral, and "natural." The natural inflorescences, which mimicked natural inflorescences of the Ss' principal food plant, were more similar to the spiral than to the linear inflorescences. The extent to which flowers were reprobed during a visit to an inflorescence was found to increase from natural to spiral to linear inflorescences.

Pyke, G. H. (1981). "Optimal foraging in hummingbirds: rule of movement between inflorescences." Animal Behaviour **29**: 889-896.

The movements of hummingbirds between inflorescences of scarlet gilia (Ipomopsis aggregata) were studied. These movements exhibited the following patterns: (1) Although the hummingbirds appeared to avoid moving to the previous inflorescence, no significant correlation was found between the directions of successive inter-inflorescence movements. (2) The frequency distribution of inter-inflorescence flight distances was found to be leptokurtic. (3) The hummingbirds were more likely to move to an inflorescence the larger and/or closer it was. (4) The hummingbirds moved to inflorescences of greatest apparent size (i.e. ratio of number of flowers available to distance from present inflorescence) more often than they moved to the largest inflorescence, the closest infloresence, or the inflorescence estimated to yield the greatest rate of energy gain. (5) The frequency distribution of moves to the inflorescence having the ith greatest apparent size is well fitted by a geometric distribution. This is consistent with the hummingbrids choosing the inflorescence of greatest apparent size (excluding the previous inflorescence) from within some scanning sector. These movement patterns are consistent with the expectations of optimal foraging theory only if the hummingbirds cannot or do not determine the directions of possible inflorescences relative to the direction of arrival at the present inflorescence and if they cannot assess independently the sizes and distances of possible inflorescences.

Pyke, G. H. (1981). "Optimal nectar production in a hummingbird pollinated plant." Theoretical Population Biology **20**: 326-343.

It is hypothesized that the average rate of nectar production per flower for a population of plants is such than an individual plant which possesses this rate has maximum fitness (i.e., is optimal). This basic hypothesis is used to develop predictions concerning nectar production in scarlet gilia (Ipomopsis aggregata), a hummingbird pollinated plant. The optimal standing crop of nectar per flower is not significantly different from the observed.

Pyke, G. H. (1982). "Foraging in bumblebees: rule of departure from an inflorescence." Canadian Journal of Zoology **60**: 417-428.

Most aspects of the bumblebees' rule of departure from an inflorescence of Aconitum columbianum are qualitatively what would be expected if the bumblebees maximize their net rate of energy gain. Flower revisitation increases with increases in the number of flowers already probed on an inflorescence and with decreases in the number of flowers available. Nectar volume per flower tends to decrease with increasing relative flower height and there is a positive correlation between nectar volumes of flowers from the same inflorescence. Consequently the bumblebees should be increasingly likely to leave an inflorescence with increases in either the number of flowers already probed or the relative height of the last flower and with decreases in either the number of flowers available or the nectar volume obtained at the last flower. The bumblebees showed all these trends.

Pyke, G. H. (1982). "Local geographic distributions of bumblebees near Crested Butte, Colorado: competition and community structure." Ecology **63**: 555-573.

It was hypothesized that the local geographic distributions of bumblebees near Crested Butte, Colorado and the community patterns exhibited by these bumblebees are the products of competition for plants. To evaluate this hypothesis several transects were established and at regular intervals throughout a summer growing season, data were collected along each transect on how many bumblebees of each species and caste were visiting the various plant species. The distributions and abundances of the plant species involved were recorded qualitatively. Seven species of bumblebees accounted for 97% of all bumblebees observed and in this paper attention is restricted to these species. Five other species were observed in very low numbers. Each bumblebee species had a different distributional pattern. The seven bumblebee species form four groups in terms of both their proboscis lengths and the corolla lengths of the plants they preferentially visit. Long—, medium—, and short—tongued groups were most often observed foraging at flowers with long, medium, and short corollas, respectively. Furthermore proboscis lengths of bumblebees tended to be very similar within each group but quite dissimilar between groups. The fourth group consisted of a single short—tongued species which has well—developed mandibles which enable it to rob nectar from many plants with long corollas. It also feeds legitimately on short—corolla flowers. Some anomalies in the above groupings are mentioned and discussed. When the data on bumblebees and plant distributions are combined with data on flower preferences, a pattern consistent with the competition hypothesis emerges. Within each proboscis—length group, bumblebee species tend to replace one another altitudinally in a manner consistent with the hypothesis. The nectar—robbing species is most abundant in areas where a plant that is usually visited by hummingbirds is most common. Other species of bumblebees are unable to gain access to the nectar of this plant. In any uniform well—isolated area, similar floristically to the present study area, only three or four species of bumblebees appear to be able to coexist. Furthermore, a bumblebee community in such an area will apparently consist of a short—, a medium—, and a long—tongued species and in some cases a short—tongued nectar—robber. These patterns are also consistent with the competition hypothesis, as similarity in proboscis length reflects similarity in diet and the intensity of competition should, for these bumblebees, be closely related to diet similarity. It is possible that the observed distributional patterns could also be explained on the basis of different distributions of suitable nest sites for each bumblebee species or different responses to local variations in climatic conditions. Neither alternative seems able, however, to explain the observed patterns.

Pyke, G. H., et al. (2011). "Activity and abundance of bumble bees near Crested Butte, Colorado: diel, seasonal, and elevation effects." Ecological Entomology **36**(4): 511-521.

1. We revisited bumble bee survey data collected by Pyke in 1974 (Pyke, Ecology, 63, 555–573, 1982) to evaluate seasonal changes in abundances of bumble bees and their floral resources, diel patterns of bumble bee activity, and elevation effects on plant and bumble bee phenology.
2. Bumble bee abundance increased during summer as spring queens founded colonies that produced workers, followed by males and autumn queens. The number of plant species visited by bumble bees increased to a peak in midsummer, then declined.
3. The number of bumble bees recorded per person-hour peaked later than the number of flowering plant species used by the bees. Few autumn queens were observed.
4. Despite species differences in emergence times of spring queens, there were no apparent phenological differences among species in worker abundances.
5. Because flowering commences later at higher elevation, abundances of workers and males are also shifted later; therefore elevational comparisons must be seasonally adjusted.
6. These analyses provide basic information about important pollinating insects, and permit future investigations of elevational shifts over time to be properly adjusted for phenological and elevation effects in survey data.

Pyke, G. H., et al. (2012). "Local geographic distributions of bumble bees near Crested Butte, Colorado: Competition and community structure revisited." Environmental Entomology **41**(6): 1332-1349.

Surveys in 1974 of bumble bee species distributions along elevational gradients (Pyke 1982) were revisited to re-evaluate the original conclusion that coexistence of bumble bee species can be ascribed to niche differentiation, primarily on the basis of proboscis lengths and the associated corolla lengths of visited flowers, and secondarily on nutritional qualities of pollen. Each bee species largely visited a few plant species,

which were preferred relative to other species. Bee proboscis length was correlated with average corolla length of visited flowers, but not when species with relatively long and short proboscises were considered separately. Bumble bee abundance was affected by presence/absence of major plant species and, contrary to Pyke's (1982) interpretation, elevation, with neither factor dominating. Multi-modal distributions of proboscis lengths and altitudinal replacement of bee species of similar proboscis length were consistent with the original hypothesis that bumble bee species compete for floral resources, especially nectar, and cannot coexist if proboscis lengths are too similar, unless one species is a 'nectar robber' and hence has exclusive use of some floral resources. However, observed overlap in elevational distributions of bumble bee species with similar proboscis length cannot be reconciled with this hypothesis unless other phenomena are invoked.

Pyke, G. H., et al. (2016). "Effects of climate change on phenologies and distributions of bumble bees and the plants they visit." Ecosphere **7**(3).

Surveys of bumble bees and the plants they visit, carried out in 1974 near the Rocky Mountain Biological Laboratory in Colorado, were repeated in 2007, thus permitting the testing of hypotheses arising from observed climate change over the intervening 33-yr period. As expected, given an increase in average air temperature with climate warming and a declining temperature with increasing elevation, there have been significant shifts toward higher elevation for queens or workers or both, for most bumble bee species, for bumble bee queens when species are combined, and for two focal plant species, with no significant downward shifts. However, contrary to our hypotheses, we failed to observe significant altitudinal changes for some bumble bee species and most plant species, and observed changes in elevation were often less than the upward shift of 317 m required to maintain average temperature. As expected, community flowering phenology shifted toward earlier in the season throughout our study area, but bumble bee phenology generally did not change, resulting in decreased synchrony between bees and plants. However, we were unable to confirm the narrower expectation that phenologies of bumble bee workers and community flowering coincided in 1974 but not in 2007. As expected, because of reduced synchrony between bumble bees and community flowering, bumble bee abundance was reduced in 2007 compared with 1974. Hence, climate change in our study area has apparently resulted primarily in reduced abundance and upward shift in distribution for bumble bees and shift toward earlier seasonality for plant flowering. Quantitative disagreements between climate change expectations and our observations warrant further investigation.

Pyke, G. H. and N. M. Waser (1981). "The production of dilute nectars by hummingbird and honeyeater flowers." Biotropica **13**: 260-270.

A survey of data from tropical and temperate regions confirms that nectars of hummingbird and honeyeater flowers are dilute, especially relative to nectars of bee flowers. We use these data, along with theoretical considerations, to examine three recently proposed hypotheses to explain low concentration of hummingbird nectars. None of the quantitative or qualitative predictions of these three hypotheses appears to be upheld. We discuss possible weaknesses of each hypothesis and then present a general framework which may be useful in generating new hypotheses to explain the evolution of nectar concentration.

Pyke, G. H. and N. M. Waser (2017). "Comment on "Cognition-mediated evolution of low-quality floral nectars"." Science **358**(6368).

Nachev et al. (Reports, 6 January 2017, p. 75) present dilute nectar in bat-pollinated plants as "paradoxical" because bats prefer concentrated nectar, but paradox disappears with realistic assumptions about nectar evolution. We argue that they make unrealistic assumptions about the cognitive abilities of bat pollinators, invoke Weber's law inappropriately, and cannot predict observed nectar concentrations of bat flowers or negative correlations between pollinator body size and average concentration.

Richman, S. K., et al. (2017). "Foraging strategy predicts foraging economy in a facultative secondary nectar robber." Oikos: n/a-n/a.

In mutualistic interactions, the decision whether to cooperate or cheat depends on the relative costs and benefits of each strategy. In pollination mutualisms, secondary nectar robbing is a facultative behavior employed by a diverse array of nectar-feeding organisms, and is thought to be a form of cheating. Primary robbers create holes in floral tissue through which they feed on nectar, whereas secondary robbers, which

often lack chewing mouthparts, feed on nectar through existing holes. Because primary robbers make nectar more readily available to secondary robbers, primary robbers facilitate the behaviors of secondary robbers. However, the net effect of facilitation on secondary robber fitness has not been empirically tested: it is unknown whether the benefit secondary robbers receive is strong enough to overcome the cost of competing with primary robbers for a shared resource. We conducted foraging experiments using the bumble bee Bombus bifarius, which can alternatively forage 'legitimately' (from the floral opening) or secondary-rob. We measured the relative foraging efficiencies (handling time per flower, flowers visited per minute, proportion of foraging bout spent consuming nectar) of these alternative behaviors, and tested whether the frequency of primary robbing and nectar standing crop in primary-robbed flowers of Linaria vulgaris (Plantaginaceae) affected foraging efficiency. Surprisingly, there was no effect of primary robbing frequency on the foraging efficiency of secondary-robbing B. bifarius. Instead, foraging strategy was a major predictor of foraging efficiency, with legitimate foraging being significantly more efficient than secondary robbing. Legitimate foraging was the more common strategy used by B. bifarius in our study; however, it is rarely used by B. bifarius foraging on L. vulgaris in nature, despite indications that it is more efficient. Our results suggest the need for deeper investigations into why bees adopt secondary robbing as a foraging strategy, specifically, the environmental contexts that promote the behavior.

Richman, S. K., et al. (2017). "Facilitated exploitation of pollination mutualisms: fitness consequences for plants." Journal of Ecology **105**(1): 188-196.

* Mutualisms are only rarely one-to-one interactions: each species generally interacts with multiple mutualists. Exploitation is ubiquitous in mutualisms, and we would therefore expect that each mutualist interacts with multiple exploiters as well. Exploiter species may also interact with one another. For example, the action of one exploiter species might open the opportunity for exploitation by a second species.
* Exploitation is common in many plant–pollinator mutualisms: 'primary' nectar robbers feed through holes they make in flowers, which can be subsequently used by 'secondary' nectar robbers unable to create holes themselves. The overall effect of nectar robbing on plant fitness is often (although not always) negative. No study has separated the effects of interacting with primary vs. secondary robbers.
* Here, we examine the effects of primary vs. secondary nectar robbing on pollinator visitation rate and female fitness in Ipomopsis aggregata. Manipulating the type of nectar robbing that flowers experienced, we found that secondary nectar robbing inflicted fitness costs to plants beyond that inflicted by primary robbing alone. Secondary nectar robbing significantly reduced pollen receipt to flowers, as well as fruit and seed production. Although the causes are elusive, the effect may be attributed to changes in pollinator behaviour at these plants.
* Synthesis. Our findings provide evidence that interacting with multiple exploiters can lead to increased negative effects for mutualists, and highlight the importance of incorporating multiple exploiters into the conceptual framework of mutualism.

Roy, B. A. (1995). "The breeding systems of six species of *Arabis* (Brassicaceae)." American Journal of Botany **82**(7): 869-877.

The ability of organisms to produce genetic variation for any trait, including resistance to pathogens, is partially determined by breeding system. I used enzyme electrophoresis, crossing experiments, and cytology to assess the breeding systems of cooccurring Arabis species that are often infected by rust fungi. The Arabis holboellii surveyed were pseudogamous apomicts with relatively high population-level allozyme variation, and variable chromosome numbers (2n, 3n, 4n). Arabis gunnisoniana (3n) and Arabis lignifera (2n) were also pseudogamous but showed no allozyme variation either at the population level, or within progeny arrays. Arabis hirsuta may be an autogamous polyploid or it may be a pseudogamous apomict; more work is needed to clarify the breeding system of this species. Arabis drummondii and Arabis crandallii were sexual, but exhibited little genetic variability due to a predominance of self-fertilization. The use of several techniques was necessary to evaluate these breeding systems. Insect exclusion indicated when pollen was necessary for seed set, but could not differentiate between sexual reproduction and pseudogamy. Electrophoresis yielded information on the degree of selfing (as evidenced by homozygosity) and apomixis (fixed heterozygosity), but could not differentiate between autogamy and apomixis in polyploids without allozyme variation. Pseudogamy was confirmed when crosses between dissimilar genotypes yielded only the maternal genotype, and cytologically by irregular meiosis.

Saavedra, F. (2001). Testing climate change predictions with the subalpine species *Delphinium nuttallianum*. Wildlife Responses to Climate Change: North American Case Studies. S. H. Schneider and T. L. Root. Washington, D. C., Island Press**:** 201-249.

Saavedra, F., et al. (2003). "Changes in flowering and abundance of *Delphinium nuttallianum* (Ranunculaceae) in response to a subalpine climate warming experiment." Global Change Biology **9**(6): 885-894.

High-altitude and high-latitude sites are expected to be very sensitive to global warming, because the biological activity of most plants is restricted by the length of the short snow-free season, which is determined by climate. Long-term observational studies in subalpine meadows of the Colorado Rocky Mountains have shown a strong positive correlation between snowpack and flower production by the forb Delphinium nuttallianum. If a warmer climate reduces annual snowfall in this region then global warming might reduce fitness in D. nuttallianum. In this article we report effects of experimental warming on the abundance and flower production of D. nuttallianum. Plant abundance (both flowering and vegetative plants) was slightly greater on warmed than control plots prior to initiation of the warming treatment in 1991. Since 1994 experimental warming has had a negative effect on D. nuttallianum flower production, reducing both the abundance of flowering plants and the total number of flowers per plant. Flower bud abortion was higher in the heated plots than the controls only in 1994 and 1999. Results from both the warming experiment and analyses of unmanipulated long-term plots suggest that global warming may affect the fecundity of D. nuttallianum, which may have cascading effects on the pollinators that depend on it and on the fecundity of plants that share similar pollinators.

Sage, T. L., et al. (2006). "Self-sterility in *Ipomopsis aggregata* (Polemoniaceae) is due to prezygotic ovule degeneration." Am. J. Bot. **93**(2): 254-262.

Based on previous studies, extreme (>99%) self-sterility in scarlet gilia (Ipomopsis aggregata) appears to be involved in late-acting ovarian self-incompatibility (OSI). Here, we confirm this suggestion by comparing structural events that follow from cross- vs. self-pollinations of I. aggregata. Growth of cross- and self-pollen tubes in the style at 11 h and growth in the ovary at 24 h was equivalent. Nonetheless, by 24 h, cross-pollen effected a significantly higher percentage of both ovule penetration and fertilization. Ovules in self-pollinated flowers showed pronounced changes, including an absence of embryo sac expansion and reduced starch in the integument, by 11 h post-pollination, well before pollen tube entry into the ovary. In addition, the integumentary tapetum and adjacent 1-3 cell layers exhibited abnormal cell division, pronounced deposition of thick, pectin-rich cell walls, and cellular collapse. Ovules and embryo sacs from cross-pollinated flowers rarely showed such features. Developmental changes in ovules from self-pollinated flowers eventually resulted in integument and embryo sac collapse, a process not observed in ovules of unpollinated flowers. We suggest that OSI involves long-distance signaling between self-pollen or self-pollen tubes and carpel tissue that reduces availability of receptive ovules for fertilization before pollen tubes arrive in the ovary.

Sardiñas, H. S. and C. Kremen (2015). "Pollination services from field-scale agricultural diversification may be context-dependent." Agriculture, Ecosystems & Environment **207**: 17-25.

Diversification of field edges is widely used as a strategy to augment pollinator populations and, in turn, supplement crop pollination needs. Hedgerow plantings, a commonly applied field-scale diversification technique, have been shown to increase wild bee richness within edges and into crop fields; however, their effects on pollination services in mass-flowering, pollinator-dependent crops typical of large-scale commercial monocultures are less well-known. We evaluated the indirect contribution of hedgerows to sunflower (Helianthus annuus) seed set vis-á-vis wild bee abundance and the interaction between wild bees and managed honey bee pollinators. Although wild bee species richness and the interaction between wild and managed pollinators were significantly associated with augmented seed set, these factors were unrelated to whether a hedgerow was present. The pollinator species foraging within crop fields differed significantly from those found within adjacent hedgerows and bare or weedy field edges, with hedgerows supporting higher species richness than crop fields or unenhanced edges. However, in an independent data set, greater numbers of sunflower-pollinating bees were found in hedgerows than in control edges. Hedgerows may therefore help these crop-pollinating species persist in the landscape. Our findings suggest

that hedgerows may not always simultaneously achieve crop pollination and wild bee conservation goals; instead, the benefits of hedgerows may be crop- and region-specific. We recommend evaluation of hedgerow benefits in a variety of crop and landscape contexts to improve their ability to meet ecosystem-service provisioning needs.

Schaeffer, R. N., et al. (2015). "Nectar yeasts in *Delphinium nuttallianum* (Ranunculaceae) and their effects on nectar quality." Fungal Ecology **18**: 100-106.

Microorganisms colonize the nectar of many angiosperms. Variable diversity and spatio-temporal dynamics of nectar-inhabiting microorganisms (e.g., yeasts) may drive variation in nectar sugar composition and subsequent plant–pollinator interactions. We assessed yeast frequency of occurrence and density in the nectar of the perennial herb, Delphinium nuttallianum, across multiple spatio-temporal scales, including flower lifetime and sex-phase transition, flowering season, populations, and years. We tested the hypothesis that pollinators vector yeasts by comparing densities between virgin flowers and those open to visitation. Finally, we identified yeasts using molecular methods and tested for an association between yeast density and nectar composition using ultra-performance liquid chromatography. Yeasts were frequent colonists of Delphinium nectar, occurring in all populations and years sampled. Yeast frequency of occurrence and density varied across most spatio-temporal scales examined. Pollinators were vectors of yeast: virgin flowers remained yeast-free, while those open to visitation became inoculated. Nectar samples were species-poor, with a majority colonized by Metschnikowia reukaufii. Finally, increasing yeast density was correlated with a decrease in sucrose and an increase in monosaccharides. Our results document that yeasts form species-poor communities in populations of this hermaphroditic perennial, in addition to highlighting their spatio-temporal dynamics and effects on nectar quality. Spatio-temporal variation in frequency of occurrence, density, and changes in nectar may have important implications for the nature and strength of interactions between Delphinium and its pollinators.

Schlessman, M. A. and L. M. Graceffa (2002). "Protogyny, pollination, and sex expression of andromonoecious *Pseudocymopterus montanus* (Apiaceae, Apioideae)." International Journal of Plant Sciences **163**(3): 409-417.

Studies of dichogamy seldom address its possible effects on other floral traits. An exception has been work on the apioid umbellifers (Apiaceae, subfamily Apioideae), which typically exhibit andromonoecy and both intra- and interfloral dichogamy. Several students of apioid umbellifers have suggested that variation in proportions of perfect and staminate flowers among sequentially blooming inflorescences (umbels) of individual plants has evolved in response to dichogamy. Here, we report a 2-yr study of dichogamy, pollination, and sex expression in Pseudocymopterus montanus, a common wildflower of the Rocky Mountains. We recorded floral phenology, determined the timing of pollination, collected and identified floral visitors, documented variation in sex expression and fecundity within and among plants, and examined possible reasons why some perfect flowers did not set fruit. Floral visitors included andrenid and halictid bees and anthomyid, muscoid, syrphid, and tachinid flies. Autogamy was prevented by strong intrafloral protogyny of the perfect flowers, which were pollinated before their anthers dehisced. Flowering individuals produced a single primary (terminal) umbel, one or two secondary (lateral) umbels, and occasionally a tertiary (sublateral) umbel. Umbels of different orders matured sequentially, producing multiple cycles of protogyny. Weak interfloral protogyny within and among umbels allowed the possibility of geitonogamous selfing. All individuals expressed the same pattern of variation in sex expression among sequentially blooming umbels. Over 90% of flowers in primary umbels were staminate, but the frequency of staminate flowers fell to 65% or less in secondary and tertiary umbels. Primary and secondary umbels differed significantly in sex expression, even though their prepollination reproductive efforts (floral biomass) were the same. We favor the view that this distinctive pattern of within-plant sex expression, i.e., decreasing proportions of staminate flowers in sequentially blooming umbels, evolved in response to protogyny. In apioid umbellifers, protogyny appears to have been derived from protandry. The selective forces involved in the derivation of protogyny may have included more specialized interactions with pollinators and the ability to geitonogamously self-pollinate if cross-pollination failed. Sex allocation theory predicts that protogyny is a sufficient condition for the evolution of increasing relative allocations to female function in sequentially blooming flowers or inflorescences. Plants with higher reproductive effort had higher absolute allocations to both male and female reproductive functions as well as higher relative allocations to female function (higher

phenotypic femaleness). The more female-biased gender of larger plants may result from higher frequencies of geitonogamy in inflorescences with more flowers.

Schmitt, J. (1980). "Pollinator foraging behavior and gene dispersal in *Senecio* (Compositae)." Evolution **34**(5): 934-943.
The foraging behavior of butterflies and bumblebees is compared on the same populations of three Senecio species. While bumblebees typically fly near-neighbor distances, resulting in very localized pollen dispersal, butterflies frequently bypass nearby plants, flying significantly greater distances between plants. Bumblebees visit significantly more heads per plant and significantly more plants per foraging bout. The flight distance distribution data are used to calculate neighborhood size and area, in the sense of Wright's isolation-by-distance model, for the observed populations under hypothetical conditions of exclusive butterfly or exclusive bumblebee pollination. It is predicted that small neighborhood sizes will typically be found in plant populations specializing on bumblebee pollination, whereas butterfly-pollinated populations will have much larger neighborhood sizes; bumblebee-pollinated plants will therefore have greater potential for local genetic differentiation. These differences in neighborhood characteristics may be augmented if plants are self-compatible or if pollen carryover occurs, since bumblebees make a higher proportion of intraplant flights. The addition of a small amount of butterfly pollination can increase gene dispersal in a plant population sufficiently to greatly reduce genetic drift and microgeographic adaptive differentiation.

Schmitt, J. (1983). "Flowering plant density and pollinator visitation in *Senecio*." Oecologia **60**: 97-102.
It has commonly been assumed that pollinator energy intake increases with flowering plant density, and visitation to flowers should therefore be higher in denser stands. I therefore investigated the relationship between flight distance and flight time for bumblebees and butterflies foraging on Senecio integerrimus and S. crassulus in the Colorado Rocky Mountains. I also compared patterns of pollinator visitation and seed set in two adjacent 15x 15 m plots in a population of S. integerrimus; one plot was experimentally thinned of flowering stalks, while the other was left at natural density. Mean flight distance had no effect on mean flight time or the number of florets or heads visited per unit time. There were no significant differences between the two plots in the rate at which plants received visits, although visitation rates varied through the season, with greatest activity at peak flowering. Pollinators were more selective in their visits to plants in the high-density plot, however. Bumblebee-visited plants in the dense plot had a lower variance in stalk height than the plant population average, while butterfly-visited plants in the dense plot had more heads than the population average. Plant density had no effect on number of heads visited per plant, but number of heads visited by bumblebees was correlated with number of heads per plant. Efficiency of visitation (percentage of flowering heads visited), declined with inflorescence size. Flowering plant density had no effect on seed production, and inflorescence size did not affect the percentage of florets setting seed. In Senecio, flowering phenology patterns and differences among pollinators in foraging behavior may have more important consequences for seed set and gene flow patterns than plant density or plant size.

Schulke, B. and N. M. Waser (2001). "Long-distance pollinator flights and pollen dispersal between populations of *Delphinium nuttallianum*." Oecologia **127**(2): 239-245.
Spatial processes in pollination biology are poorly understood, especially at levels above that of the local population. For example, little is known about how pollinators and pollen move among populations, although there is evidence that such movement can exceed what is predicted from intrapopulational movement. We explored pollination success in experimental isolates of the bumblebee- and hummingbird-pollinated wildflower Delphinium nuttallianum. We established a total of 15 arrays of potted plants isolated by 50-400 m from ten natural "source" populations, as well as control arrays embedded within each source. Flowers on potted plants were emasculated, so any pollen received could be assumed to come from source populations. A total of 69 h of observation suggested that pollinators were somewhat less abundant in isolates than in controls, but visited more plants and flowers once within an isolate. Consistent with this, 82.1% of all flowers in isolated arrays received pollen, versus 87.7% in controls. Mean receipt was more than 100 pollen grains per flower in most arrays, and seed set in isolates and controls respectively averaged 69.8% and 74.3% of ovules. Furthermore, pollen receipt in isolates declined relatively slowly with distance from the source. We conclude that pollinators of D. nuttallianum often will fly up to 400 m among populations, and that substantial pollination ensues. Thus isolated populations of this species often belong to

metapopulations in terms of pollen dispersal, with important consequences for genetic differentiation, and potential implications for the management of endangered plant species.

Sharaf, K. E. and M. V. Price (2004). "Does pollination limit tolerance to browsing in *Ipomopsis aggregata*?" Oecologia **138**(3): 396-404.

Ungulate browsing of flowering stalks of the semelparous herb Ipomopsis aggregata leads to regrowth of lateral inflorescences, a response that has been reported to yield overcompensation in some cases (browsed plants with higher reproductive success than unbrowsed), but undercompensation in others. Little is known about the mechanisms that cause such variable tolerance to herbivory. We explored one possible mechanism--variation in effects of browsing on pollination--by clipping I. aggregata inflorescences to mimic browsing, observing subsequent visits by pollinators and nectar-robbers, and adding pollen by hand to flowers of some clipped and unclipped plants. Clipping reduced floral display size and increased inflorescence branching, but neither humming-birds, the primary pollinators, nor nectar-robbing bumblebees showed any preference for unclipped versus clipped plants. Clipping delayed flowering; this shift in phenology caused clipped plants to miss the peak of hummingbird activity and to have lower per-flower visitation rates than unclipped controls in one year, but to have greater overlap with birds and higher visitation rates in the subsequent year. In three sites and 2 years, clipped plants exposed to natural pollination suffered extreme undercompensation, producing on average only 16% as many seeds as unclipped controls. This was not directly attributable to clipping effects on pollination, however, because clipped plants were unable to increase fecundity when provided with supplemental pollen by hand. Taken altogether, our results suggest that compensation was constrained less by indirect effects of browsing on pollination than by its direct impacts on resource availability and hence on the ability of plants to regrow lost inflorescence tissue and to fill seeds. Exploring the physiological and developmental processes involved in regrowth of inflorescences and provisioning of seeds is a promising future direction for research designed to understand variation in browsing tolerance.

Spear, D. M., et al. (2016). "Asteraceae pollen provisions protect *Osmia* Mason bees (Hymenoptera: Megachilidae) from brood parasitism." The American Naturalist **187**(6): 797-803.

Many specialist herbivores eat foods that are apparently low quality. The compensatory benefits of a poor diet may include protection from natural enemies. Several bee lineages specialize on pollen of the plant family Asteraceae, which is known to be a poor-quality food. Here we tested the hypothesis that specialization on Asteraceae pollen protects bees from parasitism. We compared rates of brood parasitism by Sapyga wasps on Asteraceae-specialist, Fabeae-specialist, and other species of Osmia bees in the field over several years and sites and found that Asteraceae-specialist species were parasitized significantly less frequently than other species. We then tested the effect of Asteraceae pollen on parasites by raising Sapyga larvae on three pollen mixtures: Asteraceae, Fabeae, and generalist (a mix of primarily non-Asteraceae pollens). Survival of parasite larvae was significantly reduced on Asteraceae provisions. Our results suggest that specialization on low-quality pollen may evolve because it helps protect bees from natural enemies.

Stinson, K. A. (2004). "Natural selection favors rapid reproductive phenology in *Potentilla pulcherrima* (Rosaceae) at opposite ends of a subalpine snowmelt gradient." American Journal of Botany **91**(4): 531-539.

In high altitude plants, flowering quickly ensures reproductive success within a short snow-free period, but limits maturation time and fecundity. Natural selection on prefloration intervals may therefore vary in contrasting snowmelt environments and could influence the outcome of phenological responses to climatic change. This study investigated adaptive differentiation and plasticity of prefloration intervals in the subalpine perennial Potentilla pulcherrima. Three years of in situ field observations were combined with phenotypic selection analyses and a common garden experiment. Plants from high, intermediate, and low altitudes expressed similar prefloration intervals and plasticity when grown at common altitude, indicating no evidence for adaptive differentiation. Selection on the prefloration interval was negative at both low and high altitudes before and after accounting for strong positive selection on size. Environmental differences between high and low altitudes indicated that long, dry seasons and short, wet seasons both favored rapid reproduction. Therefore, quicker reproduction was adaptive in response to late snowmelt, but slower reproduction in response to earlier snowmelt appeared to be maladaptive. Selection differed marginally

between late snowmelt years and dry ones. Plastic responses to future precipitation patterns may therefore have positive or negative effects on fitness within a single species, depending upon altitude and year.

Stout, J. C., et al. (2000). "Nectar robbing, forager efficiency and seed set: Bumblebees foraging on the self incompatible plant *Linaria vulgaris* (Scrophulariaceae)." Acta Oecologica International Journal of Ecology **21**(4/5): 277-283.

In southern England, Linaria vulgaris (common yellow toadflax) suffers from high rates of nectar robbery by bumblebees. In a wild population of L. vulgaris we found that 96 % of open flowers were robbed. Five species of bumblebee were observed foraging on these flowers, although short-tongued species (Bombus lapidarius, B lucorum and B. terrestris) robbed nectar whilst longer-tongued ones behaved as legitimate pollinators (B. hortorum and B. pascuorum). Nectar rewards were highly variable ; on average there was less nectar in robbed than in unrobbed flowers, but this difference was not statistically signifiant. The proportion of flowers containing no nectar was significantly higher for robbed flowers compared with unrobbed flowers. Secondary robbers and legitimate pollinators had similar handling times on flowers and, assuming they select flowers at random to forage on, received approximately the same nectar profit per minute, largely because most flowers had been robbed. There was no significant difference in the number of seeds in pods of robbed flowers and in pods of flowers that were artificially protected against robbing. However, more of the robbed flowers set at least some seed than the unrobbed flowers, possibly as a consequence of the experimental manipulation. We suggest that nectar robbing has little effect on plant fecundity because legitimate foragers are present in the population, and that seed predation and seed abortion after fertilization may be more important factors in limiting seed production in this species.

Suzuki, K. (1993). "Disruptive selection in flowering time of *Wyethia amplexicaulis* (Asteraceae)." Plant Species Biology **8**: 51-59.

Flowering time was earlier at a lower elevation site, where earlier-flowering flower heads produced more good seeds. At the higher elevation site later-flowering heads were more successful. Successful seed production averaged only 4% due to seed predation (Tephritid flies) and abortion.

Suzuki, K. (1994). "Pollinator restriction in the narrow-tube flower type of *Mertensia ciliata* (James) G. Don (Boraginaceae)." Plant Species Biology **9**: 69-73.

Mertensia ciliata (Boraginaceae) includes two flower types with different corolla tube widths, wide and narrow. The former is pollinated by both queens and worker bumblebees, while the latter type is pollinated exclusively by bumblebee workers. Morphological comparisons between the flowers and the bumblebees showed that the relationship between the width of the corolla tube mouth and the head width of the bumblebee is a primary factor restricting pollinators to workers in the narrow-tube type. Length of the proboscides and corolla tube are of secondary importance for this restriction, since some queens are not able to reach the bottom of a narrow corolla tube even if their proboscis is extended fully.

Thomson, J. D. (1980). "Skewed flowering distributions and pollinator attraction." Ecology **61**: 572-579.

The temporal distributions of flowering by animal-pollinated plant populations, flowering curves, can be viewed profitably as resource utilization functions. A conceptual model of plant competition for pollinators suggests that selection may favor asymmetrical, positively skewed curves, and that such skewness should be most evident in flowers which, at the initiation of flowering, are of a type unfamiliar to their pollinators. Both predictions are confirmed in an examination of 57 species of subalpine meadow plants from the Colorado Rocky Mountains.

Thomson, J. D. (1981). "Spatial and temporal components of resource assessment by flower-feeding insects." Journal of Animal Ecology **50**: 49-59.

(1) Per-flower insect visitation rates on two plant species within Rocky Mountain subalpine meadows were measured using fluorescent powders and found to be positively correlated with local flower density, suggesting that insects concentrate their foraging in dense patches of flowers. (2) Visitation rates on both species are also correlated with the presence of other plant species which share the same visitors; these correlations are positive, suggesting cooperative rather than competitive plant-plant relationships, at least insofar as visitation is concerned. (3) By correlating local visitation rates with flower densities computed over

a range of block sizes, the block sizes at which insects assessed flower density differences were estimated to be approximately 1000 m2 for solitary bees and flies visiting Potentilla spp., and 500 m2 for bumblebees visiting composites. (4) By correlating local visitation rates with flower censuses from various days before the visitation measurements, the time lags in flower density assessment were estimated to be approximately 1.5 days for the solitary bees and flies, and 0.5 days for the bumblebees. (5) Implications for plant competition and flowering strategy are discussed.

Thomson, J. D. (1982). "Patterns of visitation by animal pollinators." Oikos **39**: 241-250.

I measured the relative rates of pollination visitation at various points during the blooming periods of several species of animal-pollinated plants in Rocky Mountain subalpine meadows. The most common pattern was for visitation to be low early in the flowering period, but then to increase and remain high for the duration of flowering. Relative visitation rate on a species was uncorrelated with the amount of flowering time overlap experienced from species with similar pollinators; thus overlap is not a good indicator of competition for visits. I also measured visitation rates on three species in artificial and natural competition experiments. In one species, pressure of a potential competitor decreased visitation, but in two others increased it. Whether interspecific overlap increases or decreases visitation probably depends on floral similarity, pollinator constancy, spatial proximity of interactants, and other idiosyncratic characteristics of the interaction. I discuss the role of competition for visits as one component of competition for successful pollination service.

Thomson, J. D. (1986). "Pollen transport and deposition by bumble bees in *Erythronium*: influences of floral nectar and bee grooming." Journal of Ecology **74**: 329-341.

(1) The transport of pollen from donor flowers by bumble bees was measured by examining deposition on stigmata of sequences of recipient flowers. The rate of decay of grain deposition was estimated as a measure of pollen carryover. (2) Bombus bifarius was a much less effective pollinator of Erythronium grandiflorum than was the larger Bombus occidentalis. (3) The numbers of pollen grains deposited by bumble bees on the stigmata of Erythronium americanum vary greatly from flower to flower. (4) The time spent by a bee on a flower is positively related to the nectar concentration and volume. (5) Flowers with large volumes of nectar receive more pollen grains per visit than those with small volumes, presumably because the visits are longer. The results are insufficient to show a parallel increase in deposition with nectar concentration. (6) Measures of pollen carryover are presented. Most deposition of grains from a particular donor flower occurs on the first several recipient flowers subsequently visited by the bee, but a few grains travel much farther. (7) Pollen carryover in E. grandiflorum is reduced by bee grooming. (8) The negative effect of grooming on carryover is increased when the recipient flowers have undehisced anthers. (9) Erythronium americanum and E. grandiflorum were similar in carryover, but Linaria vulgaris showed much higher carryover. The reasons for this are discussed

Thomson, J. D. (1996). "Trapline foraging by bumblebees .1. Persistence of flight-path geometry." Behavioral Ecology **7**(2): 158-164.

By setting out arrays of potted plants of Penstemon strictus, I tested whether freely foraging bumblebee (Bombus spp.) workers would establish regular foraging routes that reflected the geometry of the array. They did, passing through an asymmetrical array in a pattern that minimized interplant flight distances. After the array was changed to a symmetrical pattern, however, the experienced bees continued to show their previous asymmetrical flight patterns. New bees without experience on the asymmetrical array showed no asymmetry on the symmetrical array. I term this persistence of flight-path geometry “trapline holdover, ” and discuss its implications for the study of animals' learning and foraging behavior.

Thomson, J. D. (2010). "Flowering phenology, fruiting success and progressive deterioration of pollination in an early-flowering geophyte." Philosophical Transactions of the Royal Society B: Biological Sciences **365**(1555): 3187-3199.

Spatio-temporal patterns of snowmelt and flowering times affect fruiting success in Erythronium grandiflorum Pursh (Liliaceae) in subalpine western Colorado, USA. From 1990 to 1995, I measured the consistency across years of snowmelt patterns and flowering times along a permanent transect. In most years since 1993, I have monitored fruit set in temporal cohorts (early- to late-flowering groups of plants) at one site. To assess ‘pollination limitation’, I have also conducted supplemental hand-pollination experiments at various times through the blooming season. The onset of blooming is determined by snowmelt, with the

earliest years starting a month before the latest years owing to variation in winter snowpack accumulation. Fruit set is diminished or prevented entirely by killing frosts in some years, most frequently but not exclusively for the earlier cohorts. When frosts do not limit fruit set, pollination limitation is frequent, especially in the earlier cohorts. Pollination limitation is strongest for middle cohorts: it tends to be negated by frost in early cohorts and ameliorated by continuing emergence of bumble-bee queens in later cohorts. This lily appears to be poorly synchronized with its pollinators. Across the years of the study, pollination limitation appears to be increasing, perhaps because the synchronization is getting worse.

Thomson, J. D. and K. S. Eisenhart (2003). "Rescue of stranded pollen grains by secondary transfer." Plant Species Biology **18**(2&3): 67-74.

Secondary transfer of pollen can occur when a second pollinator remobilizes grains that had already been transferred to a flower by a previous pollinator. We used a pollen-color dimorphism to measure components of secondary transfer by bumble bees visiting the lily Erythronium grandiflorum. Remobilization was surprisingly high, ranging from 20% of grains deposited on stigmas to 90% of grains deposited on inner tepal surfaces. Because most of the grains that are remobilized would otherwise have been stranded on non-stigmatic surfaces, secondary transfer has the beneficial effect of returning lost grains to circulation.

Thomson, J. D., et al. (2011). "Pollinator exclusion devices permitting easy access to flowers of small herbaceous plants." Journal of Pollination Ecology **4**: 24 -.

Pollinator exclusion bags for small herbaceous plants are much more convenient to apply and remove if their bottom edge is made in the form of a cloth tunnel loaded with sand to conform to the terrain. Damage and inadvertent selfing of flowers are minimized.

Thomson, J. D., et al. (1986). "Comparative studies of pollen and fluorescent dye transport by bumble bees visiting *Erythronium grandiflorum*." Oecologia **69**: 561-566.

In the Colorado Rocky Mountains the glacier lily Erythronium grandiflorum exhibits a striking dimorphism in pollen color and is commonly pollinated by the bumble bee Bombus occidentalis. We induced bees to visit sequences of flowers in a flight cage, and compared dispersal of distinctively-colored pollen and fluorescent pigment ("dye") that the bee had picked up at a single donor flower. Nonparametric and parametric analyses showed that dispersal properties of pollen and dye differed; consistently less pollen was deposited and it was carried consistently shorter distances than dye. Dye thus does not provide an accurate means of assessing exacty where or how far pollen travels in this plant-pollinator system. On the other hand, both pollen and dye responded similarly to several experimental manipulations of donor and recipient flowers. Hence dye may well be of value for a qualitative investigation of how floral traits influence pollen dispersal.

Thomson, J. D., et al. (1997). "Trapline foraging by bumble bees. II. Definition and detection from sequence data." Behavioral Ecology **8**(2): 199-210.

Trapline foraging—repeated sequential visits to a series of feeding locations—presents interesting problems seldom treated in foraging models. Work on traplining is hampered by the lack of statistical, operational approaches for detecting its existence and measuring its strength. We propose several statistical procedures, illustrating them with records of interplant flight sequences by bumble bees visiting penstemon flowers. An asymmetry test detects deviations from binomial expectation in the directionality of visits between pairs of plants. Several tests compare data from one bee to another frequencies of visits to plants and frequencies of departures to particular destinations are compared using contingency tables; similarities of repeated sequences within bees are compared to those between bees by means of sequence alignment and Mantel tests. We also compared observed movement patterns to those generated by null models designed to represent realistic foraging by non-traplining bees, examining: temporal patterns of the bee's spatial displacement from its starting point using spectral analysis; the variance of return times to particular plants; and the sequence alignment of repeated cycles within sequences. We discuss the different indications and the relative strengths of these approaches

Thomson, J. D. and D. A. Stratton (1985). "Floral morphology and cross-pollination in *Erythronium grandiflorum* (Liliaceae)." American Journal of Botany **72**: 433-437.

In bumblebee visits to flowers of Erythronium grandiflorum (Liliaceae), the ratio of self- to nonself- ("outcross") pollen grains deposited on the stigma is positively correlated with the degree of stylar exsertion beyond the anthers. Natural populations show substantial, continuous variation in stylar exsertion.

Thomson, J. D. and B. A. Thomson (1989). "Dispersal of *Erythronium grandiflorum* pollen by bumblebees: implications for gene flow and reproductive success." Evolution **43**: 657-661.

Thomson, J. D. and J. L. Zung (2015). "A restraining device to aid identification of bees by digital photography." Journal of Pollination Ecology **17**.

We developed a simple restraining chamber to hold captured bumble bees temporarily so they could be photographed in the field using inexpensive "point-and-shoot" digital cameras. The process is quick, and the resulting "digital voucher" images allowed us to correct a substantial fraction of field identifications based on visual inspection. The system can improve the accuracy of monitoring programs in which it is undesirable to kill specimens to provide traditional vouchers.

Tölke, E. D., et al. (2018). "Osmophores and floral fragrance in *Anacardium humile* and *Mangifera indica* (Anacardiaceae): an overlooked secretory structure in Sapindales." AoB PLANTS **10**(6): ply062-ply062.

Flowers of Anacardiaceae and other Sapindales typically produce nectar, but scent, often associated with a reward for pollinators, has surprisingly been mentioned only rarely for members of the family and order. However, flowers of Anacardium humile and Mangifera indica produce a strong sweet scent. The origin and composition of these floral scents is the subject of this study. Screening of potential osmophores on the petals and investigations of their anatomy were carried out by light, scanning and transmission electron microscopy. The composition of the floral fragrance was characterized by gas chromatography–mass spectrometry. In both species, the base of the adaxial side of each petal revealed specialized secretory epidermal cells which are essentially similar in structure and distinct from all other neighbouring cells. These cells also showed evidence of granulocrine secretory mechanisms and slight specific variations in their subcellular apparatus coinciding with the respective composition of the floral fragrance, predominantly composed of sesquiterpenes in A. humile and monoterpenes in M. indica. This study reports the presence of osmophores for the first time in flowers of Anacardiaceae and confirms the link between the ultrastructural features of their secretory cells and the volatiles produced by the flowers. The flowers of most Sapindales, including Anacardiaceae, are nectariferous. However, the presence of osmophores has only been described for very few genera of Rutaceae and Sapindaceae. Both the occurrence of osmophores and fragrance may have largely been overlooked in Anacardiaceae and Sapindales until now. Further studies are needed to better understand the nature and diversity of the interactions of their nectariferous flowers with their pollinators.

Waser, N. M. (1978). "Competition for hummingbird pollination and sequential flowering in two Colorado wildflowers." Ecology **59**: 934-944.

The simultaneous flowering of co—occurring plant species with similar pollinator affinities may result in interspecific pollen transfer and consequent fecundity reductions due to wastage of pollen, stigma surfaces, and effective pollinator visits. In such cases competition for pollination occurs and may lead to or maintain sequential flowering. Two common perennials in the mountains of west—central Colorado, Delphinium nelsoni and Ipomopsis aggregata, flower sequentially in the same meadows and are visited commonly by Broad—tailed Hummingbirds (Selasphorus platycercus). Hummingbirds carry pollen of both species and their exclusion from flowers leads to significant seed set reductions. During the brief period of flowering overlap between D. nelsoni and I. aggregata in natural meadows, hummingbirds visit both species, carry mixtures of their pollen, and appear to cause interspecific pollen transfer. Flowers of both species receptive during this period suffer significant seed set reductions relative to those receptive during nonoverlap periods. Interspecific pollinator flights and pollen transfer also occur in mixtures of potted plants, and seed set reductions consistently occur for both D. nelsoni and I. aggregata in such mixtures relative to single—species controls. Finally, seed set reductions occur for both species following interspecific hand pollination of potted plants. Fecundity reductions in natural and synthetic mixtures of D. nelsoni and I. aggregata indicate that the 2 species compete for hummingbird pollination and suggest that the competitive interaction

involves interspecific pollen transfer. The observed reproductive effects represent a selective force sufficient to maintain divergent flowering times of D. nelsoni and I. aggregata in nature.

Waser, N. M. (1982). "A comparison of distances flown by different visitors to flowers of the same species." Oecologia **55**: 251-257.

The morphologically complex flowers of Delphinium nelsonii, D. barbeyi, and Ipomopsis aggregata are visited by a wide variety of animals. Visitors to each species range from small insects, such as worker bumblebees and solitary bees, to hummingbirds, and thus span roughly an order of magnitude in body mass and metabolic rate while flying; they also differ in type of food collected and in their efficacy as pollinators. Despite these differences, all the visitors to a given plant species fly similar, short distances between successively visited flowers and plants. There are no significant relationships between mean flight distance and metabolic rate or body mass among the visitors to any plant species. Thus there is no evidence that flight characteristics depend on anything as straightforward as whether flower visitors have high or low energetic requirements.

Waser, N. M. (1983). Competition for pollination and floral character differences among sympatric plant species: a review of evidence. Handbook of Experimental Pollination Biology. C. E. Jones and R. J. Little. New York, USA, Van Nostrand Reinhold**:** 277-293.

Waser, N. M. (1987). "Research in plant evolution at the Rocky Mountain Biological Laboratory." Evolutionary Trends in Plants **1**: 1-3.

Waser, N. M. (1987). "Spatial genetic heterogeneity in a population of the montane perennial plant *Delphinium nelsonii*." Heredity **58**: 249-256.

The montane perennial herb Delphinium nelsonii experiences restricted pollen and seed movement and spatial environmental heterogeneity, features likely to promote genetic differentiation within populations. To explore this possibility, allele frequencies at 5 polymorphic loci were characterised along transects through a meadow. F-statistics indicated substantial heterozygote excess within transect samples (overall FIS = −0·096). Possible contributors are sex-biased gene flow, and events occurring between pollination and ovule fertilisation that favor pollen from an “optimal outcrossing distance” of 3–10 m. Higher mating success over this optimal distance should cause longer realised gene dispersal than expected from pollen and seed movement, which are

Waser, N. M. (1988). "Comparative pollen and dye transfer by natural pollinators of *Delphinium nelsonii*." Functional Ecology **2**: 41-48.

Waser, N. M. (1993). Population structure, optimal outbreeding, and assortative mating in angiosperms. The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives. N. W. Thornhill. Chicago, University of Chicago Press**:** 173-199.

Waser, N. M. (2001). Pollinator behavior and plant speciation: looking beyond the ''ethological isolation'' paradigm. Cognitive Ecology of Pollination: Animal Behaviour and Floral Evolution. L. Chittka and J. D. Thomson. the Pitt Building/ Trumpington St/Cambridge CB2 1RP/Cambs, England, Cambridge Univ Press**:** 318-335.

Waser, N. M. and W. A. Calder (1975). "Possible impairment of nest-building of hummingbirds by acetate leg-tags." Condor **77**: 361.

Waser, N. M. and D. R. Campbell (2004). Ecological speciation in flowering plants. Adaptive speciation. U. Dieckmann, M. Doebeli, J. A. J. Meta and D. Tautz. Cambridge, Cambridge University Press**:** 264-277.

Waser, N. M., et al. (2018). "Atypical flowers can be as profitable as typical hummingbird flowers." Am Nat **192**(5): 644-653.

In western North America, hummingbirds can be observed systematically visiting flowers that lack the typical reddish color, tubular morphology, and dilute nectar of "hummingbird flowers." Curious about this behavior,

we asked whether these atypical flowers are energetically profitable for hummingbirds. Our field measurements of nectar content and hummingbird foraging speeds, taken over four decades at multiple localities, show that atypical flowers can be as profitable as typical ones and suggest that the profit can support 24-h metabolic requirements of the birds. Thus, atypical flowers may contribute to successful migration of hummingbirds, enhance their population densities, and allow them to occupy areas seemingly depauperate in suitable resources. These results illustrate what can be gained by attending to the unexpected.

Waser, N. M. and M. L. Fugate (1986). "Pollen precedence and stigma closure: a mechanism of competition for pollination between *Delphinium nelsonii* and *Ipomopsis aggregata*." Oecologia **70**: 573-577.

Previous experiments showed that the sympatric herbs Delphinium nelsonii and Ipomopsis aggregata compete for hummingbird pollination and that deleterious effects of the former species on seed set of the latter involve interspecific pollen transfer. However, seed set was not reduced when pollen of both species was applied simultaneously to I. aggregata stigmas. Hence a competitive effect may require arrival of foreign pollen before conspecific pollen. To explore this possibility we subjected I. aggregata flowers to a "competition" treatment in which they received D. nelsonii pollen 6 h before I. aggregata pollen, or to a "control" in which they received only the conspecific pollen. Foreign pollen precedence decreased mean seed set by almost 50%, which is consistent with effects observed in previous experiments. Reduced seed set can be explained by the fact that foreign pollen often caused stigma lobes to close together within 1.5–6 h, reducing subsequent receptivity. Stigma closure was also elicited by conspecific pollen, but not by mechanical stimulation, and was influenced by size of the pollen load and identity of the plant being pollinated.

Waser, N. M. and D. W. Inouye (1977). "Implications of recaptures of broad-tailed hummingbirds banded in Colorado." Auk **94**: 393-395.

Waser, N. M. and J. A. McRobert (1998). "Hummingbird foraging at experimental patches of flowers: evidence for weak risk-aversion." Journal of Avian Biology **29**(3): 305-313.

Hummingbirds foraging for nectar in mountains of the western USA encounter spatially-variable rewards under energetically-demanding environmental conditions. Previous workers have found that hummingbirds respond quickly to the average quantity and quality of reward; in addition, one might expect a response to reward variance. To explore this possibility we observed free-flying hummingbirds visiting two arrays of the native plant Ipomopsis aggregata. All flowers in one array received a 1 µl aliquot of artificial nectar whereas every fifth flower in the other array received 5 µl. This yielded a mean nectar standing crop similar to that found in nature, and variances in standing crop that were only slightly more extreme than those in nature. Over 12 days we recorded 362 foraging bouts by male and female Broad-tailed Selasphorus platycercus and Rufous S. rufus Hummingbirds. The "high-variance" (HV) array received 8.9% fewer foraging bouts than the "low-variance" (LV) array, and this difference was significant statistically at the $\alpha = 5.4\%$ level. Bout frequency varied among periods of the day, but not differentially by nectar treatment. Foraging bouts tended to be longer in the LV array, though not significantly so, and hummingbirds visiting both arrays in sequence were significantly more likely to visit the LV array first. These results suggest weak risk-aversion under natural conditions. Lack of a stronger response may derive from the fact that hummingbirds sample thousands of flowers a day, so that the total profit realized over a day is not strongly influenced by flower-to-flower variation.

Waser, N. M. and R. J. Mitchell (1990). "Nectar standing crops in *Delphinium nelsonii* flowers: spatial autocorrelation among plants?" Ecology **71**: 116-123.

Several aspects of nectarivore foraging behavior have been interpreted as responses to spatial reward patchiness of the kind documented for Delphinium nelsonii floral nectar by Pleasants and Zimmerman (1979). Working with this same species over 3 yr, however, we were unable to detect substantial pattern in nectar standing volumes, either through contingency analyses or spatial autocorrelation. Although spatial outocorrelations between rewards of neighboring plants were positive in 5 or 6 samples examined, only one value was statistically significant. Spatial autocorrelations over longer distances were erratic. We used computer simulations of nectarivores foraging in a large plant population to explore factors that promote

reward patchiness. Simulations suggest that moderate patchiness will develop at all but extremely low or high flower visitation rates. Rates were intermediate at our sites and those of Pleasants and Zimmerman, however, so visitation intensity does not seem to explain the discrepancy between our results and theirs. On the other hand, reward patchiness in simulations declined substantially as nectarivores exhibited less area-restricted foraging. Hummingbirds were important visitors at our sites, and fly farther between plants than the bumble bees that predominated at Pleasant's and Zimmerman's sites. Finally, simulations suggest that spatial patchiness is lower when interplant coefficient of variation in nectar production rate is large, as in our populations.

Waser, N. M. and M. V. Price (1981). "Pollinator choice and stabilizing selection for flower color in *Delphinium nelsonii*." Evolution **35**: 376-390.

Flowers of the herbaceous perennial wildflower Delphinium nelsonii are normally deep blue. In Colorado populations we have studied, however, about 0.1% of all plants are "albinos" with pale or white flowers. This low frequency suggests an equilibrium between production of albinos through spontaneous mutation and selection against them. Indeed, selection against albinos in nature occurs in the form of consistent reductions in seed set relative to blue-flowered controls. Using field experiments with natural populations and artificial populations of potted plants, we have determined that seed set reductions correspond not to low intrinsic fecundity of albinos but rather to fixed discrimination against them by hummingbird and bumblebee pollinators. We discuss several possible reasons for discrimination, and conclude that pollinators may undervisit albino flowers because their nectar rewards are more time-consuming to extract than those of blue flowers.

Waser, N. M. and M. V. Price (1982). "A comparison of pollen and fluorescent dye carry-over by natural pollinators of *Ipomopsis aggregata* (Polemoniaceae)." Ecology **63**(4): 1168-1172.

Waser, N. M. and M. V. Price (1984). "Experimental studies of pollen carryover: effects of floral variability in *Ipomopsis aggregata*." Oecologia **62**: 262-268.

In the montane herb Ipomopsis aggregata, size and placement of stamens and pistils vary substantially among flowers within plants, among nearby plants, and among groups of plants separated by 50-100 m. We trained captive hummingbirds to feed from flowers of this species in a flight cage, and explored the effects of different degrees of floral variability on carryover of fluorescent dyes that act as pollen mimics. We found that the slopes of linear dye carryover functions generally became more shallow as floral variability increased; this led to substantially longer carryover in the treatment with greatest variability. On the other hand, total amounts of dye transferred did not appear to be sensitive to the degree of variability. Floral variability may have a subtle but important effect on plant fitness by influencing the distance of pollen transfer.

Waser, N. M. and M. V. Price (1985). "The effect of nectar guides on pollinator preference: experimental studies with a montane herb." Oecologia **67**: 121-126.

Rare "albino" morphs of the montane larkspur Delphinium nelsonii differ from common blue-flowered morphs in overall flower color, and in the strength of a contrasting color pattern at the center of the flower that presumably guides pollinators to concealed nectar. Previous studies showed that bumblebees and hummingbirds discriminate against albinos when presented with mixtures of the 2 morphs, and that it takes these pollinators longer to fly between successive flowers on albino than on blue-flowered inflorescences. To explore the link between these observations, we measured pollinator preferences and flower-to-flower flight times ("handling times") before and after painting flowers in 2 alternative ways that enhanced albino nectar guides. In all of 16 experimental replicates discrimination against albinos was reduced or eliminated after painting, and albino handling times declined toward values for blue-flowered inflorescences. This consistent result indicates that an inferior nectar guide increases the energetic cost of foraging at albinos. Increased cost in turn explains discrimination, under the reasonable assumption that hummingbirds and bumblebees are sensitive to foraging economics.

Waser, N. M. and M. V. Price (1989). "Optimal outcrossing in *Ipomopsis aggregata*: seed set and offspring fitness." Evolution **43**: 1097-1109.

Restricted gene flow and localized selection should establish a correlation between physical proximity and genetic similarity in many plant populations. Given this situation, fitness may decline in crosses between nearby plants (inbreeding depression), and in crosses between more widely separated plants ("outbreeding depression") mostly as a result of disruption of local adaptation. It follows that seed set and offspring fitness may be greatest in crosses over an intermediate "optimal outcrossing distance." This prediction was supported for Ipomopsis aggregata, a long-lived herbaceous plant pollinated by hummingbirds. In six replicate pollination experiments, mean seed set per flower was higher with an outcrossing distance of 1-10 m than with selfing or outcrossing over 100 m. A similar pattern appeared in the performance of offspring from experimental crosses grown under natural conditions and censused for a seven-year period. Offspring from 10-m crosses had higher survival, greater chance of flowering, and earlier flowering than those from 1-m or 100-m crosses. As a result, 1-m and 100-m offspring achieved only 47% and 68%, respectively, of the lifetime fitness of 10-m offspring. Offspring fitness also declined with planting distance from the seed parent over a range of 1-30 m, so that adaptation to the maternal environment is a plausible mechanism for outbreeding depression. Censuses in a representative I. aggregata population indicated that the herbaceous vegetation changes over a range of 2-150 m, suggesting that there is spatial variation in selection regimes on a scale commensurate with the observed effects of outbreeding depression and planting distance. We discuss the possibility that differences in seed set might in part reflect maternal mate discrimination and emphasize the desirability of measuring offspring fitness under natural conditions in assessing outcrossing effects.

Waser, N. M. and M. V. Price (1990). "Pollination efficiency and effectiveness of bumble bees and hummingbirds visiting *Delphinium nelsonii*." Collectanea Botanica (Barcelona) **19**: 9-20.

Delphinium nelsonii Greene is a spring-flowering perennial of the Rocky Mountains of North America. Its blue flowers conform to a classical «bee pollination syndrome», but in western Colorado they are visited by hummingbirds (mostly in the first half of the flowering season) as well as bumble bee queens (mostly in the second half of the season). Experiments with potted plants showed that a bee deposits about 10 times as much pollen while visiting a flower as does a bird, and causes about la times as many seeds to be set. In contrast, bees and birds appear similar in the «quality» of pollen they deliver, e. g., in its outcrossing distance. At the level of entire pollinator populations, hummingbird visitation rates may be over 10 times as great as those or bumble bees, in part because birds visit flowers more quickly. Thus the two visitor classes should deliver similar pollen quantities overall, which is confirmed by similar pollen loads of flowers early and late in the season, and should contribute about equally to seed set, which is confirmed by several experiments and observations. Exact relative contributions probably depend on pollinator population sizes, which in the case of hummingbirds appear to have varied 2.5 fold across 14 years. The similar contributions or birds and bees lo seed set shows that individual pollination efficiency must be distinguished from population-level effectiveness. and that the «pollination syndrome» of a flower may not indicate present-day effectiveness of its visitors.

Waser, N. M. and M. V. Price (1991). "Outcrossing distance effects in *Delphinium nelsonii*: pollen loads, pollen tubes, and seed set." Ecology **72**(1): 171-179.

Seed production of a flower depends both on the quantity of pollen received and on its "quality" as manifested in rates of germination, ovule fertilization, and seed maturation. Disentangling effects of these two factors is complicated by the fact that seed set is a decelerating function of pollen quantity. One potential element of quality is the genetic similarity of pollen donor and recipient. In ten experiments with Delphinium nelsonii, seed set varied with outcrossing distance, a correlate of genetic similarity. Over all experiments, a 10-m distance significantly ($P < .02$) outperformed shorter and longer distances by 23-33%, suggesting quality differences. To explore this possibility, we fit decelerating negative-exponential regressions to relationships among stigma pollen load, pollen tube number reaching the ovary, and seed set in a subset of experiments having 1-, 10-, and 100-m treatments. Regression parameters suggested that 10-m pollen is most efficient at producing tubes and seeds, and analysis of residuals from pooled regressions (a nonlinear ANCOVA) showed that 10-m residuals were most positive. The latter effect was significant for pollen load-seed set relationships ($P = .009$, five experimental replicates) and pollen tube-seed set relationships ($P = .021$, two replicates), but not pollen load-pollen tube relationships ($P = .128$, three replicates). These results illustrate the utility of nonlinear regression in distinguishing pollen quantity and

quality. They reinforce the conclusion that outcrossing distance affects pollen quality in D. nelsonii, and that an "optimal outcrossing distance" between 1 and 100 m maximizes seed set on average in pollinations using one donor per carpel. Insofar as quality differences persist in natural pollen mixtures that arrive on stigmas, outcrossing distance should affect reproductive success through both paternal and maternal sexual functions.

Waser, N. M. and M. V. Price (1991). "Reproductive costs of self-pollination in *Ipomopsis aggregata* (Polemoniaceae): are ovules ursurped?" American Journal of Botany **78**(8): 1036-1043.

In common with many cosexual angiosperms, the hummingbird-pollinated montane herb Ipomopsis aggregata (Polemoniaceae) is likely to experience self-pollination. Does this incur a fecundity cost even in such a highly self-sterile (presumably self-incompatible) species? Histological studies showed that self pollen germinates, and its tubes penetrate ovules almost as often as those of outcross pollen. Ovules penetrated by self tubes are especially likely to show milky callose occlusion 24-48 hr after pollination, and several observations suggest an association between occlusion and degeneration. Compared to flowers receiving only outcross pollen, seed set was reduced by 42% on average when self pollen was applied along with outcross, either by hand (in emasculated flowers) or by natural autodeposition (in unemasculated flowers). Reductions were statistically indistinguishable whether self pollen was applied 9 hr before outcross pollen, or at the same time. Unemasculated flowers accumulated substantial self pollen loads, and this autodeposition persisted when flowers were probed to mimic hummingbird visitation. Geitonogamy also is substantial, judging from field estimates of pollen transfer. Thus natural self pollen deposition may be sufficient to "usurp" ovules that otherwise could mature. In this light, late-acting self-rejection in I aggregata seems decidedly less efficient than an early-acting system that would block pollen germination or tube growth.

Waser, N. M. and M. V. Price (1994). "Crossing-distance effects in *Delphinium nelsonii*: outbreeding and inbreeding depression in progeny fitness." Evolution **48**: 842-852.

Depending on its genetic causes, outbreeding depression in quantitative characters may occur first in the free-living F1 generation produced by a wide cross. In 1981-1985, we generated F1 progenies by hand-pollinating larkspurs (Delphinium nelsonii) with pollen from 1-m, 3-m, 10-m, or 30-m distances. From the spatial genetic structure indicated by previous electrophoretic and reciprocal transplantation studies, we estimate that these crosses range from being inbred ($f \approx 0.06$) to outbred. We planted 594 seeds from 66 maternal sibships under natural conditions. As of 1992, there was strong evidence for both inbreeding depression and outbreeding depression. Progeny from intermediate crossing distances grew approximately twice as large as more inbred or outbred progeny in the first 5 yr after planting ($P = 0.013$, repeated measures ANOVA), and survived almost 1 yr longer on average (contrast of 3-m and 10-m treatments versus 1 m and 30 m; $P = 0.028$, ANOVA). Twenty maternal sibships produced flowering individuals; only four and two of these represented 1-m and 30-m crossing distances, respectively ($P = 0.021$, G-test). The cumulative fitness of intermediate distance sibships averaged about twice that of 1-m sibships, and five to eight times that of 30-m sibships ($P = 0.017$, ANOVA). Thus, even though progeny of 1-m crosses were inbred to a degree only about one-eighth that of selfing, inbreeding depression approximated 50equaled or exceeded 50

Waser, N. M. and M. V. Price (2016). "Drought, pollen and nectar availability, and pollination success." Ecology **97**(6): 1400-1409.

Pollination success of animal-pollinated flowers depends on rate of pollinator visits and on pollen deposition per visit, both of which should vary with the pollen and nectar “neighborhoods” of a plant, i.e., with pollen and nectar availability in nearby plants. One determinant of these neighborhoods is per-flower production of pollen and nectar, which is likely to respond to environmental influences. In this study, we explored environmental effects on pollen and nectar production and on pollination success in order to follow up a surprising result from a previous study: flowers of Ipomopsis aggregata received less pollen in years of high visitation by their hummingbird pollinators. A new analysis of the earlier data indicated that high bird visitation corresponded to drought years. We hypothesized that drought might contribute to the enigmatic prior result if it decreases both nectar and pollen production: in dry years, low nectar availability could cause hummingbirds to visit flowers at a higher rate, and low pollen availability could cause them to deposit less pollen per visit. A greenhouse experiment demonstrated that drought does reduce both pollen and nectar

production by I. aggregata flowers. This result was corroborated across 6 yr of variable precipitation and soil moisture in four unmanipulated field populations. In addition, experimental removal of pollen from flowers reduced the pollen received by nearby flowers. We conclude that there is much to learn about how abiotic and biotic environmental drivers jointly affect pollen and nectar production and availability, and how this contributes to pollen and nectar neighborhoods and thus influences pollination success.

Waser, N. M., et al. (2016). "Effects of road dust on the pollination and reproduction of wildflowers." International Journal of Plant Sciences **178**(2): 85-93.

Premise of research. Dust particles and pollen grains are similar in size. Dust deposition might therefore influence the pollination and reproduction of flowering plants. Little is known about such effects, however, despite more general interest in ecological effects of dust.

Methodology. We used observational and experimental methods to explore whether dust generated by traffic on unpaved roads affects the amounts of pollen received and numbers of seeds produced by four species of native wildflowers in the western United States.

Pivotal results. Flowers of Nuttall's larkspur (Delphinium nuttallianum), scarlet gilia (Ipomopsis aggregata), Lewis flax (Linum lewisii), and sulphur paintbrush (Castilleja sulphurea) growing 1-2 m from a road received substantially more dust and less pollen than those growing 40-50 m away. We observed the same pattern when we transplanted individuals of the first two species into pots and placed pots near to compared with far from a road. Experimental 'hand dusting' of scarlet gilia and Lewis flax plants also reduced stigma pollen loads to a degree that resembled the average effect of road proximity for those species. On the other hand, numbers of seeds per flower ('seed set') did not vary consistently for any species as a function of road proximity or hand-dusting treatment.

Conclusions. Several mechanisms might contribute to the different effects of dust on pollen loads and seed set. We discuss four possible mechanisms, which we refer to as pollen excess, pollen quality, resource limitation, and compensatory herbivory. These mechanisms suggest avenues for further study of dust, pollination, and plant reproduction with this and other systems.

Waser, N. M., et al. (1987). "Female mate choice in a perennial herbaceous wildflower, *Delphinium nelsonii*." Evolutionary Trends in Plants **1**: 29-33.

The success of a cross between two plants should depend in part on their genetic similarity. For example, seed set in the wildflower Delphinium nelsonii is sensitive to the physical separation of pollen donor and recipient, an apparent index of genetic similarity; the optimal separation or 'optimal outcrossing distance' is 3-10 m. Offspring of crosses over the optimal outcrossing distance also appear to survive best under field conditions. These effects of outcrossing distance on fitness components are mirrored in the performance of male gametophytes. In a series of controlled crosses, pollen of donors 10 m from the recipient had a significantly higher probability of delivering a pollen tube to the ovary than did pollen from donors 1 m or 100 m away. This suggests a physiological interaction between pollen and pistil that increases the probability of producing offspring with high viability. Such interaction can be interpreted as adaptive discrimination or 'choice' of mates on the part of female tissue.

Waser, N. M., et al. (2000). "Outbreeding depression varies among cohorts of *Ipomopsis aggregata* planted in nature." Evolution **54**(2): 485-491.

Outbreeding depression in progeny fitness may arise from disruption of local adaptation, disruption of allelic coadaptation, or a combination of these "environmental" and "physiological" mechanisms. Thus the minimum spatial scale over which outbreeding depression arises should depend on the spatial scale of gene dispersal and (with an environmental mechanism) of change in selection regimes. We previously reported substantial outbreeding depression in lifetime fitness of progeny resulting from crosses among parents separated by 100 m in natural populations of the herbaceous plant Ipomopsis aggregata. In this paper we explore the effect of crossing distance on fitness in two additional experiments begun in 1987 and 1990. We planted seed progeny derived from partial diallel crossing designs in randomized blocks in maternal environments and scored emergence of seedlings, survival, and eventual flowering of individuals over the subsequent six to eight years. Nested within each diallel design were crossing distances of 1 m, 10 m, and 100 m. Compared to 1-m and 10-m progeny, 100-m progeny of the 1987 diallel suffered a significant reduction in seedling emergence, and both 1-m and 100-m progeny that survived to flower achieved lower

lambda-values on average than 10-m progeny. Total outbreeding depression suffered by 100-m relative to 10-m progeny was approximately 10%, compared to approximately 30% in our earlier study of I. aggregata. Progeny of 10-m crosses also outperformed 1-m and 100-m progeny of the 1990 diallel by approximately 5%, but no difference among crossing distance treatments was significant. Thus, the magnitude of outbreeding depression in 100-m crosses varied among experiments. This is not surprising given likely spatial and temporal variation in gene flow and selection regimes, different population histories, and different parental and progeny environments. Characterizing outbreeding depression on the shortest spatial scales over which it is expressed, as well as its variation and causes, is worthwhile because it promises to shed light on the earliest stages of angiosperm speciation.

Waser, N. M. and L. A. Real (1979). "Effective mutualism between sequentially flowering plants species." Nature **281**: 670-672.

Since the theoretical paper of Levin and Anderson, it has been widely recognised that animal pollinators represent resources for which plants can compete. Competition for pollination might take several forms and act as a powerful selective force in establishing or maintaining sequential flowering among sympatric species. Sequential flowering in Arctic, temperate and neotropical plant assemblages has been interpreted as an evolutionary result of competition for pollination. This interpretation may prove correct in many cases, although strong evidence for competition is available for few systems. Heinrich and Raven (see also Baker and Baker et al.) pointed out that sympatric plant species may act as mutualistic partners at the same time that their sequential flowering is maintained by competition. We develop this hypothesis here explicitly and present evidence that effective mutualism occurs between two species which also compete for pollination.

Waser, N. M., et al. (1995). "Seed set and seed mass in *Ipomopsis aggregata*: variance partitioning and inferences about post-pollination selection." Evolution **49**(1): 80-88.

Events that follow pollination, such as pollen-tube growth and seed maturation, comprise an important phase of angiosperm reproduction. Differential success during this "postpollination" phase may represent phenotypic selection, including sexual selection, or interaction between parents caused, for example, by their genetic similarity. By providing a detailed partitioning of variance in success, diallel crossing designs offer great potential to determine which processes are occurring and their relative magnitudes. We performed three partial diallels with the montane herb Ipomopsis aggregata, using a large sample of parental plants (69 total). Embedded in the designs were crossing-distance treatments of 1 m, 10 m, and 100 m, reflecting a range of parental genetic similarity. We partitioned phenotypic variance in seed set per fruit into six components using restricted maximum-likelihood (REML) analysis. For one diallel, we also partitioned variance in seed mass into five components, and estimated two components of covariance between seed set and mass. Variance caused by maternal effects (Vmat) comprised 12%-35% of total variance in seed set and 62% of variance in seed mass, and there was a significant negative environmental covariance between seed set and seed mass. Parental interaction made no detectable contribution to phenotypic variance in either of our measures of postpollination success, although crossing distance did contribute slightly but significantly to fit of the model in some cases. Finally, there was no detectable paternal variance (Vpat) in seed set or seed mass. These results are in keeping with reports from other studies of natural plant populations. The finding of little or no paternal variance in particular suggests little scope for postpollination sexual selection through the male function of cosexual plants such as I. aggregata.

Waser, N. M. and Williams, C. F. (2001). Inbreeding and outbreeding. Evolutionary ecology: Concepts and case studies. C. W. Fox, D. A. Roff and D. J. Fairburn. Oxford, Oxford University Press**:** 84-98.

Watt, W. B., et al. (1974). "Nectar resource use by *Colias* butterflies." Oecologia **14**(4): 353-374.

Nectar foraging preferences of Colias butterflies in two different mountain ecosystems are examined with respect to plant distribution, nectar quantity, carbohydrate (and amino acid) content of nectar, and visual pattern of the plants utilized and avoided. Colias, and apparently numerous other small, ectothermic, low-energy-demand pollinators, “patronize” plants producing relatively dilute nectars containing a high proportion of monosaccharide sugars and significant amounts of polar, nitrogen-rich amino acids. These plants also converge on a common “target” flower pattern in ultraviolet and human-visible light. High-energy demand, endothermic pollinators, by contrast, appear to require higher concentration nectars and/or

higher proportions of di- and oligosaccharide sugars. These results are discussed in the light of water balance and energy budget demands of different pollinator classes. Questions are also raised concerning behavioral aspects of pollinator search for resources and the pertinence of these data to the concept of floral mimicry.

Williams, C. F. (2007). "Effects of floral display size and biparental inbreeding on outcrossing rates in *Delphinium barbeyi* (Ranunculaceae)." Am J Bot **94**(10): 1696-1705.

Floral display size represents a tradeoff between the benefits of increased pollinator visitation and the quantity of pollen received vs. the costs of increased self-pollination and reduced pollination quality. Plants with large floral displays often are more attractive to pollinators, but pollinators visit more flowers per plant. Intraplant foraging movements should increase self-pollination through geitonogamy, lowering outcrossing rates in large plants. Local genetic structure should also increase inbreeding and decrease outcrossing estimates, if pollinators move between neighboring, related plants. These predictions were tested in a population of larkspurs (Delphinium barbeyi) in Colorado. Allozymes were used to estimate outcrossing rates of plants varying in display size. Floral displays varied widely (2-1400 flowers; 1-26 inflorescences per plant), and outcrossing rate decreased significantly with increasing display size. Large, multistalked plants self over twice as frequently as single-stalked plants (46 vs. 21%). Local population structure is significant, and biparental inbreeding depresses outcrossing in plants surrounded by genetically similar neighbors. Protandry, coupled with stereotypical bottom-up pollinator foraging, reduces self-fertilization by autogamy or geitonogamy within inflorescences. Selfing is predominantly (>60%) by geitonogamy between inflorescences in large plants. Geitonogamy may be a significant cost to plants with large floral displays if inbreeding depression and/or pollen and ovule discounting results. If so, floral display size, particularly inflorescence number, may be under contrasting selection for pollination quantity vs. quality.

Williams, C. F., et al. (2000). "Floral dimorphism, pollination, and self-fertilization in gynodioecious *Geranium richardsonii* (Geraniaceae)." Am J Bot **87**(5): 661-669.

The selective maintenance of gynodioecy depends on the relative fitness of the male-sterile (female) and hermaphroditic morphs. Females may compensate for their loss of male fitness by reallocating resources from male function (pollen production and pollinator attraction) to female function (seeds and fruits), thus increasing seed production. Females may also benefit from their inability to self-fertilize if selfing and inbreeding depression reduce seed quality in hermaphrodites. We investigated how differences in floral resource allocation (flower size) between female and hermaphroditic plants affect two measures of female reproductive success, pollinator visitation and pollen receipt, in gynodioecious populations of Geranium richardsonii in Colorado. Using emasculation treatments in natural populations, we further examined whether selfing by autogamy and geitonogamy comprises a significant proportion of pollen receipt by hermaphrodites. Flowers of female plants are significantly smaller than those of hermaphrodites. The reduction in allocation to pollinator-attracting structures (petals) is correlated with a significant reduction in pollinator visitation to female flowers in artificial arrays. The reduction in attractiveness is further manifested in significantly less pollen being deposited on the stigmas of female flowers in natural populations. Autogamy is rare in these protandrous flowers, and geitonogamy accounts for most of the difference in pollen receipt between hermaphrodites and females. Female success at receiving pollen was negatively frequency dependent on the relative frequency of females in populations. Thus, two of the prerequisites for the maintenance of females in gynodioecious populations, differences in resource allocation between floral morphs and high selfing rates in hermaphrodites, occur in G. richardsonii.

Williams, C. F., et al. (2001). "Pollination, breeding system, and genetic structure in two sympatric *Delphinium* (Ranunculaceae) species." American Journal of Botany **88**(9): 1623-1633.

Two sympatric Delphinium species, D. barbeyi and D. nuttallianum, are ecologically and morphologically similar. However, D. barbeyi has multiple, large inflorescences while D. nuttallianum has a single, small inflorescence. These differences in floral display should result in greater intraplant pollen transfer in D. barbeyi, leading to higher rates of self-pollination through geitonogamy. Reduced gene flow by pollen should in turn produce greater population differentiation among populations of D. barbeyi relative to D. nuttallianum. We tested these predictions by comparing pollinator behavior, breeding systems, outcrossing rates, and population genetic structure of sympatric populations of the two species in Colorado. Bumble bee

and hummingbird pollinators visit more flowers and inflorescences per foraging bout in D. barbeyi than in D. nuttallianum. The species differed in breeding system; D. barbeyi produced more seeds by autogamy (9 vs. 2%) than D. nuttallianum and suffered no reduction in seed set in hand-self vs. outcross pollinations, in contrast to a 41% decline in D. nuttallianum. The outcrossing rate in one D. barbeyi population was 55%, but ranged from 87 to 97% in four D. nuttallianum populations. Genetic differentiation among population subdivisions estimated by hierarchical F statistics was >10 times greater in D. barbeyi (= 0.055–0.126) than D. nuttallianum (= 0.004–0.009) at spatial scales ranging from metres to 3.5 km. Spatial autocorrelation analysis also indicated more pronounced local genetic structure in D. barbeyi than D. nuttallianum populations. Fixation indices (FIS) of D. barbeyi adults were much lower than expected based on mating system equilibrium and suggest that differences in the degree of self-compatibility and/or the timing of postpollination selection/inbreeding depression between the two species further contribute to the genetic differences between them.

Williams, C. F. and N. M. Waser (1999). "Spatial genetic structure of *Delphinium nuttallianum* populations: inferences about gene flow." Heredity **83**(5): 541-550.

The spatial genetic structure of a plant population provides a potential record of past gene flow and mating. We used hierarchical F-statistics and spatial autocorrelation to characterize spatial genetic differentiation of allozymes in adult Delphinium nuttallianum plants within and among six natural populations separated from one another by up to 3 km. Previous direct estimates suggested that gene flow is highly localized, averaging <<10 m. Earlier studies of seed-set, pollen-tube growth and progeny fitness suggested that partial reproductive isolation exists between plants growing too close together (<3 m) and too far apart (>100 m). Thus we anticipated substantial genetic differentiation on scales of a few to hundreds of metres. However, we detected little differentiation among the six populations, among replicate study plots within populations, or among subsections of study plots, except at the smallest scale of cm to m. These results suggest that relatively rare long-distance pollen movement has gone undetected and that postpollination selection may further modify genetic structure during the life cycle. Lack of differentiation is not at odds with the observation of partial reproductive isolation, because some loci may respond to spatial variation in selection without this response being evident at marker loci.

Williams, N. M. and J. D. Thomson (1998). "Trapline foraging by bumble bees: III. Temporal patterns of visitation and foraging success at single plants." Behavioral Ecology **9**(6): 612-621.

We analyzed the temporal structure of visitation by bumble bee workers to a single Penstemon strictus plant growing in an array of conspecifics. When tested against a null distribution using a randomization model, the observed pattern of arrivals for the whole group of bees was random, but departures were clustered in time. Certain individuals visited the plant repeatedly and frequently throughout the day. These showed significantly regular arrival and departure schedules, which were likely produced by traplining. We explored whether these more frequent and regular foragers gained a higher reward than random or incidental plant visitors. Using an analytical model, Possingham predicted that a dominating forager that visited a simple, renewing resource in a regular pattern would garner higher and less variable rewards than random visitors. Inspired by these results, but interested in plant-level visitation, we constructed a simulation model of resource dynamics for a multiflowered plant with high visitation. The model incorporates the observed visitation schedules of all bees and independent reward dynamics for each flower on the plant. We calculated the rewards that observed bees would have collected given a range of resource-renewal parameters. More frequent visitors did not return to the plant when whole-plant resource levels were higher, but these visitors did get greater rewards. Their increased reward resulted from greater foraging efficiency, primarily through selecting (on average) more rewarding flowers than those selected by less frequent, random visitors.

Wolf, L. L. and F. R. Hainsworth (1986). "Information and hummingbird foraging at individual inflorescences of *Ipomopsis aggregata*." Oikos **46**: 15-22.

We studied hummingbirds foraging at single stalks of Ipomopsis aggregata near Crested Butte, CO, USA. Birds can achieve higher than average benefits by foraging at stalks that have had the longest time to accumulate nectar. Relative quality of stalks, as measured by nectar per flower, was not correlated with either plant height or number of flowers, so birds could not use this information in choosing which plants to visit.

Nectar volumes were not autocorrelated among flowers within a stalk so foraging birds could not predict volumes in nearby flowers on the same plant. Birds visiting stalks of different quality visited different numbers of flowers before they left. Foraging birds left most stalks before visiting all flowers, some after visiting only 5 or fewer flowers on stalks that had 10 or more flowers. Volumes in flowers remaining on stalks the birds left after probing only a few flowers had similar averages, but lower medians than stalks on which more flowers were visited. The primary cue for early departures from a stalk seems to be probing an empty flower(s). Stalks with higher average volumes had fewer empty flowers and empty flowers statistically were not clustered vertically on a stalk. Comparisons with the behavior of other pollinators foraging on single plants of other species suggests that interactions among inflorescence architecture, patterns of nectar production, and pollinator behavior influence the information available to make decisions about when to leave an inflorescence. Ipomopsis provides little information and birds would derive relatively little benefit from sophisticated departure decisions.

Wolf, P. G., et al. (2001). "Tests of pre- and postpollination barriers to hybridization between sympatric species of *Ipomopsis* (Polemoniaceae)." American Journal of Botany **88**(2): 213-219.

The Ipomopsis aggregata species complex (Polemoniaceae) includes species pairs that hybridize readily in nature as well as pairs that meet along contact zones with no apparent hybridization. Artificial hybrids can be made between I. aggregata and I. arizonica, yet morphological intermediates between these two species have not been observed in natural populations. This apparent lack of hybridization is perplexing given that plants of the two species often grow within a few metres of each other and both species have red flowers visited by the same species of hummingbirds. We used trained hummingbirds to examine pollen transfer within and between species. We also hand-pollinated flowers to examine paternal success of heterospecific and conspecific pollen, testing paternity with electrophoretic examination of seeds. Hummingbirds were not simply better at transferring pollen within than between species. Instead, I. arizonica was a better pollen donor so that considerable pollen transfer was observed from I. arizonica to I. aggregata, but very little in the opposite direction. Conversely, once pollen arrived at stigmas, I. arizonica pollen performed very poorly on I. aggregata pistils. However, pollen from I. aggregata could, in some cases, sire seeds on I. arizonica. We hypothesize that hybrids are scarce in nature, in part, because of asymmetric barriers to reproduction: little pollen transfer in one direction and poor pollen performance in the other.;

Wright, K. W., et al. (2015). "Turnover and reliability of flower communities in extreme environments: Insights from long-term phenology data sets." Journal of Arid Environments **115**(0): 27-34.

We used three long-term data sets from the southwestern US to investigate the reliability of flowering communities from the perspective of pollinators in extreme environments. The data sets come from three desert sites in New Mexico, two subalpine sites in Colorado, and an elevation gradient in Arizona. We used two indices to explore different temporal scales. We calculated turnover rates of species in bloom on a seasonal basis to investigate how flowering communities change from year to year. We calculated frequency of bloom in the same month over all years to determine the reliability of flowering communities in a narrow time scale. We hypothesized that communities with less reliable precipitation would have lower frequency of bloom and higher turnover rates and that annual plants would show this pattern more strongly than perennials. Flower frequency ranged from 50.3% at the highest elevation AZ site to 66.3% at a subalpine CO site. Within each site, annuals exhibited lower frequencies than perennials. On a seasonal scale, turnover rates ranged from 22.5% in Colorado to 71.4% at a NM site. Looking at the entire flower community as a resource for foraging pollinators, we found that flowers are an unreliable resource, especially in unpredictable environments.

Zimmerman, M. (1983). "Plant reproduction and optimal foraging: experimental nectar manipulations in *Delphinium nelsonii*." Oikos **41**: 57-63.

Delphinium nelsonii Greene (Ranunculaceae) individuals which were watered produced significantly more nectar per flower than did control individuals. The watered plants also set significantly more seeds per flower than did the controls. Hand pollinations suggest that the seed set difference was due to the increased nectar volume rather than to watering directly. Bumblebee pollinators' responses to the increased standing crop of reward were consistent with predictions from optimal foraging theory and explain the increase in seed set. Pollinators both visited more flowers per inflorescence and spent more time in flowers on nectar-rich plants

relative to controls. These results imply that D. nelsonii individuals could increase the female component (i.e. seed set) of plant fitness by increasing their nectar production rate. The results do not, however, allow an accurate assessment of the male component (i.e., pollen donation) of fitness.

Zimmerman, M. (1984). "Reproduction in *Polemonium*: a five year study of seed production and implications for competition for pollinator service." Oikos **42**: 225-228.

Conditions necessary for intraspecific competition for pollinator service, but not intraspecific competition itself, have previously been reported for a population of Polemonium foliossisimum Gray. As in most recent work of this sort, this conclusion was based, in part, on seed set differentials among individuals as well as between control and hand-pollinated flowers. Such differentials, when found for a single flowering season in perennial plants can, however, be misleading. Stored reserves might be drawn upon one season at the expense of the reproductive potential of later years negating an apparent reproductive increase. A five year study of seed set and flower production in a P. foliosissimum population indicates that no such reproductive tradeoff is occurring. Available information is consistent with the contention that intraspecific competition for pollinator service may have taken place and thus supports conclusions drawn from short term studies on P. foliosissimum.

Zimmerman, M. (1987). "Reproduction in Polemonium: factors influencing outbreeding potential." Oecologia **72**: 624-632.

Three measures of potential outbreeding efficiency were quantified throughout two flowering seasons in a population of the mass-flowering Polemonium foliosissimum: 1) the distances flown between plants by bumblebee pollinators; 2) the proximity of visited plants; and 3) the diversity of individuals visited. Results were consistent between years. Individual plants did not function differentially with respect to female (i.e., pollen receipt) and male (i.e., pollen donation) function. Both female and male components of outbreeding, as estimated by all three measures, were positively correlated with the number of flowers per plant. Significantly more outbreeding could occur during the final phase of flowering when most individuals had relatively few blossoms. Although the potential for outbreeding was the same at any time through an individual's blooming regime, individuals for which the peak of blooming occurred after the peak for the population as a whole have significantly more outbreeding potential via both female and male function. These factors may combine such that any pressure to increase outbreeding in this self-incompatible species manifests itself in directional selection for delayed flowering time.

Zimmerman, M. (1988). "Pollination biology of montane plants: relationship between rate of nectar production and standing crop." American Midland Naturalist **120**: 50-57.

Rates of floral nectar production and volumes of standing crops of floral nectar were measured for eight species of Colorado montane plants. Although significant positive correlations exist between the two variables, the magnitudes of the coefficients are not very great. This surprising result may be due to the large variability in rate of nectar production within each species. The patterns suggest that pollinators, by responding to standing crop volumes, may not exert very strong selective pressure on rate of floral nectar production. All species examined had similarly variable patterns of standing crop indicating that pollinators encounter a wide range of reward variability while collecting nectar, regardless of which plant species is being visited.

Zimmerman, M. and R. S. Gross (1984). "The relationship between flowering phenology and seed set in an herbaceous perennial plant, *Polemonium foliosissimum* Gray." American Midland Naturalist **111**: 185-191.

To assess the potential for evolutionary change in blooming time within a population of Polemonium foliosissimum Gray (Polemoniaceae), flowering phenology, seed set and seed predation frequencies of individual plants were monitored from 1977-1981 in the Colorado Rocky Mountains. In only 1 of 4 years was there a significant correlation between flowering rank and average seed set per flower. In contrast, there was a significant (or nearly significant, $p < .06$) positive correlation between blooming time and total plant seed set in 3 of 4 years. These positive correlations resulted from greater flower production by later-blooming plants. Thus, total seed set varied independently of seed production on a per blossom basis, indicating that seed set per flower should be used only with caution as a measure of the maternal component of plant fitness. The fairly consistent positive relationship between blooming time and total seed set indicates that

directional selection for later flowering may be occurring in P foliosissimum. Seed predation by Hylemya sp. (Anthomyiidae, Diptera) increases selection pressure more often than it offsets it. Significant, positive between-year correlations of flowering ranks of individuals, along with other inferential evidence, suggest that flowering time is likely to be heritable in P foliosissimum.

Zimmerman, M. and J. M. Pleasants (1982). "Competition among pollinators: quantification of available resources." Oikos **38**: 381-383.

In a study of the bee community of short-grass prairie Tepedino and Stanton (1981) attempted to verify several predictions of competition theory by comparing abundances of bees and flowers. Their results were inconclusive suggesting, they said, that bees do not continuously compete for floral resources. We questioned their use of the number of open flowers as a measure of resource availability to bees because it is the nectar contained within those flowers that is the resource actually utilized. In two Rocky Mountain meadows we attempted to correlate bumblebee abundance with both the number of available flowers and the number of flowers multiplied by their 24 hour nectar production rates. Our results demonstrated that merely using floral abundance as an estimate of resource availability can be very misleading. The more time consuming method of quantifying the resources actually used by bees should be performed if meaningful conclusions concerning competition are to be drawn.

Zimmerman, M. and G. H. Pyke (1986). "Reproduction in *Polemonium*: patterns and implications of floral nectar production and standing crops." American Journal of Botany **73**: 1405-1415.

Patterns of floral nectar production and standing crop were measured in four populations of the herbaceous perennial plant species Polemonium foliosissimum. Contrary to prediction (Pleasants, 1983), individual flowers in this mass-flowering species were found to produce equivalent nectar volumes every day of their lives. Alternative methods of increasing the reward variability presented to pollinators are evaluated for P. foliosissimum and the relationship between that variability and risk-aversive foraging by pollinators is discussed. Significant spatial and temporal variability in rate of nectar production was found. Populations separated by approximately 200 m exhibited different rates. Nectar production declined significantly as a function of time of the flowering season in two populations but not in a third. In spite of such variability, individual plants showed consistency in production both within a single blooming season and across successive seasons. Because of the variability found in the present study, care should be taken to design appropriate sampling protocols in future nectar studies. Patterns of standing nectar crop were consistent with those expected if pollinators were using an area-restricted searching pattern.

Zimmerman, M. and G. H. Pyke (1988). "Experimental manipulations of *Polemonium foliosissimum*: effects on subsequent nectar production, seed production and growth." Journal of Ecology **76**: 777-789.

(1) The carbon budgets of Polemonium foliosissimum individuals were experimentally manipulated. The amount of available carbon was decreased by defoliating 50% of each plant, while available carbon was enhanced by daily watering and by removing either 50% or 100% of the flower buds. (2) Rates of nectar production per flower were constant across all treatments except for a significant increase observed in the watered individuals. (3) There were no consistent changes in average seed set per flower or per plant across treatment groups. (4) Defoliated and control individuals did not differ significantly from one another with respect to average seed weight, but end-of-season underground biomass was significantly less for defoliated plants relative to controls. (5) In the year following treatment, no differences were observed in nectar production per flower, seed production per flower or seed production per plant, although a significant difference in rate of growth was observed. Defoliated plants grew least during the 2-year period, followed by control individuals. The debudded plants increased in size by the greatest margin. (6) The trade-offs between resources allocated to nectar, flowers, seeds and vegetative growth can apparently be expressed in terms of biomass

Zimmerman, M. and G. H. Pyke (1988). "Reproduction in *Polemonium*: assessing the factors limiting seed set." American Naturalist **131**: 732-738.

Evidence of pollen and/or resource limitation of seed production in the herbaceous perennial plant Polemonium foliosissimum was investigated. Large numbers of open flowers on selected individuals were hand-pollinated at regular intervals, and seed set was compared with that of control blossoms on those

same plants as well as with that of control flowers on control individuals. Experimental and control individuals were monitored and reproductive output measured during the following flowering season as well. Although hand-pollinated flowers set significantly more seeds than did either set of control blossoms, the results suggest that individual reproduction (i.e., the total number of seeds produced by a plant) was limited by resources other than pollen. Pollen availability may also have limited seed production, but to a lesser extent. A proper protocol for examining the limitation of seed set is developed, and the biases inherent in most of the simplified procedures currently in use are discussed

Zung, J. L., et al. (2015). "Bee- to bird-pollination shifts in *Penstemon*: effects of floral-lip removal and corolla constriction on the preferences of free-foraging bumble bees." Evolutionary Ecology **29**(3): 341-354.

Plants might be under selection for both attracting efficient pollinators and deterring wasteful visitors. Particular floral traits can act as exploitation barriers by discouraging the unwelcome visitors. In the genus Penstemon, evolutionary shifts from insect pollination to more efficient hummingbird pollination have occurred repeatedly, resulting in the convergent evolution of floral traits commonly present in hummingbird-pollinated flowers. Two of these traits, a reduced or reflexed lower petal lip and a narrow corolla, were found in a previous flight-cage study to affect floral handling time by bumble bees, therefore potentially acting as "anti-bee" traits affecting preference. To test whether these traits do reduce bumble bee visitation in natural populations, we manipulated these two traits in flowers of bee-pollinated Penstemon strictus to resemble hummingbird-adapted close relatives and measured the preferences of free-foraging bees. Constricted corollas strongly deterred bee visitation in general, and particularly reduced visits by small bumble bees, resulting in immediate specialization to larger, longer-tongued bumble bees. Bees were also deterred—albeit less strongly—by lipless flowers. However, we found no evidence that lip removal and corolla constriction interact to further affect bee preference. We conclude that narrow corolla tubes and reduced lips in hummingbird-pollinated penstemons function as exploitation barriers that reduce bee access to nectaries or increase handling time.