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Evidence for reward costs in two bee-pollinated *Penstemon*

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Table S1. Selection gradients and quadratic nonlinear selection for two *Penstemon* species

Table S2. Detailed statistics for linear selection gradients.

Figure S1. Partial regression plot showing the nonlinear relationship for selection on petal width in *Penstemon mensarum*.

ABSTRACT

Floral rewards are generally neglected in studies of natural selection on flowers, despite their importance in attracting pollinators. While evolution of rewards is evident from a phylogenetic and pollination system perspective, estimating selection on rewards is critical to understand how they are likely to evolve in the future. Therefore, we examined natural selection in two bee-pollinated species to compare selection on rewards between the species and among floral traits. Using *Penstemon mensarum* and *P. strictus*, we estimated phenotypic selection on several floral traits, including nectar and pollen rewards. We measured selection on plant height, flower number, petal width, corolla tube gape width, total corolla tube length, and proximal tube length, as well as rewards, to compare selection on these floral traits to nectar volume and pollen number. We also determined if any floral traits were honest signals of reward. Selection on floral traits generally differed between the two bee-pollinated species. Notably, there was selection to reduce nectar in *P. strictus* and stabilizing selection on pollen number for both species. We found little evidence for honest signalling of rewards or correlational selection on signals and traits, although our sample size limits these tests. Despite strong positive correlations in corolla tube dimensions, there was selection for longer corolla tubes but shorter proximal tube in *P. strictus*. Higher floral rewards were related to reduced female fitness in these species, suggesting costs for reward production. For nectar this pattern was linear but for pollen it was only the highest pollen grain numbers that showed a cost.

Introduction

Floral signals, pollinator fit traits, and pollinator rewards have been a focus of research into understanding angiosperm evolution and diversity (Fenster et al. 2004; Dellinger 2020). While microevolutionary processes are ultimately expected to explain these broad macroevolutionary patterns of trait convergence and divergence, few studies have examined floral rewards in this context (Parachnowitsch et al. 2019). The lack of inclusion of floral rewards in natural selection studies, makes it challenging to compare evolution of rewards with that of other floral traits. Including rewards in microevolutionary studies is particularly important because of the expected correlations with floral traits, especially signaling traits, which are often hypothesized to provide honest signals of rewards to pollinators (Wright and Schiestl 2009; Parachnowitsch et al. 2019). Pollinators likely use honest signals in foraging decisions (e.g. Knauer and Schiestl 2015) and may therefore drive selection and evolution of honest signals (Benitez-Vieyra et al. 2010, 2014; Ito et al. 2021; Eisen et al. 2023). However, since simple positive correlations between signals and rewards cannot always be assumed (Burdon et al. 2020; van der Kooi et al. 2023), the inclusion of rewards in selection studies is necessary to understand how signals and rewards evolve.

Nectar is a major nutrient source for many flower visitors (Nicolson 2022) and thus is thought to be shaped by pollinator-mediated selection (Irwin et al. 2004; Parachnowitsch et al. 2019). However, the focus of natural selection studies in pollination biology has been on floral signals and pollinator fit traits (Harder and Johnson 2009; Caruso et al. 2019; Opedal 2021), rather than the rewards themselves in part because of the challenges of qualifying nectar (Parachnowitsch et al. 2019). Only a handful of studies estimate phenotypic selection on nectar traits (reviewed in Parachnowitsch et al. 2019), although recently studies that include nectar

traits in selection analyses have rapidly increased (Lu et al. 2021; Mackin et al. 2021; Egan et al. 2022; García et al. 2023, 2024; Hou et al. 2024; Rathnayake and Parachnowitsch 2025). Selection has been detected to both increase and decrease nectar volume (Mackin et al. 2021; García et al. 2023), increase nectar sugar concentration (Kulbaba and Worley 2012), increase nectar amino acid content (Gijbels et al. 2015), reduce nectar toxins (Egan et al. 2022), as well as on nectar gradients within an inflorescence (Zhao et al. 2016; Hou et al. 2024). However, a lack of selection on nectar is also common when it has been measured (Parachnowitsch et al. 2019; García et al. 2024; Rathnayake and Parachnowitsch 2025) and the drivers of this selection when detected have been rarely tested. There was pollinator-mediated selection on the nectar gradient in *Aconitum gymnantrum* but not nectar volume (Zhao et al. 2016) and selection on nectar in *Ipomea purpurea* was driven by drought rather than pollinators (García et al. 2023) yet general patterns are difficult to extract from so few estimates. Although nectar production may differ from many other floral traits because it can respond to, for example, pollinator visitation, making it more like a behaviour than something like flower size, the growing collection of selection coefficients suggests it is possible to estimate selection on at least some of its properties.

While the function of nectar as a reward for pollinators is fairly straightforward, the role of pollen is more nuanced. Pollen can also be an important nutrient reward for bees (Lau et al. 2022) but as the male gamete, pollen's relation to fitness has quite reasonably been mainly studied from the perspective of the siring seeds (Opedal et al. 2023). Selection on pollen traits via male fitness has been examined in a few studies. For example, there was selection to increase pollen number per flower in *Raphanus raphanistrum* via male fertility (Morgan and Conner 2001) and a similar but non-significant trend in *Fragaria virginiana*, perhaps because the number of flowers had a bigger impact on overall pollen production than per flower (Ashman

and Penet 2007). Pollen traits are not only related to their gametic function because pollen is also often a reward for pollinators and can influence how bees forage on flowers (Nicholls and Hempel de Ibarra 2017; Hao et al. 2020). As a reward, it follows that pollen quantity or quality could then influence visitation in a similar way to nectar rewards (Russell et al. 2016; Nicholls and Hempel de Ibarra 2017) and thereby affect female fitness via seed set. However, the effects of pollen rewards on female fitness have rarely been studied (but see Duffy and Johnson 2011; Duffy et al. 2014). Although pollen cannot change dynamically in response to pollinator visitation as nectar can in many systems, most selection studies on nectar have been on the available reward rather than its dynamic production (Parachnowitsch et al. 2019), making it easier to compare selection between nectar and pollen. While pollinators may generally prefer higher rewarding flowers (Carr et al. 2015; Parachnowitsch et al. 2019; Burdon et al. 2020), we might not always expect selection to increase rewards from the plant's perspective. For example, nectar may be costly to produce (Pyke and Ren 2023) and in some circumstances be under selection to reduce the reward (e.g. drought, García et al. 2023). However, with no studies that have examined selection on nectar and pollen in the same species, it is difficult to predict whether these will have similar or contrasting patterns.

Using two bee-pollinated *Penstemon* species, we assessed phenotypic selection on nectar and pollen via female fitness. *Penstemon* has become a model system for understanding pollination, mainly focused on shifts from bee to hummingbird pollination (Wilson et al. 2004; Wessinger et al. 2014, 2023). Nectar rewards have evolved in response to shifts in pollination in the genus, with increased volume and decreased sugar concentration associated with the shift from insect to hummingbird pollination (Castellanos et al. 2002; Wessinger et al. 2014). Pollen release from anthers is also associated with different pollinator groups, with slower release in

bee-pollinated *Penstemons* where the pollinators actively collect pollen (Thomson et al. 2000; Rodríguez-Peña and Wolfe 2023), suggesting that nectar and pollen likely experience natural selection in these species. While evidence for natural selection on floral traits is available for two bee-pollinated species in the genus (Parachnowitsch and Kessler 2010; Parachnowitsch et al. 2012; Recart and Campbell 2021), rewards were not included in these studies. In *P. digitalis*, there is evidence for pollinator-mediated selection to increase flower size and number (Parachnowitsch and Kessler 2010), as well as selection to increase floral scent (Parachnowitsch et al. 2012). Spatiotemporal variation in selection in *P. digitalis* depends on the trait examined; on total flowers selection is positively related to failed fruits, while selection for shorter plant height is related to predispersal seed predation and selection for a smaller gape width increases with the opportunity for selection (Parachnowitsch and Geber 2026). However, selection on platform length in *P. strictus* was not detectable in the one study that examined it (Recart and Campbell 2021). For hummingbird-pollinated *P. centranthifolius*, nectar production rate was included but not under significant selection via female fitness (Mitchell et al. 1998). Thus, the role of selection on nectar and pollen rewards in *Penstemon* is generally lacking despite their importance in broader evolutionary patterns in the genus.

Interpreting selection on flower traits has benefited from comparisons among traits in these complex reproductive organs to understand where selection is likely to act (e.g. Harder and Johnson 2009; Opedal 2021). Therefore, we estimated selection on reward in the context of floral traits likely to influence pollinator attraction and effectiveness. For example, the lower lip of *P. strictus* and gape width of the corolla tube are important for interactions with bumblebees as removal and constriction experiments have demonstrated (Castellanos et al. 2004; Zung et al. 2015). Lip removal increased the number of missed stigmas in bumblebee visits, and both gape

constriction and lip removal reduce bumblebee visitation (Castellanos et al. 2004; Zung et al. 2015), suggesting these may be targets of selections. Flowers with longer lower lips (platforms) also have higher visitation rates but there was no pollinator-mediated selection for longer lower lips in *P. strictus* in one study where it was measured (Recart and Campbell 2021). Because floral traits and rewards may influence pollinators through honest signalling, we also assessed whether any floral traits were honest signals of rewards in these species to evaluate whether selection is more likely for the honest signals, rewards, or their correlation. By measuring selection in two species with similar floral form due to ancestry and shared pollinators, we could assess whether selection on floral traits was consistent or differed between the species (Eisen et al. 2020). Because the species are similar in habitat, flowers, rewards, and visitors, we had no strong expectations for similarities or differences in selection between them.

MATERIALS AND METHODS

Study species and system We studied *Penstemon strictus* Benth (Rocky Mountain Penstemon) and *Penstemon mensarum* Pennell (Grand Mesa Beardtongue) while based at Rocky Mountain Biological Laboratory (RMBL), Crested Butte, Colorado, USA. The species have overlapping ranges in Colorado and were chosen because there were accessible populations that had sufficient individuals to measure selection. Within the genus the two species are from different branches of the *Penstemon* phylogeny within the *Habroanthus* clade (Wolfe et al. 2006, 2021), although exact relationships among species are still being resolved in this rapidly diverged genus (Wessinger et al. 2016). The flowers have a "classical" bee-pollination syndrome for *Penstemon*, characterized by tubular flowers, with more prominent landing platforms and more concentrated nectar compared to hummingbird-pollinated *Penstemon* (Wilson et al. 2004).

Flowers are protandrous and visited mainly by bees that can collect nectar and pollen as food. *Penstemon strictus* has been well studied at RMBL and therefore some pollination ecology is understood. For example, *Bombus spp.* are more effective pollinators of *P. strictus* than other bee species (Recart and Campbell 2021) and hummingbirds (Castellanos et al. 2003). Nectar in *P. strictus* flowers replenishes after visitation (Castellanos et al., 2002; Thompson, 2001) and pollen is also released gradually as anthers dehisce which is hypothesized to reduce loss to hymenopteran visitors (Thompson, 2000). While nectar often varies with sexual phase in *Penstemons* (Castellanos et al. 2002; Salas-Arcos et al. 2017; Burdon et al. 2020), the inflorescence architecture and flowering maturation patterns in *Penstemon* means that there is not a predictable gradient of phases/nectar availability in the inflorescence.

In July 2019, we selected 90 *P. strictus* and 90 *P. mensarum* plants in two populations separated by approximately a kilometer. The *P. strictus* population was next to Nicholson Lake (38°54'03.3"N 107°00'25.1"W) and *P. mensarum* population on the banks of Slate River (38°54'13.9"N 107°01'06.8"W). Surrounding vegetation differed in the populations; *P. mensarum* grew with perennials such as aspen fleabane (*Erigeron speciosus*), sulfur buckwheat (*Eriogonum umbellatum*), yellow salsify (*Tragopogon dubius*), and owl's claws (*Hymenoxys hoopesii*), as well as shrubs like sagebrush (*Artemisia* sp.). In contrast, the *P. strictus* population was primarily associated with the introduced creeping thistle (*Cirsium arvense*). Both species grew taller than surrounding vegetation in relatively dense flowering patches, with a mean distance between two individuals ~0.5m. To limit bias, we used transects to select individuals choosing the closest plant along the transect every ~0.5m. Because plant distribution varied, we used a single transect for *P. strictus* and three parallel transects 5 m apart for *P. mensarum*.

In the populations, several *Bombus* species were seen visiting both *Penstemon*: *B. appositus*, *B. californicus*, *B. flavifrons*, *B. frigidus* and with additional observations of *B. bifarius*, *B. nevadensis*, and *B. pensylvanicus* on *P. strictus*. We saw a variety of unidentified solitary bees including *Anthophora* sp., wasps, and beetles visiting both species. Three butterfly species visited *P. mensarum*: *Icaricia icarioides*, *Plebejus melissa*, and *Tharsalea helloides*, while only one species, *Papilio rutulus*, was observed foraging on both *Penstemon* species. The white-lined sphinx moth (*Hyles lineata*) and rufous hummingbird (*Selasphorus rufus*) were observed on *P. mensarum* in the morning before bees began foraging.

Measurements Floral traits were measured over approximately 2 weeks starting July 16th, 2019, near peak flowering for the two species. To measure flowers, we divided the floral tube into the proximal corolla tube and throat, measuring the gape width, total corolla tube length and the width of the constriction of the proximal tube (constriction) and proximal tube length (Figure 1) because these may restrict access to rewards differently (Romero-Bravo and Castellanos 2024), especially the gape and constriction (Castellanos et al. 2004; Zung et al. 2015). We also measured the width of the middle petal of the lower lip to estimate size of the landing pad. Each plant had three flowers measured and we used the plant mean for analyses. We recorded display size at the time of measurements. The single measure of display size was positively correlated with total flower number for the two species (*P. mensarum*: $r = 0.438$, *P. strictus*: $r = 0.654$; $P_s < 0.0001$), suggesting flower number is a good approximation for overall daily display size. To assess plant size and apparency we also measured total plant height to the tip of the inflorescence.

To assess nectar volume, we used 2 mm wide filter paper strips (Whatman[®], Grade 1) for small volumes (Thomson et al., 2000). We covered inflorescences with mesh bags in the evening

to prevent pollinator access and measured three haphazardly chosen male-phase flowers the following morning. Strips were left for one minute then the distance nectar eluted measured with digital calipers. We converted nectar length to volume using a calibration from known volumes of 40% sugar artificial nectar (volume $\mu\text{L} = 0.347 \text{ mm}$, $R^2 = 0.96$) (McKenna and Thomson 1988). We restricted sampling to male-phase flowers to reduce variation in nectar due to phase differences (e.g. Burdon et al. 2020) and because female-phase flowers were not always available on all plants at the time of sampling. In *P. digitalis* and *P. speciosus*, the later female-phase flowers can have more nectar, meaning our nectar measures may underestimate availability for pollinators visiting female-phase flowers. To assess pollen, we collected one mature but unopened bud per plant. Anthers were separated into short and long stamens, dried to inhibit mold and stored in 0.5ml Eppendorf tubes (Kearns and Inouye 1993). We crushed anthers in 50 μL 1:1 Lactic acid/Glycerol solution and counted two 1 μL drops with a hemocytometer to estimate total pollen counts.

In early September 2019, we revisited the populations to estimate female fitness by the number of successful fruits developed and those that failed. We also counted seeds per fruit for 3 haphazardly chosen fruits per plant. *P. mensarum* fruits were mature and the full fruit was counted. Unfortunately, *P. strictus* fruits were not fully matured, and additional travel to the site was challenging, so we estimated fruit number by counting developing seeds. Because of the higher seed counts in *P. strictus*, we estimated seed number from half of the fruit and multiplied by two. For *P. strictus* 5 inflorescences were damaged by mammal herbivory and in *P. mensarum* 15, meaning that fitness estimates were not possible (final samples: *P. strictus* = 85, *P. mensarum* = 75).

Statistical analyses

All statistical analyses were done in R version 4.3.2 (RStudio Team 2020; R Core Team 2023), and visualization with *ggplot2* (Wickham, H 2016). We first determined whether our traits differed between species with a one-way MANOVA followed by individual ANOVAs. Dependent variables included plant height, flower number, gape width, petal width, corolla length, proximal tube length, nectar volume, pollen number, total flowers, aborted flowers, and species was treated as the independent variable. We estimated Pearson correlations among floral traits within each species using *GGally* (Schloerke et al. 2024). While correlations can be used to assess honest signals, these do not allow for testing for multiple traits together. Therefore we choose to assess whether any plant/floral traits were predictive of rewards (i.e. honest signals) using conducted stepwise regression with the MASS package using both directions (Venables and Ripley 2002). We modeled nectar and pollen separately for each species and included plant height, flower number, gape width, petal width, corolla length and proximal tube length.

We estimated phenotypic selection for each species separately following Lande and Arnold (1983). We used mean standardized phenotypic traits (Opedal 2021). We estimated female fitness as total fruit number and seeds per fruit. To estimate direct selection, we calculated selection gradients (β), using a single multivariate model for each species with standardized traits as explanatory variables and relative fitness (fruits/population mean or seeds per fruit/population mean) as the response variable. We included height, display, petal width, gape width, corolla length, proximal tube length, nectar, and pollen. We dropped the constriction width from the selection models because it was strongly correlated with gape width for both species (*P. mensarum*: $r = 0.512$, *P. strictus*: $r = 0.501$; $P_s < 0.0001$), did not have significant selection when included, and did not greatly alter the patterns of selection (not shown). We assessed variance

inflation (VIF) in the models with *vif* function the car package (Fox and Weisberg 2019). All VIF were < 2.27 . To assess whether linear selection gradients differed between the two *Penstemon* species, we used analysis of covariance (ANCOVA). Models included all traits and a term for species and all trait $\times$ species interactions as explanatory variables and relative fitness (within each species) as the response variable. A significant interaction term indicates that selection differed between the two *Penstemon* species.

To test whether there were non-linear relationships between floral traits and fitness, we estimated standardized quadratic gradients (γ). These models included both all the linear terms as above and their quadratic term. Differences in the strength of quadratic selection gradients between the *Penstemon* species were tested using ANCOVAs as described above. We doubled the estimates following Stinchcombe et al. (2008). We assessed whether there was correlational selection through models with the linear, quadratic and interaction terms for all traits. These models yielded no significant selection, likely due to a lack of statistical power. To test specifically correlational selection on honest signals of pollen and nectar reward, we tried reduced models that included the linear and quadratic terms with only the interaction terms for traits that predicted reward (see Results). For *P. mensarum* we tested the correlation between reward and petal width. For *P. strictus*, tested the correlation between reward and proximal tube length.

RESULTS

The two *Penstemon* species differed in most traits measured (Pillai = 0.831, $F_{1,158} = 66.17$, $P < 0.0001$). *Penstemon strictus* had bigger flowers and were taller than *P. mensarum* (Table 1).

Interestingly, per flower, the two species offered similar nectar reward and pollen amounts in each flower, although *P. strictus* produced more flowers than *P. mensarum*. Generally, floral dimensions were positively correlated for both species (Figure 1) and taller plants produced more flowers. For *P. mensarum*, petal width was positively associated with more nectar and pollen, suggesting it could act as an honest signal for rewards in the species (Table 2). There were no such signals positively related to rewards for *P. strictus*, however, proximal tube length was related with less nectar for *P. strictus* and fewer pollen grains in *P. mensarum* (Table 2).

We found phenotypic selection on all traits in at least one of the species except for gape width (Figure 2, Table S1, S2) and constriction width (not shown). In general, selection was similar whether we used fruit number or seeds per fruit as an estimate of fitness with one notable trait. Selection to produce more flowers via fruit number was detected for both species and of similar strength. However, selection via seeds per fruit was to reduce flower number, at least for *P. strictus* suggesting there may be trade-offs between number of flowers/fruits and seeds per fruit. Taller plants were favoured in *P. mensarum* via both fruits and seeds per fruit but not *P. strictus*. For floral dimensions, selection generally differed between *P. mensarum* and *P. strictus*, with different measures under selection in the two species. There was a trend for selection to increase petal width in *P. mensarum* (Table S1, Figure S1), where it was associated with higher rewards, but not *P. strictus*. Instead for *P. strictus*, there was selection to increase the overall corolla length but reduce the proximal tube length, which tended to be related negatively with reward in the species. Selection on these tube measures were similar in both fitness measures. There was also selection to reduce nectar rewards in *P. strictus* but not *P. mensarum*. In both species, there was significant nonlinear selection detected on pollen number via fruits, where intermediate pollen number had the highest relative female fitness (Figure 2, Table S1). For *P.*

mensarum there was also non-linear selection on petal width and plant height (Figure S1, Table S1). There was no evidence for nonlinear selection on any trait for either species via seeds per fruit. We did not find correlational selection on honest signals: nectar and petal width in *P. mensarum* (-0.441 ± 0.273 , $t = -1.61$, $P = 0.11$) and pollen and petal width (-0.237 ± 0.531 , $t = -0.45$, $P = 0.66$). In *P. strictus*, there was no evidence for correlational selection on proximal tube length and either nectar (0.036 ± 0.180 , $t = 0.199$, $P = 0.84$) or pollen (0.121 ± 0.378 , $t = 0.319$, $P = 0.75$). However, these results may reflect more a lack of statistical power than a true lack of correlational selection.

DISCUSSION

For both *P. mensarum* and *P. strictus*, there was evidence that higher rewards can be costly in terms of female fitness. Pollinators may prefer higher rewards but their subsequent prolonged visits may not be to the fitness benefit of the plant (Pyke 2016), suggesting ecological costs to rewards. Reward production likely also has direct costs (Obeso 2002; Pyke and Ren 2023), that means higher rewards will not be favoured in all conditions. For *P. strictus*, there was selection to reduce nectar volume, which differed significantly from selection in *P. mensarum*. Selection to reduce nectar has also been seen in stressful conditions such as drought (García et al. 2023), and it is possible that there were differences in water stress in the two populations. Nectar did not differ between the species and drought often also reduces nectar production (Kuppler and Kotowska 2021), although that does not preclude this population from drought stress as other populations of *P. strictus* may produce more nectar. Both *P. strictus* and *P. mensarum* also aborted similar numbers of fruit, suggesting that there may not be large differences in resources, either abiotic or in pollination (i.e. pollen limitation). Nectar is likely to have direct costs,

although they can be difficult to detect and are poorly studied (Pyke 1991; Ashman and Schoen 1997; Ornelas and Lara 2009; Pyke and Ren 2023). More nectar may also lead to changes in pollinator behaviour that leads to costs such as increased selfing (Galen and Plowright 1985; Hodges 1995). It is also interesting that we did not detect selection on nectar in *P. mensarum*, suggesting cost is not consistent, although these species appear to produce similar amounts of nectar in their male-phase flowers. Because we did not measure nectar in female-phase, where production can be higher in *Penstemon* (Castellanos et al. 2002; Burdon et al. 2020), we cannot be certain of total costs of nectar production for these species or how selection acts on nectar across the sexual phases. *Penstemon strictus* also produced ~10+ more flowers than *P. mensarum* in these populations, suggesting it may experience higher reproductive costs overall (Obeso 2002). In general, these results show that producing more nectar is not always beneficial for fitness, although the mechanism needs further investigation.

When treating pollen number as a potential reward to pollinators, we saw stabilizing selection on pollen number in both species via female fitness, suggesting reduced fitness for low and high pollen counts. The presence of pollen can influence female fitness in hermaphroditic flowers, even for incompatible species (e.g. Duffy and Johnson 2011), suggesting that too few pollen grains might reduce visitation and seed set, although pollinators are not always influenced by the presence of pollen (Li et al. 2021). We also saw selection against high pollen counts, suggesting there may be costs due to increased geitonogamy or some other trade-off, although we did only seed this effect via fruit number and not seeds per fruit. The effects of selfing has rarely been examined in the genus, however, for *P. tenuiflorus* there is some evidence for inbreeding depression (Clements et al. 1999) and genetic analyses has revealed some inbreeding for several *Penstemon* species (Kramer et al. 2011; Wolfe et al. 2016; Rodríguez-Peña et al.

2018; Stone et al. 2019). Pollen production is likely to also have costs (Delph et al. 1997; Cruden 2000), and therefore more pollen might come at the expense of developing seeds. A major caveat to the pattern of selection on pollen detected here is that we did not consider the male fitness function of pollen and measure siring success of these plants. Selection on pollen number may act very differently for siring seed and likely higher pollen counts benefit male fitness (Opedal et al. 2023).

The shape and size of tubular flowers in relation to pollinators has long been of interest in the evolution of flowers (Inouye 1980; Nilsson 1988) and selection on tube dimensions are common (Bloch and Erhardt 2008; Wu and Li 2017; Opedal 2021; Tong et al. 2024). In *P. strictus*, there was selection to increase the overall corolla length but selection to decrease the length of the tube constricted around the nectary, despite their positive correlation. Our results relate to those found in *Digitalis purpurea*, where selection was seen on the proximal corolla tube that restricts nectar access but not the overall size of tube (Mackin et al. 2021). However, for *D. purpurea* selection was mainly observed in the introduced range with a switch to hummingbird pollination from bees. In this study, *P. strictus* is growing in its native range. Hummingbirds likely visit *P. strictus* (Castellanos et al. 2003), but their role in selection on the corolla tube of these flowers is unknown and similar selection patterns were not seen for *P. mensarum* where hummingbirds were observed visiting flowers in this year. Overall, these species may face diffuse selection by a range of pollinators, although other agents may also drive selection in these systems (Caruso et al. 2019), making conclusions of mechanisms challenging without further study.

We found little evidence for selection for honest signals in these species. First, of the traits measured, only petal width in *P. mensarum* is likely to be informative of rewards with the

potential to be learned by foraging bees (Blarer et al. 2002). We saw a trend for selection to increase petal width in *P. mensarum* but no evidence for correlational selection on petal width and nectar (if anything our non-significant results suggests decoupling the positive correlation between petal width and nectar would lead to higher fitness). However, our results should be interpreted with caution because we likely lack statistical power to estimate correlational selection (Simon et al. 2022). While correlational selection on signal-reward relationships has been detected in at least one species (Benitez-Vieyra et al. 2010), how common this is within populations remains understudied. Floral traits, such as petal size, may have multiple functions in signalling and pollinator fit. Here, petal size likely also influences its function as a landing pad in these *Penstemon* and pollinators may interact with the trait differently, depending on whether they use it to land on the flower. Given that there is a community of visitors to these flowers, their effectiveness as pollinators and variation in visitation could explain differences in selection between these *Penstemon* species. Community context may also play a role in the variation in selection seen across traits (Eisen et al. 2020). For example, the co-flowering species may have influenced selection on traits.

CONCLUSIONS

Our study shows that simply increasing rewards may not be beneficial for bee-pollinated plants. Across traits, selection on rewards was also generally much weaker than apparency (height), floral display, or corolla measures, suggesting that evolution in rewards would likely lag behind these other traits depending on similarities or differences in evolvability (Romero-Bravo and Castellanos 2024). Studies of natural selection on nectar are rapidly accumulating, but pollen rewards are still often missing from this context. Plants have evolved mechanisms to reduce the costs of pollen loss through pollinator consumption such as pollen dispensing (Harder and

Thomson 1989; Thomson et al. 2000), pollen packaging (Heiling et al. 2023), and heteranthery (Vallejo-Marín et al. 2009). Studies on the effects of pollen rewards are also not novel (e.g. Duffy and Johnson 2011; Russell et al. 2016; Nicholls and Hempel de Ibarra 2017). However, our results suggest understanding the dual function of pollen as a reward and gamete in natural selection studies will deepen our understanding of how pollen evolves both through male and female function in hermaphroditic flowers. Comparison of the two similar *Penstemon* species show that patterns of selection are difficult to predict without measuring, therefore adding rewards to evolutionary ecology studies are likely to reveal new insights.

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402 No conflicts

403 **Ethics approval (include appropriate approvals or waivers)**

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405 Consent to participate (include appropriate statements)

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407 Availability of data and material (data transparency)

408 Code availability (software application or custom code)

409 Authors' contributions

410

411 AUTHOR CONTRIBUTIONS

412 ALP: initial conceptualization, data analyses, and writing. KR: conceptualization, field work,

413 data collection, initial data analyses, and writing.

414 DATA AVAILABILITY STATEMENT

415 Data is available

416 ONLINE SUPPORTING INFORMATION

417 Additional supporting information may be found online in the Supporting Information section at

418 the end of the article

419 Appendix 1: Table S1.

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687

688 Table 1. Phenotypic trait and fitness measures for two populations of *Penstemon* species growing
689 in Colorado (mean $\pm$ SD). Statistics test whether the species differ.

Trait	<i>Penstemon mensarum</i> (<i>N</i> = 75)	<i>Penstemon strictus</i> (<i>N</i> = 85)	<i>F</i> _{1,158}	<i>P</i>
Height (cm)	64.0 $\pm$ 12.3	70.4 $\pm$ 10.5	12.75	0.00047
Petal width (mm)	4.94 $\pm$ 0.81	4.54 $\pm$ 1.09	6.48	0.012
Gape width (mm)	6.72 $\pm$ 0.94	8.56 $\pm$ 1.54	80.68	<0.0001
Corolla tube length (mm)	15.87 $\pm$ 1.37	21.03 $\pm$ 1.76	419.54	<0.0001
Tube constriction (mm)	3.58 $\pm$ 0.78	3.95 $\pm$ 1.06	6.56	0.011
Proximal tube length (mm)	7.13 $\pm$ 1.04	8.52 $\pm$ 1.63	42.12	<0.0001
Nectar (μ L)	0.63 $\pm$ 0.44	0.65 $\pm$ 0.40	0.099	0.75
Pollen number	46693 $\pm$ 17773	47147 $\pm$ 17592	0.0081	0.93
Total flowers	38 $\pm$ 16	49 $\pm$ 18	17.61	<0.0001
Failed flowers	11 $\pm$ 7.7	12 $\pm$ 9.8	0.34	0.56
Fruit number	27 $\pm$ 13	37 $\pm$ 15	22.98	<0.0001
Seeds per fruit	21 $\pm$ 6.6	51 $\pm$ 16.6	206.98	<0.0001

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Table 2: Results from stepwise regressions to determine the predictors of nectar and pollen rewards in two *Penstemon* species. Full model included height, flowers, petal width, gape width, corolla length, proximal tube (PT) length; predictors from the best fit model shown. Estimates $\pm$ SE. Significant predictors are highlighted with bold.

Nectar	Predictor	estimate	<i>t</i>	<i>P</i>
<i>P. mensarum</i>	Petal width	0.262 $\pm$ 0.059	4.41	0.00036
	Corolla length	-0.050 $\pm$ 0.035	-1.42	0.16
<i>P. strictus</i>	Flowers	0.0036 $\pm$ 0.0023	1.58	0.12
	PT length	-0.092 $\pm$ 0.025	-3.65	0.00046
Pollen	Predictor	estimate	<i>t</i>	<i>P</i>
<i>P. mensarum</i>	Petal width	4669 $\pm$ 2460	1.90	0.062
<i>P. strictus</i>	Height	378 $\pm$ 182	2.08	0.041
	PT length	-1700 $\pm$ 1173	-1.45	0.15

Figure 1. Correlations and trait distributions for *Penstemon mensarum* and *P. strictus*. Drawings by KR.

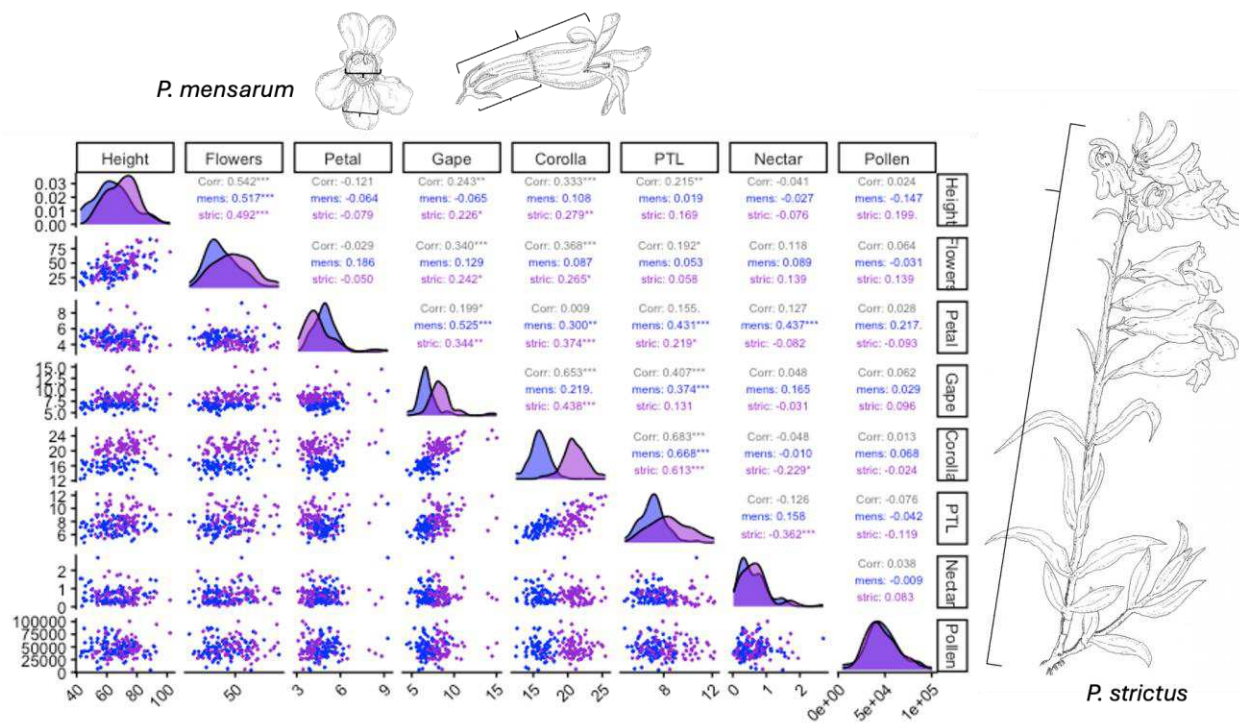
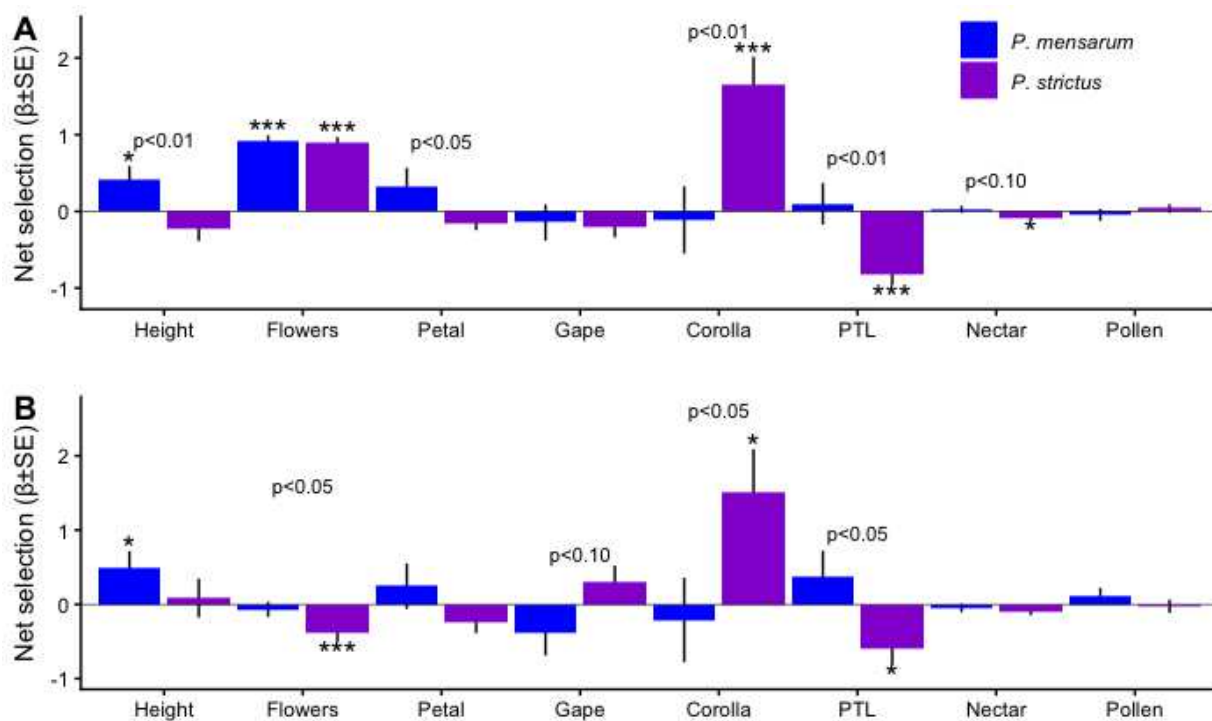
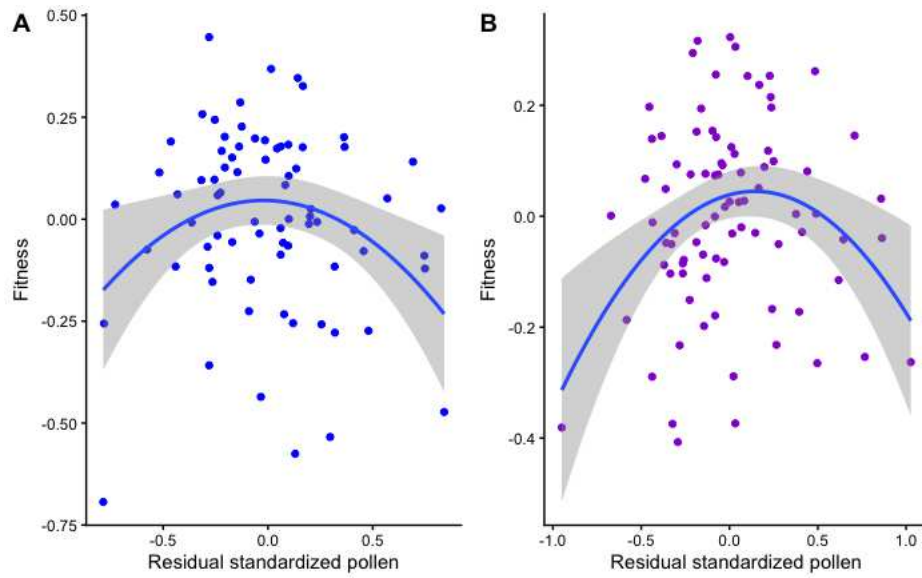


Figure 2. Linear selection gradients ($\beta \pm \text{SE}$) for phenotypic traits in two species of *Penstemon* using fruit number as a fitness measure(A) or seeds per fruit (B). Asterisk above a bar mark gradients significantly different from a slope of zero and *P*-values for gradients that differed between the species are depicted numerically. Traits = plant height (Height), number of flowers (Flowers), petal width (Petal), gape width (Gape), corolla tube length (Corolla), proximal tube length (PTL), nectar volume (Nectar), pollen number (Pollen). * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001.



713 Figure 3. Non-linear selection shown for pollen number in two species of *Penstemon*. A) *P.*
714 *mensarum* and B) *P. strictus*, both are significant (Table S1).

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719 Table S1. Selection gradients and quadratic nonlinear selection for two *Penstemon* species. * $P <$
 720 0.05, ** $P < 0.01$, *** $P < 0.001$, † $P < 0.10$

	Trait	<i>Penstemon mensarum</i>		<i>Penstemon strictus</i>	
		β	γ	β	γ
Selection via Fruit Number	Height	$0.419 \pm 0.173^*$	$1.1111 \pm 0.249^*$	-0.227 ± 0.160	0.26 ± 0.191
	Flowers	$0.914 \pm 0.079^{***}$	0.528 ± 0.250	$0.904 \pm 0.065^{***}$	0.18 ± 0.189
	Petal	0.334 ± 0.236	$-2.158 \pm 0.492^*$	-0.151 ± 0.093	-0.264 ± 0.189
	Gape	-0.145 ± 0.235	0.442 ± 0.265	-0.210 ± 0.130	0.384 ± 0.190
	Corolla tube	-0.112 ± 0.438	-0.504 ± 0.252	$1.662 \pm 0.353^{***}$	$-0.802 \pm 0.223^\dagger$
	Proximal tube	0.099 ± 0.271	-0.248 ± 0.338	$-0.814 \pm 0.034^{***}$	0.548 ± 0.220
	Nectar	0.026 ± 0.044	0.790 ± 0.441	$-0.081 \pm 0.054^*$	0.132 ± 0.194
	Pollen	-0.047 ± 0.077	$-1.044 \pm 0.202^*$	0.037 ± 0.054	$-1.096 \pm 0.184^{**}$
	Height	$0.494 \pm 0.224^*$	1.076 ± 0.400	0.086 ± 0.261	0.577 ± 0.326
Selection via Seeds per Fruit	Flowers	-0.067 ± 0.103	0.677 ± 0.341	$-0.394 \pm 0.106^{***}$	-0.326 ± 0.322
	Petal	0.245 ± 0.306	-1.394 ± 0.789	-0.235 ± 0.153	-0.818 ± 0.321
	Gape	-0.384 ± 0.305	0.360 ± 0.413	0.310 ± 0.212	-0.128 ± 0.324
	Corolla tube	-0.211 ± 0.568	0.592 ± 0.404	$1.513 \pm 0.576^{**}$	-0.826 ± 0.380
	Proximal tube	0.371 ± 0.351	0.390 ± 0.542	$-0.587 \pm 0.227^*$	0.207 ± 0.374
	Nectar	-0.048 ± 0.057	1.014 ± 0.706	-0.089 ± 0.056	0.378 ± 0.330
	Pollen	0.123 ± 0.100	0.520 ± 0.351	-0.025 ± 0.089	0.937 ± 0.313

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723 Table S2. Detailed statistics for linear selection gradients via fruit number and seeds per fruit.

	Trait	<i>Penstemon</i>		<i>Penstemon strictus</i>		Species × Trait	
		<i>mensarum</i>					
		<i>F</i> _{1,67}	<i>P</i>	<i>F</i> _{1,76}	<i>P</i>	<i>F</i> _{1,142}	<i>P</i>
Selection via fruit number	Height	2.42	0.018	1.43	0.16	7.31	0.0077
	Flowers	11.52	< 0.001	13.94	< 0.001	0.0025	0.96
	Petal	1.42	0.16	1.62	0.11	4.29	0.040
	Gape	0.61	0.54	1.62	0.11	0.05	0.82
	Corolla tube	0.26	0.80	4.71	< 0.001	9.99	0.0030
	Proximal tube	0.37	0.72	5.87	< 0.001	10.07	0.0018
	Nectar	0.59	0.56	2.36	0.021	3.73	0.055
	Pollen	0.60	0.55	0.68	0.50	0.81	0.37
Selection via seeds per fruit	Height	2.20	0.031	0.33	0.74	1.40	0.24
	Flowers	-0.65	0.52	-3.72	< 0.001	4.91	0.028
	Petal	0.80	0.43	-1.54	0.13	2.03	0.16
	Gape	-1.26	0.21	1.46	0.15	3.55	0.062
	Corolla tube	-0.37	0.71	2.63	0.01	4.55	0.035
	Proximal tube	1.06	0.3	-2.59	0.011	5.34	0.022
	Nectar	-0.84	0.40	-1.58	0.12	0.27	0.61
	Pollen	1.23	0.22	-0.29	0.78	1.23	0.27

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726 Figure S1. Partial regression plot showing the nonlinear relationship for selection on petal width
 727 (A) and plant height (B) in *Penstemon mensarum*.

