

RESEARCH ARTICLE

Modelling pollinator and nonpollinator selection on flower colour variation

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Abstract

1. Flower colour variation is ubiquitous within and between populations, which is why it has long been a focal point for studies of natural selection. This body of work has uncovered a wide range of selective agents, including pollinators, herbivores and various abiotic factors. Nevertheless, we lack an integrative framework for predicting the phenotypic outcome in terms of floral pigmentation when these forces act collectively and often in opposition.
2. We here present such a framework through a model that incorporates selection on pigmentation at the vegetative phase (i.e. through survival to reproduction) and at the flowering phase (i.e. on pollinator attraction). We focus on anthocyanins as common class of pigments although the model is equally applicable to any compounds that can be jointly expressed in vegetative tissue and in flowers. We explore the dynamics of our model in a theoretical context and in four scenarios based on classic systems for studying selection on flower colour.
3. Our model predicts that pollinators are the main driver for flower colour evolution, but selection on seedling survival plays a major role in the absence of pollen limitation, that is, if pollinator abundance is sufficiently high, or if pollinator preference is absent or weak (high variance in colour preference). In each of the case studies, our model recovered the predicted patterns of fitness for each floral morph given the strength and nature of selection.
4. This work suggests that selection at the vegetative phase must act alone or be exceptionally strong to negate pollinator preference for particular colours. Nevertheless, the influence of differential survival associated with anthocyanin production leaves a clear signature on the fitness curves, suggesting that non-pollinator agents of selection can often be detected from empirical data.
5. Synthesis: Overall, the application of this model to empirical systems will be key for understanding how flower colour diversity evolves and for predicting how changes in climate and pollinator communities may jointly alter evolutionary trajectories.

KEYWORDS

anthocyanins, fitness, flavonoids, plant life history, pleiotropy, selection across life stages

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1 | INTRODUCTION

Flower colour has long been a focal trait for understanding the role of pollinators in floral divergence and evolution. The amount and type of pigmentation together with colour patterning serve as important signals to attract pollinators and orient them with respect to the fertile (male and/or female) floral parts (reviewed in Trunschke et al., 2021). Accordingly, flower colour often experiences pollinator-mediated selection, with the precise nature of that selection depending on a range of factors (Sapir et al., 2021). For example, pollinators differ in their visual systems (e.g. Lunau et al., 2011; Shrestha et al., 2013; van der Kooi et al., 2021) and select for colours that provide the strongest detectability against the background (Giurfa et al., 1996; Koski, 2020). Flowers pollinated by multiple different pollinators (with different visual systems) may thus display stable flower colour variation within populations (Kay, 1978). The light environment and the background also play key roles in plant-pollinator interactions by affecting which colours are most visible (Altshuler, 2003; Endler, 1993; Van Der Kooi & Kelber, 2022). The interplay of these factors influences colour variation across evolutionary scales, from within and between populations (Ellis & Johnson, 2009; Sapir et al., 2021) to across species (Coetzee et al., 2021; Muchhala et al., 2014).

Despite the broad consensus that interactions with pollinators can and have shaped flower colour variation (Schiestl & Johnson, 2013; Trunschke et al., 2021; Van der Niet et al., 2014), a wide array of non-pollinator agents can also exert selection on colour. These agents can include biotic factors, such as herbivores and pathogens (Rusman et al., 2019; Strauss et al., 1996), and abiotic factors, such as temperature, precipitation, elevation and soil (reviewed in Strauss et al., 1996; Strauss & Whittall, 2006). A recent meta-analysis (Caruso et al., 2019) suggests that while biotic agents other than pollinators exert relatively weak selection on floral traits (including colour), the strength of selection of abiotic factors rivals that of pollinators. Indeed, in many systems, floral colour variation appears to be entirely controlled by the environment (i.e. *Linanthus*, *Lysimachia*, *Butomis*; reviewed in Sapir et al., 2021). Among abiotic factors, climate appears to have a particularly strong influence on floral pigmentation (e.g. Arista et al., 2013; Peach et al., 2020), as underscored by complex changes in pigmentation with global climate change (i.e. pigmentation correlated negatively with temperature and positively with aridity; Koski et al., 2020; Sullivan & Koski, 2021).

The explanation for this strong and rather counterintuitive influence of factors other than pollinators on flower colour variation likely lies in the genetic and developmental basis for colour. Three classes of pigments, flavonoids, carotenoids and betalains, are responsible for flower colour (Grotewold, 2006). Among these, the blue, purple and red anthocyanins, a class of flavonoids, are most often associated with colour variation in flowers (Grotewold, 2006; Narbona et al., 2018). Importantly, anthocyanins are also present in vegetative tissues (i.e. leaves), where they act as 'sunscreen' to protect the photosynthetic apparatus (i.e. by increasing antioxidant capability) and mitigate negative effects from drought and cold stress

(Gould, 2004; Zhang et al., 2019). In addition to these physiological roles, anthocyanins can contribute to defence against pathogens and even crypsis to avoid herbivory (Lev-Yadun & Gould, 2009; Strauss & Cacho, 2013). Importantly, the production of vegetative and floral anthocyanins is often correlated such that plants with pigmented flowers also have pigmented stems and leaves (Onslow, 1925; Warren & Mackenzie, 2001). Thus, the many documented cases of selection on flower colour by nonpollinator agents, such as herbivores or environmental conditions, may, in fact, represent direct selection on vegetative anthocyanins (or other flavonoids) with indirect (pleiotropic) effects on flower coloration (Armbruster, 2002; Del Valle et al., 2019; Landi et al., 2015).

Given this extensive literature on pollinator and nonpollinator mediated selection on flower colour, it is perhaps surprising that, to our knowledge, the empirical literature lacks comprehensive studies examining selection across life stages in relation to anthocyanin production. For example, in *Raphanus sativus*, pollinators favour pale flowers and those morphs suffer more herbivory (Irwin & Strauss, 2005; McCall et al., 2013), but it is unknown whether correlated anthocyanin production is involved. Similarly, drought stress is the primary driver of flower colour variation in *Linanthus parryae* (Schemske & Bierzychudek, 2001, 2007), but it is unclear whether this effect is mediated by vegetative anthocyanins and their physiological roles. One challenge in building such a longitudinal study of selection on colour across life stages is the lack of a theoretical framework to build hypotheses for expected outcomes depending on the nature of selection by each factor.

Here, we present a new model that predicts the fitness of flower colour morphs, integrating the effects of anthocyanin pigments on plant growth and survival with the effects on pollinator activity. We assume that anthocyanins can influence plant fitness during the vegetative phase due to their role in tolerating abiotic stressors (von Wettberg et al., 2010; Warren & Mackenzie, 2001) and mediating herbivory (Gould, 2004; Vaidya et al., 2018). In modelling fitness effects due to interactions with pollinators, we include colour preference, constancy and pollen carryover, building on the prior work of Montgomery (2009) in the context of pollinator competition. Although constancy (the tendency of an individual pollinator to specialize on a particular flowering species or morph while foraging) is typically associated with bees (Niovi Jones & Reithel, 2001; Waser, 1986), this type of nonrandom foraging is common in a range of pollinator species, including beetles, flies and hummingbirds (Amaya-Márquez, 2009; Goulson & Wright, 1998), and thus, is important to include in a general model. Finally, our framework assumes that pigment production is correlated across plant tissues, a pattern observed across many groups of flowering plants (Bate-Smith & Swain, 1962; Del Valle et al., 2019; Warren & Mackenzie, 2001) that is related to the shared biochemical pathway and regulatory architecture (Albert et al., 2014). We first explore the general dynamics of our model before using it to predict the fitness of colour morphs in four scenarios based on classic studies in colour evolution. For the first two scenarios, selection on anthocyanin production occurs only at one of the two phases while in the last two, the direction of

selection conflicts between the vegetative and flowering life stages. We expect that there is often some degree of selection on pigment production through the plant's lifespan, so the latter cases are key for predicting phenotypic outcomes depending on the strength and direction of selection in each stage.

2 | MATERIALS AND METHODS

2.1 | Model description

All of the parameters of our model are listed and described in Table 1. The first component of our model considers how anthocyanin concentration relates to survival to the flowering stage. Anthocyanins and other flavonoids are generally known for their protective functions, for example, against herbivores, pathogens, UV stress and drought (Gould, 2004), suggesting that increasing the production of these compounds will generally enhance fitness. However, higher anthocyanin production can also be associated with greater damage from herbivores, for example, if the pigments attract specialized herbivores or result in trade-offs with other defensive compounds (Frey, 2004). We assume that the range of anthocyanin production will vary across species, and therefore we modelled anthocyanin concentration (A) as ranging from some species-specific minimum

($A = 0$) and maximum value ($A = 1$), which can either increase or decrease survival probability (S):

$$S = \frac{b}{b^A}, \quad (1)$$

where S varies from b ($A = 0$) to 1 ($A = 1$) (anthocyanins increase survival)

or

$$S = 1 + d - \frac{d}{d^A}, \quad (2)$$

where S varies from 1 ($A = 0$) to d ($A = 1$), (anthocyanins decrease survival)

where b and d specify minimum survival probabilities (see Figure S1).

The second component of our model considers how pollinators will affect plant fitness in relation to anthocyanin pigmentation once the plant has survived to flowering. We assume that, as in vegetative tissue, anthocyanin concentration can vary between the minimum ($A = 0$) and maximum ($A = 1$) values. We also assume that the species requires pollinator-mediated pollen transfer for reproduction (i.e. it does not self-pollinate) and is obligately outcrossing (i.e. is

Parameters	Explanation
A	Anthocyanin concentration, ranging from zero to 1 (species-specific minimum and maximum)
S	Survival probability, which is a number between zero and 1, if survival is independent of anthocyanin concentration
b	Lowest survival probability assuming anthocyanin concentration increases survival
d	Lowest survival probability assuming anthocyanin concentration decreases survival
α	Relative abundance of focal colour of the focal species
β	Relative abundance of other colours of the focal species
$\alpha + \beta$	Relative abundance of focal species
γ	Relative abundance of other species
λ	Expected number of pollinator visits per flower
P	Probability of successful pollination
$\hat{\alpha}$	"Effective" relative abundance of preferred colour morph assuming pollinator preference can be modelled as increasing the relative abundance of the preferred colour morph
A_m	Preferred anthocyanin concentration
n	Strength of pollinator preference for A_m
$\text{var}(n(A_m))$	Variance around n
c	Increase in the probability that a pollinator visits a flower due to constancy
R	Expected pollen receipt
r	Pollen carryover rate
Z	Seed set that depends on the pollen receipt
F	Fitness associated with a given anthocyanin concentration

TABLE 1 Description of model parameters

self-incompatible); both assumptions can be softened in our model for systems where this is not the case (see the 'Claytonia' case below). To explain the model, we will focus on a particular colour phenotype, although, in practice, the model estimates fitness for all values of A .

First, in the absence of pollinator preference, the probability of successful pollination for a particular colour phenotype (a given value of A) will depend on its frequency in the population and in the community (if there are co-flowering species). If the relative frequency of a given colour phenotype is α and that of other colours from the same species in the population is β , the probability of successful pollination of that phenotype after λ visits is $P = 1 - \exp(-\lambda(\alpha + \beta))$, assuming visits follow a Poisson process (Montgomery, 2009). The two frequencies (α and β) will sum to 1 if no other species are present and will be less if any other species (with frequency γ) are flowering. Thus, all the flowering individuals in community will sum to 1, that is, $\alpha + \beta + \gamma = 1$. However, we expect that pollinators may exhibit innate colour preferences (e.g. Lunau & Maier, 1995; Raine & Chittka, 2007), and that pollination success will not be determined by raw frequency alone. In this case, pollinators will visit the preferred colour (value of A) more often than expected by chance. The lower preference at smaller or larger A values could be associated with colour contrasts being highest at intermediate anthocyanin values (Van Der Kooi, 2021). Following Kokko (2007), we can model preference as increasing the relative frequency of the preferred colour such that $\hat{\alpha} = n\alpha / (n\alpha + (1 - \alpha))$, where n specifies the strength of the preference for A (Figure S2). To include variation in pollinator preference, we assume n is normally distributed with $n > 1$, with a peak at the preferred anthocyanin concentration A_m and a variance of $\text{var}(n(A_m))$ (Figure S3). This variance will likely depend on the pollinator species and could be broad if multiple pollinators are involved. Thus, with preference, the probability of successful pollination of the preferred colour can be estimated with $\hat{\alpha}$ instead of just α as

$$P = 1 - \exp(-\lambda(\hat{\alpha} + \beta)). \quad (3)$$

(see Figure S3).

In addition to preference, constancy can increase successful pollination because it will increase the probability that a pollinator brings pollen from a flower of the same phenotype and thus of the same species (assuming individuals of the same colour morph and of the same species will have the most similar phenotype). Defining constancy as the probability c that a pollinator visits a flower of the same morph it just left (Levin & Anderson, 1970; Montgomery, 2009), transitions between flowers of the same phenotype will occur with frequency $c + \hat{\alpha}(1 - c)$ and between flowers of different phenotypes with frequency $\hat{\alpha}(1 - c)$. Thus, when c is 1, there is 100% probability of the next visit being to the same phenotype, and when c is 0, the probability of the next visit being to the same phenotype will be equal to $\hat{\alpha}$.

In the context of constancy, flowers with a 'different' phenotype can include other individuals from the same species, here occurring with relative frequency β , and flowers of other species, occurring with relative frequency γ , which equals $(1 - \hat{\alpha} - \beta)$. Pollen coming from other individuals of the same species will contribute to successful

reproduction, while pollen from other species will not. Thus, we need to calculate the probability that a pollinator, with its preference incorporated through $\hat{\alpha}$ and its degree of constancy c , will arrive at a flower of a given colour phenotype after having visited another individual of the same species (with any colour phenotype). We do that by considering the following transitions: the transition from a focal phenotype to a focal phenotype $\hat{\alpha}(c + \hat{\alpha}(1 - c))$, the transition from a different phenotype of the same species to the focal phenotype $\beta\hat{\alpha}(1 - c)$, and the transition from a different species to the focal phenotype $(1 - \hat{\alpha} - \beta)\hat{\alpha}(1 - c)$. Then, the probability that a pollinator arrives at a phenotype associated with a particular anthocyanin concentration and carries pollen from any phenotype of the same species is

$$\frac{\hat{\alpha}(c + \hat{\alpha}(1 - c)) + \beta\hat{\alpha}(1 - c)}{\hat{\alpha}(c + \hat{\alpha}(1 - c)) + \beta\hat{\alpha}(1 - c) + (1 - \hat{\alpha} - \beta)\hat{\alpha}(1 - c)},$$

which simplifies to

$$c + (\hat{\alpha} + \beta)(1 - c). \quad (4)$$

Montgomery (2009) showed that, for a model without pollinator preference but with the presence of multiple colour morphs, the probability of receiving pollen defined (Equation 4 above) holds for more than two transitions, that is, when pollinators visit several flowers prior to arriving at the focal phenotype. We can now combine this equation, with preference incorporated into $\hat{\alpha}$, to estimate the probability of successful pollination of the preferred colour A including constancy as

$$P = 1 - \exp(-\lambda(c + (\hat{\alpha} + \beta)(1 - c))). \quad (5)$$

Finally, we consider how pollen carryover from a successful visit (one which brings conspecific pollen) will contribute to fitness in terms of pollen receipt and, in turn, seed set. Even if a pollinator exclusively travels among individuals of the same species, its visits may not maximize fitness if it does not bring enough pollen from the previously visited flower to fertilize all ovules. Montgomery (2009) showed that in the absence of constancy, the expected pollen receipt (R) depends only on the relative frequency (α) and number of visits (λ), and $R = \lambda\alpha$. However, in the presence of constancy, pollen receipt also depends on the amount of pollen that pollinators move from one flower to the next, and the expected pollen receipt can be calculated as $\lambda(\alpha + cr(1 - \alpha)(1 - c + rc)^{-1})$, where r is the fraction of pollen collected from one flower and deposited on the next flower (i.e. the carryover rate). For generality, we let R vary between 0 and 1 (maximum amount of pollen receipt). If the fraction r is small, so is the amount of pollen receipt. Adding pollinator preference, we get

$$R = \lambda(\hat{\alpha} + \beta), \quad (6)$$

if constancy is absent, and

$$R = \lambda(\hat{\alpha} + \beta + cr(1 - \hat{\alpha} - \beta)(1 - c + rc)^{-1}), \quad (7)$$

if constancy is present (see Figure S4).

The amount of conspecific pollen arriving on the stigma determines, theoretically, whether all ovules in a flower will be fertilized. For this model, we do not consider post-pollination effects (i.e. pollen competition on the stigma, stigma clogging by heterospecific pollen; Minnaar et al., 2019) or aspects of female fitness (i.e. reduced seed set caused by environmental stressors despite optimal pollen receipt). We assume that seed set (Z) initially increases with the number of conspecific pollen grains on the stigma (pollen receipt) but asymptotes at some species-specific maximum number of seeds (Aizen & Harder, 2007). Here, we use the empirically estimated relationship for *Stellaria pubera* (Campbell, 1986)

$$Z = 5.05(1 - \exp(-0.015x)),$$

where x specifies the pollen receipt. Z increases for small pollen receipt and asymptotes to about five seeds per flower at about 200 pollen grains (Figure S5). Since in our model R varies between 0 and 1, where 1 specifies the maximum amount of pollen receipt, we set $x = 200R$. The exact shape of this function does not alter the qualitative model predictions because it simply maps pollen receipt to seed set.

Putting all the components together (the fitness effects of anthocyanins in vegetative tissues and in flowers), the overall fitness (F) associated with a given anthocyanin concentration A is

$$F = SZ, \quad (9)$$

where S is survival to flowering and Z is the seed set resulting from pollinator visitation.

2.2 | Empirical examples

We used our model to predict fitness of floral colour phenotypes in four case studies based on empirical systems. We chose systems where many parameters of the model (effects of anthocyanins on survival, pollinator preference for colour morphs) were available or could be roughly estimated (Table 2). Two of the scenarios involve selection on only one life stage (at least as currently known: the *Linanthus* and *Delphinium* cases), and two involve selection in opposite directions at each life stage (the *Raphanus* and *Claytonia* cases). In cases where there is preference, we chose the value for the preferred anthocyanin concentration A_m based on the qualitative descriptions from the literature. For example, if a species ranges from white to purple, but pollinators prefer the intermediate colour, pink, we would set $A_m = 0.5$. If pollinators preferred paler or darker flowers, we would choose values closer to 0 or 1, respectively. It is worth noting that additional factors beyond preference and constancy, such as competition among pollinators and learning, can influence pollinator foraging patterns (reviewed in Amaya-Márquez, 2009). Nevertheless, these would likely lead to similar effects as observed here, amplifying the fitness differences across morphs when any preference is present. For model parameters where empirical

information was lacking, we used the same values for all case studies. For example, we set the strength of preference (n) for A_m to 10 (as in Figure S1) and its variance, $\text{var}(n(A_m))$, to 0.001. This produces a distribution of preference values $A_m \pm 0.1$ (see Figure S2), an assumption that seems reasonable given the breadth of innate preference reported from experimental work (Papiorek et al., 2013). We chose 1 for the number of visits (λ) because it sets a lower bound for the pollination success of a flower that is visited at least once. We do not have precise estimates of constancy for the pollinators, but, as individual specialization has been observed in a variety of pollinator species including bees (Niovi Jones & Reithel, 2001; Waser, 1986), bumblebees (Free, 1970), beetles, flies and hummingbirds (Amaya-Márquez, 2009), we set constancy $c = 0.5$ for all scenarios, except the '*Linanthus*' case ($c = 0$), where pollinators show no preference and constancy is unlikely. We do not have data on the fraction of pollen transfer from the previously visited flower (r), so we use 0.1 as in Montgomery (2009), except in the case of *Claytonia* (see below). Finally, to obtain realistic estimates of the relative frequency of co-flowering conspecifics of different colour morphs ($\alpha + \beta$) and other species (γ), by examining photos from iNaturalist from around the original study sites (Table S1). We have listed the values of all model parameters in Table 2 and describe each of the case studies in more detail below. In identifying these parameters, we are able to calculate from the model, for every value of A , the expected pollination success (P), the expected pollen receipt (R) and associated seed set (Z), and, in combination with the survival curve, the fitness (F).

2.2.1 | Selection on vegetative phase but not on flowers: the '*Linanthus*' case

Linanthus parryae (A. Grey) Greene (Polemoniaceae) is a self-incompatible winter annual endemic to the Mojave Desert in California that presents white and blue floral colour morphs. The blue morphs have higher seed set in dry conditions, while the white morphs are favoured in wet years (Schemske & Bierzychudek, 2001). Thus, we considered fitness across anthocyanin concentrations for both scenarios (wet and dry years). We used linear relationships to reflect increasing or decreasing survival with varying anthocyanin concentration; these are just examples, not direct empirical estimates. Given the much higher seed set in wet years, we set the survival to $0.6 - 0.03A$ and for dry years, $0.1 + 0.1A$, where A is the anthocyanin concentration. In terms of pollination, *L. parryae* is exclusively pollinated by a single species of beetle (*Trichochorus* sp., Melyridae), which shows no preference for any colour morph (Schemske & Bierzychudek, 2001). It is unknown whether the beetle exhibits constancy. Thus, our model only accounts for abundance of the colour phenotype in calculating fitness. As we moved along the possible values of A (from 0 to 1), we set relative frequency of the given value of A to $\alpha = 0.8$ (since most populations are dominated by a single morph; Epling & Dobzhansky, 1942; Schemske & Bierzychudek, 2001), the relative frequency of other colour morphs $\beta = 0.084$, and the relative frequency of other species to $\gamma = 0.116$.

TABLE 2 Model parameters for case studies and scenarios

Parameter	Linanthus		Delphinium		Raphanus		Claytonia	
	Dry	Wet	Current	Novel	High frequency	Low frequency	Weak	Strong
Minimum survival probability when survival increases with anthocyanins	<i>b</i>	*	-	-	0.7	0.7	-	-
Minimum survival probability when survival decreases with anthocyanins	<i>d</i>	-	*	-	-	-	0.7	0.35
Constant survival probability independent of anthocyanins		-	-	0.7	-	-	-	-
Preferred anthocyanin concentration	<i>A_m</i>	-	-	0.1	0.1	0.1	0.5	0.5
Strength of preference	<i>n</i>	10	10	10	10	10	10	10
Variance around <i>n</i>	<i>var(n(A_m))</i>	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Relative abundance of focal colour	<i>α</i>	0.8	0.8	0.8	0.7	0.255	0.75	0.75
Relative abundance of other colours	<i>β</i>	0.084	0.084	0.062	0.255	0.7	0.25	0.25
Relative abundance of other species	<i>γ</i>	0.116	0.116	0.138	0.045	0.045	0	0
Expected number of visits	<i>λ</i>	1	1	1	1	1	1	1
Increase in visits due to constancy	<i>c</i>	0	0	0.5	0.5	0.5	0.5	0.5
Carryover rate	<i>r</i>	0.1	0.1	0.1	0.1	0.1	1.0	1.0

Each case, based on an empirical system, has two scenarios in which we vary one aspect of the model, denoted in bold font (e.g. effect of anthocyanins on survival to reproduction, pollinator preference, morph frequency). Relative abundance for other species was estimated from images online (Table S1). *See the text for survival relationship used with *Linanthus*.

These values are based on iNaturalist observations of natural populations (see Table S1).

2.2.2 | Selection on flowers but not on vegetative phase: The 'Delphinium' case

Delphinium nuttallianum Pritz. ex Walp. (Ranunculaceae) is a herbaceous, perennial native to the mountains of the western USA which produces blue and white morphs (Waser & Price, 1981, 1983). There is no information on selection on anthocyanins in vegetative tissue, and thus we set a fixed survival probability of 0.7 across all values for anthocyanin concentration (A). The flowers are pollinated by hummingbirds and bumblebees (Price & Waser, 1979; Waser, 1978), both of which show a strong preference for blue flowers (Waser & Price, 1981). Given the long history of studies on the albino morph of this species (Waser & Price, 1981, 1983), we also chose to consider a hypothetical scenario in which a novel pollinator appears that prefers white flowers (Table 2). Given that populations are typically dominated by a single morph (the blue form), we set the relative frequency of the focal flower colour (α) to 0.8, and the relative frequency of other colour morphs $\beta = 0.062$. We estimated the relative frequency of other co-flowering species (γ) as 0.138 based on iNaturalist observations (Table S1).

2.2.3 | Conflicting selection with white morphs having lower survival: The 'Raphanus' case

Raphanus raphanistrum subsp. *sativus* (L.) Domin (commonly called "Raphanus sativus", wild radish, Brassicaceae) is a self-incompatible, cultivated taxon that was introduced to the US in the 19th century and has hybridized extensively with local *Raphanus raphanistrum* in California (Hegde et al., 2006). *Raphanus sativus* has four colour morphs, varying in expression of anthocyanin and carotenoid pigments: White (none), yellow (carotenoids), pink (anthocyanins) and bronze (both pigments; Stanton, 1987). Empirical work shows that the anthocyanin-lacking morphs suffer greater herbivory (Irwin et al., 2003). Thus, we assume that anthocyanins benefit survival to reproduction, and we set the minimum survival probability (b) to 0.7 at the lowest anthocyanin concentration ($A = 0$), increasing to 1 at the highest concentration ($A = 1$). Although white and yellow flowers are selected against by herbivores, they are favoured by the primary pollinators, honeybees, which account for nearly 90% of visits (Stanton, 1987). We therefore set the preferred colour to $A_m = 0.1$. For this case, we considered two scenarios, examining how these conflicting selection pressures would play out for populations with different relative frequencies (Table S1), one where the white morph (preferred by bees) is abundant ($\alpha = 0.7$, $\beta = 0.255$) and one where it is rare ($\alpha = 0.255$, $\beta = 0.7$; Table S1). In both cases, we set the relative frequency of other flowering species to $\gamma = 0.045$, based on our iNaturalist survey (Table S1).

2.2.4 | Conflicting selection with white morphs having higher survival: The 'Claytonia' case

Claytonia virginica L. (Montiaceae) is a self-compatible but not self-pollinating perennial herb (Motten, 1986; Schemske, 1977) that is distributed throughout North America (Frey, 2004). The flowers range from white to pink to crimson. Floral colours are determined by cyanidin-derived anthocyanins and flavonols (Harborne, 1967) that are also expressed throughout the plant body (Doyle, 1981, 1983). Plants with greater anthocyanin concentrations are less prone to pathogen infection, but they receive substantially more herbivore (slug) damage (Frey, 2004). Overall, white morphs have higher survival (Frey, 2004), therefore we assumed that survival decreases with more anthocyanins. We considered two degrees of selection related to herbivores, one where survival probability declines to $d = 0.7$ at the highest anthocyanin concentrations ($A = 1$) and one where survival probability drops to $d = 0.35$ at $A = 1$ (Table 2). The primary pollinator in the northern part of *C. virginica*'s range, where Frey's (2004) study took place, is the specialist solitary bee *Andrena erigeniae* with additional visitation from the bee-mimicking parasitic fly *Bombylius major* (Parker et al., 2018). Frey (2004) used fruit set to infer that the intermediate pink morphs receive most pollinator visits, so we assume a preference for $A_m = 0.5$. There were no other co-flowering spring ephemerals in the study area (Frey, 2004), so pollinators only choose between the preferred morph, which we arbitrarily set to the relative frequency $\alpha = 0.75$, and other morphs, which we set to the frequency $\beta = 0.25$ (Table 2). We accounted for the plant's self-compatibility by maximizing the carryover ($r = 1$), assuming that deposition of self-pollen on the stigma during visitation greatly increases the expected load of compatible pollen.

2.3 | Programming language

R version 4.2.0 was used for all model simulation (R Core Team, 2022).

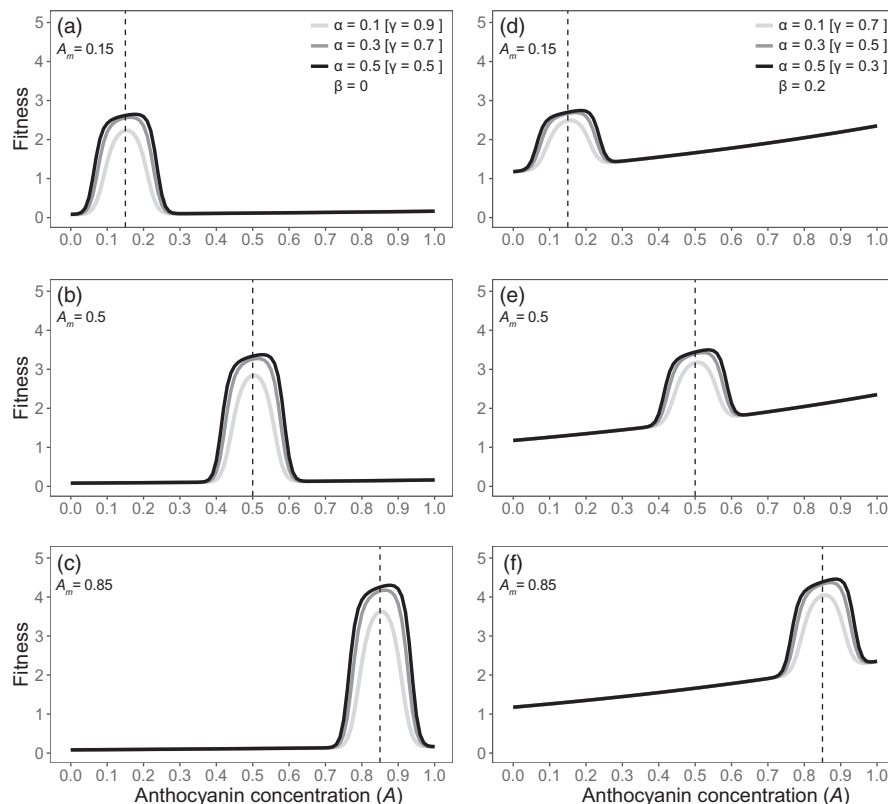
3 | RESULTS

3.1 | Theoretical findings

3.1.1 | Relative abundance and pollinator preference

We first examined how fitness varies when the preferred morph varies in abundance. For this exploration, we chose a positive relationship between anthocyanin production and survival as seems to be common for many plant species, and we set the minimum survival probability (b) at $A = 0$ to 0.5. As expected, we find that fitness closely tracks pollinator preference, with the preferred colour having the highest fitness. If pollinators prefer light flower colours with the highest preference $A_m = 0.15$ (Figure 1a),

FIGURE 1 Effect of increasing relative abundance with varying pollinator preference. The preferred anthocyanin concentration (A_m) varies from 0.15 (top row) to 0.5 (middle row) to 0.85 (bottom row). The three lines in each graph correspond to different α values for the relative abundance of the given value of anthocyanin concentration (A). In (a–c), the frequency of other morphs (β) is zero, so the remaining proportion of available flowers belongs to other species (γ). In (d–f), we increase β to 0.2 so that some fitness effects can come from incoming pollen from other morphs, and again, the remaining proportion of flowers belongs to other species. In all graphs, we set the minimum survival probability b to 0.5, the pollen carryover r to 0.1, the constancy parameter c to 0.1, the expected number of visits λ to 1, and the variance in the strength of preference $\text{var}(n(A_m))$ to 0.001. Fitness is measured in number of seeds per flower (Figure S5).



pollinators also visit flowers that are somewhat lighter or darker, that is, A -values between 0.05 and 0.25 but with lower probability. The more A deviates from A_m the less likely a pollinator visits the flower, and the probability approaches 0 for $A < 0.03$ or $A > 0.27$. With increasing relative abundance of light flowers (α), the chances of less attractive flower colours being pollinated also increases as long as A -values are between 0.05 and 0.25 (i.e. still close to A_m). However, if the relative abundance of light flowers is sufficiently high ($\alpha \geq 0.3$) the difference between the pollination successes of flowers within the preference range of pollinator ($0.05 \leq A \leq 0.25$) disappears. All of the curves hit an upper fitness limit when stigmas become saturated with pollen. In other words, once flowers produce their maximum seed set, naturally, additional pollen no longer increases fitness (see also Figure S4). In this case, the only additional factor influencing fitness is survival in relation to anthocyanin production. Since we assumed that fitness increases with higher anthocyanins, the highest fitness curves (those with higher frequencies of the preferred colour morph) show a pronounced upward tilt. Overall, relative abundance (α) only has a minor effect, noticeably increasing the fitness when the preferred morph is at 30% frequency compared to 10%, but only negligible effects comparing 30% to 50% of preferred morph frequency (Figure 1).

We also varied the availability of other colour morphs, which could contribute to successful pollination even when they are not preferred. When we add in a small proportion of additional morphs ($\beta = 0.2$ in Figure 1d–f compared to $\beta = 0$ in Figure 1a–c), the fitness for all values of A is above one. In other words, we expect at least

one seed per flower for all colours, despite the narrow range of preference of the pollinator and the sizeable proportion of individuals of co-flowering species (γ , up to 70%). As a result of this fitness benefit from more co-flowering conspecifics, there is less of a marked difference between the fitness of the preferred colour morph and other colour morphs.

Finally, we observed that the absolute peak fitness varies for different preferred colours, showing a clear effect of anthocyanins on survival to reproduction. Keeping in mind that the nature of preference is the same for each plot in Figure 1 (i.e. strength of preference (n) is 10 and variance is 0.001), we would expect all of the peaks with the same abundance values (α, β, γ) to be the same height if only pollination determined fitness. However, we see that the peak fitness is highest when pollinators prefer a high anthocyanin concentration (i.e. preferred $A_m = 0.85$, Figure 1c,f). This is because we assume that survival to reproduction increases with higher anthocyanin production, so morphs closer to 1 have higher fitness. The shape of the relationship (peaks not perfectly normal, baseline upward-trending) also shows the intersection of preference with the upward survival curve (e.g. Figure S1, b curve). Overall, these graphs demonstrate that the preference of the pollinator determines the location and width of the fitness peak, but the overall height depends on combined fitness effects of anthocyanin production during both vegetative and reproductive phases.

The predictions of our model also hold for discrete colour variation. Let us imagine a species can produce white ($A = 0.1$ – 0.2), pink (0.4 – 0.5) and purple (0.8 – 0.9) morphs. If we assume a population

consisting of exclusively white morphs of the focal species and pollinators prefer white (e.g. $A_m = 0.15$), white flowers will have a fitness between 2.4–2.6 (depending on the frequency of other co-flowering species in the area), and if by chance a seed from a pink or purple colour morph lands in that population it would have zero fitness (Figure 1a). If, on the other hand, the population consists of 20% pink and purple morphs, these will have fitness of 1.6–1.7, and 2.0–2.2, respectively, assuming pollinators exhibit some constancy ($c = 0.1$; Figure 1d). Thus, the fitness of each of the discrete morphs will depend greatly on their frequencies relative to each other and to co-flowering species.

3.1.2 | Variation in the strength of preference

We also examined the shape of the fitness curve depending on the strength of pollinator preference. Keeping the preferred anthocyanin concentration at 0.5, we relaxed the preference by increasing $\text{var}(n(A_m))$ to 0.005 and 0.01 (Figure 2). As expected, a weakening of preference broadens the peak fitness. The non-normal nature of the peak (flattened at the top with an upward slope) is even more apparent, suggesting that as pollinators are less specialized, the effect of anthocyanins on survival plays a greater role in determining fitness. In the most extreme case shown (Figure 2c,f), the peak fitness is above $A = 0.7$ for the black lines, even though the preferred colour is $A = 0.5$.

3.1.3 | Constancy and pollen carryover

In general, our model predicts that constancy has the potential to increase flower fitness. Within the range of pollinator preference, constancy acts as an amplifier of preference, leading to more visits and higher peak fitness of the preferred colour. However, this fitness advantage is only detectable when the preferred morph is at low relative abundance (Figure S6: black line within the range of pollinator preference ($0.4 < A < 0.6$) is higher than grey lines in panel A but not in panel B). These results make intuitive sense as a common morph, that also is preferred, has a high probability of pollination success (Figure S2), with little additional fitness gains to be had from constancy. It is worth noting that additional factors beyond preference and constancy, such as competition among pollinators and learning, can influence pollinator foraging patterns (reviewed in Amaya-Márquez, 2009; Goulson & Wright, 1998). Nevertheless, these would likely lead to similar effects as observed here, amplifying the fitness differences across morphs when any preference is present.

In our modelling, modifying the degree of carryover (r) had even smaller effects on fitness than constancy, barely evident even at low frequency (α) (Figure S7). While theoretically greater carryover has the potential to increase fitness (if pollen loads are composed by con-specific, genetically diverse pollen), its effect is contingent on constancy (Figure S4). When pollinators exhibit constancy, increasing carryover will increase pollen receipt (between $r = 0$ and $r = 0.5$), but as the stigma becomes saturated, additional pollen will bring no added fitness benefit.

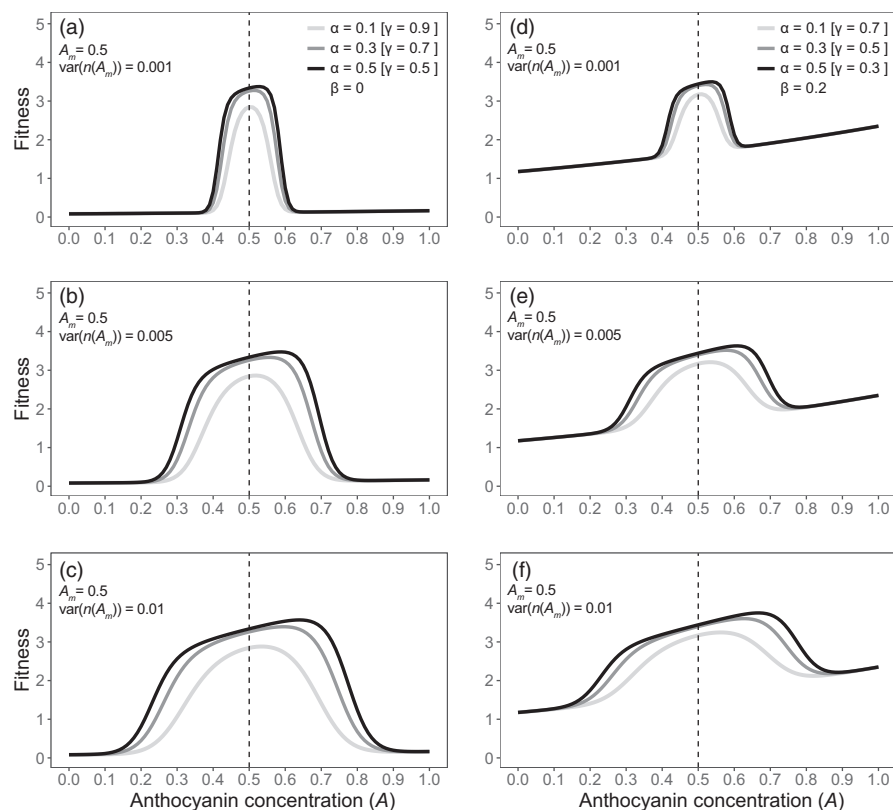


FIGURE 2 Effect of relative floral abundance and variation of pollinator preference on the fitness of flowers associated with different anthocyanin concentrations in their petals; the dashed lines indicate mean pollinator preference, $r = 0.1$, $c = 0.1$, $b = 0.5$, $\lambda = 1$. (a–c): Relative frequency of other colour morphs, $\beta = 0$, (d–f): $\beta = 0.2$, first row (a and d) $A_{var} = 0.001$, second row (b and e) $A_{var} = 0.005$, third row (c and f) $A_{var} = 0.01$.

3.2 | Empirical cases

3.2.1 | Selection only on survival in *Linanthus*

In systems like this one, where pollinators exhibit no colour preference, the evolution of flower colour is driven entirely by nonpollinator agents of selection. Based on the empirical case, we designed the model such that anthocyanin production is favoured in dry years, but the opposite in wet years. We also assumed that overall survival is much higher in wet years. The results for overall fitness perfectly mirror our input survival curves (Figure 3a). In dry years, the blue-flowered morph will have the highest fitness and in wet years, the opposite is true.

3.2.2 | Pollinator-mediated selection in *Delphinium*

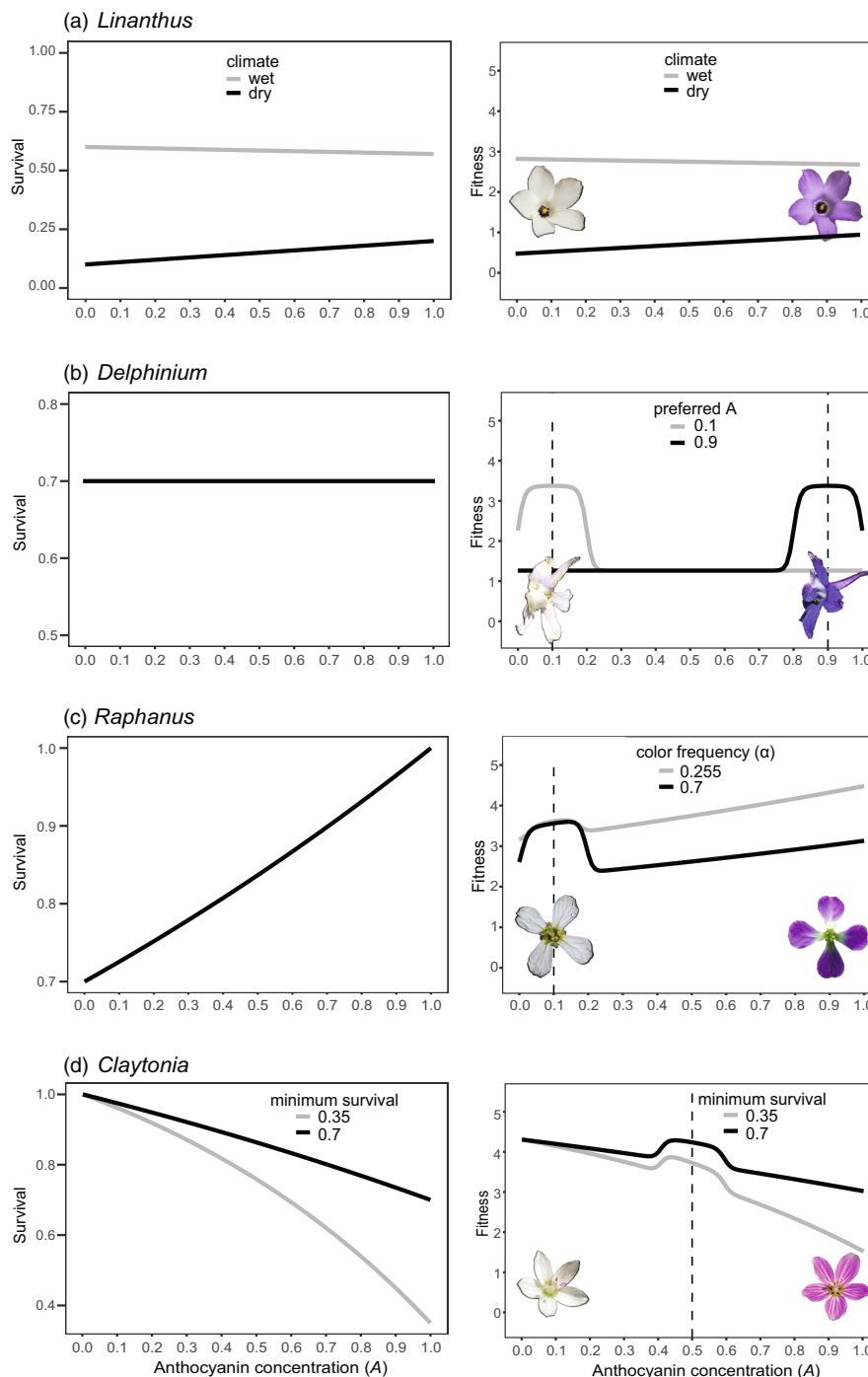
We considered two pollination scenarios, one with the current pollinators (bees, hummingbirds) preferring blue flower, and another with a novel pollinator that prefers white flowers. If pollinators prefer blue colour morphs, the fitness of the white morphs is low (represented by the black line between 0 and 0.25 in right-hand panel of Figure 3b). This value is not zero, however, because we assume a small proportion ($\beta = 0.062$, Table 2) of blue flowers even in a primarily white-flowered population. As in the theoretical case (Figure 1d–f), these additional conspecifics will boost the overall fitness of all morphs. Moreover, we assume some degree of constancy ($c = 0.5$, Table 2) so that a pollinator that visits a white flower will likely continue to visit white flowers although they are not preferred. Under a pollinator shift scenario, where the new pollinator prefers the white form, the peak fitness shifts down to $A = 0.1$ (grey line in right-hand panel of Figure 3b). In this case, the fitness peaks in both scenarios show curves that are equal in height as we assumed no

survival advantage associated with differences in anthocyanin production, and both pollinators are equal in strength of preference and other pollinator parameters (Table 2).

3.2.3 | Opposing selective forces in *Raphanus*

In this system, the anthocyanin-less morphs (white or yellow flowers) are favoured by the main pollinators (honeybees), but the anthocyanin morphs (pink or bronze) are better defended against herbivores. Given the assumed strong effect of anthocyanins on survival (Figure 3c, left-hand panel), we observed a sharply sloping baseline in the fitness curve, increasing toward higher values of A (right-hand side of Figure 3c). We examined two assumptions about frequency: one where the given value of A is never common ($\alpha = 0.255$, grey line in right-hand side of Figure 3c) and another where it is common ($\alpha = 0.7$, black line in right-hand side of Figure 3c). Our results show that within the preference range of pollinators, peak fitness is independent of the colour morph frequency (black and grey lines overlap $A = 0.025$ – 0.175). Because of the effect of anthocyanins on survival, this peak is tilted, with the highest fitness (ca. $A = 0.175$) being slightly above the value preferred by pollinators ($A = 0.1$). Outside the preference range of the pollinator, the contribution of the non-preferred colour morphs to fitness increases. Hence, the highest overall fitness across the two scenarios (black and grey) corresponds to the grey line because, in this system, the co-flowering plants are mostly conspecifics. When the dark pink morph has a low frequency (grey line, high values of A), other lighter, preferred morphs are common and will serve as a source of compatible pollen. These results highlight that the impact on differential survival related to anthocyanins interacts strongly with the composition of the population (in terms of relative abundance of different morphs and of different species).

FIGURE 3 Exploration of model performance in different empirical systems. See Table 1 for details of model parameters. (a) In wet years *Linanthus* survival decreases with anthocyanin concentration (grey line in left-hand side panel) while in dry years it increases with anthocyanin concentration (black line in left-hand side panel). Independent of anthocyanin concentration survival is higher in wet years than in dry years. Since pollinators have no preference for flower colour the predicted fitness of colour morphs in wet and dry years (right-hand panel) is explained by the effect of climate and anthocyanin concentration on survival. (b) *Delphinium* survival is independent of anthocyanin concentration (left-hand side panel). Hence, the fitness depends only on pollinator preference (preferred A , right-hand panel). If pollinators prefer light colours (preferred $A = A_m = 0.1$, grey line in right-hand panel) the fitness of colour morphs with $0.05 < A < 0.15$ is highest. In contrast, if pollinators prefer dark colours (preferred $A = A_m = 0.9$, black line in right-hand panel) the fitness of colour morphs between $0.85 < A < 0.95$ is highest. (c) *Raphanus* survival increases with anthocyanin concentration (left-hand panel). Since pollinators prefer light colours ($A_m = 0.1$) the fitness of colour morphs with $0.05 < A < 0.15$ has a peak (right-hand panel). Outside the preference range of pollinators, fitness increases with anthocyanin concentration, but how much it increases is influenced by the frequency of the preferred colour morph α : For low α -values (grey line) fitness is highest because of the high fitness contribution of other flower morphs with high survival. (d) *Claytonia* survival decreases with anthocyanin concentration (right-hand panel). We explore the effect of two different survival scenarios (black and grey lines). Within the preference range of pollinators anthocyanin has a large negative effect on survival, which is indicated by the downward slope around relatively small fitness peaks. Fitness is lower if survival decreases more sharply with anthocyanin concentration (grey line is below black line in right hand panel). Photo credits/creative commons licences images for *Linanthus*: [flickr.com/photos/jeffreysullivan/34884739990](https://www.flickr.com/photos/jeffreysullivan/34884739990/), © Jeff Sullivan; for *Delphinium*: photo by Karin Teague and commons.wikimedia.org/wiki/File:Delphinium_nuttallianum_1824f.JPG; for *Raphanus*: commons.wikimedia.org/wiki/File:Raphanus_sativus_subsp._oleiferus_flower_Bladrammena_s_bloem_%281%29_bewerkt.jpg and [flickr.com/photos/livenature/2182234124](https://www.flickr.com/photos/livenature/2182234124/); for *Claytonia*: [flickr.com/photos/treegrow/27638524318](https://www.flickr.com/photos/treegrow/27638524318) and commons.wikimedia.org/wiki/File:Claytonia_virginica_UMFS_1.jpg



3.2.4 | Opposing selective forces in *Claytonia*

This species represents the opposite situation compared to *R. sativus*; here, pollinators prefer pink flowers, but the white flowers are better defended. Accordingly, we see that fitness decreases with higher values of A , following the slope of the survival curve. In this case, we considered the impact of a two-fold difference in minimum survival, where it decreases to 0.7 at $A = 1$ or 0.35 at $A = 1$ (right-hand panel

of Figure 3d). This difference results in a reduction of plant fitness by more than 50%. We still see the effect of pollinator preference on fitness around the colour preferred by pollinators ($A_m = 0.5$), but the flat portion of the peaks trend downwards toward higher values of A with a stronger negative effect of anthocyanins on survival ($d = 0.35$) leading to lower fitness. Pollinator preference is less important in this scenario because there are no co-flowering plant species ($\gamma = 0$), and flowers can get pollinated by nonpreferred colour morphs.

4 | DISCUSSION

4.1 | Integrating modelling of selection on floral traits with correlated variation

Plant-pollinator interactions have been the subject of several modelling studies. These models have provided the framework for how pollinators forage based on expected reward (Possingham, 1992) and how various aspects of plant-pollinator interactions affect plant fitness (e.g. Campbell, 1986; Feldman et al., 2004; Rodríguez-Gironés & Santamaría, 2004). For example, pollinator specialization can, to some degree, compensate for lower efficiency (Aigner, 2001) and individual pollinator constancy can mitigate the potential fitness costs of sharing pollinators with co-flowering species (Levin & Anderson, 1970; Montgomery, 2009). Here, we build on these previous models to predict how pollinators will influence the fitness of different flower colour morphs, incorporating key aspects of pollinator behaviour, including preference and constancy. However, for flower colour, and probably other floral traits, correlated variation is likely to contribute to any observed fitness differences. We focus on anthocyanin pigment production as an example where there is broad evidence for correlated variation (e.g. Armbruster, 2002; Warren & Mackenzie, 2001) and nonpollinator agents of selection have often been cited in driving floral colour polymorphisms (Strauss & Whittall, 2006). By integrating the fitness effects of anthocyanin production across vegetative and reproductive life stages, our model reinforces the notion that diverse agents of selection can lead to a range of predicted outcomes, which could explain persistent colour polymorphism in many species (Sapir et al., 2021).

4.2 | Drivers of within-species colour variation

Our modelling results highlight the complex patterns that can arise when multiple forces act on a single trait. In a simple scenario in which pigment production is beneficial for survival and leads to increased pollinator visitation, selection will predictably favour pigmented morphs. However, as shown in our case studies (inspired by empirical systems), slight differences in aspects of community composition, survival probability, or pollinator behaviour can greatly shift the predictions about which colour will be most fit. In real systems, many of these variables will shift year to year (such as precipitation as in the *Linanthus parryae* case, Schemske & Bierzychudek, 2007) or across geographic regions (such as principal pollinators in *Claytonia virginica*, Parker et al., 2018), leading to spatiotemporal variation in colour morphs. We expect that such variation will be most common in systems where anthocyanin production is correlated across tissues, meaning that the fitness of a flower colour morph reflects the confluence of multiple selective agents. Ironically, in cases like those in Figure 3a,c,d, where pleiotropy has been implicated in the fitness differences between colour morphs, correlated production of anthocyanins in flowers and vegetative tissues has not yet been quantified. Related flavonoid compounds, such as flavonols, may also be

correlated with anthocyanin production and could underlie similar or different fitness effects (Berardi, Fields, et al., 2016a; Berardi, Hildreth, et al., 2016). Any such correlated traits, be they biochemical, developmental or otherwise, that affect survival to flowering and pollination success are perfectly amenable for study with our model. Overall, complementing the available empirical studies with quantitative biochemical analyses throughout plant life stages will be a crucial step in understanding how flower colour responds to the wide array of selective pressures beyond pollinators.

4.3 | Detecting the signal of nonpollinator agents of selection

Our study revealed that selection on anthocyanin production at the vegetative phase is likely to leave a distinct signature in the fitness curve, suggesting it can be detected even when the selective agents themselves are not known. Under many scenarios, we see that the shape of the curve relating anthocyanin production to fitness is not normally distributed but shows a slanted peak (e.g. Figures 1 and 2). Pollinator preference alone will produce a symmetrical, nearly-normal curve, (Figure S3) so this shape arises due to the effect of anthocyanin concentration on survival within the range of pollinator preference. For our theoretical work, we assumed that the vegetative anthocyanins increase survival probability, resulting in fitness curves where the peak near preference slants upward toward higher values. True survival curves may not take the form we assumed (Figure S1), but unless they are normal distributions with the same mean as pollinator preference, the combined effects of selection through both survival and pollinators will result in non-normal distributions for fitness across anthocyanin concentrations.

We also found that community composition has a strong influence on the signature of differential survival. According to our model, when a community largely comprises other co-flowering species, the focal species will be pollen-limited, and fitness differences across colours will be driven by pollinator preference (i.e. the nearly-normal grey curves in Figure 1a–c). As the focal species rises in frequency, the pollen limitation disappears and the differential survival related to anthocyanins becomes apparent (i.e. the tilted black peaks in Figure 1a–c). High abundance of co-flowering conspecifics has an even stronger effect, elevating the baseline fitness of all morphs to follow the survival curve (increasing toward higher A in Figure 1d–f). The non-normality will be even more apparent when pollinator preference is broad (Figure 2), as may be the case with generalized pollinators or systems where multiple pollinators are involved. While pollinator limitation appears to be common (Knight et al., 2005), so are systems with generalized pollination and mixed populations with multiple colour morphs, including all of the empirical cases examined here. This suggests that natural populations often present the conditions for detecting and dissecting floral and nonfloral sources of selection on flower colour. Nevertheless, the application of our model in diverse communities may require incorporating more complex dynamics, such as asymmetrical facilitation benefiting rare colour

morphs (i.e. fitness increase with increased abundance of rare morphs, Figure 1; Wei et al., 2021).

5 | CONCLUSIONS

Flower colour polymorphisms comprise one of the most ubiquitous and striking sources of variation in natural populations. Pollinators, herbivores, pathogens and abiotic factors such as climate are important drivers of this variation, with rigorous empirical evidence spanning several decades. Our model is the first attempt to link the nonpollinator and pollinator agents of selection on flower colour into a single framework. We find that while pollinators typically determine peak fitness, there are clear signatures of the effect of non-pollinator selection that emerge under biologically reasonable conditions. This framework lays the foundation for tackling outstanding questions in colour evolution, particularly in systems where multiple (e.g. different pollinators) and often opposing selective forces influence colour variation and where environmental conditions are rapidly shifting due to climate change.

AUTHOR CONTRIBUTIONS

Brigitte Tenhumberg has taken the lead in conceptualizing and programming the mathematical model; all three authors have contributed equally to writing the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

This work did not produce any data. The R-code is available at <https://doi.org/10.5281/zenodo.7391423> (Tenhumberg et al., 2022).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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