Sensory bias and signal detection trade-offs maintain intersexual floral mimicry

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Mimicry is common in interspecies interactions, yet conditions maintaining Batesian mimicry have been primarily tested in predator–prey interactions. In pollination mutualisms, floral mimetic signals thought to dupe animals into pollinating unrewarding flowers are widespread (greater than 32 plant families). Yet whether animals learn to both correctly identify floral models and reject floral mimics and whether these responses are frequency-dependent is not well understood. We tested how learning affected the effectiveness and frequency-dependence of imperfect Batesian mimicry among flowers using the generalist bumblebee, *Bombus impatiens*, visiting *Begonia odorata*, a plant species exhibiting intersexual floral mimicry. Unrewarding female flowers are mimics of pollen-rewarding male flowers (models), though mimicry to the human eye is imperfect. Flower-naive bees exhibited a perceptual bias for mimics over models, but rapidly learned to avoid mimics. Surprisingly, altering the frequency of models and mimics only marginally shaped responses by naive bees and by bees experienced with the distribution and frequency of models and mimics. Our results provide evidence both of exploitation by the plant of signal detection trade-offs in bees and of resistance by the bees, via learning, to this exploitation. Critically, we provide experimental evidence that imperfect Batesian mimicry can be adaptive and, in contrast with expectations of signal detection theory, functions largely independently of the model and mimic frequency.

This article is part of the theme issue 'Signal detection theory in recognition systems: from evolving models to experimental tests'.

1. Introduction

Mutualisms have been described as reciprocal exploitation, through which each interacting partner achieves a net benefit [1–3]. Mutualisms can thus involve cooperation but also conflict. Plant–pollinator mutualisms, in which animal pollinators visit flowers to acquire resources (floral 'rewards'; e.g. pollen or nectar) and in the process pollinate the plant, provide examples of conflict between mutualist partners. For example, pollinators sometimes exploit plants by taking resources without pollinating, as frequently happens in nectar robbing (e.g. [4]). Likewise, plants sometimes exploit pollinators by deceiving them into visiting and pollinating flowers that falsely advertise more resources than they have [5–7].

How do pollinators mitigate exploitation by plants? Pollinators such as bees alter their search behaviour after visiting unrewarding flowers and also learn to use distinctive floral cues to visit more rewarding plant species (e.g. [8–12]). For instance, bees learn to avoid the distinctively coloured flowers of unrewarding orchid species, such as *Calypso bulbosa* (e.g. [13]). It is thus not surprising that deception via mimicry of rewarding flowers—Batesian mimicry—is common and widespread among plants (greater than 32 plant families, see [6,14–16]). However, while pollinator learning is frequently proposed to mitigate
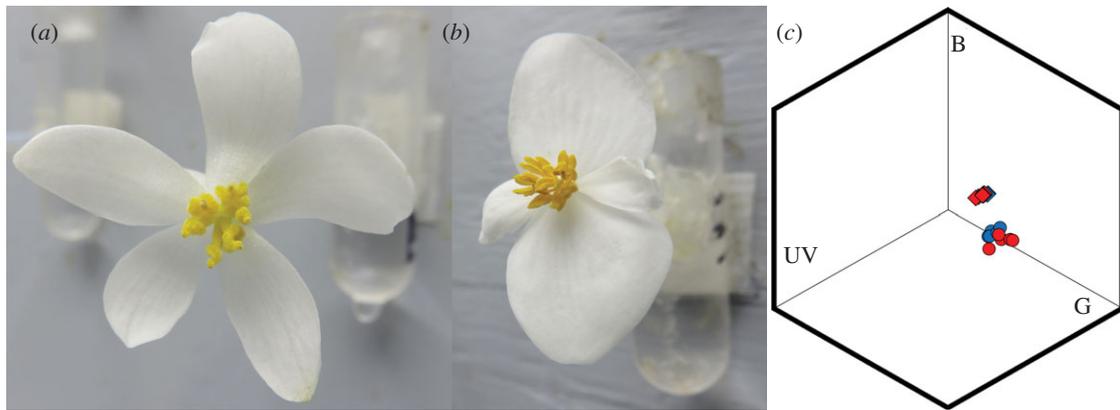
Figure 1. Imperfect mimicry among female (mimics) and male (models) in *Begonia odorata* flowers. (*a*) Female and (*b*) male flowers. (*c*) The loci in *B. impatiens* colour space (B, blue; G, green; UV, ultraviolet) of male and female petals (blue and red diamonds, respectively), stamens (blue circles) and styles (red circles) against the test arena background: female flowers resemble the colour of male flowers in terms of both reproductive and non-reproductive parts ($n = 5$ male and female flowers). On average, male and female petals differed by 0.012 colour units and reproductive parts differed by 0.065 colour units; bumblebees have difficulty discriminating targets less than 0.07 colour units apart [37].

exploitation by floral Batesian mimics [6,17–22], how this is achieved is still poorly understood (but see [18,23–25]).

Successful Batesian mimicry is thought to depend primarily on the frequency of rewarding models and unrewarding deceptive mimics, as well as on the phenotypic similarity of the mimic to the model as perceived by their receivers [26–30]. Signal detection theory predicts that a naive pollinator confronted with a rewardless mimic should, if possible, adjust its behaviour to avoid visiting mimics, even at the expense of avoiding rewarding models, such that its overall rate of correct decisions increases over time (maximizing foraging efficiency) [28,31,32]. Because naive pollinators are initially unfamiliar with the phenotypic distribution, frequency, and costs and benefits of visiting models and mimics, visitation decisions should not depend on the model and mimic frequency initially. However, with increasing experience, pollinators should optimize the avoidance of mimics versus models in relation to the model and mimic frequency [28,33–35]. Namely, as the frequency of mimics rises, the cost of visiting them rises in relation to the cost of failing to visit models. Therefore, as the frequency of mimics increases, pollinators should be more conservative and make relatively fewer visits to models to avoid mistakenly visiting mimics.

How easily pollinators can adjust their visitation behaviour should depend on how well mimics resemble models, how quickly pollinators can learn and how finely pollinators can discriminate [18,28,29,36]. In intersexual floral Batesian mimicry, male flowers typically offer a pollen reward, while female flowers of the same plant species are deceptive rewardless mimics [16] (e.g. figure 1). While we might expect that selection on the plant would favour close resemblance among female and male flowers, imperfect floral mimicry is common in intersexual floral mimicry [38–40]. Following Kikuchi & Pfennig [29], possible hypotheses include that imperfect mimicry is an anthropocentric perception and pollinators do not actually perceive mimics differently from models 'eye of the beholder' (predicts that pollinator preference is unbiased), or that mimics may be developmentally constrained in the precision of their mimicry (predicts that pollinators prefer models over mimics), or that imperfect floral mimicry is adaptive and imperfect mimics exploit pollinator sensory biases (predicts that pollinators prefer mimics over models)

[41–43]. Surprisingly, to our knowledge, no studies have disentangled the hypotheses for imperfect floral mimicry.

In this laboratory study, we tested whether generalist bumblebees (*Bombus impatiens*) used learning to mitigate exploitation by a simultaneously monoecious plant species exhibiting intersexual Batesian mimicry (in terms of overall flower colour pattern, and in terms of divided styles resembling anthers in form and colour, i.e. pseudanthery [16], *sensu* [44]; figure 1) and a flexible sex ratio (*Begonia odorata*; figure 1). We hypothesized that imperfect floral mimicry in *B. odorata* was adaptive in terms of pollination and naive bees would initially prefer to visit unrewarding female flowers (mimics) over rewarding male flowers (models) as a result of a sensory bias. We also predicted that in accordance with signal detection theory, as bees gained experience they would increase their rate of correct decisions (correct decisions: approaching and landing on models and approaching but not landing on mimics; incorrect decisions: approaching but not landing on models, approaching and landing on mimics) by increasingly avoiding both mimics and models. Finally, we assessed whether patterns of correct detections (approaching and landing on models) and correct rejections (approaching but not landing on mimics, i.e. false alarms) made by naive and experienced bees depended on the model and mimic frequency. We predicted that initially naive bees, which were initially unfamiliar with model and mimic frequency, would decrease their correct detections most when the mimic frequency was highest, owing to the higher cost of visiting mimics relative to the cost of missing models. Likewise, we predicted that experienced bees would have learned to be more conservative and would make fewer correct detections and more correct rejections when the mimic frequency was high, compared to when the frequency of mimics was low.

2. Material and methods

(a) Test subjects

We maintained nine commercially obtained (Koppert Biological Systems, Howell, MI, USA) captive colonies of the bumblebee *B. impatiens* following Russell *et al.* [45]. In brief, we allowed colonies to forage freely on 2 M sucrose solution and pulverized

honeybee-collected pollen (Koppert Biological Systems) from artificial feeders within enclosed foraging arenas (length, width, height: $82 \times 60 \times 60$ cm) set to a 14 h : 10 h light : dark cycle.

We used fresh male and female flowers with mature anthers and styles, respectively, from 10 simultaneously monoecious *B. odorata* plants raised in a university greenhouse with supplemental halogen lights to extend day length to a 14 : 10 h cycle and with monthly fertilizer applications (Miracle Gro, NPK 15 : 30 : 15). While female *B. odorata* flowers are rewardless and produce neither pollen nor nectar, male *B. odorata* flowers offer pollen, their sole reward to their principal pollinators, bees [14,36,46,47]. Female *B. odorata* flowers closely resemble male flowers in shape, size and colour (to the human eye, at minimum; figure 1); in bumblebee colour vision, both flower sexes have similar creamy white dissected petals, and the female flower's yellow and highly divided styles closely resemble the male flower's yellow numerous stamens (characterized following [48]; figure 1*c*). While petal colour resemblance probably results from a shared developmental pathway, non-homologous anthers and styles show great similarity in their colour and form, strongly suggesting selection for mimicry (see also [16,42]); furthermore. Like other *Begonia* species, *B. odorata* sex ratio on individual inflorescences is labile (e.g. [42,49]).

(b) Experiment 1: do naive bees exhibit a perceptual bias for the mimics versus the models?

Here, we tested the naive preference of bees for models (rewarding male flowers) versus mimics (unrewarding female flowers). We used 32 bees from four colonies. To initiate a behavioural trial, we set up 16 horizontally displayed freshly clipped flowers (an equal number of male and female flowers alternated by position) on the arena wall in a cleaned test arena. Flowers were spaced 7 cm apart in a Cartesian grid design. To prevent desiccation, flowers were placed into custom-made water tubes [45]. From the foraging arena, a single flower-naive worker bee was gently captured using a 40 dram vial (Bioquip) and immediately released in the centre of the test arena following Russell *et al*. [45]. We terminated the trial by capturing the bee in a 40 dram vial and euthanizing it after the bee made its first landing on a flower, or, rarely, if it did not approach any flower for a period of 5 min, whichever came first. We defined landing as the bee touching the flower with at least three of its legs simultaneously. For each trial, we used a new flower-naive bee and, for most trials, new freshly clipped flowers; when flowering was limited, we replaced only the visited flower and a random flower of the opposite sex.

(c) Experiment 2: does the frequency of models and mimics affect how naive bees learn to sample?

Here, we examined whether model (male flowers) and mimic (female flowers) frequency influenced how initially flower-naive bees learned to sample among models and mimics. We used 36 bees from three colonies. We examined three major components of sampling behaviour (visits) made by bees visiting arrays of 16 flowers: approaches, landings without sonication (on male flowers such landings typically involved the bee collecting pollen via a behaviour termed scrabbling; see [45] for a description) and landings with sonication (buzzes' or 'buzzing') (see flow diagram in the electronic supplementary material, figure S1). An approach was defined as the bee in flight greatly reducing its velocity while facing the flower within 3 cm of the flower. All landings were preceded by an approach (i.e. 'correct detections' for models; 'false alarms' for mimics) and landings on male flowers (models) nearly always involved collection of pollen. Not all approaches were followed by a landing (i.e. 'missed detections' for models; 'correct rejections' for mimics). Buzzes, which indicated an attempt at extracting pollen whether or not it was available, were identified by their distinctive sound and occurred only after a bee had landed (see [50]). Buzzing a male flower constituted a correct behavioural response and buzzing a female flower constituted an incorrect behavioural response.

We split flower-naive bees into three treatments with colonies represented approximately equally among treatments: either mimic biased (12 female and four male flowers), no-bias (eight female and eight male flowers), or model biased (four female and 12 male flowers). In the no-bias treatment, flower sexes were alternated by position. For sex-biased treatments, the rarer flower sex was assigned two central and two corner positions in either of two arrangements, which we systematically alternated across trials. We tested bees individually and never reused flowers or bees across trials. We terminated a trial after a bee made up to 60 visits (15 out of 36 bees made less than 60 visits: range 5–60 visits) and never reused flowers. Sometimes a bee visited the same flower more than once in a row, which typically involved the bee landing, hovering while within 3 cm of the same flower, and then landing again on that flower. To be conservative, we discarded these repeat visits (across all treatments, an average of 10% of visits) for all analyses, reasoning that the bee may not have had the opportunity to actively assess the other flowers.

(d) Experiment 3: does the frequency of models and mimics affect sampling by experienced bees?

Here, we determined whether sampling of models (male flowers) and mimics (female flowers) by bees familiar with the phenotypic distribution, frequency, and costs and benefits of foraging on models and mimics was influenced by model and mimic frequency. We used 24 bees from five colonies. To generate the largest possible difference in sampling behaviour, we split flower-naive bees into two treatments: mimic biased (12 female and four male flowers) and model biased (four female and 12 male flowers). We first allowed flower-naive bees to gain experience with models and mimics and their frequency by allowing them up to 40 visits (minimum: 32 visits; 6 out of 20 bees made less than 40 visits) in a training flower array with a given flower sex ratio. We used this cutoff to standardize experience across treatments and limit the potential effect of repeat sampling; by 40 visits, a bee had sampled each flower on average 2.5 times and landed on each flower at least once.

Following Russell *et al*. [11], 20 min to 1 h after training, the experienced bee was allowed to make up to 30 visits in a fresh test flower array with a frequency of models and mimics identical to the training array. Using fresh flowers in test arrays minimized the prospect that differences in sampling behaviour were a consequence of pollen depletion by test bees or learning of scent marks test bees might have added to flowers [51]. From these analyses, we excluded four bees with fewer than five visits during training and no flower landings.

(e) Data analyses
All data were analysed using R v. 3.3.2 [52].

(i) Experiments 1 and 2
To analyse flower-naive bees' naive preference for models (male flowers) versus mimics (female flowers), we pooled bees' first landing choice from experiment 1 and from the no-bias treatment in experiment 2, and used a *G*-test (DescTools package [53]). To analyse initially flower-naive bees' overall preference for one or the other flower sex from the no-bias treatment in experiment 2, we performed a paired *t*-test on the mean proportion of a bee's landings on each flower sex after checking that assumptions of normality and equal variance were met.
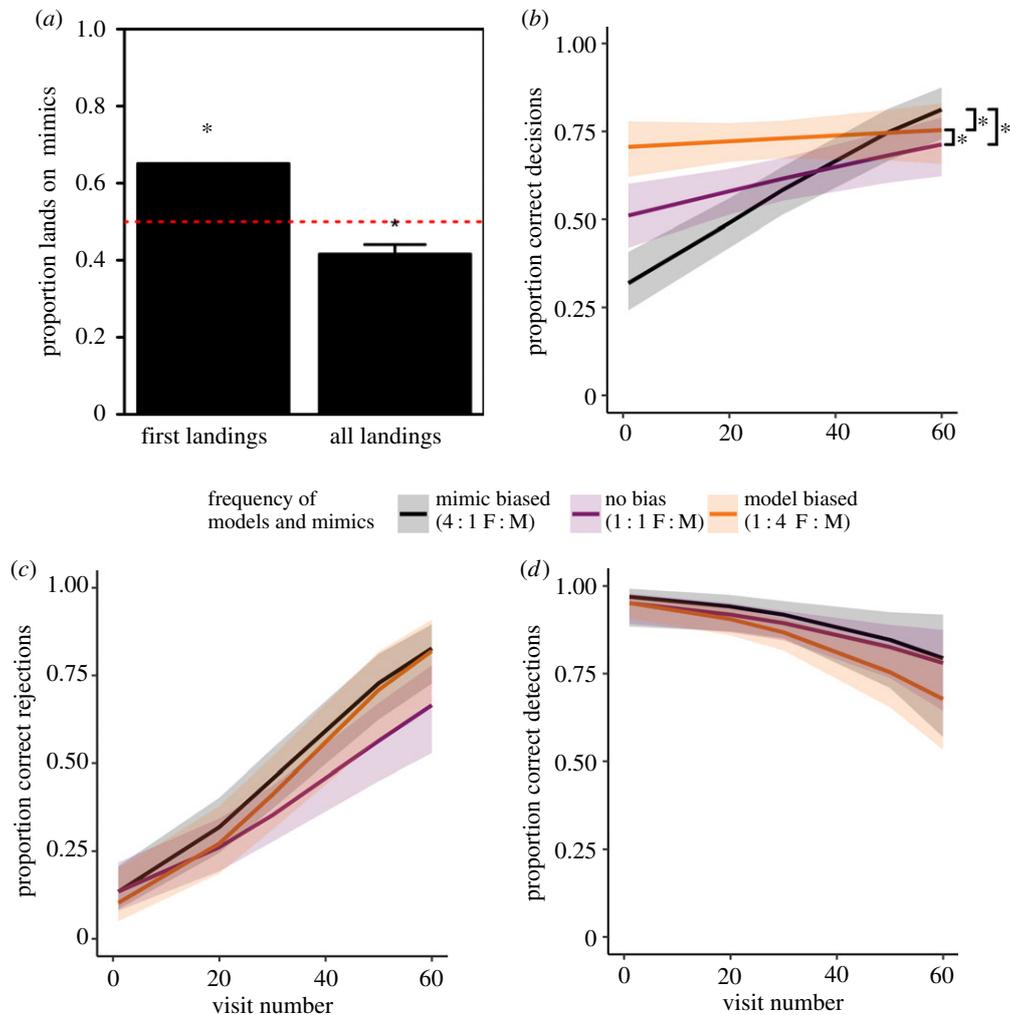
Figure 2. Sampling behaviour of initially naive bees foraging in treatments that were mimic biased (female flower biased), model biased (male flower biased) or not biased (equal number of male and female flowers). The proportion of (*a*) first landings (pooled among experiments 1 and 2) or mean proportion of landings (± s.e.) by initially naive bees on mimics (versus models) in the no-bias treatment (experiment 2). $n = 43$ and 11 bees, respectively. Mean proportion of (*b*) correct decisions, (*c*) correct rejections and (*d*) correct detections made by experienced bees making up to 60 visits. $n = 12$, 11 and 13 bees in the mimic, model and not biased treatments, respectively. Plotted lines indicate estimated means and shaded regions indicate 95% confidence intervals (CIs). Asterisks indicate significant differences in the proportion or in mean proportions among treatments at $p < 0.05$ according to a *G*-test, paired *t*-test or Tukey's *post hoc* test.

(ii) Experiments 2 and 3

To analyse how experience and the frequency of models and mimics affected sampling behaviour, we fitted generalized linear mixed models (GLMMs) with a binomial distribution using the glmer() function (lme4 package; [54]), specifying type II Wald chi-squared (χ^2)-tests via the Anova() function (car package [55]). We checked model assumptions for all models (DHARMa package [56]). The response variable was sampling behaviour (either 'correct decision', 'correct detection', 'correct rejection', 'missed detection', 'false alarm', 'landing' or 'sonication given landing') and the explanatory variables were 'treatment' (no-bias and/or female-biased and male-biased) and 'visit number'. For experiment 3, we analyzed the sampling behaviour (either 'correct decision', 'correct detection', or 'correct rejection', 'missed detection', 'false alarm') only in the test array. We included 'bee' as a random factor for all GLMMs ('bee' within 'colony' would not converge), with a single exception: random factors generated a singularity error for the correct detection model for experiment 3 and we, therefore, used a general linear model (GLM). In cases of significant effects, we ran Tukey's *post hoc* test using the glht() function (multcomp package [57]) to determine which pairs were significant. Finally, to test for a significant correlation between correct rejections and correct detections, controlling for treatment (no-bias and/or female-biased and male-biased), we used a one-way analysis of covariance (ANCOVA) using the lm() function in R.

3. Results

(a) Experiment 1: naive bees exhibit a perceptual bias for the unrewarding female flowers

When the frequency of models and mimics were equal, flower-naive bees preferred mimics (rewardless female flowers; figure 1) over models (rewarding male flowers; figure 1) on their first landing by a factor of 1.9 (figure 2*a*; *G*-test: $G = 3.99$, $p < 0.046$, $n = 43$ bees pooled from experiments 1 and 2). However, across all landings (experiment 2, equal sex ratio treatment only), bees preferred models over mimics (figure 2*a*: paired *t*-test: $t_{10} = 3.42$, $p < 0.007$, $n = 11$ bees).

(b) Experiment 2: frequency of models and mimics had little effect on how naive bees learned to sample

Initially flower-naive bees learned to make proportionately more correct decisions with experience (combining correctly rejecting mimics and correctly detecting models) (figure 2*b*; GLMM: $\chi^2_1 = 33.83$, $p < 0.0001$; $n = 36$ bees). This effect of experience was stronger as the ratio of mimics to models increased (figure 2*b*; GLMM: treatment × experience effect: $\chi^2_2 = 18.25$, $p < 0.0002$).
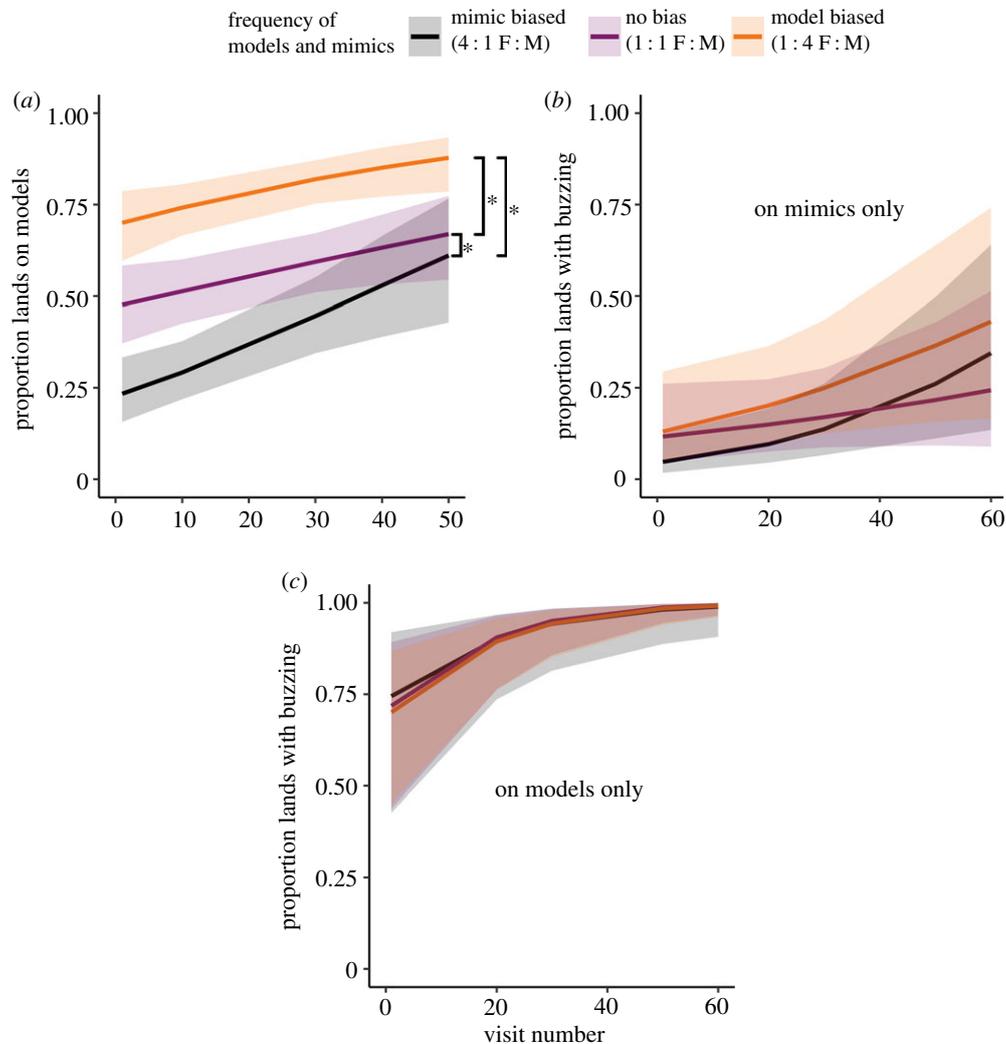
Figure 3. Landing behaviour of initially naive bees (same dataset as in figure 2, analysed for different behaviours) foraging in treatments that were mimic biased (female flower biased), model biased (male flower biased) or not biased (equal number of male and female flowers). Mean proportion of lands made on (a) models (versus mimics), (b) mimics during which the bee buzzed and on (c) models during which the bee buzzed, over the course of up to 60 visits. $n = 12$, 11 and 13 bees in the mimic, model and not biased treatments, respectively. Plotted lines indicate estimated means and shaded regions indicate 95% CIs. Asterisks indicate significant differences in mean proportions among treatments at $p < 0.05$ according to Tukey's *post hoc* test.

This effect is probably a consequence of bees having correspondingly more opportunities to reject mimics and thereby learning faster to reject, even though those bees did more poorly at first in more mimic biased treatments.

Bees improved their ability to correctly reject mimics (figure 2c), but became worse at correctly detecting models (figure 2d). As bees gained experience they increased their correct rejections by a factor of 6 (figure 2c; GLMM: $\chi_1^2 = 113.58$, $p < 0.0001$), but decreased their correct detections by a factor of 1.3 (i.e. made more missed detections) (figure 2d; GLMM: $\chi_1^2 = 29.84$, $p < 0.0001$). These patterns were not affected by model and mimic frequency (figure 2c,d; GLMMs: correct rejections, treatment effect: $\chi_2^2 = 2.88$, $p = 0.237$; treatment × experience effect: $\chi_2^2 = 2.77$, $p = 0.251$; correct detections, treatment effect: $\chi_2^2 = 2.45$, $p = 0.294$; treatment × experience effect: $\chi_2^2 = 0.41$, $p = 0.816$).

Despite making more missed detections with experience, bees improved their proportion of landings on models relative to mimics with successive visits (figure 3a; GLMM: $\chi_1^2 = 19.25$, $p < 0.0001$). Additionally, bees proportionately made significantly more landings on models in treatments with more models; this is probably a consequence of having more opportunities to land on models (figure 3a; GLMM: $\chi_2^2 = 57.14$,

$p < 0.0001$). The effect of experience did not depend on the relative frequency of models and mimics (figure 3a; GLMM: treatment × experience effect: $\chi_2^2 = 1.80$, $p = 0.411$).

Upon landing, bees sonicated both mimics and models significantly more with experience (figure 3b,c: GLMMs: sonicating mimics: $\chi_1^2 = 11.15$, $p < 0.0009$; sonicating models: $\chi_1^2 = 45.98$, $p < 0.0001$). These patterns were not affected by the relative frequency of models and mimics (figure 3b,c: GLMMs: sonicating mimics, treatment effect: $\chi_2^2 = 1.95$, $p = 0.376$; treatment × experience effect: $\chi_2^2 = 1.74$, $p = 0.418$; sonicating models, treatment effect: $\chi_2^2 = 0.03$, $p = 0.985$; treatment × experience effect: $\chi_2^2 = 0.13$, $p = 0.938$).

(c) Experiment 3: frequency of models and mimics had little effect on sampling by experienced bees

Bees experienced with the phenotypic distribution, frequency, and costs and benefits of foraging on models (rewarding male flowers) and mimics (rewardless female flowers) that had their sampling behaviour tested subsequently showed a small but significant additional improvement in correct decisions during the test (combining correctly rejecting mimics and correctly detecting models) (figure 4a; GLMM:
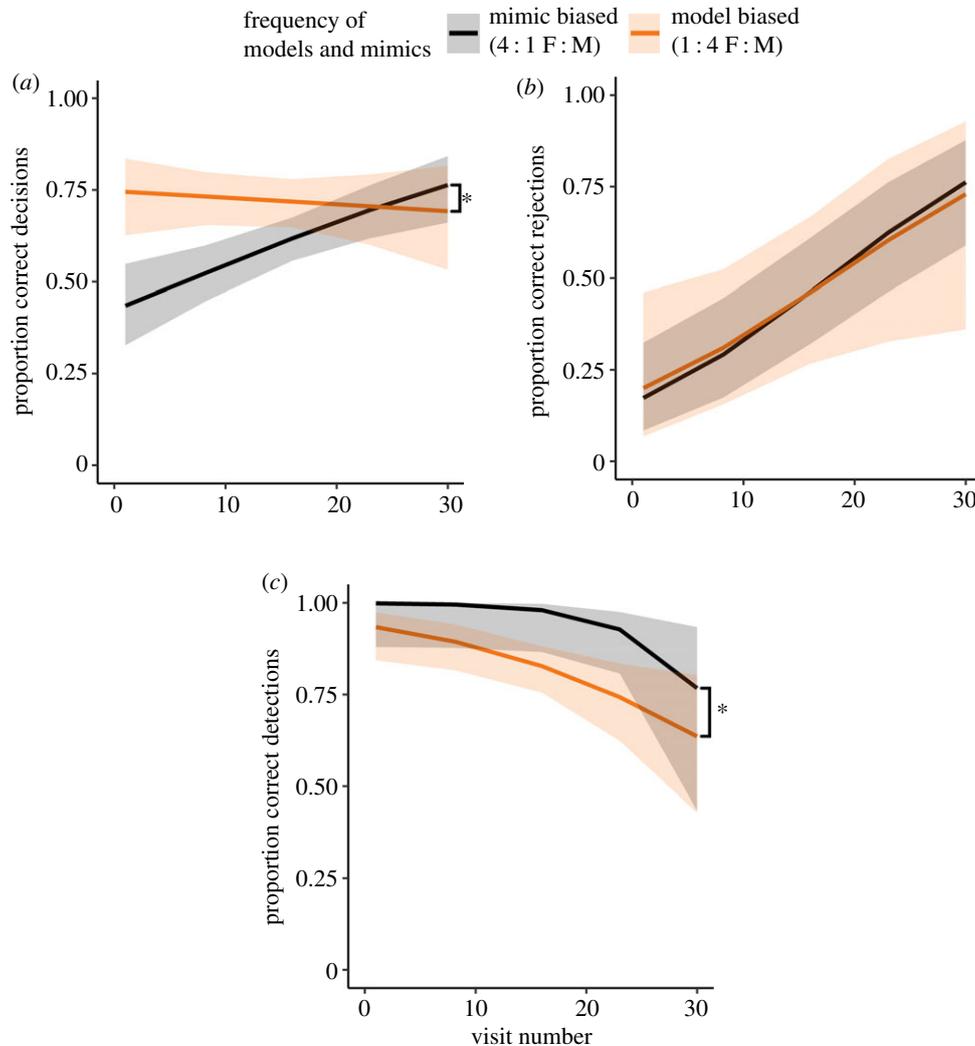
Figure 4. Sampling behaviour of experienced bees (trained previously) foraging in test treatments that were mimic biased (female flower biased) or model biased (male flower biased). Mean proportion (*a*) of correct decisions, (*b*) correct rejections and (*c*) correct detections made by experienced bees making up to 30 visits. $n = 10$ bees per treatment. Plotted lines indicate estimated means and shaded regions indicate 95% CIs. Asterisk indicates significant differences in mean proportions among treatments at $p < 0.05$ according to Tukey's *post hoc* test.

$\chi_1^2 = 5.77$, $p < 0.017$; $n = 20$ bees). This effect of experience depended on model and mimic frequency and was stronger for experienced bees in the mimic biased treatment than in the model biased treatment (figure 4*a*; GLMM: treatment × experience effect: $\chi_1^2 = 6.11$, $p < 0.014$).

We found no effect of model and mimic frequency on correct rejections and a modest, but significant effect on correct detections, examined separately (figure 4*b,c*; GLMM: correct rejections: $\chi_1^2 = 0.003$, $p = 0.956$; GLM: correct detections: $\chi_1^2 = 9.81$, $p < 0.002$). However, experienced bees still learned to make modestly more correct rejections, by a factor of 3, and decreased their correct detections by a factor of 1.3 (i.e. made more missed detections) (figure 4*b,c*; correct rejections: $\chi_1^2 = 27.03$, $p < 0.0001$; correct detections: $\chi_1^2 = 11.84$, $p < 0.0006$). Neither pattern was affected by model and mimic frequency (figure 4*b,c*; GLMM: treatment × experience effect, correct rejections: $\chi_1^2 = 0.08$, $p = 0.783$; GLM: correct detections: $\chi_1^2 = 1.85$, $p = 0.174$).

(d) Experiments 2 and 3: correct detections and correct rejections were negatively correlated

Any effect of model and mimic frequency on correct detections in experiments 2 and 3 did not depend on correct rejections (ANCOVA: naive bees: $F_1 = 2.06$, $p = 0.145$; experienced bees: $F_2 = 0.07$, $p = 0.792$). For both naive and experienced bees, there was a significant negative correlation between correct rejections and correct detections (ANCOVA: naive bees: $F_1 = 4.31$, $p < 0.046$, $R^2 = 0.152$; experienced bees: $F_1 = 5.04$, $p < 0.040$, $R^2 = 0.435$). Furthermore, for experienced bees (experiment 3), but not initially naive bees (experiment 2), there was a significant difference in the relative proportion of correct detections made among treatments, in accordance with results presented above (ANCOVA: naive bees: $F_2 = 0.89$, $p = 0.420$, experienced bees: $F_1 = 8.66$, $p < 0.0096$). However, this difference between initially naive and experienced bees is confounded with differences in experience and the number and kind of treatments.

4. Discussion

Batesian mimicry is thought to rely on the perceptual similarity of the model and mimic for the receiver [28–30,35,58]. Why then are deceptive flowers of many plant species imperfect mimics (e.g. [16,21,38–40,59])? Assuming our results are representative of Batesian floral mimicry systems, imperfect mimicry is probably an evolutionary response to the capacity of pollinators to learn and involves the exploitation of

pollinator sensory bias to overcome or diminish the effects of learning. Initially, flower-naive bumblebees strongly preferred to land on unrewarding deceptive mimics over models. However, bees rapidly learned to recognize and reject mimics. While such learning involved a trade-off, with bees also increasingly rejecting rewarding models, bees still benefitted by making increasingly more correct decisions with experience. Our results thus suggest that selection for floral traits which elicit sensory bias from pollinators has been driven more strongly by the pollinator learning to make correct rejections (i.e. sensory bias makes the mimics more difficult to reject by learning), than by the pollinator learning to positively identify the model. Following the perceptual exploitation hypothesis, Kikuchi & Pfennig [60] predicted that in interspecies Batesian mimicry, mimics should rarely be imperfect to exploit a sensory bias, because models should typically be under the strongest selection to elicit a response from receivers. However, in intersexual Batesian mimicry, a given individual plant expresses both models and mimic phenotypes. In this circumstance, selection probably favours rewardless mimics to exploit sensory bias more so than models by way of facilitating pollination.

Signal detection theory predicts that naive pollinators increase their overall rate of correct decisions (thereby maximizing foraging efficiency) by learning to avoid mimics, even if that means avoiding rewarding models [28,35]. While limited evidence from interspecies floral mimicry studies indicates that pollinators learn to avoid deceptive floral mimics (e.g. [13,18]), we compared changes in both correct rejections (avoiding rewardless mimics) and correct detections (accepting rewarding models). Consistent with signal detection theory, we found that initially naive bees adjusted their behaviour to avoid visiting floral mimics (that is, correct rejections increased), even at the expense of avoiding models (i.e. missed detections increased). In fact, correct rejections and correct detections were significantly negatively correlated: as correct rejections increased in frequency, correct detections decreased. We cannot rule out that, at least in part, bees increased their missed detections as a strategy to avoid increasingly depleted flowers; however, the number of initially rewarding flowers did not affect the pattern and missed detections also increased before bees visited depleted flowers. Despite reducing their propensity to make correct detections, the net outcome of learning was that bees made proportionally more correct decisions with experience. In addition, while we did not quantify pollen collection, bees made proportionally more pollen-collecting landings on rewarding models with experience, suggesting that they probably increased their foraging efficiency with experience.

Signal detection theory also predicts that receivers should be sensitive to the costs and benefits of foraging on models and mimics [28,34,35,60]. We found that over time, bees switched to using floral sonication to collect pollen from male flowers, a foraging behaviour used when pollen has been depleted (see [45]). The benefit to foraging on models in our study should thus decline over time; bees acquire less pollen with subsequent visits and must expend more energy per visit to collect what pollen remains (floral sonication is thought to be energetically expensive [45]). Correspondingly, the threshold should move towards being less conservative about sonicating female flowers. Consistent with this prediction, bees increasingly sonicated rewardless mimics with experience. Future work will be required to determine how manipulating the costs and benefits of models and mimics shapes the effectiveness of Batesian mimicry.

While theory predicts that successful Batesian mimicry depends on the model and mimic frequency, there have been surprisingly few empirical tests. In predator–prey systems, a handful of studies have shown that predation on mimics increased as the relative frequency of the unpalatable models decreased [26,60–64]. Conversely, we found that in this plant–pollinator mutualism, the frequency of floral models and mimics had little effect on bees' floral choice. As the frequency of unrewarding mimics increases, so should the cost of choosing a mimic [28]. We, therefore, predicted bees should be more conservative and make proportionally more correct rejections and proportionally fewer correct detections, to avoid accidentally visiting deceptive mimics. Yet for initially naive bees, model and mimic frequency did not affect correct rejections or correct detections. Given that signal detection theory assumes that receivers are familiar with the phenotypic distribution, frequency, and costs and benefits of models and mimics [28,35], we reasoned that initially naive bees may not have attained sufficient experience. We, therefore, familiarized the second set of bees with a given distribution and frequency of models and mimics, but still found no effect of model and mimic frequency on correct rejections, though there was an effect on correct detections.

Similarly, we expected that bees given more opportunities to learn mimics (i.e. when the frequency of unrewarding mimics was relatively higher) would also adjust their rate of correct rejections and correct detections more rapidly. Surprisingly, model and mimic frequency had no effect on such learning for initially naive or experienced bees. Conversely, correct decisions (combining correctly rejecting mimics and correctly detecting models) were always frequency-dependent, with bees in more mimic biased treatments making fewer correct decisions initially and learning to make correct decisions more rapidly than bees in more model biased treatments. Assuming foraging conditions in our assays reflect those found in nature, bees may be only modestly sensitive to model and mimic frequency in an intersexual Batesian mimicry system.

Uniquely, plant species with intersexual floral mimicry can potentially shape their reproductive success by controlling model and mimic frequency. Indeed, sex ratio is often labile in such plant species (e.g. [36,48,65,66]). However, prior evidence of frequency-dependence in effective intersexual floral mimicry is conflicting [14,66]. Our results strongly suggest that from a signal detection perspective, altering model and mimic frequency provides little advantage to plants. Future work will be required to determine whether other components of pollinator behaviour, such as individual differences in learning, among-plant movement or flower handing time, are shaped by floral sex ratio in ways that increase plant reproductive success (such as by increasing pollen export or outcrossing [14,18,66]).

In conclusion, by examining pollinator responses to both floral models and mimics in a signal detection framework, our study provides a greater understanding of how intersexual floral mimicry is maintained. With increasing experience, pollinators made more correct decisions and this effect was probably driven mainly by learning to avoid mimics, rather than by improvements in finding models. Indeed, bees initially made high rates of correct detections and increasingly avoided models with experience. Our results also provide evidence that imperfect floral mimicry can be adaptive and can involve the exploitation of pollinator sensory biases [29,35,59]. Our study lays the groundwork for examining floral features

contributing to this exploitation in our system and across Batesian floral mimicry systems broadly (see also [36,43]). Finally, we provide rare and valuable experimental evidence for the key role of receiver learning in Batesian mimicry.

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