

Behaviour before beauty: Signal weighting during mate selection in the butterfly *Papilio polytes*

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Funding information

National Institute of General Medical Sciences, Grant/Award Number: GM108626; National Institutes of Health, Grant/Award Number: INBRE 2 P20 GM 103443; NSF EPSCoR, Grant/Award Number: 1355423; NSF, Grant/Award Number: IOS-1452648

Editor: S. Bertram

Abstract

Mating displays often contain multiple signals. Different combinations of these signals may be equally successful at attracting a mate, as environment and signal combination may influence relative signal weighting by choosy individuals. This variation in signal weighting among choosy individuals may facilitate the maintenance of polymorphic displays and signalling behaviour. One group of animals known for their polymorphic patterning are Batesian mimetic butterflies, where the interaction of sexual selection and predation pressures is hypothesized to influence the maintenance of polymorphic wing patterning and behaviour. Males in the female-limited polymorphic Batesian mimetic butterfly *Papilio polytes* use female wing pattern and female activity levels when determining whom to court. They court stationary females with mimetic wing patterns more often than stationary females with non-mimetic, male-like wing patterns and active females more often than inactive females. It is unclear whether females modify their behaviour to increase (or decrease) their likelihood of receiving male courtship, or whether non-mimetic females spend more time in cryptic environments than mimetic females, to compensate for their lack of mimicry-driven predation protection (at the cost of decreased visibility to males). In addition, relative signal weighting of female wing pattern and activity to male mate selection is unknown. To address these questions, we conducted a series of observational studies of a polymorphic *P. polytes* population in a large butterfly enclosure. We found that males exclusively courted active females, irrespective of female wing pattern. However, males did court active non-mimetic females significantly more often than expected given their relative abundance in the population. Females exhibited similar activity levels, and selected similar resting environments, irrespective of wing pattern. Our results suggest that male preference for non-mimetic females may play an active role in the maintenance of the non-mimetic female form in natural populations, where males are likely to be in the presence of active, as well as inactive, mimetic and non-mimetic females.

KEYWORDS

male preference, mate preference, mimicry, polymorphism, sexual selection

1 | INTRODUCTION

Mating displays often consist of multiple signals, either in the same, or different, sensory modalities (Hebets & Papaj, 2005). These signals may be redundant, or may convey different information such as genetic quality or current condition (reviewed in Canolin, 2003; Hebets & Papaj, 2005). Choosy individuals, or the receivers of these complex mating displays, may differentially weigh multiple signals when selecting a mate (Johnstone, 1996). This differential signal weighting in receivers has the potential to influence how signalers behave (and display) during the mate selection process (Rowe, 1999; Ryan, 1998; Soma & Garamszegi, 2015). Recent work on signal weighting and mate selection has shown that signal weighting is not always additive, and may influence mate selection in unexpected ways—for example, visual cues help female túngara frogs (*Physalaemus pustulosus*) differentiate between male calls, but only when the male calls are similar in amplitude (Stange, Page, Ryan, & Taylor, 2017). One effect of the presence of multiple signals and variation in signal weighting may be the maintenance of polymorphisms within populations, as multiple combinations of signals may be equally attractive to choosy receivers.

Butterfly species, many of which are polymorphic, have been found to use a number of different signals during the mate selection process (Costanzo & Monteiro, 2007; Papke, Kemp, & Rutowski, 2007; Westerman et al., 2018; Wiklund & Kaitala, 1995). Some of these signals may be redundant, while others may convey unique information. For example, females use pheromone concentration to assess male age (Nieberding et al., 2012); males use female size to assess female reproductive potential (Wedell & Cook, 1999); and both sexes are known to attend to the wing patterns of the opposite sex, though the specific information conveyed through these wing patterns is often unclear (Chamberlain, Hill, Kapan, Gilbert, & Kronforst, 2009; Chouteau, Llaurens, Piron-Prunier, & Joron, 2017; Melo, Salazar, Jiggins, & Linares, 2009; Morehouse & Rutowski, 2010; Obara, Koshitaka, & Arikawa, 2008; Robertson & Monteiro, 2005) (but see Kemp & Rutowski, 2007). While females are often thought of as the choosy sex, male butterflies transfer spermatophores to females during copulation, which can be costly to the males and highly beneficial to the females (Boggs, 1995; Boggs & Gilbert, 1979; Prudic, Jeon, Cao, & Monteiro, 2011; Wiklund, Kaitala, Lindfors, & Abenius, 1993), resulting in multiple species with choosy males, in addition to (or instead of) choosy females (Ellers & Boggs, 2003; Jiggins, Naisbit, Coe, & Mallet, 2001; Kronforst et al., 2006; Prudic et al., 2011). Activity levels may also be used by both sexes in the mate selection process, and are hypothesized to convey information related to current condition (Westerman, Drucker, & Monteiro, 2014; Westerman et al., 2018). The use of multiple signals during the mate selection process coupled with an incredible diversity of visual signals, make butterflies a good group to use for the study of the role of multiple signals in the maintenance of polymorphisms.

Mimetic butterflies are particularly well known for their polymorphic wing patterns, including Müllerian and Batesian mimics.

Müllerian mimics such as *Heliconius* butterflies have been used extensively for the study of speciation and the maintenance of within species polymorphisms across wide geographic ranges in mimicry rings (Kronforst & Papa, 2015; Merrill et al., 2015). Batesian mimics such as *Papilio polytes*, *Papilio glaucus* and *Papilio dardanus*, on the other hand, have served as models for the study of female-limited polymorphism (Cook, Vernon, Bateson, & Guilford, 1994; Kunte, 2009; Kunte et al., 2014; Nishikawa et al., 2015). In many of these Batesian mimetic systems, one or many female forms mimic the wing patterns of distantly related toxic butterflies, while an additional female form is non-mimetic and male-like. The non-mimetic female form is ancestral (Zhang, Westerman, Nitzany, Palmer, & Kronforst, 2017), and the maintenance of this non-mimetic female form in populations containing mimetic females is hypothesized to be due to frequency-dependent predation pressure, male preference for the non-mimetic form and/or increased fecundity resulting from reduced male harassment (reviewed in Kunte, 2009; Westerman et al., 2018). Male *P. glaucus* have been found to prefer the non-mimetic female form (Burns, 1966; Levin, 1973), while male *P. polytes* use at least two signals when determining whom to court: female wing pattern and female activity levels (Westerman et al., 2018), suggesting that male preference may play a role in the maintenance of female-limited polymorphism in both of these Batesian mimetic species.

However, since male *P. polytes* use multiple female signals during mate selection, and previous studies did not assess male preference for the full factorial range of activity/wing pattern choice options, the relative importance of female wing pattern to mating outcome remains unclear. Male *P. polytes* prefer mimetic wing patterns when choosing between two inactive females in the presence of other males, but prefer active females independent of female wing pattern when choosing between two females allowed to move and in the absence of other males (Westerman et al., 2018). This shift in attention from female wing pattern to female activity could be due to a male preference for female activity over female wing pattern, (i.e., weighting female activity higher than female wing pattern in the mate selection process), or it could be due to competition-dependent (i.e., presence vs. absence of a sexual competitor) preferences. Males were in the absence of sexual competitors for the assays where they exhibited a preference for activity, and in the presence of sexual competitors when they exhibited a preference for the mimetic wing pattern. To assess how males weigh these two signals (female wing pattern and female activity), and whether the shift in attention towards female activity was associated with an absence of male-male competition, we conducted a series of behavioural assays in a semi-natural setting. This semi-natural setting was a large, enclosed tropical garden (described in Methods below), with an even sex ratio population of *P. polytes*. In this setting, males are in the presence of multiple mimetic and non-mimetic females, as well as other males, at all times. Since female (and male) butterflies spend some of their time flying/feeding, and some of their time basking/resting, we were able to observe males around active mimetic females, active non-mimetic females, inactive mimetic females and inactive non-mimetic

females, and test male courting preference when presented with these four types of females.

If males weigh female activity more heavily than female wing pattern when selecting a mate, we would expect to observe males courting active females more often than inactive females, independent of female wing pattern. If, however, males weigh female wing pattern and activity levels equally, we would expect males to court active mimetic females the most, followed by equal amounts of inactive mimetic females and active non-mimetic females, and the least amount of inactive non-mimetic females.

Alternatively, male courting preferences may be associated with the difficulty of differentiating between non-mimetic (male-like) females and males. One hypothesis for the maintenance of the non-mimetic female form in female-limited polymorphic Batesian mimics is the pseudo-sexual selection hypothesis, where males approach non-mimetic females more often than mimetic females because the males mistake these females for other males encroaching on their territory (Vane-Wright, 1984). Males in some species of butterflies are territorial, and approach and chase other males that enter their territory (Davies, 1978). Males are also known to approach females who enter their territories (Bergman, Lessios, Seymoure, & Rutowski, 2015; Davies, 1978), and there is current debate over whether male butterflies can differentiate between conspecific males and females from a distance (Takeuchi, Yabuta, & Tsubaki, 2016). If males cannot differentiate between other males and non-mimetic females at a distance, they may approach both. Once males are close enough to identify the non-mimetic females as females, the pseudo-sexual selection hypothesis predicts these males would then court and copulate with these females, leading to non-mimetic females receiving proportionally more male attention and copulations than mimetic females. Since this higher frequency of non-mimetic copulations is hypothesized to be the result of differences in male initiated intersexual close encounters, and not male preference for a particular female wing pattern per se, Van Wright dubbed it the pseudo-sexual selection hypothesis, to acknowledge the fact that males might exhibit a different preference in the absence of female/male mistaken identity (Vane-Wright, 1984). If pseudo-sexual selection plays a role in the maintenance of the non-mimetic, male-like form in *P. polytes*, we would expect males to court active non-mimetic females more often than active mimetic females.

Given that males attend to both female wing pattern and female activity during mate selection, and prefer active females over inactive females, and mimetic wing patterns over non-mimetic wing patterns, there is also the potential for non-mimetic females to increase their attractiveness by increasing their activity levels relative to mimetic females. We tested this hypothesis by first comparing the relative proportions of active versus inactive non-mimetic and mimetic females observed in the garden, and then comparing the amount of time mimetic and non-mimetic females spent performing different behaviours in focal watches. If non-mimetic females compensate for the unattractiveness of their wing pattern by increasing their general activity levels, we expect to observe a greater proportion of non-mimetic females exhibiting active behaviours, and for

the focal non-mimetic females to spend more time flying, feeding and fluttering their wings than the focal mimetic females.

We also compared female activity levels to those of males, and to those of the toxic model butterfly, *Pachliopta aristolochiae* to determine whether non-mimetic females exhibited activity levels more similar to males, and if mimetic females exhibited activity levels more similar to those of the toxic model, than to each other (i.e., do females behave like females? Or do females with male-like wing patterns behave like males, and females with toxic model-like wing patterns behave like toxic models?). There is some evidence that mimetic *P. polytes* females exhibit flight patterns similar to their toxic models, *P. aristolochiae* (Kitamura & Imafuku, 2015), but is it unclear if this behavioural mimicry extends to general activity levels, or influences male courting behaviour. If females with male-like wing patterns behave like males, that may increase the probability that males mistake them for males, and may increase the likelihood that they would be approached by males attempting to defend their territories against encroaching males. If females with toxic model-like wing patterns behave like the toxic models, it may be harder for males to identify them as conspecifics when in flight. If behavioural mimicry of toxic models influences male ability to identify these females as conspecifics, we might expect males to court flying mimetic females less often than either flying non-mimetic females or stationary mimetic females. However, if conspecific females behave similarly regardless of their wing patterns, they may be more identifiable as conspecific females to searching males.

To further explore the effect of female wing pattern on female behaviour and male courting frequency in *P. polytes*, we examined female resting site selection. One of the hypotheses associated with female-limited polymorphisms in Batesian mimicry is that non-mimetic females should be more cryptic than mimetic females, due to predation pressures on wing pattern evolution. This could influence both predator attack rates and male courting rates, if resting non-mimetic females are also more difficult for males to see than resting mimetic females. An associated hypothesis concerning non-mimetic and mimetic female behaviour is that mimetic and non-mimetic females will behave differently when resting, basking and flying, because mimetic females are protected from predation by their mimetic wing pattern, while non-mimetic females are not. We tested this hypothesis, and its implication on male courting rates, by comparing the foliage colour and sun exposure of the resting/basking sites of mimetic and non-mimetic females, as well as the male courting of resting/basking mimetic and non-mimetic females. We also compared resting/basking location of females to males, to assess whether there was an effect of sex on resting location.

2 | MATERIALS AND METHODS

2.1 | Study species and husbandry

Papilio polytes is a sexually dimorphic swallowtail butterfly with local female-limited polymorphism (Zhang et al., 2017). Females either exhibit a male-like wing pattern or one of several different

wing patterns that mimic the toxic *Pachliopta* genus (Kunte et al., 2014). Female mimetic and non-mimetic wing patterns are heritable, and controlled by a single locus, the gene *doublesex* (Kunte et al., 2014; Nishikawa et al., 2015). The relative frequency of non-mimetic to mimetic females is population-specific, and varies widely, with some populations in nature having close to no non-mimetic females (99% mimetic, 1% non-mimetic in a Thailand population, and 88% mimetic, 12% non-mimetic in a Malaysian population, for example), and others having much higher frequencies of non-mimetic females (70% mimetic, 30% non-mimetic in a Philippines population, and 50% mimetic, 50% non-mimetic in a Japanese population; Sekimura, Suzuki, & Takeuchi, 2017; Westerman et al., 2018; Zhang et al., 2017). While one might hypothesize that this variance in proportion of non-mimetic to mimetic female wing patterns would be associated with variance in male preference, this does not appear to be the case, as males from populations with virtually no non-mimetic females exhibit similar preferences as males from populations with up to 25% non-mimetic females (Westerman et al., 2018). The butterflies used in this study came from a population in the Philippines that consists of roughly 25% non-mimetic females (Westerman et al., 2018).

For this study, *P. polytes* butterflies were maintained and observed in a large flight arena at the Butterfly House and Aquarium in Sioux Fall, South Dakota. Individuals (pupae) were obtained from a polymorphic population in the Philippines, at the Flora Farm Butterfly in Marinduque, and allowed to emerge in the eclosion chamber at the Butterfly House and Aquarium in Sioux Falls, South Dakota, USA. *P. polytes* at the Flora Farm Butterfly are maintained as large, outbred colonies, with caterpillars reared on their native host plants (citrus), founded from a large number of wild-caught adults. After emergence in Sioux Falls, adult butterflies were numbered on their ventral forewing using a silver

sharpie marker (for details on this numbering technique, please see (Westerman et al., 2018)), and then released into a 334.45 m² (18.3 m w x 18.3 m l x 4 m h) climate-controlled (26–30°C) flight arena containing numerous tropical plants, nectaring sites and supplemental food sources (Figure 1). This flight arena was encased with 7.62 cm tempered safety glass, which transmitted all wavelengths of light. The measured irradiance of UV light was reduced relative to that found in open fields; however, it was similar to that found in rainforest understory (Endler, 1993), which is one of the native habitats of *P. polytes*. While this large flight arena housed multiple species of butterflies and plants (SI Table 1), not all of whom are native to Southeast Asia, the use of this large flight arena allowed us to observe *P. polytes* behaviour in semi-natural conditions with a known sex ratio, known non-mimetic to mimetic female morph ratio (~25:75) and known quantities of emergence and death. While different populations of *P. polytes* do contain different mimetic female wing patterns (reviewed in Zhang et al., 2017), all of the mimetic females in the population used for this study had the same wing pattern, the *P. polytes polytes* pattern (Figure 1b). The presence of both canopied and open areas within the flight arena allowed us to document resting location preferences of *P. polytes* non-mimetic females, mimetic females and males.

2.2 | Behavioural assays

To determine whether males preferentially court stationary versus active females, and mimetic versus non-mimetic females, we conducted a series of point count assays and focal watches. Over a period of 9 months (Sept. 2016–May 2017), we conducted 1–4 point counts a day for 26 days, approximately 1 day a week for the 9-month period, excluding the winter holidays. This allowed us to observe the

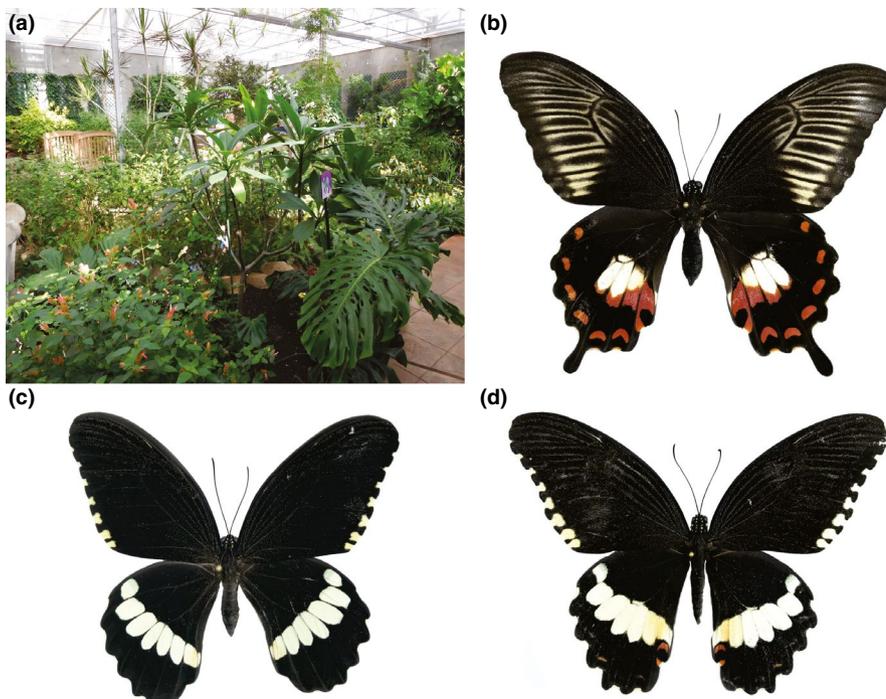


FIGURE 1 (a) Large flight arena at the Butterfly House and Aquarium, Sioux Falls, SD; (b) mimetic female *Papilio polytes*; (c) male *P. polytes*; (d) non-mimetic female *P. polytes*. Photo credit to D. Massardo for panels b–d [Colour figure can be viewed at wileyonlinelibrary.com]

butterflies under lighting conditions that ranged from 13:11 to 11:13 L:D, which is the range of L:D conditions these butterflies would experience in their natural habitat in the Philippines. Since *P. polytes* are relatively short-lived butterflies (expected lifespan in captivity is roughly 8–15 days; Fryer, 1914; Westerman et al., 2018), our weekly sampling ensured turnover of approximately half the *P. polytes* population in the butterfly flight arena between sampling events, and reduced the likelihood of exceptionally attractive (or motivated) individuals from skewing our results. Point counts were conducted at four different locations in the enclosure: near the entrance and vine-covered trestle, from which we could observe a large open area as well as the butterflies under the trestle; under full sun next to a large butterfly bush and small stream; in a shaded corner containing large nectaring plants; and on a small hill in full sun. These four spots allowed us to see the entire butterfly enclosure, and to incorporate areas containing both full sun and full shade into our observations. Observations took place between 9 a.m. and 4 p.m., always at least an hour after sunrise and before sunset, as previous observations suggest that *P. polytes* is not crepuscular and performs most of their courtship during daylight hours (Westerman et al., 2018). For point counts, we recorded the first behaviour (*fly*, *wing flutter*, *feed*, *court*, *bask* [sitting with wings open] or *rest* [sitting with wings closed]) exhibited by each *P. polytes* and *P. aristolochiae* butterfly in our line of sight during a slow (30–60 s) 360° rotation. Courtship in *P. polytes* is described as either (a) a butterfly approaching a resting or basking individual of the opposite sex and either attempting to copulate or hovering a few inches behind them, swaying from side to side, before landing and attempting to copulate, or (b) hovering a few inches below a flying individual of the opposite sex, displaying the dorsal surface of their wings (Fryer, 1914; Westerman et al., 2018). This courtship behaviour is predominantly displayed by males, although it is rarely observed in females. While we did observe copulations, we focused on courting instead of copulation for our assessment of male preference because copulation is not forced in *P. polytes*, so represents the outcome of mate preference of both males and females, while courting propensity is reflective of the preference of the individual doing the courting. In total, we conducted 102 point counts and recorded the behaviour of 561 butterflies: 37 non-mimetic, 127 mimetic and 214 male *P. polytes* butterflies, as well as 183 *P. aristolochiae*.

To obtain more detailed information on the relative activity of non-mimetic and mimetic females, as well as on male behaviour, we also conducted 1–3 10-min focal watches of these butterflies on the same days we conducted point counts. Focal butterflies were selected semi-randomly: the first butterfly we saw of the designated watch type (male, non-mimetic female, mimetic female) was used for said focal watch, if it had not already been used in a previous focal watch (which was determined by checking the number of the butterfly, and comparing that to the number of previously watched butterflies). We recorded both instances and duration of all butterfly behaviour exhibited during the 10-min focal watch. As mentioned above, all butterflies were numbered, so we could ensure that these observations were made of unique individuals. $N = 119:31$ non-mimetic females, 37 mimetic females, 51 males.

We recorded all instances of male courtship observed during each point count and focal watch, recorded the female morph the male courted, and whether the female was stationary or active. In total, we observed 22 male courtship events. As each male we observed was only ever observed courting a single female during the observation period, these 22 courtship events represent the courting behaviour of 22 different males. We also did not observe multiple males courting the same female, suggesting a low likelihood of female-driven pseudoreplication in observed male preference.

To determine whether non-mimetic, mimetic and male *P. polytes* butterflies, as well as the toxic model *P. aristolochiae*, exhibit different resting/basking location preferences, we conducted an additional point count survey of resting/basking individuals during every visit to the flight arena, and documented the location (plant colour and light environment) of every resting and basking *P. polytes* and *P. aristolochiae* butterfly observed. $N = 130:23$ non-mimetic females, 29 mimetic females, 70 males and 8 *P. aristolochiae* over 26 surveys.

2.3 | Statistical analyses

Male courting preferences for stationary versus active and non-mimetic versus mimetic females were compared using Pearson's chi-square, p -value significance threshold set at 0.025 to account for multiple testing. To determine whether non-mimetic females behaved differently from mimetic females, and whether female *P. polytes* behaved more similarly to each other than they did to either males or the toxic model respectively, we compared proportions of non-mimetic and mimetic female, and male *P. polytes*, as well as *P. aristolochiae* observed performing each behaviour during point counts using Pearson's chi-square, p -value significance threshold set at 0.0167 to account for multiple testing. We also assessed amount of time spent performing each behaviour during focal watches using a Welch's ANOVA to account for unequal variances, followed by Steel–Dwass pair-wise comparisons accounting for all tests. Basking location preferences of non-mimetic and mimetic females, and males, were compared using Pearson's chi-square, p -value significance threshold set at 0.0167. All statistical analyses were performed in JMP v13 by SASS.

2.4 | Ethical statement

All butterflies were provided with abundant food and allowed to fly freely in the large flight arena, as specified by USDA-APHIS permit P526P-16-04275 to the Butterfly House and Aquarium, throughout the course of this experiment. At time of natural death, butterflies were collected and frozen for future analyses.

3 | RESULTS

There was no effect of female wing pattern on time spent basking or resting in the large flight arena. However, non-mimetic females were observed feeding more often than mimetic females in the point count assays ($\chi^2 = 13.99$, $p = 0.012$, Figure 2). In focal watches, mimetic

and non-mimetic females flew, fluttered, walked, basked and rested equally often (Table 1). There was an effect of sex on behaviour, as males were observed flying more often than either mimetic or non-mimetic females in point counts (Pearson's chi-square test, all groups $\chi^2 = 60.53$, $p < 0.0001$, mimetic female: male $\chi^2 = 17.91$, $p = 0.012$; non-mimetic female: male $\chi^2 = 19.05$, $p = 0.004$). *P. aristolochiae*, the toxic model, was also observed flying more often than either mimetic or non-mimetic females in point counts (Pearson's chi-square test, all groups $\chi^2 = 48.28$, $p < 0.0001$, mimetic female: *P. aristolochiae* $\chi^2 = 14.25$, $p = 0.006$; non-mimetic female: *P. aristolochiae* $\chi^2 = 28.86$, $p < 0.0001$). Thus, in terms of flight, mimetic and non-mimetic females were more similar to each other than they were to either the toxic model species or to males, respectively (Figure 2).

Males were only observed courting active females ($N = 51$ focal watches, 102 point counts, 22 courting events, 0 males courting inactive females). Point count data suggest that males should be observed courting active females more often than inactive females, as 2/3 of all females observed were active. However, males courted inactive females significantly less often than the expected 2:1 active:inactive female ratio (Pearson χ^2 , $\chi^2 = 201.95$, $p < 0.0001$). While our sample size for courting instances is low, the fact that we never observed males courting stationary females, even though we observed many stationary females, suggests that our result of male preference for active females is not an artefact of sample size.

Males also courted non-mimetic females more often than expected given their relative abundance in the population (Pearson

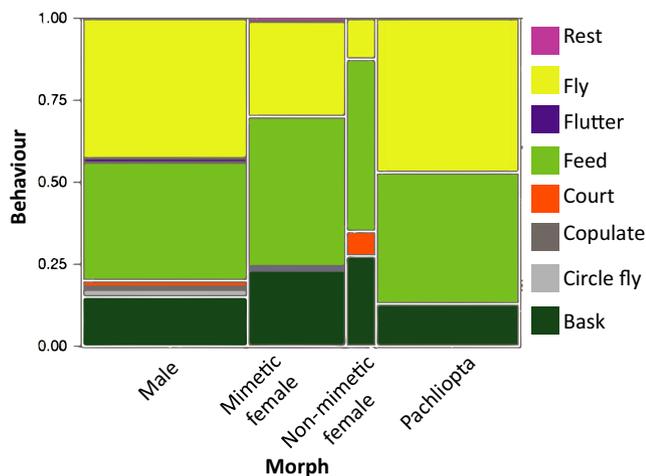


FIGURE 2 Males were more active than females, and non-mimetic females fed more than mimetic females. Proportion of individuals of each wing pattern/sex/species observed performing each behaviour (point count). Width of each column illustrates proportion of observed individuals of that wing pattern/sex/species. Length of colour within each column illustrates proportion of individuals exhibiting that behaviour within group. $N = 102$ point counts, 561 butterflies. 213 males, 127 mimetic females, 37 non-mimetic females and 183 *Pachliopta aristolochiae* (toxic model species). Pearson's chi-square² test, all groups $\chi^2 = 60.53$, $p < 0.0001$, non-mimetic versus mimetic females, $\chi^2 = 13.99$, $p = 0.012$, non-mimetic female versus male, $\chi^2 = 20.95$, $p = 0.0008$, mimetic female versus *P. aristolochiae*, $\chi^2 = 14.25$, $p = 0.0065$. [Colour figure can be viewed at wileyonlinelibrary.com]

chi-square, $\chi^2 = 6.368$, $p = 0.0116$). In our population, non-mimetic females made up approximately 23% of the female population (37 non-mimetic, 127 mimetic females; Figure 3a). However, males courted non-mimetic females in half of the observed courtship events (Figure 3b).

There was no effect of female wing pattern on female resting location. Non-mimetic and mimetic females were equally likely to be found resting or basking on light foliage, dark foliage or in full sun ($N = 52$, $\chi^2 = 2.329$, $p = 0.3121$), though males were found in dark foliage more often than either female form ($N = 122$, $\chi^2 = 17.54$, $p = 0.0075$; Figure 4). We only observed 8 resting *P. aristolochiae*, so excluded them from the above analyses due to sample size. However, of those 8, 5 were resting on light foliage, 2 were in full sun and 1 was on dark foliage.

4 | DISCUSSION

Our results suggest that male *P. polytes* butterflies exhibit a strong preference for active over inactive females, and when choosing among active females preferentially court females with non-mimetic wing patterns. These preferences for active non-mimetic females are not due to non-mimetic females exhibiting higher activity levels than mimetic females, as mimetic females exhibit similar activity levels to non-mimetic females. We had hypothesized that males would court stationary mimetic females more often than stationary non-mimetic females, and that inactive mimetic females would rest and bask in more visible areas than non-mimetic females due to reduced predation risk. However, this was not the case, as males were never observed courting inactive females, and mimetic and non-mimetic females were found equally often basking/resting in full sun, light foliage and dark foliage conditions.

Our finding of male preference for active females supports a previous finding that males prefer to court active over inactive females (Westerman et al., 2018), and demonstrates that this preference is independent of social setting (presence or absence of male competition). Our finding that males never courted stationary females in the large arena was unexpected, as males will court stationary females when given no other options. However, it does suggest that male preference for active females is stronger than previously thought. This preference for active females could either be because active females are easier to detect than inactive females, or that high activity levels are an indicator of quality. Future research should explore the effect of movement on signal detection, and the relationship between female activity and fecundity in this system. Ease of detection may also play a role in male preference for stationary mimetic females over stationary non-mimetic females in the absence of active females. Mimetic females have wing patterns that are quite distinct from those of non-mimetic females (Figure 1). While previous work has shown that mimetic and non-mimetic wing patterns are equally cryptic to avian visual systems when females are stationary and basking against a green or floral background (Zhang et al., 2017), it is unknown whether these two wing patterns are equally detectable to *P. polytes* males when

TABLE 1 Wing pattern did not affect female behaviour in focal watches

Behaviour	Non-mimetic female	Mimetic female	Male	F ratio	p-Value	NM/M Z	NM/M p value
Focal Watch (seconds)							
Flutter	0.03 ± 1.99	0.32 ± 1.82	2.49 ± 1.55	0.6348	0.532	-0.450	0.894
Fly	229.84 ± 41.3	198.05 ± 37.8	201.45 ± 32.2	0.1946	0.8234	0.807	0.698
Feed	234.26 ± 39.5	215.08 ± 36.1	108.96 ± 30.8	4.584	0.014	0.884	0.651
Walk	10.52 ± 5.35	0.43 ± 4.90	0.08 ± 4.17	1.369	0.258	-1.116	0.504
Court	11.61 ± 5.69	8.40 ± 5.21	2.20 ± 4.44	1.097	0.342	-0.396	0.917
Bask	245.13 ± 64.4	313.24 ± 58.98	361.98 ± 50.2	1.248	0.293	0.387	0.921
Rest	18.71 ± 9.59	0.27 ± 8.77	1.71 ± 7.47	1.398	0.497	0.126	0.991
Copulate	0.00 ± 20.97	24.89 ± 19.20	17.65 ± 16.35	0.401	0.670	-1.283	0.405
Circle flight	2.87 ± 1.89	0.27 ± 1.73	3.82 ± 1.47	2.844	0.067	1.518	0.282
Sit near	0.48 ± 0.31	0.38 ± 0.28	0.06 ± 0.24	0.799	0.671	-0.381	0.923

Note: Average duration of behaviour: $N = 119$, non-mimetic female = 31, mimetic female = 37, Male = 51. Welch's ANOVA, unequal variances across all groups, Steel-Dwass all pairs comparisons for female wing patterns.

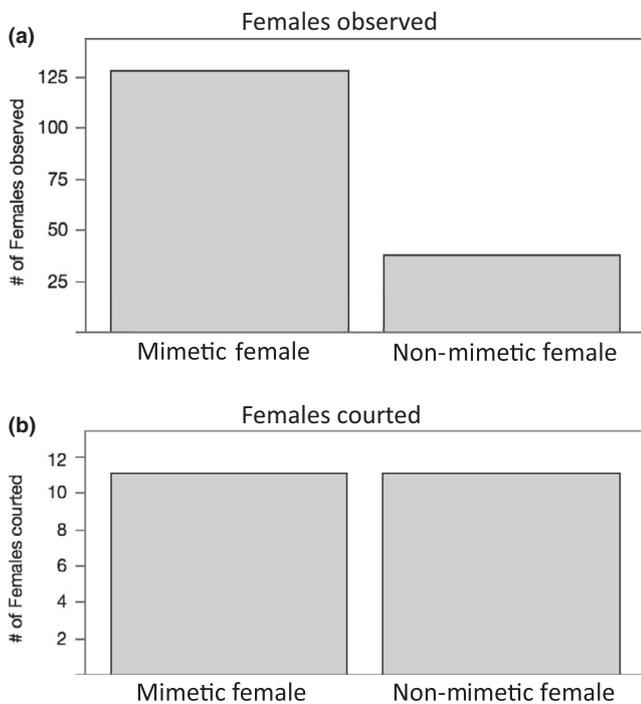


FIGURE 3 Males courted more non-mimetic females than expected. (a) There were 3× mimetic females than non-mimetic females observed in the population over the course of the study (77.44% vs. 22.56%, $N = 164$). (b) Males courted mimetic and non-mimetic females equally often ($N = 22$, $\chi^2 = 0.00$, $p = 1.00$).

females are stationary. Future work should examine the visual system of *P. polytes* butterflies to determine the role of colour detection in male courting propensity in this system, and the effect of motion on wing pattern detectability by both males and avian predators.

One of the more surprising results of this study was the preference for active non-mimetic females over active mimetic females, given the previous study demonstrating male preference for stationary mimetic females over stationary non-mimetic females. While we

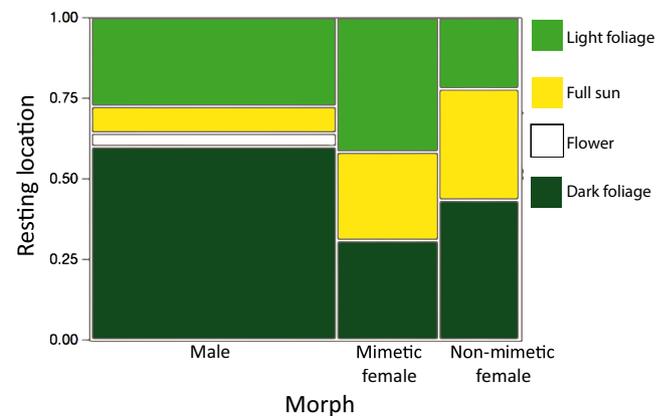


FIGURE 4 Effect of sex, not wing pattern, on resting location. Males spent more time resting in dark foliage than either mimetic or non-mimetic females ($N = 122$, $\chi^2 = 17.54$, $p = 0.0075$). Mimetic and non-mimetic females were found equally often in dark foliage, light foliage and sunny locations ($N = 52$, $\chi^2 = 2.329$, $p = 0.3121$) [Colour figure can be viewed at wileyonlinelibrary.com]

do not have a definitive explanation for this change in preference, we have a couple plausible hypotheses that, when tested, may shed further light on this finding. First, the effect of activity and wing pattern on female attractiveness may not be additive. Instead, specific combinations of signals may be more, or less, attractive, as a result of how the combined signals stimulate the sensory systems of the observing choosy individual, as seen in túngara frogs (Stange et al., 2017). Alternatively, males may not recognize non-mimetic females as females instead of males until they get close enough to court them. This would support the pseudo-sexual selection hypothesis, and help explain the heightened courtship of active non-mimetic females (Vane-Wright, 1984).

Our findings, when taken in concert with those of a previous study on male preference for female wing pattern in *P. polytes* (Westerman et al., 2018), suggest that male preference is plastic, and dependent on social environment. In even sex ratio, semi-natural conditions,

males prefer to court free-flying non-mimetic females (this study). In female-biased, individual choice trials, males prefer active females independent of female wing pattern; and in male-biased choice trials where females are held inactive, males prefer to court mimetic females (Westerman et al., 2018). The role of male preference in maintaining female-limited polymorphism in butterflies has long been a question of interest, and has inspired multiple studies, some with conflicting results (Aardema & Scriber, 2013; Burns, 1966; Cook et al., 1994; Fryer, 1914; Kunte, 2009; Levin, 1973; Westerman et al., 2018). This may be because male preference is condition dependent, as illustrated by this and previous studies in *P. polytes* (Westerman et al., 2018), and observed male preference is consequently a result of experimental design (i.e., sex ratio or number of signals available to the chooser).

One of the main goals of this study was to determine the relative signal weighting of female activity and female wing pattern in male mate selection in *P. polytes*. The exclusive courtship of active females strongly suggests that males weigh female activity more heavily than female wing pattern when determining whom to court. The subsequent preference for active non-mimetic females suggests that wing pattern does still play a role in male mate selection, albeit in an unexpected direction, given their preference for mimetic wing patterns when choosing between stationary females. One of the hypotheses for the maintenance of the non-mimetic female form in Batesian mimetic butterflies is that males prefer the non-mimetic form over that of the mimetic female form (reviewed in Kunte, 2009). This occurs in the butterfly *Papilio glaucus*, and may contribute to the maintenance of non-mimetic, male-like females in this species (Burns, 1966; Levin, 1973). An alternative hypothesis is the pseudo-sexual selection hypothesis, where males approach non-mimetic females more often than they approach mimetic females because they mistake the females for males, and then court them once they get close enough to identify the non-mimetic female as a female (Vane-Wright, 1984). Our results are consistent with both of these hypotheses. However, given that male *P. polytes* prefer mimetic females over non-mimetic females when choosing between stationary females, it seems likely that the pseudo-sexual selection hypothesis may more accurately describe the role of male preference in maintaining the non-mimetic female form in this species. Non-mimetic females appear to have a distinct sexual advantage over mimetic females in even sex ratio conditions in the presence of multiple males.

Theory suggests that non-mimetic female butterflies should behave differently from mimetic females because they have a higher predation risk. These behavioural differences should be particularly pronounced during activities such as basking, where being cryptic would be beneficial to non-mimetic individuals, and being conspicuous would be beneficial to mimetic individuals. Our finding that mimetic females were not basking in more conspicuous locations than non-mimetic females was therefore unexpected. One hypothesis as to why these two female forms did not exhibit different basking location preferences is that there were not any predators in the butterfly garden, so non-mimetic

females did not need to exhibit anti-predator behaviour. If this were the case, we would expect females to bask most often in sunny locations, instead of only a third of the time, as we observed (Figure 4). An alternative explanation for the similarities in basking location selection for non-mimetic and mimetic females is that these two wing patterns are equally cryptic against a green background to native avian predators such as the red-billed leiothrix (*Leiothrix lutea*). Recent work examining the crypsis of non-mimetic and mimetic *P. polytes* wing patterns against a green background using an avian vision model strongly suggests that these two female forms are equally difficult for birds to detect (Zhang et al., 2017). Our finding that females of these two forms exhibit similar resting location preferences further supports this hypothesis.

5 | CONCLUSIONS

Here, we show that male *P. polytes* butterflies prefer active over inactive females, and active non-mimetic (or male-like) females to active mimetic females. This finding is consistent with the pseudo-sexual selection hypothesis, where males approach non-mimetic females at higher rates than mimetic females due to their male-like appearance, and once close enough to identify them as females, exhibit courtship behaviour. This finding suggests that male preference for non-mimetic females may play an active role in the maintenance of the non-mimetic female form in natural populations. In addition, our results highlight the importance of experimental design to the study of evolutionarily relevant behaviours, as previous work examining male preference for stationary females and male preference in individual choice assays respectively observed male preference for mimetic females and an absence of male preference for female wing pattern.

ACKNOWLEDGEMENTS

We thank Audrey Willard, Becca Anderson, and the Butterfly House & Aquarium, Sioux Falls, South Dakota, USA for their generous assistance and support with butterfly husbandry and the use of their facility. We thank Darli Massardo for the use of her photographs of pinned *P. polytes* butterflies. This research was funded by the University of Arkansas, Augustana University, NIH INBRE grant 2 P20 GM103443 and NSF EPSCoR grant 1355423 to C.F.O.-M. and NSF grant IOS-1452648 and NIH grant GM108626 to M.R.K.

CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

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SUPPORTING INFORMATION

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How to cite this article: Westerman EL, Antonson N, Kreutzmann S, et al. Behaviour before beauty: Signal weighting during mate selection in the butterfly *Papilio polytes*. *Ethology*. 2019;125:565–574. <https://doi.org/10.1111/eth.12884>