Behavioural mimicry in flight path of Batesian intraspecific polymorphic butterfly *Papilio polytes*

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Batesian mimics that show similar coloration to unpalatable models gain a fitness advantage of reduced predation. Beyond physical similarity, mimics often exhibit behaviour similar to their models, further enhancing their protection against predation by mimicking not only the model's physical appearance but also activity. In butterflies, there is a strong correlation between palatability and flight velocity, but there is only weak correlation between palatability and flight path. Little is known about how Batesian mimics fly. Here, we explored the flight behaviour of four butterfly species/morphs: unpalatable model *Pachliopta aristolochiae*, mimetic and non-mimetic females of female-limited mimic *Papilio polytes*, and palatable control *Papilio xuthus*. We demonstrated that the directional change (DC) generated by wingbeats and the standard deviation of directional change (SDDC) of mimetic females and their models were smaller than those of non-mimetic females and palatable controls. Furthermore, we found no significant difference in flight velocity among all species/morphs. By showing that DC and SDDC of mimetic females resemble those of models, we provide the first evidence for the existence of behavioural mimicry in flight path by a Batesian mimic butterfly.

1. Introduction

Mimicry, in which a colour pattern of palatable individuals closely resembles that of unpalatable individuals of other species phylogenetically separated, has long attracted evolutionary biologists, mainly regarding two aspects. One aspect is its biological significance addressing whether the resemblance is actually in effect or not [1–4], and the other is the genetic basis that supports the resemblance. In the latter aspect, a 'supergene', a tightly linked cluster of loci on a chromosome, was hypothesized in butterflies [5,6], and the molecular architecture of the supergene has progressively been disclosed for Müllerian [7–11] and Batesian mimicry [12–14].

Attention to mimicry was first paid on colour patterns which are readily perceived, and then directed to similarity of movements between mimics and their models and to wing and body morphologies that support behavioural similarity.

Movement is important for prey to avoid attacks by predators. Some animals move rapidly to escape from predators, whereas others remain motionless to avoid detection by predators [15]. It is thought that sluggish movement exhibited by many unpalatable animals is also a strategy for avoiding attacks [15–17]. Sherratt et al. [18] showed in experiments with human predators attacking computer-simulated prey that unprofitable prey evolved slower movement behaviour than profitable prey if they were readily recognized as unprofitable and survived predatory attacks. This is presumably because if unprofitable prey are slow-moving, they can be more readily recognized as being unprofitable.

In butterflies, it has been thought that there are correlations between palatability and flight-related characters. Palatable species fly fast and erratically, a flight pattern that would make it difficult for predators to predict the flight path, reducing the frequency of successful attacks by predators. By contrast, unpalatable species tend to fly slowly and regularly. This flight pattern would make it easy for predators to recognize the prey as being unpalatable. Srygley and colleagues demonstrated in neotropical butterflies that unpalatable species have relatively...
smaller thoraces and longer bodies, and they fly more slowly [19–21]. By contrast, palatable species have relatively wider thoraces and shorter bodies, and they have higher maneuverability and fly faster. However, the correlation between palatability and irregularity of flight path was very weak [19]. Flight path is still poorly investigated, and skepticism remains over whether a difference in flight path exists between palatable and unpalatable species.

Golding et al. [22] showed that a Batesian mimic dronfly resembles a honeybee in its flight behaviour as well as in appearance. In butterflies, Strygley [23] found that Batesian mimics and their models perform wingbeats with slower angular velocity compared with palatable species to enhance the colour signal. Kitamura & Imafuku [24] demonstrated in a female-limited Batesian mimic butterfly, *Papilio polytes*, that mimetic females mimic their models in the minimum positional angle of their wings. As far as we know, however, there are no studies of flight path of Batesian mimic butterflies.

*Papilio polytes* is a female-limited Batesian mimic butterfly (refer to [24] for a picture of the species and morphs being investigated). Females of this butterfly show polymorphism in coloration. Non-mimetic females (*Pap. polytes* form. *cyrus*) have black wing and white spots on the hindwing, and they resemble conspecific males. Mimetic females (form. *polytes*) have black wing and white and red spots on the hindwing, and resemble an unpalatable sympatric toxic butterfly, *Pachliopta aristolochiae* [25], and thus are thought to be a Batesian mimic of this species [4,26,27]. The purpose of this study was to determine whether behavioural mimicry in flight path exists in *Pap. polytes*, on the assumption that the mimetic form shows a flight path similar to that of the model and different from that of the non-mimetic form.

### 2. Material and methods

#### (a) Subjects

We used three species of Papilioninae, *Pap. polytes* (Papilionini), *Pach. aristolochiae* (Troidini) and *Pap. xuthus* (Papilionini). *Papilio polytes* is a palatable swallowtail butterfly commonly found throughout the Oriental tropics. Males of this butterfly are monomorphic, whereas females are polymorphic. *Pachliopta aristolochiae* is an unpalatable toxic butterfly found throughout the Oriental tropics. It has warning red spots on its hindwings. Poisonous substances are resorbed from the food plant by the larva and stored by the organism during pupation and metamorphosis [25]. *Papilio xuthus*, which is not known to be either an unpalatable species or a mimic, is a swallowtail butterfly found throughout the temperate region of East Asia. *Papilio polytes* and *Pap. xuthus* use the Rutaceae group as host plants, whereas *Pach. aristolochiae* uses the Aristolochiaceae group.

Adults of *Pap. polytes* and *Pach. aristolochiae* were collected on Ishigaki Island, Okinawa, Japan (24°26′N; 124°8′E) and were brought to Minoh Park Insectary, Osaka, Japan (34°50′N; 135°28′E). Adults of *Pap. xuthus* were brought to the Insectary from Osaka Prefecture. Eggs of these butterflies were collected in the Insectary and were raised in a temperature-controlled room at 23°C. Butterflies that emerged from pupae were released into the insectary. We performed behavioural observations on butterflies kept in the insectary for at least one generation.

#### (b) Wing stroke records

During July 2007 to April 2008 and May 2009 (from 10.00 to 16.00), we recorded the flight behaviour of butterflies with two fixed digital video cameras while they were flying freely in a large cage (13 × 14 m, 10 m high) in the insectary. We constructed a rectangular framework (1.5 × 2 m, 3.5 m high) with pipes in the cage. One video camera (Sony DCR-SR100, Japan, aspect ratio 4:3) was placed at the centre of the ceiling of the framework with the lens side down to obtain a vertical view. Another video camera (Canon DM-FV M100, Japan, aspect ratio 4:3) mounted on a tripod was placed on the side of the framework at a position 1.5 m high to obtain a horizontal view. When a butterfly had passed through the framework, it was captured and the ambient temperature was immediately measured (range: 19.6–33.6°C). For synchronization of respective images from the two video cameras, the shrinking motion of a spring was recorded after every recording of butterflies. Flying behaviour of butterflies in the framework was analysed. Butterflies were used only once.

#### (c) Analysis of images

We traced the centre of the thoracic position of butterflies on images from the two video cameras (60 images per second), and the data obtained were transformed into three-dimensional coordinates, using eight reference points on the framework, by an image analysis software (FRAME-DIAS II, DKH, Japan). The change in position over time was low-pass filtered at 20 Hz. Gliding flight was excluded from the analyses. We conducted two types of analyses: one-wingbeat analysis and nine-wingbeat analysis.

One-wingbeat analysis was used to examine the up–down movement generated by wingbeats. For this analysis, we used every frame within one wingbeat (from a wing-closed posture to the next wing-closed posture) when butterflies flew linearly in a horizontal plane (figure 1a). Horizontal flight was defined as flight in which the angle between the horizontal plane (line L) and a straight line connecting the starting point (point 1) with the ending point (e.g. point 7 in figure 1a) was less than or equal to 5°. Flight in a vertical plane was defined as change in heading angle of their wings. As far as we know, however, there are no studies of flight path of Batesian mimic butterflies.

**Figure 1.** A scheme showing (a) one-wingbeat and (b) nine-wingbeat analysis of flight path. Solid circles indicate thoracic position of a butterfly. Top diagrams show lateral views of a butterfly. We used 6.60 ± 0.96 (mean ± s.d.) images in one-wingbeat analysis. Numbers indicate coordinate of a butterfly. a, b and c indicate distances between coordinates 1–2, 2–3 and 1–3, respectively. α\(_a\) indicates external angle of coordinate 2 on triangle formed by coordinate 1, 2 and 3. Bottom figures show top views of a wing-closed posture of a butterfly.
flight velocity (FVone), standard deviation of flight velocity (SDFVone), directional change (DCone) and standard deviation of directional change (SDDCone). FVone was a mean value of velocities calculated from two successive frames (FV1−2, FV2−3, ... , FVn−1−n). SDFVone was the standard deviation of all FVs within a wingbeat. Directional change (DC) was defined as the time variation of difference of heading directions in two successive frames (αn − αn−1). DCnine in figure 1a is given by

$$DC_n = \frac{1}{t} \arccos\left(\frac{a^2 + b^2 - c^2}{2ab}\right) \times \frac{1}{t},$$

where t indicates time spent from coordinate 1–3 of a butterfly. DCnine and SDDCnine were, respectively, the mean and the standard deviation of all DCs within a wingbeat. Chai and Srygley [19] used DC as the index value of irregularity, but in this study we used DC as the index value of nonlinearity and SDDC as the index value of irregularity.

Nine-wingbeat analysis was considered to examine the movement of a butterfly without wingbeat effects. For this analysis, we used 10 successive frames (totalling about 1 s, roughly corresponding to the time a butterfly stayed within the framework) that showed the butterfly with wings closed (figure 1b). We separated the three-dimensional component into an xz-plane component and a z-axis component. Four variables—flight velocity (FVnine), standard deviation of flight velocity (SDFVnine), time variation of directional change (DCnine) and standard deviation of directional change (SDDCnine)—were calculated from the xz-coordinates. Thus, we used the component of each vector in the horizontal plane. FVnine and SDfVnine were, respectively, the mean and the standard deviation of all FVs (FV1−2, FV2−3, ... , FVn−1−n) (figure 1b). DCnine and SDDCnine were, respectively, the mean and the standard deviation of all DCs in the nine wingbeats. The vertical interval (VI) was defined as the vertical distance between the highest and lowest values of the z-coordinate in the nine wingbeats.

All variables were defined as the mean value, respectively, for each species/morph.

(d) Statistical analysis

Correlation of all variables and ambient temperature was not found among species/morphs for each of sexes (p > 0.1136).

Three species were compared for males. We used Bartlett’s test to test for homogeneity of variances. If we found no significant difference in the test (p > 0.05), we conducted one-way ANOVA (three-levels for the independent variable in the ANOVA) and when we found a significant difference in one-way ANOVA (p < 0.05), we conducted multiple comparisons (Tukey’s honestly significant difference test). If we found a significant difference in Bartlett’s test (p < 0.05), we conducted Welch’s ANOVA and when we found a significant difference in Welch’s ANOVA (p < 0.05), we conducted multiple comparisons (Holm test). The significance level for experiment-wise error rate was set at p = 0.05. The females for two morphs within one species and two additional species (four levels in the ANOVA) were compared in the same manner as the males. All analysis was conducted using R (v. 3.0.2).

3. Results

In one-wingbeat analysis, 60 butterflies were used in males (Pap. xuthus n = 20, Pach. aristolochiae n = 13, Pap. polytes n = 27) and 66 butterflies in females (Pap. xuthus n = 9, Pach. aristolochiae n = 13, non-mimetic form of Pap. polytes n = 20, mimetic form of Pap. polytes n = 24). In nine-wingbeat analysis, 61 butterflies were used in males (Pap. xuthus n = 20, Pach. aristolochiae n = 13, Pap. polytes n = 28) and 75 butterflies in females (Pap. xuthus n = 14, Pach. aristolochiae n = 16, non-mimetic form of Pap. polytes n = 20, mimetic form of Pap. polytes n = 25). An example from a typical tracing of a non-mimetic and mimetic female, and the model, is shown in figure 2.

In one-wingbeat analysis, statistically significant effects of species/morph were found for DCnine and SDDCnine in both sexes, but not for FVnine or SDfVnine (table 1). In nine-wingbeat analysis, we found statistically significant effects of species/morph for SDfVnine in females and VI in males, but not for FVnine or SDfVnine in males, nor for DCnine, SDDCnine or VI in females (table 1).

In both sexes, DCnine and SDDCnine of palatable butterflies were significantly larger than those of the unpalatable species and mimetic females (figure 3c–h). In females, SDfVnine of the non-mimetic form of Pap. polytes was significantly larger than that of the mimetic form of Pap. polytes (figure 3f). For VI of males, we found statistically significant effects of species/morph between palatable species and unpalatable species in multiple comparisons (figure 3g).

In nine-wingbeat analysis, wingbeat frequency was 10.4 ± 0.84 (mean ± s.d., Pap. xuthus), 11.5 ± 0.88 (Pach. aristolochiae) and 10.4 ± 0.92 (Pap. polytes) for males, and 10.8 ± 1.20 (Pap. xuthus), 10.9 ± 0.69 (Pach. aristolochiae), 11.1 ± 1.00 (non-mimetic form of Pap. polytes) and 10.7 ± 0.92 (mimetic form of Pap. polytes) for females. Significant difference in wingbeat frequency was not found among species/morphs for each of sexes. This result coincides with that obtained previously [24].

4. Discussion

From one-wingbeat analysis, clear-cut results were obtained: the flight path, but not the flight velocity, of the control (non-toxic) species was clearly different from that of the model (toxic) species. Moreover, the flight paths of mimetic females and non-mimetic females of a Batesian intraspecific polymorphic butterfly were different from each other, and that of mimetic females was not significantly different from that of their models. This is the first evidence that shows the existence of behavioural mimicry in flight path.

As shown by the large DCnine values, butterflies of the control and non-mimetic groups performed flight characterized by large changes in heading direction. A large DC with nonlinear path could be produced by a large angular
velocity of wing and large stroke amplitude—angular difference between the most closed (ϕmax) and opened (ϕmin) minus values in many case) wing positions (see fig. 3.2 in [28])—or produced by a pause at the top of the wing stroke which causes the insect drop (flight pattern of many satyrids [29,30])—or produced by a pause at the top of the wing stroke which causes the insect drop (flight pattern of many satyrids [29,30]). However, we found no pause in our butterflies. In another study of wing motion, we found that angular velocity of wing and ϕmax were not different among these species/morphs, but ϕmin of the mimetic females and their models was larger than that of the control and the non-mimetic groups [24]. Presumably, a large ϕmin of the former group is one of the factors that cause small DCS, whereas a small ϕmin of the control and non-mimetic groups is one of the factors that cause large DCS. Further, the standard deviation of DC of the control and the non-mimetic groups was larger than that of the model and the mimicry group. This means that the former groups fly not only in a nonlinear path but also in a linear path, thus in an irregular flight path. By contrast, butterflies of the latter group continuously fly in a regular flight pattern with a linear path.

It is thought that nonlinear and irregular flight is one of the defence strategies of butterflies against aerial predation. In one classical explanation, the flight pattern functions as a warning signal indicating difficulty of capture and associated prey unprofitability [31]. However, this hypothesis has never been tested experimentally. Another explanation is that the flight pattern functions as a tactic for avoiding attack rather than as a warning signal before birds attack them. Because birds attack taking the motion of prey into consideration, a flight pattern of butterflies that makes the flight path hard to predict and makes it hard for the bird to catch them [15] will be useful for survival of butterflies. On the other hand, it is thought that linear and regular flight, characteristic of unpalatable butterflies and their mimics, increases the conspicuous effect of their warning coloration to enhance learning and avoid confusion with palatable butterflies, and decreases the chance of mistaken attacks by potential predators [19,32–34].

Additionally, Kassarov [35] considered the relationship between flight pattern of butterflies and predation by birds as follows. It is thought that aerial hawking birds are the main predators of flying butterflies. If birds find a butterfly at a distance, they must make a decision to attack or not within seconds before it flies away. At a distance, birds can differentiate the characteristic flight pattern but not the colour pattern. Thus, characteristic flight pattern becomes the factor that renders the prey ‘conspicuous’ and becomes a useful cue that signals profitability or unprofitability. Actually, at a distance, we can easily differentiate whether a butterfly is palatable or unpalatable and whether it belongs to the mimicry group by the flight pattern rather than the colour pattern in our species/morph (T.K. 2007, personal observations). When a chromatically mimetic butterfly mimics the flight pattern of its model well, it is difficult to differentiate at a distance. On the other hand, when close to the butterfly, we can first differentiate butterflies as mimics or their models by their colour pattern and morphological characteristics. Thus, at a distance from predators, unpalatable and mimic species may gain protection against aerial predation by their conspicuous flight pattern rather than by their coloration. Flight pattern may thus function as a primary defence, like aposematic coloration, for unpalatable species [15]. Further, Kassarov [35] argued that birds are not the primary selective force leading to evolution of mimicry and aposematism in butterflies, because birds are able

Table 1. The result of analysis (ANOVA) about nine parameters (FVone, SDVFone, DCone, SDDCone, FVnine, SDVFnine, DCnine, SDDCnine and VI).

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<th>ANOVA</th>
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<td></td>
<td>FVone (m s⁻¹)</td>
<td>one-way</td>
<td>ƒ, 57 = 0.328</td>
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<td></td>
<td>SDVFone</td>
<td>Welch's</td>
<td>ƒ, 34.256 = 1.008</td>
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<td>DCone (° s⁻¹)</td>
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<td>ƒ, 37.331 = 19.539</td>
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<td></td>
<td>SDDCone</td>
<td>Welch's</td>
<td>ƒ, 37.401 = 13.813</td>
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<td>FVnine (m s⁻¹)</td>
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<td>SDVFnine</td>
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<td>ƒ, 24.152 = 1.023</td>
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<td>DCnine (° s⁻¹)</td>
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<td>ƒ, 26.202 = 24.507</td>
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<td>SDDCnine</td>
<td>Welch's</td>
<td>ƒ, 25.483 = 19.085</td>
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nine wingbeat

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<td></td>
<td>FVone (m s⁻¹)</td>
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<td>ƒ, 38.274 = 2.611</td>
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<td>SDVFone</td>
<td>one-way</td>
<td>ƒ, 58 = 0.866</td>
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<td>SDDCone</td>
<td>one-way</td>
<td>ƒ, 58 = 2.494</td>
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<td>VI (m)</td>
<td>Welch's</td>
<td>ƒ, 37.138 = 9.885</td>
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<tr>
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<td>FVnine (m s⁻¹)</td>
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<td>SDVFnine</td>
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<td>DCnine (° s⁻¹)</td>
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<td>ƒ, 71 = 1.044</td>
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<td>SDDCnine</td>
<td>Welch's</td>
<td>ƒ, 34.778 = 2.566</td>
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<td></td>
<td>VI (m)</td>
<td>Welch's</td>
<td>ƒ, 33.256 = 2.096</td>
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to recognize the profitability of prey by their flight pattern not their colour pattern. However, birds attack not only flying butterflies but also butterflies landing on plants, especially in the early morning (N. Osaki & M. Ohata 2004, personal observation). They come into action earlier than butterflies, which enables them to approach butterflies closely with time enough to differentiate them by the colour pattern. It may be that aposematic coloration evolves for protection against predation in the early morning, whereas a characteristic flight pattern evolves for protection against predation in the daytime.

In the nine-wingbeat analysis, which explored horizontal movement, we found no statistically significant effects. Insectivorous birds tend to attack from a position level with the prey, and thus vertical movements of the potential prey would be easily recognized by the attacker.

It is well known that palatable butterflies generally fly fast, whereas unpalatable species fly slowly [19,32–36]. However, we could not confirm this for our species, as shown by the lack of a significant difference in \( F_{\text{Vone}} \) or \( F_{\text{Vnine}} \) among these species/morph. As energetic cost of fast and irregular flight
is high [37], butterflies may employ either of the two tactics, and our palatable species seems to adopt the latter. Alternatively, insignificant difference in flight velocity may be attributable to our experimental condition of cage confinement.

In the one-wingbeat analysis, which explored the effect of wingbeats on the flight movement, we detected a significant difference between the two morphs of the Batesian mimic species. As flight patterns are known to be related to wing and body morphologies [19,38,39], the different flight paths found in our mimic species may be caused by different morphologies of wing and body. This is an interesting possibility to be addressed in a future study. A possibility that the supergene responsible for colour patterns is associated with wing shape variation is suggested [40]. The results obtained for intraspecific variation in flight behaviour in our species will contribute to studies of superfgenes.

Data accessibility. The database for all data in this article is publicly available at Dryad http://dx.doi.org/10.5061/dryad.68b89.

Author contribution. T.K. and M.I. conceived of and designed the study. T.K. carried out the data collection and data analysis. T.K. wrote the manuscript and M.I. improved it. Both authors gave final approval for publication.

Competing interests. We declare we have no competing interests.

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