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Flower colour preferences of *Aporia bieti* (Lepidoptera: Pieridae) in the Xياما Forest Farm, Gansu, China

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ABSTRACT

In addition to their ornamental value, butterflies provide value to flowering plants through pollination, which can improve their heterosis. Studying the relationship between butterflies and flowers is useful for understanding the adaptive relationship between them. By observing the pollinating and feeding behaviour of *Aporia bieti* in the Xياما forest, Gansu, we explored the adaptive relationship between *A. bieti* proboscis size and the corolla tube length of the flowers. To eliminate interference from the butterfly's sense of smell, we used foam flowers instead of real flowers. The results indicated that (1) of the red, yellow, pink, purple, and white flowers tested, yellow flowers were preferred; (2) there was no distinct difference in the preferences of *A. bieti* for large- (45 mm) and small- (25 mm) sized flowers; and (3) *A. bieti* proboscis length was significantly related to the corolla tube length of the flowers fed on by the butterfly, which suggests co-evolution between butterfly proboscis length and the length of the pollen tube.

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1. Introduction

In addition to being important ecologically, butterflies are known as a symbol of peace, happiness, and love and are of high ornamental and economic value [1]. Globally, a great deal of work has been done on butterfly classification [2–10], habitat utilization of butterflies as a resource [11–19], and butterfly conservation [19–24]. These studies are of great significance for the conservation of biodiversity, responsible utilization of butterfly resources, understanding the feeding habits of butterflies, conservation of butterfly habitat, and artificial breeding of rare butterflies. Due to the use of both visual and olfactory cues, butterflies may choose flowers by their colour, shape, size, and/or smell. It is known that most butterflies feed on nectar, and these butterflies prefer nectar from certain plants [25]. Although some butterfly larvae are pests to agriculture, forestry, and horticulture, most of the butterfly species with which humans interact are ornamentals, and they are one of the most important and most specious ornamental insects [3]. Understanding the feeding habits of butterflies can provide a theoretical basis for the

development of butterfly habitat conservation measures and the development of artificial breeding programs for butterflies.

In biology, co-evolution occurs when two or more species reciprocally affect each other's evolution. The evolutionary interactions between flowering plants and insects were mentioned in 1859 by Charles Darwin in his most famous book *On the Origin of Species* [26]. Many of the interactions between insects and insect-pollinated flowers are examples of co-evolution. After many years of research on the evolution of the relationship between butterflies and plant species, Ehrlich and Raven proposed the concept of “co-evolution” in 1964, which they defined as the process by which one of the characteristics of a species evolves in response to a characteristic of another species, which likewise evolves in response to the former characteristic [27]. The theoretical underpinnings of coevolution are now well-developed, and demonstrate that coevolution can play an important role in driving major evolutionary transitions, such as the evolution of sexual reproduction or shifts in ploidy [28]. More recently, it has also been demonstrated that co-evolution influences the structure and function of ecological communities as well as the dynamics of infectious diseases [28]. Ehrlich and Raven aimed to examine the pattern of interactions between two major groups of organisms with close and evident ecological relationships, such as plants and herbivores. Co-evolution is the evolution of two or more species which reciprocally affect each other, sometimes creating a mutualistic relationship between the species. Such relationships can be of many different types [29–30]. Plant-pollinator

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interactions are one of the most important components of terrestrial biological communities, and the interactions between the two groups are diverse. The two most important aspects driving these interactions are that insects choose plants as food and growing sites and that they transfer pollen between plants [31–34]. Additionally, many studies have documented bees selecting flowers for feeding that have a corolla tube length matching their proboscis length. This is especially well documented by bumblebee studies [25].

The physical and chemical properties of plants affect the feeding behaviour of insects, and the quality and quantity of the secondary metabolites contained in them play a decisive role in this behaviour [34]. Insects use visual and olfactory cues to find and direct others to food when foraging. Studies have shown that butterflies visit flowers with corolla characteristics which match their proboscises and that flower colour, shape, and structure are considered to be important visual signals for attracting them [1]. Thus, studying butterflies can provide insight into the co-evolution of insects and plants. In this study, we hypothesized that the higher the degree of fit between the morphology of a butterfly's proboscis and the morphology of the flowers they pollinate, the more they will interact.

Aporia bieti belongs to the family Pieridae, genus *Aporia*, which is the dominant family in the reserve comprising 40% of all butterfly species. In the Tianzhu region, there are 52 species of butterfly, representing 8 families and 32 genera, and Pieridae comprises 28% of the total number of species and is the dominant family. Thus, it is an ideal species for studying flower colour preferences because of its large numbers. The present research aimed to (1) investigate the flower colour preferences of *A. bieti* and (2) explore the mechanisms driving these preferences.

2. Methods

2.1. Study site and butterfly population

This study was carried out in July 2014 at the Xiyama Forest Farm, an important part of the Qilianshan National Nature Reserve located in Tianzhu Tibetan Autonomous County, Northeastern Gansu Province, China (N 37°18', E 103°10'). The Xiyama Forest Farm is comprised of forest steppe habitat at an elevation of 2500–3150 m, rhododendron shrubland at an elevation of 3150–3700 m and vegetation like *Androsace* at elevations higher than 3700 m. The forest climate is cold (annual mean temperature about 0.3 °C) and semi-arid (annual precipitation about 450 mm). Mean temperature in July is 11.2 °C (0.2–32 °C), and heavy rains occur frequently in June and August. Owing to the large altitudinal gradient (2500–4074 m), natural landscapes vary from alpine scree vegetation and rhododendron thickets to *Picea crassifolia* forest, grazing meadow (consisting mainly of *Elymus nutans*, *Stipa capillata*, and *Polygonum viviparum*), and valley meadow (dominated by *Iris lacteal* and *Arenaria radix*), with some farmland dispersed among the meadows. There is a shady slope consisting of *Picea crassifolia* forest and a sunny slope and valley area mainly consisting of grassland. Our study area and the butterfly populations used were established following the criteria described in Da et al. [3].

2.2. Flower preference

We set up five butterfly observation sites in each habitat type, including grassland, roadside, riverside, slope meadow, and shrubby cinquefoil shrubland, and at each site we observed the number of *A. bieti* visiting the flowers. According to previous research [4], most Pieridae butterflies feed on cruciferous plants, particularly those containing mustard oil. To ensure that all flowers smelled the same, we used foam flowers instead of real flowers and we sprayed an equal amount of honey solution on them thereby removing flower smell from the set of cues which the butterflies used to select flowers in this experiment. Flowers of five colours—red, yellow, purple, white, and pink—were simulated by 25 mm (large flowers) and 4 mm (small

flowers) foam disks arranged 30 cm apart from one another (Fig. 1). In each habitat, flowers were placed first in single-colour blocks (four flowers per block). These blocks were then arranged in a 5 × 5 block square for a total of 100 (5 × 5 × 4) flowers per square (Fig. 1). A micro video recorder (DSJ-A5) was used at each site to record the foraging behaviour of butterflies on both natural and simulated flowers.

A 4% honey solution was used in a 10 ml cylinder. Before 09:00 each morning, we selected a *Potentilla fruticosa* shrub (because *A. bieti* is more common on this species than on other species). We sprayed each foam flower with approximately 2 ml of honey water mixture, once a day. The number of *A. bieti* individuals that visited each flower was recorded with the video recorder from 09:30 to 15:30, which coincided with peak foraging times for butterflies. The number of visits to each flower and the flower's colour and size were recorded later by watching the videos.

2.3. Butterfly measurements

We caught *A. bieti* individuals with butterfly nets and put them in a paper bag for further analysis. With electronic Vernier callipers (0.02 mm), we measured wing length, body length, and the length of the proboscis. As butterfly proboscises are curly, we stretched the proboscises with a dissecting needle to measure them.

2.4. Flower measurements

We collected 15 samples of ten plant species including *Potentilla fruticosa*, *Potentilla glabra*, *Pedicularis kansuensis*, *Pedicularis chinensis*, *Carduus nutans*, *Anaphalis lactea*, *Parnassia oreophila*, *Geranium pratense*, *Stellera chamaejasme*, and *Oxytropis ochrocephala*. In the laboratory, the plants were dissected, and the corolla tube length was measured with electronic Vernier callipers and recorded. In addition, we selected the two species of flowers that were visited most frequently, measured their corolla tube lengths, counted the number of flowers, and analysed the relationship between these variables and the morphological measurements made for *A. bieti*.

2.5. Statistical analyses

A chi-square test was used to analyse the relationship between *A. bieti* foraging frequency and flower size. Pearson's correlation coefficients were calculated to evaluate the relationship between the length of *A. bieti* proboscis and flower corolla tube length. All analyses were conducted in IBM SPSS 22 (IBM Corp., New York, USA), and a $p < 0.05$ was considered statistically significant.

3. Results

3.1. Flower preferences of *Aporia bieti*

3.1.1. Simulated flower visits

A. bieti visited the simulated flowers 371 times during 20 days of field observations, and of these visits, it visited large-diameter (45 mm) flowers 36.62% of the time and small-diameter (25 mm) flowers 63.38% of the time (Fig. 2). However, this difference was not significant ($\chi^2 = 5.000$, $df = 4$, $p = 0.287$ ($p > 0.05$)). The order of flower colour preference for large-diameter flowers from high to low was yellow (21.13%) > red (7.04%) > purple (5.63%) > pink (2.82%) > white (0.00%) and for small diameter flowers was yellow (30.99%) > red (14.08%) > purple (8.45%) > white (8.45%) > pink (1.41%) (Fig. 2).

For both large and small flowers, we observed that *A. bieti* visited yellow flowers most frequently (52.11%), followed by red (21.13%) and purple flowers (14.08%). White and pink flowers were the least frequently visited (Fig. 2).

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