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Ethogram of mating and male mate-locating strategy of *Papilio peranthus* (Lepidoptera: Papilionidae) at Gita Persada butterfly park, Lampung, Indonesia

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Abstract

Research report on the ecological and behavioral aspects of swallowtail butterfly *Papilio peranthus* is very limited. This study was aimed to deepen understanding on the reproductive behavior of *P. peranthus*. Observation of mating behavior was made by releasing virgin males and females into a flight cage. Behavioral activities of the insects were observed visually aided by video cameras. Three experimental trials were applied namely one male met one female; three males met one female; and one male met three females. The results depicted in an ethogram that involves male behavioral acts such as flying for searching female, pursuing, hovering and touching female for courting, mounting and grasping the female, flying in tandem to seek a safe perch, relaxed bellow the female upon manage to insert his clasper, and withdrew from pairs and flew away upon finishing copulation. The male mate-locating strategy developed by butterfly *P. peranthus* is patrolling.

Keywords: Swallowtail butterfly, *Papilio peranthus*, mating behavior, mate-locating strategy, courtship behavior, behavioral ethogram

1. Introduction

Majority of swallowtail butterflies species, the family Papilionidae are found in tropical latitudes with the greatest diversity in the East and Southeast Asia [1]. *Papilio peranthus* is one among the 550 species of swallowtail butterfly which are distributed in a more restricted geographical zone. There was no *P. peranthus* recorded in Taiwan [2], in Australia [3], in Pakistan [4], in West Bengal [5] and Jabalpur, and India [6]. In Southeast Asian region, *P. peranthus* was not found in Vietnam [7], in Malaysian-Borneo [8], in Philippine [9], or neither in Singapore [10-11]. Thus, the remaining area of Southeast Asia where *P. peranthus* could be found is Indonesia.

In the Indonesian archipelago, *P. peranthus* is found in the western part of Java island, precisely in the Ujung Kulon National Park [12-13], in Sulawesi [14], and in the southern part of Sumatra [15]. However, in fact, not all the time and place the peranthus butterflies can be found. Wildlife surveys conducted by Tabadepu *et al.* [16], for instance, recorded no *P. peranthus* in the Gunung Salak, a remaining natural forest ecosystem in the West Java. The most recent study conducted by Matsumoto *et al.* [17] on Lombok Island, for another instance, also showed no *P. peranthus* in their reports.

The restricted geographical distribution of *P. peranthus* is suspected to be the cause of the lack of scientific data on the ecological, physiological, and behavioral aspects of this butterfly. In most of the reputable scientific publications that can be accessed via the internet, it is very difficult, if not impossible, to find a research report on the behavior of *P. peranthus*. In fact, in the context of conservation biology, the success of animal breeding is largely determined by the knowledge of the behavioral aspects of the animals. Understanding the breeding system, for example, may improve predictions of environmental changes [18]. In butterflies, environmental changes and disturbances has proven to alter foraging and feeding pattern as well as egg-laying behavior [19-20].

In an effort to deepen knowledge and strengthen strategies of *P. peranthus* conservation, a preliminary research on the reproductive behavior of these butterflies have been conducted in the Gita Persada Butterfly Park, Lampung, Indonesia. Gita Persada Butterfly

Park is the only butterfly garden in Lampung, an Indonesian province situated in the most southern part of Sumatra that has successfully providing host plants for at least 400 species of Sumatran butterflies including *Papilio peranthus*.

There are two goals of this research. First, to construct an ethogram of the courtship behavior of the butterflies based on the behavioral act types and duration as adopted from Dinesh and Venkatesha [21]. Second, to know the male mate-locating strategy of *Papilio peranthus*, whether perching or patrolling as defined by Scott [22].

2. Materials and Methods

2.1 Site of research

The study was carried out at **Gita Persada** Butterfly Park, Lampung, Indonesia from January to March 2016. The Gita Persada Butterfly Park is located in the suburbs Bandar Lampung, on the slopes of Gunung (mount) Betung, at the coordinates of 5°25'15" S, 105°11'20"E.

2.2 Collecting and rearing the butterflies

The adult butterflies used in this research was *Papilio peranthus* Fabricius, 1787 which were provided by way of rearing the larvae. The caterpillars were collected from the host plants which in the Gita Persada, the most preferred host plant is the pink lime-berry (*Clausena excavata* Burm. f.) (Rutaceae). Caterpillars reared in a wooden box with mesh side until reaching the pupal stage (chrysalis). Upon the eclosion of chrysalis, the newly hatched butterflies were immediately separated by sex into two different captive cages with a size of 90 x 60 x 60 cm each. This separation was taken to prevent early mating between males and females that may occur on their first day of life as a imago. Male imagos distinguished from the females by a pincher-like organ, called clasper at the end of his abdomen [23].

2.3 Experimental trials and observation

Observation of mating behavior was made by releasing virgin males and females which were randomly selected from captive cages into a flight cage (observation cage) with a size of 2.5 x 2 x 2.5 m. To mimics the ambient environment of the butterflies, in the observation cage (flight cage) were provided three species of food plant of the peranthus imagos, namely Javanese ixora (*Ixora javanica* (Blume) DC., 1830), big-sage

(*Lantana camara*L.) and Indian snakeweed (*Stachytarpheta indica* (L.) Vahl).

There were three mating experimental trials applied in this study namely one male met one female as Trial-1; three males met one female as Trial-2; and one male met three females as Trial-3. Before the butterflies released into the observation cage, some individuals were marked following technique suggested by Walker and Wineriter [24] using TipEx@ correction fluid on the dorsal part of their hind wings. For the Trial-1, only the male was marked. For Trial-2, males were marked differently to distinguish male A, B and C. For Trial-3, females were marked to distinguish female A, B and C.

The experimental trials was set and carried out under outdoor sunny condition, between 08:00 and 13:00. Trial-1 was started at 8.30 AM, at the temperature of 27 °C and the relative humidity of 86 %. Trial-2 was done at 9.15 AM at the temperature of 30 °C and the relative humidity of 88 %. While Trial-3 was carried out at 9.15 AM at the temperature of 28 °C and the relative humidity of 88 %.

Courtship behavior of the insects were observed visually aided by a digital camera Canon PowerShot N100 to make video recording of the butterfly activities. Two cameras were installed at two opposite points and used simultaneously to include the entire field of view of the flight cage.

2.4 Study parameters and analysis

Observation of mating activities was started once the males and females were released into the flight cages. All activities performed by the insect, either male or female, until copulation ended (mating success) and the lasted time of each activity were recorded. The activities include flying, perching, feeding, approaching or pursuing, courting, mounting and copulation. Data of each experimental trial were presented in an ethogram which depicting the sequence, type, description and duration of activities. The mate-locating strategy of the butterflies described based on male behavioral activities before courtship rituals took place in each experimental trial.

3. Results and Discussion

3.1 The behavioral ethogram

The description, duration and the most probable goal context of the behavioral acts of *P. peranthus* in three different experimental trials are consecutively described in Table 1-3.

Table 1: Description, duration and most probable goal context of the behavioral acts performed by *P. peranthus* when one male met one female.

Behavioral Acts	Description	Duration (min)	Goal Context
Unoriented flying	Upon released in flight cage, both male and female flew in an unoriented manner	2.78	Mate location
Oriented flying	Male flew behind the female (to pursue her).	9.50	Mate recognition
Circling	Female perched on the cage mesh, male made a circle flight around the female	0.91	Courtship ritual
Hovering and touching	Male hovered above the female and lightly touching her repeatedly with his abdomen end or wings.	0.75	
Mounting and grasping	After intensive contact with each other, male mounted and grasped the female	0.66	Copulation
Moving perch	Upon pairing the butterflies flew in tandem and moved perch to another part of cage mesh.	0.55	
Genital contact	Male extend his claspers to the female abdomen end.	2.41	
In copula	Once the clasper of male contact to female's genitalia, he relaxed and dropped bellow the female and remain in copula.	55.25	
Withdrawal	Butterflies flew away from each other and perched for feeding.	Not timed	Post mating

Table 2: Description, duration and most probable goal context of the behavioral acts performed by *P. peranthus* when three males met one female.

Behavioral Acts	Description	Duration (Min)	Goal Context
Unoriented Flying	All Three Males And The Female Flew In Unoriented Manner	8.18	Mate Location
Oriented Flying	Males Flew In Group Pursuing The Female Together.	6.20	Mate Recognition

Circling	<ul style="list-style-type: none"> Female Perched, The Males (A, B, And C) Flew Alternately Around Her. Male A And C Tend To Approach The Female More Than Male B. 	1.25	Courtship Ritual
Hovering And Touching	<ul style="list-style-type: none"> Male C Successfully Hovered Above The Female and Was Touching Her Repeatedly. Male A And B Were Rejected By Her By Way Of Flapping Her Wings. 	0.83	
Mounting And Grasping	<ul style="list-style-type: none"> After Intensive Contact With Each Other, Male C Mounted And Grasped The Female. Male A Perched On A Leaf, While Male B Flew Away For Feeding. 	0.50	Copulation
Moving Perch	Upon Coupling, Male C And The Female Made A Tandem Flight And Moved Perch On The Cage Mesh.	0.75	
Genital Contact	Male C Extend His Claspers To The Female Abdomen End.	1.16	
In Copula	<ul style="list-style-type: none"> Upon Male C Managed To Insert His Claspers Into Female's Genitalia, He Relaxed And Dropped Bellow Her And Remain In Copula. 	54.22	Post Mating
Withdrawal	<ul style="list-style-type: none"> Male C And His Mate Flew Away From Each Other And Perched For Feeding. Male A Starting To Approach The Female But Rejected, While Male B Perched And Then Flew For Feeding. 	Not Timed	

Table 3: Description, duration and most probable goal context of the behavioral acts performed by *P. peranthus* when one male met three females.

Behavioral Acts	Description	Duration (min)	Goal Context
Unoriented flying	Male and all three females flew in unoriented manner	10.17	Mate location
Oriented flying	Male pursued the females (A, B or C) alternately, but more often follow female A and B.	7.18	Mate recognition
Circling	At the end of the pursuing event, the male seemed to prefers female B and made a circling flight around her.	1.08	Courtship ritual
Hovering and touching	<ul style="list-style-type: none"> Male hovered above the female B and touching her intensively. Female A and C flew around for feeding. 	0.80	
Mounting and grasping	<ul style="list-style-type: none"> After intensive contact with each other, male mounted and grasped female B. Female A flew around while female C perched on the cage mesh. 	0.70	Copulation
Moving perch	Upon coupling, male and the female B flew in tandem and moved perch on cage mesh.	0.67	
Genital contact	Male extend his claspers to the female B abdomen end.	1.03	
In copula	<ul style="list-style-type: none"> Upon male managed to insert his claspers into the genitalia of female B, he relaxed and dropped bellow her and remain in copula. 	54.05	Post mating
Withdrawal	Male and his mate (B) flew away from each other and perched for feeding.	Not timed	

Referring to the description of the behavioral activity of the butterflies in Table 1-3 above, an ethogram of mating

behavior of *P. peranthus* has been constructed as depicted in Fig. 1.

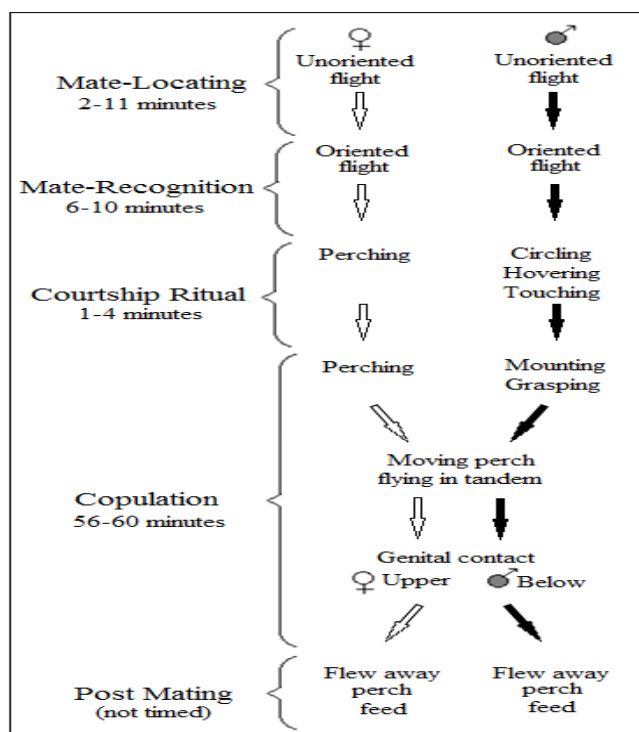


Fig 1: Ethogram of mating behavior of *Papilio peranthus*: ♀ mean female, ♂ mean male

The display of courtship and copulation rituals in the mating behavior of the butterfly *P. peranthus* illustrated in Fig. 2.



Fig 2: Photographs of behavioral acts of mating by *P. peranthus*. A) The courtship phase, male (♂) hovering above the female (♀) and repeatedly touching her. B) The copulation phase, male (♂) and female (♀) remain in copula upon genital contact.

3.2 Male mate-locating strategy

The Table 4 lists activities performed by the butterflies before courting that was put forward for categorizing the type of mate-locating strategy adopted by male *P. peranthus*.

Table 4: Behavioral acts performed by male *P. peranthus* in pre-courtsip phases.

Experimental Trials	Mate-searching phase	Mate-recognition phase
1 male met 1 female	Male made unoriented flights	Male flew pursuing female
3 males met 1 female	All males made unoriented flight	Males flew in group pursuing the female together
1 male met 3 females	Male made unoriented flight	Male tried to chase all the females

Based on the behavioral acts performed by male *P. peranthus* in all experimental trials are presented in Table 4. It is clear that all males starting to locate their mate by flying constantly in an unoriented manner. Once male detected female he oriented to pursued her immediately. None of the males involved in mating trials acted differently.

It should be recognized, indeed, that the sample size of the study is very limited so that the data obtained can only be described as it is, without a quantitative analysis. However, there are some important aspects of the mating behavior of this swallowtail butterfly that can be actualized by the research results. First, given experimental trials (Table 1-3) provide a consistent picture of the type and sequence of behavioral acts of the butterflies, then the ethogram (Fig. 1) was plausible enough to depict mating behavior of the *P. peranthus*. Second, referring to behavioral acts of butterfly listed in Table 4 it clearly implies that mate-locating method developed by male *P. peranthus* is patrolling.

The mating activities of *P. peranthus* observed in the study involved interactions between the sexes in four sequential acts—locating, recognition, courtship, and copulation. Among the four mating phases, courtship was taking the longest time (54-58 minutes) followed by mate-location (2-11 minutes), mate-recognition (6-10 minutes) and courtship rituals (1-4 minutes). The duration of copulation of the *P. peranthus* recorded in this study seem to be the same as their relatives *P. xuthus* (59.3±4.5 minutes) and *P. machaon* (55.3±2.7 minutes) as reported by Watanabe and Kobayashi [25]. A shorter courtship flight has also shown by *Papilio glaucus*, courtship flight length ranged 0-59 seconds [26].

The duration of unoriented flying of males before pursuing female was shortest when male met one female (2.78 minutes) in comparison to that of 3 males met one female (8.18 minutes) and one male met 3 females (10.17 minutes). This fact suggests that different from *P. dardanus*, *P. peranthus* tends to rely more on pheromones, rather than vision on morphs and colors, in identifying females with certainty [27]. This is reasonable, as it has been known that the effectiveness of pheromones in affecting male responses among Lepidopteran is markedly [28].

For mate-locating, butterflies have developed two type of strategies, perching or patrolling. Perching strategy is defined as a mate-locating method in which males sit at characteristic sites in search of female. While patrolling is a mate-locating method in which males fly constantly in search of females [29]. The results of the study (Table 4) clearly showed that mate-locating strategy used by male *P. peranthus* is patrolling. Such strategy is in contrast to that of their relatives, *P. polyxenes*, which obviously develops a perching method and tends to defend their territory [30]. The patrolling behavior of *P. peranthus* punctuated with the facts that all of the pre-mating and post-mating activities were aerial, no air-ground or ground phase was shown as performed by many species of Nymphalidae [31]. The difference of mate location tactics might depend on the ecological aspect such as pupation plants and larval food plant of the species [32].

In *Heliconius sara* (Nymphalidae), male shows agonistic acts against other males [33]. In a territorial butterfly, the resident males has greater change to mating than that of non resident [34]. In butterfly *Bicyclus anynana*, the presence of sexual competitor significantly make them copulated less than when in male-female pairs [35]. The results of the study, in fact, failed to show a similar activity. However, as can be seen from all experimental trials, all pairs moved perch upon coupled. This act is most likely to be an attempt to seek a safe perch from any competitor or other disturbances.

Beyond that, of course there are still many questions that cannot be answered by the resulting data and ethogram. What if *P. peranthus* was tested together with other species of Papilionoidea in one single flight cages? Will *P. peranthus* show interspecific courtship such as showed by *P. palamedes*, *P. glaucus* and *P. canadensis*? [36]. Again, due to carried out in a closed meshed cage, this research of course did not managed to account for whether the *P. peranthus* develops a hiltopping behavior for their mating ritual or not. Some species of Papilionidae namely *Papilio thoas*, *Battus polydamas*, and *Eurytides orthosilaus* have been revealed to develop a hiltopping behavior in respect to mating rituals [37].

4. Conclusion

From the male perspective, mating behavior of the butterfly *P. peranthus* involves an unoriented flight for searching a mate, made a pursuing flight to pursue upon detecting a mate, hovering and touching the perched female for courting, mounting and grasping the female, flew in tandem to seek a safe perch, relaxed below the female upon manage to insert his clasper into her genitalia, withdrew from pairs and flew away upon finishing copulation. The mate-locating strategy developed by male butterflies of *P. peranthus* is patrolling.

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