

## Diurnal foraging behavior of an aphidophagous hoverfly in an insectary plant patch

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### ABSTRACT

A series of foraging bouts of *Episyrphus balteatus* (DeGeer) females were observed and recorded throughout the day in phacelia (*Phacelia tanacetifolia* Bentham) patches. The dominant transitions of interplant movement, approaching and probing bouts were statistically more frequent in the morning than in the afternoon. In the morning, interplant movement of foragers was significantly longer and they were moving at higher places of phacelia patches. They approached and probed flowers considerably longer in morning compared to afternoon. A significant positive relationship between the number of open flowers in a patch and the patch residence time was observed in the morning. The proportion of females having swollen abdomen filled with phacelia pollen (68.2%) in the afternoon was significantly higher than that (31.82%) in the morning. In our study, *E. balteatus* females spent more time foraging in phacelia patches in morning compared to afternoon. Based on the results and by considering the dual resources requirements of a female hoverfly (flower foraging and searching for aphids to lay eggs), we will discuss the effective diurnal foraging of *E. balteatus* females in phacelia patches.

**Keywords:** Conservation biological control (CBC), *Episyrphus balteatus* (DeGeer), Foraging behavior, Insectary plant, *Phacelia tanacetifolia* (Bentham).

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### INTRODUCTION

Agricultural landscapes have been simplified at such a level that they no longer provide natural enemies with appropriate resources in time and space, resulting in the development of habitat management practices (Landis *et al.*, 2000). One of the habitat management practices is the provision of flower resources for enhancing conservation biological control (CBC) of insect pests by predators and parasitoids (e.g. White *et al.*, 1995; Irvin *et al.*, 2006). For instance, aphidophagous hoverflies larvae prey on aphids, while adults feed on flower resources such as pollen and nectar. Among the aphidophagous hoverflies, larvae of *Episyrphus balteatus* (DeGeer) (Diptera: Syrphidae) has a great potential as a biocontrol agent of aphids (Chambers and Adams, 1986). A single larva is able to feed on 660 to 1140 third-instar aphids during its development

(Tenhumberg and Poehling, 1995). However, adult hoverflies require flower resources for their survival and reproduction (Hogg *et al.*, 2011; van Rijn *et al.*, 2013; Van Rijn and Wäckers, 2016). Several flowering plant species which are experimentally evaluated as insectary plants have great potential to increase the adult hoverflies fitness. For instance, phacelia (*Phacelia tanacetifolia* Bentham) has been reported to enhance hoverfly, *E. balteatus* efficiency into agricultural and horticultural systems as this insectary plant increased its oviposition rate, lifetime fecundity and optimal reproductive potential (Laubertie *et al.*, 2012). This insectary plant strips along winter wheat field margins were found to be effective in enhancing *E. balteatus* population for controlling aphids (Hickman and Wratten, 1996). Furthermore, Amorós-Jiménez *et al.* (2014) reported that the diet of adult hoverflies

influences performance of their larvae. Moreover, aphidophagous females such as *E. balteatus* need to select suitable colony such as small and young colony consisting of nymphs and or fundatrices as their oviposition sites for the successful development of their offsprings (Kan, 1988). Therefore, the aphidophagous female adults have to forage efficiently for both flowers and aphids in a day. Hence foraging decision such as allocating time budget for each of flower and aphid resources is vital for the aphidophagous hoverflies. The time budget allocation can be governed by different extrinsic (e.g. time of the day, predation, physical structure of the environment, microclimate) and intrinsic factors (e.g. physiology, sensory and central nervous capacities) (Kramer, 2001). The few studies which have been carried out on hoverflies foraging for flower resources were based on the interaction between some of extrinsic and intrinsic factors of foraging behavior (Gilbert, 1985; Cowgill *et al.*, 1993a; Cowgill *et al.*, 1993b; Sutherland *et al.*, 1999; Pontin *et al.*, 2006; Yokoi and Fujisaki, 2009). However, to the best of our knowledge, the diurnal foraging pattern of female aphidophagous hoverflies in insectary flower patches linking their dual resource requirements (flower foraging and searching for aphids to lay eggs) has not been studied in detail. The aim of the present study is to evaluate the diurnal foraging behavior pattern of female *E. balteatus* in phacelia (*P. tanacetifolia*) plant patches. Based on the results, diurnal foraging activities of *E. balteatus* in phacelia (*P. tanacetifolia*) plant patches are illustrated and the diurnal foraging pattern of *E. balteatus* females is discussed considering their dual resource requirement.

## MATERIAL AND METHODS

### Study site, hoverfly and insectary plant

The study of foraging behavior of this hoverfly was conducted in an experimental field of 10 x 10 m<sup>2</sup> at University of Miyazaki (36° 14' N & 59 ° 40 'E) from April to May, 2014. During this period, the predominant hoverfly was *E. balteatus*. The foraging behavior study of this hoverfly species took place during the blooming period of Phacelia (*P. tanacetifolia*)

flowers. We planted this insectary plant in four plots of 3 x 2 m<sup>2</sup> and one strip 1.0 x 10 m<sup>2</sup> from south to north edge of the field. Each of the plots and strip is termed as 'patch' in the present study. In the experimental field, average temperature (°C) and relative humidity (%) were recorded to be 18.43 °C and 70.5 % respectively by the thermo recorder RTR-53 during the survey.

### Foraging bouts in the phacelia patches

We observed the foraging behavior of *E. balteatus* females following the focal sampling with continuous recording method adopted from Martin and Bateson (2007). We surveyed the phacelia patches from 7am to 5 pm on sunny days using voice recorder and stop watch to observe the foraging behavior of each individual as it entered the patch and continued until it left. The behavior was divided into foraging bouts which may be termed as a period of time spending in performing each of the foraging activities. We followed each individual during their residence in each of the patch. Whether any of the observed *E. balteatus* female re-entered in any of the phacelia patch was not considered in the present investigation as it is not possible to distinguish between each individual. The foraging bouts observed in phacelia patches were as follows: interplant movement (including hovering), approaching, probing, landing on the flower, landing on leaf, grooming and flying out. Brief description of these bouts are presented here. Interplant movement (including hovering) is the flight of *E. balteatus* from one plant to another phacelia plant in the patches. This bout includes hovering which is a slightly motioned flight. The pattern of interplant movement height of foraging *E. balteatus* in phacelia patches was determined by measuring the heights of 10 phacelia plants from the ground level in each patch. The measured height was used as a reference to estimate pattern of interplant movement height of *E. balteatus*. Approaching is the flight (hover) around <4cm of phacelia plants. Sometimes, they were touching the flower corolla. Probing is the feeding on pollen and nectar from the flower after landing

on the flower. We did not observe any nectar feeding by the *E. balteatus* females in the present study. These observation corroborate with the findings of White *et al.* (1995) in that the deep corolla of phacelia limits the nectar accessibility to hoverflies. So this insectary plant was used as pollen source for enhancement of hoverflies in their study. In our study, the probing by *E. balteatus* females is considered as the pollen feeding of phacelia flowers. Landing on the flower is the landing of *E. balteatus* on the flower without probing it. Landing on leaf is a stationary activity. Grooming is cleaning mouthparts or antennae and fly out is leaving of the patch.

We recorded the duration of each of the foraging bout. The sequence of each foraging bout was used to construct standard-style transition matrices. Such a sequence is then summarized in a two-way frequency matrix, with the preceding activities in the rows and the following activities in the columns followed after Van Der Heijden *et.al.*, 1990. For example, if we can assume that different activities can be distinguished then a frequency  $f_{ij}$  denotes the number of times that category  $i$  is followed by category  $j$ . These frequencies  $f_{ij}$  can be collected into a matrix  $F$  having  $I$  rows and  $I$  column. Thus we calculated the transition frequency for each foraging bout sequences from the transition matrices.

#### **Patch residence time**

Patch residence time was calculated as the time between entering and leaving patch (i.e. cumulative duration of all the foraging bouts). To measure whether there is a relation between patch residence time of *E. balteatus* and available flower resource in the phacelia patch, a number of inflorescences bearing open flowers were counted by randomly selecting 10 plants per patch throughout the blooming period of phacelia. Flower count was conducted in both morning and afternoon for every 3-5 days from the first week until the end of April, 2014.

#### **Abdomen status of *E. balteatus* foraging in the phacelia patches**

Upon feeding on the purple colored phacelia pollens in patches, the transparent abdomen of

*E. balteatus* becomes purple. Therefore, it was possible to use this purple colored abdomen as an indicator of the abdomen condition of the foragers. The abdomen status of *E. balteatus* was investigated by visually checking their abdomen, while they were foraging on the phacelia plants in the morning and in the afternoon. The abdomen of the observed hoverflies was termed as swollen if purple colored pollen filled the abdomen and flat when almost no pollen was present.

#### **Statistical analysis**

Tests for normality of each group were conducted using the Kolmogorov-Smirnov test. The statistically significant difference of the proportion of instances of the dominant transitions of main foraging bouts in the morning and the afternoon was evaluated by Chi-square test. Where the normality assumption was not met, the non-parametric Mann-Whitney U-test was used to compare the duration of interplant movement, approaching and probing between morning and afternoon. Interplant movement height of the foragers were checked by Kruskal-Wallis test followed by Dunn's test for multiple comparisons. A Chi-square test was used to performed the statistical significant difference of the proportion of individuals having flat and swollen abdomen between morning and afternoon during foraging. Moreover, linear regression analysis was performed to assess the relationship between patch residence time of *E. balteatus* and flowering trend of phacelia in the patches. Kruskal- wallis test followed by Dunn's test for multiple comparison test were analyzed using Graph pad prism software, version 6 and all other statistical analyses were performed with SPSS software, version 19.

## **RESULTS AND DISCUSSION**

### **Foraging bouts in the phacelia patches**

We observed the forging behavior of 126 and 90 *E. balteatus* females in the morning and in the afternoon in phacelia patches respectively. A total of 1709 sequences of the foraging bouts in the morning and 1116 in the afternoon of the foraging *E. balteatus* were observed. The matrices of transition sequences of the observed foraging bouts are given in Table 1

and Table 2 for the morning and the afternoon, while the matrices of transition frequencies of these bout sequences are presented in Table 3 for the morning and the afternoon. More than 70% of the transition sequences of the foraging bouts were interplant movement to approaching, approaching to probing and probing to approaching in both morning and afternoon (Table 1 and Table 2). Clearly, these transitions as well the foraging bouts included were predominant in our study. The probabilities of the dominant transitions of the three main foraging bouts are presented in Fig. 1. Transitions from interplant movement to approaching were significantly more frequent in the morning than in the afternoon ( $\chi^2 = 7.892$ ,  $p < 0.05$ ). However, approaching to probing transitions occurred less in the morning than in the afternoon ( $\chi^2 = 15.931$ ,  $p < 0.05$ ). Transitions from probing to the bout conducive to continued feeding (approaching to floral resources) were greater in the morning than in the afternoon ( $\chi^2 = 8.295$ ,  $p < 0.05$ ).

The mean interplant movement duration of 32.443s in the morning was significantly longer than the mean interplant movement duration of 16.163s in the afternoon (Mann-Whitney U-test,  $p < 0.05$ ; Fig.2). The heights of interplant movement varied significantly with time in the morning and in the afternoon (Kruskal-Wallis test,  $p < 0.05$ ; Fig.3). Considerably higher interplant movement was observed between 8:00 and 9:00am and between 9:00 and 10:00am than at 14:00 until 17:00 pm (Dunn's multiple comparison test). The interplant movement height of *E. balteatus* females from 8:00 until 11:00 am exceeded the average height of the phacelia plants (47.3 cm), while their heights of interplant movement were below the average height of the phacelia plants from 12:00 pm until 17:00 pm in afternoon. The mean approaching duration was 3.466s in the morning while in the afternoon it was 2.881s. This bout was significantly longer in the morning than in the afternoon (Mann-Whitney U-test,  $p < 0.05$ ; Fig. 4). The probing duration of *E. balteatus* females in the morning and in the afternoon is shown in Fig. 5). The mean

probing duration of 25.556s in the morning was significantly longer than that of 15.204s in the afternoon (Mann-Whitney U-test,  $p < 0.05$ ).

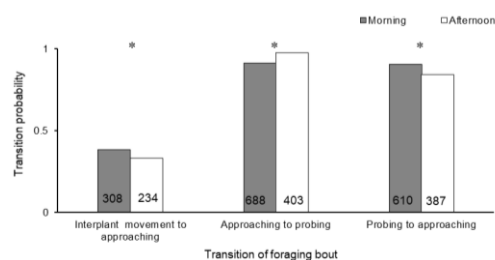
The females of *Episyrphus balteatus* were observed moving from one phacelia plant to another after entering the patch, and they approached flower resources during their flight in phacelia patches. Therefore, we divided the flying bout of *E. balteatus* in the phacelia patches into two foraging bouts namely, interplant movement and approaching. During the interplant movement in the phacelia patches, perhaps, *E. balteatus* females were searching mainly for flowers which is also supported by our result that interplant movement is transitioned in most of the cases to approaching phacelia flowers by the foraging *E. balteatus* females (Table 1 and Table 2). During approaching, *E. balteatus* females might assess phacelia flowers, because, as the results of transitions from approaching to probing, interplant movement and landing on flower during foraging by *E. balteatus* females (Table 1 and Table 2) indicating that after assessing the flowers through approaching, the observed foragers probed on the rewarding flower; however, they transitioned to interplant movement or landing on flower without probing when the flowers were unrewarding. In the foraging honey bees, approaching frequently led to probing of rewarding flowers while this transition did not occur in the unrewarding flowers (Schmitt and Bertsch, 1990). Both interplant movement and approaching includes hovering so longer interplant movement and approaching flight in the morning compared to that in the afternoon indicated longer hovering in the morning. Because, a typical feature of hoverflies flight is hovering for a longer period of time *Syrphus rebesii* is reported to be hovering 45% of its flying time (Golding *et al.*, 2001). This slightly motioned flight is an energy expensive flight (Kevan and Baker, 1973) which is utilized by the observed foragers for efficient searching and assessing. This contention is simply supported by the argument that a creature will not continue any costly activity if

it does not serve some advantage such as increased efficiency e.g Norberg (1977) found that efficiency of searching strategy is linked to energy expenditure. Therefore, longer interplant movement or prolonged searching and longer approaching or assessing of *E. balteatus* females in the morning compared to that in the afternoon (Figs. 2, 4) suggested that the observed foragers were searching and assessing actively for phacelia flowers in the morning in the phacelia patches.

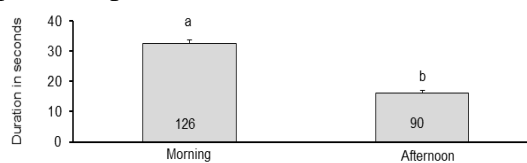
Foraging for flower seemed to be the most focused activity of *E. balteatus* females in the morning as along with longer searching and assessing, we also found longer probing of phacelia flowers by *E. balteatus* females in phacelia patches in the morning compared to that in the afternoon. This also corroborates with the finding of Gilbert (1985) in that among the observed diurnal foraging bouts, feeding (probing) was the longest for foraging *E. balteatus* in flower patches. For foraging hoverfly, *Melanostoma fasciatum* (Macquart), the focal foraging activity in flower patches is probing (Pontin *et al.*, 2006). However, the frequency of approaching to probing was lower in the morning compared to that in the afternoon and this may be explained by longer feeding which in turn caused lowered frequency resulting in the efficiency of foraging in the morning. The trade-off between the duration and the frequency of flower foraging was observed during nectar feeding by bumble bees (Jones *et al.*, 1998). Nevertheless, more frequent transitions of the observed foragers from probing to approaching (Tables 1 and Table 2) the in phacelia patches in morning compared to that in the afternoon which indicated an extensive feeding by the observed foragers because approaching in most of the cases lead to probing more frequently in the morning compared to in afternoon. The dominant transitions and duration results of the major foraging bouts such as interplant movement, approaching and probing are clear indications that the observed foragers were more inclined in phacelia patches in the morning and subsequently, *E. balteatus*

females stayed longer in the morning in the phacelia patches.

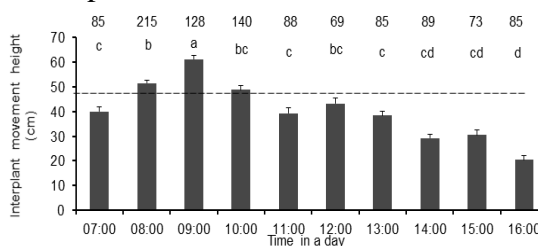
**Fig. 1.** The probabilities of the dominant transitions of the three dominant foraging bouts of *E. balteatus* in the phacelia patches



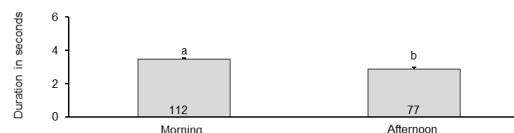
**Fig. 2.** Diurnal changes in the duration of interplant movement of *E. balteatus* in phacelia patches



**Fig. 3.** Diurnal changes in the interplant movement height of *E. balteatus* in phacelia flower patches



**Fig. 4.** Diurnal changes in the duration of approaching of *E. balteatus* in phacelia patches



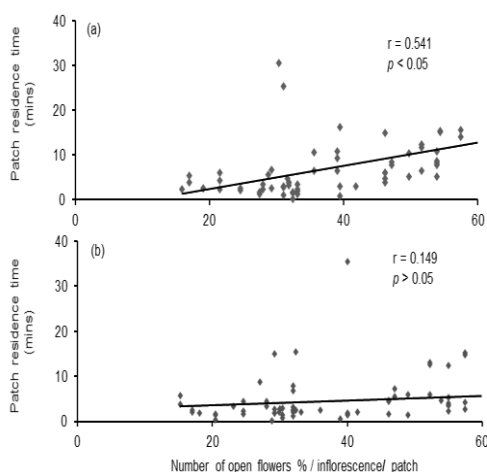
**Figure 5.** Diurnal changes in the duration of probing of *E. balteatus* in phacelia patches.



### Patch residence time

The relationship between patch residence time and the flower resources both in the morning and in the afternoon are presented in Fig. 6. The patch residence of *E. balteatus* was significantly positively correlated with percentage of open flowers inflorescence patch of phacelia in the morning ( $r = 0.541$ ,  $p < 0.05$ ; Fig. 6a). However, no such relationship was found in that in the afternoon ( $r = 0.149$ ,  $p > 0.05$ ; Fig. 6b). The average patch residence time was 7.6 min in the morning and in the afternoon it was averaged 4.6 min. Generally, flower foragers choose to stay longer in a patch of high density of flowers i.e. a patch for high reward. We found similar positive relationship between flower density and patch residence of the observed foragers in the morning, while in the afternoon, such response was not found.

**Figure 6.** Relationship between *E. balteatus*'s patch residence time and percent of number of open flowers in an inflorescence per patch of phacelia.



### Abdomen status of *E. balteatus* foraging in the phacelia patches

The abdomen condition of the foraging *E. balteatus* differed significantly ( $\chi^2 = 9.82$ ,  $p < 0.05$ ) in the morning and in the afternoon. The proportion of individuals having swollen abdomen with phacelia pollen was 31.82% in morning while the 68.2% in the afternoon. The difference in the foraging pattern of *E. balteatus* females between morning and afternoon may be explained by our findings on the abdomen condition and the interplant

movement height (Fig. 3) of these observed foragers during foraging in the phacelia patches. Firstly, the abdomen condition of the observed foragers revealed that most of the *E. balteatus* females entered phacelia patches with flat abdomen in the morning while in the afternoon they had swollen abdomen. This result indicates that in the morning, *E. balteatus* females were starved and as a result these hungry foragers must stay longer in phacelia patches to forage on the flowers more while in afternoon the observed foragers were already full fed and as a result stayed shorter in the phacelia patches. Secondly, *E. balteatus* females might shift to other activity rather than flower foraging in the afternoon. This shifting may be revealed by our foraging height result that *E. balteatus* females were foraging in the patches at higher places in the morning and in lower regions in the afternoon in the phacelia patches. Sutherland *et al.* (2001) found *E. balteatus* females in greater numbers in the field margins where no flower resources were present, suggesting that they might be searching for aphids in the herbaceous host plants in those margins. The change in the interplant movement height between morning and afternoon might be suggesting shifting of foraging resources i.e. searching for phacelia flowers in the morning to aphid colony in the afternoon. In the foraging damselflies, the diurnal shift in the habitat utilization from searching for prey to reproduction has been reported by Hykel *et al.* (2017).

Although it has already been reported that the matured hoverfly females need to travel between the flowering plants and crops to forage for flowers and searching for aphids (VanRijn *et al.*, 2006), the diurnal behavior pattern of the females which need to forage for the both resources have not been revealed. The present findings are the first to suggest that there are variations in the diurnal behavior pattern of *E. balteatus* females between morning and afternoon in the phacelia patches. We could conclude from our study that aphid ophagous female hoverfly, *E. balteatus* foraged for phacelia flowers longer to maximize energy intake in the morning, while

**Table 1.** Matrices of foraging bouts transitions of *E. balteatus* in phacelia in morning

Preceding behavior	Following behavior							Total
	Interplant movement	Approaching	Probing	Land on flower	Land on leaf	Groom on leaf	Fly out	
Interplant movement	0	118	0	0	57	36	97	308
Approaching	54	0	629	5	0	0	0	688
Probing	55	551	0	0	0	0	4	610
Land on flower	3	0	0	0	0	0	2	5
Land on leaf	46	0	0	0	0	0	11	57
Groom on leaf	29	0	0	0	0	0	12	41
Fly out	0	0	0	0	0	0	0	0
Total	187	669	629	5	57	36	126	1709

**Table 2.** Matrices of foraging bouts transitions of *E. balteatus* in phacelia in afternoon

Preceding behavior	Following behavior							Total
	Interplant movement	Approaching	Probing	Land on flower	Land on leaf	Groom on leaf	Fly out	
Interplant movement	0	77	0	0	66	19	72	234
Approaching	3	0	393	7	0	0	0	403
Probing	58	326	0	0	0	0	3	387
Land on flower	5	0	0	0	0	0	2	7
Land on leaf	66	0	0	0	0	0	0	66
Groom on leaf	6	0	0	0	0	0	13	19
Fly out	0	0	0	0	0	0	0	0
Total	138	403	393	7	66	19	90	1116

in the afternoon it spend less time in flowers and in turn might spent time in activities related to reproductive success i.e. one of the measured values of fitness which is supported by the argument that optimal utilization of time and energy of any forager is to continue any activity as long as the resulting gain in time spent for per unit of food exceeds the loss. This also fits with one of the assumption of the optimal foraging theory that fitness of any forager heavily relies on its behavior (Pyke, 1984). Nevertheless, as the aphidophagous hoverflies forage for both resources in a day, our present findings have important implication in designing CBC of aphids with hoverflies that we have to emphasize on how to reduce the foraging costs of the aphidophagous hoverflies for both flower sources and breeding site. Therefore, we suggest that insectary plants are required to be planted adjacent to the crop plants for good control of aphids by hoverflies.

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