TERRITORIAL AND MATING BEHAVIOURS OF TWO FLOWER-BREEDING DROSOPHILA SPECIES, D. elegans AND D. gunungcola (DIPTERA: DROSOPHILIDAE) AT CIBODAS, WEST JAVA, INDONESIA

Awit Suwito1, Takahide A. Ishida2, Kouhei Hattori2 and Masahito T. Kimura2

1 Zoology Division (Museum Zoologicum Bogoriense), Research Center for Biology-LIPI, Cibinong 16911, Indonesia
2 Graduate School of Environmental Earth Science, Hokkaido University, Sapporo, Hokkaido 060-0810, Japan

ABSTRACT

Drosophila elegans and D. gunungcola are closely related flower-breeding species, mainly exploiting Ipomoea flowers. Here, we report their territorial and mating behaviours in Ipomoea indica flowers at Cibodas, West Java, Indonesia. Flies of both species were almost absent from newly opened flowers in the early morning, and the number of individuals in flowers increased thereafter. Territorial males of these species fought against intruders of both species, but the frequency of fighting was significantly lower when intruders were heterospecific. Territorial males usually showed intensive courtship to conspecific females, but rarely to heterospecific females. Intensive courtship to conspecific females often led the females to desert the flowers, possibly because male’s courtship was annoying. The frequency of desertion was lower in D. gunungcola than in D. elegans. This difference may be attributable to the difference in sexual size dimorphism. Thorax size was smaller in males than in females in D. gunungcola but did not differ between the sexes in D. elegans, and therefore male courtship may be less annoying for females in D. gunungcola than in D. elegans. Copulation duration was shorter in D. elegans than in D. gunungcola, while the unreceptive period of females after copulation is shorter in D. elegans than in D. gunungcola.

Key words: body size, copulation, courtship, Drosophila, sexual size, dimorphism, territoriality

INTRODUCTION

Territorial and courtship behaviours have received much attention from evolutionary biologists since the evolution of these behaviours often shows complicated features. This is partly because these behaviours are subjected not only to natural selection, but also to sexual selection. For example, traits or behaviours attractive to individuals of the opposite sex could impose some cost for survival. The most fascinating examples are
seen in birds or mammals, but insects also show a variety of remarkable
traits or behaviours (Thorhill & Alcock 1983, Choe & Crespi 1997).
Even in Drosophila species, most of which are believed to show simple
behaviours, some are reported to exhibit complicated territorial and sexual

In this paper, we report territorial and mating activities of
Drosophila elegans Bock & Wheeler and D. gunungcola Sultana, Kimura
& Toda in Cibodas, West Java, Indonesia, to understand their behavioural
adaptations. These two species are closely related and mainly exploit
Ipomoea flowers for breeding (Lemeunier et al. 1986, Sultana et al. 1999,
elegans occurs from low to high altitudes in tropical and subtropical Asia,
while D. gunungcola has been reported only from high altitude areas of
Indonesia (Lemeunier et al. 1986, Okada & Carson, 1982, Sultana et al.
1999, Suwito et al. 2002). Our previous study revealed that males of
D. elegans hold mating territory on individual Ipomoea flowers (Kimura
& Hirai 2001), but little is known on field ecology of D. gunungcola.

MATERIALS AND METHODS

Field observation
The study was carried out at Cibodas (about 1300 m in altitude),
In the study area, Ipomoea indica (Burman) grows in bushes and around
houses, and D. elegans and D. gunungcola are the only Drosophila
species breeding on flowers of this plant. Individual flowers of I. indica
remain open for a single day; they open at dawn, close in the afternoon or
evening, and fall off on the ground at midnight or next morning.

Territorial and mating behaviour of flies was observed on I. indica
flowers growing in a bush (3 x 15 m) surrounded by open-fields and on a
fence of the Botanical Garden. Flowers occupied by single D. elegans or
D. gunungcola males were selected for observation. When other males or
females of these species arrived at the flowers, behavioural interactions
between the resident males and the arrivals were observed. The
observation was made during daytime (usually from 8:00 to 17:00). In
addition, flies were collected from flowers, preserved in 70 % alcohol and
measured for thorax length.

Laboratory experiments
The experimental stocks of D. elegans and D. gunungcola originated from
10-20 females collected at Cibodas in January 2001, maintained at 20°C
(approximate mean temperature at Cibodas) and used for experiments
within six months after the collection. To measure duration of copulation, eight-day-old virgin females and males were placed in vials. When they mated, duration of copulation was measured. In addition, females of these species were examined to see how often they re-mate. Eight-day-old females were mated with conspecific males, and then maintained in vials with *Drosophila* medium. Twelve days after the first mating, five females were placed in a vial with eight-day-old males, and monitored whether they re-mate or not for 20 min. In total, 25 females were examined for each species.

In addition, experimental flies were raised on cornmeal-malt medium at 20°C with low densities (10-20 larvae per 10 ml medium and 10-20 adult flies per 100 ml vial) and were examined for adult body weight and thorax length 14 days after eclosion.

**RESULTS**

**Daily activity**

Fig. 1 shows daily changes of the number of flies per flower at the bush site on December 19 and 20, 1999. *Drosophila elegans* was more abundant than *D. gunungcola* at this site. Flies of both species were almost absent from newly opened flowers at dawn (6:00), and the number of individuals on flowers increased thereafter. Although flowers closed in the evening, some flies remained on them until the next day. In one-day-old closed flowers, flies were observed to exhibit feeding and courtship behaviours as well as in open flowers (data not shown).

![Figure 1. Daily activity of the number of flies usually more than 30 flowers were checked at each time on *Ipomoea indica* flowers on December 19 and 20, 1999 at Cibodas, Indonesia](image_url)
Male-male interactions

When a male intruded a flower on which a conspecific male already held territory, they almost always fought and either one was expelled from the flower (Table 1). In this observation, it was not determined which of residents or intruders were expelled from the flower because they were often indistinguishable after the encounter.

In cases where a male intruded a flower on which a heterospecific male held territory, battle occurred in 29 cases out of 42 (Table 1). The frequency of occurrence of fight was significantly lower in the heterospecific encounter than in the conspecific encounter ($\chi^2$-test, $P<0.001$). In these cases, intruders were expelled in most cases.

Table 1. Consequences of male intrusion to an occupied flower (the number of occasions for each event are given)

<table>
<thead>
<tr>
<th>Occupant (male)</th>
<th>Intruder (male)</th>
<th>Event</th>
<th>Either occupant or intruder was driven out</th>
<th>No or weak Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. elegans</td>
<td>D. elegans</td>
<td>36</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>D. gunungcola</td>
<td>D. gunungcola</td>
<td>41</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>D. elegans</td>
<td>D. gunungcola</td>
<td>12*</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>D. gunungcola</td>
<td>D. elegans</td>
<td>17**</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

*D. gunungcola was driven out in 11 cases and D. elegans was driven out in one case

**D. elegans was driven out in all cases

Male-female interactions

When a female came to a flower on which a conspecific male held territory, the female usually received persistent courtship from the territorial male (Table 2): the females run away from the male, and the male chased. In consequence, females often deserted the flowers. The frequency of desertion of females was significantly higher in D. elegans than in D. gunungcola ($\chi^2$ test, $P=0.03$); i.e., 70% (14 out of 20) of females that received courtship left the flowers within 20 min in D. elegans, while 35% (7 out of 20) left the flowers in D. gunungcola. In spite of intensive courtship of territorial males, copulation was observed only twice in D. elegans.

When a female came to a flower on which a heterospecific male held territory, the male rarely showed courtship behaviour (Table 2).
Mating

Duration of copulation was much shorter in *D. elegans* (12.7 min on average) than in *D. gunungcola* (51.4 min) (Table 3). When females were placed with conspecific males 12 days after the first mating, 68% of *D. elegans* females re-mated within 20 min, but none of *D. gunungcola* females re-mated (Table 3).

Table 3. Duration of copulation and the percentage of females that remated when tested 12 days after the first mating

<table>
<thead>
<tr>
<th>Duration of copulation (min)</th>
<th>Remating (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><em>D. elegans</em></td>
<td>12.7±2.3 (10)</td>
</tr>
<tr>
<td><em>D. gunungcola</em></td>
<td>51.4±18.1 (10)</td>
</tr>
</tbody>
</table>

Numbers in parentheses refer to the number of individuals used

Body size

Table 4 shows thorax length of flies collected in the field in 1999, 2001 and 2002 and thorax length and body weight of laboratory-reared flies. Body size (thorax length and body weight) was significantly larger in *D. gunungcola* than in *D. elegans* at least in females (ANOVA, \(P<0.01\)). Thorax length was significantly larger in females than in males in *D. gunungcola* (ANOVA, \(P<0.01\) for field-collected and laboratory reared individuals), but there was no significant difference between the
sexes in \textit{D. elegans} (ANOVA, \(P=0.98\) for field collected individuals and \(P=0.37\) for laboratory-reared individuals). Laboratory reared flies were larger than field-collected ones in both species, probably because of better nutritional and environmental conditions. Body weight was significantly larger in females than in males for both species (ANOVA, \(P<0.01\)).

\textbf{Table 4.} Thorax length and body weight (mean±SD) of field-collected and laboratory-reared flies

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field-collected flies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thorax length (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(D. \text{ elegans})</td>
<td>0.87±0.07 (90)</td>
<td>0.87±0.08 (98)</td>
</tr>
<tr>
<td>(D. \text{ gunungcola})</td>
<td>0.86±0.08 (28)</td>
<td>0.94±0.09 (23)</td>
</tr>
<tr>
<td>Laboratory-reared flies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thorax length (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(D. \text{ elegans})</td>
<td>1.00±0.03 (10)</td>
<td>1.01±0.03 (11)</td>
</tr>
<tr>
<td>(D. \text{ gunungcola})</td>
<td>1.05±0.03 (14)</td>
<td>1.10±0.03 (15)</td>
</tr>
<tr>
<td>Body weight (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(D. \text{ elegans})</td>
<td>0.83±0.08 (12)</td>
<td>1.09±0.10 (12)</td>
</tr>
<tr>
<td>(D. \text{ gunungcola})</td>
<td>1.11±0.18 (16)</td>
<td>1.40±0.17 (16)</td>
</tr>
</tbody>
</table>

\textbf{DISCUSSION}

\textit{Drosophila elegans} and \(D. \text{ gunungcola}\) males hold mating territory on \textit{Ipomoea} flowers and intensively defend it against intruding males. Battles to defend territory were observed not only between conspecific males, but also between heterospecific males. In the conspecific battles, it was not determined which one was the winner since they could not be distinguished after their encounter. In heterospecific battles, occupants usually expelled intruders, suggesting superiority of occupants in territorial defense, as has been reported in many other insects (Davies 1978, Thornhill & Alcock 1983).

It is not known why males of these species often fight against heterospecific males. Fight against intruders may not be so costly, and then ability to discriminate between heterospecific and conspecific males may not be so advantageous for males. On the other hand, they can discriminate between heterospecific and conspecific females, suggesting that species-specific signals have evolved at least in females. Ability to discriminate between heterospecific and conspecific females may be more important since it directly affects the production of offspring.
In insects with male mating territoriality, males are often larger than females (Thornhill & Alcock 1983). Selection would have acted to increase the male body size since larger males are usually superior in territorial defense (Borgia 1980, 1982, Spieth 1981, Thornhill & Alcock 1983, Kimura & Hirai 2001). However, thorax size was larger in females than in males in *D. gunungcola*, although the size was not different between the sexes in *D. elegans*. Counter selection may be operating. In insects, females are often heavier than males due to egg loading. In addition, females are expected to be under selection to increase fecundity. As a result, females become much heavier than males, and then they need larger power to fly. This situation may lead to the evolution of female-biased thorax-size dimorphism (Reeve & Fairbairn 1999). The difference in the sexual size dimorphism between *D. gunungcola* and *D. elegans* may be attributable to the difference in the intensity of male-male interactions. Usually, *D. elegans* occurs at higher densities than *D. gunungcola* (Kimura & Hirai 2001, Suwito et al. 2002). Therefore, *D. elegans* males may be subjected to more intense selection for increasing body size. However, selection pressures acting on the evolution of sexual size dimorphism are usually complicated and have been little understood (Fairbairn 1990, Sih & Krupa 1992, Fairbairn & Preziosi 1994, Blanckenhorn et al. 1995, Arnqvist et al. 1996, Rowe & Arnqvist 1996, Arnqvist 1997, Rutowski 1997). Further study is needed on this topic.

Territorial males of both species usually showed persistent courtship to conspecific females, but copulation seldom occurred probably because most females were non-virgin. As a result of intensive courtship, females often deserted flowers; courtship of territorial males would be annoying for females. The rate of desertion of females was significantly higher in *D. elegans* than in *D. gunungcola*. This difference may be related to the difference in the relative size of females. In *D. gunungcola*, males have smaller thorax than females, and therefore their courtship may be less annoying for females.

Copulation duration was much shorter in *D. elegans* than in *D. gunungcola*, while the unreceptive period of females after copulation is shorter in *D. elegans* than in *D. gunungcola*. The amount of sperm transferred at copulation may be smaller in *D. elegans* due to shorter copulation, and then sperm may be depleted earlier in *D. elegans*. In *D. elegans*, it is also known that copulation duration is longer in the black form occurring in subtropical regions than in the present brown form occurring in tropical regions, although not as long as in *D. gunungcola* (Hirai & Kimura 1999). Prolongation of copulation may be associated with the adaptation to cooler climates.
ACKNOWLEDGMENTS

We thank H. Katakura, G. Takaku, S. Hartini, S. Kahono, W. A. Noerdjito for their help and encouragement during the study. This work was supported in part by Grant-in-Aid for International Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (No. 11691161) and by Japan Society for the Promotion of Science (Core University Program entitled “Environmental management of tropical wetland ecosystem in Southeast Asia”).

REFERENCES


Received: September 17, 2012
Accepted: October 5, 2012
INSTRUCTIONS FOR AUTHORS

1. General. - Manuscripts to be published in TREUBIA must be written in English, typed in Times New Roman font 12 and submitted in triplicate to the editors of TREUBIA, Division of Zoology, Research Center for Biology, Widyasatwaloka, Jl. Raya Jakarta-Bogor Km. 46, Bogor 16911, Indonesia. They should not be offered for prior or simultaneous publication elsewhere. Concise writing and omission of unessential material are recommended. After acceptance, a soft copy of the manuscript files should be sent to the editors of TREUBIA. Further correspondence can be conducted through email address: treubia@gmail.com

2. Text. - The text must be typed, double spaced throughout. Captions of tables, figures, and plates should be inserted where you want them to be inserted, or listed at the end of the manuscript. All numbers under 10 and any number forming the first word of a sentence must be spelled out. Year should be completely written. Scientific names should all be italicized. It is recommended to use metric measurements in abbreviation (e.g. kg, cm, ml).

3. Citation. - References are to be cited in the text by the author’s surname and year of publication, e.g. (Calder 1996, Carpenter 2005, Somadikarta 1986). For two authors, both names should be cited: e.g. (Ackery & Vane-Wright 1984). For three or more authors, only the first author is given followed by et al., e.g. (Foster et al. 2002).

4. Abstract. - Except for short communications, articles should be accompanied by an abstract not to exceed 250 words which clearly states the essence of the paper. Key words should be mentioned following the abstract.

5. Acknowledgements, if any, should be placed preceding the list of references

6. References. - List of references should be in alphabetical order by the first or sole author’s surname. Journal references should include author’s surname and initials, year of publication, title of the paper, full title of the journal (typed in italic), volume number (typed in bold) and inclusive page numbers. Book references should include author’s surname and initials, year of publication, title of the book (typed in italic) or/and title of the chapter and editor (if part of a book), publisher, city of publication, and page numbers.

For example:


7. Proofs and reprints. - Final proofs are given to the first or sole author for correction and approval. Twenty five reprints are supplied free of charge. Joint authors will have to divide these copies among them at their discretion. Additional reprints can be furnished at cost, the order should be placed before the final printing.
<table>
<thead>
<tr>
<th>CONTENT</th>
<th>PAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anang Setiawan Achmadi, Ibnu Maryanto and Maharadatunkamsi. Systematic and descriptions of new species of Maxomys (Muridae)</td>
<td>1</td>
</tr>
<tr>
<td>Kazuma Matsumoto, Woro A. Noerdjito and Endang Cholik. Butterflies recently recorded from Lombok</td>
<td>27</td>
</tr>
<tr>
<td>Michael S. Engel. The honey bees of Indonesia (Hymenoptera: Apidae)</td>
<td>41</td>
</tr>
<tr>
<td>Maharadatunkamsi. Morphological variation in Chironax melanocephalus (Chiroptera: Pteropodidae) from Indonesia and description of new subspecies</td>
<td>51</td>
</tr>
<tr>
<td>Djunijanti Peggie. A list of the butterflies of Ujung Kulon National Park, Java, Indonesia</td>
<td>67</td>
</tr>
<tr>
<td>Awit Suwito, Takahide A. Ishida, Kouhei Hattori and Masahito T. Kimura. Territorial and mating behaviours of two flower-breeding Drosophila species, D. elegans and D. gunungcola (Diptera: Drosophilidae) at Cibodas, West Java, Indonesia</td>
<td>77</td>
</tr>
</tbody>
</table>