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

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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 (Thornhill & 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 behaviours (Spieth 1981, Grimaldi & Fenster 1989, Burla 1990).

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, Hirai *et al*. 2000, Kimura & Hirai 2001, Suwito *et al*. 2002). *Drosophila 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), west Java, Indonesia, in December 1999, January 2001, and January 2002. 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).

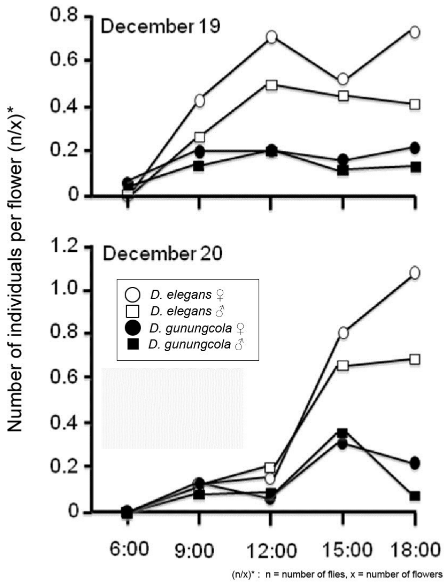

Figure 1. Daily activity of the number of flies usually more than 30 flowers were checked at each time on *Ipomoea indica* flowers on December 19 and 20, 1999 at Cibodas, Indonesia

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 (χ^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)

Occupant (male)	Intruder (male)	Event: Either occupant or intruder was driven out	Event: No or weak Interaction
D. elegans	*D. elegans*	36	0
D. gunungcola	*D. gunungcola*	41	3
D. elegans	*D. gunungcola*	12*	5
D. gunungcola	*D. elegans*	17**	8

**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* (χ^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).

Table 2. Consequences of female arrival to an occupied flower (the number of occasions for each event are given)

Occupant (male)	Arrival (female)	Event		
		Female left flower within 20 min after arrival due to persistent courtship	Continuous or intermittened courtship lasted longer than 20 min	No or weak Interaction
D. elegans	*D. elegans*	14	6	7
D. gunungcola	*D. gunungcola*	7	13	9
D. elegans	*D. gunungcola*	1	-	5
D. gunungcola	*D. elegans*	1	1	7

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

	Duration of copulation (min) (mean±SD)	Remating (%)
D. elegans	12.7±2.3 (10)	68 (25)
D. gunungcola	51.4±18.1 (10)	0 (25)

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 *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).

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

	Male	Female
Field-collected flies		
Thorax length (mm)		
D. elegans	0.87±0.07 (90)	0.87±0.08 (98)
D. gunungcola	0.86±0.08 (28)	0.94±0.09 (23)
Laboratory-reared flies		
Thorax length (mm)		
D. elegans	1.00±0.03 (10)	1.01±0.03 (11)
D. gunungcola	1.05±0.03 (14)	1.10±0.03 (15)
Body weight (mg)		
D. elegans	0.83±0.08 (12)	1.09±0.10 (12)
D. gunungcola	1.11±0.18 (16)	1.40±0.17 (16)

DISCUSSION

Drosophila elegans and *D. gunungcola* males hold mating territory on *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.

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