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# Effects of Copulation Duration on Fertility and Sexual Receptivity of Females in *Drosophila elegans*

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**ABSTRACT**—In *Drosophila elegans*, copulation duration is shorter in the brown morph (about 11 min) than in the black morph (25–35 min). In this study, it appeared that sperm transfer also occurred at an earlier stage of copulation in the brown morph than in the black morph and both sexes were responsible for the control of sperm transfer. In the HK (brown morph) strain, number of eggs produced per female decreased to some extent when copulation was interrupted 3 min after the start of copulation. In the OH (black morph) strain, the rate of sperm transfer was low when copulation was interrupted 3 or 5 min after the start of copulation. Thus, shortened (3–5 min) copulation was disadvantageous in this species. However, adaptive significance of prolonged (>10 min) copulation of the black morph was not apparent in the present experiments. Females of the HK strain recovered receptivity earlier than those of the OH strain irrespective of male-type in the previous mating: i.e., the former will mate more times in their lives than the latter. The adaptive significance of multiple mating was discussed.

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## INTRODUCTION

There is considerable variation in copulation duration among *Drosophila* species (Grant, 1983), but little is known on the adaptive background underlying this variation. In insects whose females are continuously receptive, males are expected to gain benefits by prolonging copulation because it enables them to displace sperm within the females, to transfer materials such as seminal fluids or spermatophore which may prevent sperm displacement, or to guard the females from competitor males (Parker, 1970; Thornhill and Alcock, 1983). However, females of many *Drosophila* species become unreceptive for a certain period after mating. In such species, prolonged copulation would be beneficial for both sexes if it raises the fertility of the female and also for the males if it inhibits remating (i.e., retards the recovery of receptivity) of the females. Here, we studied effects of copulation duration on fertility and sexual receptivity of females in *Drosophila elegans* Bock and Wheeler to understand the adaptive significance of copulation duration. Two morphs, brown and black, are known in this species: the brown morph is recorded from Philippines, southern China, Indonesia and Papua New Guinea, while the black one is from Ryukyu islands and Taiwan (Bock and Wheeler, 1972, Okada and Carson, 1982; Hirai and Kimura, 1997). Hirai *et al.* (1999) reported that copulation duration is shorter in the brown morph (about 11 min) than in

the black morph (25–35 min).

## MATERIALS AND METHODS

### Flies

The experimental strains originated from 10–30 females collected in Hong Kong (HK, China), Okinawa (OH, Japan) and Taipei (TW, Taiwan). The first was a brown morph strain and the last two were black morph ones. These strains were kept on cornmeal-malt medium under 15 hr light-9 hr dark at 23°C for few years before experiments. Mean copulation duration was 11.1 min in the HK strain, 34.8 min in the OH strain (Hirai *et al.*, 1999) and 26.3 min in the TW strain (per. observation).

In this study, the TW strain was used only to examine the timing of sperm transfer, while the HK and OH strains were used for all experiments. Experimental flies were reared on cornmeal-malt medium under 15 hr light-9 hr dark at 23°C and used for experiments 8–10 days after eclosion, because the egg production reaches plateau approximately at this age (pers. observation).

### Sperm transfer

Eight-day-old females were placed in vials with males and allowed to copulate. They were forced to separate 1, 3, 5 and/or 10 min after the start of copulation by shaking the vials, and examined for the occurrence of sperm in seminal receptacles under a microscope. Twenty females were examined for each datum point.

### Remating

A pair of 8–10 day-old virgin female and male was aspirated into a glass vial containing food medium without anesthesia and allowed to copulate. They were allowed to end copulation naturally or forced to separate 1, 3 or 10 min after the start of copulation. Then, the female was examined for remating immediately after the first copulation and also 1, 3, 5, 10 and 15 day(s) after the first copulation; the female was placed in a glass vial with two 8–10 day-old males and

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observed for mating for 1 hr (when copulation did not occur within 1 hr, the female was separated from the males and individually maintained to the next test). About 30 females were tested for each treatment.

In addition, remating was examined 10 days after the first mating. Eight-day-old virgin females and males were introduced into vials. They were allowed to end copulation naturally or forced to separate 10 min after the start of copulation (in the OH strain). Ten days after the first mating, the females were placed with two 8–10 day-old males in glass vials and observed for mating for 3 hr. About 30 females were tested for each treatment.

### Egg production

Eight-day-old virgin females and males were introduced into vials. They were allowed to end copulation naturally or forced to separate 3 or 10 min after the start of copulation. These females were individually transferred to new vials every day, and number of eggs laid on old medium were examined for 20 days. In addition, hatchability of eggs was examined for each female: the check of hatchability was made after eggs were left on medium for a day, because eggs of this species hatch within 24 hr after oviposition at 23°C if they are alive. This experiment was made under two different conditions, solitary or with two males that were deprived of copulatory potency by fixing the genital area with quick-drying glue (ARON ALPHA, TOAGOSEI Co. Ltd.), because our preliminary experiment suggests a possibility that the presence of males affects the fertility of females. About 10 females were used for each treatment.

### Statistical analysis

Rate of sperm transfer and receptivity of females were compared by  $\chi^2$ -test (CT). The egg production was compared by the Scheffé's method after the ANOVA (SA) and the *t*-test without correction (TT), and hatchability of eggs was compared by Kruskal-Wallis (KW) because hatchability of eggs did not always show normal distribution among the females. The SPSS package was used for these statistical analyses.

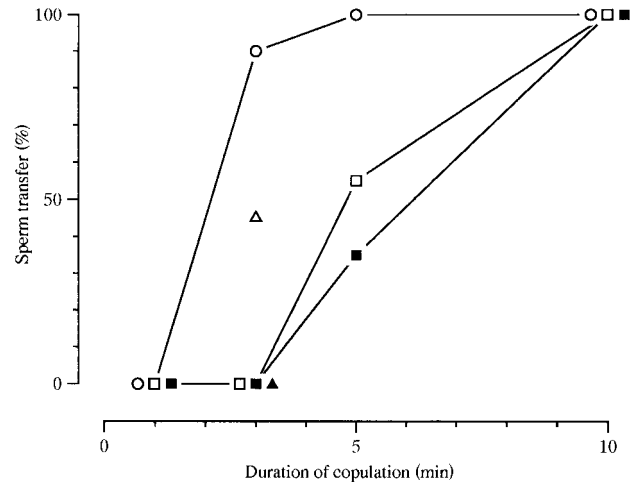
## RESULTS

### Sperm transfer

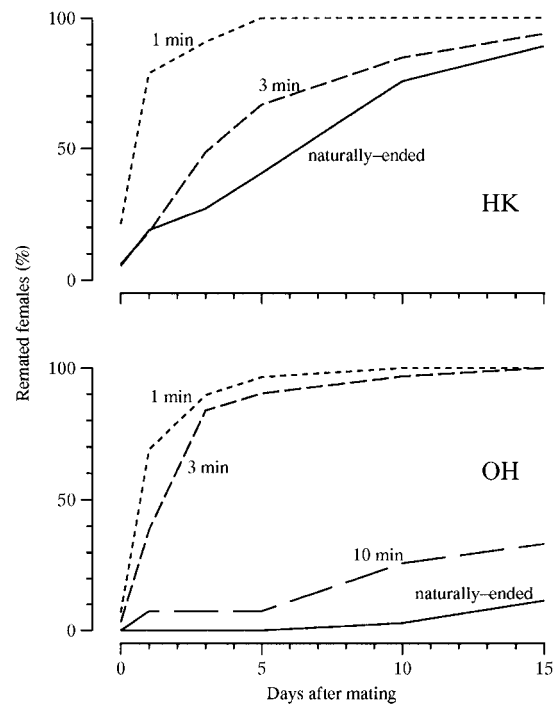
Fig. 1 shows the rate of sperm transfer when copulation was interrupted 1, 3, 5 or 10 min after the start of copulation. The sperm transfer took place earlier in the HK strain than in the OH and TW strains: 90% (18 of 20) males of the HK strain transferred sperm within 3 min, but males of the OH and TW strains did not in 3 min. When HK males were mated with OH females, about a half of them (9 of 20) transferred sperm to females within 3 min, the rate significantly lower than the rate when they were mated with HK females but higher than the rate when OH males were mated with OH females (CT,  $P < 0.01$ ). This indicates that both sexes were responsible for the control of sperm transfer. When OH males were mated with HK females, sperm transfer did not take place within 3 min.

### Remating

Fig. 2 shows cumulative percentage of remated females in the HK and OH strains. The female became unreceptive or at least reluctant to copulate immediately after copulation even when copulation was interrupted before sperm transfer (i.e., 1 min after the start of copulation in the HK strain, and 1 or 3 min after copulation in the OH strain). However, most females



**Fig. 1.** Rate of sperm transfer in the HK (○), OH (□) and TW (△) strains of *D. elegans* and in inter-morph combinations, between OH females and HK males (◻) and between HK females and OH males (◼), when copulation was interrupted 1, 3, 5 and/or 10 min after the start of copulation.



**Fig. 2.** Cumulative percentage of remated females in the HK and OH strains of *D. elegans*. Females which were forced to separate one, three, and/or 10 min after the start of copulation or allowed to end copulation naturally in the first mating were examined for remating immediately and 1, 3, 5, 10 and 15 days after the first mating.

soon recovered receptivity when copulation was interrupted before sperm transfer. When females were forced to separate after sperm transfer (i.e., 3 min after copulation in the HK strain and 10 min after copulation in the OH strain) or allowed to end copulation naturally, 70–80% of them remated within 15 days in the HK strain, but only 20–30% remated in the OH

strain. In the HK strains, receptivity recovered somewhat earlier in females which were forced to separate 3 min after the start of copulation than those which ended copulation naturally, but the difference was not significant (CT,  $P>0.05$ ).

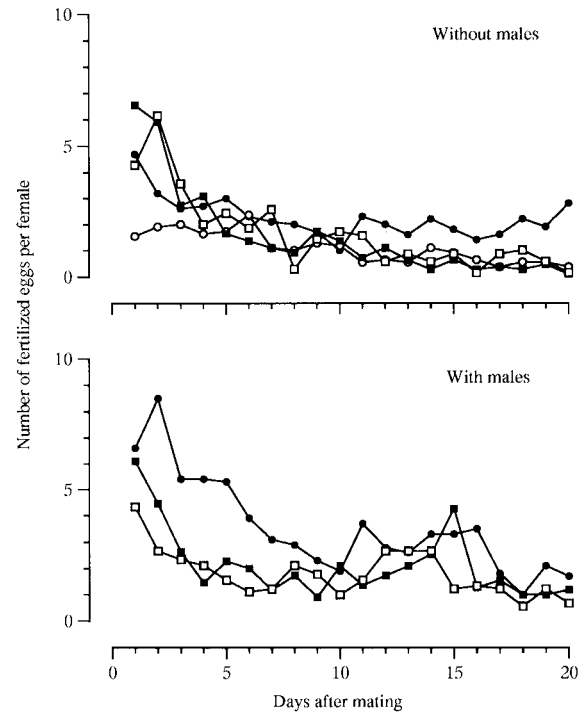
Table 1 shows the percentages of remating in females 10 days after the first copulation. In the HK strain, more than two third of females recovered receptivity in 10 days irrespective of the male-morph in the first copulation. On the other hand, 6.7% of females of the OH strain remated when the first copulation was not interrupted. The recovery rate of receptivity did not significantly increase even if females were forced to separate 10 min after the start of copulation or mated with HK males in the first copulation (CT,  $P>0.05$ ).

**Table 1.** Recovery of receptivity in HK and OH females of *D. elegans* 10 days after the first mating. At the first mating, they were allowed to end copulation naturally or forced to separate 10 min after the start of copulation.

Female	First mating		N	Receptivity (%)
	male	condition		
HK	HK	naturally-ended	30	63.3
HK	OH	naturally-ended	32	71.9
OH	OH	naturally-ended	30	6.7
OH	OH	interrupted (10 min)	30	13.3
OH	HK	naturally-ended	27	22.2

### Egg production and hatchability

Fig. 3 shows number of fertilized eggs laid per female for 20 days after copulation in experimental flies of the HK and OH strains, and Table 2 summarizes the egg productivity in these flies. Although the egg production gradually decreased with time, these strains were able to produce fertilized eggs even 20 days after mating. In the HK strains, females which were forced to separate 3 min after the start of copulation produced smaller number of eggs than those which ended



**Fig. 3.** Number of fertilized eggs laid per day by a female after copulation in the HK (■) and OH (□) strains of *D. elegans* when reared alone (upper) or with two males which were deprived of copulatory potency (lower). Females were allowed to end copulation naturally (○) or forced to separate 3 (in the HK strain) or 10 (in the OH strain) min after the start of copulation (●).

copulation naturally (the difference was not significant by SA (Table 2,  $P>0.05$ ), but significant by TT ( $P<0.01$ )). In the OH strain, the production of eggs did not significantly differ between females which ended copulation naturally and those which were forced to separate 10 min after the start of copulation by SA or TT ( $P>0.05$ ). The egg production was significantly higher in the HK strain than in the OH strain when they were allowed to end copulation naturally (SA,  $P<0.05$ ). In the HK strain, the presence of males (which were deprived of

**Table 2.** Number of total and fertilized eggs laid per day by a female of the HK and OH strains of *D. elegans* for 20 days after copulation and hatchability of eggs. Females were forced to separate three or 10 min after the start of copulation or allowed to end copulation naturally, and then maintained solitarily or with two males which were deprived of copulatory potency.

Strain	condition	No. of total eggs	No. of fertilized eggs	Hatchability Mean (SD)
		Mean (SD)*	Mean (SD)	
without males				
HK	naturally-ended	51.1 (13.2) <sup>a</sup>	45.1 (15.1) <sup>a,b</sup>	0.87 (0.12) <sup>a</sup>
HK	interrupted (3 min)	28.1 (20.3) <sup>a</sup>	21.9 (21.4) <sup>a</sup>	0.57 (0.45) <sup>a</sup>
OH	naturally-ended	35.0 (15.9) <sup>a</sup>	31.2 (13.9) <sup>a</sup>	0.89 (0.08) <sup>a</sup>
OH	interrupted (10 min)	39.3 (18.2) <sup>a</sup>	33.4 (21.9) <sup>a</sup>	0.79 (0.29) <sup>a</sup>
with males				
HK	naturally-ended	84.3 (25.6) <sup>b</sup>	71.1 (20.8) <sup>b</sup>	0.85 (0.09) <sup>a</sup>
OH	naturally-ended	50.0 (15.7) <sup>a</sup>	42.8 (12.4) <sup>a,b</sup>	0.87 (0.13) <sup>a</sup>
OH	interrupted (10 min)	49.1 (22.5) <sup>a</sup>	36.0 (26.9) <sup>a</sup>	0.68 (0.40) <sup>a</sup>

\* Same letters show no significant differences by the Scheffé's method ( $P<0.05$ ) after the ANOVA in No. of total and fertilized eggs and after the Kruskal-Wallis test in hatchability.

copulatory potency) significantly accelerated the egg production (SA,  $P < 0.05$ ). Hatchability of eggs somewhat fell when females were separated 3 min (in the HK strain) or 10 min (in the OH strain) after the start of copulation, but the difference was not significant (KW,  $P > 0.05$ )

## DISCUSSION

In *Drosophila elegans*, copulation duration is shorter in the brown morph than in the black morph and both sexes are responsible for the control of copulation duration (Hirai *et al.*, 1999). The present study revealed that sperm transfer also occurred earlier in the HK (brown morph) strain than in the OH and TW (black morph) strains. It is also revealed that both sexes are responsible for the control of sperm transfer. The female may send a signal to the male partner to commence sperm transfer.

In the HK strain, number of eggs produced per female decreased to some extent and receptivity of females recovered somewhat earlier when copulation was interrupted 3 min after the start of copulation. In the OH strain, the rate of sperm transfer was low when copulation was interrupted 5 min after the start of copulation. Thus, shortened copulation is disadvantageous in this species. It has been observed in *Drosophila* and some other insects that number of sperm and amount of seminal fluids transferred to females increase with prolongation of copulation (Riemann *et al.*, 1967; Parker and Stuart, 1976; Thornhill, 1980) and sperm and/or seminal fluids induce(s) egg production and unreceptivity in females (Manning, 1962, 1967; Hihara, 1981; Chen *et al.*, 1988; Chen and Balmer, 1989; Aigaki *et al.*, 1991; Schmidt *et al.*, 1993; Chapman *et al.*, 1995; Wolfner, 1997).

In the OH strain, however, it was not apparent copulation of 10 min is disadvantageous or not. In this strain, hatchability was somewhat lower in eggs produced by females which were forced to separate 10 min after the start of copulation than in those by females which ended copulation naturally, but the difference was not significant. In addition, recovery of receptivity was not affected by copulation duration or by male-morph in the OH strain when copulation was prolonged over 10 min. Further information is needed to understand the adaptive significance of prolonged copulation of the black morph.

In this species, HK females always recovered receptivity earlier than OH females, suggesting that the former mate more times in their lives than the latter. It has been revealed in *Drosophila melanogaster* that the recovery of receptivity of females is associated with the depletion of sperm in them (Pyle and Gromko, 1978). Females of the HK strain of *D. elegans* also seem to recover receptivity when stored sperm is not sufficient: females which were forced to separate 3 min after the start of copulation produced less number of eggs (Fig. 3) and recover receptivity somewhat earlier than those which ended copulation naturally (Fig. 2). However, the recovery of receptivity was not always associated with sperm depletion in the HK strains: females had a potential to produce fertilized eggs at least for 20 days after copulation, but two-third of them re-

covered receptivity 10 days after copulation.

Thornhill and Alcock (1983) suggested three other situations in which multiple mating is selected; 1) the female's partner provides nutritional materials or protection from other males or predators, 2) the female replaces sperm of genetically inferior males with the gamete of genetically superior individual, or increases the genetic diversity of her offspring, and 3) the female avoids the costs of trying to prevent superfluous copulation. In this study, it is not known whether seminal fluids differ in nutritional conditions between the brown and black morphs, but males of this species, whether the brown or black morph, do not protect the partners after copulation (per. observation). On the other hand, females of the brown morph may have increased the genetic diversity of their offspring by multiple mating to adapt to tropical environments where interactions among organisms are complicated. However, there are still arguments whether the genetic diversity is essential for populations to survive in tropical environments or not. The third situation is related with the sexual activity of males. It has been known that males of this species hold mating territories on *Ipomoea* flower (Kimura and Hirai, in press). Therefore, it is important to know whether the brown and black morphs differ in territorial behavior, but little information has been reported on their territorial or mating behavior.

It is noticeable that females of *D. elegans* were able to produce fertilized eggs for 20 days after copulation. In *D. melanogaster*, production of fertilized eggs is greatly reduced in 8 days after copulation (Pyle and Gromko, 1978). This difference would be related to the difference in fecundity. The maximum daily egg production per female is nearly 100 in *D. melanogaster* (Pyle and Gromko, 1978), but only 9 in *D. elegans* (Fig. 3). Therefore, sperm stored within female would be depleted much earlier in *D. melanogaster* than in *D. elegans*. The low fecundity is common in flower-breeding *Drosophila* and considered as an adaptation to use poor nutritional resources such as flowers (Kambyselis and Heed, 1971; Toda and Okada, 1983).

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