Reproductive age and copulation duration in *Drosophila* species with females with low re-mating frequency.

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In this note, we report a by-product information obtained in our research: the reproductive age, overall mating speed, and copulation duration in three *Drosophila* species reported with zero remating frequency (Markow and O'Grady, 2006): *D. acanthoptera* Wheeler 1949, *D. biarmirpes* Malloch 1924, and *D. subobscura* Collin 1936. Nevertheless, McRobert *et al.* (1997) indicated that 26% of the *D. biarmipes* females mate a second time after 8-10 days. On the other hand, Loukas *et al.* (1981) reported that 23% of the females re-mate in their life and Pitnick and Markow (1994) indicated that 46.7% of the *D. acanthoptera* females re-mate 8 days after first mating.

We obtained from the UC San Diego *Drosophila* Stock Center, *D. acanthoptera* 15090-1693.00, *D. biarmirpes* 14023-0361.06, and *D. subobscura* 14011-0131.13. We maintained *D. biarmipes* and *D. subobscura* at 20°C and *D. acanthoptera* at 24°C. We used CO_2 at 5 psi to collect virgin flies within an hour of eclosion, and we placed the flies individually in 2-ml vials until we used them in both tests. We used a modified Bloomington cornmeal recipe to handle our experiment (Castrezana, *personal communication*).

First, we look out the age in which both sexes were sexually mature. We used the following protocol: we aspirated a virgin female and a virgin male inside a vial with food. We discarded the male after 24 hours and we checked for larvae after 72 hours. For the three species, we tested pairs of flies at eight different ages (N > 15 pairs/age). We found that > 93% of the individuals for both sexes are mature at five days-old in *D. biarmipes*, and eight days-old in *D. acanthoptera* and *D. subobscura*. It is known that in some *Drosophila* species the females and the males can mature at markedly different times. Therefore, our second step was to search for possible reproductive age differences between males and females. We repeated the previous protocol but this time one of the partners used was sexually mature (five days-old in *D. biarmipes*, and eight days-old in *D. acanthoptera* and *D. subobscura*). The results are presented in the Table 1. We found a noticeable difference between the time each sex takes to mature in *D. biarmipes* and *D. subobscura*. In addition, we observed that all *D. biarmipes* males who mate successfully in the 0-24 hr period had a black patch in both wings.

Table 1. Reproductive age, by sex, in *Drosophila acanthoptera, D. biarmipes*, and *D. subobscura.* To test *D. biarmipes,* we used five days-old partners and eight days-old for *D. acanthoptera* and *D. subobscura*. Each period of time has N=15.

species	0-24 hrs	24-48 hrs	48-72 hrs	72-96 hrs	96-120 hrs	120-144 hrs	144-168 hrs	168-192 hrs
D. acanthoptera (females)	0.0%	0.0%	0.0%	13..3%	66.7%	86.7%	86.7%	100.0%
D. acanthoptera (males)	0.0%	0.0%	0.0%	0.0%	40.0%	73.3%	93.3%	93.3%
D. biarmipes (females)	40.0%	53.3%	53.3%	73.3%	100.0%			
D. biarmipes (males)	60.0%	66.6%	100.0%					
D. subobscura (females)	0.0%	0.0%	13.3%	13.3%	20.0%	40.0%	60%	93.3%
D. subobscura (males)	0.0%	0.0%	13.3%	60.0%	80.0%	93.3%	93.3%	

On the other hand, we obtained two reproductive behaviors: overall mating speed and copulation duration. Overall mating speed is defined as the sum of the male courtship latency (which is the time when the male went inside the vial until the time it started to court the female) plus the female receptivity (which is the time when the male started to court until the time female accepted to mate). Copulation duration is the time in which the female accepted to mate until the pair finished mating (Castrezana and Markow, 2008).

Table 2. Overall mating speed and copulation duration in *Drosophila acanthoptera, D. biarmipes*, and *D. subobscura* (time in minutes). Pairs were scored at five days-old for *D. biarmipes* and eight days-old for *D. acanthoptera* and *D. subobscura*.

species	Pairs mating within the first three hours encounter	Minimum-maximum overall mating speed time	Overall mating speed average time	Minimum-maximum copulation duration time	Copulation duration average time
D. acanthoptera	25/69	8.8 - 178.8	74.0 ± 11.4	9.5 - 161.3	122.1 ± 5.50
D. biarmipes	54/69	0.3 - 149.91	35.6 ± 5.2	3.2 - 37.8	21.3 ± 0.8
D. subobscura	52/69	3.6 - 179.1	52.5 ± 6.9	4.6 - 28.6	10.7 ± 0.6

For this behavioral section, we aspirated a mature virgin female and a mature virgin male inside a vial with food. Then, we set the vial on its side over a white surface. A well trained observer recorded the time when flies were introduced in the vial, the time when the flies started mating, and the time the flies finished mating. The observer watched the flies for three hours. Pairs of flies that did not mate within the first three hours of encounter were checked 48 hours later. Only two *D. acanthoptera* pairs did not lay eggs 48 hours after initial encounter. We present the overall mating speed and the copulation duration in Table 2.

Our *D. acanthoptera* copulation duration result did not differ from the data presented by Pitnick *et al.* (1991). However, we suspect that both results could be erroneous. Later in our experiment, we noticed that a *D. acanthoptera* male has a long and passive overall mating speed when it is isolated from other males. However, when we observed *D. acanthoptera* males in large population bottles, we noticed that a *D. acanthoptera* courting male is extremely aggressive with other males who tried to court the female. In fact, a male flattering a female pursued other males around the bottle for several seconds. Then, the male returned to continue the courtship. In addition, we noticed that mating pairs of *D. acanthoptera* can come apart easily after some time. We carefully observed a *D. acanthoptera* pair during copulation under 8× Leica microscope. We found that the distiphallus of the aedeagus was retracted after an hour of copulation. Nevertheless, the presisetea continued in contact with the vaginal plates. At this instant, we consider that *D. acanthoptera* may have a type of male guarding behavior. However, this male behavior should be considered "passive", because we could not observe mating pairs disturbed by other males during copulation, an opposite behavior observed in *D. pegasa* Wasserman 1962 and *D. mainlandi* Patterson 1943, where sometimes a second and third male mounted behind the back of the guarding male.

References: Castrezana, S., and T.A. Markow 2008, Behav. Genet. 38: 437-445; Loukas, M., Y. Vergini, and C.B. Krimbas 1981, Genetica 57: 29-37; Markow, T.A., and P.M. O'Grady 2006, Drosophila: *A Guide to Species Identification and Use.* Academic Press; Maynard Smith, J., 1956, J. Genet. 54: 832–842; McRobert, S.P., C.R. Adams, M. Wutjke, J. Frank, and L.L. Jackson 1997, J. Insect Behav. 10: 761-770; Pitnick, S., and T.A. Markow 1994, Am. Nat. 143: 785-819; Pitnick, S., T.A. Markow, and M. Reidy 1991, Evolution 45: 774-780.