

The Effects of Interspecific Courtship on Mating Success in *Drosophila melanogaster*

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Received: August 16, 2011 Accepted: October 13, 2011 Published: December 1, 2011
doi:10.5539/ijps.v3n2p14 URL: <http://dx.doi.org/10.5539/ijps.v3n2p14>

Abstract

Two sympatric, distantly related *Drosophila* species, *D. melanogaster* and *D. affinis* have been shown to interact sexually. Mature *D. melanogaster* males perform vigorous courtship in response to both virgin and mated *D. affinis* females, but perform no courtship in response to virgin females of another sympatric species, *D. immigrans*. To determine whether inter-specific courtship might interfere with mating success and fitness, *D. affinis* females were used as 'interference sex objects' in tests where *D. melanogaster* males had access to con-specific females. In these tests the presence of *D. affinis* females was shown to significantly reduce the mating success of *D. melanogaster* males. In comparison, the presence of mated *D. melanogaster* females or virgin *D. immigrans* females had no impact on the mating success of *D. melanogaster* males in similar tests.

Keywords: *Drosophila melanogaster*, *Drosophila affinis*, Inter-specific courtship, Mating success

1. Introduction

The mating system for *Drosophila melanogaster* appears to be based on female choice. Females, the discriminating sex, assess male courtship and control whether or not copulation occurs. Males, the indiscriminant sex, approach any fly-like object and, if sexually stimulated, perform a complex series of courtship behaviors and attempt to mate. If the fly that the male approaches is a con-specific female, the male will pursue her and repeat the courtship display until she slows down and opens vaginal plates to allow copulation to occur (reviewed in Greenspan & Ferveur, 2000). In this way males may demonstrate their fitness as an ability to perform the correct, species-specific courtship display, and females select males who have successfully caused them to become sexually receptive.

From an evolutionary perspective it would seem critical for *Drosophila* males to avoid directing their courtship toward flies from other species. Inter-specific courtship would appear to be a waste of time and energy and, if copulation ensued, gametes (Spieth & Ringo, 1983). Furthermore, since courtship subjects flies to an increased predation risk, it would seem likely that mechanisms might evolve that would reduce the amount of courtship males perform toward other species. And, indeed, this appears to be true as in many instances *Drosophila* males either do not initiate courtship of non-con-specific flies, or quickly terminate courtship of these flies (Spieth, 1974; Spieth & Ringo, 1983).

There are, however, some instances in which flies engage in inter-specific courtship. One such situation has been reported for interactions between *D. melanogaster* and *D. affinis*, the two most common *Drosophila* species in the Philadelphia area (McRobert & Tompkins, 1986a). *D. melanogaster* and *D. affinis* have been shown to engage in prolonged inter-specific courtship, with males from each species courting members of the other species (McRobert & Tompkins, 1986b; McRobert & Tompkins, 1988). Both *D. melanogaster* and *D. affinis* encounter each other in the wild, utilizing natural food sources together (McRobert, pers. notes) and both species are attracted to traps baited with bananas and yeast. The male courtship displays of these species are very similar,

with males from both species orienting toward the female, following her if she moves away, tapping at her with fore-tarsi and producing a courtship song by vibrating their wings (McRobert & Tompkins, 1986b). In addition, *D. melanogaster* perform a behavior known as ‘licking’ in which they contact the female with their proboscis. *D. affinis* males don’t perform the licking behavior (Miller, 1950). However, despite the similarities in sexual behavior, *D. melanogaster* and *D. affinis* are only distantly related and probably cannot produce viable hybrids since they have different numbers of chromosomes (Wheeler, 1981; Lakovaara & Saura, 1982).

In this report we examine the possible consequences of inter-specific courtship from the perspective of the *D. melanogaster* male. In short, do *D. melanogaster* males experience lowered mating success with con-specific females when in the presence of *D. affinis* females? In addition, we addressed the question of whether inter-specific courtship might lower the reproductive fitness of *D. melanogaster* males.

2. Methods

2.1 Stocks

Fly stocks were derived from fertilized females collected in Rosemont, Pennsylvania in 2003 (*D. melanogaster*) and 2005 (*D. affinis* and *D. immigrans*). All flies were maintained on Carolina Instant Media (Formula 4-24) at 22-26° C under a 12:12 LD cycle. For the *D. affinis* stock, liquefied opuntia cactus was added to the media, which appeared to increase the vitality of the flies.

2.2 Behavioral Observations

All behavioral observations were conducted between 8 am -12 pm, at 21-26° C. Virgin flies were collected under CO₂ anesthesia between 3 - 12 hours post-eclosion. Males were housed singly, while females were housed in groups from 2 to 10 in vials containing yeasted media. Flies were tested 3- 5 days post-eclosion.

2.3 Copulation Tests

Three separate copulation tests were conducted, including: *D. melanogaster* males with virgin *D. melanogaster* females, *D. melanogaster* males with mated *D. melanogaster* females, and *D. melanogaster* males with virgin *D. affinis* females.

For each test a single male was aspirated into a food vial containing a single female and observed for sixty minutes. During the observation period, copulation success or failure, copulation latency (the time until copulation began), and copulation duration (the time the pair stayed in copula) were timed and recorded. In tests involving mated females, the females were tested 24 hours post-mating. No individual fly was tested more than once.

2.4 Courtship Index Tests

For each test a single male was aspirated into a Plexiglas chamber (volume=0.4 cm³) along with a sex object and observed for 10 minutes. The courtship index (the fraction of the observation period during which the *D. melanogaster* male performed courtship behaviors) was timed and recorded. Behaviors classified as courtship included orientation, tapping, following, singing, licking and attempted copulation (see Tompkins, 1984; Hall, 1994; McRobert, 2004). If copulation occurred the test was ended and the courtship index was determined for the period of time leading up to copulation. Sex objects included *D. melanogaster* virgin females, mated *D. melanogaster* females, *D. affinis* virgin females, mated *D. affinis* females, and virgin *D. immigrans* females.

2.5 Courtship Interference Tests

For each test a single *D. melanogaster* male was aspirated into a food vial containing five “target” *D. melanogaster* virgin females along with nothing (for baseline), or five “interference” sex objects, and observed for ninety minutes. During each test the total number of copulations, copulation latency until the first successful copulation, and copulation duration were timed and recorded. The interference groups included five mated *D. melanogaster* females, five *D. affinis* virgin females, or five virgin *D. immigrans* females.

2.6 Offspring Production

In these tests, individual *D. melanogaster* males were mated with individual virgin *D. melanogaster* females in food vials. Following copulation the males were removed from the vials while the mated females remained in order to allow each female to lay eggs. After seven days, each female was transferred to a new food vial. This was performed twice to allow each mated female 21 days to produce eggs. Eggs and larvae were counted using a sucrose extraction technique (Frank, Adams & McRobert, 1995).

2.7 Statistical Analysis

The significance of difference in copulation frequency was determined with a 2x2 contingency chi-square test. All other comparisons were made using a one-way ANOVA followed by a post-hoc Tukey HSD test. Since courtship indices are percentages, these data were arcs in transformed before being analyzed.

3. Results

Courtship index tests were used to assess the relative degree of attraction of *D. melanogaster* males to different sex objects. *D. melanogaster* males performed significantly more courtship in response to virgin *D. melanogaster* females than in response to mated *D. melanogaster* females ($P < 0.001$), virgin *D. affinis* females ($P = 0.001$), and virgin *D. immigrans* females ($P < 0.001$). There was no significant difference in the amount of courtship *D. melanogaster* males performed toward virgin *D. melanogaster* females and mated *D. affinis* females ($P = 0.128$). There was also no significant difference between the amount of courtship performed by *D. melanogaster* males toward virgin *D. affinis* females and mated *D. affinis* females ($P = 0.3419$). Finally, *D. melanogaster* males performed significantly more courtship in response to either virgin *D. affinis* females or mated *D. affinis* females than in response to *D. immigrans* females ($P < 0.001$ and $P < 0.001$ respectively). These results are summarized in Figure I.

Copulation frequency tests were used to determine the probability of mating between *D. melanogaster* males and different types of females. *D. melanogaster* males copulated with virgin *D. melanogaster* females significantly more often than with mated *D. melanogaster* females ($P < 0.001$) or virgin *D. affinis* females ($P < 0.001$). *D. melanogaster* males copulated with virgin *D. affinis* females significantly more often than with mated *D. melanogaster* females ($P = 0.003$). These results are summarized in Figure II.

Courtship interference tests were used to determine whether the presence of certain types of flies ('interference sex objects') affected the mating success of *D. melanogaster* males with virgin *D. melanogaster* females. In these tests the presence of mated *D. melanogaster* females or virgin *D. immigrans* females caused no significant reduction in con-specific matings for *D. melanogaster* males when compared to control tests with no interference sex objects ($P = 0.9998$ and $P = 0.9970$ respectively). However, the presence of five virgin *D. affinis* females led to a significant reduction in the number of con-specific mating by *D. melanogaster* males when compared to controls ($P < 0.001$). These results are summarized in Figure III.

4. Discussion

As reported in earlier studies, the two most common *Drosophila* species in the Philadelphia area, *D. melanogaster* and *D. affinis* (McRobert & Tompkins, 1986a) interact sexually despite their relatively distant taxonomic relationship and the fact that hybridization between these species does not lead to viable offspring (McRobert & Tompkins, 1986b; McRobert & Tompkins, 1988). This raises the question of whether inter-specific courtship between these species might have negative consequences. In this report we show that the presence of *D. affinis* females leads to a reduction in their mating success by *D. melanogaster* males with con-specific females.

While *D. melanogaster* males performed the highest level of courtship toward virgin con-specific females, they performed more courtship toward both virgin and mated *D. affinis* females than toward mated females of their own species. Furthermore, *D. melanogaster* males copulated with virgin *D. affinis* females to a greater degree than with mated *D. melanogaster* females. It has been well established that *D. melanogaster* males provide females with a seminal-fluid compound known as sex peptide during copulation (Chapman, 2001). Sex peptide acts within the body of the recently mated female to reduce her production of aphrodisiac pheromone and reduce her receptivity to copulation for a period of a few days (Chen, Stumm-Zollinger, Aigaki, Balmer, Bienz, & Bohlen, 1988; Chapman, Bangham, Vinti, Seifried, Lung, Wolfner, Smith & Partridge, 2003). In this way, recently mated *D. melanogaster* females stimulate less courtship from males and are less likely to re-mate during the period of time when they are laying eggs. This system protects the sperm of the male that mated with the female, but also acts to reduce the time that other males spend courting a female who is unlikely to copulate. However, despite the fact that inter-specific matings between *D. melanogaster* and *D. affinis* never result in the production of viable offspring, no mechanism appears present to reduce the inter-specific courtship observed between these species.

The negative effects of inter-specific courtship between *D. melanogaster* and *D. affinis* were evident in interference tests. When placed into a scenario with five con-specific virgin females and either five mated *D. melanogaster* females or five virgin *D. immigrans* females (a sympatric species that does not elicit courtship from *D. melanogaster* males), *D. melanogaster* males copulated with an average of three of the con-specific virgins. This number was equivalent to the average number of copulations that occurred in the control scenario with no interference flies. These results suggest that the presence of either con-specific, mated females or virgin *D. immigrans* females had no effect on intra-specific courtship and copulation in *D. melanogaster*. However, the presence of five *D. affinis* females significantly reduced the number of copulations, indicating that inter-specific courtship between *D. melanogaster* and *D. affinis* had a detrimental impact on courtship and mating in *D. melanogaster* males.

To address the idea of how the loss of mating success might affect fitness in *D. melanogaster* males, an offspring production analysis was performed. In the laboratory, *D. melanogaster* females produced an average of 443.8 (+/-25.1) offspring from a single mating. Adding this information to the results of the interference tests, mating with three females (as males did in assays that did not include *D. affinis* females) would lead to the production of approximately 1331.4 offspring. In assays that included *D. affinis* females, males mated with an average of 2.17 females, which would lead to a production of approximately 963 offspring, a reduction of 368 offspring per session. Obviously, these numbers relate to the carefully controlled atmosphere of the laboratory, but still illustrate the point that the presence of *D. affinis* females could lead to lowered offspring production in *D. melanogaster* males.

From a traditional point of view, elaborate courtship displays prior to copulation evolve as reproductive isolation mechanisms, preventing the production of hybrids with reduced fitness (reviewed in Thornhill & Adcock, 1983). Selection would thus favor individuals that avoid the fitness costs associated with hybridization. However, due to the costs of courtship (time, energy and the risk of predation), selection should also favor animals that restrict the prolonged courtship of non-con-specifics. For example, males from the two sibling species, *D. affinis* and *D. algonquin*, do not initiate courtship or attempt copulation with non-con-specific females when placed in mixed-species groups (Miller, 1950). Similarly, *D. melanogaster* males perform little or no courtship toward two sibling species, *D. simulans* and *D. mauritiana* (Manning, 1959; Schilder & Dow, 1977; Robertson, 1983). In this study, *D. melanogaster* males performed no courtship in response to *D. immigrans* females, which are sympatric with *D. melanogaster* in the Philadelphia area.

Not all species have such firm barriers against sexual attraction and interaction, however. For example, *D. persimilis* and *D. pseudoobscura*, which overlap in territory in the western United States, will court each other and produce fertile hybrid offspring in the lab even though hybrids are rarely seen in nature (Mayr, 1946). In the closely related sibling species *D. melanogaster* and *D. simulans*, which have strong sexual isolation in nature but court in the laboratory setting, interbreeding leads to the production of sterile hybrids (Barker, 1962), and *D. melanogaster* males even prefer courting *D. simulans* females to con-specific females, though the acceptance of *D. simulans* females is quite low (Wood & Ringo, 1980).

For species that interact sexually, learning may act to reduce interactions that waste time and energy and do not lead to the production of viable hybrids. In a phenomenon known as experience-dependent courtship modification, *D. melanogaster* males reduce their courtship toward mated or virgin con-specific females following courtship of a mated female (Gailey, Jackson & Siegel, 1982; Siegel & Hall, 1979). Similar mechanisms may also be involved in inter-specific situations. For instance, in interactions between *D. persimilis* and *D. pseudoobscura*, *D. persimilis* males who had experienced courtship and rejection by *D. pseudoobscura* females consequently exhibited lowered levels of hetero-specific courtship compared to inexperienced males (Dukas, 2008). Similarly, Dukas (2004) showed that *D. melanogaster* males previously exposed to *D. simulans* females showed significantly reduced levels of courtship towards *D. simulans* females but maintained normal levels of courtship towards *D. melanogaster* females. Expanding this to a molecular level, Ellis and Carney (2009) used microarray analysis to identify altered expression of nine genes in *D. melanogaster* males that had courted *D. simulans* females. In contrast, thirty-five genes displayed altered expression in *D. melanogaster* males that had courted con-specific females. Such alterations in gene expression may represent a system that both reduces hetero-specific courtship and encourages con-specific courtship. It is possible that a similar mechanism may function to lessen interactions between *D. melanogaster* and *D. affinis* although, within the ninety minute periods utilized in our courtship interference tests, the presence of *D. affinis* females still apparently led to a reduction in con-specific mating success for the *D. melanogaster* males.

Finally, it is difficult to use laboratory studies to make definitive statements about reproductive isolation and fitness in natural populations. While the results presented here suggest that the presence of *D. affinis* may negatively impact mating success and fitness in *D. melanogaster*, other factors may occur in the wild that dramatically affect the interactions between species. However, laboratory studies like these offer an intriguing look at the effects of one species on another and provide a foundation for further work. In future two other aspects of *D. melanogaster*- *D. affinis* interaction will be addressed: First, laboratory tests will be performed to determine whether inter-specific courtship leads to courtship modification in *D. melanogaster* males. If true, *D. melanogaster* males may learn to reduce the time they spend courting *D. affinis* females. Second, field studies will be performed in an attempt to provide a detailed description of inter-specific courtship between *D. melanogaster* and *D. affinis* in the wild.

Acknowledgments

We thank Kate Buehler for help with the maintenance and testing of the flies. This project was supported, in part, by a grant from the Saint Joseph's University Sigma Xi Chapter.

References

- Amrein, H. (2004). Pheromone perception and behavior in *Drosophila*. *Curr. Opin. Neurobiol*, 14: 435-442. <http://dx.doi.org/10.1016/j.conb.2004.07.008>
- Barker, J.S.F. (1962). Sexual isolation between *Drosophila melanogaster* and *Drosophila simulans*. *Am. Nat*, 96(887): 105-115. <http://dx.doi.org/10.1086/282211>
- Chapman, T. (2001). Seminal fluid-mediated fitness traits in *Drosophila*. *Heredity*, 87: 511-521. <http://dx.doi.org/10.1046/j.1365-2540.2001.00961.x>
- Partridge, L. (2003). The sex peptide of *Drosophila melanogaster*: Female post-mating responses analyzed by using RNA interference. *Proc. Natl. Acad. Sci. USA* 100: 9923-9928. <http://dx.doi.org/10.1073/pnas.1631635100>
- Chen, P. S., Stumm-Zollinger, E., Aigaki, T. Balmer, J., Bienz, M., & Bohlen, P. (1988). A male accessory gland peptide that regulates reproductive behavior of female *D. melanogaster*. *Cell*, 54: 291-298, [http://dx.doi.org/10.1016/0092-8674\(88\)90192-4](http://dx.doi.org/10.1016/0092-8674(88)90192-4)
- Dukas, R. (2004). Male fruit flies learn to avoid interspecific courtship. *Behav. Ecol*, 15(4): 695-698, <http://dx.doi.org/10.1093/beheco/arh068>
- Dukas, R. (2008). Learning decreases heterospecific courtship and mating in fruit flies. *Biol. Letters* 4: 645-647. <http://dx.doi.org/10.1098/rsbl.2008.0437>
- Ellis, L.L., & Carney, G.E. (2009). *Drosophila melanogaster* males respond differently at the behavioural and genome-wide levels to *Drosophila melanogaster* and *Drosophila simulans* females. *J. Evol. Biol*, 22: 2183-2191. <http://dx.doi.org/10.1111/j.1420-9101.2009.01834.x>
- Frank, J. Adams, C., & McRobert, S.P. (1995). Sucrose density separation of *Drosophila* larvae from instant medium. *Dros. Info. Serv*, 76:172
- Gailey, D.A. Jackson, & F.R. Siegel, R.W. (1982). Male courtship in *Drosophila*: The conditioned response to immature males and its genetic control. *Genetics*, 102: 771-782
- Greenspan, R.J. Ferveur, J. (2000). Courtship in *Drosophila*. *Annu. Rev. Genet*, 34: 205-232. <http://dx.doi.org/10.1146/annurev.genet.34.1.205>
- Hall, J.C. (1994). The mating of a fly. *Science* 264: 1702-1714. <http://dx.doi.org/10.1126/science.8209251>
- Lakovaara, S., & Saura, A. (1982). Evolution and speciation in the *Drosophila obscura* subgroup. In M. Ashburner, H.L. Carson, and J.N. Thompson (eds.), *The Genetics and Biology of Drosophila*, Vol. 3b. Academic Press, New York, 1-59
- Manning, A. (1959). The sexual behavior of two sibling *Drosophila* species. *Behaviour* 15: 123-145. <http://dx.doi.org/10.1163/156853960X00133>
- Mayr, E. (1946). Experiments on sexual isolation in *Drosophila*. VII. The nature of the isolating mechanisms between *D. pseudoobscura* and *D. persimilis*. *Proc. Natl. Acad. Sci. USA* 32 (5): 128-137. <http://dx.doi.org/10.1073/pnas.32.5.128>
- McRobert, S.P., & Tompkins, L. (1986a). *Drosophila* species in the Philadelphia area. *Dros. Info. Serv.* 63: 95.
- McRobert, S.P., & Tompkins, L. (1986b). Incomplete behavioral isolation between two distinctly related *Drosophila* species. *Evolution*, 40(6): 1185-1190. [Online] Available: <http://www.jstor.org/stable/2408946>
- McRobert, S.P., & Tompkins, L. (1988). Two consequences of homosexual courtship performed by *Drosophila melanogaster* and *Drosophila affinis* males. *Evolution* 42(5): 1093-1097. [Online] Available: <http://www.jstor.org/stable/2408925>
- McRobert, S.P. (2004). Reproductive behavior: Sexual behavior in fruit flies – *Drosophila*. *Encyclopedia of Animal Behavior*, 930-934
- Miller, D.D. (1950). Mating behavior of *Drosophila affinis* and *Drosophila algonquin*. *Evolution*, 4: 123-134. <http://dx.doi.org/10.2307/2405389>

Robertson, H.M. (1983). Mating behavior and the evolution of *Drosophila mauritiana*. *Evolution*, 37: 1283-1293. <http://dx.doi.org/10.2307/2408848>

Schilder, F. Dow, M. (1977). Courtship behavior in *Drosophila*: Sexual isolation or sexual selection? *Z. Tierpsychol*, 43: 304-310. <http://dx.doi.org/10.1111/j.1439-0310.1977.tb00077.x>

Siegel, R.W. Hall, J.C. (1979). Conditioned responses in courtship behavior of normal and mutant *Drosophila*. *Proc. Natl. Acad. Sci. USA* 76(7): 3430-3434. <http://dx.doi.org/10.1073/pnas.76.7.3430>

Singh, S.R. Singh, & B.N. Hoenigsberg, H.F. (2002). Female remating, sperm competition and sexual selection in *Drosophila*. *Genet. Mol. Res*, 1(3): 178-215

Spieth, H.T. (1974). Courtship behavior in *Drosophila*. *Annu. Rev. Entomol*, 19: 383-406, <http://dx.doi.org/10.1146/annurev.en.19.010174.002125>

Spieth, H.T., & Ringo, J.M. (1983). Mating behavior and sexual isolation in *Drosophila*. In M. Ashburner, H.L. Carson, and J.N. Thompson (eds.), *The Genetics and Biology of Drosophila*, Vol. 2c. Academic Press, New York, pp. 224-284.

Thornhill, R., & Alcock, J. (1983). *The evolution of insect mating systems*. Harvard University Press, Cambridge, Massachusetts.

Tompkins, L. (1984). Genetic analysis of sex appeal in *Drosophila*. *Behav. Genet*, 14: 411-440, <http://dx.doi.org/10.1007/BF01065443>

Wheeler, M.R. (1981). The *Drosophilidae*: A taxonomic overview. In M. Ashburner, H.L. Carson & J.N. Thompson (Eds.). *The Genetics and Biology of Drosophila*, 3, 1-97. New York: Academic Press.

Wood, D., & Ringo, J.M. (1980). Male mating discrimination in *Drosophila melanogaster*, *D. simulans* and their hybrids. *Evolution*, 34(2): 320-329. [Online] Available: <http://www.jstor.org/stable/2407395>

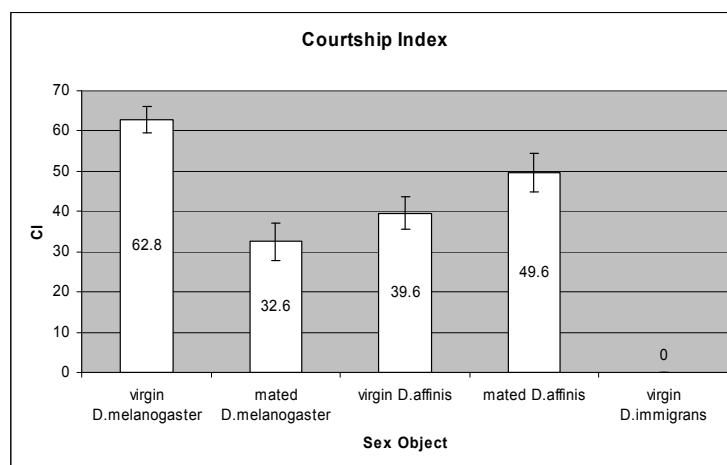


Figure 1. The courtship index of *D. melanogaster* males in response to different sex objects

N = 20 for each bar

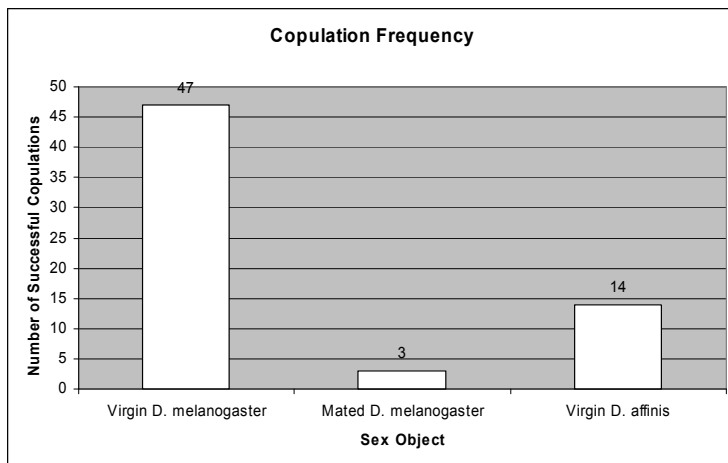


Figure 2. The copulation frequency of *D. melanogaster* males with different sex objects. N = 50 for each bar.

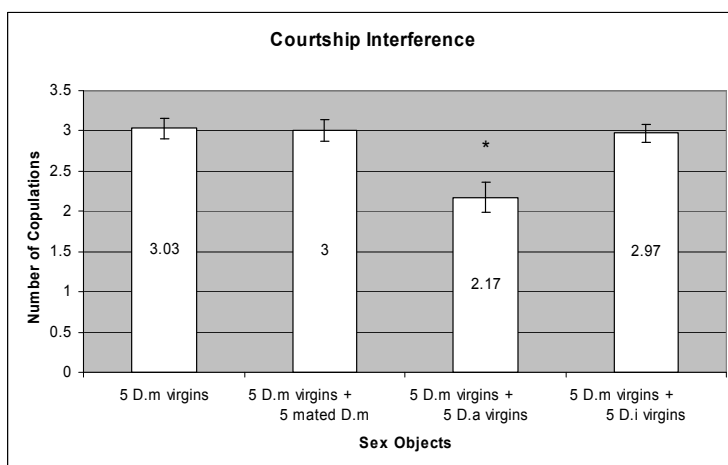


Figure 3. The effects of different “interference sex objects” on the mating success of *D. melanogaster* males with five conspecific virgin females. N = 30 for each bar. * denotes the group that was significantly different from the rest