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Evidence for minority male mating success and minority female mating disadvantage in *Drosophila ananassae*

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ABSTRACT. Frequency-dependent mating success was tested for three pairs of wild-type and mutant strains of *Drosophila ananassae*, MY and yellow body color (*y*), PN and claret eye color (*ca*), and TIR and cut wing (*ct*). The two strains of each pair were chosen for their approximately equal mating propensities. Multiple-choice experiments, using different experimental procedures, were employed. The tests were carried out by direct observation in Elens-Wattiaux mating chambers with five different sex ratios (4:16, 8:12, 10:10, 12:8, and 16:4). There was no assortative mating and sexual isolation between the strains, based on 2 x 2 contingency χ^2 analysis and isolation estimate values. One-sided rare male mating advantages were found in two experiments, one for *ca* males and the other for wild-type males (TIR). However, no advantage was found for rare males in the experiment with MY and *y* flies. Mating disadvantages for rare females were found for sex-linked mutants (*y* and *ct*). Two different observational methods (removal or direct observation of mating pairs) imparted no overall significant effects on the outcome of the frequency-dependent mating tests.

Key words: *Drosophila ananassae*, Multiple-choice experiments, Minority-mating success, Mutant flies, Wild-type flies

INTRODUCTION

At least a third of all loci are polymorphic. Various models have been postulated to explain the stability of this genetic polymorphism found in natural populations, the most commonly cited of which is heterozygote superiority. Another popular model is frequency-dependent selection, which favors the rare type for different fitness traits, including sexual activity. The most argued component of this frequency-dependent selection is minority-mating advantage, a form of frequency-dependent sexual selection. Minority-mating advantage means that a rare competing genotype is favored for mating, regardless of its type, until equilibrium is reached in a population. This model is expected to maintain genetic variation without heterosis and its accompanying genetic load, as at equilibrium frequencies the fitnesses of different types are equal (Anderson, 1969; Lewontin, 1974). Though the rare type will be favored and increase in frequency, it will never become fixed through the generations, causing extinction of the other strain, as once it is common, its advantage will be lost (Adams and Duncan, 1979). Also, it is suggested that rare male mating advantage promotes outbreeding, because an occasional male from another population is preferred for mating (Dal Molin, 1979; Grant et al., 1980). However, Ball et al. (2000) did not confirm this idea. They reported that the rare male effect had little impact on the fitness advantage of the immigrant allele in a study of the genetic contribution of single male immigrants to small, inbred populations of *Drosophila melanogaster*. Minority-mating advantage was discovered independently by Petit (1951) and Ehrman (1966). Since then, rigorous studies have been carried out by several authors, involving both empirical and statistical analyses on this phenomenon (see Knoppien, 1985a; Partridge, 1988; Singh and Sisodia, 2000). Arguments and counterarguments regarding its existence and mechanisms have made this hypothesis a most intriguing one. Despite a moderately long research history of half-a-century, the idea of minority-mating advantage has still to find a secure place in genetics test books. Evidence, found both for and against this type of frequency-dependent sexual selection, however, has kept this question open, emphasizing the need for more work on this phenomenon.

Minority-mating advantage has been reported in 12 species of *Drosophila* and also in some non-drosophilid flies, as well as in a few vertebrates (Knoppien, 1985a; Singh and Sisodia, 2000). Intraspecifically, the rare male effect has been tested using different wild-type strains, mutants, strains having different chromosome arrangements, allozyme variants, behavioral characters, the same strains reared at different temperatures (Knoppien, 1985a; Singh and Sisodia, 2000), and by isolating males and females during different developmental stages (Ehrman and Kim, 1995). Singh and Chatterjee (1989) reported a rare male mating advantage in *D. ananassae* using *se* and *cd* mutants and wild-type strains. Singh and Sisodia (1997) found evidence for a rare male effect in *D. bipectinata*. Singh and Som (2001) and Som and Singh (2004) reported a one-sided rare male mating advantage in a study made with two different karyotypic strains of *D. ananassae*; however, they found no evidence for minority-mating success in wild-type strains of the same species (Som and Singh, 2002).

We carried out experiments to study rare type mating advantage using mutant and wild-type flies of *D. ananassae*. *D. ananassae* belongs to the *ananassae* species complex of the *ananassae* subgroup of the *melanogaster* species group. Although it is cosmopolitan in distribution, it is largely circumtropical and it is commonly found in India. This species is unique in the genus *Drosophila* due to certain peculiarities in its genetic behavior, especially due to its spontaneous male meiotic recombination at an appreciable frequency, which occurs at a very low

rate in other species, including *D. melanogaster*, and for its high mutability (see, Singh, 1996, 2000). Also, like typical cosmopolitan species, *D. ananassae* males exhibit a high sexual drive, and females have a relatively high discriminating capacity (Spieth, 1966). All these characteristics make *D. ananassae* a useful model to study sexual behavior.

Multiple-choice experiments, where two types of females are confined with two types of males, give a close approach to the natural condition. They permit observations of all four combinations of matings between two types of male and female flies. Also, in this type of choice experiment, both types of females get an opportunity to choose simultaneously. In multiple-choice trials, interactions between the female types can also take place, which is not possible in other choice situations, e.g., female choice, where one type of female is kept with two types of males (Peterson and Merrell, 1983). In most of the literature non-random mating is attributed to female discrimination, while male choice has been ignored. Though males are by and large considered to indiscriminately court females, Noor (1996) suggests that mating discrimination by females and males is approximately equally frequent in *Drosophila*. Thus, multiple-choice experiments might be more informative than female choice for studying mate preference and a possible consequent rare type advantage.

MATERIAL AND METHODS

Three mutant strains of *D. ananassae*, yellow body color (y^{66}), claret eye (*ca*) and cut wing (ct^5) were studied for rare male effect against three wild-type strains, MY (Mysore), PN (Pune) and TIR (Tirupati), respectively. Each of these wild-type strains was chosen based on an initial mating propensity test, in which each strain was found approximately equal to the respective mutant strain. This precaution was taken because differences in mating propensities strongly affect the multiple-choice test, which can lead to misinterpretation of discrimination (Casares et al., 1998). Also, if one type of male is sexually more active, a one-sided rare male mating advantage favors the more vigorous type (Bryant et al., 1980).

Description of the mutant strains

y^{66} is a sex-linked recessive mutation, having a yellow body color (Tobari, 1993), with yellow wings and bristles, due to less than normal pigmentation of the cuticle. *ca* is a recessive mutation on chromosome two, with brownish eyes that darken with age (Tobari, 1993). It has homology with *ca* of *D. melanogaster*, where it was found that the claret mutation causes a reduction in the levels of both pteridines (red pigments) and ommochromes (brown pigments) (Sequeira et al., 1989). ct^5 is a sex-linked recessive mutation, with pointed wings due to marginal excisions (Tobari, 1993).

In *D. melanogaster*, most alleles of the *cut* locus are pleiotropic, exhibiting various combinations of aberrations (Johnson and Judd, 1979). In our strain of *D. ananassae* we found failure of wing expansion and sometimes a small blister associated with the *cut* wing. We only used flies with fully expanded and blisterless wings.

The wild-type mass cultures PN and TIR were established from flies collected in 1999 from Pune and Tirupati, India, respectively, and MY from Mysore, India, in 2000.

In order to understand the effects of 'rarity' on a single locus, we tried to 'randomize' the differences between the genetic backgrounds of mutant and wild-type flies by crossing the

flies of a mutant strain with the flies of a wild-type strain in reciprocal crosses. Males and females, generated from both crosses, were mixed and were maintained for several generations. Mutant and wild-type lines were established by pair mating for the *yellow* and *cut* mutations. In order to establish the wild-type line for the autosomal recessive mutant (*claret*), the vials in which *claret* flies appeared after pair mating were rejected, and only those having wild phenotypes were considered. Male and female parents of each of this type of vial were stored separately in food vials. Stored sperm in females were exhausted totally by changing the food vials every two or three days. Then females were backcrossed to test for homozygosity. Males were also tested for homozygosity. The wild-type line was established by only taking flies generated from parents found homozygous for wild type. In this way we sought to achieve randomization at the other loci, except in the vicinity of the mutant locus. However, very closely linked loci may or may not be randomized in this way. Within a few hours after eclosion, both virgin females and males were isolated under light ether anesthesia. Flies of each sex were stored in separate food vials (7.5 cm in length and 2.2 cm in diameter) in batches of 15 to avoid bias in the outcome of the rare male test due to a density effect (Knoppien, 1985b; Knoppien, 1987). The flies were aged for seven days. One day before each experiment, 20 females and 20 males were stored separately in fresh food vials at the ratios to be tested (five ratios, 4:16, 8:12, 10:10, 12:8, 16:4) using a very low dose of ether. Care was taken to avoid sampling errors (Markow, 1980). Due to the distinguishable phenotypes, there was no need to mark the flies in order to differentiate between the two types of flies. Ratios of males and females were varied simultaneously. Six replicates were carried out for each ratio. Mating success was observed directly by introducing flies into a Elens-Wattiaux (Elens and Wattiaux, 1964) mating chamber (10.5 cm in diameter), without etherization. First, females were introduced and then males. The general sex ratio was 1:1. Knoppien (1985a) has shown that the magnitude of the rare male mating advantage depends on the experimental approach. We sought to determine whether the method of observation affects the results of minority effect experiments. The mated flies were counted by the following two methods (see, Knoppien, 1985a):

1. Spiess method. Mating pairs were aspirated from the mating chamber into separate vials (Spiess, 1968). Later, mated types were identified with the help of a stereomicroscope.
2. Ehrman method. The mated flies were identified with the help of a 4X hand lens through the glass wall of the mating chamber (Ehrman, 1966). Copulating pairs were not aspirated out.

For each replicate, the first 10 matings were recorded. All the tests were conducted in a room maintained at approximately 24°C under normal light conditions from 7:30 to 10:30 am. Three experiments were carried out, involving MY and *y*, PN and *ca* and TIR and *ct*.

RESULTS

Initially, we started our experiment to study rare type mating advantage for males. As a multiple-choice procedure was used, we were also able to examine mating advantage for rare females. As assortative mating may furnish a faulty rare type effect, these two components of mating success should be separated (Bryant et al., 1980; Kearns et al., 1990). Hence, we first

analyzed the randomness of matings between the wild-type and mutant strains with pooled replicates. The χ^2 values from 2 x 2 contingency tests for assortative mating for all three pairs of strains were determined (Table 1). This approach reveals randomness in mating among females and males (Pot et al., 1980; Terzic et al., 1996). In these calculations it is presumed that matings are independent of the testing frequencies and genotypes. In the experiment involving MY and *y* flies, the 8:12 and 16:4 ratios showed significant preferential mating with the Ehrman method. In the latter case, wild-type flies showed preferential mating for their own type ($P < 0.05$). However, with the 8:12 ratio, though wild-type and mutant flies showed a tendency for homogamic matings, matings took place in all four combinations. Consequently, there was only one significant deviation (MY:*y* = 16:4), which is not enough to suggest preferential mating between these two strains. In the experiments with PN and *ca* and TIR and *ct*, significant deviations from random mating were seen in one case of 10 ($P < 0.05$, and $P < 0.01$, respectively); these can be ignored as isolated cases. However, one thing is common among these experiments (Table 1); three deviations were found when wild-type and mutant flies were placed in a 16:4 ratio. This is probably due to the fact that common flies have more access to the opposite sex of their own type at this ratio. On the whole, it can be concluded that there was no preferential mating encountered in any of the three pairs of strains. An isolation estimate (Table 1), a measurement of sexual isolation among two strains, was calculated by the formula of Merrell (1950):

$$\text{Isolation estimate (IE)} = \frac{\text{Number of heterogamic matings}}{\text{Number of homogamic matings}}$$

If IE is 1, there is no sexual isolation between the strains. If IE is zero, then isolation is complete. There was no significant sexual isolation between the mutant and the wild-type strains (Table 1). The IE values with superscript 'a', with lower IE values (all were found at the 16:4 ratio) do not necessarily indicate existence of isolation between two strains, as no overall preferential mating between two strains was found by the 2 x 2 contingency analyses. This difference between homo- and heterogamic matings is due to the fact that at these lower ratios, common males mate much more with the common females due to their higher availability within the mating chamber. Also, it is clear that at these ratios, homogamic mating percentages for the common flies are higher than the homogamic mating percentages of the rare flies (percentages not shown) irrespective of their genotypes.

The results of direct observations following both methods were recorded for all three pairs of fly strains (Tables 2, 3 and 4). χ^2 values testing the mating success of two types of flies with respect to the input frequencies were calculated. The mating percentages of both the wild-type and mutant flies, as their ratios varied, were also calculated. The expected numbers of matings were calculated on the basis of the ratios between the two types of males or females introduced into the mating chamber. There were no significant differences between observed and expected number of matings of the two types of flies in the test with MY and *y*. There was a significant disadvantage for rare females ($P < 0.01$) at a single ratio (12 MY:8*y*), in the Ehrman method observations. Mating percentages also did not indicate any mating success for minority males or females. Rather, mating percentages were lower for mutant females (*y*), when tested with both methods, and they mated much less when they were rare in the Ehrman method. In the Spiess method observation with TIR and *ct* (Table 3), there was no significant rare type

Table 1. The number of matings of *Drosophila ananassae* in three different experiments, involving three different mutant and three different wild-type flies, using two different observation methods.

Experiment/ Method	Ratio		Mating type				χ^2	IE
A MY *vs* y	MY	*y*	MY ♀ × MY ♂	MY ♀ × *y* ♂	*y* ♀ × MY ♂	*y* ♀ × *y* ♂		
	4	16	2	11	8	39	0.01	0.46
	8	12	11	20	11	18	0.03	1.06
	10	10	20	16	12	12	0.17	0.87
	12	8	23	17	15	5	1.75	1.14
	16	4	45	6	6	3	2.79	0.25[a]
B	4	16	2	15	10	33	1.00	0.71
	8	12	17	11	10	22	5.23*	0.53
	10	10	22	15	9	14	2.34	0.66
	12	8	30	16	9	5	0.00	0.71
	16	4	42	0	12	6	15.55**	0.25[a]
A PN *vs* ca	PN	*ca*	PN ♀ × PN ♂	PN ♀ × *ca* ♂	*ca* ♀ × PN ♂	*ca* ♀ × *ca* ♂		
	4	16	0	10	6	44	1.33	0.36[a]
	8	12	13	18	9	20	0.76	0.81
	10	10	11	22	10	17	0.08	1.14
	12	8	24	17	11	8	0.00	0.87
	16	4	39	9	6	6	5.00*	0.33[a]
B	4	16	2	11	9	38	0.09	0.50
	8	12	7	14	15	24	0.15	0.93
	10	10	13	16	12	19	0.23	0.87
	12	8	13	22	12	13	0.70	1.30
	16	4	38	12	8	2	0.07	0.50
A TIR *vs* ct	TIR	*ct*	TIR ♀ × TIR ♂	TIR ♀ × *ct* ♂	*ct* ♀ × TIR ♂	*ct* ♀ × *ct* ♂		
	4	16	3	9	12	36	0.00	0.53
	8	12	7	18	10	25	0.00	0.87
	10	10	24	11	14	11	0.99	0.71
	12	8	32	14	6	8	3.29	0.50
	16	4	50	5	2	3	10.27**	0.13[a]
B	4	16	7	11	11	31	0.96	0.57
	8	12	10	14	15	21	0.00	0.93
	10	10	23	12	13	12	1.14	0.71
	12	8	25	15	11	9	0.31	0.76
	16	4	47	9	2	2	2.87	0.22[a]

*P < 0.05; **P < 0.01
IE: isolation estimate = number of heterogamic matings/number of homogamic matings
a, Low IE values
MY, PN and TIR wild-type strains.
Mutant lines: y = yellow body color; ca = claret eye color; ct = cut wing.
A = Spiess (1968) method. B = Ehrman (1966) method.

mating advantage for both males and females. Also, mating percentages did not show any particular trend. However, at the 10:10 ratio, the *ca* males were more successful than the wild-type males. In the test with TIR and *ct*, employing the Spiess method (Table 4), at the 10:10 ratio the wild-type males were more successful than the *ct* males. It is generally assumed that both types of males have equal mating ability if males are equally successful in mating when they are present at equal ratio (10:10) and can be treated as a 'control' for sexual activity, as we have found in with MY and *y* (Table 2), and with the Ehrman method for PN and *ca* (Table 3) and TIR and *ct* (Table 4). As we paired the strains for mating propensity, the deviations from the 10:10 ratios (Spiess method, Tables 3 and 4) were not due to differences in mating propensity, nor were they due to assortative mating or sexual isolation (Table 1). However, the Ehrman method did not reveal significant deviations from expected mating frequencies in the 10:10 presentation ratios (Tables 3 and 4). These deviations may be due to the different observation methods. In the females, random matings were found with the 10:10 ratios with both pairs of strains. In the experiment with PN and *ca*, employing the Ehrman method, *ca* males had a significant mating advantage ($P < 0.01$) when they were rare (PN: *ca* = 12:8). At other ratios, the advantage for rare type was not apparent, independent of the observation method. The rare female advantage was also not evident in the experiment with PN and *ca*. In the tests with TIR and *ct*, employing both observation methods, there was no rare type mating advantage for males or females. Rather, when *ct* females were rare, they were at a disadvantage in three cases of four ($P < 0.05$, $P < 0.01$). They had low mating percentages when they were rare (Table 4).

This approach to study frequency-dependent advantage, using χ^2 tests, has been discussed in the literature (Ayala, 1972; Adams and Duncan, 1979). The most serious drawback considered, was that by applying the χ^2 test, a test of differential fitness is made for each input frequency separately, while there is no test for a change in fitness over frequencies, which is the

Table 2. Results of multiple-choice experiment (MY *vs* y strains) in *Drosophila ananassae* mating choice (data based on six replicates).

Ratio		Observed frequency of mating				Expected frequency				Flies per 20	Mating percentage			
MY	*y*	MY♂	*y*♂	MY♀	*y*♀	MY	*y*	χ^2♂	χ^2♀		MY♂	*y*♂	MY♀	*y*♀
Spiess (1968) method:														
4	16	10	50	13	47	12	48	0.41	0.10	4	41.66	37.50	54.16	37.50
8	12	22	38	31	29	24	36	0.27	3.40	8	45.83	45.83	64.58	41.66
10	10	32	28	36	24	30	30	0.26	2.40	10	53.33	46.66	60.00	40.00
12	8	38	22	40	20	36	24	0.27	1.10	12	52.77	52.77	55.55	40.27
16	4	51	9	51	9	48	12	0.93	0.93	16	53.12	52.08	53.12	48.95
Ehrman (1966) method:														
4	16	12	48	17	43	12	48	0.00	2.60	4	50.00	50.00	70.83	25.00
8	12	27	33	28	32	24	36	0.62	1.10	8	56.25	43.75	58.33	29.16
10	10	31	29	37	23	30	30	0.06	3.26	10	51.66	48.33	61.66	38.33
12	8	39	21	46	14	36	24	0.62	6.93*	12	54.16	45.83	63.88	44.44
16	4	48	12	54	6	48	12	0.00	3.66	16	50.00	50.00	56.25	44.79

*P < 0.05

Table 3. Results of multiple-choice mating choice experiment (PN *vs* claret eye strains) in *Drosophila ananassae* (data based on six replicates).

Ratio		Observed frequency of mating				Expected frequency				Flies per 20	Mating percentage			
PN	*ca*	PN♂	*ca*♂	PN♀	*ca*♀	PN	*ca*	χ^2♂	χ^2♀		PN♂	*ca*♂	PN♀	*ca*♀
Spiess (1968) method:														
4	16	6	54	10	50	12	48	3.75	0.41	4	25.00	62.50	41.66	50.00
8	12	22	38	31	29	24	36	0.27	3.40	8	45.83	52.08	64.58	39.58
10	10	21	39	33	27	30	30	5.40*	0.60	10	35.00	65.00	55.00	45.00
12	8	35	25	41	19	36	24	0.06	1.73	12	48.61	52.77	56.94	40.27
16	4	45	15	48	12	48	12	0.93	0.93	16	46.87	56.25	50.00	52.08
Ehrman (1966) method:														
4	16	11	49	13	47	12	48	0.10	0.10	4	45.83	58.33	54.16	41.66
8	12	23	37	20	40	24	36	0.06	1.10	8	47.91	72.91	41.66	52.08
10	10	25	35	29	31	30	30	1.66	0.06	10	41.66	58.33	48.33	51.66
12	8	25	35	35	25	36	24	8.40**	0.06	12	34.72	51.38	48.61	55.55
16	4	46	14	50	10	48	12	0.41	3.66	16	47.91	51.04	52.08	48.95

*P < 0.05; **P < 0.01

Table 4. Results of multiple-choice mating choice experiment (TIR *vs* cut wing strains) in *Drosophila ananassae* (data based on six replicates).

Ratio		Observed frequency of mating				Expected frequency				Flies per 20	Mating percentage			
TIR	*ct*	TIR♂	*ct*♂	TIR♀	*ct*♀	TIR	*ct*	χ^2♂	χ^2♀		TIR♂	*ct*♂	TIR♀	*ct*♀
Spiess (1968) method:														
4	16	15	45	12	48	12	48	0.93	0.00	4	62.50	33.33	50.00	20.83
8	12	17	43	25	35	24	36	3.40	0.06	8	35.41	45.83	52.08	29.16
10	10	38	22	35	25	30	30	4.26*	1.66	10	63.33	36.66	58.33	41.66
12	8	38	22	46	14	36	24	0.27	6.93**	12	52.77	59.72	63.88	48.61
16	4	52	8	55	5	48	12	1.66	5.10*	16	54.16	46.87	57.29	50.00
Ehrman (1966) method:														
4	16	18	42	18	42	12	48	3.75	3.75	4	75.00	45.83	75.00	16.66
8	12	25	35	24	36	24	36	0.06	0.00	8	52.08	50.00	50.00	41.66
10	10	36	24	35	25	30	30	2.40	1.66	10	60.00	40.00	58.33	41.66
12	8	36	24	40	20	36	24	0.00	1.10	12	50.00	48.61	55.55	50.00
16	4	49	11	56	4	48	12	0.10	6.66**	16	41.04	43.75	58.33	43.75

*P < 0.05; **P < 0.01

original reason for conducting the experiments. In view of this, Ayala (1972) and Ayala and Campbell (1974) proposed that it would be much more appropriate to study the overall fitness trend, by incorporating the outputs of all input frequencies in a single statistic. They suggested

application of linear regression of logarithms of output on logarithms of input ratios to study rare type effects that may reveal rare type advantage, which might not be conspicuous in χ^2 tests. So, we analyzed our data by an ordinary regression equation, $\hat{Y} = a + bx$. Logarithmic transformations were done for the values of the X, the 'input ratio' (wild type:mutant type) at which flies were introduced into the mating chamber and the values of Y, the 'output ratio' (wild type:mutant type) at which flies were mated. Then output frequencies were regressed on input frequencies. A point of equilibrium occurs, if and where the regression line crosses the diagonal, at which point individuals mate at the same frequency at which they were introduced into the mating chamber (Ayala, 1972; Ayala and Campbell, 1974). If the slope of regression is less than one ($b < 1$), the equilibrium is stable, indicating rare type advantage, while when $b > 1$, the equilibrium is unstable, which indicates an advantage for the common type. Based on the results of regression analysis rare male mating advantage was significant in two cases (Table 5), and the slopes of the least square linear regression lines (Figure 1B, C, upper panels) differed significantly from one, the origin, indicating that mating success was strongly and significantly frequency dependent. There was a rare male mating advantage for PN and *ca* males, based on the Ehrman method (see also Table 3 at the 12:8 ratio, where rare *ca* males have a mating advantage). Though no significant rare type mating advantage was evident for TIR and *ct* flies with the Ehrman method (Table 4), regression analysis did indicate a significant effect (Table 5). The mating percentages of *ca* males were high when they were rare, and they decreased with increasing input frequencies (Table 3, Ehrman method). No such trend was found for PN males. So, it can be said that the rare male mating advantage found for *ca* males is one-sided (Table 5). Similarly, higher mating percentages at lower input frequencies were found for TIR males (Table 4), which decreased when these males were common in the mating chamber, while for *ct* males no such trend was found. So, in this case, rare male mating advantage was also one-sided for TIR males. This one-sided mating advantage found with the Ehrman method PN and *ca*, and for TIR and *ct* was not due to difference in male vigor, as this was approximately equal between the two strains, as evidenced by random mating at the 10:10 ratio observed with the Ehrman method. In all the other cases (Table 5), the regression coefficients were greater than one ($b > 1$) and the corresponding Figure 1A-C shows absence of rare male as well as rare female mating advantages. In order to determine whether two regression coefficients of two different experimental methods are in agreement, ANOVA was applied to two regression coefficients of the two observation methods for a single male or female type. No overall significant differences were found between the coefficients as well as between the means of output frequencies both for males and females (values not shown here).

DISCUSSION

Ehrman et al. (1972) carried out frequency-dependent mating advantage tests using wild-type and *y* males of *D. gaucha*. Mutant males performed very poorly in comparison to the wild-type males, when they were common compared to the wild types or were equally abundant. Mutant flies performed better in competition with the wild type only when they were rare, in which case they were able to mate equally well as their wild-type counterparts. Sturtevant (1915) was the first to report that *yellow* males of *D. melanogaster* are usually unsuccessful in a competitive mating situation and that this is probably due to their reduced activity to stimulate the females. Bastock (1956) also found that *yellow* mutant males of *D. melanogaster* are less

Table 5. Relative mating success of *Drosophila ananassae* males and females of three different pairs of strains, using two different observation methods (degrees of freedom = 3).

Experiment	Sex	Method	Regression Equation	Significance
I	♂	A	$\hat{Y} = 0.02 + 1.21x$	$P < 0.001$
		B	$\hat{Y} = 0.04 + 1.0009x$	$P < 0.001$
	♀	A	$\hat{Y} = 0.14 + 1.06x$	$P < 0.001$
		B	$\hat{Y} = 0.24 + 1.16x$	$P < 0.01$
II	♂	A	$\hat{Y} = -0.16 + 1.18x$	$P < 0.01$
		B	$\hat{Y} = -0.12 + 0.90x$	$P < 0.01$
	♀	A	$\hat{Y} = 0.07 + 1.06x$	$P < 0.01$
		B	$\hat{Y} = -0.0086 + 1.06x$	$P < 0.01$
III	♂	A	$\hat{Y} = 0.08 + 1.13x$	$P < 0.05$
		B	$\hat{Y} = 0.09 + 0.85x$	$P < 0.01$
	♀	A	$\hat{Y} = 0.19 + 1.40x$	$P < 0.001$
		B	$\hat{Y} = 0.20 + 1.26x$	$P < 0.01$

Method A = Spiess (1968) method of observation. Method B = Ehrman (1966) method of observation.

active than their wild-type counterparts. Later, in studies with *D. melanogaster*, Wilson et al. (1976) found that the *yellow* mutant has a reduction in body pigmentation associated with a decrement in locomotor activity and in male competitive mating activity. They indicated that the impaired locomotor activity of *y* males might not be the general cause of their lower mating speed and reduced competitive mating ability, as they found that the stimuli that *y* males provided to females were the same as those of wild-type males, both quantitatively and qualitatively. Also, Heisler (1984) remarked that low scores of *y* males in Wilson et al.'s (1976) work might be due to inbreeding. Earlier, Barker (1962) found by employing multiple-choice experiments that sexually matured wild type and *y* males mate almost equally with the *y* females. Threlkeld et al. (1974), who used female choice experiments, reported the development of female preference for *y* males in response to selection for enhanced acceptance of the *y* males, and concluded that it is misleading to regard *y* males as offering a low level of stimulus, without carefully defining the mating system. In our initial mating propensity test, we did not observe any reduced mating ability for males in our *yellow* strain of *D. ananassae*, rather it was quite high always, similar to the wild type. However, it was expected that *y* males would not show reduced competitive mating ability compared to the MY males as these lines were selected for approximately equal mating propensities. Neither of these two strains of *D. ananassae* had mating advantages when they were rare. Nor were the *y* males found to be less acceptable by the females. However, *y* females have been found to mate less than the wild-type females and they are at a disadvantage when they are rare. The reason that *y* females are less successful in comparison with wild-type females perhaps lie in the fact that the *yellow* locus has pleiotropic effects on the structure of the female genital apparatus, known from the work of Dobzhansky and Holz, 1943 (cited in Wilson et al., 1976). Later, Burnet and Connolly (1974) suggested possible involvement of *yellow* gene in the metabolic pathway of tyrosine to 3-4-dihydroxyphenylalanine, which is utilized in the biosynthetic pathways leading to the synthesis of sclerotonin and melanin; these

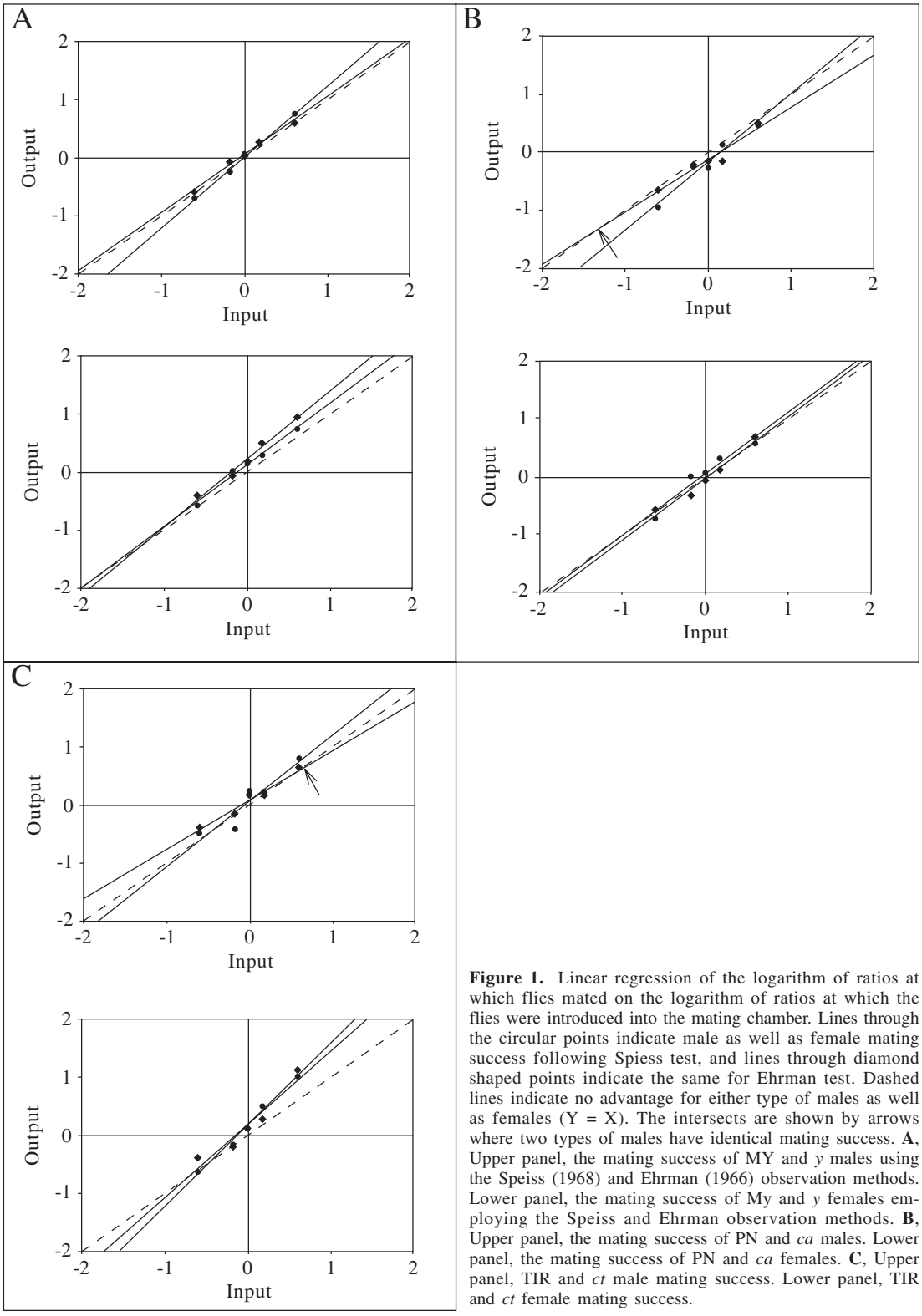

Figure 1. Linear regression of the logarithm of ratios at which flies mated on the logarithm of ratios at which the flies were introduced into the mating chamber. Lines through the circular points indicate male as well as female mating success following Spiess test, and lines through diamond shaped points indicate the same for Ehrman test. Dashed lines indicate no advantage for either type of males as well as females (Y = X). The intersects are shown by arrows where two types of males have identical mating success. **A**, Upper panel, the mating success of MY and *y* males using the Speiss (1968) and Ehrman (1966) observation methods. Lower panel, the mating success of My and *y* females employing the Speiss and Ehrman observation methods. **B**, Upper panel, the mating success of PN and *ca* males. Lower panel, the mating success of PN and *ca* females. **C**, Upper panel, TIR and *ct* male mating success. Lower panel, TIR and *ct* female mating success.

substances are involved in the hardening and pigmentation of the cuticle. They suggested that mutation in the *yellow* gene causes a change in some mechanical properties of the integument, which may result in functional impairment of the genitalia. This may be the reason for our finding of mating disadvantage of mutant females, as males mate with *y* females less frequently, especially when they are rare, as more acceptable females are easily available (as males can also be choosy).

Rare male experiments, using eye color mutants and wild-type strains, are not uncommon in the literature. Rare male advantage was first discovered in the *white* mutant (Petit, 1954). Thereafter, several research papers providing evidence for both advantages and disadvantages of rare eye color mutants were published (Knoppien, 1985a; Sondergaard, 1986; Spiess and Bowbal, 1987; Lichtenberger et al., 1988; Depiereux et al., 1990; Cakir and Kence, 1999). Both-sided rare male advantages were reported by Singh and Chatterjee (1989), who tested *sepia* (*se*) and *cardinal* (*cd*) eye color mutants against a VN-ST wild-type strain of *D. ananassae*. The *ca* eye color mutation that we used causes a reduced level of red and brown pigments. Many studies have evidenced the lower sexual fitness of eye color mutants, due to a positive correlation between pigment intensity and mating success, as vision plays a critical role in the courtship and mating of *Drosophila* (see Ochando, 1981), Just and Markow (1989) found almost equal mating success of *vermilion* mutant flies (sex-linked recessive mutation lacking brown pigment) with Canton-S wild-type flies at equal ratios (1:1). In our experiment, *ca* males of *D. ananassae* did not show lower mating success, even when in competition with the wild-type flies. Instead, one-sided rare male mating advantage was found for rare *ca* males. Since randomization was carried out to remove the differences in the residual genetic background outside the *ca* locus in both strains, it is likely that the advantage of *ca* males is a function of the mutant phenotype. Though rare *ca* females did not have a mating advantage, they were not at a disadvantage in mating in competition with wild-type females. Ochando (1981), based on quantitative analysis of eye pigments in mutants of *Drosophila*, reported that eye pigmentation is not necessarily related to visual ability. He indicated that the visual process is extremely complex and visual acuity is not necessarily directly related to greater pigment content (ommochromes and pteridines). Consequently, it can be concluded that the *ca* eye color mutation does not weaken the mutant males due to lower visual ability, nor does it prevent the females from accepting visual stimuli from the males.

We previously found evidence for both-sided rare male mating advantage using wild-type and *ct* wing strains of *D. bipectinata* (Singh and Sisodia, 1997) in a female-choice test. In the present experiment, using wild-type and *cut* wing flies of *D. ananassae*, a one-sided rare male mating advantage was found for TIR males, though rare *ct* males were not privileged; this was not found in the individual ratios, employing the χ^2 test, but was revealed when mating success was compared for changing ratios in a single statistic (Table 5). TIR and *ct* flies had approximately equal mating propensities, and no impairment in the mating ability of mutant males was observed. Earlier, Singh and Sisodia (1996) compared the mating ability of wild-type and *ct* wing flies of *D. bipectinata* and found that the wild- and mutant type strains were equally successful in mating, which is in agreement with what we found here with strains of *D. ananassae*. The ability to distinguish a rare from a common type implies discriminatory capacities (Ehrman, 1990). At the same time, another established fact is that the mate recognition system is polygenic in *Drosophila*. So, it is difficult to say exactly what caused this one-sided minority advantage for TIR males. Still, it can be said, since randomization was done in the

genetic background outside the *cut* locus, that this advantage is related to this locus. Minority advantage for TIR females was absent and mutant females had disadvantages when they were rare. In *Drosophila*, *cut* wing is a complex locus and is known for its pleiotropic effects. It was found in *D. melanogaster* that much of this locus is devoted to tissue- and stage-specific activation of the structural element (Johnson and Judd, 1979). This may somehow makes *ct* females less receptive to both the males and thus disadvantaged when they are in a minority.

Anderson and McGuire (1978) concluded that "Mating success is probably important as a component of fitness in both sexes, but in most experiments it can be determined accurately only for males".

Rare female mating advantage has not been discussed in the literature as exhaustively as it has been for males, though a few references are available regarding this aspect (Knoppien, 1985a; Cereghetti et al., 1987; Lichtenberger et al., 1988; Depiereux et al., 1990; Dernoncourt-Sterpin et al., 1991; Singh and Sisodia, 2000). In our experiment, a rare female mating disadvantage was found in the case of *y* and *ct* females, both sex-linked recessive mutations, but not in the *ca* females (autosomal). In *Drosophila* males, X-linked genes are hyperactivated, by which means total X-linked gene activity in male and female is approximately equalized. However, this is not always true, e.g., we observed failure of wing expansion more frequently in females than in males in the *ct* strain. So, it is quite possible that degree of expression of pleiotropic effects of *y* and *ct* loci differs in males and females, which might have led to minority mating disadvantages in females but not in males in the sex-linked mutants.

In our experiment, two observational methods were employed, the Spiess method and the Ehrman method (see Material and Methods). The Spiess method, in which mating pairs are aspirated out, furnishes a minimal value for rare male mating advantage, as there is no chance of remating in males (females generally do not remate as quickly). But it is clear that as copulating pairs are aspirated out, changes in the initial 'relative' sex ratio (ratio of X male to Y females or Y males to X females) occur. However, Dernoncourt-Sterpin et al. (1991) showed that the relative sex ratio factor plays only a very minor role in determining the rare type advantage found in the genotype ratio experiments. However, one male can take advantage of the courtship stimulation of another male type when both forms are courting the same females (Chatterjee and Singh, 1989). In these strains of *D. ananassae*, one or more males could often be observed surrounding a copulating pair. So, removing the copulating pair may disturb the second male's sexual activity. This may affect the outcome of a rare male experiment. On the contrary, in the Ehrman method, as copulating flies are not removed, rare males may gain much of their advantage from the possibility of mating more than once (Knoppien, 1985a). However, this should not be considered as a source of erroneous results showing strong rare male mating advantage, as repeated matings reinforce sexual selection, favoring males that mate repeatedly (Singh and Singh, 2001). In our experiment, rare male advantages as well as rare female disadvantages were more prominent with the Ehrman method than with the Spiess method. So the Ehrman method appears to be a more effective measure than the Spiess method. However, based on the ANOVA analysis, differences between the two methods did not significantly affect the outcome of the rare male experiments.

In summary, we found a one-sided rare male mating advantages for an eye color mutant (*ca*) and for a wild-type strain of *D. ananassae* in two different experiments, giving further evidence in support of minority-mating advantage and indicating influence of a mutant locus on this phenomenon. However, this could not be detected in another experiment employing wild-

type flies and *yellow* flies. This, and one-sided male mating success in two other experiments indicate that this might not be a universal phenomenon in *Drosophila* and perhaps it is not the only mechanism maintaining genetic polymorphisms in *Drosophila*. Moreover, we found minority mating disadvantages for rare females in *y* and *ct* strains, though they performed well when they were common or in equal proportion with the wild-type females in the mating chamber. To our knowledge, this is the first report for rare female mating disadvantage in *Drosophila*, though a disadvantage for rare males was reported earlier by Peterson and Merrell (1983). We found all these non-random matings in the absence of assortative mating or sexual isolation or a difference in mating propensities between two experimental strains, which, in agreement with Bryant et al. (1980), we think should be ensured to demonstrate a real minority effect. Moreover, our results indicate that application of different observational methods do not significantly alter the outcome of frequency-dependent experiments.

Finally, the mechanism of rare type advantage is not clearly understood. On the presumption that female choice is the key factor for producing a rare male advantage, different models regarding minority-mating advantage have been postulated, e.g., the sampling and habituation hypothesis (Ehrman and Spiess, 1969), the avoidance hypothesis (Spiess and Kruckeberg, 1980), constant female preference for one male type (O'Donald, 1977), and female discrimination capacity among different male phenotypes (Spiess and Bowbal, 1987). However, a female's ability to discriminate rare males is not fully understood, as Ayala and Campbell (1974) said that "When the whole genome is considered, every individual *Drosophila* has a unique genotype and thus is a rare type. Moreover, it is not likely that every single gene difference could be "recognized" by the flies. Yet either the females or the males, or both, must recognize a difference for the females to identify the rare males and prefer them as mates, or for the rare-type males to become sexually more active." Another major factor in mate recognition has been highlighted by Cobb and Ferveur (1996), "One of the major problems that bedevil behavior genetic studies is pleiotropy, or the production of multiple phenotypic effects from one genotypic effect. .....for studies that seek to pin down single, unitary effects, however, pleiotropy can lead to erroneously simple interpretations." A more recent concern of male choice for their females (Noor, 1996; Blows and Allan, 1998; Van Gossum, 2000; Bonduriansky, 2001) is expected to be included in minority-mating advantage experiments in the future.

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