

Lack of evidence for rare male mating advantage in wild type strains of *Drosophila ananassae*

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Experiments were conducted to test the rare male mating advantage in wild type strains of *Drosophila ananassae* derived from different geographic localities, by employing different experimental designs as well as different marking procedures. Mating success of two types of males was scored by direct observation in Elens–Wattiaux mating chamber at 5 different ratios. The results show that the differences between observed and expected numbers of matings of two types of males at 5 different ratios are not significant in all the experiments. Thus, there is lack of evidence for rare male mating advantage in wild type strains of *D. ananassae*. Further, there is consistency in the results with different experimental designs as well as different marking procedures, which suggests that different experimental designs and marking procedures have no effect on the results. The comparison of mating frequencies of two types of males shows that both types of males are equally successful in mating and thus have similar mating propensity. Further, there is no evidence for assortative mating as both types of females are equally receptive to two types of males.

RARE male mating advantage, abbreviated here as RMMA, is one of the widely studied examples of frequency-dependent selection, which suggests that fitness of a given genotype depends on its frequency. The term 'rare male mating advantage' implies that when two variants (phenotypic or genotypic) of the same species are present together, the relative mating success of males of each of the two variants is inversely related to that variant's relative abundance in the population, i.e. rare male type has a higher rate of success in mating than the common type. Since its first demonstration by Petit¹ in 1951 in *Drosophila melanogaster* and later by Ehrman² in 1966 in *D. pseudoobscura*, it has been reported in twelve species of *Drosophila* till date and subsequently in other genera also (for references see the reviews by Knoppin³ and Singh and Sisodia⁴). RMMA may explain how high level of genetic variability is maintained in natural population without genetic load at equilibrium and is thus more acceptable than the heterozygous model. It is also supposed to promote outbreeding^{5,6}.

In spite of its general acceptance, rare male effect hypothesis has been questioned many times regarding its causes. However, the more serious moot point is: whether the rare male effect exists or it is due to experimental artifact? And the debate is ongoing, emphasizing much more research regarding this intriguing subject of population genetics.

D. ananassae, a cosmopolitan and domestic species, belongs to the *ananassae* sub-group of the *melanogaster* species group and possesses a number of genetic peculiarities^{7,8}. A number of studies have been documented on certain aspects of behaviour in *D. ananassae*^{9–19}. Earlier, RMMA has been reported in this species using *cd* and *se* mutants with wild-type flies¹⁴.

During the present study, rare male effect experiments were conducted in two wild type strains of *D. ananassae*. Rare male effect experiments were carried out employing female choice technique, by direct observation in Elens–Wattiaux mating chamber²⁰. Further, different experimental methods as well as different marking procedures were also used to know their effects on rare male experiments. The results of these studies are reported in this communication.

Two different mass-culture wild-type strains of *D. ananassae* were used during the present study: (1) TIR-established from flies collected from Tirupati (Andhra Pradesh) in 1990, and (2) ELEN-raised from flies collected in Elenthikara (Kerala) in 1993. A preliminary test of mating propensity was carried out previously in different geographical strains and finally these two strains of the same mating propensity were chosen to avoid any preferential mating. To test frequency-dependent mating advantage, 'female choice' technique was used in which one type of females was placed with two different types of males. It would be more informative than 'male choice', 'multiple choice' or 'no choice' tests²¹. Two experimental methods were followed.

In the Spiess²² method, copulating pairs were aspirated out of the mating chamber and then the type of mated males was identified under binocular microscope. One male can mate once in this method.

In the Ehrman² method, mated male type was detected directly in the mating chamber, without taking out the copulating pair. Individual males may mate more than once.

Though Spiess, Ehrman and others in different experiments followed different experimental designs (e.g. multiple choice or female choice technique, taking flies in different ratios and numbers or different sex ratios, etc.), for convenience, the two terms 'Spiess method' and 'Ehrman method' are used in this paper, which differ basically in the way of observation. In the present experiment, a plan was chalked out according to the objective of the test, i.e. testing the mere existence of rare male effect in wild type strains and any possible

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effect of different experimental methods and marking procedures on this phenomenon.

Stocks were maintained on simple culture medium. Virgins were collected within few hours of eclosion, as previous exposure of the females to males, of a particular type lowers the mating advantage of males, when this type is rare²³. Virgin females and males of both the geographic strains were separated. Virgin females and males were stored separately in food vials, 15 flies per vial. The number was fixed to overcome the bias due to density effect²⁴. Flies were aged for seven days. One day before the experiment, virgins from more than one vial were taken and females of one type were kept in batch of 20 in a fresh food vial. Males of two types were kept in separate vial in the ratios to be tested and in a total of 20. Males were taken in five different ratios (4:16, 8:12, 10:10, 12:8 and 16:4). Care was taken to avoid any sampling error which could result from aspirating flies from upper part of the storage vial, since flies close to the top can be more active than the flies nearer to the bottom²⁵. Before keeping in the fresh vials, males of one type were marked in order to distinguish them from the other type. For each ratio, in half of the replicates, minority males and in the other half, common males were marked. Marking was done in two ways on lightly etherized males: (1) clipping the distal part of the right wing; and (2) putting a small drop of nail polish on the thorax.

Both the marking procedures were used for both the experimental methods, Spiess and Ehrman. Thus four sets of experiments were carried out based on two methods and two marking procedures: (1) Spiess method and marking the flies by wing clipping; (2) Spiess method and marking the flies by nail polish; (3) Ehrman method and marking the flies by wing clipping; (4) Ehrman method and marking the flies by nail polish.

Each of the above four sets of experiments was carried out for two types of females separately (female

choice). With each type of females, six replicates were run at each of the five ratios.

All the tests were performed from 7.00 to 10.30 am in a temperature-controlled room (24°C approx.) under normal light conditions. The differences between the number of matings were tested by calculating χ^2 values.

Results of four sets of experiments are presented in Table 1 (Spiess method and two marking procedures) and Table 2 (Ehrman method and two marking procedures). Number of matings of TIR and ELEN males with both types of females, in first 10 matings at different ratios are shown. Totally, 2400 matings (10 matings \times 6 runs \times 5 ratios \times 2 female types \times 4 sets of experiments) were recorded. The expected number of matings was calculated on the basis of the ratio between two types of males introduced into the mating chamber. χ^2 values show no significant difference ($P > 0.05$) between observed and expected number of matings of two types of males in all the cases, even when they were present in minority. The mean mating percentages of both TIR and ELEN males at different ratios were calculated from the data presented in Tables 1 and 2, by combining the data of both the females. These are shown in Tables 3 and 4.

During the observation, the sequence of matings was also recorded. In order to see if there is any preferential mating, the number of matings of TIR and ELEN males in the first five matings in each replicate was counted from the same data. The number of each type of males out of 600 matings in four sets of experiments with each type of females was calculated and the data are presented in Table 5. The χ^2 values ($P > 0.05$) show that TIR and ELEN males are equally successful in mating and both types of females are equally receptive to the two types of males, i.e. there is no evidence for preferential mating between two geographic strains tested during the present study.

Table 1. Results of female choice experiments following Spiess method applying two marking procedures, wing clipping (wc) and nail polish (np) (data based on 6 replicates)

Female type	Male ratio		Observed frequency (wc)		Observed frequency (np)		Expected frequency		χ^2 (wc)	χ^2 (np)
	TIR	ELEN	TIR	ELEN	TIR	ELEN	TIR	ELEN		
TIR	16	4	44	16	48	12	48	12	1.66	0.00
	12	8	35	25	40	20	36	24	0.06	1.10
	10	10	29	31	31	29	30	30	0.06	0.06
	8	12	23	37	25	35	24	36	0.06	0.06
	4	16	15	45	13	47	12	48	0.93	0.10
ELEN	16	4	46	14	46	14	48	12	0.41	0.41
	12	8	35	25	35	25	36	24	0.06	0.06
	10	10	34	26	32	28	30	30	1.06	0.26
	8	12	19	41	17	43	24	36	1.73	3.40
	4	16	12	48	12	48	12	48	0.00	0.00

Table 2. Results of female choice experiments following Ehrman method applying two marking procedures, wing clipping (wc) and nail polish (np) (data based on 6 replicates)

Female type	Male ratio		Observed frequency (wc)		Observed frequency (np)		Expected frequency		χ^2 (wc)	χ^2 (np)
	TIR	ELEN	TIR	ELEN	TIR	ELEN	TIR	ELEN		
TIR	16	4	47	13	50	10	48	12	0.10	0.41
	12	8	30	30	30	30	36	24	2.50	2.50
	10	10	24	36	31	29	30	30	2.40	0.06
	8	12	22	38	18	42	24	36	0.27	2.50
	4	16	9	51	8	52	12	48	0.93	1.66
ELEN	16	4	45	15	46	14	48	12	0.93	0.41
	12	8	36	24	32	28	36	24	0.00	1.10
	10	10	25	35	29	31	30	30	1.66	0.06
	8	12	17	43	30	30	24	36	3.40	2.50
	4	16	8	52	11	49	12	48	1.66	0.10

Table 3. Mean mating percentage of TIR males at different ratios, calculated by combining the data of both TIR and ELEN females (shown separately for four sets of experiments; I and II are based on the data presented in Table 1 and III and IV those on Table 2)

Number of males per 20	Mean mating percentage			
	I (wc)	II (np)	III (wc)	IV (np)
4	56.25	52.08	35.41	39.58
8	43.75	43.75	40.62	50.00
10	52.50	52.50	40.83	50.00
12	48.61	52.08	45.83	43.05
16	46.87	48.95	47.91	50.00

The results obtained during the present study clearly demonstrate that there is no evidence of RMMA in wild type strains of *D. ananassae* tested. Further, there is consistency in the results with both methods (Spiess and Ehrman) and also with both marking procedures. Based on these observations, it is suggested that marking procedures and experimental techniques have no effect on the results.

Robertson²⁶ found that clipping only a small part of the wing has practically no influence on mating success, at least in *D. melanogaster*. Before starting the experiments, one preliminary test was conducted to see whether mating propensity in *D. ananassae* might be altered by marking procedures. It was found that clipping the distal tip of one wing or putting a small drop of nail polish on the scutellum does not change mating propensity in this species²⁷. However, following multiple choice technique and marking by wing clipping in housefly, *Musca domestica*, Bryant *et al.*²⁸ found that if males are harmed during marking for identification such that their mating success is subsequently affected, rare male effect will be enhanced. Hence during the present study, wing clipping as well as nail-polish markings were followed and found to have no effect on rare male experiments. A small drop of nail polish was put on the

Table 4. Mean mating percentage of ELEN males at different ratios, calculated by combining the data of both TIR and ELEN females (shown separately for four sets of experiments; I and II are based on the data presented in Table 1 and III and IV those on Table 2)

Number of males per 20	Mean mating percentage			
	I (wc)	II (np)	III (wc)	IV (np)
4	62.50	54.16	58.33	50.00
8	52.08	46.87	56.25	60.41
10	47.50	47.50	59.16	50.00
12	54.16	54.16	56.25	50.00
16	48.43	49.47	53.64	52.60

thorax, as it was easier to mark a large number of flies rather than putting it on the minute scutellum.

It may be possible that favoured rare males may gain much of their advantage from the possibilities of mating more than once, as may occur in Ehrman method, in which copulating pairs are not aspirated out of the mating chamber. In that case, Spiess method, in which copulating flies are taken out, will diminish rare male effect but it obtains a minimal value for rare male advantage, as there is no chance of remating. Again, taking out the flies changes the male ratio taken at the beginning, which may affect the result. So both the experimental methods were followed. But throughout the work no evidence for significant RMMA was found for these two wild type strains of *D. ananassae*.

It is clear from Knoppien's review³ that though there are several studies supporting RMMA, there are also several reports which show lack of evidence for this phenomenon even when repeated in the same species following the same experimental criterion²⁹. Peterson and Merrell²¹ reported the disadvantage for rare males.

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Table 5. Number of mated males of TIR and ELEN strains in four sets of experiments (serial followed as mentioned in the text) with both types of females (calculation based on first five matings in each replicate)

Type of females	Type of males	Number of matings				Total number of mated males	χ^2
		I	II	III	IV		
TIR	TIR	76	80	74	64	294	0.24
	ELEN	74	70	76	86	306	
ELEN	TIR	71	75	67	74	287	1.12
	ELEN	79	75	83	76	313	

More recently, Cakir and Kence³⁰ have commented that RMMA is probably not an important factor maintaining genetic variation in natural populations of *Drosophila*. The inconsistency in the results of rare male experiments is due to the fact that mating success of *Drosophila* is dependent on many factors^{31–33}, which makes it difficult to interpret a real RMMA. In this context, one should ensure at least some points²⁸: competing male types are equally vigorous, marking for identification has no effect upon mating preference, the number of pairs per strain is sufficient to override sampling effects and females (or some of them) do not show a preference for one male type or the other. Though here the term 'vigour' is replaced by 'mating propensity', as vigour implies an intrinsic property and is a broad term. It has been mentioned earlier that these two wild type strains have more or less equal mating propensity and the results show that two types of males were equally successful in mating when present in an equal ratio (10:10). According to Bryant *et al.*²⁸, though the two strains have the same sexual vigour, when flies are sampled from the population, the difference in sexual vigour between the sampled lines would not be zero; so the number of pairs per strain should be sufficient, which may counteract this sampling effect. In these female choice experiments, minority males are taken in numbers of 4 or 8. Thus there is less chance of error due to sampling effect, if any. And it is clear from Table 5 that females are equally receptive to both types of males, showing no preference for any particular type of male. Thus the males of the two geographic strains of *D. ananassae* with equal mating propensity, are exposed to near equality of factors without chances of preferential mating. RMMA was found in geographic strains of *D. pseudoobscura*, but not in geographic strains of *D. paulistorum*^{2,34} or *D. immigrans*³⁵, due to intraspecific sexual isolation. But the absence of RMMA in geographic strains of *D. ananassae* tested during the present study is not due to ethological isolation, as there is no preferential or assortative mating between the strains tested.

Different experiments by Ehrman and her colleagues seem to implicate pheromone as a basis of communica-

tion in *D. pseudoobscura*. In their sampling and habituation hypothesis, Ehrman and Spiess³⁶ suggested that females experience a greater degree of sensory habituation to the cues of common type of male, and the rare type of male can break through this habituation by its slightly different cue and has an advantage in mating. This hypothesis has been supported by Averhoff and Richardson^{37,38} and by Van den Berg *et al.*³⁹, who suggested that genetic variation existed for pheromone. In view of this, absence of RMMA in the present experiments might be surmised as follows: probably there is no distinctive difference in cues of males of these two wild type strains of *D. ananassae*, as a result of which females are unable to discriminate the rare and common males. These results emphasize the need for more work in this interesting field of study.

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Plio-Pleistocene pedogenic changes in the Siwalik palaeosols: A rock magnetic approach

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Thirty-one pedogenic horizons of the Upper Siwalik (Plio-Pleistocene) sequence near Haripur, Himachal Pradesh (HP) in NW Himalaya are studied using rock magnetic properties to investigate their climatic and stratigraphic significance. The palaeosols are characterized by low initial susceptibility (χ_{ir} -mean = 9×10^{-8} m³/kg), high coercivity of remanence (B_{0Cr} -mean = 447 mT), and low frequency-dependent susceptibility (X_{fd} -max = 6%) as a result of predominance of the canted-antiferromagnetic minerals of SD-PSD range. Stepwise acquisition of isothermal remanent magnetization (IRM) to a forward field of 7000 mT and backfield of 1000 mT indicates the presence of high coercivity minerals (goethite and hematite). Rock magnetic properties of pedogenic levels are inferred with reference to parental horizon of each palaeosol profile. Based on selective saturation levels of induced magnetic field, inorganic and organic carbon content and Rb/Sr ratios, new parameters are attempted to infer the relative variation in pedogenic changes of oxidation, hydroxylation and humification. These parameters record dynamic changes in the soil development processes of the Indo-Gangetic foreland basin, suitable for high-resolution stratigraphic correlations and reconstruction of climate change during the Plio-Pleistocene time.

THE Siwalik Group of the Indo-Gangetic Foreland Basin (IGFB) in Himalaya preserves a long record (~18 to

0.5 Ma) of alluvial fan sedimentation, giving rise to rapid lateral lithofacies variations. This resulted in a problem of time-stratigraphic correlation of the exposed sequences throughout the basin. Scanty occurrence of datable fauna restricted an ideal biostratigraphic approach. Whereas magnetostratigraphy has helped only to a limited extent in solving the stratigraphic problems¹, due to less abundance of suitable rock types that could ideally preserve the records of depositional remanence. The sediments of Siwalik province, although deposited in high-energy conditions, have developed thick and multi-storied soil profiles (or palaeosols) that are untapped yet, to investigate their stratigraphic and climatic relevance.

Rock magnetism presents a rapid, economic, non-destructive and quantitative technique that has recently been applied successfully for climatic and stratigraphic studies worldwide^{2,3}. We report here an independent rock magnetic approach on pedogenic horizons from a well-documented section of Upper Siwalik sub-Group (Plio-Pleistocene) in the IGFB of NW Himalaya (Figure 1), to investigate its climatic and stratigraphic applications.

The Siwalik Group represents the most continuous and near complete record of mammalian faunal evolution in South Asia and is well-known for its hominoid primate fossils^{4,5}. Biostratigraphic records indicate a remarkably high bio-diversity during the Siwalik era⁵ and the Plio-Pleistocene transition records a major faunal change related to climate⁶. Thus it is quite relevant to study the soil supporting such healthy co-existence of eco-system and its relation to climate change.

Johnson⁴ described the Siwalik palaeosols as low-grade oxisols to represent alternate wet and dry seasons, proposing its vegetative similarity to the alluvial soils of Ghana, Sudan and Brazil. Using magnetostratigraphic ages, Behrensmeier *et al.*⁷ derived 13,000–23,000 years as the maximum time for soil formation during Siwaliks. The characteristic ‘ferruginous-carbonate’ association of these palaeosols is described

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