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Mating success and morphometric traits in *Drosophila ananassae*

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Mating success was scored in two geographical strains of *Drosophila ananassae* by direct observation for sixty minutes in an Elens–Wattiaux mating chamber. In each replicate 15 pairs were tested and in total twenty replicates were run in each strain. After one hour of observation, mated and unmated flies of both sexes were kept separately and various morphometric traits such as face width, head width, thorax length, wing length and wing width were measured. Mean values for all the morphometric traits measured are higher in mated flies compared to unmated ones in both the strains of *D. ananassae*. Variations were tested by two-way ANOVA and highly significant variations were found for mating status and sex in both the strains. Significant positive correlation was also found among different phenotypic traits. Based on these results, it is suggested that body size of males and females of *D. ananassae* is correlated with mating success.

EVOLUTIONARY response to selection depends on the amount of genetic variation expressed in the population. Because of this, the effect of environmental changes on the expression of genetic variation in the quantitative traits has important evolutionary implications[1]. Body size and reproductive performance are known to be correlated in both male and female insects[2]. In *Drosophila*, as in many other insects, body size has been found to be positively correlated with male mating success. However, body size itself may not necessarily be the direct target of sexual selection, because such an association could be the result of selection on one or many traits correlated with it[3]. Body size is a fitness character that can be easily measured. The experimental measurable trait has produced a large amount of data relating size with other life-history traits, particularly in *Drosophila*[4–7].

Drosophila ananassae is a cosmopolitan and domestic species and occupies unique status among *Drosophila* species due to certain peculiarities in its genetic behaviour[8]. It has been extensively used for genetic studies, particularly population and behavioural genetics[9]. Because sexual selection could occur on many traits correlated with body size, it is of interest to examine the relative importance of size-related traits that could be related to mating success. Yet no work has been done on morphometric traits and their relation to mating success in *D. ananassae*. In view of this, the aim of the present study is to examine the reproductive performance of laboratory stocks of *D. ananassae* by measuring thorax length, wing and head traits.

In order to test mating success in *D. ananassae*, two mass culture stocks, established from flies collected from different geographic localities, were used: (i) PC-Pondicherry, established in 1999; (ii) CA-Calcutta, established in 1999. Both these stocks were maintained on simple culture medium under normal laboratory conditions by transferring about 50 flies (females and males in equal number) to fresh culture bottles in each generation. Mating was scored by direct observation in Elens–Wattiaux mating chamber kept in a room maintained at approximately 24°C temperature under normal laboratory light condition during 7.00 to 11.00 AM. In each stock, virgin females and males were collected and aged for seven days. Fifteen virgin females and males were introduced into the Elens–Wattiaux mating chamber. Mating was observed for 60 min. All mated pairs were removed with the help of an aspirator. The flies persisting in the mating chamber after one hour were collected and designated 'unmated', while copulating flies were designated

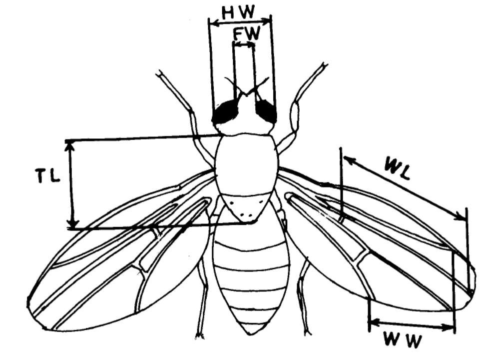

Figure 1. Schematic diagram of a *Drosophila* showing the measured traits: FW, face width; HW, head width; TL, thorax length; WL, wing length; WW, wing width.

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Table 1. Mean ± SE for five measured traits of mated and unmated flies of PC strain of *D. ananassae*

	Female				Male			
	Mated		Unmated		Mated		Unmated	
Trait	*N*	Mean ± SE	*N*	Mean ± SE	*N*	Mean ± SE	*N*	Mean ± SE
FW	156	18.929 ± 0.176	115	17.347 ± 0.240	156	17.262 ± 0.163	115	16.330 ± 0.200
HW	156	52.057 ± 0.256	115	47.826 ± 0.503	156	49.166 ± 0.277	115	45.678 ± 0.379
TL	156	60.237 ± 0.262	115	57.591 ± 0.363	156	54.564 ± 0.215	115	51.191 ± 0.374
WL	156	81.557 ± 0.319	115	78.582 ± 0.367	156	73.903 ± 0.272	115	70.565 ± 0.387
WW	156	57.109 ± 0.258	115	54.539 ± 0.311	156	52.647 ± 0.217	115	49.626 ± 0.326

N, Number of individuals; 1 unit (one division of ocular) = 16.67 μ.

Table 2. Comparison of five measured traits between mated and unmated individuals of PC strain of *D. ananassae* by two-way ANOVA

Trait	Source	df	MS	F
FW	Mated status	1	63.39	51.53*
HW		1	280.72	82.80*
TL		1	308.85	46.79*
WL		1	234.23	74.59*
WW		1	239.64	66.75*
FW	Sex	1	34.36	27.94*
HW		1	171.11	50.47*
TL		1	791.46	119.91*
WL		1	1271.73	405.00*
WW		1	395.07	110.04*
FW	Interaction	1	2.28	1.85
HW	Mated status × sex	1	0.181	0.053
TL		1	2.58	0.39
WL		1	1.11	0.35
WW		1	2.65	0.73
FW	Error	76	1.23	
HW		76	3.39	
TL		76	6.60	
WL		76	3.14	
WW		76	3.59	

*$P < 0.001$.

as 'mated'. Twenty replicates were run for each of the two strains of *D. ananassae*. They were also stored in 70% alcohol separately. They were also scored for five metric traits (Figure 1): face width, head width, thorax length, wing length and wing width. Face width (FW) is the smallest distance between eyes. Head width (HW) is the distance between the left and the right side of head capsule. Thorax length (TL) was scored from the anterior margin of the thorax to the posterior tip of the scutellum. Wing length (WL) was measured from anterior crossvein to distal tip of vein III. Wing Width (WW) was scored as the distance between distal tips of veins II and V. All measurements were performed under a compound microscope fitted with an ocular micrometer at 5X × 10X magnification. The ocular micrometer is of 100 divisions and one division of micrometer is equivalent to 16.67 μ measured with the help of stage micrometer. To measure HW and FW, the head was removed and observed. The TL was scored from lateral view.

Table 3. Results of Pearson correlation test for morphometric traits for PC strain

	FW	HW	TL	WL	WW
Female					
FW	–	0.579**	0.458**	0.412**	0.401**
HW		–	0.508**	0.420**	0.383**
TL			–	0.679**	0.701**
WL				–	0.650**
WW					–
Male					
FW	–	0.550**	0.294**	0.250**	0.225**
HW		–	0.455**	0.355**	0.327**
TL			–	0.669**	0.539**
WL				–	0.672**
WW					–

**Correlation is significant at the 0.01 level (two-tailed).

Mean ± SE for all five measured traits of mated and unmated females and males of PC strain are given in Table 1. Mean for all the five traits are higher in mated flies than unmated flies in both females and as well as in males. This suggests that mated flies are larger than unmated flies. Differences for all the five traits between mated and unmated females and males are tested by two-way ANOVA (Table 2). Differences are significant for mated status and sex ($P < 0.001$), but interaction between mated status and sex is not significant. To correlate all the five morphometric traits with each other for PC strain, Pearson correlation test was performed (Table 3). It has been found that there is significant positive correlation among all the five traits in males and females of PC strain. Table 4 presents mean ± SE for all the five morphological traits of mated and unmated individuals of CA strain of *D. ananassae*. Comparison of five measured traits between mated and unmated individuals of CA strain is made by two-way ANOVA (Table 5). The differences are highly significant for both mated status and sex ($P < 0.001$). The interaction between mated status and sex is not significant. To correlate all the five morphometric traits with each other for CA strain, Pearson correlation test was performed (Table 6). It has been found that there is significant positive correlation among all the five traits

Table 4. Mean ± SE for five measured traits of mated and unmated flies of CA strain of *D. ananassae*

	Female				Male			
	Mated		Unmated		Mated		Unmated	
Trait	*N*	Mean ± SE	*N*	Mean ± SE	*N*	Mean ± SE	*N*	Mean ± SE
FW	188	18.840 ± 0.257	87	17.62 ± 0.310	188	17.255 ± 0.145	87	16.080 ± 0.208
HW	188	52.500 ± 0.295	87	49.367 ± 0.434	188	48.893 ± 0.271	87	46.379 ± 0.419
TL	188	60.760 ± 0.339	87	56.977 ± 0.558	188	54.239 ± 0.272	87	50.931 ± 0.469
WL	188	81.883 ± 0.354	87	77.344 ± 0.694	188	71.590 ± 0.284	87	69.344 ± 0.535
WW	188	56.691 ± 0.269	87	53.597 ± 0.409	188	52.244 ± 0.222	87	48.540 ± 0.425

N, Number of individuals; 1 unit (one division of ocular) = 16.67 μ.

Table 5. Comparison of five measured traits between mated and unmated flies of CA strain of *D. ananassae* by two-way ANOVA

Trait	Source	df	MS	F
FW	Mated status	1	21.69	13.06*
HW		1	163.30	24.33*
TL		1	205.79	24.21*
WL		1	213.67	17.85*
WW		1	175.94	29.27*
FW	Sex	1	49.96	30.09*
HW		1	170.81	25.45*
TL		1	804.22	94.61*
WL		1	1702.75	142.25*
WW		1	573.52	95.42*
FW	Interaction	1	0.096	0.057
HW	Mated status × sex	1	0.336	0.05
TL		1	1.01	0.11
WL		1	5.51	0.47
WW		1	0.252	0.041
FW	Error	76	1.66	
HW		76	6.71	
TL		76	8.50	
WL		76	11.97	
WW		76	6.01	

$*P < 0.001$.

Table 6. Results of Pearson correlation test for morphometric traits in CA strain

	FW	HW	TL	WL	WW
Female					
FW	–	0.293**	0.264**	0.221**	0.216**
HW		–	0.694**	0.634**	0.550**
TL			–	0.851**	0.706**
WL				–	0.752**
WW					–
Male					
FW	–	0.460**	0.299**	0.231**	0.185*
HW		–	0.700**	0.641**	0.633**
TL			–	0.864**	0.733**
WL				–	0.812**
WW					–

*Correlation is significant at the 0.05 level (two-tailed).
**Correlation is significant at the 0.01 level (two-tailed).

in males and females of CA strain. For all the measured traits the results are similar in both the strains.

The present results suggest that mating males and females were larger than unmated males and females in both the strains of *D. ananassae*. Noory *et al.*[3] reported significant difference between mated and unmated males, but difference was not significant for females in *D. buzzatii*. Bateman[10] suggested that there is sexual difference in fitness variances for mating success in *D. melanogaster*, largely due to the fact that mating success is more variable in males than in females (for mathematical demonstration see Wade and Arnold[11]). Male reproductive success is determined by number of matings achieved, whereas female reproductive potential is determined by number of eggs laid. Singh and Mathew[12] reported that females and males with high number of sternopleural bristles in *D. ananassae* are more successful in mating than those with low number of bristles. Further, Singh and Mathew[13] tested the effect of sternopleural bristle phenotypes on fitness in *D. ananassae* and results suggested that flies with higher number of sternopleural bristles show greater fertility than the flies with lower number of bristles. This provides evidence for positive correlation between sternopleural bristle number, mating propensity and fertility in *D. ananassae*. Santos *et al.*[14] reported that larger flies of *D. buzzatii* mate more often in nature. In *Drosophila*, wing plays an important role in courtship, because species-specific auditory signals (courtship song) are produced by the male's wing vibration[15]. Aspi and Hoikkala[16] showed the importance of male song in mating success in *D. littoralis* and *D. montana*. A male with long wings produces more effective courtship song than one with short wings. Using wing as an index of body size, Monclus and Prevosti[17] and Naseerulla and Hegde[18] have shown that fast-mating flies have larger wings than slow- and non-mating flies in *D. subobscura* and *D. malerkotliana*, respectively, in multiple-choice situation. Thus most of the studies have indicated the advantage of large size in males. Dow and Schilcher[19] while studying aggression and mating success in *D. melanogaster* showed that large male was successful in mating with females. The larger male could win over the smaller ones in getting the female partner. Though there is no physical fight in *Drosophila*, larger males by virtue of their higher vigour would be successful in obtaining their mate.

Krishna and Hegde[20,21] studied reproductive success of large and small flies in *D. bipectinata* complex by various choice methods. Preferential mating occurred between large males and large females as well as small males and small females which demonstrated that large males have higher re-mating ability and longevity than small males, as a result of which they can inseminate more females in their life time than small males. Large females also have more number of ovarioles, lay more eggs and produce more fertile offspring than the small females and also mate with more males in their life time than small females, due to higher longevity. These findings suggest that large flies have higher reproductive success than small flies. Higher reproductive success of large flies can also be accounted for from their longevity.

Earlier studies on body size and male reproductive performance have generally demonstrated that larger males have an advantage with respect to one or more measures of reproductive performance. In *Drosophila*, larger males are more likely to mate than smaller males when held in mixed cultures of large and small males[22–24]. Furthermore, field studies indicate that wild mating males of *D. melanogaster*, *D. pseudoobscura* and *D. buzzatii* are larger than wild males sampled at random[7,14].

During the present study in *D. ananassae*, larger males and females are advantageous. Large males seem to have higher mating success by virtue of their aggression to mate with females and larger females may have higher success because they can discriminate the size of their mates. The finding by Ewing[25] that small males are less preferred than large males when both are present would confirm the above statement. Our results could be best interpreted as due to vigour or general activity levels of larger flies which are more likely to encounter suitable mates than smaller ones. From our observations, we can conclude that males and females with larger body size are found to engage more often in mating than flies with smaller body size. So there is sexual selection acting on body size, either directly or indirectly.

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Polymelia in the tadpoles of *Bufo melanostictus* (Anura: Bufonidae)

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In this communication we report vitamin A-induced multiplication of forelimbs and hind limbs in the tadpoles of *Bufo melanostictus* (Anura: Bufonidae). Different types of limb multiplication, based on the skeletal elements have also been investigated. Hind limb bud-stage tadpoles were exposed to vitamin A (Palmitate, 10 IU/ml) solution for 24 (set I), 48 (set II) and 72 h (set III), respectively. Vitamin A caused an exposure-dependent toxic effect on survival of the tadpoles because 10, 30 and 60% tadpoles died prior to the emergence of forelimbs from sets I, II and III, respectively. Metamorphosis was delayed in the experimental tadpoles.

THERE is a marked difference between the regenerating and developing limbs of amphibians in response to vitamin A. Niazi and Ratnasamy[1] reported that the regenerating limbs produce proximo-distal (PD) duplication, while simultaneously the developing limbs become hypomor phic in response to vitamin A in *Bufo melanostictus*.

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