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Mating success is not correlated with fluctuating asymmetry in *Drosophila ananassae*

C. Vishalakshi and B. N. Singh*

Genetics Laboratory, Department of Zoology,
Banaras Hindu University, Varanasi 221 005, India

Sexual selection results from differential mating success among individuals within a population. In the present study, the relationship between the mating success, and size and asymmetry of different morphological traits, viz. thorax length, wing length, sternopleural bristle number, wing-to-thorax ratio, sex-comb tooth number and ovariole number was investigated in two geographical strains of *Drosophila ananassae*. Mating success was scored in an Elens-Wattiaux mating chamber for 60 min. After 1 h of observation, mated and unmated flies of both sexes were kept separately and different traits were measured. The size of all the morphological traits was higher in mated than unmated flies. The level of fluctuating asymmetry was similar in mated and unmated flies for all the traits, except sternopleural bristle number in females. Positional fluctuating asymmetry, a sensitive measure of developmental instability was also significant for bristle number in females. When fluctuating asymmetry (FA) across all traits was combined into a single composite index (CFA), the magnitude of CFA was similar in mated and unmated flies. Interestingly, there is significant difference in males and females for CFA values in both mated and unmated flies. Males show higher FA in comparison to females, suggesting that males are more prone to developmental perturbations. The results suggest that the size of the sexual trait is a more reliable indicator of individual quality in sexual selection rather than fluctuating asymmetry in *D. ananassae* and thereby weakens the hypothesis that FA is a cue of overall ‘genetic quality’.

Keywords: Developmental stability, *Drosophila ananassae*, fluctuating asymmetry, morphological traits, sexual selection.

SEXUAL selection arises due to variation in mating success among individuals of a population due to competition for mates¹. Two distinct processes that are responsible for this variation are intrasexual and intersexual selection². The former is contest interaction between members of the same sex for access to mate, e.g. male–male competition to gain access to reproductive females. The latter is mate selection by individuals of one sex among members of the opposite sex, e.g. females prefer to mate with potential mating preferences in order to get fitness benefits from being choosy through direct (for themselves) and

*For correspondence. (e-mail: bnsingh@bhu.ac.in)

indirect (for their offspring) benefits³. Direct benefit may be derived from enhanced fertility, fecundity or parental care obtained from mating with preferred males, while indirect benefits arise from arbitrary male attractiveness or viability signalled by secondary sexual trait^{3,4}. Under the direct and indirect benefit models of female preferences evolution, females need to obtain cues to the potential benefits of accepting a given male through phenotypic conditions indicating male quality⁵, reflected by developmental stability (DS)³, which is the outcome of corrective mechanism that buffers the effects of the minor, intrinsic perturbations to developmental processes^{6,7}. It reflects the overall ability of the individual to cope with genetic and environmental stress⁸. However, when an organism failed to buffer itself against epigenetic perturbations, thereby disrupting its developmentally programmed phenotype, this is called developmental instability⁹. Thus, lack of developmental fidelity incurs physiological costs; reduced DS has been predicted to compromise individual fitness components^{7,10,11}. Therefore, DS may be a target of female choice because females mating with males showing greater DS should produce offspring that are better equipped to handle environmental stress and also acquire Fisherian benefit, i.e. greater attractiveness¹².

A population parameter to measure developmental (in) stability is fluctuating asymmetry (FA), which refers to subtle random deviations from perfect bilateral symmetry¹³. Beside FA, there are two other types of asymmetry, directional asymmetry (DA) and antisymmetry (AS). DA occurs when there is normally greater development of a character on one side of the plane of symmetry than on the other. AS occurs when one side of the plane of symmetry is consistently larger than the other, but the larger side may be either right (*R*) or left (*L*) at the random, resulting in a bimodal distribution of *R*–*L* differences about a mean of zero¹³. Both DA and AS are developmentally controlled and have adaptive significance, whereas FA is not likely to be adaptive as symmetry is expected to be the ideal state^{13,14}. The levels of FA are affected by various types of genetic and environmental stresses and seem to increase with increased homozygosity, hybridization, inbreeding, mutation, extreme physical conditions, pollution and habitat deterioration, but their response appears to be taxon- and trait-specific⁸. High levels of FA are assumed to reflect reduced developmental stability, i.e. reduced fitness¹⁵. Both intra and intersexual selection are situations in which individuals make decisions based on the quality of their conspecifics. The link between FA of an individual and its ability to cope with stress encountered in the environment and/or its genetic quality reflects that FA could be an ideal phenotypic cue for choosy females and competing males⁴. The association between developmental instability (DI) and fitness, and their role in sexual selection has been extensively reviewed¹⁶, but due to publication bias and lack of general patterns^{17,18}, our understanding of the forces behind DI–fitness association is

hampered. In the present communication, we investigate the association between FA and a component of fitness, i.e. mating success using a drosophilid fly as test organism.

Drosophila ananassae, a cosmopolitan and domestic species, occupies a unique status among the *Drosophila* species, due to certain peculiarities in its genetic behaviour¹⁹. There is evidence for both correlation of mating success with body size²⁰ and size-dependent sexual selection²¹ in *D. ananassae*. Yet no work has been done on FA of morphological traits and their relation to mating success in *D. ananassae*. In view of this, we tested whether mating success is connected to the putative higher genetic quality of more symmetrical individuals in *D. ananassae*. To test the sensitivity of FA to sexual selection, levels of FA in different morphological traits, viz. wing length, sternopleural bristle number, wing-to-thorax ratio, sex-comb tooth number and ovariole number in mated and unmated flies of *D. ananassae* were compared. Further, we have tried to investigate whether FA indeed is a reliable cue for potential mate.

Two different wild-type strains of *D. ananassae* kept in mass cultures established from flies collected from different geographic localities were used: (i) PC – Pondicherry, established in 2005, (ii) MB – Mumbai, established in 2006. Both strains were maintained on simple yeast – agar culture medium under normal laboratory conditions at approximately 24°C by transferring about 50 flies (females and males in equal number) to fresh culture bottles in each generation.

Virgin females and males of both strains were separated under anesthesia within 2–4 h of eclosion to avoid previous exposure of females to male courtship, which may otherwise affect the results²². Virgin females and males were kept in separate batches of ten and this number was fixed to avoid bias due to density effects²³. Flies were aged for seven days. One day before the experiment, virgin females and males were taken and examined under anesthesia for any obvious morphological distortions that might have taken place during the period of ageing. Only normal flies were taken.

Fifteen males and females were introduced into an Elens–Wattiaux mating chamber²⁴, without etherization; females were introduced first. Mating success was directly observed for 60 min. All the mated pairs were removed with the help of an aspirator and kept in separate empty vials. Flies persisting in the mating chamber after 1 h were collected and designated as ‘unmated’, while copulating flies were marked as ‘mated’. Five replicates were carried out for each strain. The tests were performed in a temperature-controlled room maintained at approximately 24°C under normal laboratory light conditions from 7.00 to 11.00 a.m.

Different morphological traits (thorax length (TL), wing length (WL), sternopleural bristle number (SBN), wing-to-thorax ratio (W/T), sex-comb tooth number (SCTN) and ovariole number (ON)) were scored in mated and

unmated flies on both the left and right sides. TL was measured from the anterior end of the thorax to the posterior end of the scutellum. For WL, absolute length between the anterior crossvein to the distal tip of the third longitudinal vein was measured under a microscope at 50X magnification using ocular micrometer (1 unit = 16.67 μm). The W/T ratio was calculated from data on WL and TL. On the sternopleuron of males and females, two sets of bristles are present. Anterior bristles (A) occur in an oblique row from the forecoxa towards the midline, whereas the transverse bristles (T) run in a thin line towards the centre of the fly just anterior to the middle leg. The anterior and transverse sternopleural bristles were counted under stereo binocular. In females, ovaries were dissected in insect saline (0.67% NaCl), stained with 2% acetocarmine stain, mounted in 45% acetic acid and ovariole number was counted under a microscope at 50X magnification. In males of *D. ananassae*, there are several transverse rows of stout, blackish bristles on the ventral surface of the first, second and third tarsal segments of prothoracic legs, known as sex-comb teeth. Forelegs of males were dissected and mounted in insect saline and the number of the teeth on the first (C1), second (C2) and third tarsal segments was counted under a microscope at 50X magnification. As measurements of morphological traits showed no significant deviation from normality in the Kolmogorov–Smirnov test for goodness-of-fit, no transformation was used for the traits studied. To test the difference between the mated and unmated flies of the two strains, Student's *t* test was performed for all traits. To correlate all the five morphological traits in males and females, Pearson's correlation was employed.

The framework laid by Palmer¹⁴ was followed. Measurement error (ME) artificially inflates the estimates of FA. For ME, 28 flies randomly collected from the cultures and two replicate counts were made for different traits per fly, each on a different day. ME was assessed using two-way mixed model ANOVA in which sides were entered as fixed factor and individuals as a random factor. The tests for FA differences were only justified if interaction (side \times individual) variance was significant. Repeatability estimated the true FA variation as a proportion of total between-sides variation, including ME.

For directional asymmetry, one sample *t* test on the signed differences ($R - L$) for each trait was performed to determine whether the mean values differed from zero¹⁴. For antisymmetry, we checked departures from normality of the distribution of the signed differences ($R - L$) using Kolmogorov–Smirnov test. We also examined the normality of distributions determining whether skewness and kurtosis coefficients of all the traits deviated from zero, which is the expected value for normal distribution by one-sample *t* test. A significant negative kurtosis (platykurtosis) indicated the presence of antisymmetry. Individual asymmetry was measured as $D = R - L$, where R is the value of the trait on the right side and L the value on the

left side. FA1 (which is the FA measure reported in most studies) has been calculated for a given trait as the mean of absolute value of the difference in trait size between the right and left side of the body, $|R - L|$ ¹⁴. Thus FA for SBN, WL, W/T ratio, ON and SCTN was calculated (data not shown). Positional fluctuating asymmetry (PFA)²⁵, a measure of the difference between the two sides of the body in the way in which components of a meristic trait are arranged or positioned, was calculated for both SBN and SCTN²⁵. For SBN, positional fluctuating asymmetry (PFA_B) was calculated as $|(Right\ A/Right\ T) - (Left\ A/Left\ T)|$ and for sex-comb tooth number (PFA_S) as $|(Right\ C1/Right\ C2) - (Left\ C1/Left\ C2)|$. Trait size for each trait was measured by the average value of right and left side $(R + L)/2$. To know whether trait FA co-varies with trait size, we obtained non-parametric Spearman's correlation coefficient for all traits between absolute trait asymmetry $|R - L|$ and trait size $(R + L)/2$.

We calculated a size-corrected or relative-trait FA index $(|R - L|)/(R + L)/2$ for those traits where trait asymmetry was significantly correlated with trait size. To make sure that the correction was successful, we again correlated the size-corrected indices with size. To test the differences in relative-trait FA index of mated and unmated flies in each strain, *t* test was performed in males and females separately. FA across the multiple traits was calculated by pooling all the information in composite fluctuating asymmetry analyses (CFA1)²⁶. CFA was calculated as a summation of absolute FA values across traits (j) for each individual (i) and then compared by single-factor analysis of variance for both populations and sexes $\{CFA_i = |FA_{ij}| j = 1 \text{ to } k; k \text{ represents the number of traits per individual}\}$.

Table 1 summarizes the results of five morphological traits in mated and unmated flies of each strain. There is significant difference between mated and unmated flies for TL and WL in both strains and sexes. However, in the PC strain there was significant difference in mated and unmated flies for SCTN in males and ON in females. There was no difference in W/T ratio between the mated and unmated flies. To correlate all the five morphological traits with each other, Pearson's correlation test was performed. In males, thorax length was positively correlated with WL ($r = 0.467$, $P < 0.001$), SBN ($r = 0.014$, $P = 0.868$) and SCTN ($r = 0.396$, $P < 0.001$), but negatively correlated to W/T ratio ($r = -0.777$, $P < 0.001$). Similarly, in females, TL was positively correlated to WL ($r = 0.692$, $P < 0.001$), SBN ($r = 0.009$, $P = 0.913$) and ON ($r = 0.121$, $P = 0.139$), and negatively correlated to W/T ratio ($r = -0.595$, $P < 0.001$).

In ANOVA (not shown), the interaction between side and individual was highly significant ($P < 0.001$), indicating that measurement error in all traits was negligible compared with the variation between sides.

One-sample *t* test revealed that mean values of each trait did not differ significantly from zero ($P > 0.05$). Also, none of the frequency distributions of the signed differ-

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Table 1. Comparison of morphological traits between mated and unmated flies of *Drosophila ananassae*

Trait	PC strain		<i>t</i> value	MB strain		<i>t</i> value
	Mated (<i>n</i> = 41) Mean ± SE (CV)	Unmated (<i>n</i> = 34) Mean ± SE (CV)		Mated (<i>n</i> = 55) Mean ± SE (CV)	Unmated (<i>n</i> = 20) Mean ± SE (CV)	
Male						
TL	54.58 ± 0.38 (0.045)	52.00 ± 0.54 (0.06)	3.996**	55.53 ± 0.23 (0.016)	54.25 ± 0.523 (0.044)	2.605*
SBN	16.54 ± 0.28 (0.047)	16.35 ± 0.25 (0.089)	0.489	15.47 ± 0.18 (0.086)	14.95 ± 0.19 (0.037)	1.626
WL	73.95 ± 0.32 (0.037)	71.62 ± 0.42 (0.038)	4.518**	73.36 ± 0.28 (0.004)	71.3 ± 0.33 (0.021)	4.118**
W/T	1.357 ± 0.01 (0.032)	1.381 ± 0.01 (0.05)	-1.589	1.32 ± 0.01 (0.039)	1.317 ± 0.01 (0.048)	0.400
SCTN	65.61 ± 1.08 (0.105)	61.5 ± 0.89 (0.084)	2.866*	68.62 ± 0.84 (0.09)	69.15 ± 1.38 (0.089)	-0.329
Female						
TL	60.66 ± 0.40 (0.042)	54.47 ± 0.64 (0.058)	8.485**	62.84 ± 0.20 (0.024)	60.4 ± 0.48 (0.035)	5.471**
SBN	17.19 ± 0.27 (0.044)	16.91 ± 0.25 (0.087)	0.751	16.33 ± 0.22 (0.100)	16.0 ± 0.36 (0.052)	0.769
WL	83.12 ± 0.52 (0.042)	76.5 ± 0.84 (0.064)	6.457**	83.82 ± 0.33 (0.031)	81.45 ± 0.83 (0.045)	3.179*
W/T	1.37 ± 0.01 (0.006)	1.407 ± 0.01 (0.055)	-1.786	1.33 ± 0.01 (0.032)	1.348 ± 0.01 (0.026)	-1.401
ON	24.95 ± 0.60 (0.155)	22.5 ± 0.58 (0.149)	2.895*	23.11 ± 0.29 (0.093)	23.85 ± 0.48 (0.089)	-1.327

* $P < 0.01$; ** $P < 0.001$.

TL, Thorax length; SBN, Sternopleural bristle number; WL, Wing length; W/T, Ratio of wing length and thorax length; ON, Ovariole number; SCTN, Sex-comb tooth number.

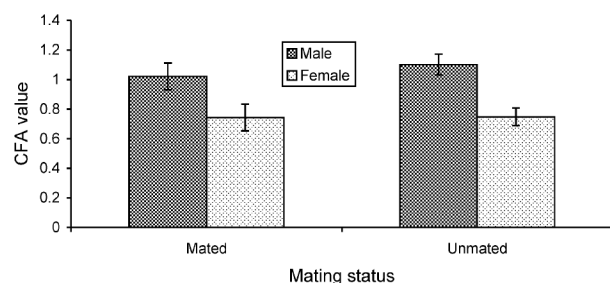


Figure 1. Mean and SE (shown by error bars) of composite fluctuating asymmetry (CFA) of mated and unmated *Drosophila ananassae*.

ences ($R - L$) showed normal distribution in the Kolmogorov–Smirnov test for normality. Moreover, none of the skewness and kurtosis values differed from zero ($P > 0.05$) for all the traits (data not shown). This indicates that we observe true FA rather than directional asymmetry and antisymmetry in our data. FA has been calculated as the mean of absolute trait asymmetry ($|R - L|$) for males and females. Absolute trait asymmetry ($|R - L|$) was correlated with trait size $(R + L)/2$. In females, positive correlation was found for SBN ($r = 0.219$, $P = 0.007$); ON ($r = 0.026$, $P = 0.754$); PFA_B ($r = 0.323$, $P < 0.001$), and negative correlation for WL ($r = -0.120$, $P = 0.143$) and W/T ratio ($r = -0.002$, $P = 0.977$). Similarly, in males

positive correlation was found for SBN ($r = 0.162$, $P = 0.048$), SCTN ($r = 0.065$, $P = 0.432$), PFA_B ($r = 0.171$, $P = 0.037$), PFA_S ($r = 0.095$, $P = 0.248$), WL ($r = 0.035$, $P = 0.669$) and W/T ($r = 0.233$, $P = 0.004$). Thus, where significant relationship exists between FA and trait size, the FA data have been corrected as the relative index of FA. No significant correlation was found between relative index of FA and trait size in any of the traits studied after correction (data not shown).

We tested the difference between mated and unmated flies of each strain for the relative FA of each trait by using *t* test (Table 2). This revealed that the levels of FA were similar in both mated and unmated flies in both strains, except SBN and PFA_B in females of the MB strain. We then calculated a composite FA index (CFA) for all traits in males and females separately by pooling data of the two strains. There was a difference in the levels of CFA values (Figure 1) in the mated and unmated males and females. To determine whether the difference was significant, *t* test was employed. The magnitude of CFA was similar in mated and unmated males ($t = 0.565$, $df = 598$, $P = 0.572$) and females ($t = 0.046$, $df = 598$, $P = 0.963$). But there was significant difference in the levels of CFA between males and females in both mated ($t = 3.269$, $df = 598$, $P = 0.001$) and unmated ($t = 2.634$, $df = 598$, $P = 0.009$) flies.

Table 2. Comparison of differences in fluctuating asymmetry {corrected for trait size, $(|R - L|)/(R + L)/2$ } of morphological traits between mated and unmated flies of *D. ananassae*

Trait	PC strain			MB strain		
	Mated (n = 41)	Unmated (n = 34)	t value	Mated (n = 55)	Unmated (n = 20)	t value
Male						
SBN	0.092 ± 0.015	0.103 ± 0.015	-0.498	0.095 ± 0.01	0.099 ± 0.02	-0.200
WL	0.006 ± 0.001	0.0103 ± 0.003	-1.547	0.011 ± 0.002	0.012 ± 0.004	-0.394
W/T	0.00147 ± 0.0039	0.0059 ± 0.002	2.285	0.0089 ± 0.002	0.011 ± 0.004	-0.52
SCTN	0.0901 ± 0.117	0.069 ± 0.01	1.421	0.089 ± 0.01	0.08 ± 0.014	0.496
PFA _B	0.169 ± 0.028	0.185 ± 0.025	-0.421	0.159 ± 0.02	0.166 ± 0.03	-0.176
PFA _S	0.168 ± 0.02	0.123 ± 0.015	1.877	0.162 ± 0.015	0.132 ± 0.03	0.973
Female						
SBN	0.117 ± 0.14	0.098 ± 0.17	0.825	0.109 ± 0.01	0.160 ± 0.03	-2.150*
WL	0.005 ± 0.001	0.007 ± 0.002	-1.182	0.0105 ± 0.003	0.0062 ± 0.001	0.980
W/T	0.007 ± 0.002	0.0042 ± 0.001	1.412	0.0102 ± 0.003	0.0058 ± 0.001	0.984
ON	0.129 ± 0.022	0.163 ± 0.02	-1.151	0.103 ± 0.02	0.061 ± 0.02	1.504
PFA _B	0.180 ± 0.027	0.220 ± 0.03	-1.000	0.183 ± 0.02	0.284 ± 0.04	-2.758**

* $P < 0.05$, ** $P < 0.01$.PFA_B, Positional fluctuating asymmetry for SBN; PFA_S, Positional fluctuating asymmetry for SCTN.

It is evident from the results that the traits related to body size (i.e., TL and WL) of mated flies of *D. ananassae* were larger than unmated ones, supporting the previous findings that body size appears to be related to mating in *Drosophila*^{20,27,28}. It has been shown that larger flies have higher reproductive success than smaller flies, as they mate more frequently and also more rapidly²⁸⁻³¹. This is because large males perform different courtship acts more quickly, through which they are able to convey sexual signals and stimulate the female to reach the threshold for copulation, and also are able to withstand a rejection response for longer, resulting in female acceptance than smaller males³¹. On the other hand, larger females showed more rejection response and fecundity than smaller females³². But the relationship between body size and mating success is not a universal phenomenon in *Drosophila*, as there are many cases where body size is influenced by various environmental conditions, e.g. larval density³³⁻³⁵, nutrition^{36,37} and temperature³⁸ along with genetic component³⁹. Thus, environmentally induced variance in body size may be important in determining the mating success of *Drosophila* species in the wild, as suggested by Joshi *et al.*³⁴.

There was positive correlation between the body size (TL and WL) and other morphological traits (SBN, SCTN, and ON), suggesting its relation with adult fitness, such as mating success²⁰ and fecundity⁴⁰. The positive association of TL with SBN and ON in the present study supports the previous finding that there was positive correlation between SBN, mating propensity and fertility in *D. ananassae*⁴¹. Wings play an important role in courtship, because of species-specific auditory signals that are produced by male wing vibration in *Drosophila*⁴². It has been observed that fast-mating flies have larger wings

than slow and non-mating flies in *D. subobscura* and *D. malerkotliana* under multiple-choice situation^{43,44}.

Sexual traits, i.e. ON in females and SCTN in males were more variable than those of other morphological traits (Table 1) reflected through higher coefficient of variation (CV). The traits more closely related to fitness are expected to be better buffered against environmental effects because deviations from the optimal phenotype will be strongly selected against⁴⁵⁻⁴⁷. This may explain the relatively lower CV in the wing traits (WL and W/T ratio) than bristle number in both sexes, because the wing traits are under direct selection for aerodynamic stability, whereas the bristle number is likely to be under weaker selection⁴⁶.

Developmental stability may play an important role in sexual selection because it represents a general health certificate of an individual under given environmental conditions⁴⁸. FA was used to measure the developmental instability levels in mated and unmated flies. In males, the levels of FA were similar in unmated flies for all morphological traits in both strains (Table 2). Similarly, in females the levels of FA were similar in mated and unmated flies in both strains for all traits, except for SBN. PFA is a sensitive measure for developmental instability, which reflects the fidelity of buffering mechanisms operating at the interface of interconnected developmental pathways⁴⁹. The magnitude of PFA in mated and unmated flies was similar for SBN in males and females (except MB strain) and SCTN in males. Moreover, when FA across all the traits was combined, the levels of CFA were similar in mated and unmated males and females. The values of CFA were higher in males than females in both mated and unmated flies (Figure 1), supporting the previous findings in *D. ananassae* that males were more prone to

disruption of developmental homeostasis⁵⁰, which is due to hemizygoty of loci on the X-chromosome in males⁵¹.

It has been documented that FA in the sexual traits was higher than the non-sexual trait⁵². However, in the present study, there were similar FA values of the sex-comb (a sexually selected trait) and SBN (a nonsexually selected trait) in males, which is inconsistent with the FA-sexual selection hypothesis. Although the reason for the relatively low level of FA variation in the sex comb is unknown, perhaps this may be due to the history of selection that has reduced the variation that existed in the past on this trait, as suggested by Polak *et al.*²⁵. Therefore, the trait size of sexual traits is a better indicator of stress than FA, supporting earlier findings^{15,25,50}.

From our observations, we conclude that sexual selection acts directly or indirectly on the body size in *D. ananassae*. Further, the size of the sexual traits seems to be a far better indicator of individual quality than fluctuating asymmetry in *D. ananassae*.

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A cDNA-AFLP approach to look for differentially expressed gene fragments in dioecious pointed gourd (*Trichosanthes dioica* Roxb.) for understanding sex expression

Subhash Kanti Roy, Gaurab Gangopadhyay, Kaushik Ghose, Sanjukta Dey, Debabrata Basu and Kalyan Kumar Mukherjee*

Cytogenetics Laboratory, Department of Botany, Bose Institute, 93/1, APC Road, Kolkata 700 009, India

Transcript profiling of unopened male and female floral buds of pointed gourd (*Trichosanthes dioica* Roxb.), a dioecious cucurbit, at uniform developmental stage was done through cDNA-AFLP to look for the differentially expressed unique and/or up-regulated gene fragments associated with sex expression. Thirty-one such fragments, twenty-three from male and eight from female, were selected, cloned, sequenced and assigned putative protein functions after database searching and obtaining GenBank accessions. The annotation of one male-derived clone (TDM16) as polygalacturonase with remarkably high level of homology as revealed from database searching is a promising finding, since this protein has direct involvement with pollen development, germination and tube growth with particular function in depolymerization of pectin.

Keywords: cDNA-AFLP, gene fragments, sex determination, *Trichosanthes dioica*.

POINTED GOURD (*Trichosanthes dioica* Roxb., Cucurbitaceae) is a tropical summer vegetable crop with origin in the Indian subcontinent. The plant is a perennial, dioecious and grows as a vine. The pointed gourd is usually propagated through vine cuttings and root suckers. This age-old human selection pressure perhaps has resulted in its poor seed germination. Since the sex of plants cannot be determined before flowering, crops established from seeds may contain 50% non-fruiting male plants (<http://www.hort.purdue.edu/newcrop/proceedings1999/v4-397.html>), thus causing loss in the farmers' field. The pointed gourd breeding programme has recently been initiated to develop new cultivars. Dioecy represents an inconvenience in pointed gourd breeding. Currently there is no method for distinguishing between male and female plantlets prior to flowering in *T. dioica*, though a female sex-associated RAPD marker has been proposed¹. ISSR-based approach to detect hermaphrodite and female plants of *Carica papaya*, family Caricaceae, a related family of Cucurbitaceae, in the pre-flowering stage has been successfully undertaken by our group². At present, most of the mark-

*For correspondence. (e-mail: kalyan@bosemain.boseinst.ac.in)