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Resistance to meiotic drive at the M^D locus in an Indian wild population of *Aedes aegypti*

By S. G. SUGUNA,*† R. J. WOOD,‡ C. F. CURTIS,‡§
A. WHITELAW,‡ and S. J. KAZMI‡||

* *WHO/ICMR Research Unit on Genetic Control of Mosquitoes, New Delhi, India*

‡ *Department of Zoology, Manchester University, Manchester M13 9PL, U.K.*

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SUMMARY

Females from an Indian wild population of *Aedes aegypti* were crossed to males carrying the sex ratio distorter factor M^D which shows meiotic drive. Progenies from F_1 males were tested for sex ratio distortion, i.e. the chromosomes from the wild females were screened for their resistance to the action of M^D. The distribution of sex ratio in the progenies of different F_1 males indicated a polymorphism in the wild population for resistant and sensitive variants of the *X* chromosome. Seven discrete categories of *X* appear to exist, associated with sex ratios ranging from 50 % ♀ to less than 1·25 % ♀. The overall level of resistance varied slightly but significantly in different parts of a town. The results are discussed in relation to the use of sex ratio distortion for genetic control of mosquitoes.

1. INTRODUCTION

A major attraction of meiotic drive factors as agents to be used for the genetic control of insect pests is that, unlike most other kinds of deleterious genetic factor, they could increase spontaneously in their frequency in a wild population even while exerting a deleterious effect upon it (von Borstel & Buzzatti Traverso, 1962; Hamilton, 1967). In mosquitoes, meiotic drive causing distortion of the sex ratio in favour of males is particularly attractive as a control measure because only the female sex of mosquitoes is harmful. Craig, Hickey & VandeHey (1960), McClelland (1960) and Wood (1961) each reported a sex ratio distorting factor in *Aedes aegypti* inherited through the male parent. Hickey & Craig (1966*a*, *b*) showed that the distortion was due to meiotic drive in the male controlled by alleles at, or close to, the sex determining locus; the distorter type of male having the genotype M^Dm^d and the other combinations, M^Dm^D, M^dm^d and M^dm^D all giving normal sex ratios. The m^D or m^d gene is inherited by a male from its female parent and the relative

† Present address: V.C.R.C., 20 Sham Nath Marg, Delhi 110054, India.

§ Present address: London School of Hygiene and Tropical Medicine, London WC1E 7HT, U.K.

|| Present address: V.C.R.C., Kosapalyam, Pondicherry, India.

frequency of these two alleles in a wild population will determine the extent to which sex ratio distortion would be expressed in the progeny of the F_1 and subsequent generations after a release of distorter males. Hickey and Craig also showed that several populations of African and American origin varied in their relative frequencies of m^D and m^d, i.e. in their level of 'resistance' to the expression of sex ratio distortion.

Evidence was produced by Hickey & Craig (1966*b*) and Hickey (1970) suggesting two kinds of m^d: the highly sensitive $m^{d'}$ and the less sensitive $m^{d''}$. More recently Wood (1976) has identified six kinds of m 'allele' differing in sensitivity to M^D. These are listed as follows with the modal sex ratios associated with them in parentheses: m^{r2} (57·5% ♀), m^{r1} (47·5% ♀), m^{s1} (40% ♀), m^{s2} (32·5% ♀), m^{s3} (12·5% ♀), m^{s4} (< 10% ♀). The alleles m^{r1} and m^{r2} are together approximately equivalent to m^D of Hickey and Craig; m^{s1} and m^{s2} correspond with $m^{d''}$ and m^{s3} and m^{s4} with $m^{d'}$.

The present paper describes a study of resistance to sex ratio distortion in *A. aegypti* in a wild population from the town of Sonepat in Haryana State in northern India.

2. MATERIALS AND METHODS

Stocks

The following *A. aegypti* stocks were used:

T30. A Trinidad strain carrying M^D, and polymorphic for sensitivity to it (Wood, 1976).

TSD. Carrying the M^D gene derived from the T30 stock, m alleles sensitive to distortion and with Delhi genetic background (Suguna & Curtis, 1974).

DT_1/T_1. Homozygous for the T_1 translocation between chromosomes 1 and 3 (Lorimer, Hallinan & Rai, 1972) and with the distorter genotype and Delhi genome as in TSD; the effects of the linkage of M^D to T_1 are reported by Suguna *et al.*

Wild material. Eggs were collected in black jar ovitraps (Fay & Eliason, 1967; Reuben *et al.* 1976), laid out in a grid pattern (Reuben, in preparation). The data for different ovitraps have been grouped into nine zones, generally of 400 × 400 m as illustrated in Fig. 1.

3. METHODS

The crossing scheme for assaying the distortion resistance/sensitivity of wild females was basically that described by Hickey & Craig (1966*a*, *b*). Some of the test matings were carried out in Delhi and others were made in Manchester with eggs mailed from India.

The methods used in Delhi were as follows: females derived from the eggs collected in the wild were mated to males of one of the Distorter stocks and each female was egged individually. A few of the egg papers were picked at random for hatching and further study. Because of this random sampling procedure it is unlikley that the progenies chosen for further study had any close genetic relationship with each other. The larvae from the chosen egg papers were reared and the

male progeny were mated with wild-type females in single pairs. Eggs were obtained from the first oviposition cycle of each female, and the larvae were carefully reared in 500 ml bowls on a diet of powdered dog biscuit and yeast and the pupae were sexed by size. With the rearing conditions used the survival from 1st instar to pupae averaged 97·8 % (range 88·0–100 %). There is therefore little chance of appreciable differential mortality between the sexes during larval life and the pupal sex ratios are assumed to be an accurate reflexion of the sex ratio at the time of eclosion of eggs. With the non-competitive rearing conditions used, pupal size was a very reliable criterion for distinguishing the sexes. In a few doubtful cases the genitalia were examined under the microscope.

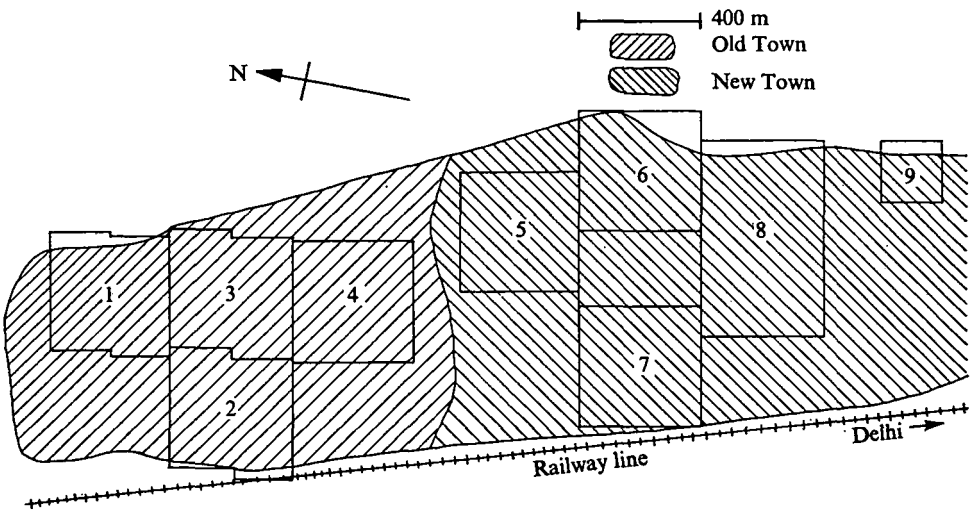

Fig. 1. Sketch map of Sonepat showing the zones where the samples were collected.

The methods used in Manchester were as follows: females derived from eggs collected in Sonepat and sent to Manchester by post, were mass-mated to T30 males. A random sample of F_1 eggs was hatched and the larvae reared to adults which were inbred as pair matings. Eggs obtained from these pairs for as many oviposition cycles as possible, to maximise the 'clutch' size, were hatched as soon as possible in an infusion of hay and the larvae reared in 500 ml bowls on a diet of dog biscuit. The yield of pupae per 100 eggs averaged 76·0 (range 11·0–98·6). In a few cases high yield was associated with extreme sex ratio distortion, e.g. 87·5 % yield with 3·4 % ♀ (147 pupae), 79·5 % yield with no females (66 pupae). It is evident that differential mortality in the egg or larval stages could not account for these.

4. RESULTS AND DISCUSSION

The sex ratio of the Sonepat strain reared in Delhi was 50·1 % ♀ (from 42 633 eggs); in Manchester it was 48·5 % ♀ (n = 11 464 pupae). Each count covered several generations. The progenies of 100 single pair matings were studied with respect to their sex ratio and no cases of distortion were found. More than 75 wild

males were mated to $m^{s4}m^{s4}$ females and the male progeny tested for sex ratio distortion, which was not found. In routine use of a laboratory stock of Sonepat origin for single pair test matings no cases of distortion were found. Thus there is strong evidence that the M^D gene is absent from the Sonepat wild population.

The object of the study was to assess the Sonepat X chromosomes for sensitivity to M^D. To do this it was necessary to examine the sex ratios from individual $m^D m^{Sonepat}$ males. The data on the sex ratios in the progeny of the $M^D m^{Sonepat}$ males (F_1 descended from females from each of the zones in Sonepat mated to

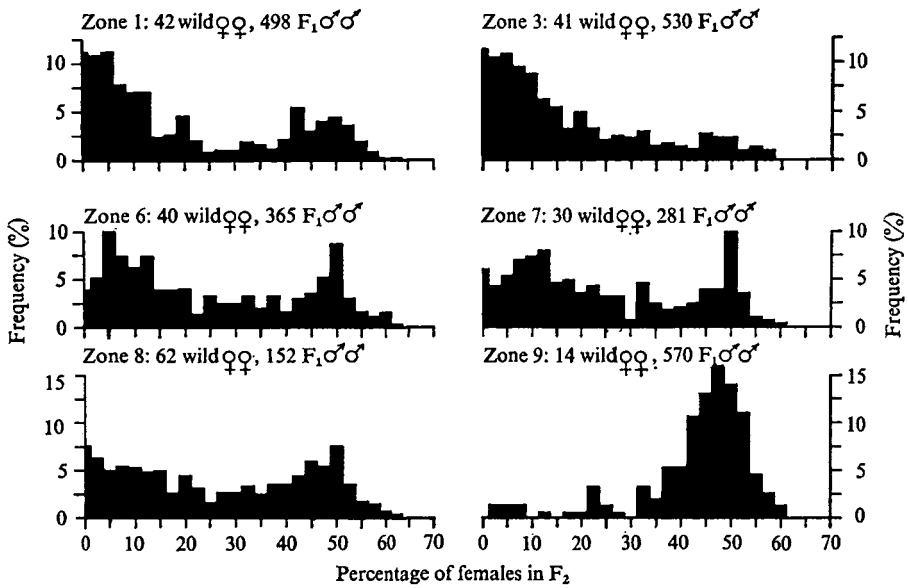

Fig. 2. Distribution of percentage female among the progeny (F_2) of single $M^D m^{Sonepat}$ males (F_1), themselves derived from a cross Sonepat (wild) females × M^D males. Samples from six zones of Sonepat are compared. The M^D males were of the DT_1/T_1 strain.

distorter males) are summarized in Figs. 2 and 3 in the form of frequency distributions of families (F_2's) with different proportions of females. The dotted histograms are from males carrying M^D derived from TSD or T30, the solid ones from males deriving M^D from DT_1/T_1. In most cases the distribution is broadly bi-modal. However, there were always some families with intermediate sex ratios and in some cases (e.g. zone 2) these were so common as to completely obscure the 'bi-modality'. The intermediate cases do not appear to be due to sampling error in the estimates of the sex ratio of each individual progeny as can be shown as follows. In, for example, zone 6 the two main modes are at approximately 7·5 % and 50 % females. The numbers in the families from individual F_1 males averaged 54 and seldom fell below 30: a conservative estimate of 36 will be adopted. Assuming that only sampling error occurred about the two modes the 95 % confidence limits may be calculated (Fisher & Yates, 1963) for the distorted and non-distorted

families as 0·5–21 % and 32–67 %. Thus 2·5 % of each type of family would have been expected to fall in the interval 21–32 % female. In fact, however, 10·8 % fell in the interval 20–30 %. Thus the variation between the two modes cannot be explained by sampling error. Hickey & Craig (1966*b*) and Hickey (1970) suggested that three different forms of the *m* allele might exist with different degrees of sensitivity/resistance to distortion. However, the data in Figs. 2 and 3 show no sign of tri-modality and it seems necessary to postulate either more numerous forms of the *m* allele as found by Wood (1976) in the T30 strain or else polygenic modifying factors.

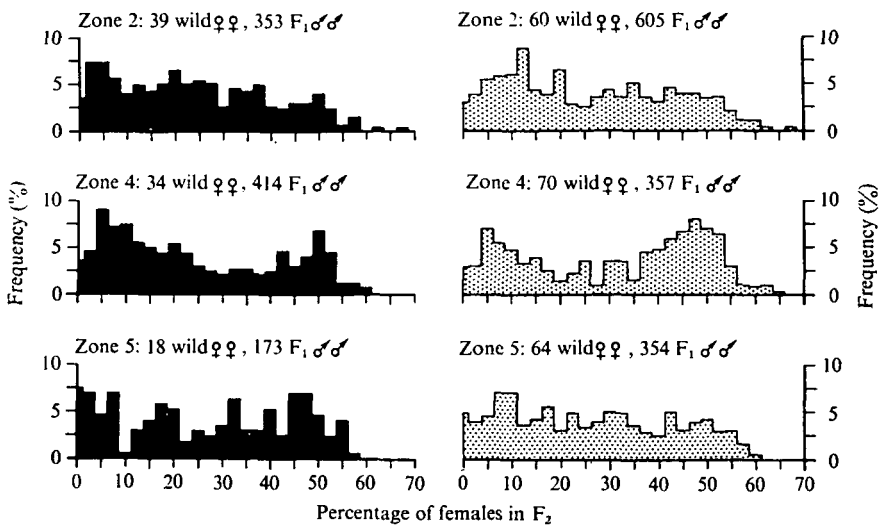

Fig. 3. Distribution of percentage female among the progeny (F_2) of single $M^D m^{Sonepat}$ males (F_1), themselves derived from a cross Sonepat (wild) females × M^D males. Samples from three zones of Sonepat are compared. In the solid histograms, the M^D males were of the DT_1/T_1 strain: in the dotted histograms, the M^D males were of the TSD or T30 strains.

A number of submodes appear in the distributions and it is of interest to see whether these correspond in the different zones. All are listed, zone by zone, in Table 1, from which it is evident that modes were most commonly observed at < 1·25 % ♀, 5 % ♀, 20 % ♀, 32·5 % ♀ and 50 % ♀. In addition it should be noted that a mode at 12·5 % ♀ was very distinct in some samples (zone 2, TSD; zone 6, DT_1/T_1; zone 7, DT_1/T_1). The same holds true for a mode at 42·5 % ♀ (zone 4, DT_1/T_1; zone 5, TSD; zone 1, DT_1/T_1).

All zones are combined in figure 4 (total sample size of 4652). Clear modes now appear at 5 %, 20 %, 32·5 % and 50 % together with 'shoulders' corresponding to < 1·25 %, 12·5 % and 42·5 %. The fact that there is not a mode at < 1·25 % is probably attributable to the method of plotting which was designed to provide a 'bar' of the histogram precisely at 50 % ♀ but, in arranging the histogram this way, the lowest sex-ratio category is made half the width of all the others.

Summarizing, there is evidence for seven sex ratio categories in the progeny of

Table 1. *'Modal' values appearing in the frequency distributions plotted in Figs. 2 and 3 of percentage females found in the progeny of individual* $M^{D}m^{Sonepat}$ *males: a comparison of all samples from nine zones*

(The data marked (T_1) are from translocation carrying males which derive from DT_1/T_1 parents; the remainder are from males derived from TSD or T30 males.)

	'Modal' values of percentage female														
Zone	1·25	3·75	5	7·5	8·75	10	12·5	15	17·5	20	22·5	25	27·5	30	31·25
1 (T_1)	*	.	*	.	.	.	.	.	.	*	.	.	.	.	.
2 (T_1)	.	*	.	.	.	.	*	.	.	*	.	*	.	.	.
2	.	.	.	.	.	.	*	.	.	*	.	.	.	*	.
3 (T_1)	*	.	*	.	.	.	.	.	.	*	.	.	*	.	.
4 (T_1)	.	.	*	.	.	*	.	.	.	*	.	.	.	.	.
4	.	.	*	.	.	.	.	*	.	.	.	*	.	.	*
5 (T_1)	*	.	.	*	.	.	.	.	*	.	.	*	.	.	.
5	*	.	.	.	*	.	.	.	*	.	*	.	.	*	.
6 (T_1)	.	.	*	.	.	.	*	.	.	*	.	*	.	.	.
7 (T_1)	*	.	.	.	.	.	*	.	*	.	*	.	.	.	.
8 (T_1)	*	.	.	*	.	.	.	*	.	*	.	.	.	.	.
9 (T_1)	.	.	*	.	.	.	.	.	.	.	*	.	.	.	.
Total	6	1	6	2	1	1	4	2	3	7	3	4	1	2	1

	'Modal' values of percentage female														
	32·5	33·75	35	37·5	40	42·5	45	46·25	47·5	50	52·5	55	57·5	60	62·5
1 (T_1)	*	.	.	.	.	*	.	.	.	*	.	.	.	.	.
2 (T_1)	*	.	.	*	.	.	.	.	.	*	.	.	*	.	.
2	.	.	*	.	.	*	.	.	.	.	*	.	.	.	.
3 (T_1)	*	.	.	*	.	.	*	.	.	*	.	*	.	.	.
4 (T_1)	.	*	.	.	.	*	.	.	.	*	.	.	.	.	.
4	.	.	.	.	.	.	.	.	*	.	.	.	.	.	*
5 (T_1)	*	.	.	.	*	.	.	*	.	.	.	*	.	.	.
5	.	.	.	.	.	*	.	.	.	*	.	.	.	.	.
6 (T_1)	*	.	.	*	.	.	.	.	.	*	.	.	.	*	.
7 (T_1)	*	.	.	.	.	.	.	.	.	*	.	.	.	.	.
8 (T_1)	*	.	.	.	.	.	*	.	.	*	.	.	.	.	.
9 (T_1)	*	.	.	.	.	.	.	.	*	.	.	.	.	.	.
Total	8	1	1	3	1	4	2	1	2	8	1	2	1	1	1

$M^{D}m^{Sonepat}$ males: < 1·25% ♀, 5% ♀, 12·5% ♀, 20% ♀, 32·5% ♀, 42·5% ♀ and 50% ♀.

The results may now be compared with the study of Wood (1976) on the T30 and 64 strains (Table 2). The revised classification of *X* chromosomes into eight categories extends that given by Wood (1976). It remains possible that the m^{r1} and m^{s1} categories may each have to be subdivided.

In many cases the genotype of individual females cannot be identified with certainty from the sex ratios produced by their male progeny because of sampling error. Therefore in studying the distribution of sensitivity to distortion within the

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town of Sonepat no attempt was made to determine the frequencies of the *m* alleles in the different zones of the town and, instead, sensitivity to distortion was treated as a quantitative character. The histograms in Figs. 2 and 3 suggest differences in sensitivity in the populations in the different zones of Sonepat with, perhaps, greater sensitivity in the old (Northern) part of the town. To test the significance of these apparent zonal differences an analysis of variance was carried out on the proportions of females (arcsine transformed) in the progeny of males deriving from each female from the wild population which was mated to a DT_1/T_1 male. The F ratios were calculated of the variance between the different zones of the town and that within the zones between the descendents of each of the wild females collected. The results of the analysis of variance indicate a significant difference between the zones but no difference between old and new towns (Table 3). The zones remain significantly heterogeneous even if one excludes zone 9, which showed an exceptionally high degree of resistance to distortion based on a small sample of wild females.

Table 2. *Classification of the X chromosomes of three strains of* A. aegypti *according to the sex ratio found in the progeny of males carrying these chromosomes paired with the* M^D Y *chromosome*

Strain	Sex ratio category (% ♀)								Reference
T30	57·5	47·5	40	32·5	—	12·5	—	—	Wood (1976)
64	—	—	—	—	—	—	< 10	—	Wood (1976)
Sonepat	—	50	42·5	32·5	20	12·5	5	< 1·25	Present study
X chromosome (*m* variant)	m^{r2}	m^{r1}	m^{s1}	m^{s2}	m^{s5}	m^{s3}	m^{s4}	m^{s6}	

In the apparent absence of the M^D gene from Sonepat, the adaptive significance, if any, of the distortion resistant and sensitive genes must presumably be due to pleiotropic effects on adaptation to local ecological conditions, and there is a possibility of heterozygous advantage in females. But, at present, no hypothesis can be proposed to 'explain' the variations in distortion resistance frequency in terms of correlation with ecological conditions. The *A. aegypti* population in Sonepat fluctuates very markedly with season (Reuben *et al.* 1975) and the possible random effects of the 'founder principle' should not be overlooked.

The histograms in Fig. 3 for females from the same zones tested with DT_1/T_1 and with TSD or T30 males are similar although in the former case the crosses were made in Delhi and in the latter case partly in Manchester and partly in Delhi. A marked effect of the T_1 translocation on the level of distortion when combined with the *m* chromosome of a standard inbred distortion-sensitive line has previously been reported by Suguna *et al.* (1977). However, when the M^DT_1 chromosome is combined with wild chromosomes, many of which prevent distortion, the overall percentage of females at the F_2 generation was only slightly higher in the TSD derivatives than the DT_1/T_1 derivatives (Fig. 4) and a two-way analysis of variance showed that the difference was not significant (Table 4).

Table 3. *Mean percentage of females in the F_2 generation from females collected in each zone of Sonepat and mated to DT_1/T_1 males and analysis of variance*

	Old town				New town				
Zones ...	1	2	3	4	5	6	7	8	9
Mean % of females at F_2	21·0	23·5	17·0	25·3	27·8	25·6	25·6	28·0	41·4
No. of wild females tested	42	39	41	34	18	40	30	62	14

Variance ratios:

Comparison	D.F.	*F*	*P*
(*a*) Between zones 1–8	7; 298	2·30	$<0·05$
(*b*) Between zones 1–9	8; 311	4·92	$<0·001$
(*c*) Excluding zone 9:			
Between old and new town	1; 6	4·99	0·05–0·1
Between zones, within old and new town	6; 298	1·46	N.S.
(*d*) Including zone 9:			
Between old and new town	1; 7	3·69	N.S.
Between zones, within old and new town	7; 311	3·68	$<0·01$

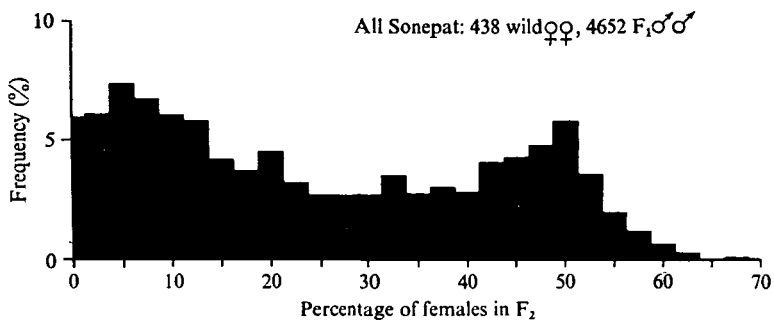

Fig. 4. Distribution of percentage female among the progeny (F_2) of single $M^D m^{Sonepat}$ males (F_1), themselves derived from a cross Sonepat (wild) females × M^D males. Samples from all zones (as shown in Figs. 2 and 3) combined.

The discovery of such a high level of resistance to meiotic drive in the Sonepat population indicates that the idealized concept of 'seeding' a wild population with distorter males to be followed by effortless control of the female population would certainly not be realized. However, the resistance genes in wild populations do not affect the distortion in matings by released males bred from a distortion-sensitive maternal stock. In a field cage test with a 'target' population of Sonepat origin the proportion of females among emerging pupae was reduced to less than 10% while releases of distorter males continued, and after termination of releases there was a considerable degree of inherited sex ratio distortion (Curtis *et al.* 1976). There was evidence for a decline in the level of distortion with time, as would be expected from the argument of Fisher (1930) that natural selection will, other things being equal, favour a 1:1 sex ratio. However, after the release of M^D the rate and com-

Table 4. *Comparison of the percentage of females at the* F_2 *generation derived from* DT_1/T_1 *and from TSD males*

	Zone 2	Zone 4	Zone 5
(1) From DT_1/T_1:			
% female	23·5	24·5	27·8
No. wild females tested*	39	30	18
(2) From TSD:			
% female	26·2	32·6	29·3
No. wild females tested*	39	30	18

* In order to avoid complications in two-way analysis of variance with unequal sample sizes, data from an appropriate number of wild females chosen at random was omitted so that the sample sizes tested with TSD and DT_1/T_1 within each zone were equal (Sokal & Rohlf, 1969).

Variance ratios:	D.F.	*F*	*P*
Between DT_1/T_1 and TSD, within zones	1; 163	1·80	N.S.
Between zones, within DT_1/T_1 and TSD	2; 163	1·51	N.S.
Interaction	2; 163	0·61	N.S.

pleteness with which a population would return to a 1 : 1 sex ratio is not yet known. Many populations carrying M^D show a stable sex ratio of 38-44 % ♀ (Craig *et al.*, 1960; Wood, 1962; Hickey & Craig, 1966*b*) with the *X* chromosomes remaining polymorphic (Wood, 1976), which suggests that balanced forces of natural selection may affect the *m* allele frequencies.

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