GENETIC AND SYSTEMATIC STUDIES ON INDIAN DROSOPHILA 1171

I. Description of two new species of Drosophila: Lifehistory and preliminary studies on the Genetic constitution of Drosophila emulata, Sp.Nov.

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(Communicated by D. Mukherji)

The studies on the genetic structure of free-living Drosophila species initiated by Tschetwerikoff (1926) and developed by Dubinin (1934), Sturtevant and Dobzhansky (1936), Dobzhansky (1939) and their co-workers have opened a new field of research on the problem of organic evolution. Genetical studies in reference to the question of species formation have been neglected in India. With a view to exploring the possibilities of genetic research on these flies in India, the present authors started collecting the wild species of Drosophila in Calcutta and encountered two new species, which were the subject of enquiry for the mutation of genes. In this paper descriptions of these two new species, together with an account of the life-history of one of them (D. emulata, sp.nov.), are given. Descriptions and mode of inheritance of certain mutations obtained in a heterozygous condition in the wild population of this species are also given in brief.

Material and Technique

In their larval stages most species of Drosophila feed on fruit or fungi, or are leaf-miners. It is easy to attract the commoner species to over-ripe bananas kept in fruit stores. The flies thus caught were brought to the laboratory and etherized. Males and females were first separated and a pair were transferred to separate small glass vials, containing standard maize-meal-agar medium as per technique described in Drosophila Information Service, Vol. 6. Pure cultures of both the species were thus separately raised and the systematic descriptions given here are based on specimens from such stock cultures. The data on the ratios of different bristles and wing-venation indices were calculated from measurements of the different structures selected from a large number of individual of different stock cultures of the same species. The range of variation of the wing-vein indices is also given. The measurements of the size of the flies and their wings were made from mature flies (3-4 days old) bred on the standard medium at a constant temperature of 25° ± 2° C. Living stocks of wild and mutant flies of the two species described in this paper are being continuously kept on standard culture medium in the Zoological Laboratory of the Calcutta University.

1. Systematic Description

The only endemic species of Drosophila so far recorded from India is D. prasadi Pruneti (1925), the type-locality of which is Calcutta. Six other species reported from India are more or less cosmopolitan in distribution D. melanogaster Meigen, D. ananassae Doleschall, D. montium de Meijere, D. tristipennis Duda, D. bipectinata Duda and D. repleta Wallaston.

The first four species were collected from Nungambaukam, Madras (Sturtevant, 1927). Of the remaining two species, D. bipectinata, the type specimen of which is in the Hungarian National Museum, was described by Duda (1923), and its type-locality is Darjeeling. D. repleta was recorded from Calcutta by Bezzi (Sturtevant, 1921). It may be noted here that D. melanogaster and D. repleta are cosmopolitan in the true sense, whereas D. ananassae and D. montium appear to be limited to the oriental region. [illegible]

Sophophora

R2/6 Drosophila brunetti, sp.n. Chaudhuri and Mukherjee. 1941

Male.- Arista with 4 branches above and 3 below, excluding the bifurcated terminal part of the main axis. Antennae light brownish yellow, the 3rd joint grey. Front about 1/3 the width of the head, wider above, yellow. 2nd orbital bristle about 1/2 the size of the other 2. 2nd oral bristle nearly as long as the 1st. Face pale yellow. Cheeks extremely pale yellow. Acrostichal hairs in the front of the anterior dorsocentral bristles in 8 rows; no prescutellars. Mesonotum and scutellum pale brownish yellow. Pleurae and legs pale yellow. Preapical bristles on the 1st leg, apical and preapicals on the 2nd leg, preapicals on the 3rd. Wings clear. Costal index 1.5; 4th vein index 2.4; 5x index 1.9; 4c index 1.5. Length of the body and wing 2.0 mm.

Female.- Length of the body and wing 2.2 mm.

R2/6 Drosophila emulata sp.n. Chaudhuri and Mukherjee. = melanogaster

Male.-Arista with 5 branches above and 3 below, excluding the terminal part of the main axis which is bifurcated. Antennae pale brown, 3rd joint grey. Front about 1/2 the width of the head, wider above. 2nd orbital bristles about 1/2 the size of the toher 2. 2nd oral bristle as long as the first. Face pale brown. Cheeks extrememly pale yellow. Acrostichal hairs in 8 rows. Mesonotum and scutellum shining reddish-brown. Pleurae and legs pale brown. Preapical bristles on the 1st leg, apicals and preapicals on the 2nd and preapicals on the 3rd. A comb-like row of about 10 short curvedblack bristles on the inner distal surface of the basal tarsal segment of the 1st leg. Dorsal side of the last 2 segments of the abdomen shining black. Three other conspicuous black bands on the lower margin of the remaining segments, not extending to more than middle of each segment. Wings clear. Costa index 2.5; 4th vein index 2.15; 4c index 1.1; 5x index 1.6. Length of the body 2.6 mm. ength of the wing 2.3 mm.

Female.- Length of the body 3.0 mm. Length of the wing 2.6 mm.

FROM: Ind. Jour. Ent. 3:215-224. Descriptions on page 216.

The former has been recorded from Java, Formosa, Sumatra, New-Guinea and India and the latter from Java, Formosa, India and West Indies.

Drosophila brunettii, sp. nov.

Male.- Arista with four branches above and three below, excluding the bifurcated terminal part of the main axis. Antennae light brownish-yellow the third joint grey. Front about one-third thewidth of the head, wider above yellow. Second oral bristle nearly as long as the first. Face pale yellow. Cheeks extremely pale yellow. Acrostichal hairs in the front of the anterior dorso-central bristles in eight rows; no prescutellars. Mesonotum and scutellum pale brownish-yellow, Pleurae and legs pale yellow. Preapicals on the first and third. Wings clear. Costal index 1.5; fourth vein index 2.4; 5x index 1.9; 4c index 1.5. Length of the body and wing 2.0 mm.

Female. - Length of the body and wing 2.2 mm.

Drosophila emulata, sp. nov.

Male.- Arista with five branches above and three below, excluding the terminal part of the main axis which is bifurcated.

Antennae pale brown, third joint grey. Front about one-half the width of the head, wider above. Second orbital bristle about one-half the size of the other two. Second oral bristle as long as the first. Face pale brown. Cheeks extremely pale yellow. Acrostichal hairs in eight rows. Mesonotum and scutellum shining reddish-brown, Pleurae and legs pale brown. Preapical bristle on the first leg, apicals and preapicals on the second and preapicals on the third. A comb-like row of about ten short curved black bristles on the inner distal surface of the basal tarsal segment of the first leg.

TABLE 1. Range of variation of the wing-vein indices in D.brunetti, sp. nov.

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No. of individuals	III	Costal index	No. of individuals	4th vein index	No. of individuals.	4c. index	No.of individuals	5x index
2		1.3	6	2.3	14	1.5	4	1.7
7		1.4	10	2.4	4	1.6	3	1.8
9		1.5	5	2.5	6	1.7	10	2.0
5		1.6	3	2.6	1	1.8	7	2.1
3		1.7						
Total		Average	Total		Average	Total	Average	Total Average
26		1.5	24		2.4	25	1.5	24 1.9

Dorsal side of the last two segments of the abdomen shining black.

Three other conspicuous black bands on the lower margin of the remaining segments not extending to more than the middle of each segment. Wings clear. Costal index 2.5; 4th vein index 2.15; 4c index 1.1; 5x index 1.6 Length of the body 2.6 mm. Length of the wing 2.3 mm.

Female. - Length of the body 3.0 mm. Length of the wing 2.6 mm.

Range of variation of certain specific characters.- It is desirable to know the range of variation within the species of the different measurable characters used for taxonomic purposes. Sturtevant (1921) gives the range of variation in the number of dorsocentral bristles, wing-vein indices and number of rows of acrostichal hairs. Tables 1 and 2 show the variation in the wing-vein indices of D. brunettii and D. emulata respectively.*

Discussion.- D. brunettii, sp. nov. seems to be allied to D. bipectinata. The latter possesses two oblique combs of black stout bristles on the inner surface of first tarsal segment of the prothoracic leg but the former is without any such structure. The wing venation indices are about the same in both the species. Second orbital bristle is proportionally smaller in D. bipectinata than in D. brunettii.

D. emulata, sp. nov. resembles D. melanogaster Meigen closely. It differs from the latter, however, in the general colouration of the body, wing-venation indices and size. The body and the wings are of equal size. in D. melanogaster that is 2.0 mm., but in D. emulata the wing is definitely smaller than the body both in the male and the female.

TABLE 2. Range of variation of the wing vein indices in D. emulata, sp. nov.

No. of individuals	Costal index	No. of individuals	4th vein index	No. of individuals	4c index	No. of individuals	5x index
6	2.5	8	2.1	5	1.05	8	1.6
7	2.6	8	2.2	7	1.10	3	1.7
1	2.7			2	1.15	5	1.8
2	2.8			2	1.2		
Total	Average	Total	Average	Total	Average	Total	Average
16	2.5	16	2.15	16	1.1	16	1.6

D. prashadi Brunetti was described from Calcutta and the two species described in this paper are quite distinct from it as explained below:

The description of D. prashadi as given by Brunetti, unfortunately refers mostly to generic characters and consequently applies to all the species of Drosophila. Two species characters of taxonomic importance mentioned by him are (i) the number of bristles present on the arista and (ii) the bandings on the hind margins of the abdominal segments. The number of bristles on the arista, however, is shown by him to be a variable character and this might be anything from 2-4. Moreover he had two flies in the same collection

* In the description of the species referred above we have given the average values of the different wing-vein indices.

which had 8 bristles on the arista, 5 above and 3 below. If we take into consideration these two exceptional flies the range of variation in the number comes to 2-8. As to the bandings of the abdomen he writes: "The hind margins of the abdominal segments are more or less narrowly black, the colours sometimes extending to the middle of the segments, sometimes hardly visible or quite absent". According to this description, the markings of the abdomen are also quite variable in D. prashadi. We have examined a large number of flies in our collection from the same locality to ascertain the range of variation of both these characters. In most of our flies the number of bristles on the arista is quite constant. Occasionally, however, one gets one or two flies in which the number deviates from the normal, sometimes by one more or one less. But the range of variation never assumes such porportions as to be anywhere between 2 and 8. We have not been able to find any reference to the descriptions of any one of the hundreds of Drosophila species so far described, where such variation as given by Brunetti for D. prashadi is usual. The black banding on the abdominal segments is very conspicuous in D. emulata and extends to the middle of the segments but this is not so marked in D. brunettii. If one has got, without his knowledge a mixed collection of these two species, he runs the risk of inferring that the bandings are more or less narrowly black, sometimes extending to the middle of the segments and sometimes are hardly visible, as was possibly done by Brunettii. This seems to be more likely, since D. emulata has 8 bristles on the antenna (5 above and 3 below) and if we take into consideration his two exceptional flies which have exactly this number, we are justified in concluding that he had a mixed collection of different species and did not distinguish them. An examination of the type specimens of this species in the Indian Museum, Calcutta, was also done but unfortunately the state of preservation was found to be so bad that important characters could not be studied. The description of the species given by him is applicable to all the common species of Drosophila found in Calcutta and it is difficult to say which of the species he really meant; it is, therefore, useless to retain the species created by him. We have called one of our species as D. brunettii after the name of Brunetti, since he was the first to describe an endemic species of Drosophila from India.

2. Life-History of Drosophila emulata

Eggs, larvae and pupae of all the species of Drosophila, although very similar, show certain variations, which afford excellent specific differences (Sturtevant, 1921; Kikkawa and Peng, 1938). Detailed study of these variations were not done during the present work. General desriptions of the various stages and references to literature can be obtained from Kikkawa and Peng's (1938) monograph on Drosophila species of Japan and adjacent localities.

The females usually lay eggs about 48 hours after emergence. The number of eggs laid by different females even of the same stock varies to a great extent. They continue to lay eggs for a month or more, at the rate of 50-60 per day, afterwards the rate of re-production gradually diminishes.

We have determined the duration of successive stages from the egg up to the emergence of the adult flies.

The following procedure was adopted in studying the life-history:

An ordinary glass-stoppered, wide-mouthed jar was taken and standard food medium was put into the cup-like depression of the hollow glass stopper About 200 impregnated females were put in the jar and these were passed over to the glass stopper containing the food. Flies were allowed to lay eggs only for one hour and 30 minutes. Nearly 400 eggs were thus obtained within this period. The adults were then discarded and the eggs were allowed to hatch. The culture

was regularly examined and the durations of various stages were noted. The whole life-history was worked out at a constant temperature of $26^0 \pm 2^0$ C.

The first larva came out 17 hrs. 15 mins. after the starting of the egg-laying and all the larvae crawled out within 20 hrs. 30 mins. of the commencement of oviposition. The time which elapsed between the hatching of the first and the last larvae is, therefore, 3 hrs. 15 mins. Had the rate of development or the growth-rate been the same in all the individual eggs, the difference in time between the first and the last larvae to come out of the eggs, should not have exceeded 1 hr. 30 mins., that is, by a period of more than the age difference between the first and the last egg laid.

The first pupa was formed 94 hours after the first larva came out of the egg shell. All of them pupated within eight hours. We find here a discrepancy of 4 hrs. 45 mins., if we assume the rate of growth of all the larvae to be uniform.

The first imago emerged from the pupal case 72 hrs. after pupation. The emergence of all the individuals was completed within 22 hrs.

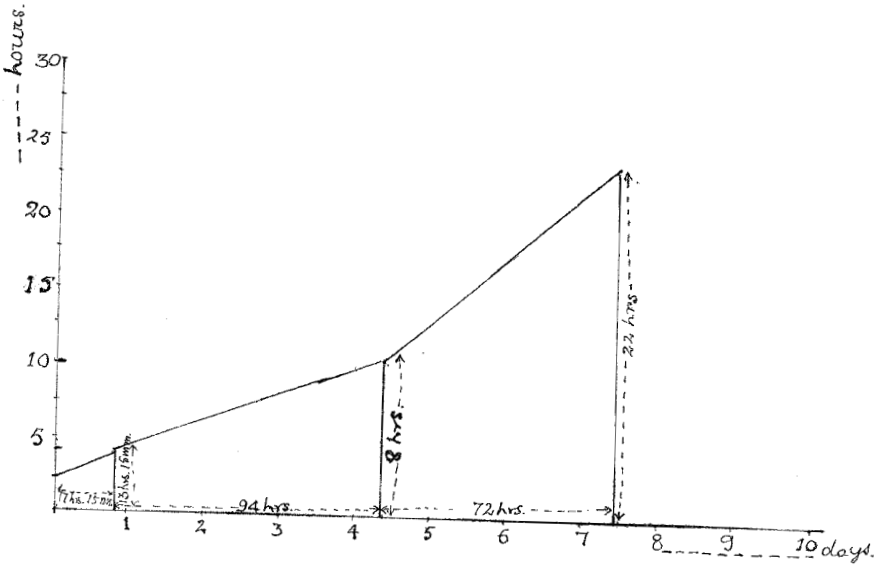
We started with an age difference of not more than 1 hr. 30 mins. between the first egg and last egg laid but at the end of the development we found the age difference between the first and last imago to be as high as 22 hrs. This is remarkable since this difference was obtained in a species in which the life-history is completed within such a short period.

The above results are graphically represented where the abscissa gives the time interval between the first appearances of successive stages and the ordinates stand for the duration of laying, hatching, pupation, and emergence. From the graph it is obvious that the growth rate is not the same throughout. If the growth rates at the different stages varied uniformly we would, within limits of experimental error, get a straight line joining the tops of the ordinates. The pattern, however, shows that this is not the case. The variation in the growth rates at successive stages of the life-history is different. From the data given in the graph we can calculate the extreme growth rates at the various stages.

We do not know exactly which one of the eggs laid within the period of 1 hr. 30 mins. hatched out first. In an extreme case, therefore, it is possible that an egg laid at the end of the laying period might be the one which hatched out first. Therefore, the shortest possible period in which an egg can hatch is 17 hrs. 15 mins minus 1 hr. 30 mins., viz., 15 hrs. 45 mins. Similarly the longest period is 17 hrs. 15 mins. plus 3 hrs. 15 mins., viz., 20 hrs 30 mins. The ratio of the two extreme growth rates for this stage is 20.5 hours: 15.75 hours or 1.0 : 0.77. Therefore the dispersion is

$$\frac{1.0-0.77}{\frac{1}{2}(1.77)} \cdot 100 = 26\%$$

Similarly for the larval and pupal stages the dispersions are 11% and 38% respectively.



Thus it appears that the variation in growth-rate is the maximum in the pupal stage and minimum in the larval stage. Since all the eggs were under identical environmental conditions, we can only interpret our results by postulating variations in the genetic make-up of the eggs. These variations are probably due to a certain gene or genes which control the rate of growth. The longest period (94)hrs. in the life-cycle intervenes at the larval stage. The larvae have an independent existence and move about freely in search of food If the environment had any significant role in our experiment, in causing variation in growth rates, we should expect the dispersion in the larval stage to be the maximum. On the contrary we find it to be the lowest, being only 11%. Evidently the genes which cause these variability in the rate of development at various stages must have acted during the intra-embryonic period and reaching its height in the pupal stage.

3. Gene Mutation Obtained In Drosophila emulata

About 50 flies were trapped near a market in Ballygunge in Calcutta and 24 pair-matings were done in small glass vials. F_1 individuals were obtained from progeny in each vial and about 6 brother-sister matings were done for each family to obtain F_2 . Flies of each generation were carefully examined for visible mutations. It is easy to see how a recessive autosomal mutation present in a hetrozygous condition in the wild population, can manifest themselves in F_2 by this process of enforced homozygosis through close inbreeding. No attempt was made, however, in this preliminary survey to determine quantitatively the frequancy of mutated gene present in the wild sample. Three definite mutations and a probable fourth were obtained amongst the F_2 progeny. They are described below:

(i) 'garnet'- An autosomal recessive mutation affecting the eye colour of the flies. The garnet- eye-colour is much lighter and is very pron-ounced in the newly emerged flies. With the age of the flies the colour becomes darker, but it is always possible, with a little practice, to distinguish them from the wild type.

(ii) 'curved'- An autosomal recessive mutation affecting the shape of the wing. Homozygous curved flies have the tip of their wings curved up-wards instead of being flatly placed on the back.

(iii) 'posterior-cross-vein'- An autosomal recessive mutation affecting wing venation. Homozygous 'posterior-cross-vein flies have a small longitudinal branching of the posterior cross-vein in the 2nd posterior cell of the wing. The manifestation of this character is very irregular. Accurate data about its mode of inheritance are being worked out.

(iv) Several flies, both males and females, appeared in some of the F_2 vials with abnormal longitudinal veins. A few pair matings were done with these flies but none of them produced any offspring.

TABLE 111

F_2 progeny obtained out of the F_1 between wild type and garnet flies

Wild type			Garnet			Wild garnet ratio
NO. of males	NO. of females	Total	NO. of males	NO. of females	Total	
246	281	527	85	92	176	2.99

Neither sex-linked recessive nor autosomal dominant mutation was

found.

In Tables 111, 1V and V are given the number and kind of progeny obtained in course of determining the mode of inheritance of these mutations.

The F_2 ratios of wild and mutant flies (Tables 111 and 1V) show that 'curved' and 'garnet' are two simple autosomal recessive genes. The ratios of the different types of F_2 progeny of curved-garnet cross are not much different from the expected 9:3:3:1 ratio, and therefore they are situated on two different chromosomes. We could not decide, as yet, whether posterior cross-vein is situated on the curved chromosome or on the garnet chromosome. It might be as well on a different chromosome altogether.

The results obtained show definitely that 'garnet' and 'curved' are two simple Mendelian autosomal recessive genes situated on two different chromosomes.

TABLE 1V

F_2 progeny obtained out of the F_1 between wild type and curved flies

Wild type			Curved			Wild curved ratio
No. of males	No. of females	Total	No. of males	No. of females	Total	
95	87	182	22	26	48	3.79

TABLE V

F_2 progeny obtained out of the F_1 between curved and garnet flies

Wild type	Curved	Garnet	Curved & garnet
292	77	90	25

A more detailed study of the genetics of the two species described in this paper and also a quantitative genetical analysis of the wild population will be undertaken in winter, because culture of these flies can more easily be handled in large numbers during the colder months. Salivary gland chromosomes and other cytological studies of these species are in progress.

SUMMARY

Two new species of Drosophila are described from Calcutta: reasons are given to show why the only other species so far described from the same locality, cannot be retained.

Life-history of D. emulata, sp. nov. has been worked out in detail. In course of this investigation it was found that in spite of identical environmental conditions, under which the individual eggs, larvae and pupae were reared, they differed considerably in their rates of development. The existence of genes controlling the rates of development, acting at particular stages of the life-history, has been presumed to explain the differences in the rates of growth.

Three definite and a probable fourth autosomal recessive mutations have been found in heterozygous condition in 24 wild females caught near a market in Ballygunge, Calcutta.

ACKNOWLEDGMENTS

The authors are indebted to Dr. H. K. Mookerjee, D. Sc. (Lond.), Head of the department of Zoology, Calcutta University, for facilities and to Mr. D. Mukerji, Lecturer, Calcutta University, for constructive criticism.

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(1941)

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Material and Technique

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I. SYSTEMATIC DESCRIPTION

The only endemic species of *Drosophila* so far recorded from India is *D. prashadi* Brunetti (1923), the type-locality of which is Calcutta. Six other species reported from India are more or less cosmopolitan in distribution : *D. melanogaster* Meigen, *D. ananassæ* Doleschall, *D. montium* de Meijere, *D. tristipennis* Duda, *D. bipectinata* Duda and *D. repleta* Wallaston.

The first four species were collected from Nungambaukam, Madras (Sturtevant, 1927). Of the remaining two species, *D. bipectinata*, the type-specimen of which is in the Hungarian National Museum, was described by Duda (1923), and its type-locality is Darjeeling. *D. repleta* was recorded from Calcutta by Bezzi (Sturtevant, 1921). It may be noted here that *D. melanogaster* and *D. repleta* are cosmopolitan in the true sense, whereas *D. ananassæ* and *D. montium* appear to be limited to the oriental region. The former has been recorded from Java, Formosa, Sumatra, New-Guinea and India and the latter from Java, Formosa, India and West Indies.

Drosophila brunettii, sp. nov.

Male.—Arista with four branches above and three below, excluding the bifurcated terminal part of the main axis. Antennæ light brownish-yellow, the third joint grey. Front about one-third the width of the head, wider above, yellow. Second orbital bristle about one-half of the size of the other two. Second oral bristle nearly as long as the first. Face pale yellow. Cheeks extremely pale yellow. Acrostichal hairs in the front of the anterior dorso-central bristles in eight rows; no prescutellars. Mesonotum and scutellum pale brownish-yellow. Pleuræ and legs pale yellow. Preapical bristles on the first leg, apical and preapicals on the second leg, preapicals on the third. Wings clear. Costal index 1.5; fourth vein index 2.4; 5x index 1.9; 4c index 1.5. Length of the body and wing 2.0 mm.

Female.—Length of the body and wing 2.2 mm.

Drosophila emulata, sp. nov.

Male.—Arista with five branches above and three below, excluding the terminal part of the main axis which is bifurcated.

Antennæ pale brown, third joint grey. Front about one-half the width of the head, wider above. Second orbital bristle about one-half the size of the other two. Second oral bristle as long as the first. Face pale brown. Cheeks extremely pale yellow. Acrostichal hairs in eight rows. Mesonotum and scutellum shining reddish-brown. Pleuræ and legs pale brown. Preapical bristle on the first leg, apicals and preapicals on the second and preapicals on the third. A comb-like row of about ten short curved black bristles on the inner distal surface of the basal tarsal segment of the first leg.

TABLE I: *Range of variation of the wing-vein indices in* D. brunettii, *sp. nov.*

No. of individuals	Costal index	No. of individuals	4th vein index	No. of individuals	4c index	No. of individuals	5x index
2	1.3	6	2.3	14	1.5	4	1.7
7	1.4	10	2.4	4	1.6	3	1.8
9	1.5	5	2.5	6	1.7	10	2.0
5	1.6	3	2.6	1	1.8	7	2.1
3	1.7						
Total	Average	Total	Average	Total	Average	Total	Average
26	1.5	24	2.4	25	1.5	24	1.9

Dorsal side of the last two segments of the abdomen shining black. Three other conspicuous black bands on the lower margin of the remaining segments, not extending to more than the middle of each segment. Wings clear. Costal index 2.5 ; 4th vein index 2.15 ; 4c index 1.1 ; 5x index 1.6. Length of the body 2.6 mm. Length of the wing 2.3 mm.

Female.—Length of the body 3.0 mm. Length of the wing 2.6 mm.

Range of variation of certain specific characters.—It is desirable to know the range of variation within the species of the different measurable characters used for taxonomic purposes. Sturtevant (1921) gives the range of variation in the number of dorsocentral bristles, wing-vein indices and number of rows of acrostichal hairs. Tables I and II show the variation in the wing-vein indices of *D. brunettii* and *D. emulata* respectively.*

Discussion.—*D. brunettii*, sp. nov. seems to be allied to *D. bipectinata*. The latter possesses two oblique combs of black stout bristles on the inner surface of first tarsal segment of the prothoracic leg but the former is

*In the description of the species referred to above we have given the average values of the different wing-vein indices.

without any such structure. The wing venation indices are about the same in both the species. Second orbital bristle is proportionately smaller in *D. bipectinata* than in *D. brunettii*.

D. emulata, sp. nov. resembles *D. melanogaster* Meigen closely. It differs from the latter, however, in the general colouration of the body, wing-venation indices and size. The body and the wings are of equal size in *D. melanogaster* that is 2.0 mm., but in *D. emulata* the wing is definitely smaller than the body both in the male and the female.

TABLE II. *Range of variation of the wing vein indices in* D. emulata, *sp. nov.*

No. of individuals	Costal index	No. of individuals	4th vein index	No. of individuals	4c index	No. of individuals	5x index
6	2.5	8	2.1	5	1.05	8	1.6
7	2.6	8	2.2	7	1.10	3	1.7
1	2.7			2	1.15	5	1.8
2	2.8			2	1.2		
Total	Average	Total	Average	Total	Average	Total	Average
16	2.5	16	2.15	16	1.1	16	1.6

D. prashadi Brunetti was described from Calcutta and the two species described in this paper are quite distinct from it as explained below :

The description of *D. prashadi* as given by Brunetti, unfortunately refers mostly to generic characters and consequently applies to all the species of *Drosophila*. Two specific characters of taxonomic importance mentioned by him are (*i*) the number of bristles present on the arista and (*ii*) the bandings on the hind margins of the abdominal segments. The number of bristles on the arista, however, is shown by him to be a variable character and this might be anything from 2-4. Moreover he had two flies in the same collection which had 8 bristles on the arista, 5 above and 3 below. If we take into consideration these two exceptional flies the range of variation in the number comes to 2-8. As to the bandings of the abdomen he writes : " The hind margins of the abdominal segments are more or less narrowly black, the colours sometimes extending to the middle of the segments, sometimes hardly visible or quite absent". According to this description, the markings of the abdomen are also quite variable in *D. prashadi*. We have examined a large number of flies in our collection from the same locality to ascertain the range of variation of both these characters. In most of our flies the number of bristles on the arista is

quite constant. Occasionally, however, one gets one or two flies in which the number deviates from the normal, sometimes by one more or one less. But the range of variation never assumes such porportions as to be anywhere between 2 and 8. We have not been able to find any reference to the descriptions of any one of the hundreds of *Drosophila* species so far described, where such variation as given by Brunetti for *D. prashadi* is usual. The black banding on the abdominal segments is very conspicuous in *D. emulata* and extends to the middle of the segments but this is not so marked in *D. brunettii*. If one has got, without his knowledge, a mixed collection of these two species, he runs the risk of inferring that the bandings are more or less narrowly black, sometimes extending to the middle of the segments and sometimes are hardly visible, as was possibly done by Brunetti. This seems to be more likely, since *D. emulata* has 8 bristles on the antenna (5 above and 3 below) and if we take into consideration his two exceptional flies which have exactly this number, we are justified in concluding that he had a mixed collection of different species and did not distinguish them. An examination of the type specimens of this species in the Indian Museum, Calcutta, was also done but unfortunately the state of preservation was found to be so bad that important characters could not be studied. The description of the species given by him is applicable to all the common species of *Drosophila* found in Calcutta and it is difficult to say which of the species he really meant; it is, therefore, useless to retain the species created by him. We have called one of our species as *D. brunettii* after the name of Brunetti, since he was the first to describe an endemic species of *Drosophila* from India.

2. Life-History of *Drosophila emulata*

Eggs, larvæ and pupæ of all the species of *Drosophila*, although very similar, show certain variations, which afford excellent specific differences (Sturtevant, 1921; Kikkawa and Peng, 1938). Detailed study of these variations were not done during the present work. General descriptions of the various stages and references to literature can be obtained from Kikkawa and Peng's (1938) monograph on *Drosophila* species of Japan and adjacent localities.

The females usually lay eggs about 48 hours after emergence. The number of eggs laid by different females even of the same stock varies to a great extent. They continue to lay eggs for a month or more, at the rate of 50-60 per day, afterwards the rate of re-production gradually diminishes.

We have determined the duration of successive stages from the egg up to the emergence of the adult flies.

The following procedure was adopted in studying the life-history :

An ordinary glass-stoppered, wide-mouthed jar was taken and standard food medium was put into the cup-like depression of the hollow glass stopper. About 200 impregnated females were put in the jar and these were passed over to the glass stopper containing the food. Flies were allowed to lay eggs only for one hour and 30 minutes. Nearly 400 eggs were thus obtained within this period. The adults were then discarded and the eggs were allowed to hatch. The culture was regularly examined and the durations of various stages were noted. The whole life-history was worked out at a constant temperature of 26° ± 2°C.

The first larva came out 17 hrs. 15 mins. after the starting of the egg-laying and all the larvæ crawled out within 20 hrs. 30 mins. of the commencement of oviposition. The time which elapsed between the hatching of the first and the last larvæ is, therefore, 3 hrs. 15 mins. Had the rate of development or the growth-rate been the same in all the individual eggs, the difference in time between the first and the last larvæ to come out of the eggs, should not have exceeded 1 hr. 30 mins., that is, by a period of more than the age difference between the first and the last egg laid.

The first pupa was formed 94 hours after the first larva came out of the egg shell. All of them pupated within eight hours. We find here a discrepancy of 4 hrs. 45 mins., if we assume the rate of growth of all the larvæ to be uniform.

The first imago emerged from the pupal case 72 hrs. after pupation. The emergence of all the individuals was completed within 22 hrs.

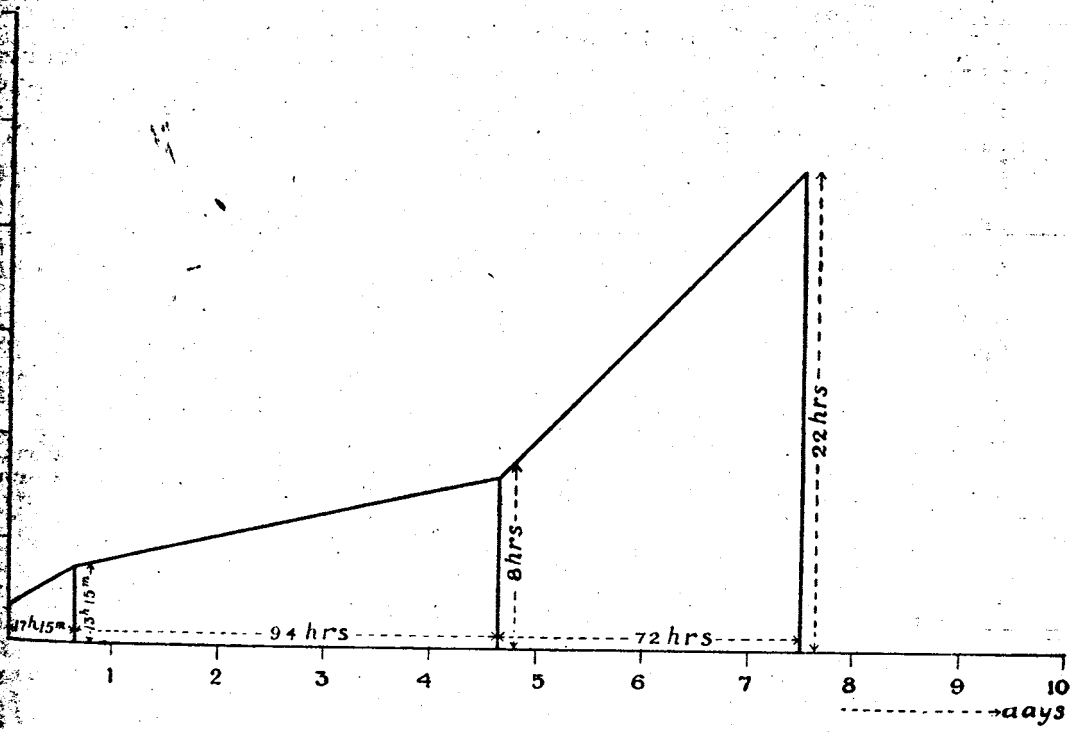
We started with an age difference of not more than 1 hr. 30 mins. between the first egg and last egg laid but at the end of the development we found the age difference between the first and last imago to be as high as 22 hrs. This is remarkable since this difference was obtained in a species in which the life-history is completed within such a short period.

The above results are graphically represented where the abscissa gives the time interval between the first appearances of successive stages and the ordinates stand for the duration of laying, hatching, pupation and emergence. From the graph it is obvious that the growth rate is not the same throughout. If the growth rates at the different stages varied uniformly we would, within limits of experimental error, get a straight line joining the tops of the ordinates. The pattern, however, shows that this is not the case. The variation in the growth rates at successive stages of the life-history is different. From the data given in the graph we can calculate the extreme growth rates at the various stages.

We do not know exactly which one of the eggs laid within the period of 1 hr. 30 mins. hatched out first. In an extreme case, therefore, it is possible that an egg laid at the end of the laying period might be the one which hatched out first. Therefore, the shortest possible period in which an egg can hatch is 17 hrs. 15 mins *minus* 1 hr. 30 mins., *viz.*, 15 hrs. 45 mins. Similarly the longest period is 17 hrs. 15 mins. *plus* 3 hrs. 15 mins., *viz.*, 20 hrs. 30 mins. The ratio of the two extreme growth rates for this stage is 20.5 hours: 15.75 hours or 1.0 : 0.77. Therefore the dispersion is

$$\frac{1.0-0.77}{\frac{1}{2}(1.77)} \cdot 100 = 26\%$$

Similarly for the larval and pupal stages the dispersions are 11% and 38% respectively.



Thus it appears that the variation in growth-rate is the maximum in the pupal stage and minimum in the larval stage. Since all the eggs were under identical environmental conditions, we can only interpret our results by postulating variations in the genetic make-up of the eggs. These variations are probably due to a certain gene or genes which control the rate of growth. The longest period (94 hrs.) in the life-cycle intervenes at the larval stage. The larvæ have an independent existence and move about freely in search of food. If the environment had any significant role in our experiment, in causing variation in growth rates, we should expect the dispersion in the larval stage to be the maximum. On the

contrary we find it to be the lowest, being only 11%. Evidently the genes which cause these variability in the rate of development at various stages must have acted during the intra-embryonic period and reaching its height in the pupal stage.

3. GENE MUTATION OBTAINED IN *DROSOPHILA EMULATA*

About 50 flies were trapped near a market in Ballygunge in Calcutta and 24 pair-matings were done in small glass vials. F_1 individuals were obtained from progeny in each vial and about 6 brother-sister matings were done for each family to obtain F_2. Flies of each generation were carefully examined for visible mutations. It is easy to see how a recessive autosomal mutation present in a hetrozygous condition in the wild population, can manifest themselves in F_2 by this process of enforced homozygosis through close inbreeding. No attempt was made, however, in this preliminary survey to determine quantitatively the frequency of mutated gene present in the wild sample. Three definite mutations and a probable fourth were obtained amongst the F_2 progeny. They are described below :

(*i*) '*garnet*'—An autosomal recessive mutation affecting the eye colour of the flies. The garnet eye-colour is much lighter and is very pronounced in the newly emerged flies. With the age of the flies the colour becomes darker, but it is always possible, with a little practice, to distinguish them from the wild type.

(*ii*) '*curved*'—An autosomal recessive mutation affecting the shape of the wing. Homozygous curved flies have the tip of their wings curved upwards instead of being flatly placed on the back.

(*iii*) '*posterior-cross-vein*'—An autosomal recessive mutation affecting wing venation. Homozygous 'posterior-cross-vein' flies have a small longitudinal branching of the posterior cross-vein in the 2nd posterior cell of the wing. The manifestation of this character is very irregular. Accurate data about its mode of inheritance are being worked out.

(*iv*) Several flies, both males and females, appeared in some of the F_2 vials with abnormal longitudinal veins. A few pair matings were done with these flies but none of them produced any offspring.

TABLE III

F_2 progeny obtained out of the F_1 between wild type and garnet flies

Wild type			Garnet			Wild garnet ratio
No. of males	No. of females	Total	No. of males	No. of females	Total	
246	281	527	85	92	176	2.99

Neither sex-linked recessive nor autosomal dominant mutation was found.

In Tables III, IV and V are given the number and kind of progeny obtained in course of determining the mode of inheritance of these mutations.

The F_2 ratios of wild and mutant flies (Tables III and IV) show that 'curved' and 'garnet' are two simple autosomal recessive genes. The ratios of the different types of F_2 progeny of curved-garnet cross are not much different from the expected 9 : 3 : 3 : 1 ratio, and therefore they are situated on two different chromosomes. We could not decide, as yet, whether posterior cross-vein is situated on the curved chromosome or on the garnet chromosome. It might be as well on a different chromosome altogether.

The results obtained show definitely that 'garnet' and 'curved' are two simple Mendelian autosomal recessive genes situated on two different chromosomes.

TABLE IV

F_2 progeny obtained out of the F_1 between wild type and curved flies

Wild type			Curved			Wild curved ratio
No. of males	No. of females	Total	No. of males	No. of females	Total	
95	87	182	22	26	48	3.79

TABLE V

F_2 progeny obtained out of the F_1 between curved and garnet flies

Wild type	Curved	Garnet	Curved and garnet
292	77	90	25

A more detailed study of the genetics of the two species described in this paper and also a quantitative genetical analysis of the wild population will be undertaken in winter, because culture of these flies can more easily be handled in large numbers during the colder months. Salivary gland chromosomes and other cytological studies of these species are in progress.

SUMMARY

Two new species of *Drosophila* are described from Calcutta; reasons are given to show why the only other species so far described from the same locality, cannot be retained.

Life-history of *D. emulata*, sp. nov. has been worked out in detail. In course of this investigation it was found that in spite of identical environmental conditions, under which the individual eggs, larvæ and pupæ were reared, they differed considerably in their rates of development. The existence of genes controlling the rates of development, acting at particular stages of the life-history, has been *presumed* to explain the differences in the rates of growth.

Three definite and a probable fourth autosomal recessive mutations have been found in heterozygous condition in 24 wild females caught near a market in Ballygunge, Calcutta.

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