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SHORT PAPER

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ABSTRACT

Two different species which were originated from India and Indochina have been called *Drosophila jambulina* as the same species. They were compared genetically, cytologically and electrophoretically, as well as morphologically. From these results it was concluded that the Indian species was the real *D. jambulina* and that the Indochinese species seemed to be a new species and was tentatively named *D. punjabiensis-like* because it was closer to the sibling species, *D. punjabiensis*. *D. jambulina* showed a color dimorphism in a terminal tergite of female. A light-type gene controlling the dimorphism was dominant over a dark-type gene. The heterozygote segregated into 3 light: 1 dark in the next generation.

Drosophila jambulina was described as a new species belonging to the *D. montium* subgroup (Parshad and Paika 1964) which is a common and widespread species in India. The metaphase chromosomes consist of two pairs of metacentric autosomes (2nd and 3rd) and one pair of submetacentric autosomes (4th), in addition to one pair of telocentric X chromosomes in female or the X and submetacentric Y chromosome in male (Singh and Gupta 1980). However, when Baimai (1980) examined a stock from the University of Texas (Cambodia origin: Bock and Wheeler 1972), its metaphase karyotype was different from the above constitution especially in the 4th chromosome (dot) and the Y chromosome (metacentric), suggesting that the stock could be a different species from *D. jambulina*.

In 1979, we (Scientific expedition for the collection of *Drosophila* by the Tokyo Metropolitan University) collected many flies of *D. jambulina* and the sibling species, *D. punjabiensis* from India. They were crossed each other and two groups of mutually crossable strains were obtained (*jambulina*-TMU and *punjabiensis*-TMU). On the other hand, laboratory strains were obtained from Dr. B.K. Singh of the Banaras Hindu University, India (*jambulina*-BHU), and from Dr. M.R. Wheeler of the University of Texas, U.S.A. (*punjabiensis*-Malaysia, Texas stock no. 3033.4). *D. punjabiensis-like* were

Table 1. *All possible crosses between six strains. O is F_1 progeny obtained, X is no F_1 progeny obtained*

Female		Male					
		j-TMU	BHU	Thai.	Camb.	*p*-TMU	Malay.
jambulina	(TMU, India)	O	O	X	X	X	X
jambulina	(BHU, India)	O	O	X	X	X	X
punjabiensis-like	(Thailand)	X	X	O	O	X	X
punjabiensis-like	(Cambodia)	X	X	O	O	X	X
punjabiensis	(TMU, India)	X	X	O	X	O	O
punjabiensis	(Malaysia)	X	X	O	X	O	O

supplied as *D. jambulina* from the University of Texas (Thailand, Texas stock no. 3116.11 and Cambodia, Texas stock no. 3120.5), but as shown in the following sections, they were tentatively named *D. punjabiensis-like.*

Table 1 shows the results of the all possible crosses between the above six strains. From the successful crosses, they could be classified into three groups; *D. jambulina* (TMU and BHU), *D. punjabiensis-like* (Thailand and Cambodia) and *D. punjabiensis* (TMU and Malaysia). An interesting exception was found in the successful crosses between the *D. punjabiensis-like* of Thailand males and *D. punjabiensis* females. This suggests that the two species, *D. punjabiensis-like* and *D. punjabiensis,* are genetically closer to each other than to *D. jambulina.*

Next we examined the mitotic metaphase chromosomes from the brain ganglion of the 3rd instar larvae. The results are shown in Fig. 1. Three species were clearly distinguished by the shape of the Y chromosome and the 4th chromosome. *D. jambulina* was different from *D. punjabiensis-like* in the following manner: The former species had medium sized submetacentric 4th chromosomes and a submetacentric Y chromosome, while the latter had the dot shaped 4th chromosomes and a metacentric Y chromosome. The difference between *D. punjabiensis-like* and *D. punjabiensis* was found only in the shape of Y chromosome; a metacentric Y for the former and a submetacentric Y for the latter. The difference between *D. punjabiensis* and *D. jambulina* was the 4th chromosome, dot and submetacentric, respectively. The short arm of the X chromosome of *D. punjabiensis* may be slightly longer than those of other species which needs more data to confirm it. These results are almost the same as those of Singh and Gupta (1980) and Baimai (1980), when we regard the *D. jambulina* of Baimai as *D. punjabiensis-like.* The karyotypes of two strains of each species (*jambulina*-TMU and BHU; *punjabiensis-like* Thailand and Cambodia; *punjabiensis* TMU and Malaysia) were identical.

Two kinds of electrophoretical studies were employed: Two-dimensional

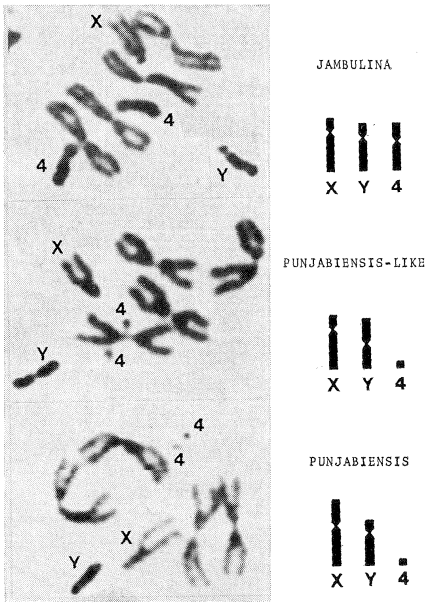


Fig. 1. Karyotypes of *D. jambulina*, *D. punjabiensis-like* and *D. punjabiensis*.

electrophoresis (2DE) of proteins and Standard starch gel electrophoresis (SDE) of 20 allozyme loci (*Acph-1*, *Adh*, *Dip-A* and *B*, *Est-6*, *Est-C*, *Fum*, *Got*, *G6pdh*, *αGpdh*, *Hex-A* and *C*, *cMdh*, *mMdh*, *Men*, *Odh*, *6Pgdh*, *Pgi*, *Pgm* and *Xdh*) according to the method described by Ohnishi *et al.* (1982). Genetic distance (D) for the 2DE data was calculated by Aquadro and Avise (1981)'s equation: $D=1-F=1-2n_{xy}/(n_x+n_y)$, where n_{xy} is the number of shared loci in both species and n_x or n_y is the total number of scored loci in each species. The genetic distance for the SDE data was calculated by Nei (1972)'s equation: $D=-\log_e I$, in which I is the normalized identity of genes (or proteins) between strains.

Phylogenetic trees of the six strains were constructed according to Nei (1975)'s procedure as shown in Fig. 2. Distance between strains (pairs of each species) was clearly shorter than that between species (other pairs) in both 2DE and SDE, although the distance calculated by 2DE method always showed lower value than that by SDE. *Drosophila punjabiensis-like* was closer to *D. punjabiensis* than to *D. jambulina* in the both methods. These results seem to correspond to the previous genetical and cytological studies.

The morphology of *D. jambulina*, *D. punjabiensis-like* and *D. punjabiensis* was described by Parshad and Paika (1964) and Bock and Wheeler (1972) using different strains which have caused the confusion of the name of *D.*

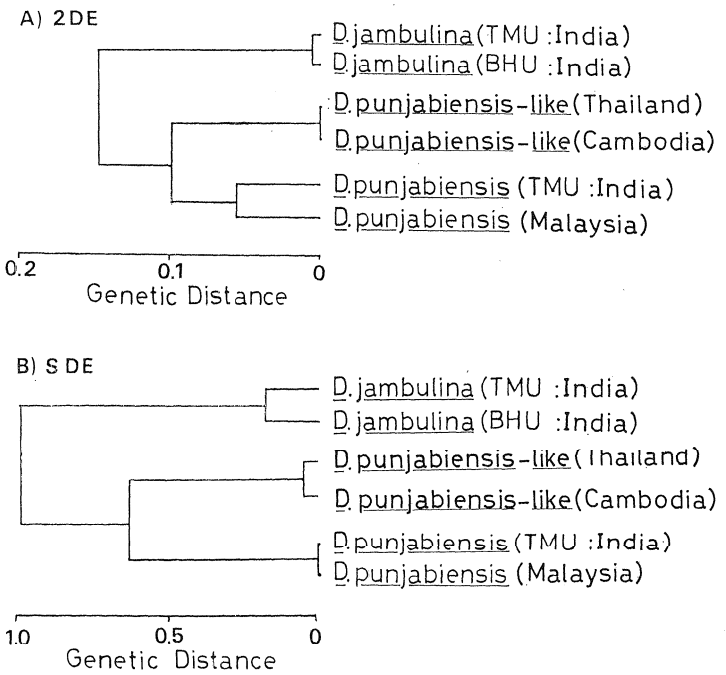
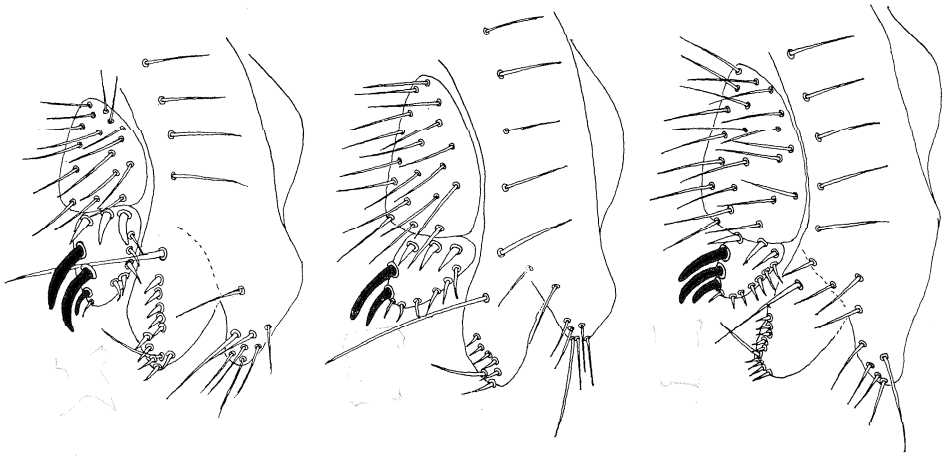


Fig. 2. The phylogenetic relationship among six strains of three species by two-dimensional electrophoresis (2DE) and starch gel electrophoresis (SDE).

jambulina. For this reason we give here a comparative description to classify the above three species. Superficially *D. punjabiensis-like* resembles *D. punjabiensis* in characters such as phallic organs and color of carina of male. The former species, however, differs from *D. punjabiensis* and *D. jambulina* with respect to the following characters:

	D. jambulina	*D. punjabiensis-like*	*D. punjabiensis*
(1) Carina of male	milky white	pale	pale
(2) Wing indices (mean of 10 samples)			
Costal index	2.3	2.1	2.0
4C-index	1.5	1.7	1.4
4th-index	3.0	3.2	2.5
5X-index	3.0	3.6	2.7
(3) Teeth of sex comb (mean of 10 samples)			
Proximal (range)	21(19-23)	26(24-27)	29(28-31)
Distal (range)	16(15-18)	19(18-20)	19(18-20)
(4) Teeth of secondary clasper (in general)	Two large one smaller	Two large	Three large

The present observation of periphallic organs of both *D. jambulina* and *D.*



Jambulina Punjabiensis-like Punjabiensis

Fig. 3. Male genitalia of *D. jambulina*, *D. punjabiensis-like* and *D. punjabiensis*.

Table 2. *Cross tests between light-type (L) and dark-type (D) of D. jambulina*

Female×Male	Cross	No. of progeny	
		L	D
L × L	9	688	0
D × D	7	0	384
L × D	7	283	0
D × L	7	292	0
L/D × L/D	7	253	77
D/L × D/L	11	437	124
L/D × D	6	196	181
D × L/D	23	627**	533

**deviated from the 1:1 ratio significantly.

punjabiensis agrees for all its details with that described by Parshad and Paika (1964). Morphological differences of the periphallic organs among the two species and *D. punjabiensis-like* are comparatively presented in Fig. 3.

Twelve isofemale strains of *D. jambulina* collected from India in 1979 showed dimorphic variation in color of a terminal tergite only in females. Two were dark-type, two were light-type and eight were the mixture of dark- and light-types. From each mixtured strain, two subisofemale lines were taken and they were pair-mated for several generations to establish typical strains of dark- and light-types. Nine light type strains and seven dark-type strains were finally obtained. When those light- and dark-types

were reciprocally crossed, F_1 females were exclusively light-typed ones (Table 2). In the F_2 generation, light and dark flies segregated in 3 light and 1 dark ratio. In the backcross series, we obtained almost 1 to 1 ratio, although the light-typed females were slightly exceeded than the expected. These results indicate that the color dimorphism is controlled by a singe locus with two alleles and that a light color controlling gene is dominant over a dark gene.

Drosophila females especially the *D. montium* subgroup often showed color dimorphism: *D. kikkawai* (Freire-Maia 1949), *D. rufa* (Oshima 1952) and *D. auraria* (Lee 1963). A gene controlling dark-color was always dominant over a light one in these species. It is a great surprise to find a paper (Parkash and Sharma 1978) showing that in *D. jambulina* from India a dark allele was dominant over a light allele. The dominance of the color alleles was perfectly reversed from the present study. We could not test the stocks from Parkash and Sharma. But, when two additional stocks sent from India by Dr. B. K. Singh which were the mixture of light- and dark-types were examined, the light-type was completely dominant over the dark-type, which confirmed aforementioned results.

It is still in question why our stocks of *D. jambulina* (TMU, BHU) showed an exceptional behavior in the dominance of color type. If the *D. jambulina* examined by Parkash and Sharma (1978) was really the same species as studied by us, it should be a great problem existing in a simple genetic phenomenon.

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