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On the hybrid tumor in the cross of *Drosophila melanogaster* females and *D. simulans* males.

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Drosophila melanogaster and *D. simulans* are quite similar in morphology, but they are reproductively isolated. When *D. melanogaster* females are crossed with *D. simulans* males, hybrid females appear and hybrid males die at the larval stage. This type of cross is generally more successful than the reciprocal cross of *D. simulans* females and *D. melanogaster* males, in which hybrid males eclose and hybrid females die at the embryonic stage. Survivors are sterile in the both reciprocal crosses (Sturtevant, 1920). Uenoyama and Inoue (1995) found the S2 line of *D. simulans*, an isofemale line from a natural population collected in l988 at Mishima, Japan, and females of this strain showed remarkably high mating success with *D. melanogaster* males. This is the mutant for premating reproductive isolation. The high crossability was caused by at least two genes that act additively, one on the second and one on the third chromosome, but the X chromosome showed no effect.

In the present study, the cross of *D. melanogaster* females and males of the *D. simulans* S2 line were analyzed. The wild strains used for *D. melanogaster* were Oregon-R (OR). For *D. simulans* the S-2 line and the yellow mutant y^{NS} line, being as the control, were used. The experiments were carried out as follows. Virgin females and males were collected within four hours after eclosion without anesthesia and aged separately for two days. Then each ten females and males were placed together in a 50 ml vial containing fresh medium. After two days the females were individually transferred to new vials and were allowed to lay eggs. The number of examined females [A] and number of females producing hybrid progenies [B] were examined, and the frequency [B/A] was calculated. A total of 253 females were analyzed in the present study. Table 1 shows the higher crossability of the *D. simulans* S-2 line to *D. melanogaster* by the frequency of females producing hybrid progenies at 24^{o}C. The S-2 line females showed the higher frequency of 0.696 to the *OR* males, whereas the y^{NS} line showed zero value. The progenies, being all males, were morphologically normal. These results confirmed the previous experiments (Uenoyama and Inoue, l995) in which the insemination ability was analyzed. Although the frequencies were much lower than the reciprocal cross, the S-2 line males also showed significantly higher frequency of 0.200 to the *OR* females in comparison with the y^{NS} line which showed zero value. Thus the S-2 lines showed the higher crossability in both reciprocal crosses. Moreover, the hybrid progenies were all females, which had frequently melanotic tumor on their legs when the S-2 line males were used for mating. The tumor was found around the connection part between femur and tibia (Figures 1 and 2).

In Table 2, pupa to adult lethality and frequency of hybrid pupae with melanotic tumor from crosses of the *OR* females and S-2 line males were examined by temperature shift experiment. The experiments were carried out as follows. To get the same developmental stage of the hybrids, each parental flies were placed in medium vials and were allowed to lay eggs in a short period of several

Figure 1. Hybrid progenies with a tumor from the cross between *OR* females and S-2 line males.

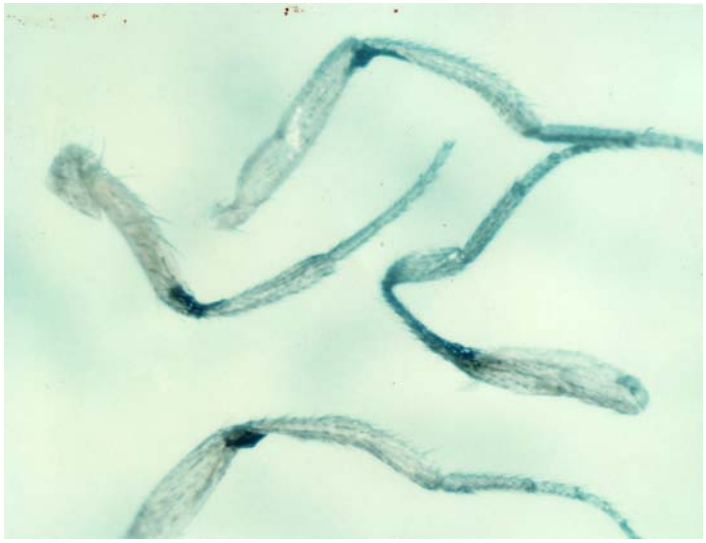

Figure 2. Tumors found around the connection part between femur and tibia of hybrid progenies between the *OR* females and S-2 line males.

hours. Temperatures were shifted at the following three stages during development; (A) early stage of the third instar larva, still being in medium surface, about 3 days of age; (B) late stage of the third instar larva, climbing glass wall just before pupation, about 5 days of age; and (C) two days after pupation, about 7 days of age. A total of 1606 pupae were analyzed. At 24^{o}C, the tumor frequency was high at 0.833 and lethality was 0.682, indicating that all dead progenies were not caused by melanotic tumor, and there were some survivors with melanotic tumor. Both lethality and tumor frequency were very low at 0.029 at 23^{o}C. The viable hybrid pupae were almost without tumor, and most dead pupae had tumor. Sturtevant (1929) reported that the hybrid females from the cross between *D. melanogaster* females and *D. simulans* males failed to emerge from puparium or died within a few hours after emergence at 25^{o}C. At 15^{o}C to 22^{o}C, they emerged normally and were fully viable. The borderline of the temperature sensitivity was found to be between 23^{o}C and 24^{o}C in the present study. Also the hybrid tumor seemed to have the same borderline of temperature sensitivity. Under shift down condition from 24^{o}C to 23^{o}C at early stage of the third instar larvae, all hybrid pupae were viable without tumor, indicating that the temperature sensitive period was after early

stage of the third instar larvae. Under shift up condition from 23°C to 24°C at the same developmental stage, tumor frequency was 0.586 and lethality was 0.224, both of which were unexpectedly lower in comparison with those of 24°C condition. The reason was unknown, probably being related to imperfection of the 24°C condition. More than half of tumor pupae seemed to be viable. On the other hand, the tumor frequency was 0.476 and lethality was 0.317 under shift down condition from 24°C to 23°C at late stage of the third instar larvae. These values were about half of the tumor frequency and lethality at 24°C condition. Under shift up from 23°C to 24°C at the same stage, tumor frequency was 0.390 and lethality was 0.309, both of which were slightly lower than the reverse shift condition. In addition, the tumor frequency and lethality were relatively high, at 0.645 and 0.667, respectively, under shift down from 24°C to 23°C at the stage of two days after pupation. Although the tumor frequency was somehow a little lower, lethality was almost the same as the case of the 24°C condition. In this case, all tumor pupae seemed to be dead. Under shift up from 23°C to 24°C at the same stage, the lethality was 0.130, being slightly higher, and tumor frequency was 0.037, being almost the same, in comparison with those of 23°C condition. In this case more than half of tumor pupae survived. These results indicated that the temperature sensitive period for lethality almost ceased at the stage of two days of pupation.

Table 1. The reciprocal crossability between *D. melanogaster* and *D. simulans* at 24° C condition.

Cross		No. of females examined [A]	No. of females producing progenies [B]	Frequency [A/B]
D. simulans female X *OR* male				
S-2	OR	58	40	0.696
y ^{NS}	OR	53	0	0
OR female X *D. simulans* male				
OR	S-2	65	13*	0.200
OR	*y* ^{NS}	77	0	0

* Most of hybrid progenies had melanotic tumor (see Table 2).

Table 2. Pupa to adult lethality and tumor frequency in hybrids between *D. melanogaster OR* females and *D. simulans* S-2 line males under temperature shift experiment between 23° C and 24° C.

Temperature shift	No. of pupae examined [A]	No. of pupae with tumor [B]	Freq. of pupa with tumor [B/A]	No. of adults[C]	Pupa to adult lethality [1- C/A]
+++ (A)+++ (B) +++ (C) +++	466	388	0.833	148	0.682
=== (A)=== (B) === (C) ===	627	18	0.029	609	0.029
+++ (A) === (B) === (C) ===	40	0	0	40	0
=== (A) +++ (B) +++ (C) +++	58	34	0.586	45	0.224
+++ (A) +++ (B) === (C) ===	145	69	0.476	99	0.317
=== (A) === (B) +++ (C) +++	123	48	0.390	85	0.309
+++ (A) +++ (B) +++ (C) ===	93	60	0.645	31	0.667
=== (A) === (B) === (C) +++	54	7	0.130	52	0.037

+++ : 24° C condition, and === : 23° C condition. (A) Early stage of the third instar larva, still being in medium surface, about 3 days of age. (B) Late stage of the third instar larva, climbing glass wall just before pupation, about 5 days of age. (C) Two days after pupation, about 7 days of age.

Thus the limited number of hybrid progenies emerged and were frequently accompanied with melanotic tumor at 24°C in the cross between the *OR* females and S-2 line males. The hybrid lethality and appearance of the hybrid tumor were strictly temperature sensitive; the 24°C condition was critical and 23°C was permissive. The sensitive developmental stages were found to be from the early third instar larvae to early pupa in both cases. Before and after the late third instar larva stage, both lethality and tumor seemed to appear in almost the same degree (Table 2). The present hybrid tumor seemed to be highly linked with reproductive isolation between these two sibling species, for the correlation coefficient between tumor frequency and hybrid lethality was calculated by the data of Table 2 to be 0.931, showing statistically significant positive correlation (d.f. = 6, $p < 0.01$).

References: Sturtevant, A.H., 1920, Genetics 5: 488-500; Sturtevant, A.H., 1929, Carnegie Inst. Wash. Publ. 399: 1-62; Uenoyama, T., and Y. Inoue 1995, Jpn. J. Genet. 70: 365-371.

Availability of the University of Texas Publications Dealing with *Drosophila*

Marshall R. Wheeler

From 1940 to 1972 many research articles were published by the University Press in the series, "Studies in the Genetics of *Drosophila*" with J.T. Patterson as editor and later (from 1957-1972) with M.R. Wheeler as editor. In 1960 the series title was changed to "Studies in Genetics." There were also a few special issues. Many of these are now out of print (OOP); all known copies of the remaining issues have been made available by Dr. Wheeler. The copies are available from the office of the Editor, *Drosophila Information Service*; contact Dr. James N. Thompson, jr., (jthompson@ou.edu) for details.

Some issues were given titles and subtitles, but the Publication Number (*e.g.*, UTP 4213) is the best reference. This is the complete list of all the publications:

1940: UTP 4032 (OOP). 1942: UTP 4213 (OOP). 1942: UTP 4228 (OOP). 1943: UTP 4313, "Drosophilidae of the Southwest" (OOP). 1944: UTP 4445, with "Drosophilidae of Mexico" (OOP). 1947: UTP 4720, "Isolating Mechanisms" (OOP). 1949: UTP 4920 (OOP). 1952: UTP 5204 (25 copies). 1954: UTP 5422 (OOP). 1957: UTP 5721 (45 copies). 1959: UTP 5914, "Biological Contributions." Dr. Patterson's 80th birthday issue (59 copies). 1960: UTP 6014 (16 copies). 1962: UTP 6205 (63 copies). 1966: UTP 6615, Morgan Centennial Issue (28 copies). 1968: UTP 6818 (24 copies). 1969: UTP 6918, W.S. Stone Memorial Issue (12 copies). 1971: UTP 7103 (22 copies). Final volume, 1972: UTP 7213 (29 copies).

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