The Number of Bristles and the Pairing of

The Chromosomes in Hybrids between

Drosophila melanogaster and Drosophila simulans

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INTRODUCTION

The hybrids of Drosophila melanogaster and D. simulans were found to have certain bristles missing from the body (Sturtevant 1920) that are normally present in both parent species. Sturtevant (1929a) found that the average number of bristles present (counting dorsocentrals) in these hybrids varies with the melanogaster stock used, and that the simulans male apparently produces no effect on bristle number. This suggested that the melanogaster egg cytoplasm might cause the effect, but a test gave negative results.

It was at Prof. Sturtevant's suggestion that I have tried to discover whether individual genes which caused the bristles to be missing from the hybrids could be identified in the melanogaster stocks.

I wish to express my appreciation to Prof. A. H. Sturtevant for his direction of the work, and to Prof. T. H. Morgan and Prof. Th. Dobzhansky for their helpful suggestions.

MATERIAL AND METHODS

The various melanogaster and simulans stocks which were used are listed alphabetically in tables 1 and 2. Stocks inbred for many generations were used when possible to insure a condition homozygous for any modifiers present. An attempt was made to control the whole chromosome, as far as possible, by using crossover-suppressors preventing the loss of modifying genes through crossing over in the female parent.

In making the crosses small mass-cultures were used consisting

TABLE 1
List of D. melanogaster stocks used in the experiments

Symbol	Meaning of symbol	Chromosome
$\dfrac{b\ gp\ a}{Cy\ R}$	black gap arc over the crossover-suppres- sor dp^2 Cy C_{IILCy} pr C_{IIR} 1 px sp	II
$\dfrac{Bl}{Cy\ sp}$	Bristle (1) over the crossover-**suppressor** Cy C_{IILCy} cn^2 C_{IIRCy} sp	II
$\dfrac{cg}{dp^2Cy}$	comb-gap (5) over the crossover- suppressor dp^2 Cy C_{IILCy} cn^2 C_{IIRCy}	II
ff b pr c se h	stock containing attached X chromosomes bearing forked, and homozygous for black purple curved and sepia hairy	I, II, III
$\dfrac{fr\ sp}{Cy}$	fringed speck over the crossover- suppressor Cy C_{IILCy} cn^2 C_{IIRCy}	II
$\dfrac{fu}{ClB}$	fused over the crossover-suppressor sc t^2 v C_I 1 sl B	I
$\dfrac{gt\ bb"}{ClB}$	giant (2) bobbed[11] over the crossover- suppressor ClB	I
$\dfrac{lz}{ClB}$	lozenge over the crossover-suppressor ClB	I
$\dfrac{pn^2}{ClB}$	stock containing prune[2] males and yellow females with attached X's	I
$\dfrac{px\ bw\ mr\ sp}{Cy}$	plexus brown morula speck over the crossover-**suppressor** Cy C_{IILCy} cn^2 C_{IIRCy}	II
$\dfrac{res}{C_{FL}\ C_{FR}}$	"res" over a crossover-suppressor (5)	III
res	homozygous stock: roughoid hairy thread scarlet peach curled stripe sooty	III
$\dfrac{res}{Df\ 2C\ ca}$	"res" over the crossover-suppressor 1 C_{IIILP} Df 1 C_{IIIRP} ca (3)	III
rucuca	homozygous stock: roughoid hairy thread scarlet curled stripe sooty claret	III
$\dfrac{s^2\ f}{ClB}$	sable[2] forked over the crossover- suppressor ClB	I
$\dfrac{sc\ cv\ v\ f\ bb^1}{ClB}$	scute crossveinless vermilion forked bobbed[1] over the crossover-suppressor ClB	I

TABLE 1 (cont)

Symbol	Meaning of Symbol	Chromosome
se h	homozygous stock; sepia hairy. There was also a stock $\dfrac{se\ h}{C_{FL}\ C_{FR}}$	III
st sr e^4 ro ca	homozygous stock: scarlet stripe ebony^4 rough claret. There was also a stock $\dfrac{st\ sr\ e^4\ ro\ ca}{C_{FL}\ C_{FR}}$	III
$\dfrac{st\ sr\ e^4\ ro\ ca}{C_{IIILP}\ C_{IIIRP}}$ [1]	scarlet stripe ebony^4 rough claret over a crossover-suppressor	III
$\dfrac{ta}{yy}$	stock containing twisted-abdomen males and yellow females with attached X's	I
thes	homozygous stock: thread scarlet peach curled stripe sooty. There were also stocks $\dfrac{thes}{C_{FL}\ C_{FR}}$ and $\dfrac{thes}{C_{IIILP}\ C_{IIIRP}}$ [1]	III
$\dfrac{v\ g^3\ sl\ f}{ClB}$	vermilion garnet^3 small-wing forked over the crossover-suppressor ClB	I
$\dfrac{y\ U\ cv\ v\ f}{sc\ cv\ v\ f}$	stock producing females with U-shaped X containing yellow crossveinless vermilion forked over scute crossveinless vermilion forked (4)	I
$\dfrac{y\ U\ cv\ v\ f}{sc\ ec\ ct^6\ g^2}$	stock producing females with y U cv v f over scute echinus cut^6 garnet^2	I
$\dfrac{y\ U\ cv\ v\ f}{y\ U\ ec\ ct^6\ g^2}$	stock producing females with y U cv v f over y U ec ct^6 g^2	I
$\dfrac{y\ U\ ec\ ct^6\ g^2}{sc\ ec\ ct^6\ g^2}$	stock producing females with y U ec ct^6 g^2 over sc ec ct^6 g^2	I

For a more complete description of the characters see Morgan, Bridges and Sturtevant 1925

(1) For Bristle, see King 1927
(2) For giant, see Morgan, Sturtevant and Bridges 1927
(3) For Df 2C ca, see Morgan, Sturtevant and Bridges 1928
(4) For y U, see L. V. Morgan 1926
(5) No published data for cg or $C_{FL}\ C_{FR}$.

TABLE 2

List of D. simulans stocks used

Symbol	Meaning of Symbol	Chromosome
b	homozygous stock: black	II
g bb	homozygous stock: garnet bobbed	I
Metz Fla. y	homozygous stock: yellow collected in Florida by C. W. Metz	I
Mrstwn.	wild stock from Morristown, New Jersey	---
N. Orl.	wild stock from New Orleans, Louisiana	---
Pas.	wild stock from Pasadena, California (1)	---
se	homozygous stock: sepia	III
St. Aug.	wild stock from St. Augustine, Florida	---
st	homozygous stock: scarlet	III
st p	homozygous stock: scarlet peach	III
y pn	homozygous stock: yellow prune	I
y w	homozygous stock: yellow white	I

For a more complete description of the characters and the stocks see Sturtevant 1929a

(1) No published data for this stock.

of 4 or 5 melanogaster females with 8 or 10 simulans males, or 8 to 10 simulans females with 4 or 5 melanogaster males. When the combination was made in this way the cross of simulans ♀ by melanogaster ♂ caused less difficulty than had been experienced previously by Sturtevant, Morgan, Bonnier and others. The more frequent failure of the latter combination is due to the lower viability of simulans flies in wet and acid food conditions. The females may become weakened and die before laying many eggs or even before mating. To overcome this difficulty the flies were mated and kept in vials with a small amount of food for one day before transferring them to the culture bottles. The bottles were kept in the incubator, set at 25.5°C., until pupae had formed. The parents were then removed and the bottles kept at room temperature (20-22°C.).

The chance that non-virgin females may be present increases with the number of females used in a mating. Since a large number of simulans females were used in each cross greater care was required in selecting virgins. There was the added difficulty that simulans females are sometimes fertilized a few hours after emerging (Morgan 1929). Hybrid cultures generally consist of only one sex (see Sturtevant 1920). This served as a check, but in addition all the crosses were made in such a way that flies which were not hybrids could be detected immediately by other characteristics.

In counting the bristles the four dorsocentrals and four scutellars were considered. To save time in making and recording the counts it was decided to include flies with more than four of the eight bristles missing in the class of "four missing". This tended to lower the computed mean number of missing bristles in cases where many bristles were absent, but did not affect the cases where a smaller number were absent. If only four of the eight bristles had been considered the

low classes would have been **reduced** by approximately half and **the**
differences between them would hardly be apparent. The differences between
the various high classes are sufficient, as recorded, to distinguish them
from one another. Recording all eight bristles would make the differences
greater instead of less. In some of the later counts all the eight
bristles were recorded.

Preliminary crosses

It was first necessary to find various melanogaster stocks
that produced different bristle effects on the hybrids. Females from
stocks containing genes in the three large linkage groups were crossed to
males from various simulans stocks. In the case of the X and the second
chromosome it was possible to use stocks containing recessive genes as
well as a dominant gene with linked crossover-suppressors such as
$\frac{gt\ bb''}{Cl\ B}$ or $\frac{b\ gp\ a}{Cy\ R}$. Thus in the hybrids the effect on the bristles of each
of the homologous chromosomes could be determined by comparing the normal
hybrids with those showing the character due to the dominant gene. There
was no suitable third chromosome stock containing a dominant gene which
could be used since any gene affecting the bristles had to be avoided.
In this case stocks were used that contained a gene or genes corresponding
to the simulans genes. These melanogaster stocks were first balanced
against a third chromosome crossover-suppressor and then crossed to
homozygous simulans males. The hybrids consisted of two classes and the
effect of each chromosome could be observed.

The successful crosses using melanogaster stocks involving
the X, second and third chromosomes are shown in tables 3, 4, and 5 with
the mean number of bristles missing for each class. The columns headed
by B, Cy, st, etc. represent the hybrids which showed Bar eye, Curly
wing, scarlet eye, etc., but the actual melanogaster chromosome re-

TABLE 3

Preliminary crosses involving chromosome I

Melanogaster ♀	Simulans ♂	Number of Flies	Mean number of bristles missing	
			B	+
$\dfrac{fu}{ClB}$	N. Orl.	125	1.09	.85
$\dfrac{gt\ bb''}{ClB}$	Mrstwn.	31	.00	.52
$\dfrac{gt\ bb''}{ClB}$	St. Aug.	361	2.11	2.47
$\dfrac{gt\ bb''}{ClB}$	se	284	.31	.47
$\dfrac{lz^4}{ClB}$	N. Orl.	838	1.02	.73
$\dfrac{lz^4}{ClB}$	St. Aug.	40	2.72	2.68
$\dfrac{s^2\ f}{ClB}$	se	282	.25	.18

TABLE 4

Preliminary crosses involving chromosome II

Melanogaster ♀	Simulans ♂	Number of Flies	Mean number of bristles missing	
			Cy	+
$\dfrac{b\ gp\ a}{Cy\ R}$	Mrstwn.	58	1.00	.80
$\dfrac{b\ gp\ a}{Cy\ R}$	N. Orl.	460	1.82	1.06
$\dfrac{cg}{dp^2\ Cy}$	N. Orl.	394	.73	.99
$\dfrac{fr\ sp}{Cy}$	Mrstwn.	433	.65	1.82
$\dfrac{px\ bw\ mr\ sp}{Cy}$	Mrwtwn.	363	.82	1.96

TABLE 5

Preliminary crosses involving chromosome III

Melanogaster ♀	Simulans ♂	Number of Flies	Mean number of bristles missing	
			st	+
$\dfrac{rucuca}{C_{FL}\ C_{FR}}$	st	328	.69	.82
$\dfrac{st\ sr\ e^4\ ro\ ca}{C_{IIILP}\ C_{IIIRP}\ ^1}$	st	418	.88	.50
$\dfrac{st\ sr\ e^4\ ro\ ca}{C_{FL}\ C_{FR}}$	st	147	1.86	.93
			st p	+
$\dfrac{thes}{C_{IIILP}\ C_{IIIRP}\ ^1}$	st p	200	.49	.25
$\dfrac{thes}{C_{FL}\ C_{FR}}$	st p	100	.94	.96
$\dfrac{res}{C_{FL}\ C_{FR}}$	st p	337	.86	.71
			se	+
$\dfrac{se\ h}{C_{FL}\ C_{FR}}$	se	910	.23	.33
se h	se	355	.28	--

sponsible for this is different in most cases as will be seen by examination of the stocks used in each cross.

It is evident that the same melanogaster stock gave different averages with the different simulans males used in the crosses. This had not been expected, and further tests were made that will be described below.

Crosses to test cytoplasmic differences
in melanogaster stocks

In order to test the effect of the cytoplasm on the bristles reciprocal matings were made between melanogaster stocks producing high and low numbers of missing bristles. Stocks involving ClB could not be used in this way since ClB males do not live. Two stocks were obtained which could be used reciprocally since the recessive genes present would not affect the viability of the males. Females having the constitution $\frac{y\ U\ cv\ v\ f}{sc\ c\bar{v}\ v\ f}$ and $\frac{y\ U\ ec\ ct^6\ g^2}{sc\ ec\ ct^6\ g^2}$ were crossed to males of several different simulans stocks but none of the cultures produced flies (see table 17). Many different numbers of males and females per culture were tried*, and the wings and legs of the females were cut in

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*Sturtevant (1915) has shown there may be fighting between the males if two court the same female.

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the hope of making mating easier, but all the cultures failed (see Sturtevant 1929a). It is probable that mating did not take place, since many eggs were laid but none hatched. Some of the females were dissected, but no sperm could be found in their receptacles. This does not, however, prove conclusively that no mating took place, since the females were at least nine days old and the sperm may have been used up after a possible mating, but males were still alive in the bottles when the females were

dissected and sperm should be found if a recent mating had occurred. Males of the constitution y U ec ct^6 g^2 were crossed to simulans females, but again all the matings failed.

Of the second chromosome stocks $\frac{cg}{dp^2 \ Cy}$ males were crossed with $\frac{gt \ bb''}{ClB}$ females and the $\frac{ClB}{+} \ \frac{dp^2 \ Cy}{+}$ F_1 females crossed with Morristown simulans males (table 6). The low values - much lower than those for either parent - are probably due to the loss or addition of modifiers through crossing the two stocks. The high average stock $\frac{b \ gp \ a}{Cy \ R}$ was crossed reciprocally with the low average stock $se \ h$ and the $\frac{Cy}{+} \ \frac{se \ h}{+}$ F_1 flies used in crosses with sepia simulans males and females.

$$\frac{b \ gp \ a}{Cy \ R} \ \female \ \times \ \frac{se \ h}{se \ h} \ \male \longrightarrow \frac{Cy}{+} \ \frac{se \ h}{+} \ \female \text{ and } \male \quad \textbf{(A)}$$

$$\frac{se \ h}{se \ h} \ \female \ \times \ \frac{b \ gp \ a}{Cy \ R} \ \male \longrightarrow \frac{Cy}{+} \ \frac{se \ h}{+} \ \female \text{ and } \male \quad \textbf{(B)}$$

The F_1 females (A) and (B) have exactly the same chromosome constitution, but (A) females came from eggs with Cy cytoplasm and (B) females came from eggs with se h cytoplasm. The males are alike except for the X and Y. The **(A)** males receive their X from the Cy stock and their Y from the se h stock; the **(B)** males receive their X from se h and their Y from Cy. The different Y chromosomes can be seen to have no effect on the bristles of the hybrids, since the classes from both (A) and (B) crosses show practically the same values (table 7). This table also shows the hybrids from (A) and (B) females crossed with se simulans males differ from each other slightly or not at all. If the cytoplasm of the Cy stock was responsible for the high number of missing bristles we would expect the hybrids from (A) to show a higher number missing than those from (B). The striking difference is that between the number of bristles missing in males compared with females and the separation of these values into four classes in case of the males.

TABLE 6

$$\frac{gt\ bb''}{ClB}\ ♀ \times \frac{cg}{dp^2\ Cy}\ ♂ \longrightarrow \frac{ClB}{+}\ \frac{dp^2\ Cy}{+}\ ♀\ mel.\ \times\ Mrstwn\ ♂\ sim$$

Culture	Number of bristles missing.															
	Cy B				Cy +				+ B				+ +			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
112	86	3	2	0	77	7	3	0	62	7	1	0	55	4	1	0
121	51	10	5	0	57	5	1	0	32	5	2	1	33	4	3	0
122	30	6	1	0	27	5	3	0	22	7	1	0	33	7	1	2
Total	167	19	8	0	161	17	7	0	116	19	4	1	121	15	5	2
Mean Values	.18				.17				.21				.22			

TABLE 7

Mean number of bristles missing in crosses between se sim and Cy se h mel.

Melanogaster parent	Simulans Parent									
	se ♂ producing ♀ hybrids					se ♀ producing mostly ♂ hybrids				
	Number of Flies	Cy se	Cy +	+ se	+ +	Cy se.	Cy +	+ se	+ +	Number of Flies
$\frac{Cy\ se\ h}{+\ +\ +}$ from (A)	3372	.25	.24	.18	.20	♂ 1.24 ♀ .56	1.62 .55	.39 .15	.63 .28	5078 379
$\frac{Cy\ se\ h}{+\ +\ +}$ from (B)	1731	.23	.18	.16	.17	1.29	2.01	.40	.66	2436
$\frac{Cy\ se\ h}{II\ +}$ from (D)	1099	.22	.31	.12	.19	1.64	2.45	.44	.83	2088

TABLE 8

Mean number of bristles missing using yy $\frac{Cy}{II}\ \frac{se\ h}{Df}$ ♀ mel. (C)

Simulans male used	Number of Flies.	Cy + .	Cy . Df	+ +	. + Df
se	589	2.97	3.12	1.21	1.72
N. Orl.	661	2.82	2.91	1.25	1.60
Pas.	652	2.95	3115	1.44	1.65
Mrstwn	679	1.49	1.52	.51	.57

This difference is shown to be due to the sex of the hybrid, since males and females which hatched from the same cultures (A σ x se simulans $\female$) gave decidedly different values (table 7).

The use of melanogaster attached-X stocks

Since the hybrid males have more bristles missing than the females it was decided to study them in particular and to compare the hybrid males obtained by using melanogaster attached-X stocks with hybrid males from simulans mothers. A melanogaster stock was made up as follows:

$$yy\ \female\ \text{from}\ \frac{pn^2}{yy}\ x\ \frac{Bl}{Cy\ sp}\ \sigma \longrightarrow yy\ \frac{Cy}{+}\ \circ$$

$$yy\ \frac{Cy}{+}\ \female\ x\ \frac{res}{Df\ 2C\ ca}\ \sigma \longrightarrow yy\ \frac{Cy}{+}\ \frac{Df}{+}\ \female$$

$$yy\ \frac{Cy}{+}\ \frac{Df}{+}\ \female\ x\ \frac{II}{II}\ \frac{se\ h}{se\ h}\ \sigma \longrightarrow yy\ \frac{Cy}{II}\ \frac{se\ h}{Df}\ \circ\ \text{and}\ \frac{Cy}{II}\ \frac{se\ h}{Df}\ \sigma\ (C)$$

The stock was kept by selecting the yy Cy Df females and crossing to se h males. Thus the stock contained the crossover-suppressor including Cy over the normal second chromosome from se h and the third chromosome from se h over the crossover-suppressor including Df. Males and females are alike except for the X-chromosome, but this does not enter into the constitution of the hybrids, since only male hybrids will be produced when either males or females from this stock are crossed to simulans. Many male hybrids were obtained by using yy $\frac{Cy}{II}\ \frac{se\ h}{Df}$ females, but unfortunately the crosses (thirty-eight attempts) involving $\frac{Cy}{II}\ \frac{se\ h}{Df}$ males failed.

Table 8 shows the results of crosses between (C) females and males from four different simulans stocks. The hybrids from Morristown males are seen to give lower values than the others in all four classes. In this table the class labeled Cy+ corresponds to the Cy se class of table 7. Cy Df corresponds to the Cy+ class, and so on, since the Df chromosome now corresponds to the : third chromosome of table 7. Of

course sepia appeared in the hybrids from the cross with se simulans.

Another melanogaster stock was made up as follows:

$$\frac{Bl}{Cy\ sp}\ ♀\ x\ \frac{II}{II}\ \frac{se\ h}{se\ h}\ ♂ \longrightarrow \frac{Cy}{II}\ \frac{se\ h}{+}\ ♀\ and\ ♂\ (D)$$

These $\frac{Cy}{II}\ \frac{se\ h}{+}$ flies were like the yy $\frac{Cy}{II}\ \frac{se\ h}{Df}$ flies with regard
to the Y, to one third and to both second chromosomes. The male hybrids
from $\frac{Cy}{II}\ \frac{se\ h}{+}$ melanogaster males would differ from the hybrids from
yy $\frac{Cy}{II}\ \frac{se\ h}{Df}$ females only in the chromosome containing Df. They serve in
a partial comparison of male hybrids derived from eggs containing different
cytoplasm. Females from (D) were also compared with females from (A)
and (B) in table 7. Little difference was expected in the comparison
of the females, since they generally give low values with slight variations.
The males from (C) and (D) (tables 7 and 8) differ in regard to missing
bristles in each case except when Morristown male is crossed to (C)
female. Considering the first three crosses it is seen that the differ-
ence in each class is consistent. Since the values for (D) males are
with se simulans females, a comparison with the hybrids from (C) females
by se males alone would be more complete. Here the differences suggest
that something is active during development causing one group of hybrids
to have more bristles missing than the ohter. This comparison is not
exact, however, because the hybrids from (C) contain the Df chromosome
while the others contain the normal third chromosome. Considering the
four classes of hybrids produced in these crosses and comparing the ratio
of Df to se (2.42 to 2.11) with that of + to se (in the cross with (D):
1.65 to .99) it appears that any effect on the bristles by Df is in the
direction of reducing the number missing, thus reducing the differences
in our comparison of crosses with (C) and (D) instead of increasing them.

A more direct comparison was made by using the following stocks:

$$yy \; ♀ \; \text{from} \; \frac{pn^2}{yy} \; x \; \frac{b \; gp \; a}{Cy \; R} \; ♂ \longrightarrow yy \; \frac{Cy}{+} \; ♀ \; \text{and} \; \frac{Cy}{+} \; ♂ \; (E)$$

$$yy \; ♀ \; \text{from} \; \frac{ta}{yy} \; x \; \frac{b \; gp \; a}{Cy \; R} \; ♂ \longrightarrow yy \; \frac{Cy}{+} \; ♀ \; \text{and} \; \frac{Cy}{+} \; ♂ \; (F)$$

The males and females of (E) and of (F) have the same chromosome (autosome) constitution and when crossed with simulans females or males should produce only **male** hybrids. The hybrids will have different melanogaster Y, but this has been shown to have no effect on the bristles (see discussion page 10 and table 7). These hybrids should be exactly alike except that one type developed from simulans egg and the other from melanogaster egg. Any difference between the two types should be due to the effect of the egg cytoplasm on the development. The results of the crosses are shown in table 9. This table shows that in each case hybrids developing from melanogaster eggs had a higher number of bristles missing than hybrids of the corresponding class developing from simulans eggs. The chromosome constitution of the hybrids from the two crosses is identical.

Another test was made which shows a definite maternal effect on the bristles of the hybrids. An attached-X stock was made up in such a way that the male and female flies were known to have identical Y-chromosomes. A double yellow female from $\frac{pn^2}{yy}$ was mated to a $\frac{b \; gp \; a}{Cy \; R}$ male and an F_1 yy Cy female and Cy male crossed. An F_2 Cy male was then crossed back to an F_1 yy Cy female. This stock will be designated as (G).

$$\overline{XX}\underline{Y} \; x \; XY \; \frac{b \; gp \; a}{Cy \; R} \longrightarrow \overline{XX}\underline{Y} \; \frac{Cy}{+} \; x \; X\underline{Y} \; \frac{Cy}{+} \longrightarrow \overline{XX}\underline{Y} \; \frac{Cy}{+} + XY \; \frac{Cy}{+}$$

$$\overline{XX}Y \; \frac{Cy}{+} \; x \; XY \; \frac{Cy}{+} \longrightarrow \overline{XX}Y \; \frac{Cy}{+} + XY \; \frac{Cy}{+} \quad (G)$$

A similar stock was made up using a female from $\frac{ta}{yy}$ and will be designated as (H).

TABLE 9

Reciprocal crosses

Simulans stock	Melanogaster stock					
	yy Cy ♀ from (E)			Cy ♂ from (E)		
	Number of Flies	Mean number of missing bristles		Number of Flies	Mean number of missing bristles	
		Cy	+		Cy	+
yw ♂ - ♀	58	2.70	1.50	135	2.51	.47
se ♂ - ♀	--	----	----	286	2.81	1.18
	yy Cy ♀ from (F)			Cy ♂ from (F)		
yw ♂ - ♀	146	3.50	1.65	207	2.10	.37
se ♂ - ♀	78	2.95	1.78	315	2.36	1.11
	yy Cy ♀ from (G)			Cy ♂ from (G)		
b ♂ - ♀	314	5.04 ± .17	3.52 ± .19	1693	2.65 ± .07	1.20 ± .05
	yy Cy ♀ from (H)			Cy ♂ from (H)		
b ♂ - ♀	583	4.32 ± .15	3.10 ± .12	1030	3.22 ± .10	1.07 ± .06

Reciprocal crosses were made between each of the stocks (G) and (H), and black simulans. The hybrids were examined and a record kept of all the dorsocentral and scutellar bristles instead of recording no more than four as was done previously. These results are included in table 9. The differences between classes are in the same direction as those found with stocks (E) and (F).

The Effect of melanogaster
cytoplasm.

The results of all the crosses in which the hybrids from reciprocal matings have their chromosome constitution identical or nearly so are arranged in table 10. The sex chromosomes are marked Xm for melanogaster X and Xs for simulans X. The Y comes from the melanogaster parent in each case. Cy indicates that the hybrids contained the melanogaster Curly chromosome, and + indicates the presence of the not-Curly chromosome. The other chromosomes are identical in each case and are not represented. The terms low, medium, high, etc. are purely arbitrary, based on the mean values shown.

The female hybrids developing from simulans eggs show a slightly higher mean value than those from the reciprocal cross. However, in other crosses using females from (A) values were obtained which approach .55 and in crosses with females from (D) values higher than this were obtained. (see table 19). The male hybrids derived from (C) were not identical with those derived from (D) since one parent contained the Df chromosome. As shown above the Df chromosome influenced the bristles even less than the homologous normal chromosome. However, the best comparisons for the males are those involving hybrids derived from (E), (F), (G), and (H). In (E) and (F) the two groups are identical in constitution except for the origin of the melanogaster Y as mentioned

TABLE 10.

Results from reciprocal crosses

Hybrids from melanogaster eggs			Hybrids from simulans eggs		
Melano-gaster ♀	Chromo-some compos-ition	Mean number of missing bristles	Melano-gaster ♂	Chromo-some compos-ition	Mean number of missing bristles
$\frac{Cy\ se\ h}{+\ +\ +}$ from (A)	Xm Xs Cy	very low .24	$\frac{Cy\ se\ h}{+\ +\ +}$ from (A)	Xm Xs Cy	low .55
	Xm Xs +	very low .19		Xm Xs +	very low .21
yy $\frac{Cy\ se\ h}{II\ Df}$ from (C)	Ym Xs Cy	high 2.97	$\frac{Cy\ se\ h}{II\ +}$ from (D)	Ym Xs Cy	medium 1.95
	Ym Xs +	medium 1.46		Ym Xs +	low .52
yy Cy from (E) and (F)	Ym Xs Cy	high 3.19	Cy from (E) and (F)	Ym Xs Cy	medium 2.48
	Ym Xs +	medium 1.65		Ym Xs +	low .89
yy Cy from (G) & (H)	Ym Xs Cy	very high 4.57	Cy from (G) and (H)	Ym Xs Cy	high 2.86
	Ym Xs +	high 3.25		Ym Xs +	medium 1.15

TABLE 11.

Comparison of males from reciprocal crosses

Melanogaster stock used	Type of Hybrid	♂ hybrids from mel. ♀	♂ hybrids from sim. ♀	Difference	Number times the probable error
G	Cy	5.04 ± .17	2.65 ± .07	2.39 ± .18	13.3
	+	3.52 ± .19	1.20 ± .05	2.32 ± .20	11.6
H	Cy	4.32 ± .15	3.22 ± .10	1.10 ± .18	6.1
	+	3.10 ± .12	1.07 ± .06	2.03 ± .13	15.6

above. The differences between the mean values 3.19 and 2.48, and the values 1.65 and .89 are certainly significant. In (G) and (H) the two groups of hybrids are identical in chromosome constitution since the stocks were made up in such a way that hybrids from both melanogaster males and females would contain the same chromosomes. Here the values for each class are higher, since all eight of the bristles were recorded. A comparison of these values with the difference between them is shown in table 11.

In each case the hybrids from a melanogaster mother have more bristles missing than those from a simulans mother. The difference between these classes range from 6.1 to 15.6 times the probable error.

In every cross producing males the hybrids which developed from melanogaster eggs showed a higher mean value than those which developed from simulans eggs. The females showed a difference in only one class and that in the opposite direction, but this disagreement has been explained above, partially at least.

Influence of simulans stocks

It has been mentioned above that different results were obtained when various simulans stocks were crossed to the same melanogaster stock.* While most of the simulans stocks gave nearly equal numbers of missing bristles one in particular (Morristown) gave a lower mean value. This difference is not easily detected in the female hybrids, but can be seen plainly in case of the males. Further crosses were made in order to compare male hybrids from various simulans stocks.

Males from **four** different simulans stocks were crossed with $yy\frac{Cy}{II}\frac{se\ h}{Df}$ females from (C) and the male hybrids compared. These values

*Sturtevant (1929a) did not find this difference possibly because the simulans stocks he used produced a medium effect and also because he dealt mainly with female hybrids.

are shown in table 8.

Males from eight different simulans stocks were crossed with yy females and from $\frac{pn^2}{yy}$ and $\frac{ta}{yy}$ melanogaster stocks. The results are shown in table 12. For comparison the actual numbers are shown for the cross of yy females from $\frac{ta}{yy}$ to the black and Morristown stocks (table 13).

The hybrids from simulans females also differ in the number of birstles missing according to the simulans stock used. Females from six simulans stocks crossed to $\frac{Cy\ se\ h}{II\ +}$ males from (D) produced male hybrids which differed from each other just as in the previous crosses. The results are given in table 14. This shows that the difference between the simulans stocks is due to the constitution of the stock. The presence in the stock of inhibitors or modifiers of some sort causes the hybrids to develop with different numbers of missing bristles.

A comparison of the simulans stocks is shown in table 15. The melanogaster parent is listed at the top of each column and the various simulans stocks are arranged in the order of their effect on the bristles of the hybrids. The stock causing the greatest effect means that one causing most bristles to be missing.

In each cross except one the values for the Morristown stock were the lowest. The difference in this one case may be due to the small number of hybrids which were obtained. When black was used in the crosses it produced the greatest effect in all except one case where a small number of hybrids from St. Augustine stock showed a higher value. The other stocks were distributed at random between the highest and lowest values which suggest that they are parctically equal in their effect on the hybrids. It is surprising that the black and Morristown stocks should differ so widely, since they are the most closely related of all the stocks used. The black mutation was found in the Morristown stock (Sturtevant 1929a) and was never outcrossed. Some change in one

TABLE 12.

Influence of simulans male on bristle number

Simulans male	$\bar{y}\bar{y}$ ♀ from $\frac{pn^2}{yy}$		yy ♀ from $\frac{ta}{yy}$	
	Number of flies	Mean number missing bristles	Number of flies	Mean number missing bristles
b	438	2.92	552	2.73
N. Orl.	870	2.26	770	1.93
se	528	2.28	1409	1.83
Pas.	948	1.81	634	1.89
St. Aug.	524	2.19	532	1.32
Mrstwn.	1424	.78	1358	.74
y w	----	----	118	2.53
y pn	----	----	173	1.38

TABLE 13.

Comparison of black and Morristown stocks

Simulans male	Number of cultures	Average-- flies per culture	Number of bristles missing					Mean value
			0	1	2	3	4	
b	4	138	72	56	80	83	261	2.73
Mrstwn.	10	136	791	282	164	76	45	.74

TABLE 14.

Influence of simulans female on bristle number
$\frac{Cy}{II} \frac{se\ h}{+}$ ♂ from (D) used in each case.

Simulans female.	Number of flies	Mean number missing bristles	
		Cy	+
St. Aug.	115	3.36	1.32
b	1270	2.70	.79
se	2088	2.06	.62
N. Orl.	844	1.84	.29
Pas.	347	1.60	.41
Mrstwn.	438	1.20	.15

TABLE 15.

Simulans stocks arranged in order of effect on the bristles of hybrids.

Effect on the bristles	$\dfrac{Cy \; se \; h}{II} \; \dfrac{\circ}{+}$		$\dfrac{Cy \; se \; h}{II} \; \dfrac{\sigma}{+}$		$yy \; \dfrac{Cy \; se \; h}{II} \; \dfrac{\circ}{Df}$		$yy \; \dfrac{\circ}{+}$ from $\dfrac{pn^2}{yy}$	$yy \; \dfrac{\circ}{+}$ from $\dfrac{ta}{yy}$
	Cy	+	Cy	+	Cy	+		
Greatest	b	b	St. Aug	St. Aug	Pas	Pas	b	b
	Mrstwn.	Mrstwn.	b	b	se	se	se	N. Orl.
	N. Orl.	N. Orl.	se	se	N. Orl.	N. Orl.	N. Orl.	N. Orl.
	se	se	N. Orl.	Pas	Mrstwn.	Mrstwn.	St. Aug	se
	Pas	Pas	Pas	N. Orl.	------	------	Pas	St. Aug
Least	------	------	Mrstwn.	Mrstwn.	------	------	Mrstwn.	Mrstwn.

TABLE 16.

Tests of Morristown stock

Type of cross	Mean number missing bristles.	
	· b	+
Mrstwn. $\circ$ x b σ -----o + σ sim. x ff b pr c se h $\circ$ mel.	2.59 ± .18	1.82 ± .14
b $\circ$ x Mrstwn σ -----o + σ sim. x ff b pr c se h $\circ$ mel.	3.82 ± .23	3.26 ± .19
Difference	1.23 ± .29	1.44 ± .24
	se	+
Mrstwn. $\circ$ x se σ -----o + σ sim. x ff b pr c se h $\circ$ mel.	2.10 ± .16	2.26 ± .19
se $\circ$ x Mrstwn. σ -----o + σ sim. x ff b pr c se h $\circ$ mel.	3.56 ± .18	3.48 ± .19
Difference	1.46 ± .24	1.22 ± .27

or both of the stocks must have taken place since then, or else the black mutation affects the bristles also. To test this pair matings were made of black simulans flies and the bristles counted. Of 391 offspring not one had less than four dorsocentral and four scutellar bristles, while some had extra scutellars. The mean number of bristles was $8.67 \pm .04$. It is probable that this excess of bristles is not directly related to the absence of them in the hybrids since here only the scutellars are duplicated, while in the hybrids both dorsocentrals and scutellars are decreased in number.

The morristown stock was then tested to see if a definite chromosome was the cause of the low bristle effect on the hybrids. It was compared with black to test the second chromosome and with sepia to test the third. A melanogaster stock (ffb pr c se h) was made up which had attached-X chromosomes bearing forked, and was homozygous for the second chromosome characters black, purple, curved and for the third chromosome characters sepia and hairy. Reciprocal matings were made between Morrostown and b and Morristown and se, and the F_1 males in each case crossed to ff b pr c se h melanogaster females. This allowed a comparison of the normal second chromosome of Morristown with the b chromosome in one case, and of the normal thrid chromosome of Morristown with the se chromosome in the other. The effect of the Morrixtown X was tested in both cases. In these crosses all eight of the bristles were recorded. The results are shown in table 16.

The third chromosome of the Morristown stock does not differ from the se chromosome in its effect on the bristles of the hybrids since the values for these two classes are equal. The second chromosome of the Morristown stock is very little different from the b chromosome, since the values are nearly equal. In one case the difference between the black

and normal classes is .77 ± .23 or 3.3 times the probable error while in
the other case it is only .56 ± .30 or 1.9 times the probable error.
But both these differences are in the same direction, so it is possible
that these two chromosomes differ slightly in their effect on the hybrids.
The greatest difference between the Morristown and b and se stocks is due
to the difference of the Morristown X chromosome. The values from the
reciprocal crosses differ in each class and show that the X chromosomes
of the b and se stocks cause more bristles to be missing in the hybrids
than the Morristown X chromosome. The differences are 4.2 and 6.0 times
the probable error for the b stock and 6.1 and 4.5 times the probable
error for the se stock.

Bristles of the parent species

Bristles were missing from some of the flies of every hybrid
culture, regardless of the stocks which were used in the cross. Some
melanogaster stocks gave higher numbers of missing bristles and some lower,
also different results were found to be due to the presence of different
chromosomes. The chromosome containing Curly produces a greater effect
than its homologue and sepia hairy chromosome seems to produce less effect
than its homologue. If specific genes acting on the bristles cause this
effect in hybrids, it would be suspected that the Curly chromosome contains
more of these, or else certain ones producing greater effect than those
in the sepia hairy chromosome. In that case the Curly chromosome might
be expected to affect the bristles in melanogaster cultures. To test this
$\frac{Bl}{Cy\ sp}$ melanogaster females were mated to normal melanogaster males and
683 offspring examined. The mean number of bristles present for curly
flies was 8.01 ± .001 and for normal flies was 8.02 ± .008. Only one
fly had less than eight bristles and in that case it looked as though

the bristle had been present, but was broken off. Homozygous stock of
sepia hairy would be expected to have some of the bristles absent, since
it would be homozygous also for the genes causing the missing bristles in
the hybrids. The bristles of 304 sepia hairy flies (from pair matings)
were examined and the mean number of bristles present was found to be
8.64 $\pm$ .04. Not one fly had less than eight bristles and none had extra
dorsocentrals. The extra bristles were scutellars and in most cases
anterior scutellars. Duplication of these bristles was found to be one
manifestation of the gene for hairy (Mohr 1922). The black simulans
stock was tested in the same way, and the mean number of bristles present
was found to be 8.67 $\pm$ .04. The cause of the extra bristles in this
stock is not known. The black simulans stock showed the greatest number
of bristles missing from the hybrids.

If specific genes cause missing bristles in the hybrids they do
not produce a noticeable effect on melanogaster flies either when in
heterozygous or in homozygous condition. The simulans flies which were
examined had extra bristles, but this is believed to be due to some other
cause not related to the hybrid effect.

Success of Crosses

It is easier to make the cross using melanogaster females than
when simulans females are used. This had been found previously by Sturte-
vant, Morgan and others, but the difficulty with simulans females was not
as great in this work as in their experiments. This may have been due to
the methods that were used. Sturtevant (1929a) and Morgan (1929) expressed
the opinion that the yellow stocks gave a greater percent of successful
crosses since the females were less vigorous and offered less opposition
to mating. In the present experiment the yellow simulans stocks gave poor
results and the yellow melanogaster stocks gave about the average number

of successful matings. Table 17 shows the percent of matings with melanogaster females which produced hybrids. Only those stocks are included which were crossed with two or more simulans stocks.

In cases where the cross was one hundred percent successful no more than four or five crosses were attempted, but in some cases as many as fifty unsuccessful attempts at crossing were made. The figure for all the crosses, 44%, is really not a true index since an equal number of attempts at each cross was not made. The values range from 0% to 86% for the simulans males, and from 0% to 100% for the melanogaster females. The stock which gave 100% included only six cultures; the next highest value is 68%.

Table 18 shows the percent of matings with simulans females which produced hybrids. Here the values range from 8% to 100% for the simulans females and from 0% to 83% for the melanogaster males. The figure for all crosses, 45%, is again not a ture index as to the success of the matings. It must be remembered than an average of ten simulans females were used for each culture, while only five melanogaster females were used in the crosses.

Considering all the matings the black simulans stock was found to give the highest percent of successful crosses, 79%. The New Orleans stock came next with 68%. In the melanogaster stocks yy Cy from (F) was first with 70% and $\frac{Cy\ se\ h}{+\ \ +}$ from (B) second with 67% of successful crosses.

A summary of most of the crosses with the mean number of bristles missing is given in table 19.

Table 20 shows the number of hybrids produced by all the crosses. the 41 males produced by regular melanogaster females were due to non-disjunction in females containing ClB. The 3 females produced by attached-X melanogaster females were due to separation of the attached-X's in the mother.

TABLE 17.

Percent of matings which produced hybrids from melanogaster females

Sim stock ♂	Cy se h (A)&(B) / + + / +	Cy se h (D) / + / II	yy Cy se h (C) / II Df	yy ♀ from pn2 / yy	yy ♀ from ta / yy	y U cv v f / sc cv v f	y U ec ct6 g2 / sc ec ct6 g2	sc ec ct6 g2 / y U cv v f	yy Cy (E)	yy Cy (F)	gt bb" / ClB	lz / ClB	s2 f / ClB	v g3 sl f / ClB	b gp a / Cy R	Total percent
se	.53	.44	.64	.29	.67	.00	.00	---	.00	.25	.60	---	.40	.00	.00	.48
Mrstwn	.67	.60	1.00	.83	.77	.00	.00	---	---	---	.40	---	---	---	.25	.63
N.Orl.	---	1.00	.67	.80	.67	---	---	---	---	---	---	1.00	---	---	1.00	.80
St.Aug	1.00	---	---	.83	1.00	---	---	---	---	---	.50	1.00	---	---	---	.86
Pas.	---	.64	.59	.67	.67	---	---	---	---	---	---	---	---	---	---	.63
b	---	.88	---	.89	.60	---	---	---	.46	.90	---	---	---	---	---	.72
yw	---	---	---	.00	.16	.00	.00	.00	.25	.50	.00	---	.00	.00	---	.05
y pn	---	---	---	.00	.33	.00	.00	.00	.00	.00	---	---	---	---	---	.04
Metz Fla y	---	---	---	---	---	.00	.00	---	---	---	---	---	---	---	---	.00
Total %	.58	.68	.67	.50	.56	.00	.00	.00	.29	.60	.57	1.00	.18	.00	.29	.44

TABLE 18.

Percent matings which produced hybrids from simulans females

Simulans stock ♀	Cy se h (A)&(B) / + + / +	Cy se h (D) / + / II	Cy se h (C) / II Df	pn2	ta	y U ec ct6 g2	Cy (E)	Cy (F)	gt bb"	g gp a / Cy R	Total percent
se	.84	.80	.00	---	.00	.00	.75	1.00	---	.57	.67
Mrstwn.	.20	.45	.00	.25	.00	---	---	---	.75	.00	.21
N. Orl.	---	.67	.00	---	---	---	---	---	---	---	.38
St. Aug.	.00	.50	---	---	---	---	---	---	.50	---	.33
Pas.	---	.14	.00	.00	.00	---	---	---	---	---	.08
b	---	1.00	---	---	---	---	1.00	1.00	---	---	1.00
y w	---	---	---	.00	.00	.00	.80	.83	---	.00	.34
y pn	---	---	---	---	---	---	.50	.00	---	---	.25
Tot. %	.75	.47	.00	.09	.00	.00	.83	.82	.63	.22	.45

TABLE 19.

Mean number of bristles missing in hybrids between

Drosophila melanogaster and Drosophila simulans.

Simulans stock	Melanogaster stock											
	Cy se h ♀ / + + + from (A)(B)		Cy se h ♂ / + + + from (A)(B)		Cy se h ♀ / II + from (D)		Cy se h ♂ / II + from (D)		yy Cy se h / II Df ♀ from (C)		yy ♀ from pn²/yy	yy ♀ from ta/yy
	Cy ♀	+	Cy ♂	+	Cy ♀	+	Cy ♂	+	Cy ♂	+	♂	♂
se	.23	.18	♂1.50 / ♀.55	.52 / .21	.27	.15	2.06	.62	3.04	1.44	2.28	1.83
N. Orl.	----	----	----	----	.59	.23	1.84	.29	2.85	1.38	2.26	1.93
Pas.	----	----	----	----	.19	.07	1.60	.41	3.04	1.55	1.81	1.89
Mrstwn.	.40	.28	1.25	.13	.85	.45	1.20	.15	1.51	.54	.78	.74
St. Aug.	.36	.33	----	----	----	----	3.36	1.32	----	----	2.19	1.32
b	----	----	----	----	1.26	.46	2.70	.79	----	----	2.92	2.73
y w	----	----	----	----	----	----	----	----	----	----	----	2.53
y pn	----	----	----	----	----	----	----	----	----	----	----	1.38

TABLE 20.

Hybrids produced by all crosses

Type of cross	Female Hybrids	Male Hybrids	Totals
mel. ♀ x sim. ♂	20,388	41	20,429
XXY mel. ♀ x sim ♂	3	15,045	15,048
sim. ♀ x mel. ♂	437	16,870	17,307
Totals	20,828	31,956	52,784

Chromosomes of the hybrids

Pairing of the chromosomes in the Diptera was first found by Miss Stevens (1907, 1908). She stated (1908) that there is "pairing of chromosomes in cells somewhat removed from the sphere of the reduction process". Metz (1914, 1916 a, b) found this to be the case in many more species in addition to the ones studied by stevens. The pairing was uniform and complete except in occasional displacements of one or two pairs, and was found to be characteristic of somatic as well as germinal tissues.

In their studies on the chromosomes of the various species of Drosophila Metz and Moses (1923) placed melanogaster and simulans in the same group as having like chromosome figures in the female. The chromosomes of the males were thought to be similar also until Sturtevant (1929b) showed that the simulans male has a short rod-shaped Y chromosome instead of the hooked Y of melanogaster.

Bonnier (1924) studied the chromosomes of hybrids between melanogaster and simulans and in a drawing of one oogonial plate showed the chromosomes to be paired perfectly as in both parent species. Metz had suggested (1916a, p. 259) that "chromosome pairing is dependent upon the qualitative nature of the chromosomes, - and more specifically upon a qualitative (physico-chemical) similarity between associating members". From this Bonnier concluded (1924, p. 63) that "the same force within the chromosomes, which in melanogaster causes them to lie in pairs close beside each other, is likewise to be found in simulans". He objected to the view of Sturtevant (1921) that species differ from each other in many genes which have arisen through mutations of the same kind as those giving rise to different mutant races. He believed that pairing chromosomes in the hybrids served as proof that at least not many genes differed between the two species. Later he discussed species-differences again (Bonnier 1927) and suggested that two species may be different in a single

cellular structure other than the genes, that is transferred by the sperm as well as by the ovum.

The difficulty in studying the chromosomes of the hybrids has been to find enough material for study since the ovaries never develop to normal size and are sometimes so rudimentary as to be difficult to find. The work with males is even more difficult. The method used by Frolowa (1926), Stern (1929) and others of studying the nerve ganglia of the larvae gave a better opportunity to study the chromosome group of the hybrids. A good many large cells with distinct chromosome figures have been found, though they are not as numerous as in larvae from either pure species.

The method of fixation was that used by Stern (1929), Frolowa (1926, 1929) and others, and the sections cut at 5 micra were stained with gentian violet.

When the first chromosome figures of the hybrids were seen it was found that they gave little evidence of pairing. As more of them were found there was the impression that there was no definite arrangement in pairs. Since no work had been done on pairing of the chromosomes in nerve cells it was necessary to study these cells in both parent species in order to be sure that the same pairing occurs here as in the gonial cells.

Larvae of each species were dissected and the nerve cells examined. All cells in which the chromosomes could be seen distinctly were counted and the chromosome groups examined. Each limb of the V-shaped chromosomes was considered separately so that a cell with perfect pairing would be given a score of six: two for each V-shaped chromosome pair and one each for the sex chromosomes and IV chromosomes.

The results of this comparison are shown in figures 1 and 2. Figure 1 shows the curves for pairing of all chromosomes of the hybrids

and parent species. Figure 2 shows the curves with the sex chromosomes omitted from the count since the X and Y chromosomes might not be expected to pair. These results are from 15 cells of hybrids (all ♂ from yy mel. ♀), 12 cells of melanogaster (4 ♀, 8 ♂) and 50 cells of simulans (males and females were not distinguished).

In both figures the curve for the hybrids is seen to resemble a normal probability curve, while those for the pure species are very asymmetrical and reach the highest point at complete pairing. It must be rememberd, in case of the hybrids, that when one limb of one V- shaped chromosome was beside that of another it was considered pairing of homologous limbs of identical chromosomes. Since the second and third chromosomes have not been identified in simulans it was impossible to distinguish them in the hybrids.

These results show that in ganglion cells of the hybrids the chromosomes are not paired beyond what might be epxected through random arrangement. The percent of pairing when the sex chromosomes are omitted is 48% in hybrids, 81.7% in normal melanogaster and 87.2% in normal simulans. There is stronger pairing of chromosomes in oögonia of the pure species, but statistical study of these has been made, that the agents that bring about pairing in the two parent species are different and do not cause pairing of the chromosomes in the hybrids.

Discussion of the results

The effect on the bristles cannot be due to a weakened condition of the hybrids because flies with missing bristles do not necessarily show other developmental abnormalities and abnormal flies may have all bristles present. Many females appear to be abnormal in one way or another, but few bristles are missing. Males generally have an normal appearance, but have many bristles missing. The females often have rough
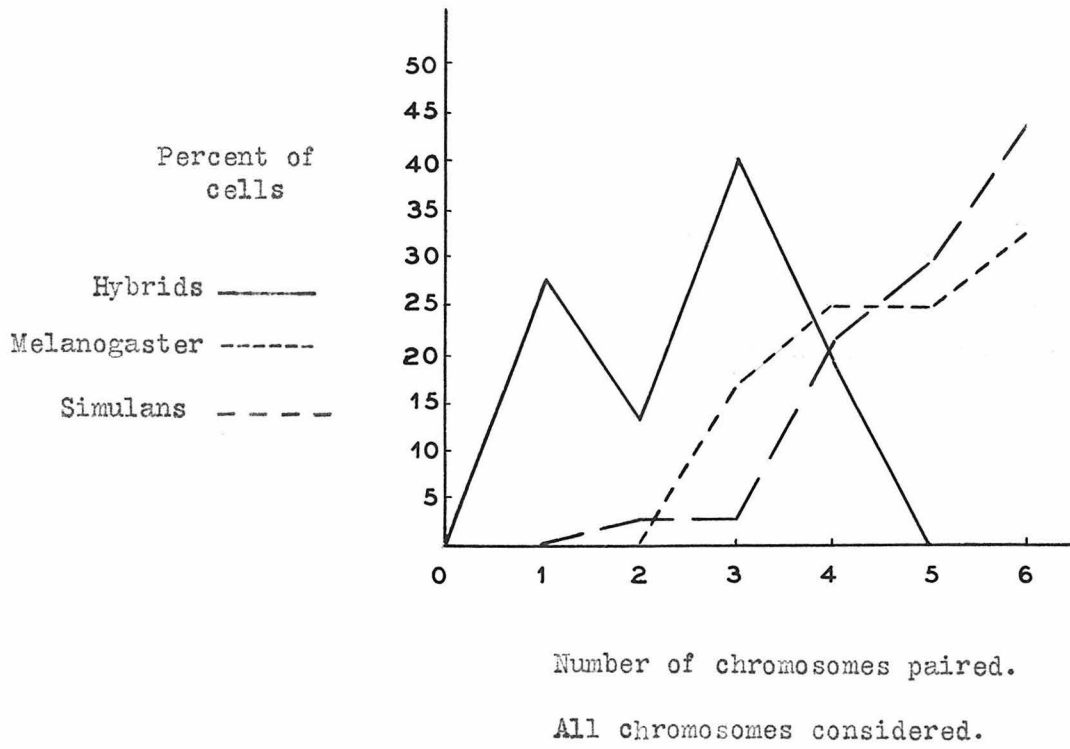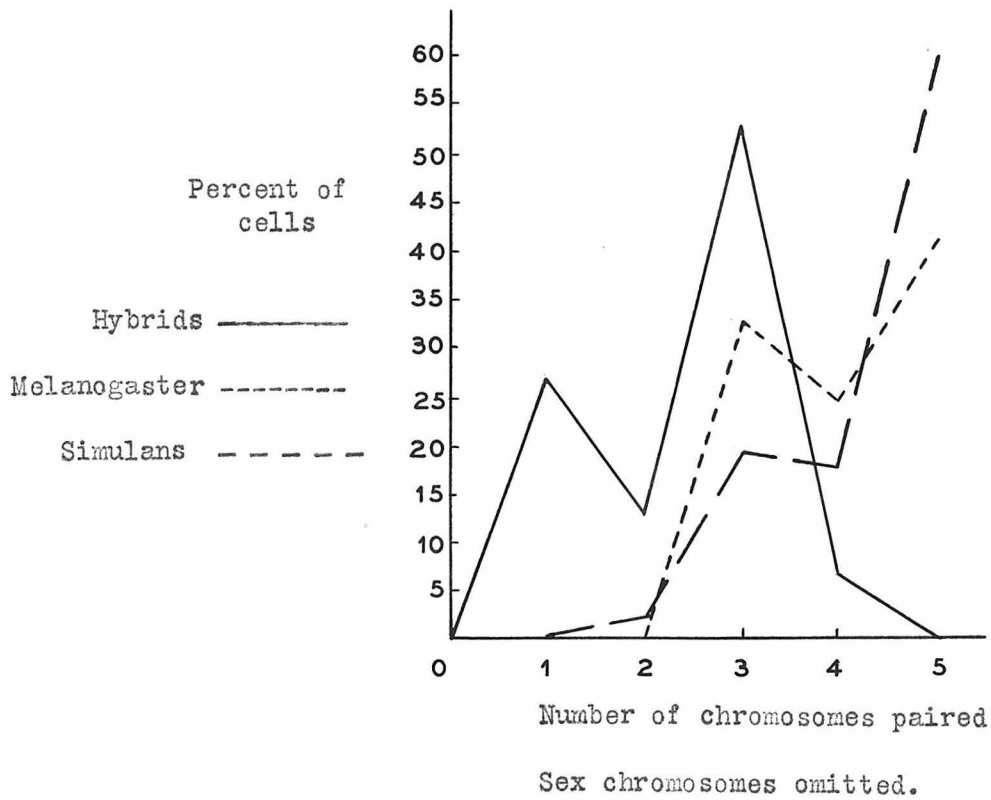
FIGURE 1.

Percent of
cells

Hybrids ———

Melanogaster ------

Simulans — — —

Number of chromosomes paired.

All chromosomes considered.

FIGURE 2.

Percent of
cells

Hybrids ———

Melanogaster --------

Simulans — — —

Number of chromosomes paired

Sex chromosomes omitted.

eyes (especially those from certain simulans stocks) while the eyes of
the males are normal or practically so. Females hatching from the same
culture with males may have rough eyes and the eyes of the males appear
perfectly normal. Under conditions of high temperature (29°C) female hybrids
rarely develop past the pupal stage, while males emerge and appear normal.
Males emerging at 29° have fewer bristles missing than those emerging at
22° C. The abdomen of the females is more often abnormal than the abdomen
of the males, and flies with abnormal abdomens may have all the bristles
present.

The missing bristles are evidence of imperfect coördination of
the genes of the two species. Genetic differences between the species are
shown also in the manifestation in the hybrids of the dominant melanogaster
characters Bar and Lobe (Morgan 1929) and Delta (Sturtevant 1929a). In
respect to facet number Bar differs only slightly from normal eye in
the hybrids and Lobe cannot be distinguished at all. The larger eye of
simulans is probably responsible for part of this difference. The Delta
character due to the melanogaster gene is less extreme in the hybrids than
in pure melanogaster, and that due to the simulans Delta gene is more extreme
in the hybrids than in pure simulans. The failure of the hybrid chromo-
somes to pair in somatic stages is taken as evidence of gene difference
between the chromosomes of the parent species. The inversion of part
or the right limb of chromosome III of simulans (Sturtevant and Plunkett
1926, Sturtevant 1929a) compared with melanogaster, would tend to prevent
pairing of that limb. It is possible that other shorter inversions may
also be present.

In general hybrids from reciprocal crosses between species do
not differ from each other, but in some cases they do and the difference
has sometimes been attributed to the effect of the cytoplasm of the egg.
Difficulties often encountered in cross-mating land animales are not met

with in many marine forms in which the eggs and sperm can be brought together outside the body. These forms have been largely used in hybridization experiments. The earlier experiments involving reciprocal crosses were made with different species of echinoderms, especially sea-urchins. Since the hybrids did not develop very far, the results were confined to larval characters. Vernon, Driesch, Boveri, Tennent and others studied hybrids from many sea-urchin crosses and reported reciprocal larvae that resembled the female parent in several respects. Some of the early larval characters were ascribed to the cytoplasm of the egg. The sperm was not supposed to exert an immediate influence on these stages. A complete discussion of these experiments, with the conclusions that were reached, is given by Morgan (1927).

In adition to effects of this kind there have been found other conditions in the cross-fertilized eggs that are supposed to effect the characters of the embryo. Fro example, Baltzer (1910) discovered that some of the paternal chromosomes are eliminated at the first cleavage of the egg in the cross between Strongylocentrotus, Arbacia and Echinus females and Sphaerechinus males, but that chromosome distribution is normal in the reciprocal crosses. Elimination of one or more chromosomes was found by Tennent in crosses between Toxopneustes and Arbacia (1912) and Cidaris females and Lytechinus male (1922) and by Doncaster and Gray (1913) in crosses between species of Echinus. In each case the larvae developed abnormally and when maternal characters were present it was suggested that this is due to the elimination of one or more paternal chromosomes.

Crosses have also been made between closely related species of fishes and even between species of different sub-orders. Reciprocal crosses have given cases of intermediate and also matroclinous hybrids.

The mitotic divisions were studied in several cases to see if chromosomes were eliminated as in the sea-urchin. Morris (1914) working with hybrids between Fundulus and Ctenolabrus, and G. and P. Hertwig (1914), working with species of Gobius, Crenilabrus and Box, found abnormalities in the divisions with some chromosomes lagging behind, but reported no elimination of chromatin. The larvae often died early in development when the cross was made in one direction, but lived longer when the other cross was made. Pinney (1918) made a study of three reciprocal crosses between Ctenolabrus and Fundulus, Stentotomus and Menidia and compared the early mitotic divisions with the type of hybrid which developed. She reported that hybrids of maternal type may have normal as well as abnormal mitoses in early divisions and that hybrids with mitoses abnormal as well as "prevailing normal" may die during gastrulation before definite characters can be distinguished. She concluded that the cause of abnormal chromosome behavior in the hybrids is the real cause of abnormal development, but that normal chromosomal behavior in the early stages is not closely correlated with normal development. At least some of the reported "maternal in- heritance" must be due to something else besides irregular chromosome behavior in early mitoses.

Many reciprocal crosses have been attempted in Lepidoptera and most of the hybrids were found to be intermediate between the parent forms. In some cases they resembled one parent more than the other, Harrison (Harrison and Doncaster 1914) reported hybrids between species of the Bistoninae that showed "the superior influence of the male", but suggested no explanation of the fact. Others found hybrids that resembled the mother in greater or less degree, or that behaved differently when the cross was made in a particular way. In the cross Pygaera curtula female by P. anachoreta male Federley (1911) obtained almost cross-fertilization if mating occurred, but only a few hybrids reached the adult stage. In the

reciprocal cross it was easy to obtain matings, but only about 30 percent
of the eggs were fertilized. From these most of the mal s and some of the
females developed into adult hybrids. Bytinski-Salz and Günther (1930)
found that hybrids from crosses of Celerio porcellus female by C. euphorbia
and C. elpenor male and of C. galii female by C. euphorbia male developed
normally, but the reciprocal crosses produced adult males and an equal
number of female pupae that practically never developed further. The
chromosome numbers and divisions were the same in the two types of hybrids
as in the parent species. They concluded that the different results must
be due to differences in the egg cytoplasm or to a disturbance of the balance
between the paternal X and maternal Y chromosomes.

In crosses between races of Lymantria dispar Goldschmidt obtained
hybrids of various kinds. Many intersexes were found and these were
explained by assuming the presence of sex-determining factors outside of
the sex chromosomes. He at first thought (Goldschmidt 1916, 1917) that the
determiner for femaleness was inherited through the egg cytoplasm, but
later (1922) concluded it was located in the Y or W chromosome. Goldschmidt
(1924) also reported cytoplasmic inheritance in characters of caterpillars
from inter-racial crosses, but his results have been disputed by Kosminsky
(1929) who reached quite opposite conclusions. Kisminsky states (p. 59)
"The influence of the maternal or paternal cytoplasm cannot be convincingly
confirmed by Goldschmidt's experiments. It is very difficult to draw
definite conclusions from the facts which have been discussed by Goldschmidt
since he has not taken into consideration the factors for chitin color
was well as the circumstance that each "race" represents a mixture of
lines with different factors for chitin coloring. Nevertheless Gold-
schmidt's observations can be explained completely on the basis of my
results, while my principles explain some of these facts better than
Goldschmidt's hypotheses."

Toyama (1912, 1913) reported several characters in the silk-worm moth which seemed to show non-Mendelian inheritance. Since then maternal inheritance in the silkworm has been quite thoroughly discussed. Tanaka (1924) thinks true maternal inheritance can occur only in the cases of brown and white egg colors and voltinism. It is quite probable that the egg-color or serosa color is due only to the egg because the sperm has not had time to exert an influence on such early structures. Uda (1923) believes changes in voltinims are due to temperature effects. If there are definite characters for univoltinism and divoltinism he thinks the irregularity can still be due to the "time of action" of the sperm. In any case the characters depend on the chromosomes though there may be a delay of a generation in the production of the effects. Miss Pellew (1925) explains and discusses these results briefly and concludes there is some maternal influence.

A "prematuration" (maternal) effect was considered by Morgan (1912, 1915) as a cuase of the irregularities in the inheritance of rudimentary wing in Drosophila Melanogaster. Miss Lynch (1919) found this to be a cause of the partial sterility of homozygous rudimentary or fused females. Miss Redfield (1924, 1926) reported a sex-limited lethal effect which acted only when the mother was homozygous for a second chromosome gene. The females died chiefly in the egg stage due to the influence of the maternal genetic composition on the eggs, before they left the mother's body. Gabritchevsky and Bridges (1928) found an enhancer of giant which showed a maternal effect on the eggs of females homozygous for the enhancer. Sturtevant (1934) explains the inheritance of coiling in Limnaea as being due to the genetic complex of the unreduced egg regardless of the constitution of the offspring.

In the present work hybrids developing from eggs containing melanogaster cytoplams have more bristles missing than hybrids developing

from eggs with simulans cytoplasm, even though the chromosomes in each case are identical. This may be interpreted as meaning that the chromosomes in the melanogaster egg have produced some effect on the cytoplasm before fertilization. Development might then be slightly different from that of a similar gene complex developing in a simulans egg. Whether the cytoplasm itself (independently of the chromosomes) causes any difference in development cannot be tested, since the hybrids have been found to be completely sterile (Sturtevant 1920). Purely cytoplasmic inheritance is not probable, however, since the results show that the differences among the melanogaster stocks can all be accounted for in terms of chromosomes.

The failure of hybrids to show pairing chromosomes supports the view that the two species differ from each other in having many genes which have become changed, although not enough with striking somatic effects to change greatly the appearance of the species itself. This, of course, is based on the assumption that similarity of genes is responsible for pairing of the chromosomes in the parent species.

SUMMARY

1. In crosses between Drosophila melanogaster and D. simulans some of the hybrids have bristles missing that are present in both parents.

2. The melanogaster stocks differ in their effect on the bristles in the hybrids, but each stock is constant when crossed to a given simulans stock.

3. Male hybrids show the bristle effect more than do female hybrids, and males and females hatching from the same culture show this difference.

4. Male hybrids from attached-Xmelanogaster females were compared with male hybrids from simulans females. Hybrids from melanogaster mothers

differed from hybrids from simulans mothers in regard to the number of missing bristles, although the genetic constitution was identical. This difference is considered to be due to the effect of the chromosomes on the cytoplasm of the egg before the egg leaves the mother's body.

5. The simulans stocks differ in their effect on the hybrids. Black produced the greatest effect and Morristown the least. The other stocks which were tested gave generally the same values for missing bristles and are considered to be practically equal in their effects on the hybrids.

6. The difference between the black and Morristown stocks was found to be due chiefly to the X chromosomes of the two stocks.

7. No specific genes were identified which caused the bristles to be absent in the hybrids.

8. The chromosomes of the hybrids were examined to determine if somatic pairing could be observed. The nerve ganglia of the larvae were used for this purpose. The chromosomes showed no pairing beyond what would be expected through random arrangement.

9. The failure to pair is considered as a partial demonstration of gene differences between the chromosomes of the two species. This gene difference is considered as the cause of the missing bristles in the hybrids.

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