INTERCHROMOSOMAL CONTROL OF KARYOTYPE FITNESS IN THE TUMOROUS-HEAD STRAIN OF DROSOPHILA MELANOGASTER[1]

CHARLES M. WOOLF[2] AND BARBARA B. KNOWLES

Department of Zoology, Arizona State University, Tempe

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CHROMOSOMAL dimorphism exists among adult flies in the tumorous-head (*tu-h*) strain of *Drosophila melanogaster* (WOOLF and PHELPS 1960). Two types of third chromosome are present, symbolized 3A and 3B. Chromosome 3A contains the semidominant gene *tu-3* in the right arm which produces abnormal growths in the head region. Chromosome 3B contains *tu-3*, but in addition, the Payne inversion (*In(3L)P*) and the recessive gene for scarlet eyes (*st*) in the left arm. Penetrance of the *tu-3* gene is increased by a maternal effect which is controlled by a sex-linked recessive gene (*tu-1*). Even though chromosome 3B is homozygous lethal, over 80 percent of the flies in laboratory bottles and population cages are heterozygous for this chromosome. Heterokaryotypes (3A/3B) of both sexes are more viable than homokaryotypes (3A/3A), and female heterokaryotypes produce more offspring than female homokaryotypes. No such difference in productivity exists for males (WOOLF and CHURCH 1963). Investigations are now in progress to determine whether differential female productivity is a matter of fecundity, fertility, or both. LI (1963) has presented the equilibrium formulae for this situation when selection favors the heterozygote but acts differentially in the two sexes.

WOOLF and CHURCH (1963) pointed out the difficulty of maintaining a *tu-h* strain free of chromosome 3B. Although such strains can be synthesized, few flies are produced each generation and usually the strains must be discarded after one or more generations because of uncontrollable mold or bacteria. This is mainly attributable to the small number of offspring produced by female homokaryotypes. However, by outcrossing to laboratory stocks and introducing new genetic variability, successful strains can be obtained that are homozygous for *tu-1* and *tu-3*, but lack chromosome 3B. Several possibilities were proposed to account for this observation: (1) Productivity in the *tu-h* strain is influenced by heterozygosity per se. Outcrossing and recombination provides the level of heterozygosity normally maintained by the Payne inversion. (2) Reduced productivity of female homokaryotypes is due to homozygosity for a gene arrangement in the left arm of chromosome 3A. Outcrossing and crossing over replaces this segment. (3) Gene arrangements exist on chromosomes other than 3A and 3B which influence productivity. Outcrossing and recombination replaces these chromosomes.

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[2] Present address: Department of Genetics, University of Utah, Salt Lake City.

The objective of this investigation was to test these various possibilities. Further motivation for this investigation was the unique opportunity of studying the genetics of an important fitness character contributing to balanced chromosomal dimorphism among adults in a laboratory population.

EXPERIMENTAL PROCEDURE

All matings to test female productivity were made in 25 × 95 mm vials containing the standard cornmeal-yeast-agar-molasses (or sucrose) medium. One female was placed in a vial with three brown scarlet (*bw;st*) males or four *rucuca* (*ru h th st cu sr* e^s *ca*) males. All vials were kept at room temperature (about 25°C). The parents were removed on the fifth day and the counting of offspring took place on the 15th day. If scarlet offspring occurred, the female parent was classified as a heterokaryotype; if not, she was classified as a homokaryotype.

Nonparametric statistical tests were used to analyze the measurements since there was no assurance that the assumptions basic to a parametric test, such as normal distributions and equal variances, were fulfilled. The median and interquartile range were used as the measures of central tendency and variation, respectively (SNEDECOR 1959).

In those experiments where both chromosomes were segregating, more of the female parents were 3A/3B than 3A/3A. The vials were selected at random until the first one with no scarlet offspring (3A/3A parent) was observed. The progeny were then counted. The flies in the next vial showing scarlet offspring (3A/3B parent) were counted and used as a control. Many hours were usually required to complete the counts in one experiment. The paired measurements were obtained within a few minutes of each other. Since there is an increase of emergence from pupa cases with time, the Friedman two-way analysis of variance was utilized to correct for covariance between paired measurements. The comparison of data from different experiments was made by Kruskal-Wallace one-way analysis of variance. The statistics χ_r^2 and H, obtained from the Friedman and Kruskal-Wallace tests, respectively, are distributed approximately as chi-square (SIEGEL 1956).

Classifying the parents on the basis of a progeny test introduces a bias. Female heterokaryotypes are more productive than female homokaryotypes although each exhibits variability. If a 3A/3B female were to produce only four or five wild-type offspring, she would be incorrectly classified as 3A/3A. Including all low numbers in an analysis falsely magnifies the differential between karyotypes whereas excluding all low numbers minimizes the differential. Experience showed that including or excluding low numbers (less than ten) did not alter the decision to reject or accept the null hypothesis at the 0.05 level in any of the experiments. Low numbers were not omitted in the results presented here.

RESULTS

Differential female productivity in the tu-h *strain:* For comparative purposes, the productivity of female homokaryotypes and heterokaryotypes from the *tu-h* strain was studied. A total of 381 matings was made (one *tu-h* females × three *bw;st* males). Heterokaryotypes produced a median number of 105 offspring, while the median number produced by homokaryotypes was 16. The null hypothesis that these two samples come from the same population is rejected ($\chi_r^2 = 135$; $P < 0.001$). The results confirm those of WOOLF and CHURCH (1963) that female heterokaryotypes are more productive than homokaryotypes.

The effect on female productivity of heterozygosity for chromosomes 1 and 2: A strain of flies was synthesized that was segregating for chromosomes 3A and 3B as well as the Muller-5 (M-5) and Curly balancer (*Cy*) chromosomes. From this strain, females were selected that were (a) 1/1; 2/2, (b) M-5/1; 2/2, (c) 1/1;

Cy/2, and (d) M-5/1; *Cy*/2. Arabic numerals refer to chromosomes from the *tu-h* strain. Each female was mated with three *bw;st* males. The results are given in Table 1. It is clear that heterozygosity imposed by M-5 and *Cy* for the first and/or second chromosome, respectively, does not influence the differential productivity of the two karyotypes. The median number produced by the homokaryotypes in each experiment was low (range 5 to 8), while that for the heterokaryotypes was much higher (range 55 to 91). The results in the four experiments were consistent (heterogeneity $\chi^2 = 0.262$; 3 degrees of freedom; $0.98 > P > 0.95$). The comparison of medians from experiment to experiment is not too meaningful as the experiments were carried out at different times and laboratory variables had an influence. Modifications were being made in the medium during the time many of the experiments were in progress.

It can be concluded from these experiments that heterozygosity per se for chromosomes 1 and 2 does not alter female productivity.

The effect on female productivity of a modified 3A chromosome: Segments were substituted by crossing over into the left arm of chromosome 3A by first synthesizing females of the constitution: 1/1; 2/2; *ru h* D^3 *st ri InRC e* 13e/3A, and mating them to males with the same types of chromosomes. A male was selected from the progeny that was phenotypically roughoid (*ru*), hairy (*h*), and Dichaete (D^3). This male resulted from the union of a sperm containing *ru h* D^3 *st ri InRC e* 13e and an egg containing a modified 3A chromosome resulting from a crossover between *h* at position 26.5 and D^3 at position 40.4. The crossover was more likely closer to *h* than D^3 since the small Dichaete inversion reduces crossing over in its vicinity. This modified 3A chromosome with the end of the left arm replaced by a segment containing *ru* and *h* is symbolized *ru h* 3A. The male (*ru h* D^3 *st ri InRC e* 13e/*ru h* 3A) was then mated to *tu-h* females, and from the progeny non-Dichaete females were selected that were either *ru h* 3A/3A or *ru h*

TABLE 1

The effect of heterozygosity for the first chromosome and/or the second chromosome on female productivity

Mating	Total mated	Karyotype of female parent 3A/3A	3A/3B	Median number of offspring and (interquartile range) 3A/3A	3A/3B	χ^2_r
1 ♀ 1/1; 2/2 × 3 ♂ ♂ *bw;st*	228	9.5%	90.5%	5 (8)	83 (91)	14 (P<0.001)
1 ♀ M-5/1; 2/2 × 3 ♂ ♂ *bw;st*	230	13.1%	86.9%	8 (10)	55 (29)	31.21 (P<0.001)
1 ♀ 1/1; *Cy*/2 × 3 ♂ ♂ *bw;st*	200	23.3%	76.7%	8 (7)	91 (60)	30.12 (P<0.001)
1 ♀ M-5/1; *Cy*/2 × 3 ♂ ♂ *bw;st*	234	18.6%	81.4%	6 (13)	71 (52)	41.14 (P<0.001)

3A/3B. These females were then mated singly to three *bw;st* males. The results are shown in Table 2. While a large differential productivity existed between the two karyotypes in the controls, which was expected (3A/3A median = 16, 3A/3B median = 72.5), this was not the case when a modified 3A chromosome was present. The *ru h* 3A/3A females produced a median number of 60 offspring, whereas the median number produced by *ru h* 3A/3B females was 69. Even though the hypothesis that the two samples come from the same population must be rejected ($\chi^2 = 6.94$; $P < 0.01$), it is clear that productivity is increased in *ru h* 3A/3A females. The null hypothesis is rejected ($H = 18.0$; $P < 0.001$) when the productivity of *ru h* 3A/3A females (median = 60) is compared with the control 3A/3A females (median = 16) and accepted ($H = 0.49$; $0.50 > P > 0.30$) when the productivity of *ru h* 3A/3B females (median = 69) is compared with the control 3A/3B females (median = 72.5).

As a follow-up experiment, *tu-h* females were mated with *ru h* 3A/3A males. Four types of progeny occurred, all phenotypically wild type: 3A/3A, *ru h* 3A/3A, 3A/3B, and *ru h* 3A/3B. These females were mated individually with *rucuca* males. On the basis of the phenotypes occurring in the offspring (i.e., wild type, roughoid-hairy, scarlet, roughoid-hairy-scarlet), the female parents were classified as to the chromosomes they carried. The median numbers of offspring (Table 3) produced by the four types of females were: 3A/3A, 24; *ru h* 3A/3A, 47; 3A/3B, 57.5; *ru h* 3A/3B, 64. The 3A/3A females were the least productive and, importantly, they were significantly less productive than the *ru h* 3A/3A females ($\chi^2_r = 11.0$; $P < 0.001$). The null hypothesis of no difference is accepted when *ru h* 3A/3B and 3A/3B females are compared ($\chi^2_r = 0.02$; $0.90 > P > 0.80$). Females may be ranked as to productivity in the following ascending order: 3A/3A, *ru h* 3A/3A, and 3A/3B. The results are compatible with the hypothesis that genes

TABLE 2

A comparison of the productivity of females containing the ru h *3A chromosome with females from the tumorous-head strain*

Mating	Total matings	Median number of offspring and (interquartile range)	H
1 ♀ *ru h* 3A/3A × 3 ♂♂ *bw;st*	83	60 (42)	18 ($P<0.001$)
1 ♀ 3A/3A × 3 ♂♂ *bw;st*	28	16 (30)	
1 ♀ *ru h* 3A/3B × 3 ♂♂ *bw;st*	110	69 (41)	0.49 ($0.50>P>0.30$)
1 ♀ 3A/3B × 3 ♂♂ *bw;st*	65	72.5 (39)	

TABLE 3

A comparative study of the productivity of the wild-type progeny from the cross tu h *females* × ru h *3A/3A males*

Mating	Total matings	Median number of offspring and (interquartile range)	χ^2_r
1 ♀ *ru h* 3A/3A × 4 ♂ ♂ *rucuca*	61	47 (29)	11.0 (P<0.001)
1 ♀ 3A/3A × 4 ♂ ♂ *rucuca*	47	24 (30)	
1 ♀ *ru h* 3A/3B × 4 ♂ ♂ *rucuca*	61	64 (44)	0.02 (0.90>P>0.80)
1 ♀ 3A/3B × 4 ♂ ♂ *rucuca*	60	57.5 (51)	

reducing productivity are distributed along the left arm of chromosome 3A. Chromosome *ru h* 3A covers the genes less well than chromosome 3B, although coadaptation between chromosomes 3A and 3B cannot be ruled out.

Comparison of the effect on productivity of ru *3A*, h *3A, and* ru h *3A chromosomes:* Females and males of the constitution *ru h* 3A/3A were mated. Four different types of gametes were produced by the females: noncrossovers *ru h* 3A and 3A, and crossovers *ru* 3A and *h* 3A. From the offspring, wild-type females were selected and mated to *rucuca* males. The female parent was classified as 3A/3A, *h* 3A/3A, *ru* 3A/3A, and *ru h* 3A/3A, according to the progeny she produced. The *ru* 3A chromosome contains a smaller substituted segment in the left arm than the *ru h* 3A chromosome, while the *h* 3A chromosome has a segment, containing *h*, inserted into the left arm. Since each of these modified chromosomes arose by crossing over somewhere between *ru* and *h*, the exact boundaries of these segments are unknown. Each of the *ru* 3A and *h* 3A chromosomes was likely different, and because of possible double crossing over within the region, any two chromosomes labeled as *ru h* 3A, or 3A, could have different constitutions.

Even though the previous experiments suggested that genes reducing productivity might be distributed along the left arm of chromosome 3A, the results (Table 4) of the present experiment can be used as evidence for a primary region close to *ru*, near the tip of the left arm. A dichotomy occurs among the four medians: *h* 3A/3A, 30; 3A/3A, 33; *ru h* 3A/3A, 75.5; *ru* 3A/3A, 80. Females carrying the *ru* 3A chromosome were comparable in productivity, on the average, to those heterozygous for the *ru h* 3A chromosome with the larger substituted segment. Females heterozygous for the *h* 3A chromosome were no more productive, on the average, than 3A/3A females; however, *h* 3A/3A females were extremely variable as evidenced by a relatively large interquartile range. Variability is ex-

TABLE 4

A comparative study of the productivity of the wild-type female progeny from the cross ru h *3A/3A females* × ru h *3A/3A males*

Female parent × 4 *rucuca* males	Total matings	Median number of offspring and (interquartile range)	χ^2_r
3A/3A	36	33 (39)	0.00 (P = 1)
h 3A/3A	8	30 (75)	
ru 3A/3A	17	80 (91)	0.059 (0.90>P>0.80)
ru h 3A/3A	120	75.5 (54)	

pected if a primary region exists near the left end of the chromosome, since some of the *h* 3A chromosomes could include this region.

The effect on productivity of wild-type second chromosomes: Suggestive evidence for second chromosome involvement was the observation that heterokaryotypes are not too productive when the *tu-h* second chromosomes are replaced by *Cy/Pm* chromosomes (*Pm* = Plum eyes). From a total of 493 matings, the median number of offspring produced by 1/1; *Cy/Pm*; 3A/3A females was 15, while that for 1/1; *Cy/Pm*; 3A/3B females was only 19 ($\chi^2_r = 13.4$; $P < 0.001$). Reduction in productivity of heterokaryotypes was not observed when only the Curly chromosome was substituted (*Cy/2*), suggesting an interacting effect of the Plum chromosome.

A strain was then synthesized with wild-type second chromosomes by substituting second chromosomes from the laboratory stock *H³/In3RC Sb e* 13e for the *Cy/Pm* chromosomes. Females from this strain are symbolized: 1/1; +/+; 3A/3B and 1/1; +/+; 3A/3A. These females were mated individually to three *bw;st* males. The results (Table 5) demonstrate clearly that a genetic mechanism on the second chromosomes of the *tu-h* strain influences productivity. The productivity of the homokaryotypes is increased and the differential between karyotypes is reduced as shown by the following medians: 3A/3A, 50; 3A/3B, 63 ($\chi^2_r = 4.74$; $0.05 > P > 0.02$).

TABLE 5

The effect of wild-type second chromosomes on female productivity

Mating	Total mated	Karyotype of parent 3A/3A	Karyotype of parent 3A/3B	Median and number of offspring (interquartile range) 3A/3A	Median and number of offspring (interquartile range) 3A/3B	χ^2_r
1 ♀ 1/1; +/+; × 3 ♂ ♂ *bw;st*	242	25.8%	74.2%	50 (30)	63 (26)	4.74 (0.05>P>0.02)

DISCUSSION

The results of the present study provide experimental evidence that the fitness of flies carrying different chromosomal arrangements may be influenced not only by the gene arrangements on the involved chromosomes, but also by those on other chromosomes. Coadaptation of some inversion and translocation systems is thus a function of interacting genetic mechanisms involving different chromosomes.

Reduced productivity of female homokaryotypes in the *tu-h* strain is influenced by a genetic system involving, at least, the left arm of chromosome 3A and one or more regions on the second chromosome. Females homozygous for chromosome 3A produce few offspring in comparison to heterokaryotypes (3A/3B), if they also possess the second chromosomes (2/2) from the *tu-h* strain. Substituting wild-type second chromosomes increases productivity. Heterozygosity for the left arm of chromosome 3A imposed by using a modified 3A chromosome (*ru h* 3A) derived by crossing over, also increases productivity.

The genetic constitution of the second chromosomes from the *tu-h* strain is unknown. Heterozygosity for the Curly chromosome (*Cy*/2) does not increase the productivity of 3A/3A females, suggesting the action of a dominant gene, or a polygenic system with the threshold being reached with the presence of a single *tu-h* second chromosome. The nature of the genic system on the second chromosome is now under investigation.

Since the *ru h* 3A chromosome covers the 3A chromosome less well than the 3B chromosome, it is concluded that genes reducing productivity of female homokaryotypes are distributed along the left arm of chromosome 3A. Females of the constitution *ru h* 3A/3A are still homozygous for the proximal region of the left arm. Coadapted heterosis of 3A/3B types is an alternative explanation for the superiority of 3A/3B types; it is possible that both phenomena are operating. In all experiments, the most productive females were those heterozygous for chromosome 3B.

It is now understandable why a strain of homokaryotypes is difficult to maintain, but successful *tu-1*; *tu-3* strains, free of chromosome 3B, can be synthesized (WOOLF and CHURCH 1963). Outcrossing to laboratory stocks can bring about recombination for chromosome 2 and the left arm of chromosome 3A. In fact, substitution for the left arm of 3A is all that is needed, as evidenced by the observation that *ru h* 3A/*ru h* 3A and *ru* 3A/*ru* 3A strains succeed well in the laboratory.

The evolution of the complex genetic mechanism in the *tu-h* strain is an intriguing problem. The strain originated from a sample of a natural population made, in 1941, in Acahuizotla, Mexico, by investigators from the University of Texas (NEWBY 1949). In the spring of 1945 abnormal growths in the head region were first noticed and, in 1946, the strain was sent to the University of Utah. Since the Payne inversion (BRIDGES and BREHME 1944) and *tu-1* (GARDNER and GARDNER 1953) are found segregating in some natural populations, it is likely that both were present in the natural population at Acahuizotla, Mexico. The

tu-3 gene probably originated by mutation in the laboratory at the University of Texas. At the University of Utah, Utah State University, and Arizona State University, where research on the *tu-h* strain has been carried out, selection has been made for flies showing abnormal head growths. Modifiers are present in natural and laboratory populations that increase penetrance (GARDNER and GARDNER 1953). The penetrance is about 90 percent (25°C) in the strain used in these experiments at Arizona State University.

The abnormal growths, many of which appear to be homoeotic in nature, vary in size from a small protuberance, affecting a small area of the eye or antenna, to massive amorphous growths. A large upset in normal developmental processes brings about the expression. Only about 50 percent of the eggs develop into adults at 25°C, and only 6 percent at 30°C (GARDNER and RATTY 1952). At higher temperatures females are much less viable than males and the penetrance increases in both sexes (GARDNER and WOOLF 1950).

How can the gene complexes reducing the productivity of homokaryotypes be accounted for? Although chance fixation or inbreeding degeneration may have played a role, it is, perhaps, more likely that the system arose as a result of selection. Because of the detrimental action of the genes giving the tumorous-head abnormality, there would be strong natural selection for a genic system increasing viability. A working hypothesis is that some of the genes increasing viability (and perhaps penetrance) through pleiotropic action, also reduce female productivity. A major region, containing one or more of these genes, is near the tip of the left arm of chromosome 3A and chromosome 2 also contains one or more of these genes. In this complex interchromosomal system, reduced productivity of the female homokaryotypes is part of the segregation load, and thus the price the population pays for the genes leading to the tumorous-head trait. Investigations are now in progress to test this hypothesis.

SUMMARY

Two different types of third chromosomes (3A and 3B) are segregating in laboratory bottles and population cages of the tumorous-head strain of *Drosophila melanogaster*. Chromosome 3B, containing the Payne inversion, is homozygous lethal. Selection favors the heterozygote, but operates differentially in the two sexes. Female heterokaryotypes (3A/3B) produce significantly more offspring than female homokaryotypes (3A/3A). No such difference exists between the two karyotypes in males.

Experiments were designed to investigate the genetic mechanisms leading to reduced productivity of female homokaryotypes. Heterozygosity per se for chromosomes 1 and 2 does not increase productivity. However, productivity is increased in the absence of chromosome 3B if the second chromosomes are replaced by wild-type chromosomes, or if by crossing over a segment is substituted into the left arm of chromosome 3A. It is concluded that reduced productivity of female homokaryotypes is determined by an interchromosomal effect. Experimental evidence is, therefore, available that coadaptation of an inversion system

in Drosophila is a function of interacting genetic mechanisms involving different chromosomes.

LITERATURE CITED

BRIDGES, C. B., and K. S. BREHME, 1944 The mutants of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **522**.

GARDNER, E. J., and M. D. GARDNER, 1953 Further evidence for maternal effect and modifiers of tumorous head genes in natural populations of *Drosophila melanogaster*. Cancer Res. **13**: 689–693.

GARDNER, E. J., and F. J. RATTY, 1952 Penetrance and expressivity of tumorous head in *Drosophila melanogaster* and relative viability of flies carrying tumorous head genes. Genetics **37**: 49–61.

GARDNER, E. J., and C. M. WOOLF, 1950 The influence of high and low temperatures on the expression of tumorous head in *Drosophila melanogaster*. Genetics **35**: 44–55.

LI, C. C., 1963 Equilibrium under differential selection in the sexes. Evolution (in press).

NEWBY, W. W., 1949 Abnormal growth on the head of *Drosophila melanogaster*, J. Morphol. **85**: 177–196.

SIEGEL, S., 1956 *Nonparametric Statistics for the Behavioral Sciences*. McGraw-Hill, New York.

SNEDECOR, G. W., 1959 *Statistical Methods*. 5th edition. Iowa State College Press, Ames.

WOOLF, C. M., and K. CHURCH, 1963 Studies on the advantage of the heterokaryotypes in the tumorous head strain of *Drosophila melanogaster*. Evolution (in press).

WOOLF, C. M., and L. J. PHELPS, 1960 Chromosomal polymorphism in the tumorous head strain of *Drosophila melanogaster*. Science **132**: 1256–1257.