V. ADDITIONAL TESTS WITHIN THE QUINARIA SPECIES-GROUP OF DROSOPHILA

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INTRODUCTION

Although the quinaria species-group represents an extremely interesting complex, extensive genetic and cytological studies have never been fully carried out on the group. Sturtevant (1942) and Spencer (1942) have classified the members of this group as fungus-feeders. It is therefore somewhat difficult to culture them on laboratory food. Moreover, the salivary gland chromosomes tend to remain knotted when smears are made and are thus not well suited for analysis. Wharton (1943) has worked most of the metaphase configurations. Hybridization between members of this group has been reported by Spencer (1942) and Sears (1947), and the present study augments some of their data.

The distribution of the members of this group has been discussed by Spencer and by Patterson and Wagner (1943). According to the data of Patterson and Wagner, none of the species is very numerous in the population. Nevertheless, the group is very wide spread on the North American Continent. In addition, members of the group have been reported from Japan by Kikkawa and Peng (1938, from Switzerland by Burla (1948), and from China by Tan, Hsu, and Sheng (1949). The quinaria group now includes the following species: *palustris, subpalustris, occidentalis, sub-occidentalis, munda, subquinaria, suffusca, tenebrosa,* and *innubila,* all of which have been described by Spencer. Other species are: *quinaria* Loew, *transversa* Fallen, *deflesta* Malloch, *phaletrata* Meigen, *limbata* van Rosen, *mutandis* Tan, Hsu, and Sheng, and *nigromaculata* Kikkawa and Peng. Of the sixteen species mentioned, there is some doubt about the status of *nigromaculata.*

The members of the group utilized in this investigation, the source from which they were obtained, and the laboratory stock number are given below.

D. subquinaria, 1202.3, Manitou Springs, Colorado.
D. munda, 929.8, Cave Creek, Arizona.
D. transversa, 1062.6, Great Smoky Mount. Nat. Park, Tenn.
D. innubila, 1324.6, Bonita Canyon, New Mexico.
D. palustris, 1757.13, Lake Bemijdi State Park, Minn.
D. tenebrosa, 1795.16, Zamora, Mexico.
D. suboccidentalis, 1763.5, Glacier Nat. Park, Montana.
D. quinaria, Wooster, Ohio.

In order to obtain the data for the experiments to be discussed, the following procedure was used. Mass and pair matings of virgin flies from four to ten days old were made in half-pint bottles and in shell vials. The

current medium of banana-malt-yeast-agar of this laboratory was employed. The progeny from these matings was used to test for hybrid fertility, and the larvae and pupae from the same matings were used to determine the salivary chromosome morphology. Acetic-orcein (45%) was employed to stain both the salivary and metaphase chromosomes. Enough matings were made to insure a sufficient number of flies for dissection in order to determine the presence of motile or immotile sperm and a possible "insemination reaction" in the female reproductive tract.

Five days after the matings were made the vials were examined, and those in which one or both parents were dead, or were contaminated with mold, were discarded. At this time twenty-five of the exposed females were dissected and the results recorded. The remaining flies were then transferred to new food vials. The old vials were retained for five days, after which they were discarded and the above procedure repeated at five day intervals until 100 pairs had been dissected for each combination. The character of the insemination reaction was determined by dissecting and examining the reproductive tract of the female at varying intervals after copulation. This was done by observing the matings, and thus a timed series of dissections could be obtained.

RESULTS AND DISCUSSION

Insemination reaction. Ever since the discovery of the insemination reaction by Patterson (1946), he (1947) and Wheeler (1947) have made studies on the possible function of this reaction in both homogamic and heterogamic matings, and its bearing on the problem of speciation and isolation in Drosophila. Wheeler reported on this reaction in seven members of the quinaria group. The results obtained in the present study on three other matings and some additional information on one reported by Wheeler are given. In addition, results on three heterogamic matings are reported.

Drosophila tenebrosa. Three pairs of flies of this species were observed copulating. The average mating time was about six minutes. The first female was dissected fifteen minutes after the end of copulation. The vagina was very much enlarged and contained a reaction mass. The second specimen dissected at thirty minutes was similar to the first, except for the presence of motile sperm in the ventral receptacle and the spermathecae. Not much change was observed in the third specimen, which was dissected one hour after copulation.

Drosophila palustris. Four pairs of flies were observed copulating. The average mating time was about seven minutes and thirty seconds. The first specimen was dissected thirty minutes after copulation, and her vagina was very much enlarged with the presence of a reaction mass, which was being extruded from the median oviduct that was full of motile sperm. Two specimens were dissected eight hours after copulation. One had no reaction mass, but motile sperm were in the spermathecae and ventral receptacle; the other had an egg in the vagina and a soft reaction

mass was being ejected. Another female dissected at ten hours contained motile sperm in the spermathecae.

Drosophila suboccidentalis. Six matings of this species were obtained with flies eleven days old. The average time of mating was six minutes and thirty seconds. The first female was dissected at fifteen minutes, and her vagina was greatly enlarged and contained a very large reaction mass. The vagina of a second female dissected at thirty minutes was slightly more pear-shaped than that of the first. The ventral receptacle and spermathecae were teeming with sperm, and the reaction mass and sperm were coming out of the broken end of the oviduct. Three more females were dissected at one, two, and four hours respectively, but showed no changes from number one. The last specimen was dissected at twelve hours after mating, and the vagina was beginning to clear. It was still large with a central mass surrounded by motile sperm, which were also present in the ventral receptacle and spermathecae.

Drosophila transversa. It can be added to Wheeler's data that after four hours the reaction mass is no longer present in the female of this species. This conclusion is based on the dissection of four feltilized females. Three were dissected four hours after mating, and one eight hours after. All four had sperm in the ventral receptacles and spermathecae, but the dissection of a female one hour after copulation still had a large reaction mass in the vagina.

tenebrosa ♀ × *palustris* ♂. Two pairs of copulating flies were isolated, and for one of them the mating lasted six minutes. The first female was dissected thirty minutes after the end of mating, and the vagina was found to be greatly enlarged and filled with the dense granular reaction mass, with motile sperm around the edge of this mass. Sperm were also present in the ventral recptacle and spermathecae. The second female was dissected two hours after mating, and motile sperm were present in the ventral receptacle and spermathecae, but no reaction mass was evident. The female had previously been observed expelling the semen and excess sperm, and this accounts for the absence of a reaction mass.

suboccidentalis ♀ × *palustris* ♂. Three complete copulations were observed for this cross, with the average length of mating being about seven minutes and thirty seconds. The mating behavior of the female was very antagonistic, for she constantly kept fighting off the male. The vagina of one of the females, dissected after one hour, was much enlarged and contained a reaction mass, with sperm in the ventral receptacle and spermathecae. A second female was dissected at nine hours, but the conditions were the same as in the first specimen. The third female was dissected at twelve hours, and although the copulation time was eight minutes and thirty seconds, yet no reaction mass or sperm was found in her reproductive tract.

suboccidentalis ♀ × *transversa* ♂. Many unsuccessful attempts at mating between these two species were observed, and while the male might

succeed in mounting the female, he was usually dislodgd within thirty seconds. The reproductive tract of one female dissected five minutes after copulation contained no sperm or reaction mass.

Cytology

Wharton (1943) states that *tenebrosa* has a rod-shaped X with a proximal constriction, two rod-shaped autosomes, one of which had a proximal constriction, a J-shaped autosome, and a dot. She used a strain from Mexico. I also used a strain from Mexico, but from a different locality, and found a somewhat different configuration. Instead of a J it has a V-shaped element, and no proximal constriction in either of the two autosomal rods. I also checked a strain of *suboccidentalis*, and found that it had the same configuration as reported by Wharton.

The salivary gland chromosomes of this group are quite long, which adds to the difficulty of obtaining good preparations for analysis. All of the species examined showed five long arms and a dot in the salivary gland nuclei, and thus confirms Wharton's observations. The only species checked which showed almost complete synapsis was *munda*. There were, however, a few gene differences and a tiny deletion or inversion at the tip of one arm. *Drosophila tenebrosa* showed one inversion, and possibly others. The chromosomes of this species are very difficult to spread, and the stock was lost before a complete analysis could be made. *Drosophila palustris* has inversions in at least two arms; and *innubila, transversa, quinaria, subquinaria* and *suboccidentalis* all showed an inversion in one arm. A study of the salivary gland chromosomes in interspecific crosses gave the following results:

innubila ♀ × *palustris* ♂. Chromosomes from pupae showed complete synapsis between these two species.

suboccidentalis ♀ × *munda* ♂. The salivary gland chromosomes from this cross are very irregular. The preparations from four pupae and two larvae were not very good.

transversa ♀ × *innubila* ♂. The salivary gland chromosomes from this cross were comparatively easy to prepare, with the result that good preparations were obtained for analysis. Synapsis occurs only occasionally, and but a few genes seem to be homologous, as the rest of the chromosomes show no tendency to pair. This may account for the sterility of these hybrids. According to Sears (1947), and the present tests, the initial cross is quite fertile, but the F_1 hybrids are completely sterile.

Fertility Tests

Fertility percentages of the controls in the present experiments were much higher than those reported by Sears. This increase is undoubtedly due to improvement in the food used, and to the absence of mites and mold infection. Percentages of present fertility tests and those reported by Sears are given below:

Species	Present	Sears
transversa	84%	63%
tenebrosa	75%	—
subquinaria	67%	46%
quinaria	78%	45%
palustris	71%	27%
suboccidentalis	81%	80%
innubila	37%	9%
munda	92%	75%

An inspection of the results recorded in Tables 1 and 2 will show that ten out of twenty-six possible combinations were fertile. The types of hybrids that were obtained will be considered in the order given in Table 1.

TABLE 1

Crosses	No. of hybrids			Types of hybrids*		$F_1 \times F_1$
♀ ♂	♀	♂	total	♀♀	♂♂	
1. tenebrosa × palustris	24	7	31	fert.	st.	-------
2. tenebrosa × innubila	27	19	46	fert.	st.	sterile
3. tenebrosa × transversa	1	---	1	-------	-------	-------
4. tenebrosa × munda	19	14	33	fert.	st.	sterile
5. munda × tenebrosa	2	2	4	-------	-------	-------
6. tenebrosa × quinaria	26	30	56	fert.	fert.	fertile
7. tenebrosa × suboccidentalis	9	3	12	-------	-------	-------
8. suboccidentalis × tenebrosa	14	34	48	st.	st.	sterile
9. suboccidentalis × palustris	62	21	83	fert.	st.	sterile
10. suboccidentalis × munda	4 pupae, 2 larvae					

*F_1 hybrids discussed in text.

In crosses numbers 1 and 2 the F_1 female was fertile in one direction only, back to *tenebrosa* males.

The female obtained in cross 3 was mated to *transversa* males, but no offspring was obtained.

The females from cross 4 were fertile only to *tenebrosa* males. The F_1 male from this cross was dark like the female parent, and the F_1 female was slightly lighter than the female parent. In the reciprocal cross (5) the F_1 male was lighter and the female somewhat darker. These F_1 flies were inbred but died without producing offspring.

The progeny from cross 6 when backcrossed were fertile only to *tenebrosa*.

All of the progeny from cross 7 died before they could be adequately tested. Cross number 8 was 2.5% fertile, and here again the F_1 male was darker than the female. Although the F_1 flies were kept together for a period of thirty-five days, no offspring was obtained. When these males were dissected it was found that only sperm bundles and an occasional aberrant sperm were found.

Cross 9 was eleven % fertile and when backcrossed the F_1 females were fertile only to *palustris* males. From this backcross the progeny consisted of two kinds of males and three kinds of females. An F_1 female mated to *suboccidentalis* male was observed in copula, but no progeny was produced. Dissections of F_1 males showed that only a few abnormal sperm and sperm bundles were found. All F_1 crosses were held for forty days.

Cross 10 produced four pupae and two larvae from mass matings. These were used for the study of the salivary gland chromosomes.

Sexual Isolation

Examination of the results given in Table 2 indicates several interesting possibilities. From twenty-six different crosses, five days after ex-

TABLE 2

Fertility Relationships in P_1 crosses of members of the quinaria group

Crosses ♀ ♂	5 days* sperm	5 days* egg	10 days* sperm	10 days* egg	15 days* sperm	15 days* egg	20 days* sperm	20 days* egg	per cent inseminated	eggs
tene. × palu.	2	----	3	2	2	1	4	1	----	11	4
palu. × tene.	----	----	----	----	----	----	----	----	----	----	----
tene. × innu.	1	4	----	4	----	7	----	----	----	1	15
innu. × tene.	----	----	----	1	----	2	----	----	----	----	3
tene × trans.	----	----	----	1	----	2	----	----	----	----	3
trans. × tene.	----	1	----	----	----	2	----	----	----	----	3
tene. × munda	1	----	----	----	1	5	----	4	----	2	9
munda × tene.	----	----	----	----	----	10	----	2	----	----	12
tene. × subqu.	----	4	----	2	----	1	----	----	----	----	7
subqu. × tene.	----	----	----	2	----	----	----	1	----	----	3
tene. × quin.	----	----	5	----	2	7	2	5	----	9	12
quin. × tene.	----	----	----	----	----	1	----	2	----	----	3
tene. × subocc.	2	3	----	1	----	3	----	1	----	2	8
subocc. × tene.	----	4	----	2	----	----	1	1	----	1	7
subocc. × palu.	7	2	7	1	4	1	1	----	----	19	4
palu. × subocc.	----	1	----	5	----	6	----	5	----	----	17
subocc. × quin.	----	1	1	2	----	----	----	----	----	1	3
quin. × subocc.	----	1	1	2	----	2	----	1	----	1	6
subocc. × subqu.	----	1	----	----	----	1	----	----	----	----	2
subqu. × subocc.	----	----	----	----	----	----	----	1	----	----	1
subocc. × munda	----	1	----	----	----	4	----	3	----	----	8
munda × subocc.	----	6	----	2	----	12	----	9	----	----	29
subocc. × trans.	----	2	----	----	----	2	----	2	----	----	6
trans. × subocc.	----	3	----	----	1	1	----	1	----	1	5
subocc. × innu.	----	2	----	2	----	2	----	2	----	----	8
innu. × subocc.	----	1	----	1	----	3	----	2	----	----	7

*25 dissected for each cross.

posure to the males, the females from five different matings contained motile sperm in their reproductive tracts. Ten days after exposure, again females from only five combinations showed motile sperm. Three of these combinations were different from those of the first five. Fifteen and twenty days after exposure the females from only five and four combinations, respectively, were found to contain motile sperm.

The number of combinations in which eggs were present in the vagina of the female is also relatively constant. If the retention and disintegra-

tion of the eggs in the vagina is due to the effects of the insemination reaction, then it can be assumed that mating has taken place. Although no motile sperm was detected in the reproductive tracts of these females, over fifty per cent of them appeared to have been inseminated.

The results given in Table 2 indicate that the flies must have mated during the first five days. After fifteen days exposure the total number of females with sperm in their reproductive tracts was beginning to decrease, but the total number with eggs in the vagina was highest at this time. This of course would be expected if the insemination reaction is operative, which it obviously is. In every case the males were dissected, but no results were presented, as all of the males were found to be normal.

The cross, *munda* ♀ × *suboccidentalis* ♂, was most interesting. The results listed in Table 2 show that twenty-nine out of the 100 females dissected contained eggs in their vaginae. In over half of these females more than one egg was present. This was particularly true in the lot dissected after fifteen days exposure. In this group the most frequently observed number of eggs per individual was four. One contained seven eggs in various stages of disintegration. In some females the eggs had reached a stage of disintegration that made it difficult to determine their exact number. The observation recorded on one female at the time of dissection is as follows: In this female the disintegrated eggs could be seen through the wall of the abdomen. The posterior part of the abdomen was dark-orange in color, swollen and turgid. Upon dissection, the vaginal contents were found to be orange and brownish in color, and the number of partially disintegrated eggs could not be determined. The spermathecae and ventral receptacle were intact. This was the most unusual case observed. Obviously in this case the insemination reaction definitely played an important role as an isolating mechanism. Although no motile sperm was ever found in the reproductive tract of this female, yet there is apparently no mating barrier, but a possible gene exchange between the two species was prevented by the insemination reaction. A large number of mass matings were set up but no offspring was ever obtained from this cross. After numerous mass matings the reciprocal cross (Table 1) was only slightly fertile.

The results tabulated in Tables 1 and 2 reveal that *tenebrosa* crosses to over half of the species to which it was tested, but it crosses more readily when used as the female parent. Several factors may be responsible for this. An examination of Table 2 indicates that complete sexual isolation is operative in the *palustris* ♀ × *tenebrosa* ♂ cross. If sterility is due to the effects of the insemination reaction, some females with disintegrating eggs in the vagina should have been found, since this is true of the other crosses producing no progeny. Fertilization is apparently not necessary for egg laying, as *suboccidentalis* females lay virgin eggs when only three days old. Evidently most of the species of this group mature earlier than was formerly believed. Most of the difficulty with this group,

as has been pointed out, is due to the lack of proper food, but this obstacle is gradually being eliminated.

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