# DROSOPHILA MIRANDA, A NEW SPECIES

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## INTRODUCTION

The species of Drosophila to be described in the present paper has certain properties that make it particularly interesting from the point of view of genetics. This species, to be designated *Drosophila miranda* Dobzhansky, is related to, and can be crossed with, a species previously known and rather extensively used for genetic purposes, namely *Drosophila pseudoobscura* Frolowa. The two species are so similar in appearance that most taxonomists would hesitate to separate them on the basis of the morphological differences alone. They represent, however, two different reaction systems, as shown by the presence of some clear-cut physiological differentials. Furthermore, the structure of the chromosome apparatus in *Drosophila miranda* is unique among the known species of Drosophila, and is, in fact, rare elsewhere. Sex determination in *Drosophila miranda* is complicated by the presence of three instead of two sex-determining chromosomes, and is based on a precise functioning of a singular mechanism that insures the proper distribution of the sex-determining chromosomes at the meiotic divisions.

Certain morphological differences between *miranda* and *pseudoobscura* were first noticed by Dr. A. H. STURTEVANT, who has also contributed a number of very valuable suggestions and criticisms; to him the writer wishes to express his sincerest appreciation.

## MATERIAL

Several strains presumed to be *Drosophila pseudoobscura* were brought by Mr. R. D. BOCHE from Seattle, Washington in 1932. Each strain was derived from a single fertilized female caught outdoors. In one strain, designated "Seattle-7," the flies were much larger than in others. The Seattle-7 strain died off before any tests could be made. In 1934 the writer collected a number of strains in the same geographic region. Among these, three strains produced very large flies. The three strains are Cowichan-1, Cowichan-7 (derived from single females collected at Cowichan Lake, Vancouver Island), and Olympic-1 (collected in the Olympic Mountains, near Brinnon, Washington). These strains proved to belong to *Drosophila miranda*, a species different from *Drosophila pseudoobscura*.

*Drosophila miranda* was not encountered in various localities where collecting was made in Alaska, eastern British Columbia, and the western United States. Its geographic distribution is probably restricted to the region around Puget Sound.

## MORPHOLOGY

*Drosophila miranda* is similar to *pseudoobscura* in most external characteristics used for distinguishing species of Drosophila (STURTEVANT 1921). The differences observed are summarized in the following description.

### *Drosophila miranda, sp. nov.*

Differs from *Drosophila pseudoobscura* Frolowa by a larger size (living females of *miranda* developed at 24½°C are about 3.1 mm long, males 2.8 mm, *pseudoobscura* females 2.7 mm, males 2.5 mm), a darker coloration of the whole body, less distinct grayish stripes on the thorax, and slightly wider cheeks in relation to the longer diameter of the eye (the ratio being about 1:3 in *miranda*, and about 1:4 in *pseudoobscura*). Both species have eight rows of acrostichals between the dorsocentral bristles, two sex combs on the anterior tarsi in the males, and two egg filaments; the chaetotaxy, the facial carina, the number of branches in the arista, and the genitalia of both species (studied in a material cleared in a KOH solution and glycerine) are alike. The type is in the collection of the AMERICAN MUSEUM OF NATURAL HISTORY.

Accurate measurements of the length of the body are difficult in Drosophila on account of the telescopic movements of the abdomen and the looseness of the joints between the head and the thorax and the thorax and the abdomen. The length of the hind tibiae was therefore measured with the aid of an ocular micrometer (in cleared flies). The results are summarized in table 1. The differences between *miranda* and *pseudoobscura* are clearly statistically significant.

TABLE 1

*Length of the hind tibiae in (at 24½°).*

| SPECIES | RACE | STRAIN | MALES | | | | FEMALES | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | M±m | $\sigma$ | LIM | n | M±m | $\sigma$ | LIM | n |
| *pseudoobscura* | A | Okanagan-3 | 715 | | 661–748 | 43 | 746±2.9 | 16.2 | 714–782 | 32 |
| | A | Olympic-2 | 707 | | 678–748 | 24 | | | | |
| | A | Average | 712±2.8 | 22.6 | 661–748 | 67 | 746±2.9 | 16.2 | 714–782 | 32 |
| | B | Cowichan-2 | 644 | | 609–678 | 19 | | | | |
| | B | Shelter Cove-1 | 663 | | 626–695 | 39 | | | | |
| | B | Average | 656±2.9 | 21.9 | 609–695 | 58 | | | | |
| *miranda* | | Olympic-1 | 771 | | 713–818 | 41 | | | | |
| | | Cowichan-1 | 779 | | 713–818 | 60 | | | | |
| | | Cowichan-7 | 777 | | 731–818 | 35 | 831±2.4 | 15.3 | 800–870 | 37 |
| | | Average | 777±2.1 | 25.2 | 713–818 | 136 | 831±2.4 | 15.3 | 800–870 | 37 |

Another difference between *miranda* and *pseudoobscura* involves the number of teeth in the sex combs (table 2).[1] *Miranda* has larger sex combs than *pseudoobscura.* The proximal sex comb (located on the first tarsal joint) is considerably larger in *miranda* than the distal comb (on the second tarsal joint).

TABLE 2

*Number of teeth in the sex combs (at 24½°).*

| SPECIES | RACE | STRAIN | DISTAL COMB | | | | PROXIMAL COMB | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | M± m | σ | LIM | n | M± m | σ | LIM | n |
| *pseudoobscura* | A | Okanagan-3 | 5.14 | | 4–7 | 43 | 6.60 | | 6–8 | 43 |
| | A | Sequoia-6 | 5.35 | | 4–7 | 46 | 6.89 | | 6–9 | 46 |
| | A | Chehalis-5 | 5.08 | | 4–6 | 25 | 6.48 | | 6–8 | 25 |
| | A | Portland-2 | 5.04 | | 4–6 | 26 | 6.19 | | 6–7 | 26 |
| | A | Olympic-2 | 5.46 | | 4–7 | 25 | 6.42 | | 6–8 | 25 |
| | A | Average | 5.22 ± .05 | .60 | 4–7 | 165 | 6.57 ± .05 | .65 | 6–9 | 165 |
| | B | Cowichan-2 | 4.58 | | 4–5 | 19 | 5.84 | | 5–6 | 19 |
| | B | Klamath-5 | 4.83 | | 4–6 | 29 | 5.93 | | 5–7 | 29 |
| | B | Shelter Cove-1 | 4.90 | | 4–6 | 40 | 5.63 | | 5–6 | 40 |
| | B | Average | 4.81 ± .06 | .60 | 4–6 | 88 | 5.77 ± .06 | .54 | 5–7 | 88 |
| *miranda* | | Olympic-1 | 5.81 | | 5–8 | 41 | 8.71 | | 7–10 | 41 |
| | | Cowichan-1 | 5.73 | | 5–7 | 60 | 8.33 | | 7–10 | 60 |
| | | Cowichan-7 | 5.90 | | 5–7 | 31 | 8.29 | | 6–10 | 31 |
| | | Average | 5.80 ± .06 | .68 | 5–8 | 132 | 8.44 ± .07 | .81 | 6–10 | 132 |

There is no difficulty in distinguishing *Drosophila miranda* from *pseudoobscura* by body size, provided both species have developed under similar environmental conditions. However, body size in Drosophila is rather strongly affected by temperature and food conditions (ALPATOV 1930), and *miranda* developed at 25° in crowded cultures is not sufficiently different from *pseudoobscura* developed at 15° in cultures well provided with food to permit a clear separation. In fact, the original *miranda* females caught in nature struck the eye by their large size, but at least one other female that seemed to be as large as they were produced offspring that proved to be race B of *pseudoobscura.*

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[1] Data presented in tables 1 and 2 suggest the existence of a morphological difference between race A and race B of *pseudoobscura.* Up to now no such difference has been recorded. It remains to be ascertained whether this difference persists at all temperatures. Temperature of 24½° is less advantageous for race B than for A, and the smaller size of race B may be merely an expression of the unfavorable effects of heat on race B. KOLLER (1934) has stated that race A has longer and narrower testes than race B. I can not confirm this observation.

## PHYSIOLOGICAL CHARACTERS

According to POULSON (1934) the development of *Drosophila pseudoobscura* takes, at 25°, thirteen to fourteen days (from oviposition to the emergence of the adult), about ten hours more in males than in females, and about ten hours more in race B than in race A. The development of *miranda* females takes, at the same temperature, about fifteen days, and males are very much slower than females. If parents are allowed to oviposit for only 24 hours, the emergence of the adult female offspring ends before the emergence of the males begins. For a day, or even two, no flies at all emerge in the cultures, and this period is followed by three or four days during which the males emerge. If the parents are allowed to oviposit for a longer time in the same culture, the times of the emergence of the males and females naturally overlap, but it remains apparent that the sexual dimorphism in the time of development is stronger in *miranda* than in any other species of Drosophila with which the writer is familiar.

Newly emerged females have undeveloped ovaries in both *miranda* and *pseudoobscura*, but in the former the full sexual maturity is reached at least two days later than in the latter (at room t°, about 22°). *Miranda* is more sensitive to high temperatures than *pseudoobscura;* at 25° *miranda* females are mostly sterile. At temperatures around 20° both species, however, seem to be about equally vigorous.

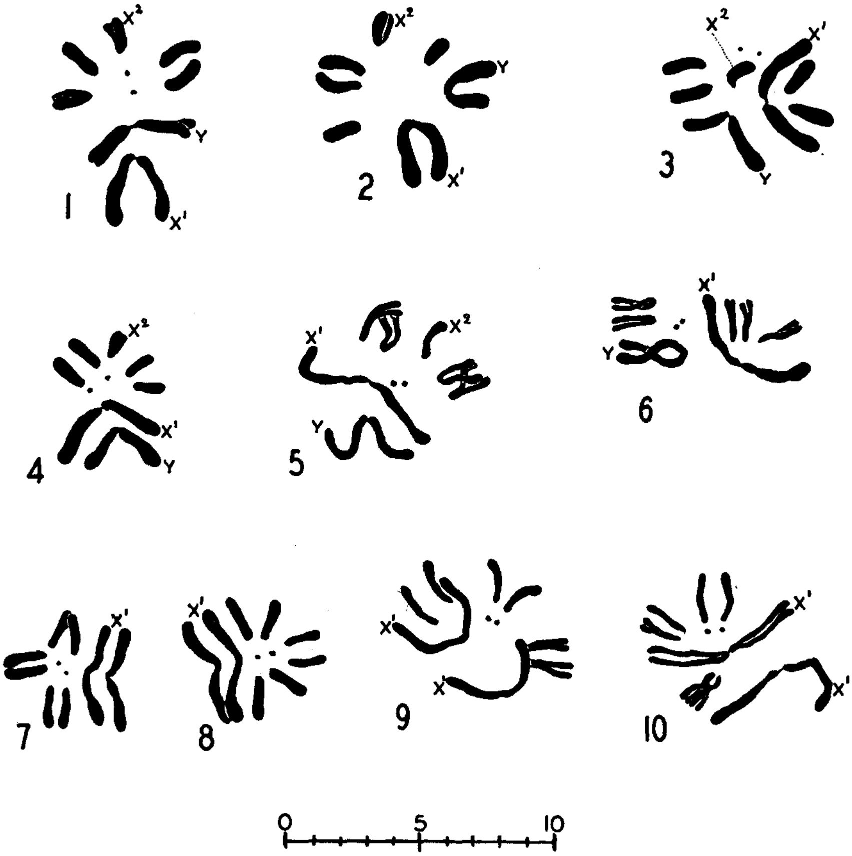
## CHROMOSOMES

Chromosomes of *Drosophila miranda* were examined in the spermatogonial divisions (figures 1–4), in the nerve cells of the larval ganglia (figures 5, 6, 9, 10), and in the oogonia (figures 7 and 8). Spermatogonial divisions were seen in all three strains available (Olympic-1 Cowichan-1, Cowichan-7). The aceto-carmine smear method was used in most cases, but sections of the testes were also studied. As a supplement, the chromosomes were investigated also in the larval salivary glands, following the technique of PAINTER (1934).

*Drosophila miranda* females have a chromosome group resembling that of *Drosophila pseudoobscura* both as to the number and as to the shapes of individual chromosomes (figures 7–10). A pair of V-shaped equal armed chromosomes ($X^1$) appear similar to the X chromosomes, and the three pairs of rods and one pair of very small dots (so small that in some plates they are invisible) seem similar to the autosomes of *Drosophila pseudoobscura.* Females of both species have $2n=10$. *Drosophila miranda* males (figures 1–6) are, however, unique among the known species of Drosophila in having an odd number of chromosomes ($2n=9$).

Two pairs of rod-shaped and one pair of dot-shaped chromosomes are similar in males and in females. These are, then, the autosomes of *Droso-*

*phila miranda.* Instead of the third pair of rods present in the females, the males show a single rod-shaped chromosome ($X^2$) having no partner. Only one V-shaped equal armed chromosome is present in the male ($X^1$); its partner is a somewhat shorter, V-shaped, more or less clearly unequal-armed chromosome (Y). The latter chromosome appears similar to the Y chromosomes of some strains of race B of *Drosophila pseudoobscura* (DOBZHANSKY and BOCHE 1933, DOBZHANSKY in press). In the spermatogonia the chromosomes tend to form a definite pattern (figures 1 and 4). The V-shaped chromosomes and the unpaired rod occupy the two ends of a diameter, the rod-shaped autosomes lie between the unpaired rod and the V's, and the dots are at the center.



FIGURES 1–10.—Cameralucida drawings of chromosomes of *Drosophila miranda.* FIGURES 1–4.—Spermatogonial metaphase plates. FIGURES 5 and 6.—Nerve cell divisions in the ganglia of male larvae; FIGURES 9 and 10.—Same, in female larvae. FIGURES 7 and 8.—Oogonial divisions. $X^1$—the $X^1$ chromosome; Y—the Y chromosome; $X^2$—the $X^2$ chromosome having no mate. The scale below represents 10 micra.

The only possible interpretation of the chromosome complex of the *miranda* male is that this species possesses two distinct X chromosomes, namely the equal-armed V (to be referred to as the $X^1$ chromosome), and the rod which has no mate in the male (the $X^2$ chromosome). The female has, then, $X^1X^1+X^2X^2+$three pairs of autosomes, and male is $X^1+X^2+Y$ $+$three pairs of autosomes.

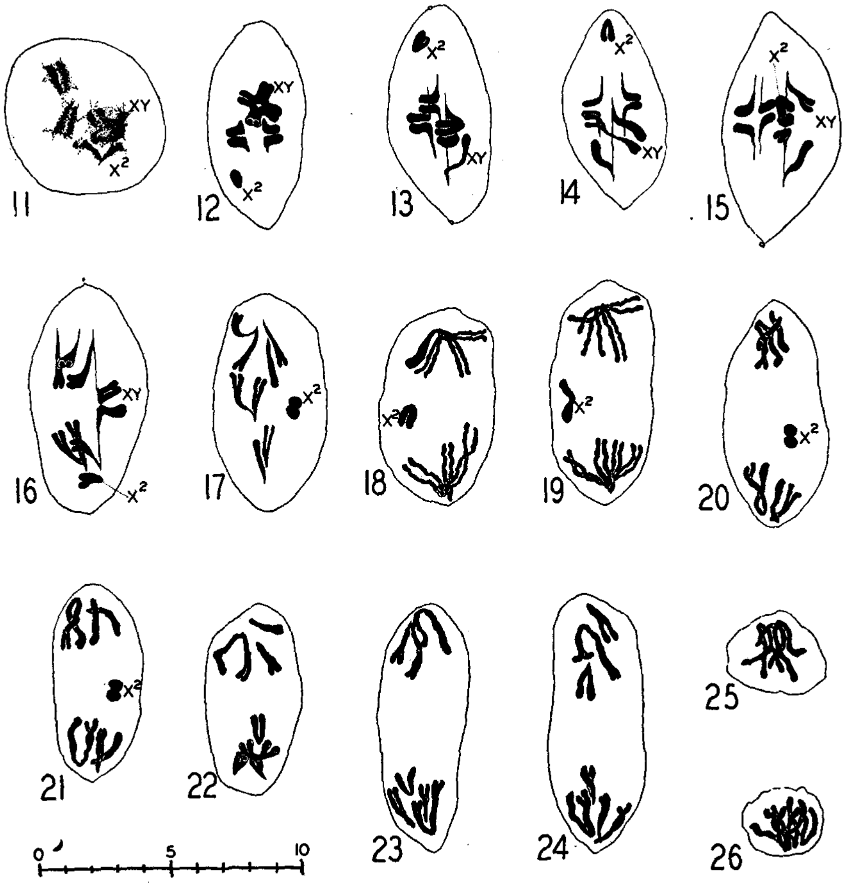
Such a situation seemed so extraordinary in a species of Drosophila that care was taken to discover any possible mistake. In some plates (figures 1, 2, 4) the $X^2$ chromosome shows the equational split more clearly than the rest of the chromosomes. Is it possible that the $X^2$ is not really single but represents two separate chromosomes showing an unusually intimate somatic pairing? This question is to be answered in the negative, because: (1) the $X^2$ in the nerve and the oogonial cells shows no more doubleness than the rest of the chromosomes, (2) in no case does the $X^2$ chromosome manifest a quadripartite structure, (3) the behavior of $X^2$ in the spermatogenesis (see below) is incompatible with the above assumption; and finally, (4) the observations on the salivary gland chromosomes afford conclusive proof of the singleness of the $X^2$. In the salivary glands of the fully grown larvae and young pupae the chromosomes undergo pairing, and the paired homologs appear as cross-striped worm-like bodies. If a chromosome has no homolog in the diploid group, as in the cases of the X chromosomes of *melanogaster*, *simulans*, and *pseudoobscura*, it appears in the salivary gland cells as a body that is paler and narrower than the paired chromosomes. Disregarding the very small dot-like autosomes, the salivary gland cells of *miranda* females contain five darkly staining strands (two limbs of the $X^1$ chromosome$+$two autosomes$+$the $X^2$ chromosome). In *miranda* males two dark strands (the two autosomes) and three light strands ($X^1$ and $X^2$) are visible. In *pseudoobscura* males three dark (three autosomes) and two light strands (two limbs of the X chromosome) are observed.

## SPERMATOGENESIS

The above information on the chromosome group of the *Drosophila miranda* male makes an investigation of the spermatogenesis in this species a logical necessity. For a mechanism must be present, whereby a proper distribution of the two X chromosomes ($X^1$ and $X^2$) at the reduction division is effected, thus allowing the peculiar method of sex determination observed in this species to perpetuate itself. Testes of young adult males were fixed in Flemming's and Navashin's fluids, and sections ($7\mu$ thick) were stained with iron haematoxylin or gentian violet.

Spermatogenesis of *Drosophila pseudoobscura* has been studied by METZ (1926), KOLLER (1933, 1934), DOBZHANSKY (1934) and DARLING-

TON (1934). Spermatogenesis in *miranda* is in general similar to that in *pseudoobscura*, thus permitting us to make a comparative description. The general structure of the testis, of the spermatogonia, and of the first spermatocytes is similar. The stainability of chromosomes in prophase stages of meiosis is even poorer in *miranda* than in *pseudoobscura*. At stages up to and including diakinesis the chromosomes appear as more or less pale brown clumps. At late diakinesis (figure 11) four bodies can be seen in the nucleus; one of them, the largest, is probably the bivalent composed of the $X^1$ and the Y chromosomes; two others are the autosomal tetrads, and the fifth is the $X^2$ chromosome. The $X^2$ does not seem to occupy any definite position with respect to the $X^1Y$ bivalent.



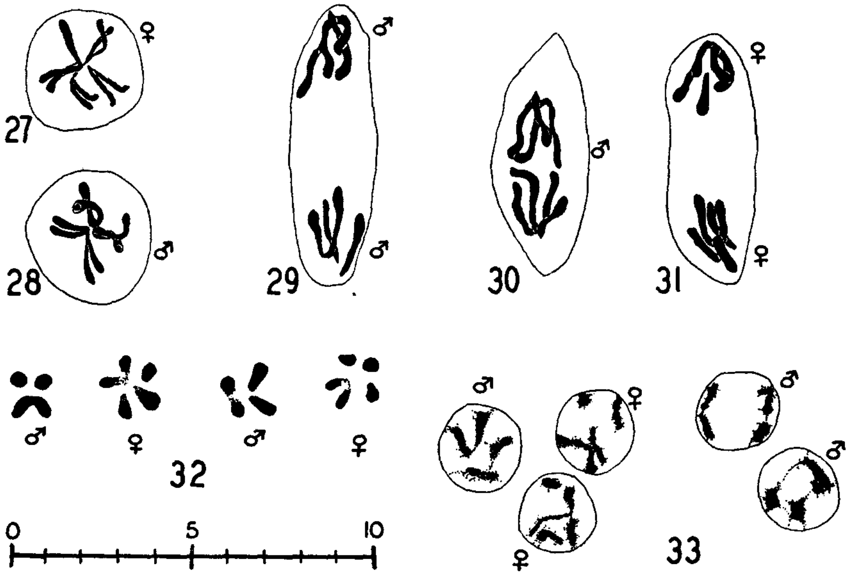
FIGURES 11–26.—Diakinesis and the first meiotic division in *Drosophila miranda*. XY—the $X^1Y$ bivalent; $X^2$—the $X^2$ chromosome (the chromosomes are marked only in the figures in which they can be identified with certainty). The scale below represents 10 micra.

The first meiotic division (figures 12–24) is frequently very clear in *miranda*. The chromosomes are somewhat longer and more slender than in *pseudoobscura.* At metaphase and early anaphase the $X^2$ may lie anywhere on the spindle, in the equatorial region (figure 15) or near one of the poles (figures 12–14, 16). This chromosome is very much condensed, the equational split shows clearly in most cases (figures 13–16), and its spindle fibre attachment is directed toward (figure 14) or away from (figures 13, 16) the nearest pole. It is to be emphasized, that there is no apparent physical connection between the $X^2$ chromosome and the $X^1Y$ bivalent, nor does it occupy any definite position with respect to the latter. The two diads composing an autosomal bivalent are frequently separated from each other by a much wider space than is the case in *pseudoobscura* (see especially figure 15). This corroborates the observations of DARLINGTON (1934) and DOBZHANSKY (1934) according to which no chiasmata are formed in the autosomal bivalents in Drosophila males. On the other hand, the $X^1Y$ bivalent assumes shapes that were interpreted by DARLINGTON (l.c.) as indicating the presence of two chiasmata in this bivalent (figure 12, also figures 13–16).

At late anaphase and early telophase the autosomes and the $X^1$ and Y may be seen lying at the poles of the spindle (figures 16–21). Their bodies are long and slender, and the equational split is frequently very clearly visible (figures 18 and 19; it is not certain whether figures 18 and 19 represent an earlier stage than figures 20 and 21, or *vice versa*). The $X^2$ comes to lie in the equatorial region of the spindle, its body having the shape of a dumb-bell or of a very short and stout V, and its spindle fibre attachment still showing no definite orientation with respect to the poles (figures 17–21). At the next stage (figures 22–26) the $X^2$ chromosome is included in one of the telophasic groups, its spindle attachment now clearly pointed toward the nearest pole, and its shape no longer different from that of the rest of the chromosomes.

The first meiotic division is, consequently, reductional both for the $X^2$ chromosome and, probably, for the $X^1Y$ bivalent. It is frequently possible to count the chromosomes in both telophasic groups in the same cell, and to ascertain that one of these groups may contain four and the other three chromosomes, a V-shaped chromosome being present in each. Since, however, the $X^1$ and Y chromosomes are not distinguishable at this stage, it is impossible to determine whether the $X^2$ goes to the same pole with the $X^1$ or with the Y chromosome.

In contradistinction to *Drosophila pseudoobscura,* the second spermatocytes (figures 25, 26) in *Drosophila miranda* seem to exist only for a very short time. No resting stage intervenes between the first and the second meiotic divisions, and the chromosomes pass onto the spindle of the second division without disentangling themselves from the telophasic snarls. Because of this, the metaphases of the second divisions are not clear in *miranda*, and counting the chromosomes is possible only in exceptional cells (figures 27, 28). The anaphases and telophases are again clear enough (figures 29–31), and it is easy to see that two kinds of cells are present. In some cells (figures 28-30) three chromosomes, including one V-shaped one, migrate to each pole, while in other cells (figures 27, 31) four chromosomes are involved. Evidently, the former kind is devoid of $X^2$ chromosome and is destined to produce male-determining sperms, and the latter kind contains the $X^2$ chromosome and gives female-determining sperms. Counting chromosomes is especially easy in telophasic groups observed in polar view (figure 32). In 102 groups so counted, 53 contained three chromosomes and 49 four chromosomes, a close approximation to a 1 : 1 ratio.



FIGURES 27–33.—The second meiotic division (figures 27–32) and the nuclei of young spermatids (figure 33) in *Drosophila miranda*. Cells giving rise to female- and male-determining spermatozoa are marked ♀ and ♂ respectively. The scale below represents 10 micra. All drawings are made with the aid of a camera-lucida.

The spermatids and the spermiogenesis are apparently alike in *miranda* and *pseudoobscura*, with the distinction that in the former the chromosomes in the spermatids do not fuse into a single body immediately after the second division as they do in the latter. The nuclei of the young spermatids (figure 33) frequently show separate and countable chromosomes; some nuclei contain three, and others four chromosomes. The bundles of spermatozoa consist of a number of threads approaching 128, the same number as observed in *pseudoobscura*. No spermatids or spermatozoa are visibly abnormal.

The result arrived at is an anomalous one. At no stage of meiosis is the

$X^2$ chromosome paired with either $X^1$ or Y, and at the first division the $X^2$ proceeds to one of the poles seemingly undirected by the distribution of the $X^1$ and Y. An independent distribution of the $X^2$ with respect to the $X^1$ and Y must, however, give rise to four kinds of spermatozoa: (1)—$X^1X^2$, (2)—Y, (3)—$X^1$, (4)—$X^2Y$. Since the female is the homozygous sex, all eggs must be $X^1X^2$. Accordingly, four classes of zygotes should be produced: (a)—$X^1X^1X^2X^2$, (b)—$X^1YX^2$, (c)—$X^1YX^2X^2$, and (d)—$X^1X^1X^2$. Among the adult individuals we find only classes (a) and (b). Hence, gametes (3) and (4) are either not formed at all, or give rise to inviable eggs, or some chromosomes are eliminated in the eggs after fertilization, perhaps in a manner similar to that described by METZ (METZ, MOSES and HOPPE (1926), METZ and SCHMUCK (1931)) in Sciara. To discriminate between these possibilities, observations on the sex ratio and the viability of the eggs were made.

## THE SEX RATIO AND THE VIABILITY OF THE EGGS

Sex of the offspring from pair matings is recorded in table 3. No unisexual progenies are produced. The sex ratio in the total is 100 ♀ ♀ to 75.3 ♂ ♂. The deficiency of males is probably due to their slow developmental rate (see above) which puts them at a disadvantage in the cultures. It may be noticed that cultures producing few offspring give a sex ratio approaching unity.

TABLE 3

*Sex ratio in Drosophila miranda.*

| CULTURE NO. | ♀♀ | ♂♂ | CULTURE NO. | ♀♀ | ♂♂ |
|---|---|---|---|---|---|
| 1 | 56 | 45 | 7 | 43 | 29 |
| 2 | 65 | 40 | 8 | 45 | 33 |
| 3 | 15 | 16 | 9 | 100 | 56 |
| 4 | 95 | 66 | 10 | 68 | 63 |
| 5 | 90 | 81 | | | |
| 6 | 17 | 18 | Total | 594 | 447 |

*Drosophila miranda* females were allowed to deposit eggs on paper spoons with food and a small amount of yeast. The number of larvae produced and the number of eggs that failed to hatch were recorded. The result is shown in table 4.

In another experiment (table 5) the spoons with a known number of eggs were placed in regular culture bottles, and the adults obtained were counted.

The mortality in the offspring of *Drosophila miranda* is obviously insufficient to account for the elimination of the 50 per cent of male gametes and the resulting zygotes that may theoretically be produced. In fact, the ob-

TABLE 4

*Viability of the eggs.*

| SPOON NO. | EGGS LAID | LARVAE HATCHED | PERCENT OF HATCHING | SPOON NO. | EGGS LAID | LARVAE HATCHED | PERCENT OF HATCHING |
|---|---|---|---|---|---|---|---|
| 1 | 16 | 12 | 75 | 6 | 58 | 52 | 89½ |
| 2 | 96 | 92 | 96 | 7 | 47 | 45 | 96 |
| 3 | 18 | 18 | 100 | 8 | 47 | 47 | 100 |
| 4 | 92 | 86 | 93½ | 9 | 36 | 36 | 100 |
| 5 | 90 | 83 | 92 | | | | |
| | | | | Total | 500 | 471 | 94.2 |

TABLE 5

*Number of adults derived from a given number of eggs.*

| CULTURE NO. | NUMBER OF EGGS | ♀♀ | ♂♂ | TOTAL | PERCENT OF SURVIVAL |
|---|---|---|---|---|---|
| 1 | 62 | 30 | 22 | 52 | 84 |
| 2 | 19 | 8 | 8 | 16 | 84 |
| 3 | 13 | 9 | 4 | 13 | 100 |
| 4 | 29 | 14 | 12 | 26 | 89½ |
| 5 | 33 | 16 | 10 | 26 | 79 |
| 6 | 26 | 13 | 9 | 22 | 84½ |
| 7 | 27 | 10 | 8 | 18 | 66½ |
| 8 | 13 | 6 | 4 | 10 | 77 |
| Total | 222 | 106 | 77 | 183 | 82.4 |

served morality is only slightly greater than necessary to account for the deviation from the 1:1 sex ratio among the adults.

The conclusion is forced on us that the two classes of gametes not represented among the adults are either not produced at all, or are somehow eliminated before fertilization. Since there is no indication that the latter possibility is realized, it seems probable that only $X^1X^2$ and Y gametes are produced in spermatogenesis. To put it another way, the disjunction of $X^2$ is not independent of that of the $X^1Y$ bivalent, but on the contrary, the $X^2$ chromosome always passes to the same pole of the spindle of the first meiotic division as does the $X^1$ chromosome. The *modus operandi* of the mechanism by which such a determinate disjunction is brought about is, however, not at all clear.

## HYBRIDS BETWEEN DROSOPHILA MIRANDA AND DROSOPHILA PSEUDOOBSCURA

A preliminary account of the results of hybridization of the two species under consideration may be given here, a fuller account being reserved for a separate publication. There is no great difficulty in crossing *Drosophila miranda,* either as a female or as a male, either to A or to B race of *Droso-*

*phila pseudoobscura.* The results of crosses to A and B races are apparently similar. The cross *miranda* ♀ × *pseudoobscura* ♂ produces adult offspring of both sexes in approximately equal numbers. Hybrid females are normal morphologically, slightly closer in size to *miranda* than to the other parent, and, after a rather long delay in the maturation of the ovaries, produce numerous eggs none of which however hatches. The eggs are normal externally. Hybrid males hatch late, and are visibly abnormal—short, squat, somewhat flattened bodies, rough eyes, spread wings, misshapen legs. Their testes are very small and contain nothing resembling mature sperm. The cross *pseudoobscura* ♀ × *miranda* ♂ produces mostly females (about 1 ♂♂ : 1,000 ♀♀). Hybrid females are similar to those from the reciprocal cross. The occasional males are less abnormal morphologically than hybrid males from the reciprocal cross, but still have very small testes and are completely sterile.

## DISCUSSION

The morphological differences between *Drosophila miranda* and *pseudoobscura* are relatively slight, and subject to a rather considerable variation due to environmental influences. The result is that classifying a single individual developed under unknown external conditions as belonging to one or the other species may be hazardous. To be certain, if the individual in question is a living male, its species can be securely established by an examination of its chromosomes. A taxonomist accustomed to determining species of dead and dried specimens will, however, derive little comfort from this consideration, and few geneticists will seriously argue that he should change his habits in this respect. Is, then, the elevation of *Drosophila miranda* to specific rank justified? In the opinion of the writer this question should be answered in the affirmative.

The category of the species is basically different from all other taxonomic categories. The latter are "natural" only insofar as they reflect the discontinuities actually existing in the living world at a given time level, but the general scheme of classification is determined essentially by its expediency. Species possesses all the phenomenological and all the pragmatic attributes of the rest of the taxonomic categories, plus an attribute peculiar only to itself. A species is a system the members of which, because of their physiological properties, do not interbreed with members of other similar systems. (Races that are prevented from interbreeding by geographic isolation, but which are fully inter-fertile when brought together by natural or artificial means, are, thus, not to be considered distinct species.) Considered dynamically, the species represents that stage of evolutionary divergence, at which the once actually or potentially interbreeding array of forms is segregated into two, or more, separate arrays that are physiologically incapable of interbreeding. It would be out of place here to discuss in detail how widely the above criterion can be consistently applied. Its application is admittedly not universal; for example, it is restricted to habitually cross-fertilizing organisms. It is, however, apparent that *Drosophila miranda* and *pseudoobscura* must be considered specifically distinct, since they are isolated by the complete sterility of their hybrids. Non-transgressive morphological differences are not a necessary prerequisite of the specific distinction, since the genetic factors preventing interbreeding (that is, factors determining the inviability of the hybrid offspring, or the hybrid sterility, or the sexual isolation may or may not be reflected in morphological structures. Closely related species of Drosophila (for example, *melanogaster* and *simulans*) frequently show a paucity of morphological differentials.

The nature of the specific difference between *miranda* and *pseudoobscura* constitutes an interesting problem. A cycle of physiological differences makes *miranda* an ecological type separate from *pseudoobscura.* Spermatogenesis in *miranda* deviates from that in *pseudoobscura* not only in those features that are clearly related to the presence of an unpaired chromosome in *miranda*, but also in a number of other respects (the failure of the meiotic prophase chromosomes to stain, the elimination of the interphase between the two meiotic divisions, the visibility of chromosomes in the spermatids). The most striking peculiarity of *miranda* is the $X^1$-$X^2$-Y mechanism of sex determination. *Miranda* has one pair of autosomes less, and one sex chromosome more, than *pseudoobscura.* It is very unlikely that the $X^2$ chromosome of *miranda* is completely homologous to one of the autosomes of *pseudoobscura.* The superficial similarity of the chromosomes in the females of both species is undoubtedly deceiving, concealing, as it does, a far reaching remodeling of the chromosome apparatus. A preliminary study of the salivary gland chromosomes in the hybrid larvae, performed by Mr. C. C. TAN and the writer, fully corroborates this conclusion. (Note added in proof: it has been found that not a single chromosome of *miranda* is identical with any chromosome of *pseudoobscura;* even the small dot-like chromosomes are different. Besides numerous inverted sections, frequently very small in extent and covering only a few bands, a number of translocations between the chromosomes are observed. The, $X^2$ chromosome of *miranda* contains at least one section that in *pseudoobscura* is located in the X chromosome, and the fourth chromosome of *pseudoobscura* includes at least one section whose homolog is found in the $X^1$ chromosome of *miranda.*)

The origin of *pseudoobscura* and *miranda* from their hypothetical common ancestor must have involved a series of elaborate changes in the mechanisms whose precise functioning is essential if the race is to endure.

Not only has the arrangement of the genic material in the chromosomes been altered, but a mechanism of determinate disjunction of heterochromosomes at the reduction division has been evolved. In fact, such a mechanism is, to the writer's knowledge, not found in any other form with the possible exception of the insufficiently established case of a race of *Gryllotalpa vulgaris* described by VOINOV (1914). Multiple X's and Y chromosomes are, of course, not infrequent in various groups of animals and also in plants (a review in SCHRADER 1928), but at least in most of such cases the multiple heterochromosomes pair with each other at meiosis, and this may be presumed to insure their regular disjunction. The spermatogenesis of Sciara (METZ 1929, 1933) constitutes the only real, though very remote, parallel to that in *Drosophila miranda*.

## SUMMARY

1. *Drosophila miranda* occurs in the state of Washington and in the adjacent part of British Columbia.

2. *Drosophila miranda* differs from *Drosophila pseudoobscura*, a species to which it is very closely related, by a somewhat larger body size, larger sex combs in the male, longer period of development, and a greater sensitivity to heat. The classification of dead specimens is hazardous.

3. The male of *Drosophila miranda* has an odd number of chromosomes ($2n=9$), among which one heteromorphic pair (the $X^1$ and Y chromosomes), and one unpaired chromosome ($X^2$) are present. The female has ten chromosomes and no heteromorphic pairs.

4. This condition is interpreted as meaning that the species has two distinct X chromosomes, $X^1$ and $X^2$. The male is $X^1YX^2$, and the female $X^1X^1X^2X^2$.

5. The $X^1$ and Y chromosomes form a bivalent at meiosis. The $X^2$ chromosome remains unpaired. During the first meiotic division the $X^2$ lags on the spindle, and finally passes undivided in one of the telophasic groups. The $X^2$ divides normally at the second division.

6. Evidence is presented to show that the $X^2$ passes at the first division to the same pole with the $X^1$ chromosome. Two, instead of the possible four, types of male gametes are formed. The nature of the mechanism insuring this determinate disjunction is unknown.

7. Spermatogenesis in *Drosophila miranda* differs from that in *pseudoobscura* in some further particulars not clearly related to the heterochromosome situation. Thus, in *miranda*, the interphase between the first and the second divisions is practically eliminated, and the chromosomes remain for a certain time visible in the spermatids.

8. *Drosophila miranda* can be crossed to *pseudoobscura*. The cross *miranda* ♀ × *pseudoobscura* ♂ produces offspring of both sexes, while the re-

ciprocal cross produces predominantly females, and only occasionally a few males. The hybrids of both sexes are completely sterile. In addition, the male hybrids are somatically abnormal.

9. The nature of the specific difference between *miranda* and *pseudoobscura* is discussed.

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