A COMPARATIVE ANALYSIS OF THE CHROMOSOMES OF THE GUARANI GROUP OF DROSOPHILA

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INTRODUCTION

In some families of plants and animals there is great uniformity of karyotype; in other families, sometimes even within a single genus, there is considerable variation. Where such variation exists, it is variation on an established theme, for karyotype like macromorphology arises as the result of various tendencies which preserve a somewhat unstable equilibrium within the organism. When it varies, it does not do so capriciously or arbitrarily. In groups where the usual taxonomic characters differ very slightly from species to species, chromosomal configuration may be a better guide to specific differences than the characters of external morphology.

Within the genus Drosophila there is considerable variation of karyotype from species to species. The haploid number of chromosomes varies from three to six, and individual chromosomes may be rods, dots, V's or J's in varying sizes and proportions. Certain general configurations are found widely spread throughout the genus; again, two species scarcely differing morphologically may have very distinct chromosomal complements. The several very similar species of the *virilis* group were first satisfactorily separated as a result of chromosomal analysis (Patterson, 1943).

The *guaraní* group consists of six species which on the basis of morphology are very closely related. They clearly belong to the subgenus Drosophila but are not closely related to any other known species of the genus. The present paper deals with the chromosomal configuration of these six species and the light which this chromosomal analysis sheds on the interspecific relationships within the group. Another paper dealing with the general interspecific relationships aside from the chromosomal configurations is in preparation and will be published shortly.

The six species of the *guaraní* group are the following: *guaraní* Dobzhansky and Pavan, *guarú* Dobzhansky and Pavan, *guaramunú* Dobzhansky and Pavan (Dobzhansky and Pavan, 1943), *griseolineata* Duda (Duda, 1925), *subbadia* Patterson (Patterson, 1943), and *guarajá*, species nova, described below.

Drosophila guarajá, species nova

Male. Arista with 9 branches, sometimes with as many as 4 or 5 more very small ones. Second segment of antennae dull tan with darker area around bristles, third segment darker, more corneous in color. Front dull grayish tan, lightly pollinose, area between ocelli almost black, frontal lines velvety yellow-brown, converging and almost joining anteriorly. Anterior orbitals $\frac{2}{3}$ posterior, middle orbital minute. Two prominent oral bristles of approximately equal length. Face dull corneous tan, lightly pollinose. Carina very large, broad, flattened, not sulcate. Cheeks dull corneous tan, their width about $\frac{1}{6}$ greatest diameter of eye. Eyes deep maroon with a fine brown pile.

Achrostical hairs in six rows, very irregular. No prescutellars. Anterior scutellars divergent. Thorax dull dark tan, finely pollinose, without pattern. Pleurae much darker, almost walnut, less pollinose than dorsum of thorax. Anterior sternopleural $\frac{2}{3}$ posterior and much finer, middle about as long as anterior but still finer. Legs corneous tan shading to darker proximally. Prominent apical and preapical bristles on midtibiae; scarcely differentiated on fore and hind. One or two strong bristles at base of each tarsal joint. Some fine recurved hairs on fore tarsi.

Abdominal tergites shining black, deeper caudad of segment 4. On tergites 3 and 4 a corneous tan stripe along anterior margin about half width of tergite, far from reaching lateral margins, extended posteriorly on mid-dorsal line. On tergite 2 a narrower similar stripe without extension along mid-dorsal line. Abdominal sternites deep brownish gray. Membranous conjunctivae pale corneous.

Wings with slightly brownish tinge; cross veins very lightly clouded. Two prominent bristles of equal length at apex of first costal section. Third costal section with heavy bristles on its basal ½. Costal index 3.3–3.7; 4th vein index 1.5–2.1; 5th vein index 1.1–1.4.

Length of body 2.8–3.1 mm., wing 2.3–2.4.

Female. No recurved hairs on fore tarsi. Costal index 3.2–3.9; 4th vein index 1.5–1.8; 5th vein index 1.1–1.3. Length of body 3.1–3.5 mm., wing 2.4–2.5 mm.

Anterior Malpighian tubes two, simple, short common stalk; posterior two with ends apposed but lumen not continuous.

Testes pale amber yellow with 7 or 8 coils, sperm pump with two long diverticula. Ventral receptacle with about fifty kinks, diameter gradually decreasing distally. Spermatheca pear-shaped, purplish brown, heavily chitinized, especially in old flies, no indentation.

Eggs—four slender filaments: an anterior pair and a posterior pair arising about ⅛ of length of egg from anterior end, latter about length of egg, anterior pair slightly shorter. Length of egg about 0.5 mm.

Puparia—yellow-brown, often with an orange tint. Horn slender, almost invariably bent making measurement difficult, horn index between 5 and 6. Anterior spiracle with 18–20 very slender branches.

Chromosomes—metaphase plates show five pairs of chromosomes; one pair of large equal-armed V's, three pairs of rods of slightly different lengths, and one pair of small dots. The two pairs of longer rods often show satellites and appear to have subterminal centromeres. The sex chromosomes are evidently the smallest rods, as in plates of male larvae one chromosome of this pair shows a large satellite. The X never shows this satellite. The V's are necessarily autosomes. In this respect the species differs from all known others of the group, having five rather than six centromeres.

Salivary chromosomes show five long euchromatic strands and a dot. The chromocenter is very small.

Type—deposited at the American Museum of Natural History.

Distribution—the species has been collected only at the type locality, Campos de Jordão near the border of the states of São Paulo and Rio de Janeiro, Brazil.

Relationships—closely related to *D. guaramunú* and *D. griseolineata*, forming with these two species a natural sub-group within the *guaraní* group. The sub-group is characterized by the following among other characters: dark color of body and eyes, spheroidal rather than ellipsoidal abdomen in both sexes of well fed and well developed flies, and structure of Malpighian tubes—anterior with a short common stalk and posterior with ends apposed but without continuous lumen.

Materials and Methods

Cultures of *guaraní, guarú, guaramunú* and *griseolineata* were obtained from the laboratory of Professor Dobzhansky who brought them from Brazil in 1943. A culture of *subbadia,* collected in Mexico, was obtained from Professor Patterson of the University of Texas. The culture of *guarajá* was obtained from the University of São Paulo in October, 1945. At the same time and from the same source an additional culture of *guaraní* and one of *guarú,* both from different localities than the original cultures, were also received.

All the species grow reasonably well in the standard Drosophila culture bottles, but the adult flies are very adversely affected by an advanced state of fermentation in the culture medium. To start a successful culture it is necessary to age flies for three or four days in one bottle and then transfer them to a fresh bottle; otherwise oviposition will probably not occur. Sometimes a second transfer in another three or four days is necessary. Flies left for more than three or four days in one bottle usually become stuck on the surface of the food medium. A group of flies which have begun to oviposit can be transferred from bottle to bottle every two or three days and will produce satisfactory numbers of eggs and larvae for from six weeks to two months. The addition of grated yeast to the bottles when the larvae are about half grown greatly increases the number of larvae which pupate. In culture bottles the flies breed best at relatively low temperatures, 17–20° C.

Aceto-orcein squash preparations were

made from brains of nearly full grown, feeding larvae. In these, metaphase and prophase figures of somatic mitoses were studied. The salivary chromosomes were studied in similar squash preparations of glands taken from larvae ready for pupation which had been raised at 17–18° C. in bottles provided with extra yeast.

The Mitotic Chromosomes

guaramunú sub-group

Drosophila guaramunú.—Metaphase plates (figures 1 and 2) show five pairs of rods and a pair of dots. Four pairs of rods are rather short but with some variation in length among them. All give evidence of subterminal centromeres and one pair often displays distinct satellites. The fifth pair of rods is more than twice as long as the others. These are sometimes alike and sometimes heteromorphic and are without much doubt the sex chromosomes. The X has two constrictions not far from the middle, setting off a central segment of about $\frac{1}{5}$ the total length. Sometimes the chromosome is bent at these constrictions, giving it the appearance of a V, but apparently the centromere is at one end or very near it and not at either median constriction. The Y, as observed in the plates, is a long rod often curved rather than bent. It contains one constriction about $\frac{1}{3}$ of its length from one end, and at the tip of the smaller segment there is a small but distinct satellite or bead. Prophases (figures 3 and 4) show the heterochromatin to be very largely concentrated in the two long rods, especially in the Y. The constrictions in the sex chromosomes visible at metaphase can be distinctly seen in most prophases.

Drosophila griseolineata.—In metaphase figures of this species (figures 5 and 6) there are also five pairs of rods and a pair of dots. The dots are distinctly smaller than those of *guaramunú*. Four pairs of rods are of approximately equal length and all are rather short. None bears a satellite and there is little evidence that the centromeres are anything but terminal. The fifth pair of rods is about twice as long as the others. Careful study shows these rods to be distinctly, if somewhat subtly, heteromorphic. One usually shows a submedian constriction and never a satellite. The other does not show any one definite constriction and does show a very definite satellite. The long rod with the satellite never occurs twice in the same cell; it is always paired with the one showing the constriction and having no satellite. The latter does often occur twice in the same cell. Therefore the long rod with the satellite is the Y, its homologue the X.

Prophase figures (figures 7 and 8) show the Y to be entirely heterochromatic and the X to contain some heterochromatic segments. The autosomes in general seem to contain very little heterochromatin. Thus the general chromosomal configuration of *guaramunú* and *griseolineata* is very similar although the total amount of heterochromatin seems to be smaller in the latter than in the former.

Drosophila guarajá.—The metaphase figures (figures 9 and 10) of this hitherto undescribed species differ from those of all other species of the group in showing five rather than six pairs of chromosomes. There are three pairs of rather short rods of slightly different lengths, a pair of V's and a pair of dots. The sex chromosomes must be the smallest pair of rods for while these are sometimes identical, they are also sometimes distinctly heteromorphic, one of them showing a very large, distinct satellite. This short rod with the large satellite never occurs twice in the same cell although its homologue often does. The short rod with the satellite must, therefore, be the Y and its partner the X. Both pairs of autosomal rods usually show very distinct satellites. Since the sex chromosomes have been identified as rods, the pair of V's must necessarily be autosomal. This offers an explanation of the reduced number of chromosomes in *guarajá*. Two autosomal rods have evidently become fused by translocation with the resulting loss of one centromere.

Prophases (figures 11–12) bear out the deductions made from the metaphase figures. The Y appears to be the only chromosome which contains any appreciable amount of heterochromatin. It appears to be wholly heterochromatic and shows up with its large satellite clearly visible. The X appears to contain little heterochromatin which is reasonable because of its small size. The satellites of the two pairs of autosomal rods are also clearly in evidence. The dots appear in some figures to have condensed precociously, but whether or not they con-

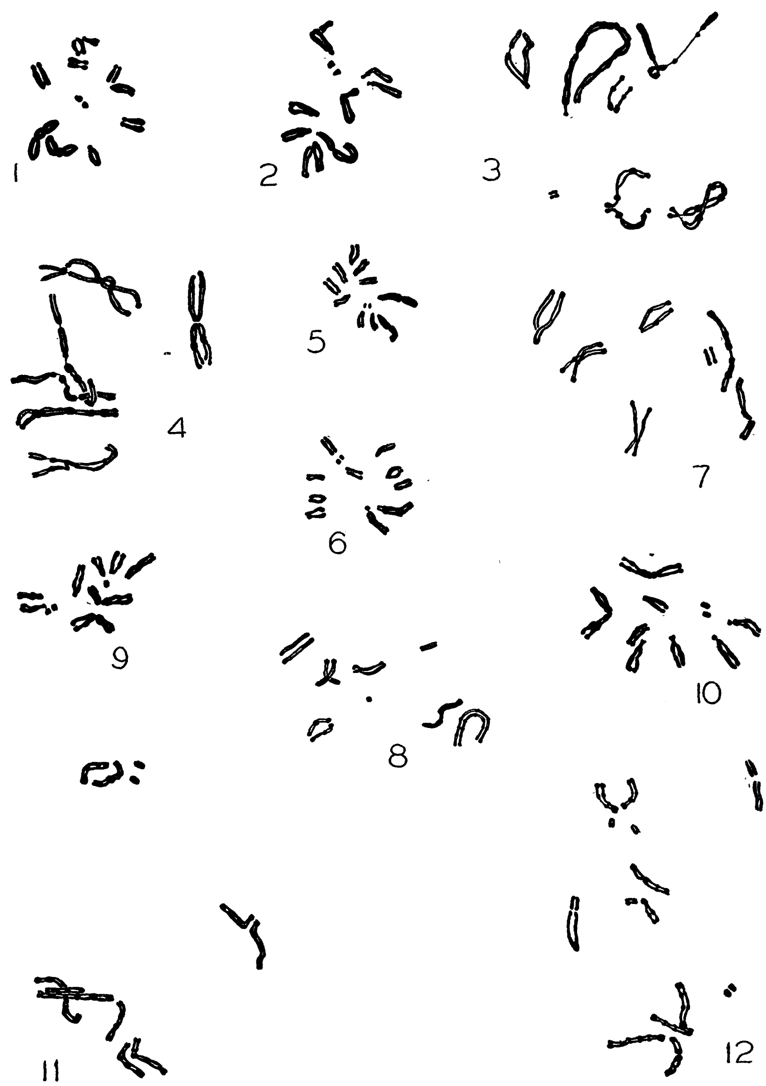

Fig. 1. *guaramunú* female metaphase.—Fig. 2. *guaramunú* male metaphase.—Fig. 3. *guaramunú* male prophase.—Fig. 4. *guaramunú* female prophase.—Fig. 5. *griseolineata* female metaphase.—Fig. 6. *griseolineata* male metaphase.—Fig. 7. *griseolineata* female prophase.—Fig. 8. *griseolineata* male prophase.—Fig. 9. *guarajá* male metaphase.—Fig. 10. *guarajá* female metaphase.—Fig. 11. *guarajá* female prophase.—Fig. 12. *guarajá* male prophase.

tain a large proportion of heterochromatin, is not at all clear. What appear to be dots in figure 11 are more probably the satellites of the adjacent pair of rods.

As was noted in pointing out the relationships of *D. guarajá,* the three species just discussed form a natural sub-group. They can be distinguished from each other morphologically, although the differences are rather slight, especially between *guaramunú* and *guarajá.* As to chromosomal configuration there are similarities between these species which set them off from the three species still to be discussed. In all of them the sex chromosomes are rods and in all the Y bears a distinct satellite. Passing now to *guarú, guaraní* and *subbadia* we come to three species which likewise form a distinct sub-group. They are set off morphologically from the species of the other sub-group, but among themselves they are extremely similar in morphology; and genically, as we shall see later, they are much more nearly alike than are *guaramunú, griseolineata* and *guarajá.*

guaraní-sub-group

Drosophila guarú.—Metaphase figures (figures 17 and 18) show six pairs of chromosomes: a pair of V's, four pairs of rods and a pair of dots. One pair of rods is perceptibly shorter than the others which are of approximately equal length. The rods all give evidence of having satellites or subterminal centromeres. The V's are of two kinds: one approximately equal-armed, one arm of which usually shows the equational split before the other, and one unequal-armed in a ratio of between 1 : 3 and 1 : 4 with often a secondary constriction in the long arm about one third of the way from the distal end. These heteromorphic V's are undoubtedly the sex chromosomes. Known male larvae always show one unequal-armed V and no cell has been seen which contained two unequal-armed V's. The equal-armed V is, therefore, the X and the unequal the Y.

Prophases (figures 19 and 20) confirm the subterminal location of the centromeres in the rods and show three pairs of rods with satellites. They also show the X chromosome with large heterochromatic sections, especially in one arm, and show the Y, the unequal-armed V, as entirely heterochromatic.

Drosophila guaraní.—This species is so similar in appearance to *guarú* that even one who has great familiarity with both species finds it extremely difficult to determine individual flies. Nevertheless, their chromosomes are unmistakably different. Metaphases of *guaraní* (figures 13 and 14) show six pairs of chromosomes: one pair of V's and five pairs of rods. The rods vary somewhat in length. Usually one pair is distinctly shorter than the others. All the rods appear to have satellites or slightly subterminal centromeres, and one pair seems to have the centromere so distinctly subterminal as sometimes to appear as a "J" with a short hook. The V's are about equal-armed, the arms being about as long as the longest rods. Often one arm shows a small satellite, and there is a secondary constriction in each arm a short distance from the centromere. There is nothing in the metaphase plates to indicate which pair of chromosomes constitutes the X and Y.

Prophase configurations (figures 15 and 16) bring to light two very interesting facts. First, the pair of V's appears to be composed largely of heterochromatin. Sometimes one V is apparently heterochromatic throughout while the other appears to be so in one arm only. This makes it clear that the V's are the sex chromosomes, that the Y is entirely heterochromatic and that the X is euchromatic in one arm only. Second, one of the shorter pairs of rods—whether or not the shortest is not clear—appears to be wholly heterochromatic with a dot-like satellite at one end. This observation is of particular significance as it suggests an explanation for the absence of a pair of dot-like chromosomes which are present in *guarú* and are so common among species of Drosophila. If a segment of heterochromatin had been translocated to the pair of dot-like chromosomes of *guarú,* we should have a configuration of two V's and five pairs of rods, precisely what we have in the metaphase plates of *guaraní.*

Drosophila subbadia.—This species closely resembles *guaraní,* but is more easily distinguished from it than is *guarú.* Its metaphase figures (figures 21 and 22) are very similar to those of *guaraní.* There are five pairs of rods and sometimes a pair of equal-armed V's. Other cells show one equal-armed V paired with

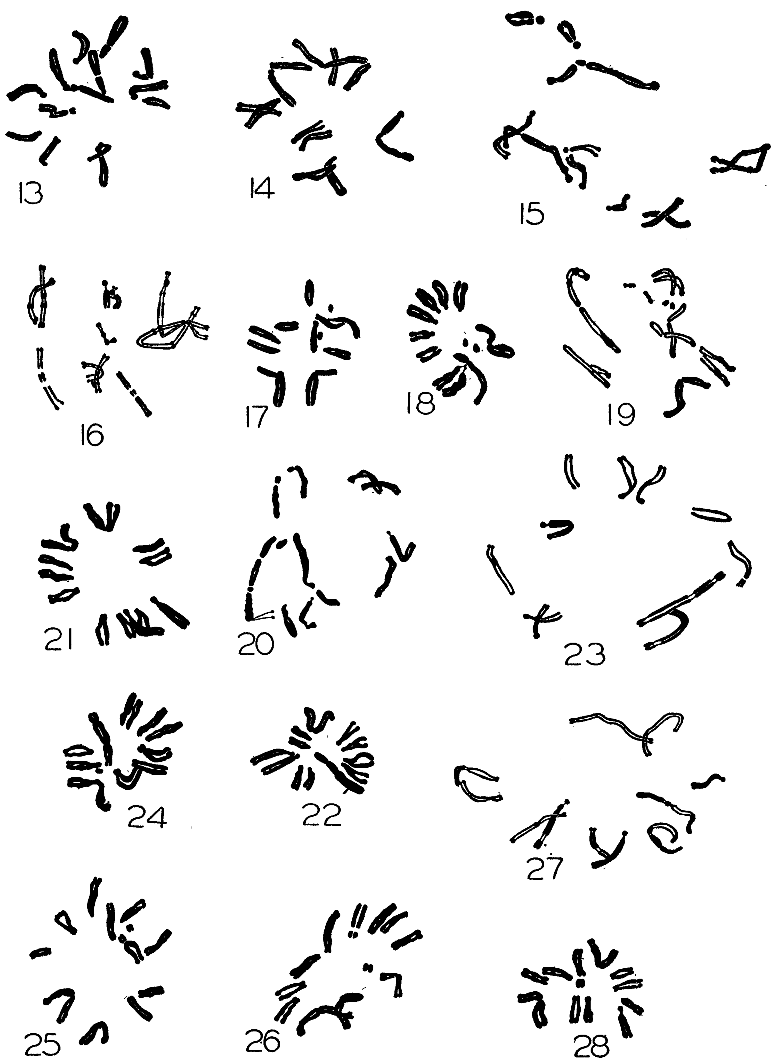

Fig. 13. *guaraní* metaphase.—Fig. 14. *guaraní* metaphase.—Fig. 15. *guaraní* prophase.—Fig. 16. *guaraní* prophase.—Fig. 17. *guarú* female metaphase.—Fig. 18. *guarú* male metaphase.—Fig. 19. *guarú* male prophase.—Fig. 20. *guarú* female prophase.—Fig. 21. *subbadia* female metaphase.—Fig. 22. *subbadia* male metaphase.—Fig. 23. *subbadia* prophase.—Fig. 24. *guarú* X *subbadia* male metaphase.—Fig. 25. *guarú* X *subbadia* female metaphase.—Fig. 26. *guarú* X *subbadia* female metaphase.—Fig. 27. *subbadia* X *guarú* female prophase.—Fig. 28. (*guarú* X *subbadia*) X *guaraní* male metaphase.

a very long rod with two constrictions, one at about the middle and the other about half way between this and one end. The long rod is evidently the Y chromosome as it is found in cells of larvae known to be male. Sometimes it is bent at the constriction nearest one end making it an unequal-armed V similar to the Y of *guarú*. The chromosomes of *subbadia* differ from those of *guaraní* in that the sex chromosomes are definitely heteromorphic. They also differ in that one pair of rods in *subbadia* is very distinctly longer than the four others. As in *guaraní* and *guarú* all rods show indications of subterminal centromeres. No detatched satellites have been observed.

In prophase figures (figure 23) the X and Y have not been distinguished, but the sex chromosomes can easily be distinguished as being more heterochromatic than four of the five pairs of autosomes. The fifth pair of autosomes, as in *guaraní,* appears to be wholly heterochromatic with a dot-like satellite at one end.

Thus the members of the *guaraní* subgroup show chromosomal similarities in spite of the fact that *guarú* possesses a pair of dots which is replaced in *guaraní* and *subbadia* by a pair of rods. In all three species the X chromosomes are large V's, and in two, *guarú* and *guaraní,* the Y is also a large V. In general the autosomal rods are longer than those in the *guaramunú* sub-group. The total chromosomal material appears to be greater and this seems to be largely the result of an increased amount of heterochromatin.

Mitotic Chromosomes of Hybrids

Interspecific hybrids may easily be obtained in large numbers by mating *guarú* females and *subbadia* males. The reciprocal cross produces a few offspring. The chromosomal configuration in these hybrids is of great interest for here we are able to see chromosomes of the two species side by side in the same cell. The most striking characteristic of the metaphases (figures 24, 25 and 26) is that they show an uneven number of rods. Just as would be expected from the separate analysis of the chromosomes of the two species, one dot has as its homologue a rod. In some cases the dot element of this rod appears slightly separated from the heterochromatic portion of the chromosome; in other cases the dual make-up of this chromosome is merely suggested. In plates from known male larvae (figure 24) it is easy to recognize the long tripartite Y of *subbadia*. The V which accompanies it must, of course, be that of *guarú*. When the two X's appear together in the cells of female larvae (figures 25 and 26), it is possible to distinguish the two. The *subbadia* X is more nearly equal-armed and tends to have a secondary constriction near the distal end of each arm. Finally, the longest pair of rods is also heteromorphic: the longer stemming from the male *subbadia,* the slightly shorter from the *guarú* female.

Favorable prophase figures (figure 27) also demonstrate these differences exactly as would be expected. Since dot chromosomes often fail to show up in prophases, it often happens in these hybrids that the heterochromatic rod which contains the dot element in *subbadia* appears without a partner giving the effect of an eleven chromosome figure.

It has been found impossible to obtain hybrids between *guaraní* and *subbadia* or between *guaraní* and *guarú,* even though in many respects *guaraní* appears to be morphologically intermediate between the two. Since for reasons of chromosomal study it was very desirable to involve *guaraní* in some sort of liaison, an attempt was made to mate *guaraní* males to female *guarú-subbadia* hybrids which were known to be fertile in back-crosses. This highly unconventional arrangement does produce some off-spring, and both somatic mitoses and salivary gland chromosomes of such triple hybrid larvae have been studied.

Metaphases of these hybrids (figure 28), which are almost invariably males, show necessarily a *guaraní* Y. In all plates sufficiently favorable to make determination possible, the X appears to be that of *guarú*. All the plates which have been examined show one dot, which must have come from *guarú,* paired with a rod which must be attributed to *guaraní*. In some cases one of the autosomal rods appears perceptibly longer than its homologue and is probably the very long autosomal rod seen in metaphases of *subbadia*. The fact that no females are produced in this cross suggests that there is an incompatibility between the *guaraní* X and the X of either *guarú* or *subbadia*. Furthermore, the absence from male larvae of the *subbadia* X and of that *subbadia* autosome which is largely heterochromatic strongly suggests that only a restricted number of the possible chromosomal combinations is viable and that the others fail to develop.

Salivary Chromosomes

Salivary gland cell preparations of all species of the group are unfavorable for detailed observation. Often the nuclei of the cells do not break when the squash preparations are made, and when they do, the salivary chromosomes generally remain snarled around each other and many of the strands are snarled back upon themselves making it very difficult to find the free ends. If enough trials are made, however, some favorable cells can be found.

The salivary chromosomes of the species of the *guaramunú* subgroup have been investigated only for general configuration and all three species show five long strands and a dot. This is what one should expect from the metaphases of *guaramunú* and *griseolineata,* one long strand for each paid of rods and a euchromatic dot corresponding to the pair of dots. The case of *guarajá* is particularly interesting because it shows that both arms of the V's must be euchromatic and also because its chromocenter is strikingly small. This bears out the evidence of the prophases that there is very little heterochromatin in the entire chromosomal complement of the species. Since what little heterochromatin there is seems to be in the Y, one would expect the chromocenter in the cells of male larvae to be distinctly larger than those in the cells of female larvae; but this has not been definitely established.

In general configuration the salivary chromosomes of the *guaraní* sub-group also show five long strands and a euchromatic dot. When the chromocenter is broken, four strands pull free with a small amount of heterochromatin adhering to their bases and the rest of the chromocenter remains attached to one strand which is associated with the nucleolus. This latter strand undoubtedly represents the X for it is paler in the cells of male larvae. The euchromatic dot is usually embedded in or attached to the large mass of heterochromatin adhering to the X. These observations on the salivary chromosomes of this subgroup confirm the deductions that the metaphase V's are the sex chromosomes, that the X is euchromatic in only one arm, and that in *guaraní* and *subbadia* the fifth pair of rods is largely heterochromatic and contains only a dot-like euchromatic element.

Of all the pure species of the group, direct and intensive study of the salivary chromosomes has been carried out only in the case of *guarú*. Here it has been possible to identify the tips and bases of the five long strands, although the identification of more than two strands in any one cell is possible only rarely.

The most interesting thing about the *guarú* salivaries is the number of inversions which they show. It has not been possible to make an exhaustive list of these because the chromosomes are so infrequently disentangled. However, certain of them have been definitely identified. The diploid X appears to contain several inversions, but this chromosome is almost invariably snarled and contorted to such an extent that only one small inversion near the base has been definitely isolated. The four long autosomes afford somewhat more favorable observations. They have been lettered A, B, C and D as identified by their tips and bases. Chromosome A (figure 29) is usually spread out more favorably than any other. It has a very distinctive tip and a subterminal bulb. It often contains the large double inversion shown in the figure. It lies in the basal half and has one large and one small loop. This inversion has been seen many times. Chromosome B (figure 32) is much less frequently favorable for observation, but the complicated double inversion shown near the tip and the large single inversion to be seen near the base have been identified. Chromosome C is also usually unfavorable for observation, but apparently it contains a subterminal single inversion. No inversions have been definitely identified in chromosome D, and no evidence of rearrangement has been observed in

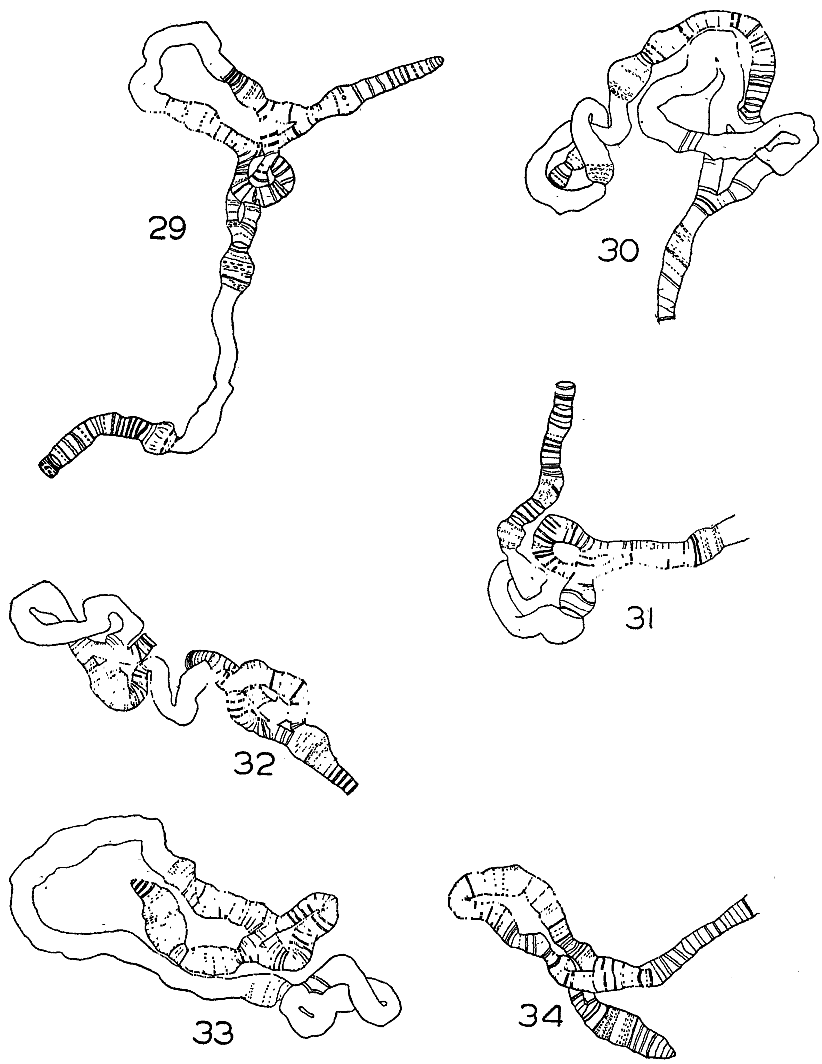

Fig. 29. *guarú* salivary chromosome A.—Fig. 30. *guarú* × *subbadia* salivary chromosome A.—Fig. 31. *guarú* X *subbadia* salivary chromosome A.—Fig. 32. *guarú* salivary chromosome B.—Fig. 33. *guarú* X *subbadia* salivary chromosome B.—Fig. 34. *guarú* X *subbadia* salivary chromosome B.

the dot, chromosome E. There are undoubtedly other rearrangements. Some of them have been observed, but under circumstances when it was impossible to tell in which chromosome or in what part of it they lay or when they were not sufficiently clear to make identification possible. The number and persistence of these inversions are the more remarkable since they have all been found in the strain of *guarú* descended from a single female collected at Amalia.

A second strain of *guarú* collected at Bertioga has not been intensively studied for inversions. But examination of the glands of four larvae showed inversions in chromosomes A, B and C. Not one of these appeared to be identical with any inversion known in the Amalia strain. One of them, a small subterminal inversion in chromosome A including the bulb, was particularly clear and unmistakable.

Another interesting phenomenon observed in the salivary chromosomes of *guarú* concerns the boundary between the euchromatin and the heterochromatin at the base of the X. This is not the same in all cells. A rather sizeable segment which shows discrete discs in some cells is entirely assimilated to the chromocenter in others, and various intermediate conditions can also be found. This is precisely the condition described by Dobzhansky (1944) in the salivary chromosomes of *D. pallidipennis,* in this case also in the X.

Salivary Chromosomes of the Hybrids

The salivary chromosomes of the *guarú-subbadia* hybrids are even more interesting than the mitotic ones. Perhaps the most striking fact is that these chromosomes show excellent pairing, quite as intimate as in either pure species, and there is no evidence that any part of any chromosome fails to find its effective homologue. As must follow from these observations, the general configuration in these salivaries is precisely the same as in either pure species.

Since numerous inversions occur in the salivaries of the pure species *guarú,* they must also appear in the hybrids. Actually many inversions are found there. Some of them cannot be identified with any known inversions occurring in *guarú.* This is not at all surprising since the chromosomes of each of two species might be expected to contain gene arrangements not shared by both. Some inversions in the hybrids, however, can be identified with some which exist in the pure species of *guarú,* and these are of very great interest. The large double inversion which is often seen in the basal half of chromosome A of *guarú* has never been found as such in the hybrids. Nevertheless, the two elements of this inversion can be found separately in the hybrids as single inversions (figures 30 and 31). These facts can have but one meaning. The laboratory strain of *guarú* (Amalia) contains two gene arrangements of chromosome A, neither of which could have come into being from the other without the existence of an intermediate arrangement which apparently is not present. This missing arrangement is, however, present in the laboratory strain of *subbadia.* An exactly analogous situation occurs in the subterminal segment of chromosome B. The double inversion found there in pure *guarú* turns up in the *guarú-subbadia* hybrids as two separate single inversions (figures 33 and 34).

The intimacy of pairing and the facts concerning gene arrangements in the *guarú-subbadia* hybrids establish incontrovertibly that the two species have at some time been parts of one interbreeding population.

The salivaries of the triple hybrids, unlike those of the pure species or of the *guarú-subbadia* hybrids, show a substantial amount of failure of pairing. However the pairing is surprisingly complete, more so than is commonly the case in hybrids between two species of Drosophila. Providentially, one cell was found in which all the chromosomes were well spread out and could be identified. It

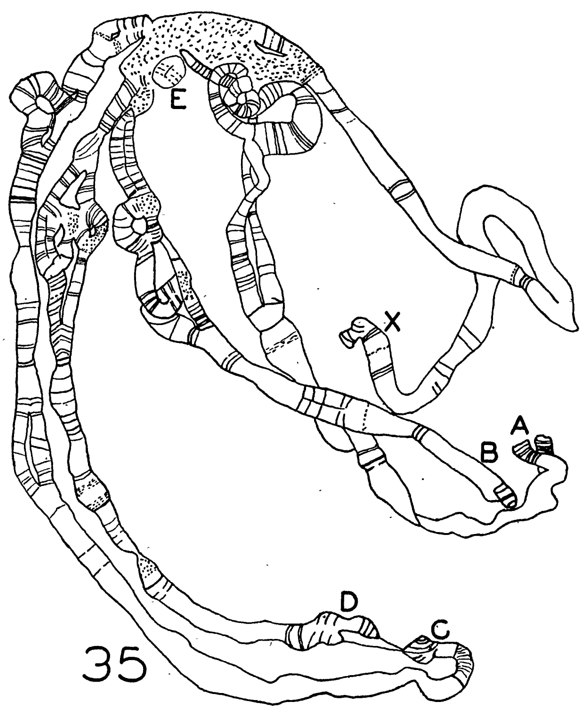

FIG. 35. (*guarú* X *subbadia*) X *guaraní*. Drawing of salivary gland cell.

was drawn and photographed (figures 35 and 36) and shows surprisingly few rearrangements. In chromosome A there appear to be three inversions in the basal portion, the same region where cells of *guarú* often show a double inversion. In chromosome B there seems to be a double inversion in the basal region near the area where a single inversion has been found in *guarú*. Chromosome C shows a subbasal single inversion, very possibly the same as has been found in *guarú-subbadia* hybrids. Chromosome D shows a double inversion in the basal portion. This is the only case in which a rearrangement has been seen definitely in this chromosome.

In the triple hybrids the tip of chromosome A shows a very interesting formation. This is the tip which is most easily identified in both *guarú* (figure 37) and *subbadia* and it appears unchanged in hybrids between them. In the triple hybrids (figure 38) this tip is very often unpaired for a short distance. Sometimes, however, as in the figure, it is paired at the extreme tip and then unpaired for a very short distance immediately behind. Careful examination of this unpaired region brings to light some minute differences in the bands there. These differences may be the result of a very short inversion, a very small duplication, or some other alteration of a few bands in this restricted area. At any rate while the tip of this chromosome is identical in *guarú* and *subbadia*, the *guaraní* tip differs in some very subtle way. The differences observed here are very similar to those discussed by Metz (1937 and 1938) in the salivary chromosomes of Sciara where they seem to be very common. As Metz notes, indications of similar structures have been found in certain other cases of Drosophila hybrids, although they seem to be much less common there than in Sciara.

Thus the chromosomal analysis of

FIG. 36. Photograph of same cell figured in figure 35.

these three species demonstrates that *guarú* and *subbadia* which differ most in general appearance are actually more nearly identical genically than either is with *guaraní,* although in general aspect the latter appears to be intermediate between the two. Nevertheless, the three species are so similar genically that an individual containing a chromosome complement drawn from all three species can carry its development successfully to the adult state. One must conclude that not only *guarú* and *subbadia* but all three species have at some time constituted one single interbreeding population.

DISCUSSION

The chromosomal configuration at metaphase which is most widely distributed throughout the genus Drosophila consists of five pairs of rods and a pair of dots. This configuration is commonly

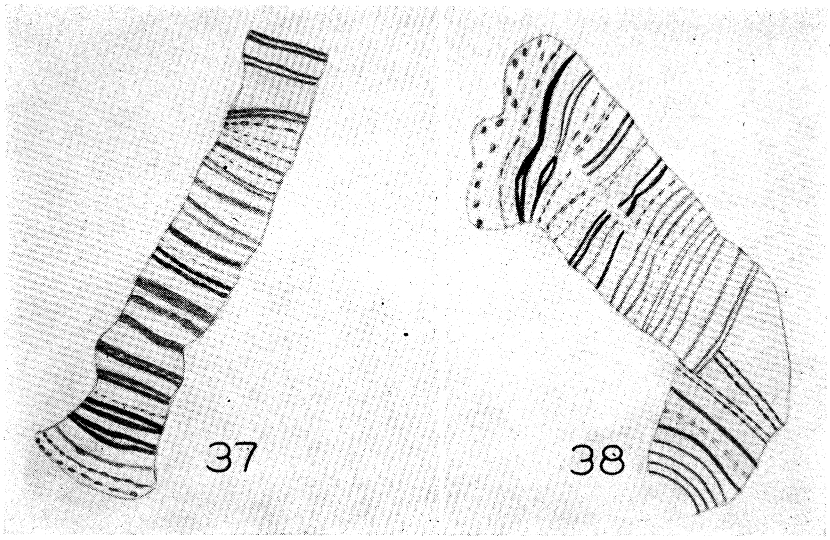

FIG. 37. *guarú*. Tip of salivary chromosome A.
FIG. 38. (*guarú* X *subbadia*) X *guaraní*. Tip of salivary chromosome A.

believed to be a generalized one because, among other reasons, all other known configurations can be derived from it through a series of plausible steps and because the most obviously specialized and aberrant forms usually depart from it.

Of the six species in the *guaraní* group this primitive configuration is seen in both *griseolineata* and *guaramunú*. The chief difference between the two lies in the length of the pair of rods which constitutes the sex chromosomes. In *guaramunú* both the X and the Y are distinctly longer than they are in *griseolineata*. This appears to be merely a difference in the amount of heterochromatin. The Y in both species seems to be entirely heterochromatic; the X of *guaramunú* shows a substantial heterochromatic segment while that of *griseolineata* gives evidence of very little. It is easy to imagine the origin of the *guaramunú* configuration from that of *griseolineata*. The Y could be lengthened by one or more duplications; the X by one or more translocations to it of heterochromatic sections from the Y. Or the *griseolineata* configuration could arise from that of *guaramunú* by the loss of heterochromatic segments from both the X and Y.

The chromosomes of *guarajá* are actually very similar in general configuration to those of *griseolineata* even though *guarajá* is the one species of the group possessing five rather than six centromeres. The autosomal V of *guarajá* must have arisen through a translocation of one rod which had lost its centromere to another rod at a point on the opposite side of its subterminal centromere. The occurrence of such a translocation in *griseolineata,* if accompanied by a lengthening of the X and Y through the addition of heterochromatic segments, would give a configuration almost identical with that found in *guarajá*.

It is interesting to note that the three species *guaramunú, griseolineata* and *guarajá,* which are very similar in so many respects, all have definite satellites on the Y chromosome.

Of the three remaining species, *guarú* is less different than the other two from the three just discussed. The chief difference lies in the fact that here the sex chromosomes are V's. A long rodshaped Y could become a V by the simple expedient of a pericentric inversion. Such has evidently occurred in *guarú* giving a V with unequal arms. The X could become a V either by a pericentric inversion or by the translocation of a heterochromatic segment from the Y to the short side of a subterminal centromere.

Passing to *subbadia,* the chief difference between this species and *guarú* is the replacement of the dot chromosomes by another pair of rods. We know that this was achieved by the translocation of a heterochromatic segment to the dots. Again, the most likely explanation is a translocation of an acentric fragment from the Y. An interesting point is provided by the pair of autosomal rods of *subbadia* which are so definitely longer than the corresponding pair in *guarú* and in *guaraní.* It may be that these rods have been lengthened in *subbadia* by another translocation of heterochromatin. The prophase (figure 27) of the *guarú-subbadia* hybrid suggests this explanation by the heteromorphism of the pair of chromosomes at six o'clock. But the pair at seven o'clock is also distinctly heteromorphic, and the prophase of *subbadia* (figure 23) does not clearly show any pair of autosomes, except those replacing the dots, as definitely more heterochromatic than the others.

The chromosomes of *guaraní* differ from those of *subbadia* largely in the greater total length of the Y which has become an equal-armed V. This change could easily have come about either by duplication or by translocation.

Within the six species of the group all major differences of chromosomal configuration can, with one exception, be explained by differences in amount and arrangement of heterochromatin. This is true for example of two species of which the metaphase plates present such completely different pictures as do those of *griseolineata* and *guaraní.* In fact the six species can be arranged according to the increasing relative amount of heterochromatin in the following order: *guarajá, griseolineata, guaramunú, guarú, subbadia* and *guaraní.* The one exception is the presence of the autosomal V in *guarajá;* and this is explainable as a comparatively simple translocation of euchromatic rather than heterochromatic material.

From the above analysis it seems safe to make the generalization that differences of chromosomal configuration between two species indicate little as to differences of genic balance. Differences of general configuration are evidence of degree of relationship only to the extent that one can estimate the probability of the occurrence of the chromosomal breaks which must be postulated to account for the differences. Two species having very different chromosomal configurations may nevertheless have very nearly identical genic make-up, *viz. guarú* and *subbadia.* The converse, that two species with apparently identical chromosomal configurations may be worlds apart in genic balance, is, of course, a truism.

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Summary

The chromosomes of the six species of the *guaraní* group of Drosophila are described and compared in metaphase and prophase of somatic mitoses and in salivary cells. Noticeable differences in metaphases and prophases can, with one exception, be explained as differences in amount and arrangement of heterochromatin. In one case the difference is the result of the loss of a centromere and the

fusion of two chromosomes. The salivary chromosomes bear out these conclusions.

Three of the species can be involved in crosses producing hybrids which make possible a more exact comparison of the chromosomes of these three species in metaphases, prophases and salivaries.

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