ON THE DOT CHROMOSOMES OF DROSOPHILA REPLETA AND D. HYDEI

A. H. STURTEVANT

William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena

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INTRODUCTION

DROSOPHILA HYDEI Sturtevant and *D. repleta* Wollaston[1] are closely related species, both frequent in association with man in many regions—at least throughout the United States, where both species are evidently of relatively recent introduction. There are numerous other species of the same group, mostly found in the Neotropical region and in the southern portion of the United States. All these agree in having a pale gray pollinose dorsal surface of the mesonotum, each hair and bristle arising from a dark blackish-brown non-pollinose spot. This spotted pattern has furnished the basis for most of the studies reported here.

D. repleta has the chromosome configuration that is most characteristic for the subgenus Drosophila to which it belongs—five pairs of rods (one of which is X) and a pair of dot-like chromosomes. It is, of course, probable that each of these chromosomes also has a short heterochromatic arm (see STURTEVANT and NOVITSKI 1941). *D. hydei* differs in its metaphase configuration only in that there is an additional arm visible on the X; this arm appears to be wholly heterochromatic. *D. neorepleta* Patterson and Wheeler, from Guatemala, resembles *repleta* in its external characters even more than does *hydei;* it differs from *repleta* cytologically in that one of the rod-shaped autosomes has become J-shaped—presumably through the occurrence of a pericentric inversion. For our present purposes the significant point is that the dot chromosome, or element F (STURTEVANT and NOVITSKI 1941), is present in all three species (for *neorepleta* see footnote on p. 261).

D. REPLETA

DR. E. NOVITSKI subjected *D. repleta* to X-rays and obtained a dominant mutant gene, called "Stubble" (symbol *Sb*). The Stubble phenotype somewhat resembles the character bearing the same name in *melanogaster*, but is less extreme. The bristles are shortened and taper less than do those of wild type. There is never any difficulty in classifying for *Sb*. *Sb* is lethal when

[1] This name is well established in the literature, but may be incorrect. The type specimens, from Madeira, I saw at the British Museum in 1922. At that time I was aware of the existence of only four species in this group, and these specimens most resembled the species now given this name; they were, however, surprisingly small and dark. I am now inclined to suspect that they represent some other species—perhaps *D. buzzatii* Patterson and Wheeler. However, if this be so, the correct name to apply here will still be in doubt, since there are several supposed synonyms that will be difficult to identify with certainty. For the present it seems best to retain the name *repleta.*

homozygous, and was long kept in a selected stock, but has now been balanced by a recessive lethal that arose in a *Sb*/+ stock.

On outcrossing *Sb* to wild type, I have found that a new phenotype is regularly produced. This type, which may be called "Diminished" (*Dm*), is characterized as follows: darker than wild type, owing to an extension of the dark spots surrounding the bases of the hairs and bristles; smaller than wild type; bristles rather blunt, but by no means Stubble; furrow between mesonotum and scutellum deep; both sexes wholly sterile. Table 1 shows the frequencies with which this type was produced.

TABLE 1

Offspring of Stubble flies.

MATING	*Sb*	+	*Dm*	*Dm Sb*	TOTAL
+ ♀ × *Sb* ♂	485	446	55	0	986
Sb ♀ × + ♂	197	207	49	1	454
Sb ♀ × *Sb* ♂	338	120	20	15	493

Taking into account the X-ray origin of Stubble, these results leave little doubt that we are dealing with a translocation. The Diminished flies then are aneuploid, and there is presumably a complementary class that is inviable. Since males and females are approximately equally frequent among both *Sb* and *Dm* flies in all three crosses, the two chromosomes involved are evidently both autosomes. The most likely situation then is that one of them is the dot, most of the genes of which have been exchanged for a small portion of one of the four rod-shaped autosomes. The *Dm* flies then are haploid for most of the dot, and triploid for the small portion of a rod that is now attached to the dot centromere. This interpretation also accounts for the *Dm Sb* flies; they carry a normal rod, both translocated chromosomes (two of the shorter one, one of the longer one; the latter is responsible for the *Sb* phenotype), but no normal dot. In the second cross the individual of this type was due to primary non-disjunction of the dot in the wild type father; presumably it had only one small translocated chromosome. This interpretation is confirmed by the observation that sterile individuals having this phenotype are occasionally found in pure wild type stocks, both of *repleta* and of *neorepleta*, and among the hybrids discussed below.

The frequency with which the "irregular" type of segregation occurs in *Sb* flies can hardly be determined, since the *Dm* flies seem to be distinctly less viable than their + or *Sb* sibs; it may be noted, however, that the segregation seems to be more often regular in the males (5.6 percent *Dm*) than in the females (10.8 per cent *Dm*).

It was first shown by NOVITSKI (unpublished data) that the mating *Drosophila neorepleta* ♀ × *D. repleta* ♂ occasionally produces a few offspring. Neither NOVITSKI nor I have ever been able to get any offspring from the reciprocal mating. WHARTON (1942, 1944) reports the same result, though the table in the latter paper (p. 177) gives the reverse result. Miss WHARTON in-

forms me (private communication) that her observations agree with ours and with her text, not with her table.

The F_1 hybrids are rarely produced, and the males are all wholly sterile, with poorly developed testes; but the females occasionally produce a few offspring when mated to *repleta* males—never, in my experience, when mated to *neorepleta* males. Some of the females from this backcross are moderately fertile, and a study of their genetic behavior will be reported elsewhere. For present purposes the significant point is that hybrid strains from such a source have repeatedly produced a light-colored type that behaves as though it were due to a dominant gene, and have also occasionally produced a sterile dark type that closely resembles the one derived from Stubble.

The Light (symbol *Lt*) specimens may be supposed to have three dot chromosomes, since their pattern differs from that of wild type in the opposite direction from that of *Dm*. Presumably when first produced they carry two dots derived from *repleta* and one from *neorepleta*.[2] After repeated backcrossing to *repleta* it is more likely that all three are of *repleta* origin; no phenotypic difference has been observed that could be attributed to such a difference in origin of the dots.

When *Lt*×+ or +×*Lt* matings are made, there result *Lt* and + flies in approximately equal numbers in both sexes. The mating of *Lt*×*Lt* gave 576 *Lt*: 229+; but it was observed that some of the Light flies from such matings are more extreme than the usual type. The dark spots have almost disappeared, and the groove between the mesonotum and scutellum is very shallow —again a character opposite in sign to that found in *Dm*. Such extreme Light flies are sterile. They evidently carry four dot chromosomes. In the crosses where their identification was attempted, the count was: *Lt* ♀ × *Lt* ♂ → 81 Extreme *Lt*, 337 *Lt*, 161+. Evidently the viability of tetra-dot is about one-half that of triplo-dot or of wild type.

Lt and *Sb* were crossed, and it was observed that flies showing both characters had the Stubble phenotype fully developed. Unfortunately the genetic tests of such *Lt Sb* flies were not carried out extensively, nor were the matings planned to give the simplest analysis; accordingly it does not seem necessary to present what data were obtained.

In the absence of these critical crosses, and in the absence of all cytological data, the evidence for the interpretation of the *Dm* and *Lt* types (as being haplo-dot and triplo-dot, respectively) is not conclusive. There can, I think, nevertheless be no doubt that such is their nature, since their properties are in such good agreement with the data from *hydei*, now to be described.

D. HYDEI

D. hydei is closely related to *D. repleta* and resembles it in all essential respects that concern characters used in identifying the *Dm* and *Lt* types of

[2] WHARTON (1942) shows that there is no dot-shaped chromosome in *neorepleta;* her observations are confirmed by DR. NOVITSKI. It is evident, however, from the chromosome numbers and from WHARTON'S report on the salivary gland chromosomes, that element F is present as a separate chromosome, but has acquired enough heterochromatin to make it impossible to identify it in metaphase figures.

repleta; it was to be expected, therefore, that the corresponding types of *hydei* could be worked with. This expectation has been partially fulfilled for *Lt*, but *Dm* has not been observed. The chief reason for turning to *hydei* is the existence of strains carrying mutant genes located in the dot chromosome. The two types, both discovered and located in the dot by DR. W. P. SPENCER, but not yet described, were supplied by him, along with his generous permission to use them and to publish his localization of them.

One of these types, Extended (symbol *Ex*), is characterized by an extension of the dark spots at the bases of the hairs and bristles. In homozygous Extended the gray ground of the mesonotum and scutellum is wholly obliterated, producing a blackish-brown fly, and the head and thorax are also somewhat abnormal in shape. In *Ex*/+ the shape is normal, but there is a marked and easily identified darkening of the mesonotum, especially along its lateral margins. A sample count from *Ex*/+ × *Ex*/+ gave 51 *Ex*/*Ex*: 100 *Ex*/+ : 45 + —indicating approximately equal viability for all three classes.

The other mutant strain received from SPENCER apparently carries two mutant genes in the same dot chromosome—though the two have not been separated by crossing over. One is a recessive, grooveless, very similar to the dot chromosome character of the same name in *melanogaster* and *pseudoobscura.* This character is rarely seen, since most flies homozygous for the chromosome in question fail to survive; no use was made of the grooveless character in the present studies. The other mutant gene in this chromosome is a dominant called Cubitus interruptus (*Ci*). In *Ci*/+ flies, the fifth vein is interrupted between the posterior crossvein and the anal crossvein—this section is in fact usually less than half present. In *Ci*/*Ci* flies, when they occasionally survive, the character is similar, but distinctly more extreme, and includes a slight effect on the fourth vein. The parallelism to the cubitus interruptus of *melanogaster* may be questioned, since that character involves the fourth vein much more markedly than it does the fifth.[3] *Ci*, however, does closely resemble the description and figure given by CHINO and KIKKAWA (1933) for the dominant Gap in the dot chromosome of *virilis.*

From two cultures of *Ci*/*Ex* mated together the counts were: 214 *Ci*/*Ex*, 86 *Ex*/*Ex*. The *Ci* chromosome here, as usual, acted as a lethal, and the expected ratio therefore is 2:1. There is a slight deficiency of *Ex*/*Ex*, which was shown above to have (in another experiment) normal viability. It may be concluded that heterozygosis for *Ci* does not decrease the viability. It should be added that I have never detected crossing over between *Ci* and *Ex*, though no extensive tests have been made.

The first tests for non-disjunction of the dot were carried out with *Ex*. Since *Ex*/+ resembles the haplo-dot of *repleta*, the most hopeful test seemed to be that based on the assumption that +/+/*Ex* would be more nearly wild type in pattern than is +/*Ex*—an expectation that was in fact realized. Wild type

[3] This discrepancy raises a question as to the appropriateness of the name applied. It would appear, however, that the fifth vein, rather than the fourth, is best considered the cubital. The type with the questionable name then is the *melanogaster* one rather than the *hydei* one; and the name is now too well established in the genetic literature on *melanogaster* to be changed.

strains were crossed, to produce +/+ flies in which the dot chromosomes were of diverse geographical origins, and such flies were then mated to *Ex/Ex*. All the regular offspring are expected to be +/*Ex*, but if non-disjunction occurs in the +/+ parent, +/+ gametes will give +/+/*Ex* flies. The results obtained are shown in table 2.

TABLE 2

Tests for primary non-disjunction of dot chromosomes in D. hydei.

SOURCE OF + CHROMOSOMES	SEX OF WILD TYPE PARENT	+/*Ex* OFFSPRING	+/+/*Ex* OFFSPRING
Alabama; Iowa	♀	755	0
Alabama; Iowa	♂	511	0
Yucatan; Iowa	♀	892	9

The nine +/+/*Ex* were all from a single pair mating (among six of this composition) and may perhaps have been due to an irregular somatic division, rather than to nine separate meiotic ones. They had 141 +/*Ex* sibs. Phenotypically these flies were intermediate between +/+ and +/*Ex*. Later experience has indicated that they can be separated from the latter with little difficulty, but not always with complete certainty from the former—though it is usually possible to be reasonably certain of the composition of a few individuals of each class. Accordingly, in table 3 three classes are recorded: "+," "+/*Ex*," "*Ex*." The two latter are both diplo-dot in the first four crosses; the first includes +/+/+, +/+, and +/+/*Ex*; and the "+/*Ex*" class from the last cross also includes +/*Ex*/*Ex*.

TABLE 3

Offspring of triplo-dot hydei carrying Extended.

♀ PARENT	♂ PARENT	"+"	"+/*Ex*"	"*Ex*"	TOTAL	% *Ex* GAMETES
+/+/*Ex*	+/+	167	28	0	195	14.4
+/+	+/+/*Ex*	193	91	0	284	32.1
+/+/*Ex*	*Ex*/*Ex*	105	924	164	1193	13.8
Ex/*Ex*	+/+/*Ex*	70	145	68	283	24.1
+/+/*Ex*	+/+/*Ex*	576	318	47	941	—

The +/*Ex*/*Ex* class is darker than +/*Ex*, but it has not been found possible to distinguish the two in every case—though again it is possible to select individuals that are clearly of either composition. The +/+/+ class corresponds to the Light type of *repleta* and agrees with it in having smaller dark spots. It is, however, less sharply different from wild type, and here also it has been found possible only to identify some of the more extreme individuals.

To recapitulate: the order of increasing darkness (or increasing size of

dark spots) is: +/+/+, +/+, +/+/*Ex*, +/*Ex*, +/*Ex*/*Ex*, *Ex*/*Ex*. The *Ex*/*Ex*/*Ex* class has not been identified. In this seriation only two of the intervals are sharp enough to allow certain separations—that between +/+/*Ex* and +/*Ex* and that between +/*Ex*/*Ex* and *Ex*/*Ex*.

Triplo-dot specimens from these experiments were crossed to the *Ci* strain, and it was found that *Ci*/+/+ is intermediate between +/+ and *Ci*/+; unfortunately it overlaps both of these classes, so that counts were found to be quite unreliable—though once again some individuals could be identified, as was shown by genetic tests involving *Ex*. The *Ci*/*Ci*/+ class was also produced; it showed the *Ci* character in extreme form, but could not be sharply separated from *Ci*/+. Because of these classification difficulties it does not seem desirable to record the crosses that were made. These experiments, however, were adequate to confirm the interpretation of the *Ex* experiments as being due to an extra dot chromosome, rather than to an independently inherited inhibitor of *Ex*.

The final proof of the correctness of the interpretation, however, was obtained by cytological methods. DR. K. W. COOPER examined the neuroblast cells of three larvae from a mating of +/+/*Ex* × +/+/*Ex* and found two of them to have three dot chromosomes (fig. 1a), while the third had four (fig. 1b). This tetra-dot specimen raises the problem of whether or not such individuals survive to the imaginal stage, as they evidently do in *repleta*. No specimens were so identified, and the data of table 3 suggest that they are not fully viable; the question whether or not they ever survive cannot yet be answered.

The data of table 3 indicate that, in *hydei* as in *melanogaster*, segregation is not random in triplo-dot flies. With random segregation the mating between +/+/*Ex* and *Ex*/*Ex* (in either direction) should give 2+/*Ex*/*Ex*: 2+/*Ex*: 1+/+/*Ex*: 1*Ex*/*Ex*—or, grouping the classes that cannot be distinguished, 1"+": 4"+/*Ex*": 1"*Ex*." Mating of +/+/*Ex* to +/+ should give, after similar grouping, 5"+": 1"+/*Ex*." Examination of the table shows that this result is approximated by the triplo-dot females, though there is probably a real deficiency of ++−*Ex* segregation as compared to +−+*Ex*. In the males, however, there is a distinctly more marked *excess* of ++−*Ex* segregation. Unfortunately the "+" chromosomes in the present experiments came from a variety of sources, with the result that a detailed analysis of the preferential segregation, similar to that which has been made for *melanogaster* (STURTEVANT 1936), cannot be made until more carefully designed experiments are carried out. Such experiments were planned, but when triplo-dot *Ci* turned out to be unclassifiable it was evident that no satisfactory test of the segregation-frequency interrelations could be made, and the experiments were not carried out. It should be indicated, however, that the observed sex difference in preference values is opposite in sign to that found in *melanogaster*, where males, rather than females, give nearly random segregation in triplo-dot experiments. In *hydei* this difference has been consistently present and of the same sign in experiments where the "+" chromosomes differed in origin from one experiment to another, but were alike in the males and females compared.

COMPARISON OF D. REPLETA AND D. HYDEI

As indicated above, triplo-dot is more easily distinguished from wild type (diplo-dot) in *repleta* than in *hydei*. This statement, however, needs some qualification. Several wild stocks of *repleta* (from Pasadena; Austin, Tex.; St. Louis, Mo.; Detroit, Mich.; Newcastle, Pa.) have been found to be variable in the extent and intensity of the dark thoracic markings. Selection within these strains has easily established rather uniformly dark types that are perhaps

FIGURE 1.—Ganglion cells from two female larvae of *Drosophila hydei*. Triplo-dot (left) and tetra-dot (right). Preparations and drawings by Dr. K. W. COOPER.

slightly darker than the usual *neorepleta* or *hydei* types; the work here recorded has involved only such dark strains—which represent the most usual form of the species. Selection of the lighter types has resulted in strains lighter than the triplo-dot individuals here described. It is clear that numerous genes are involved here, but few if any of them appear to be in the dot chromosome. In any case, it is necessary to avoid such strains if clear-cut separations for triplo-dot flies are desired.

These extreme light selected strains bear a superficial resemblance to the sex-linked recessive described by STURTEVANT (1915) (see also MORGAN, BRIDGES, and STURTEVANT 1925), but that gene has not been found in any of the stocks used in these experiments. The extreme light types also bear a phenotypic resemblance to the form of *hydei* described by SPENCER (1940) as subspecies *yucatanensis*. It happens that one of the +chromosomes used in the *hydei* experiments was derived from *yucatanensis* (see table 2); this chromosome had been transferred to a California background by repeated crosses to an Extended stock, and the results show that little if any of the lightness of *yucatanensis* is due to its dot-chromosome. With the exception of the strains of *yucatanensis* (from Chichen-Itza), I have not found *hydei* to be particularly variable in its thoracic pattern—certainly the strains I have had are much less so than are most wild strains of *repleta*.

The greater ease of separation of triplo-dot in *repleta* is therefore present in spite of a somewhat greater variability of the wild type with which it is compared.

Two other differences between the two species are probable but less certain —namely, that both tetra-dot and haplo-dot are more viable in *repleta*. The former was identified in a *hydei* larva, but not in adults; the absence of the latter may be due only to lack of efficient methods of identifying it.

COMPARISON WITH SPECIES OF DROSOPHILA NOT IN THE REPLETA GROUP

Haplo-dot individuals have been identified and described in *melanogaster* (BRIDGES 1921), *simulans* (STURTEVANT 1929), *ananassae* (KIKKAWA 1938), and *virilis* (CHINO and KIKKAWA 1933; CHINO 1937).

In all these species the type is characterized by "minute" bristles—that is, by small thin, tapering ones—rather than by the rather heavy blunt ones of *repleta*.[4] In *melanogaster* and in *ananassae* the haplo-dot individuals are relatively fertile; in *simulans* as in *repleta* they are almost completely sterile. In *virilis* the fertility is so low that stocks are difficult to maintain. The gray pollinosity of the mesonotum that is affected by this chromosome in *repleta* and *hydei* is absent in the other species concerned, so this character could not show in them. The deepening of the groove between the mesonotum and scutellum, present in haplo-dot *repleta*, has not been recorded in these other species.

In *simulans* the haplo-dot individuals usually show a weakening or partial absence of the last section of the fourth vein. This character, which has not been observed in the other species, gains interest from the observation by MULLER and PONTECORVO (1942) that a *simulans* dot, transferred to *melanogaster* by an ingenious technique, acts as though it carried an incompletely dominant allele of cubitus-interruptus. It should be added, however, that my observations indicate that *melanogaster* cubitus-interruptus is fully recessive in diplo-dot F_1 hybrids between these species. I am inclined to suspect that the single transferred chromosome studied by MULLER and PONTECORVO may have undergone mutation at the time it was heavily X-rayed in their initial technique for transferring it to *melanogaster*.

In *melanogaster* (BRIDGES 1921) and in *ananassae* (KIKKAWA 1938) triplo-dot has been identified by cytological methods, but has not been found to show any clear-cut phenotypic difference from wild type—which would also be the case in *repleta* and *hydei* if, as in *melanogaster* and *ananassae*, one were unable to make use of the thoracic pattern.

It appears, then, that these four species resemble each other and differ at least from *repleta* (and probably also from *neorepleta* and *hydei*) in the phenotype of haplo-dot individuals. *Melanogaster*, *simulans*, and *ananassae* are closely related members of the subgenus Sophophora, while *virilis* and *repleta* are members of the subgenus Drosophila. I have, however, suggested (STURTEVANT 1942) that *virilis* is nearer to Sophophora than is *repleta;* the present result is consistent with that view.

The differences in phenotypic effects of the haplo-dot condition raise the question of the degree of homology between the dot-chromosomes of the species of Drosophila. Mutant genes located in the dot have been recorded in *affinis*, *ananassae*, *melanogaster*, *simulans*, and *pseudoobscura* of the subgenus Sophophora, and in *hydei* and *virilis* of the subgenus Drosophila. I have an

[4] It may be remarked here that in *melanogaster* most triploid strains of long standing come to have two, rather than three, dot-chromosomes. Such diplo-dot triploid individuals show definite suggestions of the Minute character; it is my impression that, with practice, one could learn to distinguish them from triplo-dot triploids by their slightly smaller and thinner bristles.

unpublished mutant type in *robusta*, also of the subgenus Drosophila. The references for the previously recorded types are given by STURTEVANT and NOVITSKI (1941).

The homology between the dots of *melanogaster* and *simulans* is clear. *Simulans* has produced a dominant allele of shaven, and a Minute-4 that resembled that of *melanogaster* and was shown to be a deficiency for *melanogaster* abdomen-rotatum. The experiments of MULLER and PONTECORVO (1942) with a *simulans* dot transferred to *melanogaster* furnish further and more detailed confirmation of the homology.

In the case of *ananassae* the dot has become attached to part of X and is a small V rather than a dot at metaphase (KIKKAWA 1938). That it still contains the characteristic genes of the dot of other species (element F) is indicated by the occurrence of a dominant Shaven and by the phenotypes of haplo-dot and triplo-dot.

In *affinis* and *pseudoobscura* no haplo-dot or triplo-dot has been identified. The homology is indicated by the occurrence of grooveless in *pseudoobscura;* and of abdomen-rotatum, a possible allele of shaven, and a dominant that closely resembles the Cell of *melanogaster*,[5] in *affinis*.

These are all the mutant genes known in the element F of members of the subgenus Sophophora (other than *melanogaster*); since all of them can reasonably be supposed to parallel types known in *melanogaster*, it seems probable that the element has retained much the same genetic makeup and properties in all of them.

In the subgenus Drosophila the picture is not so clear. The one mutant known in the dot of *robusta* does not bear a close resemblance to any type known to me in any other species. The Extended of *hydei* would not be recognizable except by its recessive effect on the shape of the head and thorax in most species, and I know of no other type with those characteristics. The grooveless of *hydei* does agree very well with those of *melanogaster* and *pseudoobscura*, and the Cubitus-interruptus with the Gap of *virilis*. These two latter may also be compared to the cubitus-interruptus of *melanogaster* (which has a dominant allele), though with less confidence (see remarks above). In *virilis* there is also abdomen-rotatum that is a good parallel, and a reasonably satisfactory representative of shaven—an allele of which especially resembles the presumed shaven of *affinis*. There is also an eye mutant, glossy, that has been compared to *melanogaster* eyeless but which seems to me not at all a convincing parallel. The phenotype of haplo-dot virilis also resembles that of *melanogaster*, as indicated above, but that of *repleta* (and of *hydei?*) does not.

These comparisons may be taken as indicating that the dot of *virilis* is much like that of Sophophora, and that those of *repleta* and *hydei* (and perhaps of *robusta*) are somewhat less similar—though essential homology is still probable.

[5] The dominant in *affinis*, called Fused, was described by STURTEVANT (1940). Cell, discovered by GLASS, was described by BRIDGES and BREHME (1944).

SUMMARY

An X-ray induced dominant mutant in *D. repleta* is probably associated with a translocation between the dot and another autosome.

An aneuploid type produced by this translocation in interpreted as being haplo-dot.

A type phenotypically the opposite (as compared to wild type) is produced by *repleta-neorepleta* hybrids, and is interpreted as triplo-dot.

Triplo-dot × triplo-dot gives a more extreme type, probably tetra-dot.

Haplo dot is dark-colored, sterile, and has blunt but not Minute bristles; triplo-dot is light-colored and fertile; tetra-dot is still lighter and is sterile.

In *D. hydei* triplo-dot was detected by the use of the mutant Extended, and its composition was verified by cytological study.

Here also triplo-dot is lighter in color than wild type.

Preferential segregation occurs in triplo-dot *hydei;* it is more evident in males than in females.

A discussion of the available genetic data on the dot chromosome leads to the conclusion that it is similar in its properties in all the species of the genus studied, but among these is perhaps most different in *repleta* and *hydei.*

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