THE RELATION BETWEEN THE COLOR OF TESTES AND VASA EFFERENTIA IN DROSOPHILA*

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IN *Drosophila melanogaster*, as in the diptera in general, the genital ducts are independent in origin from the gonads. The ducts are outgrowths of imaginal discs, located at the posterior end of the larval body, which join during the pupal metamorphosis with the gonads which themselves at this stage lie embedded in the fat body in the body cavity. In adult males of the wild race of *D. melanogaster*, as well as in most mutant races, both the testes and the vasa efferentia are yellow or orange colored. On the other hand, in stocks where the eye pigmentation is very light or where no pigment is formed, both testes and vasa are colorless or nearly so.

In order to study the origin of male sterility due to absence of a complete Y chromosome we have transplanted sterile and fertile testes into fertile and sterile larval hosts respectively (STERN and HADORN 1938). For purposes of identification, fertile testes from a nearly colorless race and sterile testes from a colored race were used. During these studies a dependence of the coloration of the vasa derived from the host upon the testis derived from the donor became apparent. This was confirmed in experiments especially devoted to the problem. The following forms an analysis of the testis-vas interrelationship (STERN and HADORN 1937).

METHODS

The transplantations were made with the technique of EPHRUSSI and BEADLE, generally by using larval hosts of the last instar and testes from slightly younger donors but still of the same instar (see STERN and HADORN 1938). The main body of evidence was obtained in *Drosophila melanogaster*. In the original experiment the colored testes were of the constitution "carnation" (*car* at I, 62.5) and the nearly colorless testes "apricot vermilion" (w^a at I, 1.5; v at I, 33.0). Later only testes of "wild" constitution were used for "colored" gonads and either the slightly tinged w^av or white (w) testes for colorless ones. It will be seen from tables 1–4 that in regard to the color of the vasa carnation behaves like wild and apricot vermilion like white. This latter result was somewhat unexpected since transplantation experiments (EPHRUSSI and BEADLE 1937) have shown differences between w and w^av with respect to

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eye color. The reason for the divergence in regard to vas and eye coloration will become clear in the discussion.

In the first series the operated specimens were dissected about the fifth day of adult life. Later all dissections were made 24–48 hours after emergence from the pupa. No significant differences in coloration were noticed between the earlier and the later dissected cases. In the following account "colorless" and "not-colored" refer to vasa and testes of both w and $w^{a}v$ constitutions.

Our experiments with *D. melanogaster* have been supplemented by results on *D. pseudoobscura*, kindly put at our disposal by DR. TH. DOBZHANSKY and by interspecific transplantations between some other Drosophila species.

COMPOUND TESTES

After a successful operation the implant either develops "free," that is, without becoming attached to a vas, or it replaces a host testis by joining with a vas, or finally it fuses with one or rarely both host testes, thus forming compound gonads. These were always found to be connected with a vas. In the stage in which the testes were transplanted the gonads are ellipsoid bodies. A free testis retains its larval oval shape in spite of increase in size while attachment to a vas leads to a transformation of the testis into a long spiral (DOBZHANSKY 1931; STERN 1938). DOBZHANSKY and BEADLE (1936), transplanting testes in *D. pseudoobscura*, found a percentage frequency of the three main types of implant development of 61.1 ± 4.5 free; 27.3 ± 4.2 separately attached; 11.5 ± 3.0 compound. In our work with *D. melanogaster* the first series gave the percentage proportions 66.2 ± 5.6 : 15.5 ± 4.3 : 18.3 ± 4.5 (data given in STERN and HADORN 1938, table 1) while in the later and larger series (this paper, tables 1 and 3) nearly all implants which were not "free" had formed compound gonads (58.6 ± 4.3 : 1.5 ± 1.1 : 39.9 ± 4.3). Whether these diverse results are due to genetic differences in the races employed, or to differences in age of host and donor or to other causes, is unknown.

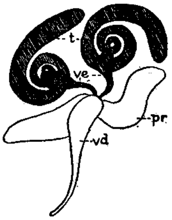

FIGURE 1.—Internal male genitalia of *Drosophila melanogaster*. Semidiagrammatic after SHEN. *t* = testes; *ve* = vasa efferentia; *pr* = paragonia; *vd* = vas deferens.

The diagnosis of a testis as a compound is of importance for the later analysis and a description of the criteria, therefore, is in order. Except for a few special cases, a gonad was considered to be compound (1) whenever its membrane was a mosaic of colored and uncolored areas and (2) when no free implant was found simultaneously. In addition, in the very great majority of cases such a gonad indicated its compound nature also morphologically by its larger size when compared with the normal testes of the same individual and by its obviously double structure—from a nearly separate round testis attached on the side of its spiral partner, to a uniform spiral gonad with two separate apical endings. That the two parts which make up compound testes actually enclose a common cavity was seen both in some fresh dissections as well as in sections of a few selected cases. This was also apparent from the fact that in compounds of sterile and fertile testes motile spermatozoa in the appropriate vas were present even when the fertile part, as judged by its color, was mainly restricted to the apical end. However, it must be pointed out that the distribution of the colored and uncolored areas on the surface of a testis may not necessarily permit deductions as to its internal structure. This will be discussed below.

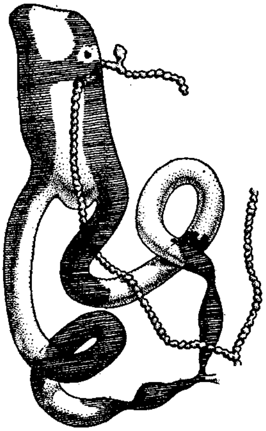

FIGURE 2.—*D. melanogaster*. Triple compound gonad consisting of two colored (+) host testes and a white donor testis. A malpighian tubule is partly enclosed by the compound structure.

The exact mode of compound formation is unknown. For its understanding it may become of importance to point out that in four cases out of a total of 50 compounds, a triple structure was formed. Here the two original host testes had become connected with each other by the implant which had fused with each of them. One such case is shown in figure 2. In addition to the general characteristics of a triple compound, this case is distinguished by a special feature. A malpighian tubule obviously has been "caught" during the process of fusion of the gonads so that it penetrates the compound structure. The tubule was found to lie free within a tunnel-like duct formed by the walls of the gonad. It seemed probable that the walls of the duct were partly provided by one yellow host testis and partly by the white implant. The formation of this strange structure points to the assumption that the initial fusion of the testes into compounds may occur independently in more than one region of the two joining gonads.

In addition to these triple compounds there were two cases in which the implant had become divided into two parts each of which had gone into compound formation with one host testis. Perhaps these cases are derived from triple compounds. In another instance two separate (but attached) gonads, obviously compound, had been formed by the implant and one host testis while the other host testis had remained free.

IMPLANTATIONS OF WILD TYPE OR *car* TESTES INTO w OR w^av HOSTS

In the great majority of cases *a free implanted testis of wild constitution does not exert any visible influence on the pigmentation of either* w^av *or* w *vasa.* Table 1 indicates this for 45 out of 47 cases, if we consider the first two

TABLE 1
Testis transplantation experiments in D. melanogaster (colored testis into colorless host).

TYPE OF TRANSPLANTATION	TOTAL	I		II	III			IV
		IMPLANT FREE		IMPLANT ON TIP OF ONE HOST TESTIS; PROBABLY NO COMPOUND	ONE COMPOUND ATTACHED			BOTH TESTES COMPOUND OR TRIPLE COMPOUND
		VASA COLORLESS	ONE VAS WITH COLORED SPOTS	VASA COLORLESS	BOTH VASA COLORLESS	1 VAS COLORLESS; 1 VAS COLORED	BOTH VASA COLORED	BOTH VASA COLORED
$+\rightarrow w^av$	25	17*	—	—	1	2‡	4	1
$+\rightarrow w$	42	17	2†	11	—	1¶	10	1

* In one individual one host testis and its vas were degenerate.
† One case: one spot; one case: three spots.
‡ One case doubtful if really compound testis.
¶ One case doubtful if really compound testis (figure 2).

classes together. In reality the first group only carries the designation "Implant free," while the second group is labelled "Implant on tip of one host testis, probably no compound." This distinction had not been drawn carefully in the series $+ \rightarrow w^a v$, so that it is likely that the 17 cases of the column "implant free" in reality contain some specimens which belong to the next column. The importance of distinguishing the two groups comes from indications of a closer relation of host and implant gonad in these cases of rather loose attachment to the tip of the former. In nine of the 11 individuals yellow pigmentation like that of the attached implant was found to cover narrow adjacent parts of the otherwise colorless host gonad and at the same time small colorless areas or islands were found on the otherwise colored implant. A case of this nature, as far as the gonads were concerned and disregarding the vas, is shown in figure 3. In the remaining two of the 11 individuals no yellow pigment was found on the host gonad, but an unpigmented region occurred on the implant. As stated in table 1 none of the 11 implants and host testes which were attached to each other had formed a real compound gonad. The criterion on which this judgment is based was the lack of a communication between the two gonadal cavities. It is, however, possible that the mosaic pigmentation in the cases of the two gonads indicates a limited compound formation between their membranes. It must be added that in two cases the host testis of the connected pair possessed a large yellow island somewhere along the middle portion of its otherwise colorless spiral. A discussion of these interrelations of the pigmentation of the testes will be given later.

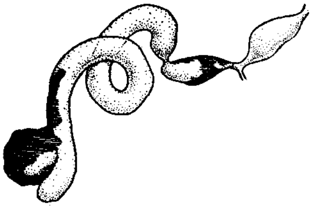

FIGURE 3.—*D. melanogaster*. Host: white; donor: +. Implant externally attached to host gonad. Colored area on host and uncolored area on donor testis. One vas partly colored.

There were two exceptions to the rule that the vasa remain uncolored in the presence of a free colored implant. In these two individuals one of the two vasa still fitted the rule by having remained unpigmented; the other vas, however, possessed in one case one, in the other case three yellow spots. The single spot was located near the middle of the vas, on one side of it. Of the three spots two were situated at that end of the vas into which the gonad opened and the third at the opposite end. The pigmented areas in the two exceptional vasa covered considerably less than 25 percent of the total surface of each vas.

When we consider the ducts to which true compound gonads had become attached, we find more numerous and more striking examples of *yellow* *pigmentation on vasa of colorless constitution.* In only one of 18 cases which have been classified in the third group of table 1 was there no trace of pigment to be found on either vas. In three of the 17 positive cases the pigment was seen only on that vas efferens which connected the compound gonad with the vas deferens and in the remaining 14 cases both vasa efferentia were colored. To these last cases may be added two more, recorded in the next (fourth) column of table 1.

An analysis of the types of compound testis discloses that a correlation exists between presence of pigmented testicular areas near the vas and the amount of coloration on the vas itself. In the individual both of whose vasa were colorless the compound gonad consisted of a long spiral colorless part and a smaller club-shaped colored part joined to the former in its apical half only. No pigment was present on any region of the lower half of the compound gonad. There is a slight possibility that no true compound gonad had been formed in this case. This possibility is greater in two of the three cases where one vas had remained completely colorless while the other exhibited pigmentation. In one case the implant was located at the tip of a host testis which itself was completely colorless, one small spot excepted. Only the uppermost, in this case unusually constricted portion of the vas appeared clearly orange colored. The second doubtful specimen exhibited the condition shown in figure 2. Again a very large lower section of the possibly compound testis was unpigmented. This time a considerable area of the vas *distal* to the connection with the testis was pigmented. In the third case we meet for the first time a completely yellow vas. It was attached to a compound testis the lowest region of which was yellow and which alternated with more apical colorless and yellow regions.

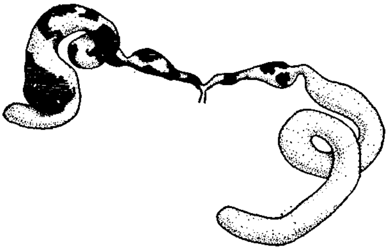

FIGURE 4.—*D. melanogaster.* Host: white; donor: +. Compound gonad. Both vasa spotted, the left to a higher degree than the right.

When we come to the 14 specimens in which pigment was found in both vasa though a compound gonad was attached to only one of them, we find in 10 cases that the amount of pigmentation was greater in that vas which belonged to the compound testis than in the other vas (figure 4). In the four other cases no striking differences in total size of pigmented area were found; in one of these individuals both vasa were completely colored. In no case was the vas, which was connected with the uncolored host testis more colored than the compound attached vas. Among the 10 pairs with differences in amount of color one case was found with the first vas about 50 percent colored, and the second vas about 10 percent colored; one case with the first vas about 80 percent colored, and the second vas less than 30 percent colored; and eight cases with one vas fully colored (in two cases perhaps only 95 percent), and the other vas from about 10 to about 50 percent colored (in one case about 80 percent).

Finally all vasa of each of the two individuals with double or triple compound gonads were colored in from 25 to 90 percent of their area.

These findings concerning wild-type implants are supplemented by six specimens from the series *car*→w^av (table 2) where the implanted testis had become attached to a vas separately, that is, without fusion with a host testis. In each case the vas belonging to the colored implanted gonad was fully pigmented (figure 5). Moreover, in two individuals the coloration was also partly extended over the other vas.

TABLE 2

Testis transplantation in D. melanogaster.

TYPE OF TRANSPLANTATION	IMPLANT ATTACHED WITHOUT COMPOUND FORMATION	
	VAS ON IMPLANT COLORED	BOTH VASA COLORED
car→w^av	4*	2

* In one case the two host testes were free, and only one vas was present to which the implant had become attached.

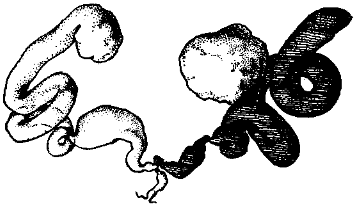

FIGURE 5.—*D. melanogaster.* Host: w^av; donor: *car.* Donor gonad attached to colored vas.

In summing up, the most significant result of the transplantations of testes of "colored constitution" into hosts of "uncolored constitution" is that a ***genetically colorless vas efferens acquires pigmentation when the colored testis has become attached to it*** either by itself or in the form of a compound gonad. In many cases the coloration extends over both vasa though to a different extent. A few cases were found also in which colored spots occurred on a vas of an individual where the implanted testes had not become attached at all.

The location of colored areas on the vasa

There does not seem to be any definite preference in regard to location of the pigmented areas. The colored regions were found scattered at random over the whole surface of the ducts both on the duct which was in direct connection with the implanted testis and on the other duct (figures 3, 4, 6). This should be pointed out especially, as certain selected cases might give the impression of a diffusion-like spreading of the coloring effect. Thus a single colored spot occurs occasionally next to the entrance place of the colored compound testis, or a single colored area may be seen on the lower end of a vas adjoining the totally pigmented vas of the other side. However, other cases where the colored spots are as far away as possible from the larger colored areas show this impression to be erroneous.

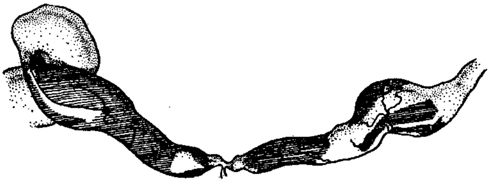

FIGURE 6.—*D. melanogaster*. Host: white; donor: +. Vasa efferentia. Attached to the left was a compound gonad the lowermost part of which was uncolored as indicated in the figure. To the right a white testis was attached. Note different total size of colored areas on the two ducts. The fine lines indicate tracheoles. A tracheole forms the border of the unpigmented area farthest to the left.

In this connection it may be mentioned that in those individuals in which the two vasa efferentia open into the vas deferens by means of a short common stalk containing the two ducts this stalk and the immediately adjacent parts of the vasa proper are frequently unpigmented even when the whole remainder of one vas, that is, nearly the complete vas and a large lower section of the other vas are colored.

The outline of spots both on the compound testes and on the vasa was quite definite and without intergrading. Frequently the limiting borders formed sharp angles. There were no outlines which pointed to a simple diffusion of color. In some cases it was seen that a limit of an area coincided exactly with the course of a tracheole in the testicular sheath; however, in many other cases no such relation existed (figure 6).

IMPLANTATIONS OF $w^{a}v$ AND w TESTES INTO WILD-TYPE AND *car* HOSTS

In all 31 specimens with a free implant both vasa were completely pigmented (table 3, I). The implanted testis generally remained devoid of color and the host testes were fully colored. However, in a number of cases in which the implant had become connected with the tip of a host testis small colored areas on the implanted testis were visible near the border between the two gonads.

The majority of specimens with one compound testis (22 out of 28 in table 3, II) also possessed two completely colored vasa (see figure 1a in STERN and HADORN 1938). In nearly all of these cases the lowermost region of the compound gonad possessed pigmented areas. In three of the six specimens with colorless areas on that vas, into which the compound gonad opened, an extensive unpigmented region formed the lower end of the testes.

TABLE 3

Testis transplantation experiments in D. melanogaster (colorless testis into colored host).

TYPE OF TRANSPLANTATION	TOTAL	I IMPLANT FREE OR ON TIP OF ONE HOST TESTIS	II ONE COMPOUND ATTACHED		III TWO COMPOUNDS ATTACHED	IV TRIPLE COMPOUND		V IMPLANT ATTACHED WITHOUT COMPOUND FORMATION	
		BOTH VASA COLORED	1 VAS SPOTTED	BOTH VASA COLORED	BOTH VASA COLORED	1 VAS SPOTTED	BOTH VASA COLORED	1 VAS SPOTTED	1 VAS COLORLESS
$w^{a}v\rightarrow+$	15	8	1*	5	1†	—	—	—	—
$w\rightarrow+$	48	23	5‡	17¶	1	1**	2	1††	1

* Colorless areas about 50% of total vas.

† Colored testis free.

‡ Three cases: one small colorless spot in middle of vas; two cases: colorless area about 50% of total vas.

¶ One case: compound-nature doubtful.

** Only small colorless spots on yellow background

†† Only small colorless spot on yellow background.

There were five individuals with either two compound testes each, or one triple compound (table 3, III and IV). In four of them both vasa were completely pigmented and in the fifth only small spots had remained colorless. In all five cases the testes possessed yellow areas near or directly adjacent to the vasa.

Of greatest interest in these series are the seven individuals in which the colorless implants had become attached to a vas without having entered into a compound formation (table 3, V, and table 4). In two cases this vas was largely colorless and in five it was completely so. In three of the latter cases the other vas had not become pigmented, once apparently due to generally poor development and twice in connection with the fact that no gonad had become attached to it at all, leaving both host testes free. The two other cases, however, showed a complete development of the uncolored vas.

The general conclusion from the results of implantations of colorless gonads into colored hosts is that *the vasa efferentia of the genetic constitution "colored" are usually not able to exhibit pigmentation when connected with an unpigmented gonad.*

TABLE 4

Testis transplantation in D. melanogaster.

TYPE OF TRANSPLANTATION	IMPLANT ATTACHED WITHOUT COMPOUND FORMATION	
	ONE VAS SPOTTED	ONE VAS COLORLESS
$w^a v$→*car*	1*	3†
w →*car*	—	1‡

* One colored spot of less than 30% of total area.

† One case: other vas colored.
Two cases: no testes attached to other vas, *colorless!*

‡ Other vas very underdeveloped. Attached to a yellow host testis but itself colorless.

TRANSPLANTATION IN DROSOPHILA PSEUDOOBSCURA

Extensive unpublished data relating to *Drosophila pseudoobscura* were obtained by DR. TH. DOBZHANSKY and with his permission are presented here. Three different stocks of this species were used: (1) the wild type which is characterized by deep orange-red coloration on testes and ducts, (2) the prune mutant exhibiting a pale yellow testis-duct color, and (3) an eosin magenta strain with colorless testes and ducts. The two last named stocks behaved so similarly in transplantation experiments that they have been combined in tables 5 and 6 which were compiled from DOBZHANSKY'S original notes. It seems unnecessary to discuss the results in detail. A study of the tables makes it obvious that a dependence of the coloration of the vasa efferentia upon that of the attached testes exists, very similar to that in *D. melanogaster.* Special attention should be paid to the recorded distribution of pigment in compound testes or in testes which are externally attached to each other. Irregular splashes or spots of color are frequently found on genetically unpigmented gonads.

INTERSPECIFIC TRANSPLANTATION

Interspecific transplantation, made reciprocally between the eosin magenta stock of *D. pseudoobscura* and normal *D. azteca,* and reciprocally between the same *D. pseudoobscura* stock and *D. virilis,* again demonstrate the relation between testis and vas color. The testes and vasa of *D. azteca* and *D. virilis* are reddish-orange. In more than 20 cases spots of this color were found on testes and vasa of genetically unpigmented constitution where attachment of donor gonad to host duct, compound formation or close external junction of host and donor testes had occurred. It is noteworthy that in these cases the interrelation of tissues is based upon transplants derived from a species different from the host species.

TABLE 5

Transplantation experiments in D. pseudoobscura. Donor: wild; host: prune or eosin magenta. Color of implant: red.

RESULT OF EXPERIMENT	HOST		
	TESTES	VASA	NO. OF CASES
free or only touching a host testis	uncolored	uncolored	27
compound	part of compound red	one or both fully or spotted red	8
attached	uncolored	both fully or spotted red	9
attached	part of loosely touching host testis spotted red	both fully or spotted red	5
attached (small)	part of loosely touching host testis spotted red	adjoining vas red, the other uncolored	1
attached to both vasa	uncolored	red	1
not found	uncolored	1 red spot	1

TABLE 6

Transplantation experiments in D. pseudoobscura. Donor: prune or eosin magenta; host: wild. In all cases testes and ducts of host: red. Implant slightly tinged or uncolored unless stated otherwise.

IMPLANT		NO. OF CASES
free	uncolored	13
loosely touching a host testis	red spot near contact	5
compound	nearly fully red	2
attached	uncolored	1
attached	red spots near contact zone with host testis and near vas	1

THE DEVELOPMENTAL RELATION BETWEEN TESTES AND DUCTS

If it is true that the testes and ducts originate completely independently from each other, then the observed interaction has to be interpreted as a non-autonomous behavior of the pigment-producing cells of host and donor. However, such an interpretation meets difficulties. It involves the assumption of diffusion of a specific substance from an attached colored testis to account for the pigmentation of the vasa. Such a diffusion process would have to depend on more or less direct contact of a vas with a pigmented region of an attached testis, for the vasa generally do not acquire color when the colored implant is free or when the lower parts of the attached testes are colorless. Special assumptions are necessary in order to explain the facts pointed out earlier that the outlines of colored areas are clear-cut without intergradations of color and that they often form sharp angles and straight lines. Further difficulties arise in adapting a simple diffusion hypothesis to certain specific mosaic color conditions. If the source of diffusion is postulated to be at the junction of testis and duct it is hard to see why occasionally only the opposite end of the vas is colored or why sometimes only distant parts of the other vas are pigmented when the nearer regions are transparent.

It might appear possible to overcome the objections raised by the addition of further assumptions to the diffusion hypothesis. It seemed more appropriate, however, to open once more the question of the ontogeny of testes and vasa and their supposed independence. Clearly most difficulties would disappear and the results would become fully intelligible if the origin of the vasa could be traced back to the gonads.

The independent origin of the genital ducts of flies and of the gonads was first demonstrated by BRÜEL (1897). In *Drosophila melanogaster* these observations were corroborated by DOBZHANSKY (1930) who made a complete series of pupal dissections showing the gradual transformation of the genital imaginal disc into the various ducts and accessory parts of the internal genitalia and their gradual joining up with the gonads which do not appear to contribute to the ducts. On the other hand, at nearly the same time, GEIGY (1931) expressed the view that contrary to BRÜEL'S observations, the vasa efferentia are derived from a special group of cells in the testes and that only the vas deferens owes its origin to the imaginal disc.

Although GEIGY has failed to document his statements by means of illustrations, while DOBZHANSKY has produced a clear series of drawings, it seemed desirable to reinvestigate the problem once more. MR. H. D. STALKER, in our laboratory, prepared and studied paraffin-sections of pupae of consecutive stages between 0 and 52 hours after pupation, kept at about 22.5°C. His conclusions agree in the main with those of DOBZHANSKY reached by total dissections. It can be seen without doubt that the imaginal disc produces, among other structures, two hollow tubes which grow forward till they meet the testes and transform into the vasa efferentia.

These observations of the normal development are further substantiated by observations of occasional abnormalities. It happens sometimes in gynandromorphs that normal vasa are present in spite of the absence of testes (*D. simulans*, DOBZHANSKY 1931). Similarly in transplantation experiments cases occur in which both vasa are present although only one testis is found attached to a vas while the other testis or testes are found free or are lacking. On the other hand, in transplantation work no individual has ever been found with more than two vasa though three or even four testes were present in the adult, either partly free or as compound gonads.

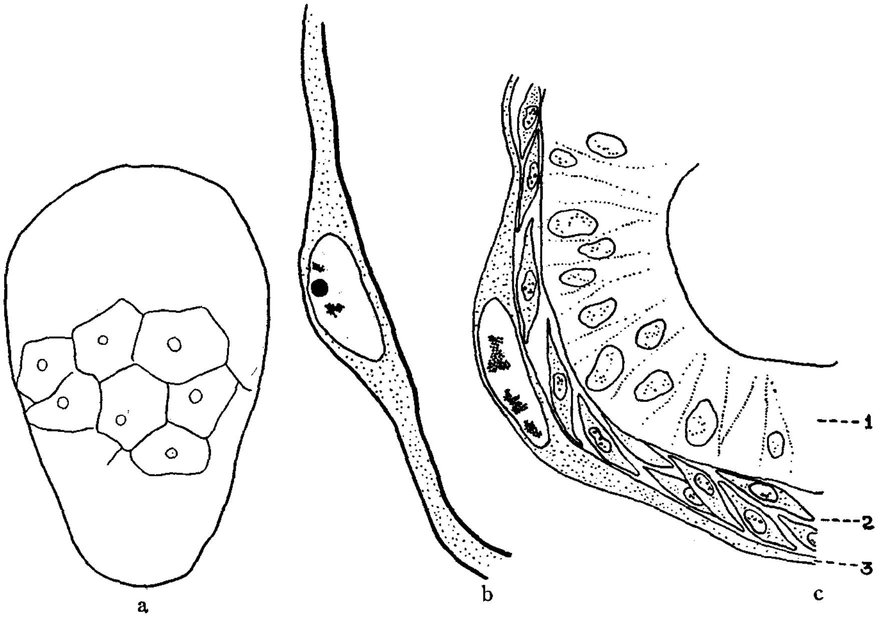

FIGURE 7.—(a) *D. melanogaster*. Adult, unattached testis from stock "sx" showing clearly the single layer of epithelial cells. Only some of the cells are indicated. Drawn from living gonad. 360×.

(b) *D. pseudoobscura*. Cross section through a sheath cell of adult testis. Permanent preparation. 1560×.

(c) *D. melanogaster*. Cross section through vas efferens of 40 hour pupa about 10 hours after attachment of testis to vas. Permanent preparation. 1560×.

(1) inner epithelium of vas
(2) outer epithelium of vas
(3) sheath cell.

While thus a study of the gonads and ducts as whole organs seemed to demonstrate their independence, a closer histological investigation finally has established that certain cells of the vasa efferentia are derived from the testes. An examination of the testes of adult wild males showed that the yellow color is caused by a single layer of pigment granules which are located in the external epithelial covering of the gonads. This covering consists of very characteristic, large, flat cells, the nuclei of which bulge out over the otherwise low cytoplasmic epithelial level (figure 7a, b). It was found that the same type of cell covers the unpigmented testes but that these cells lack pigment. Furthermore, it became obvious that the external cover of the vasa efferentia consists of precisely the same type of cells as that of the testes and that no such cells are found in other parts of the genital tract.

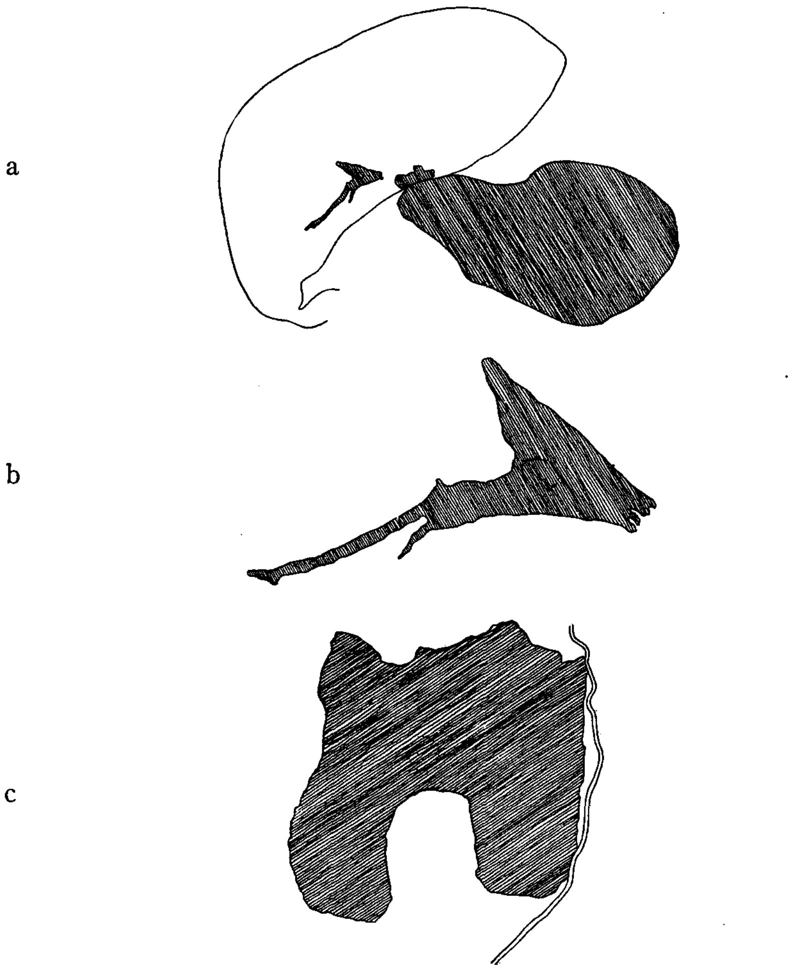

FIGURE 8.—Host: *D. pseudoobscura* (eosin magenta). Donor: *D. virilis.*

(a) Donor gonad externally attached to host gonad. Two colored areas on host testis, one consisting of single cell.

(b) This cell more highly magnified.

(c) Single cell forming a colored spot in a similar case. To the right a tracheole.

The ontogenetic history of these cells during the pupal stages was studied in MR. STALKER'S slides. At the time of pupation the typical though colorless epithelial coverings of the testes are present while no similar cells occur in the imaginal disc of the ducts. This condition persists through the following stages. No striking changes occur in regard to the testes. The vasa efferentia, however, become differentiated as outgrowths of the imaginal disc. At this time they consist of small cells which form a columnar layer on the inside and a squamous layer outside. At the time of close approach between the outgrowing ducts and the testes, stages are found which indicate clearly a migration of the large cells of the external testicular covering toward the vasa which in a slightly later stage are covered completely down to the region of junction of the two ducts (figure 7 c).

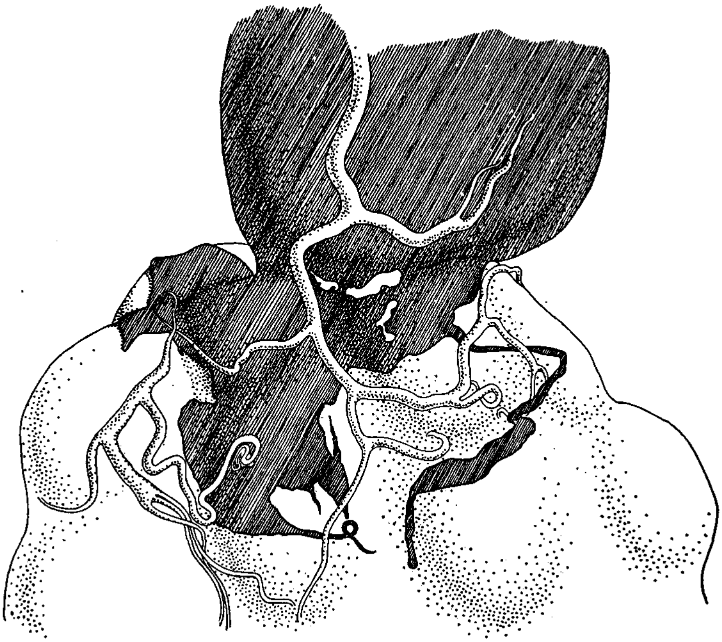

FIGURE 9.—Host: *D. pseudoobscura* female. Donor: *D. azteca* male. Colored testis (above) attached to uncolored ovary (below). Only parts of the gonads are indicated. Pseudopodial overgrowth of pigment from testis to ovary. The light tubes represent tracheae.

The observations regarding the derivation of the pigment cells on the vasa efferentia by migration from the pigment cells of the testes provide a simple basis for the interpretation of our transplantation experiments. Accordingly, no question of genetic cell autonomy or depedence in regard to coloration is involved but an ontogenetic derivation of the external epithelium of the ducts from that of the testes.

Further light is thrown on the migration of epithelial cells derived from the testes by observations on adult individuals in the experimental class "transplantation of *D. virilis* or *D. azteca* testis into *D. pseudoobscura* hosts." In these species the sheath cells are very clearly visible in freshly dissected living organs. It was found that isolated spots of pigment on genetically uncolored host testes sometimes represent single epithelial cells. These cells have an irregular, sharply defined outline suggesting pseudopodial motion (figures 8a, b, c). A still more striking picture was presented in a case of implantation of a *D. azteca* testis into a *D. pseudoobscura* female. The orange-red male gonad had become connected to the tip of one of the unpigmented ovaries (figure 9). From the region of attachment a strikingly pigmented sheet of cells had "flowed" over to the ovary which covered the surfaces of egg-strings and sent out a number of peculiar, long pseudopodial processes.

It would be interesting to study the histology of vasa neither of which has joined a testis in order to check whether the large celled external layer is absent. No such material is available at present.

DISCUSSION

Migration of pigment-producing epithelial cells enables us to understand the general dependence of the duct coloration upon that of the testes, the often irregular distribution of spots, the extension of coloration to both vasa in case of attachment of a colored testis to one of them only, as well as the frequently sharp and irregular outlines of spots. It also explains the few cases of pigmented spots on unpigmented host testes in the presence of a completely free implanted testis as due to migration of some pigment cells from one testis to another possibly during an early pupal stage in which a direct contact may have occurred.

Finally it makes intelligible the occurrence of "tongues" of unpigmented areas reaching from a colorless testis into the pigmented surface of an attached colored testis and also the rare occurrence of unpigmented islands within a pigmented testis cover. Obviously not only the pigmented cells but also their unpigmented homologues are able to move.

This latter point calls for further consideration. In the cases of two vasa to one of which is attached a pigmented gonad to the other a non-pigmented one, it is found that on the average more than 50 percent of the total area of the two vasa is colored. As a general rule it may be stated that in transplantation cases the pigmented cells cover a relatively larger area of either vasa or testes than the corresponding unpigmented cells. Two explanations of this fact may be suggested. It is possible that the initial number, the growth rate and the migratory activity of both cell types is equal and that some of these cells come to lie on top of each other in more than a single layer. Under these conditions a multilayer of pigmented and unpigmented cells might appear as a normally colored area and not reveal the presence of the colorless epithelial cells. This hypothesis, however, does not seem to be corroborated by observation. Though perhaps not fully conclusive, inspection seems to disprove the presence of more than a single layer of external cells covering the testes and ducts. In this case we should find different grades of coloration, since two or more layers of yellow cells would be darker than one single layer. This is contrary to observation. Furthermore, in favorable instances the cell boundaries can be seen and no overlapping of cells is found. An alternative hypothesis may ascribe different degrees of activity to cells of different genetic constitution. This means with regard to our *D. melanogaster* experiments that wild type cells either have a higher growth or a higher migration rate than genetically white cells. If the growth rate is equal for both kinds of cells, differential migration rates may still lead to different sizes of colored areas provided that the area taken up by single cells varies with the amount of spreading of the cytoplasm.

The general interpretation of the relation between colored and colorless areas seems consistent with the facts described in this paper. However, it does not fit certain facts described by DOBZHANSKY (1931) in *D. simulans* gyandromorphs which possessed a "white" constitution in their male parts and were "wild" in their female parts. These facts are: (1) gradual pigmentation with age of genetically "white" testes in partly "wild" gynandromorphs, (2) colored spots on testes which became attached to oviducts, the pigment being restricted to the surroundings of the point of junction, (3) pigmentation of vasa efferentia in the absence of any testes.

Whether these results are due to the fact that the male organs were present in wild type female tissue from the very beginning of development instead of from late larval stages or whether wild type XX cells can differentiate into pigmented epithelial cells can not be decided at present.

SUMMARY

1. Reciprocal transplantations of larval testes between races with pigmented and unpigmented adult gonads show that the color of the vasa efferentia is dependent on that of the testes attached to them and not on the genetic constitution of the ducts themselves.

2. The specific character of this dependence, namely the frequent occurrence of irregular, sharply outlined spots of pigment, makes an explanation based on the assumption of diffusible substance unlikely.

3. A histological study of the pupal development of testes and vasa efferentia provides the basis for the understanding of the experimental facts: Although otherwise of independent origin, the vasa efferentia obtain their external epithelial covering by a migration of the external covering cells from the testes.

4. The experiments were performed mainly on *Drosophila melanogaster*. Corresponding results appeared in transplantations involving *D. pseudoobscura* (experiments of DR. TH. DOBZHANSKY), *D. virilis* and *D. azteca*.

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