

EXPERIMENTAL EVIDENCE ON THE PRODUCTION OF THE MUTANT "ARISTAPEDIA" BY A CHANGE OF DEVELOPMENTAL VELOCITIES

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THE recessive mutant aristapedia (ss^a) of *Drosophila melanogaster* was first described by BALKASHINA (1929). It represents a remarkable case of homoeosis, in which the bristle on the antenna (arista) is transformed into a homologous organ, a tarsus (figure 1a and 1b). BALKASHINA has shown that in aristapedia flies segmentation of the antennal disk starts at two and one-half days of larval age, at the same time that segmentation of the leg disks begins, while in normal flies the segmentation of the antennal disks does not start until four and one-half days of larval age. GOLDSCHMIDT (1938) tried to explain these data in terms of developmental physiological processes. He assumed that the action of the ss^a gene consists of shifting the initiation of an embryological process to a different point in the time of development. He explained that if an evocator which determines leg segmentation is present in the germ at two and one-half days of larval age all disks in the proper stage of development will react to this stimulus by formation of a tarsus. The antennal disk of normal flies is far behind in differentiation at this time and, therefore, will not react to this evocator. According to BALKASHINA the differentiation of the antennal disk of ss^a starts at two and one-half days. This suggests that the differentiation of the ss^a antennal disk is speeded up and that it is so mature at the time when a "leg evocator" is present that it will react simultaneously with the leg disk in starting tarsal segmentation.

In 1937 two sets of experiments were started by the present author in order to bring forward experimental evidence for this view. The first part of these investigations was based on the consideration that if GOLDSCHMIDT's explanation is correct, a combination of ss^a with different mutants affecting leg structure should exhibit the mutant effect on the antennae of aristapedia as well as on the legs. On the other hand, mutants influencing the arista should show no effect on aristapedia. The second part of the investigations attempted to prove the existence of a difference in developmental velocities by transplanting ss^a disks into normal larvae and normal antennal disks into ss^a larvae.

A short summary of the results obtained was published recently (BRAUN 1939). A more extended discussion will be given here.

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COMBINATIONS OF ss^a WITH DIFFERENT LEG AND ANTENNAL MUTANTS

For the crosses involving mutants which affect the leg structure the recessive mutations *dachs* (*d*) and *thickoid* (*tkd*) were chosen. They are both located in the second chromosome. *Dachs* reduces the segments of the tarsus and results in shorter legs which are held close to the body. *Thickoid* exhibits especially short legs. *tkd* and *d* flies were crossed to ss^a flies, the factor for which is located in the third chromosome. The stock of ss^a used exhibits a rather constant appearance of the tarsuslike appendage of the antenna (figure 1b).

First ss^a and *d* were crossed and the F_1 flies inbred. It was possible to clearly distinguish $ss^a d$ flies from the ss^a flies by the shortened leglike appendage on the antenna, thus showing the leg effect on the tarsuslike part of the antenna (figure 1c and 1d). However, the double recessive hatched about two days later than the normal flies and the number of homozygous $ss^a d$ flies was actually smaller than 1/16 (243 +, 72 ss^a , 66 *d*, 13 ss^a). Most F_2 ss^a flies from these crosses exhibited a strange modification of the tarsuslike part of the antenna. The proximal half was tarsuslike, while the distal half represented an arista (figure 1e).

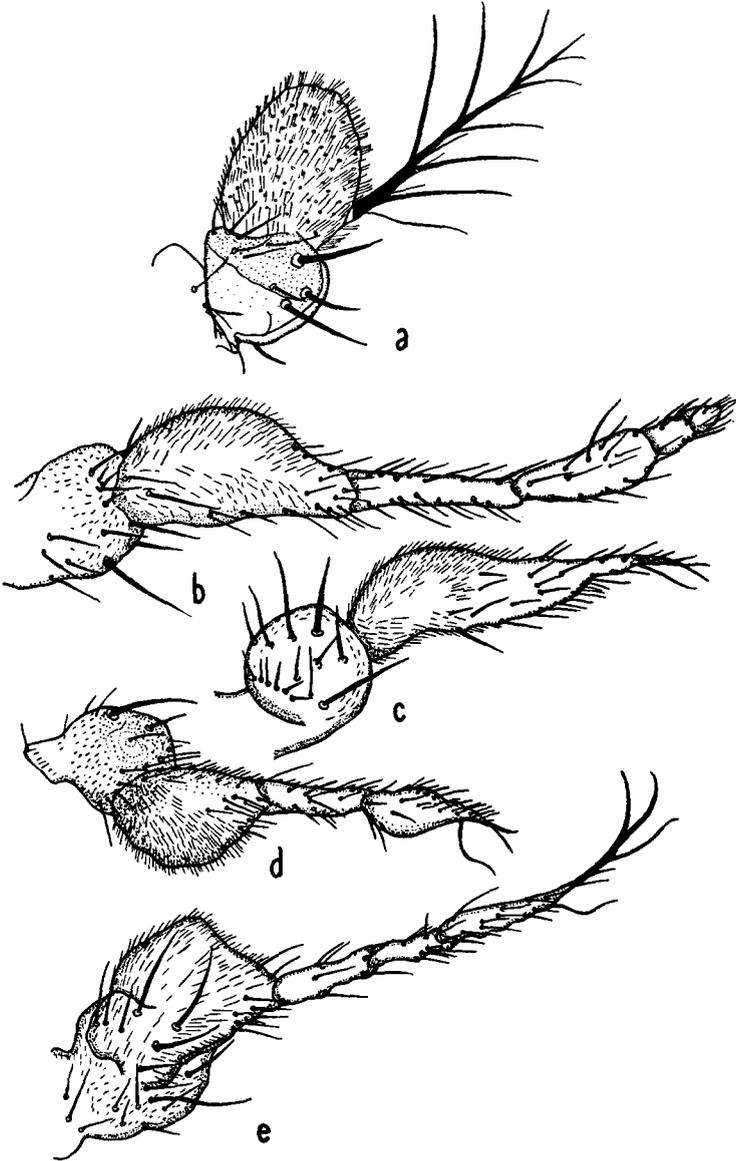
A possible explanation of this strange modification may be suggested. One might assume that a determination stream for arista development starts proximally in the antennal anlage of ss^a and progresses distally in a wavelike fashion. This determination process is interrupted at an early developmental point in these flies due to some as yet undetermined influence of the *dachs* stock. Therefore, it does not progress completely through the anlage. The distal part of the antennal anlage is thus left unchanged in these particular ss^a flies and will be determined in subsequent development by the "arista evocator." The result would be as observed, that is, an arista with a proximal leglike base.

This type could be easily selected and was kept as a true-breeding stock for several months before it was accidentally lost. It can be reproduced, however, at any time from a cross of ss^a and *d* and it deserves some extended and careful investigation.

The homozygous *tkd ss^a* flies from the F_2 of a cross between ss^a and *tkd* also showed a leg effect on the modified tarsuslike arista. These flies hatched only one day later than the normal ones and were more viable than the $ss^a d$ flies of the cross previously described (312 +, 185 ss^a , 103 *tkd*, 44 ss^a *tkd*).

These combinations showed convincingly that the modified antenna of ss^a is affected by the factors which modify leg structure. It now had to be observed whether a factor which influences the normal arista would also change the modified arista of ss^a . Therefore, in the next set of experiments the mutant aristaless (*al*), which modifies the normal arista, was used. Aristaless is a recessive mutant, located in the second chromosome; it strongly reduces the arista and causes the posterior scutellars to become

erect and divergent. The count of eight bottles of F_2 flies of the cross $ss^a \times al$ gave the following results: 1052 +, 312 ss^a , 264 al . Only these three classes were distinguished. However, it was immediately recognized that the excessively large ss^a class probably included the "missing" $ss^a al$ flies. This meant that the factor al showed no effect on aristapedia flies, at least as regards the altered antennae. In succeeding crosses attention was paid



FIGURES 1a-e.—a, normal antenna; b, antenna of aristapedia; c and d, antennae of " $ss^a d$ " flies; e, antenna of ss^a fly from the F_2 of $ss^a d$.

to the posterior scutellars which are modified by the *al* factor. In this way the fourth class "*ss^a al*" could be distinguished. Four cultures of the F₂ (*ss^a × al*) gave the following result: 424 normal, 105 aristaless, 115 *ss^a*, 50 *ss^a al*. The "*ss^a al*" flies showed no effect of *al* on the antennae of arista-pedia, but its usual effect on the scutellar bristles was clearly present.

TRANSPLANTATION EXPERIMENTS

Upon the suggestion of DR. R. GOLDSCHMIDT, normal and *ss^a* antennal disks of approximately 400 larvae were transplanted into *ss^a* and normal larvae respectively. The technique developed by EPHRUSSI and BEADLE (1936) was used. Transplantations were performed between larvae of *ss^a* and normal at different stages of larval age from two and one-half days to pupation. The age of the larvae was determined by allowing the mother to lay eggs for 12 hours only. Donor and host were not always of same age. As a preliminary experiment normal antennal disks had been transplanted into normal larvae, in order to check whether the structure of an arista could be observed in a mature transplant. The antennal disks were usually transplanted together with the eye disk to which they are closely attached. This simplified the localization of the transplant in the mature fly. The transplant (when dissected from the adult fly) always presented the appearance of a rather disorderly mass of tissue, bristle, and chitin grown together. In about 30 percent of these control transplants, however, it was possible to locate the arista among the many bristles. The arista of such a transplant was well developed.

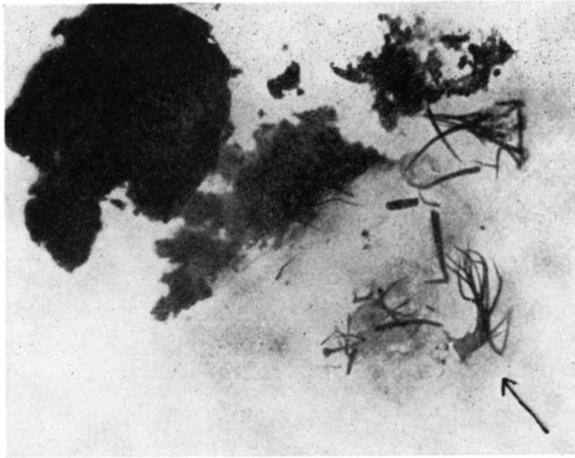
Several (nine) transplantations of normal leg disks into normal larvae were performed. No clear segmentation could be observed in the mature transplant. A spiral structure of the width of a tarsus but longer, with bristles on the inside (transplant not everted during development) was the most pronounced structure in the transplant.

After these preliminary tests were concluded, it was evident that the finding of well-developed aristae in antennal disk transplants and the spiral structure always found in leg transplants would help in analyzing the results of transplantations of disks between *ss^a* and normal larvae.

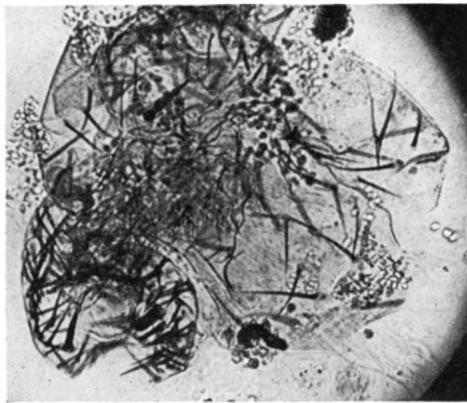
When the antennal disks of normal larvae were transplanted into *ss^a* larvae, at stages varying from two and one-half days of larval age to pupation, it was possible to locate a well developed arista in several cases (figure 2a). The spiral structure was never found in these transplants.

Transplants of *ss^a* antennal disks into normal larvae, (again at stages varying from two and one-half days to pupation) always showed the spiral structure, which is assumed to be an undeveloped tarsus (fig. 2b).

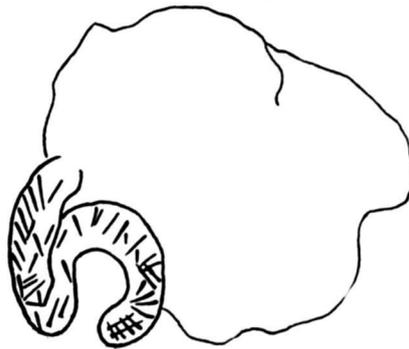
Transplantations between larvae younger than two and one-half days



2a



2b



2c

FIGURES 2a, b, c.—a, (above) a mature transplant of normal antennal disk into 55^{th} larva; b, (center) a mature transplant of 55^{th} antennal disk into normal larva. Sketch (below) shows location of spiral structure.

were not attempted, since they are extremely difficult on account of the small size of the larvae at this early stage.

DISCUSSION

These results can easily be explained on the basis of GOLDSCHMIDT'S interpretation of the development of aristapedia, and they furnish the first experimental evidence for his views.

It is to be expected that factors influencing the leg structure should also effect the leglike antenna of ss^a if the antennal disk of ss^a starts its differentiation at the same time that the leg disks begin their differentiation. This expectation has been fulfilled by the results of experiments in which the antennal structure of ss^a was observed in combinations of ss^a and different leg mutants.

On the other hand, factors influencing the structure of the arista should not be able to modify the tarsuslike antenna of ss^a . If the antennal disk of ss^a has started its differentiation at the time leg differentiation starts (at two and one-half days of larval age) it will already be far advanced in development at the time when any factors influencing the differentiation of an arista are active in the developing germ (fourth day of larval age). The already differentiated antenna of ss^a , therefore, cannot respond to such factors. This assumption has been proven in the combination of ss^a and a factor influencing the size of the arista; no effect of the aristaless factor could be observed on the antenna in homozygous " $ss^a al$ " flies.

What seems at first glance to be a negative result of our transplantation experiments may be additional evidence for accelerated development of the antennal disk in ss^a . The youngest larvae used for our transplantations were somewhat older than two and one-half days, and all ss^a , therefore, had already differentiated antennal disks. The fact that ss^a antennal disks developed into tarsuslike antennae in normal larvae suggests that they may have been differentiated prior to the time of transplantation, namely at two and one-half days of larvae age. The fact that normal antennal disks developed aristae in ss^a larvae does not allow us to draw any conclusions as to the time point of determination for the arista. Determination antedates differentiation and experiments of a different nature have to be carried out in order to find the exact determination point for development of aristae. However, it has to be pointed out that these disks may be autonomous from very early stages and thence transplantation may not test their potencies. The validity of the first interpretation could be checked only if it would be possible to transplant normal antennal disks into a ss^a host prior to two and one-half days of larval age.

From these results we conclude that the ss^a factor acts by shifting the initiation of the differentiation of the antennal disks to an earlier point in

development by speeding up the development of the antennal disks. Thus it happens that the antennal disks of *ss^a* will be mature enough to respond to a "leg-evocator" by starting tarsus segmentation at a larval age of two and one-half days.

As GOLDSCHMIDT has already pointed out, the same explanation will be valid for other cases of homoeosis, like the mutant proboscipedia (BRIDGES and DOBZHANSKY 1933) and the mutations bithorax and tetraptera (ASTAUROFF 1929). In the latter cases the metathorax exhibits characteristics of the mesothorax; winglike structures are present instead of halteres. In the case of proboscipedia the mouth organs assume the characters of tarsi. The same simple experiments which we used to demonstrate the validity of GOLDSCHMIDT's explanation for the *ss^a* case, should also be performed with the mutants just mentioned.

SUMMARY

1. Homozygous combinations of *dachs* or thickoid with *aristapedia* showed that the factors influencing leg structure affected the tarsuslike part of the antenna of *ss^a* flies as well.
2. Homozygous combinations of *ss^a* with *aristaless*, a factor influencing the size of the arista and the position of the posterior scutellar bristles showed no effect of *aristaless* on the tarsuslike part of the antenna of *ss^a* flies, but its usual effect on the posterior scutellars.
3. Transplantations of *ss^a* antennal disks into normal larvae at stages from two and one-half days of larval age till pupation resulted in development of structures which were recognized as leglike.
4. Transplantations of normal antennal disks into *ss^a* larvae at stages from two and one-half days till pupation resulted in development of aristae.
5. These results furnish experimental confirmation of views first expressed by GOLDSCHMIDT concerning the physiology of development of the mutant *aristapedia*, which acts by shifting the initiation of an embryological process to an earlier point in development.

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