Expression of homeotic mutations in duplicated and regenerated antennae of *Drosophila melanogaster*

By WALTER J. GEHRING¹ AND GEROLD SCHUBIGER²

From Biozentrum, University of Basel and the Department of Zoology, University of Washington

**SUMMARY**

Three homeotic mutants, *aristapedia (ss¹ and ss⁻¹⁻¹*) and *Nasobemia (Ns)* which involve antenna-leg transformations were analyzed with respect to their time of expression. In particular we studied the question of whether these mutations are expressed when the mutant cells pass through additional cell divisions in culture. Mutant antennal discs were cultured *in vivo* and allowed to duplicate the antennal anlage. Furthermore, regeneration of the mutant antennal anlage was obtained by culturing eye discs and a particular fragment of the eye disc. Both duplicated and regenerated antennae showed at least a partial transformation into leg structures which indicates that the mutant gene is expressed during proliferation in culture.

**INTRODUCTION**

Extensive studies on transplantation and extirpation of imaginal discs of *Drosophila melanogaster* have shown that each disc during metamorphosis gives rise to a specific area of the adult epidermis with its cuticular structures. By transplanting defined fragments of discs from mature larvae into host larvae of the same age and allowing them to metamorphose, the various adult structures can be ‘mapped’ within each disc in great detail (for a review see Gehring & Nöthiger, 1973). Each fragment forms its particular area of the adult cuticle. However, if the fragment is allowed to proliferate, it can exhibit a larger developmental potential: it may give rise to duplicated adult structures, it may regenerate structures which normally are derived from other areas of the respective disc, or after extensive growth, it may undergo transdetermination, whereby it can form additional structures which are normally derived from another kind of disc (allotypic structures). Proliferation of disc fragments can best be achieved by culturing them in adult hosts and subsequent transplantation into host larvae in order to obtain metamorphosis (Hadorn, 1963). The developmental response depends essentially on the kind of fragment which is

¹ Author’s address: Biozentrum, University of Basel, 4056, Switzerland.
² Author’s address: Department of Zoology, University of Washington, Seattle, Washington 98105, U.S.A.
Fig. 1. Normal and homeotic antennae in situ. (a) Aristapedia (ss⁺); (b) aristapedia-UCI (ss⁺UCI); (c) wild-type (ss⁺); (d) Nasobemia (Ns); A I, A II, A III = first, second and third antennal segment. Ar, Arista; Cl, claws; Co, coxa; Fe, femur; St, sternopleura; Ta, tarsus; Ti, tibia; Tr, trochanter.
Fig. 2. Fragments of the eye-antennal disc used for the experiments. A, Antennal disc; E, eye disc; F, fragment of the eye disc.

used. Certain kinds of fragments tend to give rise to duplicated structures, others are capable of regeneration (Gehring, 1966a; Schubiger, 1971; Bryant, 1971; Gehring & Nöthiger, 1973). For transdetermination usually more extensive proliferation is needed.

The kind of allotypic structures which are obtained after transdetermination again depends on the specific fragment which is cultured (Hadorn, 1966; Gehring, 1966a). Each fragment can give rise to one or a few types of allotypic structures. Transdetermination of one cell type into another has also been demonstrated in clones of genetically marked cells (Gehring, 1967), for example palpus cells can give rise to wing cells, or cells of the third antennal segment can become transdetermined and form tarsal cells.

Similar ‘transformations’ are also observed in situ in homeotic mutants. However, the relationship between transdetermination and homeotic mutation is not understood. In the present study we have analyzed discs from three homeotic mutants with respect to their capacity to duplicate and regenerate homeotic structures. Two of the mutants are alleles of spineless-aristapedia, ssα (Balkaschina, 1929) and ssα-UCl (Vyse & James, 1972), which transform the arista and adjacent parts of the third antennal segment into a four-jointed tarsus (Fig. 1). These two mutants have complete penetrance and constant expressivity which makes them highly suitable for such studies. Experiments on a temperature-sensitive allele of aristapedia will be reported separately (Schubiger, 1975). The third mutant Nasobemia (Gehring, 1966b) in the extreme case transforms the antenna and adjacent parts of the head into a complete mesothoracic leg. This mutant also has complete penetrance, but it is much more variable in its expression and normally gives only partial transformation (Fig. 1).

The head structures of the fly are derived from three pairs of imaginal discs: the eye-antennal discs, the imaginal cells of the clypeo-labrum, and the labial discs (Gehring & Seippel, 1967). The eye-antennal disc (Fig. 2) is composed of two morphologically distinct parts, the eye disc (E in Fig. 2) which gives rise to
one half of the head capsule including one compound eye, and the antennal disc (A in Fig. 2) which forms the antenna and the maxillary palpus. The two parts can easily be separated mechanically with a tungsten needle. The wild-type antennal disc has been studied extensively with respect to its capacity for duplication and regeneration (Gehring, 1966a). It was found that in cultured antennal discs, the antennal anlage frequently duplicates and forms two approximately symmetrical antennae. A disc fragment which contains only the antennal anlage is capable of regenerating a palpus, but in no case was it found that the antennal disc can regenerate eye structures. On the other hand, cultured eye discs frequently regenerate an antenna (Gehring & Nöthiger, 1973). Therefore, by using the homeotic mutants mentioned previously, we can study the duplication of a homeotic antenna from a preexisting antennal anlage and its regeneration from cells of the eye disc. Previous work with in vivo cultures of antennal discs homozygous for aristapedia has shown that after prolonged culturing the mutant tissue is capable of producing both tarsal structures as well as aristae (Gehring, 1966a). This observation indicates that ss a cells contain the genetic information for forming an arista which normally is not expressed in situ. Since tarsal structures can also arise by transdetermination, the earlier work did not answer the question of whether the ss a mutation is also expressed in disc cells which undergo additional divisions in culture. This latter question will be examined in the present paper.

MATERIALS AND METHODS

The donor larvae were taken from homozygous aristapedia (ss a and ss a'UC1), Nasobemia (Ns), and Oregon-R wild-type stocks (Lindsley & Grell, 1967; Vyse & James, 1972). As hosts ss a and two wild-type stocks (Oregon-R and Sevelen) were used. The Sevelen stock was marked with multiple wing hairs (mwh) and ebony (e). The stocks were reared on standard Drosophila medium at 25 °C.

In the control experiments the disc material was taken from donor larvae at about 100 h after egg deposition and transplanted into larvae of the same age, using the technique developed by Ephrussi & Beadle (1936). The eye-antennal disc was dissected from the donor larva in tricine-buffered Ringer's solution or balanced saline (Chan & Gehring, 1971) and cut with fine tungsten needles (Fig. 2).

In the duplication and regeneration experiments the discs from 'mature' larvae (100 h) were transplanted into newly eclosed adult females, cultured for five days or about 2 weeks, and subsequently transferred into larval hosts (80 h after egg deposition). The metamorphosed cuticular structures were dissected in Ringer's solution and mounted directly in Gurr's water mounting medium or Faure's solution.
RESULTS

Control experiments

The effect of transplantation on developing antennal discs from aristapedia and Nasobemia mutants was examined by transplanting them into wild-type larvae (see Materials and Methods). It has been shown previously by several authors that aristapedia is expressed autonomously in wild-type hosts (Braun, 1940; Vogt, 1946) and also in genetic mosaics (Roberts, 1964). This was confirmed by our control experiments summarized in Table 1: both ss\textsuperscript{a} and ss\textsuperscript{a-UCI} transplants form antennal tarsi. However, the number of tarsal bristles is reduced particularly in ss\textsuperscript{a-UCI} and to a lesser degree in ss\textsuperscript{a}. This effect might be due to a partial non-autonomy of the mutant discs in the wild-type hosts. However, a reduction in the number of tarsal bristles is observed, when ss\textsuperscript{a} discs are transplanted into ss\textsuperscript{a} hosts. Both in wild-type and in ss\textsuperscript{a} hosts, the number of tarsal bristles is not only reduced, but it also is much more variable as indicated by the large standard errors. However, the first and second antennal segments do not show any reduction in the number of bristles and only slightly larger standard errors. Furthermore, the ss\textsuperscript{a} transplants, especially in the area of the third antennal segment and the tarsus, show signs of incomplete metamorphosis and necrosis much more often than is observed in the corresponding area of wild-type discs. Therefore, we may conclude that the aristapedia discs are more sensitive to handling in vitro and transplantation than wild-type discs. In addition, the mutant expression may be somewhat reduced under the experimental condition, i.e. in transplants there probably are more cells which differentiate into arista structures than in situ. However, the mutant expression is constant enough for the study of duplication and regeneration of the homeotic antenna. In this context it is interesting to note that in situ the most distal part of the arista is not always transformed into tarsal structures.

In starved animals from crowded cultures of ss\textsuperscript{a-UCI} a strong reduction of the aristapedia phenotype is observed. Under these conditions fewer leg bristles are formed (Table 1) and the distal part of the appendage differentiates as an arista. In transplantation experiments nerves and tracheas are disconnected, which might lead to a similar effect as starvation.

The mutant expression in Nasobemia is much more variable, but it also behaves autonomously in transplants (Gehring, 1970). Ns shows a similar sensitivity to experimental manipulation as mentioned previously for the aristapedia alleles.

Duplication experiments

When wild-type antennal discs (A in Fig. 2) are cultured first in an adult host for 13–17 days and subsequently transplanted into a larva of the early third instar, the antennal anlage frequently duplicates and forms two symmetrical antennae (Gehring, 1966\textsuperscript{a}). Under these conditions transdetermination to tarsal
<table>
<thead>
<tr>
<th>Donor genotype</th>
<th>Experimental condition</th>
<th>Host genotype</th>
<th>Culture period (days)</th>
<th>Number of cases</th>
<th>Number of bristles</th>
</tr>
</thead>
<tbody>
<tr>
<td>ss&lt;sup&gt;a&lt;/sup&gt;</td>
<td>In situ</td>
<td>—</td>
<td>—</td>
<td>18</td>
<td>4.3 ± 0.4</td>
</tr>
<tr>
<td>ss&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Transplanted</td>
<td>ss&lt;sup&gt;+&lt;/sup&gt;</td>
<td>—</td>
<td>6</td>
<td>3.4 ± 1.1</td>
</tr>
<tr>
<td>ss&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Transplanted</td>
<td>ss&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
<td>9</td>
<td>3.2 ± 0.8</td>
</tr>
<tr>
<td>ss&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Duplicated</td>
<td>ss&lt;sup&gt;+&lt;/sup&gt;</td>
<td>17</td>
<td>12</td>
<td>3.8 ± 1.8</td>
</tr>
<tr>
<td>ss&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Regenerated</td>
<td>ss&lt;sup&gt;+&lt;/sup&gt;</td>
<td>16</td>
<td>11</td>
<td>1.7 ± 0.6</td>
</tr>
<tr>
<td>ss&lt;sup&gt;a&lt;/sup&gt;-UCI</td>
<td>In situ</td>
<td>—</td>
<td>—</td>
<td>46</td>
<td>5.2 ± 0.7</td>
</tr>
<tr>
<td>ss&lt;sup&gt;a&lt;/sup&gt;-UCI</td>
<td>In situ starved</td>
<td>—</td>
<td>—</td>
<td>22</td>
<td>5.4 ± 0.7</td>
</tr>
<tr>
<td>ss&lt;sup&gt;a&lt;/sup&gt;-UCI</td>
<td>Transplanted</td>
<td>ss&lt;sup&gt;+&lt;/sup&gt;</td>
<td>—</td>
<td>27</td>
<td>4.3 ± 0.9</td>
</tr>
<tr>
<td>ss&lt;sup&gt;a&lt;/sup&gt;-UCI</td>
<td>Transplanted</td>
<td>ss&lt;sup&gt;a&lt;/sup&gt;-UCI</td>
<td>—</td>
<td>16</td>
<td>4.3 ± 1.4</td>
</tr>
<tr>
<td>ss&lt;sup&gt;a&lt;/sup&gt;-UCI</td>
<td>Regenerated</td>
<td>ss&lt;sup&gt;+&lt;/sup&gt;</td>
<td>5</td>
<td>6</td>
<td>3.6 ± 0.5</td>
</tr>
</tbody>
</table>

**Table 1. Number of bristles on homeotic antennae formed under given experimental conditions**
Table 2. Phenotype of duplicated antennae in cultures of fragment A (in Fig. 2)

<table>
<thead>
<tr>
<th>Genotype of cultured fragment</th>
<th>Number of cultures</th>
<th>Culture period (days)</th>
<th>Number of duplications</th>
<th>Phenotype of duplicated antennae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>24</td>
<td>16</td>
<td>9</td>
<td>Normal 9, Homeotic 0</td>
</tr>
<tr>
<td>ss&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30</td>
<td>17</td>
<td>12</td>
<td>Normal 0, Homeotic 12</td>
</tr>
<tr>
<td>Ns</td>
<td>29</td>
<td>14</td>
<td>14</td>
<td>Normal 10, Homeotic 4</td>
</tr>
</tbody>
</table>

* An antenna is classified as homeotic if it carries at least 2 tarsal bristles.

structures is rare and in our experiments it was never observed. In 9 out of 24 cultures the antenna duplicated and formed two normal antennae (Table 2). The same experiments performed with ss<sup>a</sup> yielded 12 duplicated antennae, all of which showed the homeotic phenotype. However, in some cases only a few tarsal bristles were found at the base of a fairly normal arista. Nevertheless, this result clearly indicates that the ss<sup>a</sup> mutant phenotype is also expressed in duplicated antennae. In Ns four duplications were obtained in which both partners were homeotic, and in 10 pairs only one of the partners was homeotic whereas the other appeared to be normal. This observation can be explained by the variable expression of Ns, and the 4 positive cases are in agreement with the result obtained with ss<sup>a</sup>.

Regeneration experiments

The eye disc (fragment E in Fig. 2) when transplanted into a host larva of the same stage as the donor, gives rise to structures of the head capsule and the compound eye only. However, if we allow it to proliferate extensively in an adult host, it will regenerate a complete antennal disc with an antenna and a palpus anlage. If we compare wild-type and homeotic mutants with respect to their capacity for this type of regeneration (Table 3), we find that wild-type discs always regenerate normal antennae, whereas aristapedia (ss<sup>a</sup> and ss<sup>a-UCI</sup>) discs give rise to homeotic antennae only (Fig. 3). In ss<sup>a-UCI</sup>, antennal regeneration is obtained after five days in culture, a culturing period which does not allow transdetermination. Ns is again intermediate presumably because of its variable expression.

In order to make sure that the cultured fragment does not contain any antennal cells which accidentally have not been removed, we cultured F fragments (Fig. 2) in the same way as E fragments. Regeneration in F fragments was much rarer, but again the regenerated antennae differentiated according to their genotype (Table 3).
DISCUSSION

Homeotic mutants alter the determination of particular groups of cells such that they differentiate according to a different developmental pathway. In the present study we are primarily interested in the time of gene action of these mutants or their wild-type alleles.

Several lines of evidence indicate that the bithorax (bx) gene complex which is
Table 3. Phenotype of regenerated antennae in cultures of fragments E and F (Fig. 2)

<table>
<thead>
<tr>
<th>Cultured fragment</th>
<th>Genotype</th>
<th>Number of cultures</th>
<th>Culture period (days)</th>
<th>Phenotype of regenerated antenna</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>E</td>
<td>Wild-type</td>
<td>40</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>ss^a</td>
<td>31</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>ss^a*ucr</td>
<td>36</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Ns</td>
<td>28</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>F</td>
<td>Wild-type</td>
<td>16</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>ss^a</td>
<td>28</td>
<td>16</td>
<td>0</td>
</tr>
</tbody>
</table>

involved in the determination of the thoracic and abdominal segments is already expressed in embryonic stages of development. First, it was observed that homozygous Ultrabithorax larvae and several mutant combinations of the bx complex have, in addition to the normally present mesothoracic pair of spiracles, both a metathoracic and a first abdominal spiracle pair (Lewis, 1964). Since these spiracles are formed during embryogenesis, this observation suggests an early action of these genes. Furthermore, the sensitive period for phenocopying bx was found to be the blastoderm stage around 3 h of development (Henke & Maas, 1946; Gloor, 1947), which strongly suggests that the bx gene complex is involved in the initial determination of the thoracic segments during embryogenesis. Using the technique of induced somatic recombination, Lewis (1964) has been able to show that the wild-type allele of bx is still functioning during larval stages, as late as the late third instar, which indicates that determination is under continued genetic control until metamorphosis sets in.

In aristapedia there is no evidence available on gene action in early development. Somatic recombination studies indicate that the wild-type allele must function at least until 20 h before puparium formation (Roberts, 1964; Postlethwait & Schneiderman, 1973; Postlethwait & Girton, 1974). The temperature-sensitive period (TSP) for various aristapedia alleles also falls into the third larval instar (Villee, 1943; Vogt, 1946; Grigliatti & Suzuki, 1971). The TSP presumably corresponds to the time interval during which the gene product controlled by the temperature-sensitive allele is biologically active, and does not indicate the time of transcription or translation (Suzuki, 1970), but we can assume that transcription and translation occur prior and/or during the TSP. Whether the gene ceases to function after the TSP is an open question.

Our experiments aimed at the question of whether the homeotic genes are active when metamorphosis is delayed and the imaginal disc cells go through additional mitoses in culture. Both in duplicated and in regenerated antennae, the antennae are at least partially transformed into leg structures. For the
duplicated antennae one might argue that the homeotic leg cells pass their determination on to their daughter cells in the course of duplication (cell heredity). However, a recent study on the duplicating antennal disc of a temperature-sensitive homeotic mutant (Schubiger, 1975) clearly rules out cell heredity of the state of determination as the mechanism for duplication in these experiments. Furthermore, the interpretation which assumes cell heredity cannot be applied to the regeneration experiments in which the cultured fragment does not contain any homeotic leg cells. In this case we have to assume that determination is under continued genetic control by the homeotic genes or their wild-type alleles during proliferation in the adult host. The hypothesis of stable determinative factors synthesized early in development and passed on in the course of proliferation is very unlikely since the regenerated antenna contains several thousand cells and such factors would be greatly diluted in the course of cell multiplication. It seems much more probable that the gene product is synthesized during regeneration.

The biochemical nature of the gene product remains unknown. The observation that after prolonged culture in vivo, ss<sup>a</sup> antennal discs besides producing tarsal structures, can also produce normal antennae with aristae (Gehring, 1966a) indicates that the mutant cells do not lack any of the structural genes for forming an antenna, but rather that ss<sup>a</sup> is a controlling gene involved in the alternative differentiation of antenna or leg structures. The present experiments indicate that the ss<sup>a</sup> gene is expressed during proliferation in culture, but its expression becomes more variable, and antennal as well as leg structures are produced.

This work has been supported by the following grants: GB-17267 from the National Science Foundation and 258 from the Jane Coffin Childs Memorial Fund for Medical Research to W. Gehring and GB-38375 from NSF to G. Schubiger.

REFERENCES


Mutations in antennae of Drosophila 469


(Received 14 July 1974)