Developmental Biology 449 (2019) 63–82

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Commentary

Commentary on "Regeneration, duplication and transdetermination in fragments of the leg disc of *Drosophila melanogaster*": Schubiger, G. (1971)

To celebrate the Society for Developmental Biology's 80th anniversary, *Developmental Biology* is reaching into its archives to republish a set of editorially-curated classic papers. For my part, I have chosen to highlight some of Gerold Schubiger's seminal work on *Drosophila* imaginal discs, published in 1971 while he was working in the lab of Howard Schneiderman at UC Irvine. As Gerold was my PhD mentor from 1996 to 2001, it's difficult to separate a deep personal regard for the author from a purely professional perspective. Nevertheless, my time in his lab left me with a unique appreciation for the classic imaginal disc studies he carried out together with a group of his esteemed colleagues. Performed in the decades prior to a brewing revolution in *Drosophila* developmental genetics, their body of work helped establish the logical foundation for a molecular-genetic approach to pattern formation that blossomed nearly 20 years later. As we now know, that golden era yielded profound insights into signaling pathways, growth regulation, morphogen gradients, and more.

In the decade prior to the 1971 paper highlighted here, a group of *Drosophila* luminaries including Ernst Hadorn, Walter Gehring and Antonio Garcia-Bellido were leveraging the transplantation methods of Ephrussi and Beadle (1936) to provide a progressively more complex view of imaginal disc pattern formation and cell determination, as well as the associated process of transdetermination. These experiments began with simple fate-mapping studies, including earlier works by Schubiger as a student of Hadorn, wherein fragments of imaginal discs were injected into larval hosts in order to determine which adult cuticular structures were differentiated. Because specific structures (bristles, bracts, and sensillae for example) were stereotypically differentiated from distinct regions, highly detailed fate maps could be generated for each imaginal disc. As a further experimental evolution to explore the maintenance of determined cell states, the same imaginal disc fragments were also cultured under growth-permissive conditions in adult female hosts, explanted, and then re-transplanted into larval hosts for differentiation and analysis. These challenging manipulations led to the stunning discovery that given time, certain imaginal disc fragments could grow and exhibit unexpected regulative behaviors, producing more cuticular structures than expected from the fate map. Even more intriguing, some cultured fragments could even differentiate structures associated with other imaginal discs (such as wing tissue growing from a leg), a phenomenon known as transdetermination.

Dismissed by some regeneration purists as phenomenological folly, we can imagine that analyzing the mysterious regulative behaviors of cultured disc fragments could be regarded as a distraction from the central issues of developmental biology. Nevertheless, today Schubiger's painstakingly rigorous 1971 analysis of regeneration, duplication and transdetermination in the leg disc stands as a classic *Developmental Biology* manuscript remarkable for both its simplicity and its conceptual impact on the field. Reading the paper with a modern eye offers a fascinating window into the logical underpinnings of imaginal disc development, as Schubiger exploits careful observation to probe the elemental relationships between cell proliferation and cell determination, and between growth and regeneration. Although similar experiments had been performed in other insect species, because this paper and a host of related studies were performed in *Drosophila* they collectively set the stage for future genetic analyses catalyzed by the paradigm-shifting screens of Wieschaus and Nusslein-Volhard that would be published 10 years later. Careful, curiosity-driven descriptive work is the foundation upon which modern Developmental Biology is built, and refreshingly free from either mechanism or artifice, this classic paper reflects a pure essence of the field.

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https://doi.org/10.1016/j.ydbio.2019.03.012

DEVELOPMENTAL BIOLOGY 26, 277-295 (1971)

Regeneration, Duplication and Transdetermination in Fragments of the Leg Disc of *Drosophila melanogaster*[1]

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When fragments of the foreleg disc are injected into old larval hosts, they differentiate structures according to the anlage plan. When a short additional time for growth is allowed, they can also duplicate patterns, regenerate, or form multiple copies of units of markers. Transdetermination is observed only when fragments are cultured for longer periods.

The type of differentiation which is produced is dependent on the origin of the fragment. Medial halves regenerate, show multiplication of units, and later on transdetermine very frequently; pattern duplications are rare. Under the same conditions, lateral halves do not form allotypic structures but often duplicate their patterns.

Regeneration occurs in a proximodistal as well as a mediolateral direction. It was shown that a specific quarter of a disc is capable of regenerating a complete leg.

It seems that structures are successively replaced predominantly from one cutting region.

INTRODUCTION

When imaginal discs or disc fragments from mature larvae of *Drosophila melanogaster* are transplanted into mature host larvae, they metamorphose with the host shortly after the operation. The adult differentiations obtained from such discs or fragments are predictable: a specific region of the disc gives rise only to certain structures of the adult fly. Thus it has been possible to localize in a disc the anlagen for structures such as groups of sensilla, clasper teeth or sexcomb bristles, arista, claw organ, and so on. Experiments of this kind provide an anlage plan, or fate map, for a specific imaginal disc. Such anlage plans have been made for the male and female genital disc (Hadorn and Gloor, 1946; Hadorn, *et al.*, 1949; Ursprung, 1957, 1959), the wing disc (Hadorn and Buck, 1962), the eye-antennal disc (Vogt, 1946; Gehring, 1966; Ouweneel, 1970), the haltere disc (Loosli, 1959), and the male first leg disc (Schubiger, 1968). It has been concluded from these studies that an imaginal disc in a late third instar larva is a mosaic of determined anlagen, such that a small number of cells is determined to form, for example, a group of sensilla or even a single bristle, such as an edge bristle (Schubiger, 1968). The last step to the final determination seems to occur after puparium formation, as was indicated by Tobler (1966), who showed that, after dissociation and reaggregation of leg discs from mature larvae, bracts were formed only in association with bristles, never in isolation. It was concluded that bracts were not yet determined at the time of dissociation.

There still remained the problem of whether the mosaic determination of the disc is maintained when fragments are given additional time to grow, either by culturing them in adult flies or by transplanting them into young larvae before metamorphosis. That disc fragments can grow in larval and adult hosts has been shown by Ursprung (1962) and Wildermuth (1968a). Generally, additional growth of the fragment leads to duplication of the anlagen already present in the original piece (Vogt, 1946; Ursprung, 1959; Hadorn and Buck,

[1] This investigation was supported by the following grants to Dr. Howard A. Schneiderman: GB-16690 from the National Science Foundation and HE-13194-01 from the National Institutes of Health.

1962; Gehring, 1966; Nöthiger and Schubiger, 1966). Such duplications have also been described in *Ephestia* (Kroeger, 1958) and in *Tenebrio* (Hadorn *et al.*, 1969). Cytological (Kroeger, 1958) and autoradiographic (Wildermuth, 1968b) studies have shown that mitoses are a prerequisite for these structure duplications. Thus a disc fragment, during growth, usually produces copies of the anlagen that were already present.

Growth of disc fragments can also lead to structures that are normally formed by other discs (allotypic structures; Hadorn, 1965b). Hadorn (1965a) proposed the term "transdetermination" for this change.

A third process, which may occur after growth, has been reported by Spinner (1969), who examined how missing parts of the leg disc of *Culex* were replaced. He found that fragments that contained only the prospective material for proximal leg structures could regenerate the missing distal parts if cultured *in vivo* for sufficient time before metamorphosis. The same phenomenon was also found in *Tenebrio molitor* (Hadorn *et al.*, 1969). A high regeneration capacity of the Lepidoptera, for structures such as the wing disc, is well known (Meisenheimer, 1908; Ubisch, 1911; Bodenstein, 1935; Pohley, 1960; Madhavan and Schneiderman, 1969). Here the extirpation of the complete anlage is followed by regeneration and all wing structures are differentiated, provided that the time between the operation and the pupation is long enough.

The present study supplies information on the behavior of imaginal disc fragments of *Drosophila* after growth. We were especially interested to find out whether regeneration also occurs in disc fragments of higher Diptera as it does in lower Diptera, such as *Culex*, and, if such regeneration occurs, whether it is restricted to a certain region in the disc. Furthermore we have investigated the relation between growth and regeneration, pattern duplication and transdetermination.

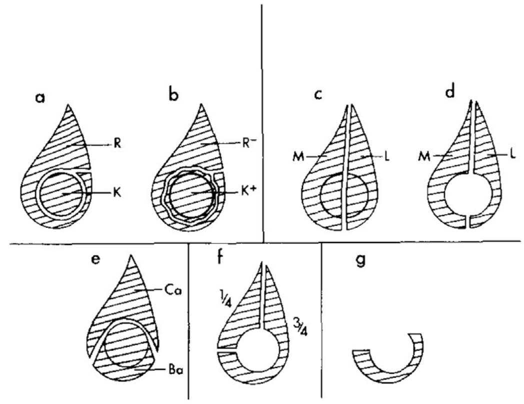


FIG. 1. Fragments from mature leg discs used to locate regions with regenerative capacities. (a) Isolated endknob (K), remainder piece (R). (b) Endknob with additional material (K^+), corresponding remainder piece (R^-). (c) Disc halves, medial (M), lateral (L). (d) Disc halves without endknob material. (e) Cap (Ca), basal (Ba) fragments. (f) "Upper medial" quarter piece (¼) and the three-quarter piece (¾) (g) Modified basal piece without the endknob. (d, f, g) Experiments where the endknob was not used.

MATERIALS AND METHODS

In all experiments we used the wild-type *Oregon-R* stock of *Drosophila melanogaster* both as donors and as hosts. The animals were reared on standard medium (cornmeal, sugar, agar, and yeast) at 25°C. The age of the larval donors and hosts is given in hours after egg deposition. The time of puparium formation was about 115 hr after egg deposition. The adult hosts were 2-day-old fertilized females.

The first leg disc of mature male larvae were dissected in a Ringer solution (Ephrussi and Beadle, 1936) containing 1.79 gm of tricine buffer (CalBiochem) per liter and adjusted to pH 6.8. Thin tungsten needles (diameter at the point about 10 μ) were used to cut the discs into different fragments as illustrated in Fig. 1. Electron microscopical observations of discs cut in this manner show that cell death usually does not extend more than two cells beyond the cut edge (Poodry, personal communication). The pieces were than transplanted into larvae or adults by the technique of Ephrussi and Beadle (1936). In control experiments the larvae formed puparia within 8 hr after injection. In the regeneration experiments the fragments were given three different time periods for growth. In the first series fragments were injected into late second instar larval hosts (68 hr) where they grew and underwent metamorphosis with the host. In two other series the fragments were cultured in adult females as described by Hadorn (1963). After 3 or 8 days the fragments were isolated from the adult host and injected into larvae (78–85 hr old), where they metamorphosed simultaneously with the host. The results from all three series of experiments were similar and indicated that differences in hormonal milieu between larvae and adult were not important for the phenomena under investigation. Differentiated implants were retrieved from the hosts, dissected, and mounted in Gurr's water mounting medium.

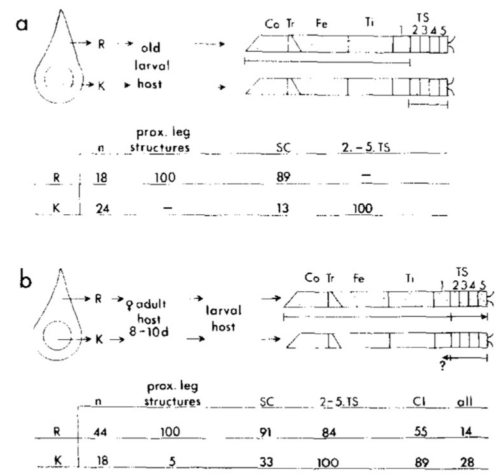
RESULTS

Regeneration in the Proximodistal Direction

In a previous publication (Schubiger, 1968), we examined the structures produced after metamorphosis when particular parts of a mature leg disc were implanted into old host larvae for metamorphosis. These experiments showed that in a male foreleg imaginal disc of a mature larva, the endknob (K) represents the anlage for tarsal segments 2, 3, 4, and 5 including the claw organ. A disc from which the endknob is removed, called hereafter the remainder piece (R), differentiates only proximal leg structures when implanted into an old larval host (Figs. 1a and 2a). This fragment usually produces some sexcomb teeth, which are structures of the first tarsal segment. These easily recognizable bristles are also differentiated at a low frequency from the K pieces. We therefore concluded that the anlage for the sexcomb lies at the junction of the R piece and the K piece (Schubiger, 1968).

In the first of the present experiments we cultured the R and the K pieces separately in adult females for 8–10 days before letting them metamorphose. The results (Fig. 2b) show that the R pieces differentiated not only proximal leg structures, but also claws and all the tarsal segments in 55% of the implants. In 84% of the cases we were able to recognize some tarsal segments distal to the basitarsus. The K pieces almost always differentiated, as we expected, tarsal segments (100%) and claws (89%). Sexcomb teeth were observed in a lower frequency than from the R pieces. One preparation also showed some tibial bristles. These results indicate that K pieces rarely regenerate proximal structures, if at all, whereas R pieces regenerate distal leg parts frequently.

The formation of the tarsal structures by the R piece might result from a transdetermination to the distal parts of the meso- or metathoracic leg. Indeed in this experiment we observed allotypic wing structures in 6 cases out of 44. However, when R fragments from old third instar donors were implanted into second instar larval hosts (68 hr after oviposition) no allotypic structures



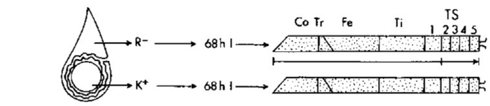
a

	n	prox. leg structures	SC	2.–5. TS
R	18	100	89	–
K	24	–	13	100

b

	n	prox. leg structures	SC	2.–5. TS	Cl	all
R	44	100	91	84	55	14
K	18	5	33	100	89	28

FIG. 2. Differentiations of remainder pieces (R) and endknobs (K). (a) After transplantation into old larval hosts. (b) After culturing for 8–10 days in adult before passing metamorphosis. Leg segments: Co, coxa; Tr, trochanter; Fe, femur; Ti, tibia; 1–5 TS, tarsal segments, all, allotypic structures. Cl, claw; d, days; *n*, number of cases; SC, sex comb. Differentiated segments are stippled.



	n	prox. leg structures	SC	2–5.TS	Cl	all
R^-	14	100	64	86	43	14
K^+	15	87	93	100	87	0

FIG. 3. Differentiations of the endknob with additional material (K^+) and the corresponding remainder piece (R^-), after transplantation into 68-hour-old larvae (*l*). Abbreviations as in Fig. 2.

arose, but in 12 cases (71%) we recognized several tarsal segments besides the first segment and in 5 cases (29%) we found a fully differentiated leg with a whole claw organ. Evidently R pieces can regenerate without first forming allotypic structures.

To exclude the possibility that the R fragment included parts of the K anlage, we fragmented discs in such a fashion that the K pieces also had some R material. Such fragments are referred to as R^- and K^+ pieces (Fig. 1b). They were injected into larval hosts 68 hr old. Figure 3 shows that even under these conditions the R^- pieces were able to differentiate the 2nd–5th tarsal segments (86%) and the claws (43%). The very high percentage of proximal differentiations from the K^+ pieces indicates that these fragments now contained proximal anlagen. Furthermore both the R^- and the K^+ fragments of individual discs were followed together in this experiment. Of 11 paired R^- and K^+ fragments, 5 differentiated claws in both pieces of the same disc. This rules out the explanation that a mistake in cutting is responsible for the apparent regeneration.

All these observations lead us to conclude that distal leg segments can regenerate from the proximal anlagen, if the fragments have enough time to grow before differentiating.

Regeneration of Claws from Half Discs

Figure 4 compares the frequencies with which claws differentiated in various experiments. The first pair of columns on the left side (a) shows that when the lateral halves (L) of leg discs were injected into old larval hosts they made claws much more frequently (82%) than did the medial halves (M, 11%). This control experiment confirms the results from earlier work (Schubiger, 1968) in which we concluded that the primordium of the claw is in the lateral half of the endknob.

When the medial and the lateral halves were cultured in adult flies for 3 days prior to metamorphosis, the frequency of claw formation in lateral pieces remained the same (86%) as in controls. However, the medial halves were now able to differentiate claws far more often than controls. This is shown in the second pair of columns in Fig. 4.

In the next experiment we cut discs in half and removed the endknob parts from both fragments, before they were transplanted into young larvae (68 hr old). The result of this experiment is represented in the third pair of columns (c); medial halves now differentiated claws far more fre-

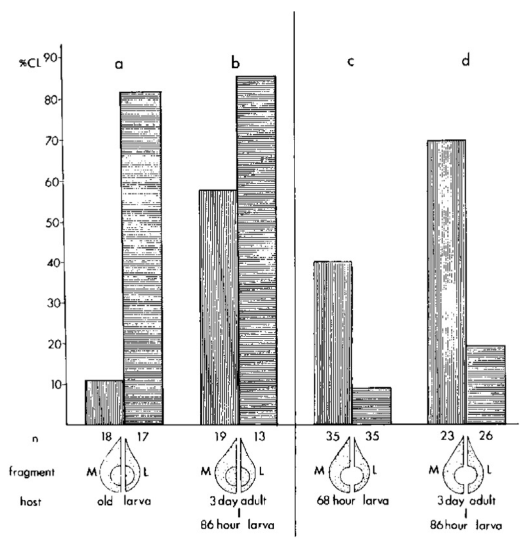


FIG. 4. Frequencies of claw (Cl) differentiation of half leg discs with (a and b) or without (c and d) endknob after injection directly into larval hosts or after culturing for 3 days in adults before passing metamorphosis. M, medial; L, lateral halves; *n*, number of cases.

quently than in the controls (40%). This is not the case for the lateral halves, where claws only rarely differentiated (9%). The medial halves also regenerated claws much more frequently than the lateral halves even after a 3-day culture in an adult female before differentiation (last pair of columns in Fig. 4). Therefore, it seems that the ability to regenerate a missing part is restricted to proximal anlagen and, furthermore, to the medial half of the disc.

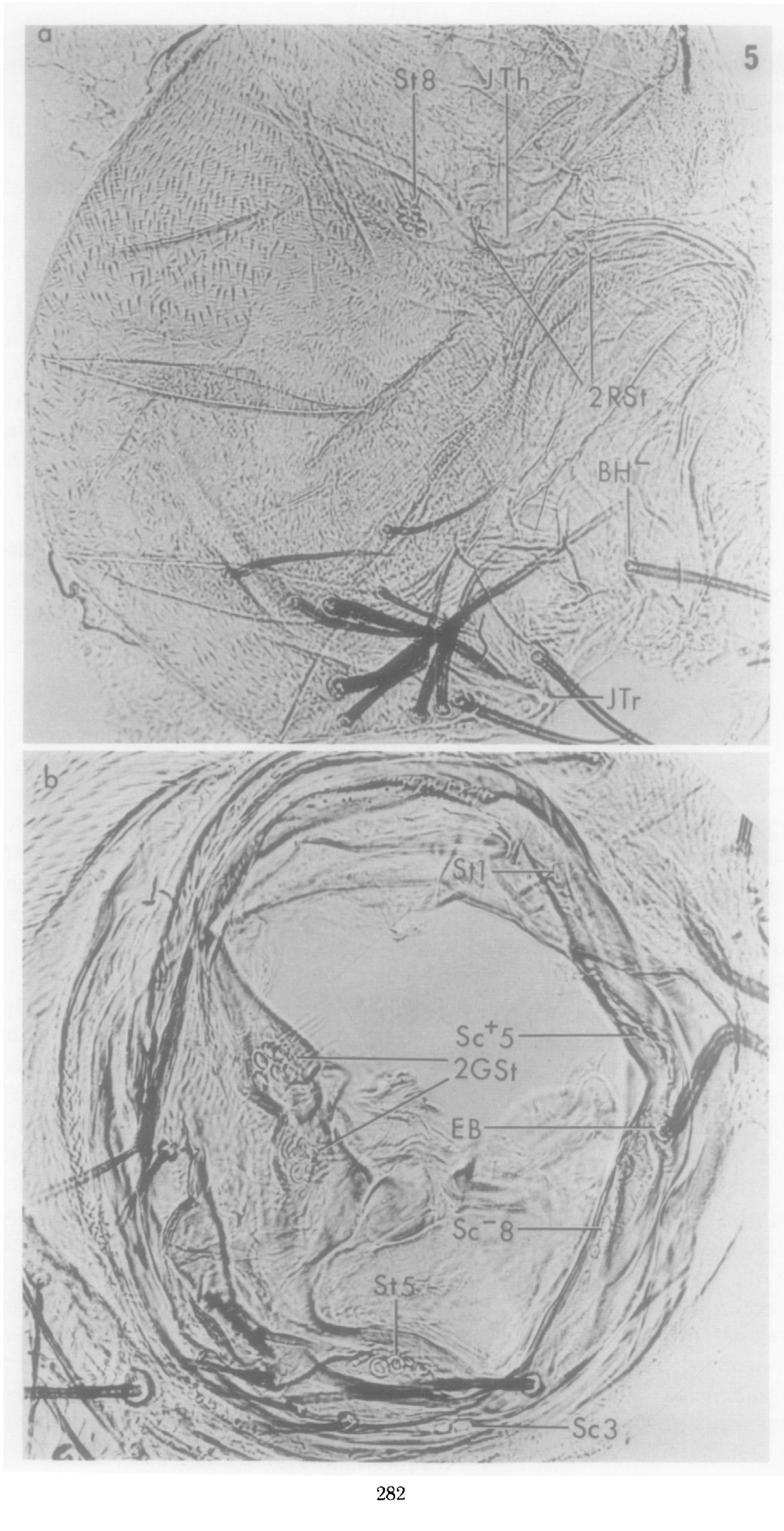
Regeneration within Leg Segments

Nöthiger and Schubiger (1966) demonstrated that disc fragments, for example half a leg disc, usually do not regenerate missing structures even after additional growth. Instead, culturing commonly resulted in an enlargement or duplication of the anlagen already present. But in several cases it was observed that some fragments differentiated structures of the leg other than those expected from the anlage plan. Similar results were found by Gehring (1966) working with the antennal disc. In his experiment, the posterior half of the antennal disc in some cases was able to regenerate the missing palpus, when it was cultured for 8 days in an adult host before differentiating. The palpus anlage is located in the anterior half of the disc which, after prolonged growth, shows only duplications of the anlagen already present and no regeneration of missing structures.

In the present study we have investigated this regeneration-like phenomenon in more detail for the leg disc. The results are summarized in Table 1. The markers which we studied are listed in Table 1 and are illustrated in Fig 5. In the control experiment (C) we implanted medial and lateral halves from longitudinally cut discs into old larval hosts which metamorphosed within 8 hr after injection. This experiment gives us the frequencies with which different marker structures occur in each half (Table 1, C). The data confirm earlier work (Schubiger, 1968): structures missing from the medial half were formed from the lateral half and vice versa.

For further studies we cut leg discs in the following way: in experiment 1 we made longitudinal halves (medial and lateral); in experiments 2 and 3 we also removed the endknobs from the halves. Then pieces either were injected directly into young larvae (68 hr old, experiment 2) or were cultured for 3 days in adult females before metamorphosing (experiments 1 and 3). We compared the frequencies with which different markers occurred in metamorphosed implants and in controls: in Table 1 all structures are underlined which appeared rarely (less than 50%) or are missing from controls. These results show that after a period of *in vivo* culture many medial halves were able to replace missing parts, but that lateral halves did not have this capacity.

We next compared the developmental capacities of the medial fragments in experiments 1–3 (Table 1) with the medial control fragments which metamorphosed immediately. Table 1 shows that in control coxa, the joint to the prothorax (JTh) occurred rarely (6%). However, this structure was formed eight times more often if the fragment had time to grow (experiments 1–3: 47–51%). Also the frequency of the two rows of sensilla trichodea (RSt) and of the isolated bristle BH^- is markedly increased. Coxal structures which were usually differentiated in controls (St8 and JTr) were found in experiments 1–3 with the same frequency. For the trochanter, the control experiment shows that the medial half never differentiated the edge bristle (EB) nor the group of sensilla campaniformia on hairless cuticle (Sc^-8). Also the row of 3 sensilla campaniformia (Sc3) and the row of 5 sensilla trichodea (St5) were observed in 11% and 44%, respectively, of the cases. However, all these structures occurred much more frequently after further proliferation (EB, 26–30%; Sc^-8, 49–58%; Sc3, 80–95%; St5, 94-100%).


a
5
St8
JTh
2RSt
BH
JTr
b
St1
Sc+5
2GSt
EB
Sc−8
St5
Sc3

Most of the remaining markers in the trochanter were found with equal frequencies in controls and experiments 1–3. Similar observations were made for the markers in the femur (Sc11), the tibia (transverse bristle rows), and the first tarsal segment (sex comb) (Table 1).

From these results we conclude that medial halves can regenerate structures which in controls are differentiated only by the lateral halves. We found 14 preparations from medial halves which formed all of the markers and bristles of a complete leg, so that it was not possible to distinguish them from implants from injected whole discs.

The results obtained with lateral halves were different. The missing markers in control halves were never replaced in experiments 1–3. This holds true without exception for the group of sensilla trichodea in the prothorax (GSt) and the joint between coxa and trochanter (JTr). In the trochanter we never observed the single sensillum trichodeum (St1), the group of sensilla campaniformia on hairy cuticle ($Sc^{+}5$) or the two groups of sensilla trichodea (GSt). The transverse rows of the tibia as well as the sex comb teeth in the first tarsal segment also were always missing. The structures which normally differentiate from lateral halves (e.g., lateral markers of the trochanter) were found with no higher frequency in experiments 1–3 than in controls. Even after culture, the lateral halves gave rise to more or less the same structures as controls. There seems to be a reduction in the frequencies of certain markers (EB, St5) after culture (e.g., in experiment 3, lateral halves). The cause of this is unknown. At first glance it seems that the second of the two rows of sensilla trichodea (2RSt) in the lateral half of the coxa can be regenerated (control 18%, experiments 1–3, 64–78%). But in implants we are often not able to distinguish between rows 1 and 2. Since many of the lateral halves show pattern duplications, and because we found no significant increase in the frequency of the marker JTh near these two rows of sensilla, it is more likely that this is a duplication of an anlage which was already present rather then a regeneration of the second row of sensilla.

The one case where St8 in the coxa was differentiated from lateral halves (experiment 3) could possibly be an exception to the above results. This may indicate a low regeneration capacity of lateral halves, but could also be explained by an occasional error in cutting the disc.

Regeneration (R) from medial halves is not the only phenomenon of interest which occurs in fragments after prolonged culture. We also found multiplication of units in the markers (M. Table 2), transdetermination (T), pattern duplication (D), and differentiation according to the anlage plan (A) (Fig. 6, Table 2).

A typical case of multiplication of units is illustrated in Fig. 6b. The figure shows that the group of 8 sensilla trichodea (St8) in the coxa has increased to about 20. This multiplication of units (e.g., sensilla) was a common occurrence. Figure 6f shows an example of a pattern duplication. Here the markers St5, Sc3, and one GSt in the trochanter appear twice, all completely separated from each other. The number of units in the duplicated sensilla may be the same as in controls or may be smaller in one of the two groups. The fragments may also show the above phenomenon in various combinations. In Table 2 we have recorded for each implant the types of

FIG. 5. Coxa (a) and trochanter (b) of control implant. Markers of the coxa: JTr, joint between coxa and trochanter; St8, group of 8 sensilla trichodea; JTh, joint between coxa and prothorax; 2 RSt, two rows of sensilla trichodea; BH^{-}, isolated bristle in a hairy island. Markers of the trochanter: 2 GSt, two groups of sensilla trichodea; $Sc^{+}5$, five sensilla campaniformia on hairy ground; St1, one single sensillum trichodeum; EB, edge bristle; $Sc^{-}8$, eight sensilla campaniformia on naked ground, Sc3, row of three sensilla campaniformia, St5, row of five sensilla trichodea. (a) × 370; (b) × 540.

TABLE 1
DIFFERENTIATION OF LEG DISC HALVES AFTER TRANSPLANTATION[a]

[a] Differentiation of leg disc halves (black) after transplantation into old larval hosts (C, control), after additional growth in young larvae 68 hr old (2) and after 3 days (d) adult (A) culture (1, 3). exp., experiment, l, larva, *n* number of cases; Pt, Prothorax; GSt, group of sensilla trichodea; Sc11, group of 11 sensilla companiformia (femur); Sc1, single sensillum campaniforme (femur); Ti, tibia; TR, transverse bristle rows; SC, sex comb; 2–5, tarsal structures of the last 4 tarsal segments; Cl, claw organ; all, allotypic structures. Markers of the coxa and of the trochanter as in Fig. 5. Structures which occurred in less than 50% of the control cases and their occurrence in the experimental series 1–3 are underlined.

	exp	fragment	host	n	Pt	Coxa						Trochanter								Femur		Ti	Tarsus			
					GSt	JTr	St8	JTh	RSt 1	RSt 2	BH^-	St1	Sc^+5	EB	Sc^-8	Sc3	St5	GSt 1	GSt 2	Sc11	Sc1	TR	SC	2-5	Cl	all
medial	C		old l	18	100	100	94	6	28	0	22	89	100	0	0	11	44	100	100	100	72	100	100	100	11	0
medial	1		3d A l	19	100	100	95	47	58	32	79	84	100	26	58	95	100	100	100	100	79	100	100	95	58	58
medial	2		68 h l	35	100	94	100	51	54	37	71	89	97	29	49	80	94	97	94	97	71	100	97	80	40	?3
medial	3		3d A l	23	100	100	96	48	48	43	83	87	96	30	57	91	96	100	100	96	48	100	100	78	70	52
lateral	C		old l	17	0	0	0	77	100	18	29	0	0	88	100	88	53	0	0	59	6	0	0	100	82	0
lateral	1		3d A l	14	0	0	0	57	100	64	29	0	0	79	100	71	64	0	0	64	0	0	0	100	86	0
lateral	2		68 h l	35	0	0	0	83	100	77	20	0	0	89	100	63	31	0	0	66	9	0	0	94	9	0
lateral	3		3d A l	27	0	0	4	89	96	78	15	0	0	52	100	67	22	0	0	56	7	0	0	74	19	0

differentiations that occurred. In this table we used the results from experiments 1, 2, and 3 (Table 1) and combined the data from experiments 1 and 3, since in both cases fragments were cultured for 3 days in an adult host before metamorphosing. In column A all preparations are listed which differentiated according to the anlageplan; that is, they formed the same markers with the same number of units as

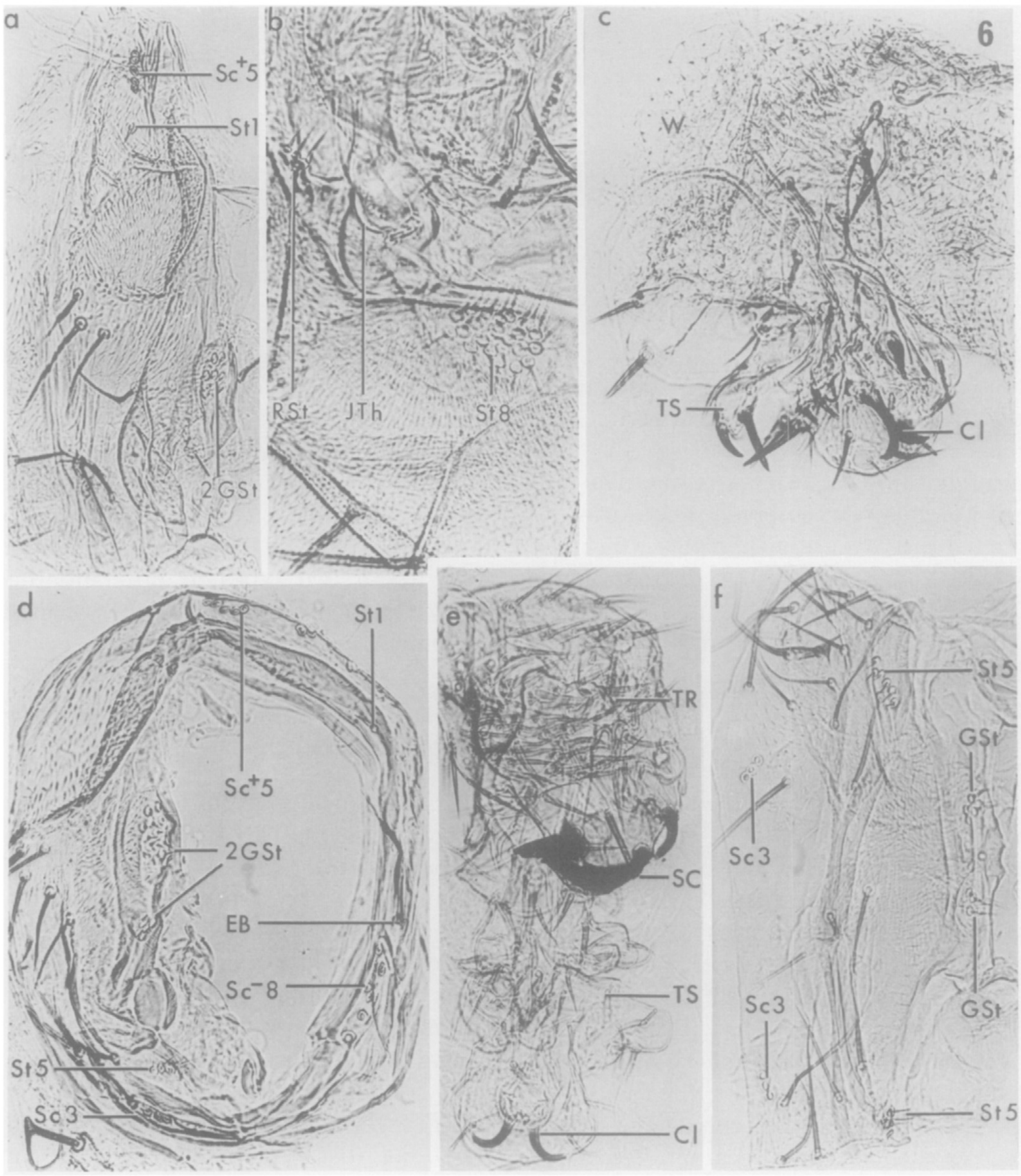


FIG. 6. Types of differentiations. (a) Structures of the trochanter differentiated from a medial half disc, according to the anlage plan. (b) Multiplication of units. The group of 8 sensilla trichodea (St8) in the coxa is enlarged to about 20 sensilla (units). The row of sensilla trichodea (RSt) and the joint to the thorax (JTh) are normal. (c) Transdetermination from leg to wing (w), direct contact between tarsal segments (TS) and the wing spread. Cl, claw organ. (d and e) Regeneration. (d) A complete trochanter differentiated from a quarter piece (Fig. 1) after 8 days of culturing in an adult. (e) In the same preparation as (d), tarsal segments (TS) and claw organ (Cl) also regenerated. SC, sex comb; TR, transverse bristle rows of the tibia. (f) pattern duplication. This differentiation originates from a basal piece (Fig. 1) after injection into 68-hour-old larvae. Markers of the trochanter as in Fig. 5. (a, and c–f, × 280; (b) × 450.

TABLE 2
TYPES OF DIFFERENTIATION OF DISC FRAGMENTS AFTER ADDITIONAL GROWTH[a]

Host	Fragment	n	A	R	M	D	RM	MD	RMD	MT	RMT	MDT	RMDT	RT	DT	T
68 hr l	M	35	1	10	9	0	10	5	0	0	0	0	0	0	0	0
3 day A l	M	42	1	6	3	0	9	1	1	8	7	1	2	1	1	1
68 hr l	L	35	1	0	1	21	0	12	0	0	0	0	0	0	0	0
3 day A l	L	41	7	0	1	25	0	7	1	0	0	0	0	0	0	0

[a] Medial (M) and lateral (L) disc fragments; host: l, larva; A, adult. The following types were distinguished: A, according to anlageplan; R, regeneration, M, multiplication; D, pattern duplication; T, transdetermination. The observed combinations are also given.

controls. From Table 2 it is evident that medial halves never showed pattern duplication alone (D). However, in lateral fragments, pattern duplication was the most common kind of differentiation. The only cases where we observed duplication in medial pieces were in implants which also showed multiplied (MD) or regenerated (RMD) structures.

Regeneration alone (R) and with multiplication (RM) was found only in medial halves.

The two halves also show differences in the tendency for multiplication of units. This phenomenon (M) occurred alone much more frequently in medial halves than in lateral halves. Furthermore, in medial halves multiplication tended to occur together with regeneration or transdetermination whereas in lateral halves it occurred almost exclusively together with duplication.

These data lead us to believe that when a medial half of a disc grows, the first recognizable difference from the anlage plan is regeneration or multiplication. When lateral halves grow, they seem to show duplication first, multiplication only later, and regeneration extremely rarely.

A striking fact recorded in Table 2 is that transdeterminiation was never observed in lateral halves, and in medial halves it was observed only after a period of culture in the adult. When transdetermination occurred we always found it combined with multiplication or regeneration. There is a correlation between transdetermination, multiplication, and regeneration. From our results, it seems likely that multiplication is closely related to transdetermination, since these two types of differentiations appear more frequently together than do the other combinations.

Localization of a Region with High Regenerative Capacity

In the previous experiments, regeneration from medial halves was best observed in the trochanter. Since the anlage of this segment is larger in medial halves than in lateral halves, it seemed possible that the size of the trochanter anlage which was initially transplanted could be responsible for the different capacities to regenerate. To exclude this possibility the disc was cut in such a manner that one piece (cap piece Ca, Fig. 1e) had little trochanter material, the other (basal piece Ba, Fig. 1e) contained a large portion of the trochanter anlage. First these fragments were injected into mature larvae for metamorphosis. The results of these transplantations are given in Table 3 (controls). The markers which were studied are the same as in Table 1, and the structures which appeared

TABLE 3

DIFFERENTIATION OF CAP (Ca) AND BASAL PIECES (Ba) AFTER TRANSPLANTATION INTO OLD OR YOUNGER (68 HR) LARVAL HOSTS (l)[a]

Fragment	Host	n	Pt	Coxa						Trochanter								Femur			Ti	Tarsus		
			GSt	JTr	St8	JTh	RSt 1	RSt 2	BH⁻	St1	Sc⁻5	EB	Sc⁻8	Sc3	St5	GSt 1	GSt 2	Sc11	Sc1	br. no. $\bar{x}$ ± SE	TR	SC	2.–5.	Cl
Ca	old l	14	100	14	100	100	100	79	0	79	100	71	36	0	0	43	0	0	71	45.9 ± 3.8	14	0	0	0
Ca	68 hr l	17	100	53	100	94	94	88	12	94	100	71	82	35	35	88	47	41	65	89.7 ± 5.8	71	18	12	12
Ba	old l	14	0	86	7	0	0	0	86	0	0	0	93	100	100	86	50	100	7	34.7 ± 3.3	100	100	100	93
Ba	68 hr l	14	36	71	50	7	0	0	86	0	0	7	28	100	100	64	57	100	0	88.1 ± 7.1	100	100	100	93

[a] Abbreviations as in Table 1 and Fig. 5. br. no., bristle number; $\bar{x}$, mean value; SE, standard error.

rarely in controls (less than 50%) are underlined. This experiment shows, as did the previous one, that structures which were missing from one fragment were differentiated by the other. On the basis of these controls we were able to compare the frequency of structures which were produced when the fragments were injected into young larval hosts (68 hr old) and given time to grow. Under these conditions in some cases the cap pieces were able to regenerate the missing structures: in the coxa, the isolated bristle (BH⁻, 12%); in the trochanter, the row of 5 sensilla trichodea (St5, 35%), the 3 sensilla campaniformia (Sc3, 35%), both groups of sensilla trichodea (2GSt, 47%); and in the femur, the group of 11 sensilla campaniformia (Sc11, 41%). Also, the structures which were rare in the controls now appeared far more frequently: for example, in the coxa, the joint to the trochanter (JTr) was formed in 53% compared to controls with 14%. In the trochanter the appearance of Sc⁻8 increased from 36 to 82%. In 8 out of 18 cases the tibia was fully differentiated, whereas in controls only a few bristles were formed (10 or less). Two preparations showed completely regenerated legs, with all markers, including the claw organs. Thus, the cap piece can regenerate all structures of the leg. This result demonstrates that differences in the amount of trochanter anlage in the fragments are not responsible for the differences in regeneration capacity.

The basal piece was never able to differentiate a complete leg; in the trochanter we found the same structures as in controls, although in one exceptional case an edge bristle was differentiated. A comparison between controls and the pieces which were injected into 68-hr-old hosts shows that in the latter the group of sensilla in the prothorax (Pt, GSt) differentiated in 36% of the cases whereas in the former this structure was not observed. In the coxa the group of 8 sensilla trichodea (St8) was formed more often (50%) than in controls

(7%), and in one case we observed the joint between coxa and prothorax (JTh). This indicates a low capacity for regeneration in the basal piece.

We found an increase in the bristle number (compare Table 3) in fragments which regenerate (Ca) and in those which duplicate (Ba), indicating that in order to duplicate or regenerate the fragment has to grow.

After additional growth, either in young larvae or in adult flies cap and medial pieces from old donor larvae showed a high ability to regenerate. This is in contrast to the basal and lateral fragments, which usually failed to regenerate. This observation suggests that the region which both cap and medial pieces have in common, namely the upper medial quarter (Fig 1f), is responsible for regeneration. To test this, we injected upper medial quarter discs without the endknob into 4 different types of hosts (Fig. 7). In the control experiment (C) the pieces were caused to metamorphose without additional growth. In three other experiments, the fragments were allowed to proliferate before metamorphosis either in young larval hosts (68 hr old) or for either 3 days or 8 days in adult females. Figure 7 represents the frequency at which different markers occurred. The innermost circle shows the frequencies for the control experiment (C). These data agree well with those from our earlier work (Schubiger, 1968). The circle labeled 68h l, shows that when the same fragments were implanted into young larval hosts (68hr) only little regeneration was observed and limited to structures situated near the region of the cut, for example the joints of the coxa to the trochanter (JTr) and to the prothorax (JTh) and also the two rows of sensilla trichodea (2 RSt). This low capacity for regeneration is further expressed in the trochanter, where we found both groups of sensilla trichodea (2 GSt) in 12% of the preparations, and in the femur, where the 11 sensilla campaniformia (Sc11) were formed in 6%. One case with tarsal segments also indicates regeneration of distal structures, but this seems to occur rarely. No transdetermination was observed in this experiment.

The next circle, labeled 3d A, l, shows that the amount of regeneration increased when fragments were cultured for 3 days in adult flies. The missing anlage of the coxa appeared in 11–21% of the preparations and those of the trochanter in 11–32%. The marker Sc 11 in the femur regenerated in 37% of cases. In 2 of the 19 preparations a complete leg was differentiated. Allotypic structures were noted in 4 cases.

After culturing for 8 days in an adult host, some of the fragments resembled normal mature leg discs with all folds including the endknob. At metamorphosis these 4 implants differentiated complete legs, which contained all the markers and bristles that are formed from a transplanted whole disc. In 4 other cases almost an entire leg was produced and some of the other preparations showed partially regenerated leg segments. The outermost circle in Fig. 7 records the frequency of occurrence of regenerated structures such as those just described. In the coxa the missing markers were noted in 33–60% of cases, in the trochanter in 23–67%, and in the femur in 70%. The distal parts were also regenerated and in 47% we found the claw organ. These results demonstrate that the upper medial quarter of the leg disc is able to regenerate and differentiate a complete leg.

Furthermore, it is interesting that when pieces have only a short time to grow, only those markers regenerate which, according to the anlage plan, are located nearest the region of the cut. After prolonged proliferation (8 days in adult) the frequencies of regenerated markers are not equal within a segment. For example, the edge bristle and the $Sc^{-}8$ in the trochanter have the lowest values (23% and 40%), whereas the markers Sc3, St5, and BH^{-} more often regenerated (60–67%). It was also observed that in all cases where the markers $Sc^{-}8$ and EB were regenerated, the Sc3, St5, BH^{-} and Sc11 were also found. But there

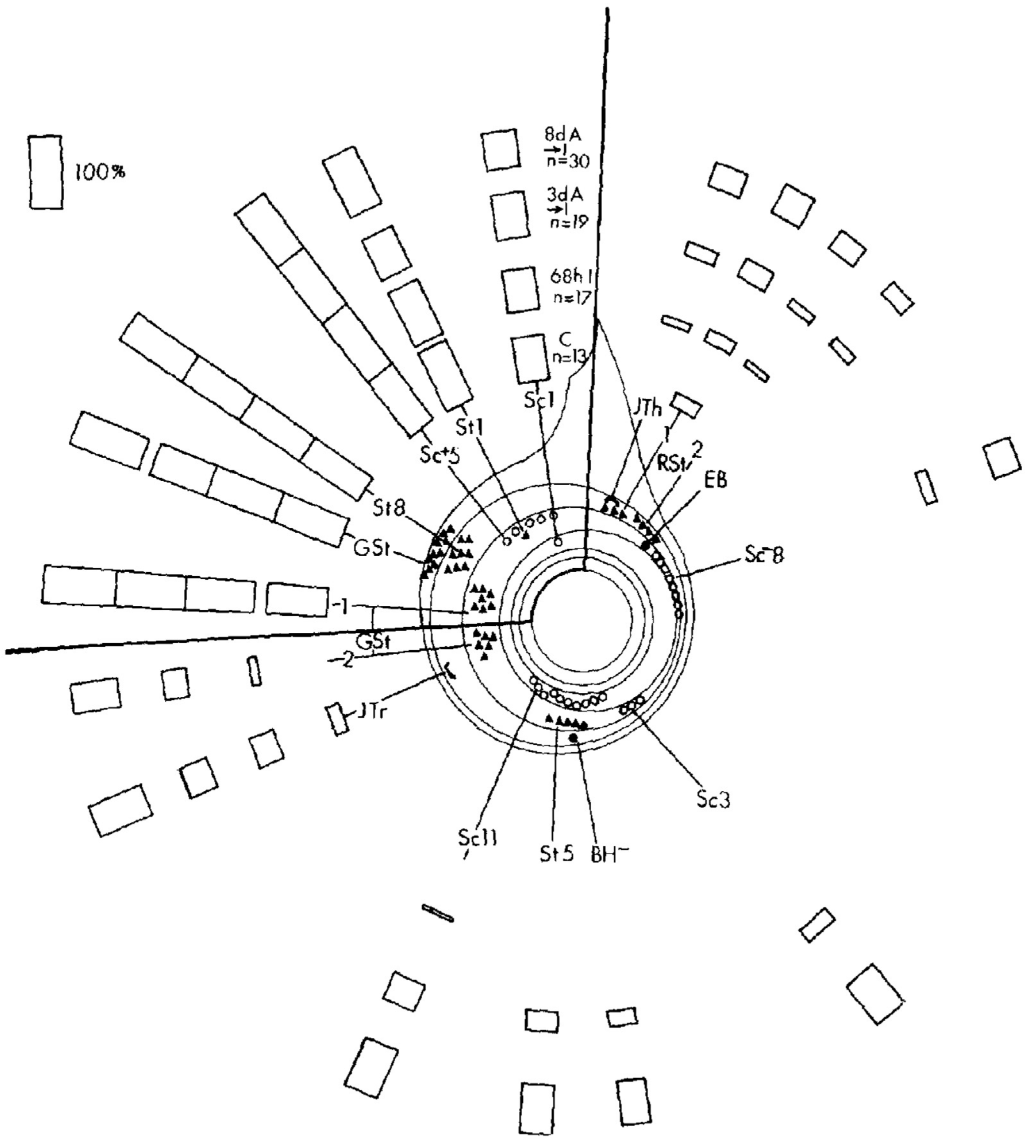


FIG. 7. Percentage of cases in which specific markers were differentiated from the upper medial quarter after different culture periods (four series). The percentage is represented by the length of the column. Data from each series are arranged on a circle. *n*, number of cases; l, larval hosts; A, adult host; C, control, mature larval host. Markers as in Fig. 5. The disc is drawn from a simplified anlage plan after Schubiger (1968).

are cases where regeneration was incomplete, and ended for example with Sc3, no Sc-8 being found. This indicates that regeneration occurs sequentially from the horizontal cut (Fig. 7). However Fig. 7 also suggests that there may be limited regeneration from the vertical cut surface, for example of the markers JTh and RSt, but this is always much less pronounced than the regeneration from the horizontal cut.

As in previous experiments, transdetermination occurred from quarter fragments after prolonged growth in adult females (3 days, 21%; 8 days, 53%).

The complementary three-quarter disc fragments (lateral and basal part without the endknob: Fig. 1f) after 3 days of adult culture produced pattern duplications in mirror image arrangement in 15 out of 16 cases (Fig. 6f). None of these cases formed a complete leg. However, a partial regeneration was observed; in the prothorax, the group of sensilla appeared twice, in the coxa, the St8 was found three times; in the trochanter, the St1 was differentiated in 4 and $Sc^{+}5$ in 3 cases; and the marker Sc1 in the femur was found in one implant. All these markers are mapped in the missing part. Hence, the capacity for regeneration of upper medial structures is low in this fragment. However the claw was differentiated in 14 out of 16 cases. This indicates that the three-quarter pieces have the ability to regenerate missing distal structures (derivatives from the endknob), but a low ability to regenerate proximal structures (derivatives from the medial quarter). Allotypic structures were found in 4 cases.

Pieces smaller than one quarter of a disc failed to show regeneration because they were difficult to culture and differentiated only those structures found in control experiments. This indicates that these fragments grow very little.

In the last set of experiments, we cut the disc as shown in Fig. 1g in order to obtain more information on the developmental capacities of the basal portion. Such fragments were cultured for 7 days in adults and then injected into larval hosts. We obtained 24 metamorphosed pieces, none of which had transdetermined. Twenty-two showed pattern duplications, most evident in the trochanter. The remaining 2 implants had regenerated some of the missing structures, for example $Sc^{+}5$ and St1 in the trochanter. This again demonstrates the low regeneration capacity and the frequent duplication of structures from the basal piece.

DISCUSSION

Regeneration

The results described in this paper for the leg disc show that culture of fragments for brief periods results in duplication of anlagen (see Fig. 6f), multiplication of units such as sensilla (see Fig. 6b), and/or regeneration of the missing parts of the disc (Fig. 6d and e). Only after prolonged growth do we observe a high incidence of transdetermination. Similar results for transdetermination were reported by Wildermuth (1968a) working with the labial disc. Furthermore we found that different fragments behaved in different ways. The differences in developmental capacities of fragments lie in their original position in the discs, and probably not in their size or in the degree of damage from cutting. Regenerative ability seems to be restricted to the upper medial quarter of the disc, whereas other regions show a high tendency to duplicate the anlagen already present in the fragment. Similar results were obtained by P. Bryant (personal communication) using a technique of *in situ* surgery. Bisection of a 96-hr-old leg disc into cap and basal portion results in a regeneration from the cap piece and a duplication in the basal piece. Regional differences in developmental capacities have also been reported from other imaginal discs. For example Gehring (1966) found that in the antennal disc a certain portion of the fragments regenerated other regions of the disc.

Our experiments also showed that proximal anlagen regenerated distal structures. The regeneration process of the leg seems to be polarized in proximodistal direction just as in *Pyrameis*, (Bodenstein, 1937), *Culex* (Spinner, 1969), and *Tenebrio* (Hadorn *et al.*, 1969). When proximal anlagen (R pieces) are transplanted into young larvae, they regenerate the tarsal segments in 71% of the cases and only rarely the most distal elements, the claw organ (29%). But the frequency of claw formation can be enhanced (to 55%) if the growth period is prolonged by an 8–10-day period of culture in an adult. This indicates that regeneration occurs successively, perhaps from the cutting region.

Fragments of proximal anlagen (e.g., medial halves, quarter pieces) not only regenerate distal structures but are also capable of completing the entire segment, that is, lateral elements are formed by the progeny of the medial cells of the disc anlage. But we found no cases where lateral pieces differentiated medial structures. However, it cannot be excluded that, under certain circumstances (e.g., extremely long culture period), they could replace missing regions. This seems possible because in a few cases lateral halves without endknob material differentiated claws, indicating that lateral fragments had a low but measurable capacity for regeneration of distal structures.

Quarter pieces (upper medial quarter, Fig. 1f) can sometimes regenerate complete legs if they are given a long time to grow. The degree of regeneration therefore depends on the amount of time allowed for growth between the operation and the onset of differentiation. The observation that after adult culture medial fragments produce pieces which resemble a complete leg disc in size and form, indicates that determination in regenerated parts takes place during culturing. The data from regenerating quarter fragments (Fig. 7) demonstrate a successive replacement of structures from the cutting region. A similar finding was reported by Lüönd (1961), who studied the "regulation process" in fragments of the genital disc. He found a sequence in appearance of the different structures which were regenerated. Even quarter pieces of the genital disc could produce a qualitatively normal genital apparatus, when transplanted into young larvae.

In quarter leg discs (Fig. 1f) coxa, trochanter, and femur regenerate synchronously. Hence regeneration does not start in the coxa and then complete the trochanter and femur. There are two possible mechanisms for the observed regeneration in proximal segments: (1) each segment is regenerated from cells of that specific segment; that is, coxa cells only replace coxa structures, for example. (2) A blastema of rapidly dividing cells is formed at the cutting region, so that cells from one segment might contribute material for other segments. These cells would be arranged in a sector that would grow in a mediolateral direction. Determination within the sector would occur along the proximodistal axis only later on. We favor the second hypothesis for the following reasons: first, Gehring (1967) and Postlethwait *et al.* (1971) concluded that the rate of cell divisions in a cultured blastema varies to a large extent among different cells. According to the first hypothesis, we might therefore expect that the different segments would not regenerate at the same rate. This is not the case. Second, preliminary results from clonal analysis of regenerates, using X-ray induced somatic crossing over, showed that the progeny of a single cell can differentiate structures from different segments.

The process of regeneration demonstrates that, under our experimental conditions, cell heredity of a specific determination cannot always be the rule in the leg imaginal disc. On the other hand, there is evidence that in some cases areal specific determination is replicated and passed on to daughter cells. Hadorn (1966) observed that long-term cultures of genital disc fragments had a tendency to produce monocultures. The inventory of differentiations became gradually reduced, and finally, for example, only anal plates and rectum were found. These two structures were continuously observed during 70 transfer generations. This indicates that an areal specific state of determination can be propagated over many cell generations. A monoculture of anal plates derived from an eye-antennal disc for more than 100 transfer generations was also observed by E. Gateff in this laboratory. Further evidence for the cell heredity of a specific determination is given by Nöthiger and Schubiger (1966). When certain anlagen in half a genital disc were eliminated with a UV microbeam, the grown and metamorphosed fragment differentiated to form a symmetrical but incomplete genital apparatus. The eliminated structure (e.g., the anal plate) was missing in both mirror images.

How can we explain the different behavior of cells which pass their areal specific determination on to their progeny and those which can regenerate? All regions of the imaginal disc show areal specific determination when fragments are transplanted into mature larvae and caused to metamorphose immediately. Consequently it is possible to establish anlage plans of the discs as mentioned previously. If pieces of imaginal discs are caused to grow, they are capable of forming other structures, depending on the origin of the fragment. Let us postulate two populations of cells in a disc. One consists of cells that replicate their specific state of determination. Such cells would more frequently be found in the lateral and base regions of the leg disc. The seond cell population proliferates rapidly and propagates only the general quality of "legness." This type of cell is located mainly in the upper medial quarter of the disc, and is also capable of regenerating. Whether specifically determined cells as well as cells carrying only the quality for a certain disc exist together in a mature imaginal disc or whether these pluripotent cells arise only after extensive proliferation, must remain an open question.

Multiplication of Units

In an implant either a single marker can be multiplied and all the others differentiate according to the anlage plan or several markers can differentiate an increased number of units. It is important to distinguish between the multiplication of units and pattern duplication. By multiplication of units we refer, for example, to an enlargement of the group of 8 sensilla trichodea in the coxa such that up to 20 sensilla will be differentiated. In pattern duplication the anlage of the entire fragment appears twice and the two duplicates separate, so that in the end a complete mirror image is formed of the structures originally in the fragment.

Wigglesworth (1940) observed a process in *Rhodnius*, which resembles the multiplication of units we have observed in *Drosophila*. He showed that cauterized cells in the abdominal tergites were replaced by cells migrating from the periphery, which underwent rapid divisions. In this process the original determination for marginal spines of the surrounding cells was maintained and was expressed by an increased number of such spines as these cells covered the burned spot. In the case of marginal spines there was a multiplication of units. In the present experiments, it could be that multiplication originated from regions with frequent cell divisions in which cells passed on to daughter cells the determination for a specific marker.

Pattern Duplication

Pattern duplications and the occasional triplications are composed only of elements for which the anlagen were present in the original fragment (Nöthiger and Schubiger, 1966). Furthermore, lateral, basal and three-quarter pieces (Fig. 1) show pattern duplications far more frequently than do medial fragments.

Similar results were obtained in this laboratory by Bryant and Postlethwait, using a completely different technique. They X-irradiated animals at 24 hr after egg deposition and found flies after metamorphosis with structure duplications. Twenty-nine of 30 cases with leg duplicates had double incomplete trochanters, and the anlagen of the missing structures all mapped in the medial half of the disc.

Gehring (1966) studied the formation of mirror images from cultured antennal disc fragments and found that duplicated palps arose when a primordium enlarged, exceeded the normal size, and divided into two equal anlagen. Each of the symmetrical palps differentiated about the normal number of bristles. This phenomenon has been called arealization (Gehring, 1966). The following observations indicate that the multiplication of units does not precede pattern duplication: (1) in a certain leg segment one marker alone can be multiplied, leaving the remaining markers of the pattern unchanged; (2) multiplication is frequently observed from medial halves, but these pieces rarely produce pattern duplications; (3) multiplication can lead to areas with a greatly increased number of units, e.g., 45 sex comb teeth in one comb, or 30 sensilla in the coxal group of 8 sensilla trichodea (St8). Pattern duplication, on the other hand, involves an ordered doubling of all structures. Evidently, in multiplication of units, arealization is disturbed.

The mechanism which leads to pattern duplication is not known. Ulrich (1971) investigated the cell lineage in pattern duplications from half genital discs by marking clones of cells genetically by X-ray-induced somatic crossing over. He found that the new halves, formed after culture, contained after differentiation larger spots than the old halves did. He concluded that the duplicate is formed from a small number of progenitor cells, indicating that the process of duplication does not depend on cell-by-cell replication of specific states of determination. Similar results were reported by Postlethwait *et al.* (1971).

It may be possible to obtain conclusive information on the mechanism of structure duplication by performing clonal analysis on lateral fragments, which after transplantation into 68-hr-old larvae never regenerate.

Transdetermination

Several authors have demonstrated that, after proliferation, cells from certain regions of a disc transdetermine more frequently than others (Gehring, 1966; Wildermuth, 1968a; Mindek, 1968; Schubiger, 1968). This observation was also made in the present experiments. After 3 days of adult culture, medial disc halves differentiate allotypic structures in 52–58% of the cases (Table 1). Under the same conditions lateral halves do not transdetermine. Therefore the average rate of transdetermination in all half discs (medial and lateral) is 26–29%. In a previous paper (Schubiger, 1968), we reported a frequency of 34% transdetermination in disc halves cultured for a period of 14 days. In the present experiments an even higher rate of transdetermination (53%) was exhibited by quarter (upper medial) fragments cultured for 8 days. This frequency of transdetermination is comparable to that for disintegrated leg discs (25–57% depending on the genotype, Tobler, 1966).

On the one hand, there appears to be a correlation between the extent of proliferation and the frequency of transdetermination (Schläpfer, 1963; Gehring, 1966; Tobler, 1966; Garcia-Bellido, 1966; Wildermuth, 1968a; Mindek, 1968); but on the other hand, certain disc regions transdetermine more frequently than others. This leads to the tentative conclusion that extensive proliferation is not the only requirement for transdetermination. To permit transdetermination, much extensive proliferation of cells must occur in a certain specific region. This conclusion is strengthened by the observation that some long-term cultures tend to form monocultures (e.g., anal plates, Hadorn, 1966; E. Gateff, personal communication) even though they proliferate well (Mindek, 1968). Apparently growth cannot be the sole prerequisite for transdetermination. We have already suggested that either multiplication of units or regeneration precede transdetermination. We prefer the first possibility since multiplication of units is often found together with allotypic structures. Multiplication of units might be the first recognizable consequence for rapidly dividing cells. Surrounding cells would not divide or would divide only slowly and would differentiate structures according to the anlage plan. Wildermuth (1968b) found that, when cultured discs give rise to mirror-image copies, rapid proliferation occurs only in the new part. Moreover allotypic structures form only in the new part. The original part always differentiates structures according to the anlage plan. Our results also demonstrate that in the cases where transdetermination occurred, the elements expected from the fate map differentiated next to allotypic structures. But this does not mean that allotypic patterns appear only in direct contact with differentiations from the medial quarter; it is possible that a fragment can regenerate first and that only later do cells from the newly formed regions start to proliferate intensively and subsequently transdetermine.

In conclusion, the foregoing experiments demonstrate that fragments of imaginal discs develop in different ways depending on the position they occupied in the original disc. One specific area of the disc is capable of complete regeneration, whereas in the other areas growth generally leads to pattern duplication, and regeneration occurs only rarely, if at all. This fact has several important consequences for future experiments on imaginal discs. For example, in experiments dealing with processes such as determination and transdetermination, it will be necessary, in order to interpret the data, to specify precisely what part of the disc is used. Also, it may turn out that an understanding of processes like transdetermination will require a prior understanding of the process of regeneration. Finally, the results demonstrate that even though imaginal discs show mosaic determination, this determination can be altered during a period of additional growth and they can undergo regeneration as observed in other insects.

The author would like to thank Dr. Howard A. Schneiderman, Dr. Peter J. Bryant, and Dr. Clifton Poodry for many helpful comments during the course of this work.

REFERENCES

BODENSTEIN, D. (1935). Beintransplantationen an Lepidopterenraupen. III. Zur Analyse der Entwicklungspotenzen der Schmetterlingsbeine. *Wilhelm Roux Arch. Entwicklungsmech. Organismen* **133,** 156–192.

BODENSTEIN, D. (1937). Beintransplantationen an Lepidopterenraupen. IV. Zur Analyse experimentell erzeugter Bein-Mehrfachbildungen. *Wilhelm Roux Entwicklungsmech. Organismen* **136,** 745–785.

EPHRUSSI, B., and BEADLE, G. W. (1936). A technique of transplantation for *Drosophila*. *Amer. Natur.* **70,** 218–225.

GARCIA-BELLIDO, A. (1966). Changes in selective affinity following transdetermination in imaginal discs of *Drosophila melanogaster*. *Exp. Cell Res.* **44,** 382–392.

GEHRING, W. (1966). Uebertragung und Aenderung der Determinationsqualitäten in Antennenscheiben-Kulturen von *Drosophila melanogaster*. *J. Embryol. Exp. Morphol.* **15,** 77–111.

GEHRING, W. (1967). Clonal analysis of determination dynamics in cultures of imaginal discs in *Drosophila melanogaster*. *Develop. Biol.* **16,** 438–456.

HADORN, E. (1963). Differenzierungsleistungen wiederholt fragmentierter Teilstücke männlicher Genitalscheiben von *Drosophila melanogaster* nach Kultur *in vivo*. *Develop. Biol.* **7,** 617–629.

HADORN, E. (1965a). Ausfall der Potenz zur Borstenbildung als "Erbmerkmal" einer Zellkultur von *Drosophila melanogaster*. *Z. Naturforsch. B* **20,** 290–292.

HADORN, E. (1965b). Problems of determination and transdetermination. *Brookhaven Symp. Biol.* **18,** 148–161.

HADORN, E. (1966). Konstanz, Wechsel und Typus der Determination und Differenzierung in Zellen aus männlichen Genitalanlagen von *Drosophila melanogaster* nach Dauerkultur *in vivo*. *Develop. Biol.* **13,** 424–509.

HADORN, E., and BUCK, D. (1962). Ueber Entwicklungsleistungen transplantierter Teilstücke von Flügel-Imaginalscheiben von *Drosophila melanogaster*. *Rev. Suisse Zool.* **69,** 302–310.

HADORN, E., and GLOOR, H. (1946). Transplantationen zur Bestimmung des Anlagemusters in der weiblichen Genital-Imaginalscheibe von *Drosophila*. *Rev. Suisse Zool.* **53,** 495–501.

HADORN, E., BERTANI, G., and GALLERA, J. (1949). Regulationsfähigkeit und Feldorganisation der männlichen Genital-Imaginalscheibe von *Drosophila melanogaster*. *Wilhelm Roux Arch. Entwicklungsmech. Organismen* **144,** 31–70.

HADORN, E., HAURI, H., MÜLLER, P., and STEINER,

E. (1969). Transplantationsexperimente mit Imaginalanlagen von *Tenebrio molitor* L. *Rev. Suisse Zool.* **76,** 642–649.

KROEGER, H. (1958). Ueber Doppelbildungen in die Leibeshöhle verpflanzter Flügelimaginalscheiben von *Ephestia kühniella Z. Wilhelm Roux Arch. Entwicklungsmech. Organismen* **150,** 401–424.

LOOSLI, R. (1959). Vergleich von Entwicklungspotenzen in normalen, transplantierten und mutierten Halteren-Imaginalscheiben von *Drosophila melanogaster. Develop. Biol.* **1,** 24–64.

LÜÖND, H. (1961). Untersuchungen zur Mustergliederung in fragmentierten Primordien des männlichen Geschlechtsapparates von *Drosophila séguyi. Develop. Biol.* **3,** 615–656.

MADHAVAN, K., and SCHNEIDERMAN, H. A. (1969). Hormonal control of imaginal disc regeneration in *Galleria mellonella* (Lepidoptera). *Biol. Bull.* **137,** 321–331.

MEISENHEIMER, J. (1908). Ueber Flügelregeneration bei Schmetterlingen. *Zool. Anz.* **33,** 689–701.

MINDEK, G. (1968). Proliferations- und Transdeterminationsleistungen der weiblichen Genital-Imaginalscheiben von *Drosophila melanogaster* nach Kultur *in vivo. Wilhelm Roux Arch. Entwicklungsmech. Organismen* **161,** 249–280.

NÖTHIGER, R., and SCHUBIGER, G. (1966). Developmental behaviour of fragments of symmetrical and asymmetrical imaginal discs of *Drosophila melanogaster* (Diptera). *J. Embryol. Exp. Morphol.* **16,** 355–368.

OUWENEEL, W. J. (1970). Developmental capacities of young and mutant, wild-type and *opht* eye imaginal discs in *Drosophila melanogaster. Wilhelm Roux Arch. Entwicklungsmech. Organismen* **166,** 76–88.

POHLEY, H. J. (1960). Experimentelle Untersuchungen über die Steuerung des Häutungsrhythmus bei der Mehlmotte *Ephestia kühniella* Zeller. *Wilhelm Roux Arch. Entwicklungsmech. Organismen* **152,** 183–203.

POODRY, C. A., SCHNEIDERMAN, H. A., and POSTLETHWAIT, J. H. (1971). Cellular dynamics of pattern duplication in imaginal discs of *Drosophila melanogaster* cultured *in vivo. Develop. Biol.* **26,** 125–132.

SCHLÄPFER, TH. (1963). Der Einfluss des adulten Wirtsmilieus auf die Entwicklung von larvalen Augenantennen-Imaginalscheiben von *Drosophila melanogaster. Wilhelm Roux Arch. Entwicklungsmech. Organismen* **154,** 378–404.

SCHUBIGER, G. (1968). Anlageplan, Determinationszustand und Transdeterminationsleistungen der männlichen Vorderbeinscheibe von *Drosophila melanogaster. Wilhelm Roux Arch. Entwicklungsmech. Organismen* **160,** 9–40.

SPINNER, W. (1969). Transplantationsversuche zur Blastemgliederung, Regenerations- und Differenzierungsleistung der Beinanlagen von *Culex pipiens* (L.). *Wilhelm Roux Arch. Entwicklungsmech. Organismen* **163,** 259–286.

TOBLER, H. (1966). Zellspezifische Determination und Beziehung zwischen Proliferation und Transdetermination in Bein- und Flügelprimordien von *Drosophila melanogaster. J. Embryol. Exp. Morphol.* **16,** 609–633.

UBISCH, V. L. (1911). Ueber Flügelregeneration beim Schwammspinner *Lymantria dispar. Wilhelm Roux Arch. Entwicklungsmech. Organismen* **31,** 637–653.

ULRICH, E. (1971). Cell lineage, Determination und Regulation in der weiblichen Genitalimaginalscheibe von *Drosophila melanogaster. Wilhelm Roux Entwicklungsmech. Organismen* **167,** 64–82.

URSPRUNG, H. (1957). Untersuchungen zum Anlagemuster der weiblichen Genitalscheibe von *Drosophila melanogaster* durch UV-Strahlenstich. *Rev. Suisse Zool.* **64,** 303–311.

URSPRUNG, H. (1959). Fragmentierungs- und Bestrahlungsversuche zur Bestimmung von Determinationszustand und Anlageplan der Genitalscheiben von *Drosophila melanogaster. Wilhelm Roux Arch. Entwicklungsmech. Organismen* **151,** 504–558.

URSPRUNG, H. (1962). Einfluss des Wirtsalters auf die Entwicklungsleistung von Sagittalhälften männlicher Genitalscheiben von *Drosophila melanogaster. Develop. Biol.* **4,** 22–39.

VOGT, M. (1946). Zur labilen Determination der Imaginalscheiben von *Drosophila.* I. Verhalten verschiedenartiger Imaginalanlagen bei operativer Defektsetzung. *Biol. Zentralbl.* **65,** 223–238.

WIGGLESWORTH, V. B. (1940). Local and general factors in the development of "pattern" in *Rhodnius prolixus* (Hemiptera). *J. Exp. Biol.* **17,** 180–200.

WILDERMUTH, H. (1968a). Differenzierungsleistungen, Mustergliederung und Transdeterminationsmechanismen in hetero- und homoplastischen Transplantaten der Rüsselprimordien von *Drosophila. Wilhelm Roux Arch. Entwicklungsmech. Organismen* **160,** 41–75.

WILDERMUTH, H. (1968b). Autoradiographische Untersuchungen zum Vermehrungsmuster der Zellen in proliferierenden Rüsselprimordien von *Drosophila melanogaster. Develop. Biol.* **18,** 1–13.