

REGENERATION IN LARVAL LEGS OF SILKWORMS.

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WITH 10 FIGURES.

As far as mentioned by Morgan¹ and Brindley,² which are the only two recent accounts, known to me, that attempt to refer in an inclusive way to the recorded observations and experiments on regeneration in insects, all³ the work done on regeneration of the legs in insects with complete metamorphosis has been limited to making mutilations of the larval legs and noting what, if any, effect was apparent in the legs of the imago. There are several accounts of such observations, and some of these accounts are, curiously enough, of comparatively recent date. I say "curiously," for it has been known now for a score of years and more, that the legs (as also the wings, antennæ, etc.,) of insects of complete metamorphosis are derived (at least in all the higher forms, such as the *Lepidoptera*, *Diptera* and *Hymenoptera*) not by a transforming of the larval legs (if present) into the imaginal ones, but from new centers called imaginal discs or histoblasts. These histoblasts are developed from an invagination of the larval cellular skin layer (hypoderm) and only in comparatively late larval life do the new developing imaginal legs lie within the larval ones. It follows from this that if a larval leg be cut off in early larval life the imaginal leg is in no way mutilated, and that if it

¹Regeneration, 1901.

²On Certain Characters of Reproduced Appendages in *Arthropoda*, particularly in the *Blattidae*. Proc. Zool. Soc., Lond., 1898, pp. 924-958.

³Tornier describes (Zool. Anzeiger, Vol. XXIV, pp. 634-664) certain experiments on regeneration in the meal beetle, *Tenebrio molitor*, in which account he states that cut-off larval legs are regenerated before pupation, if young larvæ are used as subjects. This statement of Tornier's I have only found since sending my paper to press.

appears of full size and normal character in the adult insect, this is not due to restorative regeneration, but simply to its normal growth and development. If a leg be cut off in late larval life, the developing imaginal leg may or may not be at the same time mutilated. If mutilated, however, it will always be by a removal of much less of its extent than of the extent of the larval leg taken off. A cut which severs the larval leg near its base (for example, through the base of the femur,) will not take off more than the tarsus or perhaps part of the tibia and tarsus of the imaginal leg, which, in its development, is beginning to extend into the larval one. Thus if the imaginal leg be found, when the imago issues, to lack a tarsus but to possess a complete femur and tibia, this is no indication that there has been a partial regeneration; there may have been none whatever.

To make a definitive test of the capacity of an insect with complete metamorphosis to regenerate lost parts I have cut off legs, both thoracic and abdominal (prop-legs) of the larvæ of the silkworm moth, *Bombyx mori*, at various ages, and have noticed whether or not regeneration of these legs took place before pupation, and if so in what degree and whether normally, *i. e.*, so as to produce an exact replica of the lost leg, or not. The life of the silkworm larva is about 50 days (in the races which I have used for study, and under the conditions attending their rearing in my laboratory), and is divided by four moultings into five approximately equal, active, feeding periods. In the first and second period the larvæ are too small to operate upon satisfactorily, but after the second moulting the legs can be taken off at any particular level desired. In the experiments silkworms of several races, viz: Japanese white, Chinese white, Italian yellow, Chinese crossed, etc., were used but the regenerative phenomena in all were alike.

Regeneration of Legs.

The results of the experiments may be stated and illustrated (see Figs. 1 to 10), as follows: In the first lots of individuals, mostly of six each, one thoracic leg or one abdominal (prop-) leg or one of each group of legs was cut off of larvæ about 15

day sold; that is, between the first and second moulting. Colloidionization of the wounds was first tried, as it was believed that with such a turgid body and with the kind of circulation possessed by the silkworm the loss of blood-lymph would be considerable. It was soon noted that the blood loss was small and the wound

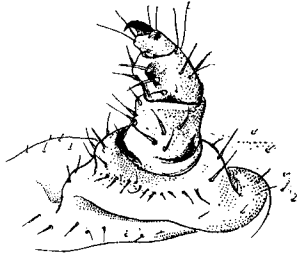


FIG. 1.



FIG. 2.

Fig. 1. Normal leg of third thoracic segment of full-grown larva.
Fig. 2. Normal abdominal prop-leg of full-grown larva.

quickly closed in nearly all cases. Most of the worms, roughly about 80 per cent, lived, and went through their subsequent moultings normally. After the second moulting, which was the first moulting after the loss of the legs, the wounds were always cleanly covered over by new skin, and no sign of regeneration nor of scar was apparent. After the next moulting, however, some specimens would show a certain obvious degree of regeneration, both thoracic and prop-legs being replaced more or less nearly completely as regards number of segments, size, and character of the distal tip. Some specimens would however show no regeneration at all. Nor would these non-regenerating individuals show any change after the later (last) moulting. This was also apparently true of the regenerating individuals also; that is, the amount or character of regeneration shown after the second moulting after mutilation was not increased or changed in the later life of the larva, which has regularly another moulting before the time of spinning up and pupation.

The unevenness of the results in these cases, both in degree of regeneration and in the regular occurrence of a few cases of no

regeneration at all led me to modify the later experiments as follows: In one lot of worms one prop-leg and one thoracic leg were cut off in the case of each individual halfway between base and tip, or at least always somewhat above the base, while in another lot of larvæ taken at the same time, at the same age,

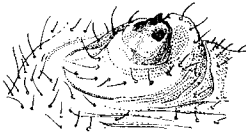


FIG. 3.

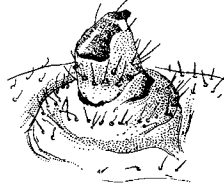


FIG. 4.

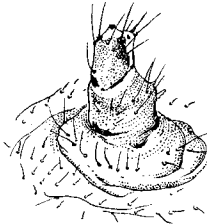


FIG. 5.

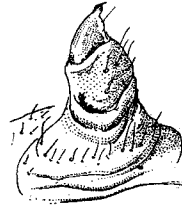


FIG. 6.

Fig. 3. Regenerated thoracic leg of full-grown larva from stump of leg cut off just above base after the second moulting.

Fig. 4. Regenerated thoracic leg of full-grown larva from stump of leg cut off just above base after the second moulting.

Fig. 5. Regenerated thoracic leg of full-grown larva from stump of leg cut off just above base after the second moulting.

Fig. 6. Regenerated thoracic leg of full-grown larva from stump of leg cut off just above base after the second moulting.

the legs were cut off as close to the body as possible. The results indicated that a condition, which I expected would be revealed, actually does exist. The larvæ with legs cut so as to leave a stump in all cases regenerated the leg more or less nearly completely, although in practically all cases of smaller size than the original; while those larvæ whose legs had been cut off as near the body as possible, *i. e.*, wholly removed, in no case regenerated a leg or any part of one. That is, the silkworm's leg can regener-

ate any part of itself, but the silkworm's body (trunk) cannot regenerate a leg wholly lost.

The structural characteristics of the normal thoracic legs and normal prop-legs, are shown in Figs. 1 and 2, respectively, while Figs. 3 to 8 illustrate cases of regeneration selected to show various

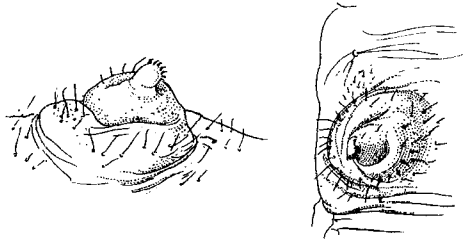


FIG. 7.



FIG. 8.

Fig. 7. Regenerated abdominal (prop-) leg of full-grown larva from stump of leg cut off just above base after first moulting; at left profile view; at right, ventral view.

Fig. 8. Regenerated abdominal (prop-) leg of full-grown larva from stump of leg cut off just above base after first moulting; at left, profile view; at right, ventral view.

degrees of it. Figs. 9 and 10 illustrate examples of no regeneration. In no case of regenerated leg was there a complete reproduction of the original in all details, but in all cases the evident tendency is plainly toward a replica of the original. In the case of the segmented legs (the thoracic) the original number of segments was usually reached and a small terminal claw was produced although always in reduced condition. In the case of the unsegmented prop-legs the terminal half circlet of hooks characteristic of the normal leg was in no case of regeneration completely reproduced, but in all cases a few at least of these terminal hooklets reappeared.

To sum up the results of the experiments, we may say, (a) that the larva of the silkworm moth, *Bombyx mori*, has the capacity of regenerating its thoracic and abdominal (prop-) legs from stumps of these legs, but not from the body (trunk), *i. e.*, that each leg has the capacity to regenerate any distal part from

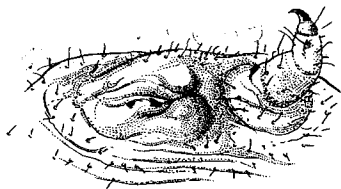


FIG. 9.

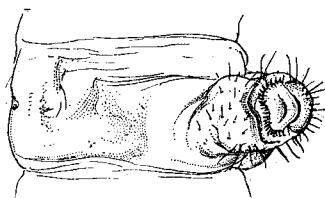


FIG. 10.

Fig. 9. Ventral aspect of third thoracic segment of full-grown larva, which had the left leg cut off at base after the second moulting; no regeneration.

Fig. 10. Ventral aspect of abdominal segment of full-grown larva, which had the left leg cut off at base after the first moulting; no regeneration.

any proximal part, but that the body cannot produce a wholly new leg; (b) that this regeneration shows externally not after the first moulting after the mutilation but after the second moulting, and that the regenerative processes are completed with the appearance of the new parts after this second moulting succeeding the mutilation.

Regeneration of Caudal Horn.

The small caudal horn, a pointed non-segmented, but movable, process projecting upward from the dorsal surface of the penultimate abdominal segment was cut off in many larvæ (silkworms) of various ages, and in no case was there the slightest regeneration. After the first moulting succeeding the mutilation the new skin always extended smoothly over the place where the horn had been, without any sign of scar.

The function of this horn, which occurs on some other lepidopterous larvæ, notable and characteristically on the larvæ of the Sphingid moths, is unknown. It has been explained by some entomologists as an ornament, by others as a "terrifying

organ." It is not a sting nor in any way an effective weapon of defense, as even where long and conspicuous ($\frac{1}{8}$ in. long) it is weak and easily bent. Nor does it secrete an acrid or ill-smelling fluid. Certainly in the silkworm it has had for many hundreds of generations no possible function as a weapon. It is interesting to note that this useless organ is not regenerated.

Relation of Regeneration to Natural Selection.

This suggests to us a consideration of the relation of regeneration, as we have observed it in the silkworm, to its causes, or at least to natural selection as an explaining cause. If the caudal horn is now a useless organ in the silkworm body its lack of capacity to regenerate (loss of capacity, if it ever had it) would seem to favor the theory of the natural selectionists concerning regeneration. At first glance, also, the retaining of the regenerative capacity of the legs, useful organs, may seem to favor this theory. But it must be borne in mind that the silkworm has been for approximately 5000 years a domesticated animal cared for under such conditions as to make the natural loss of legs almost an impossible occurrence.

Perfectly protected against such natural enemies as bite off legs, there has certainly been nothing of that sharp necessity, during all the life of countless successive generations of silkworms, which is supposed to be the basis for maintaining the advantageous capacity for regeneration. There has been a clear field for panmixia. But the regenerative capacity still exists in effective degree. The silkworm offers little aid and comfort to those who would explain regeneration wholly as a phenomenon fostered and maintained by natural selection on a basis of utility.