

Tibia to Femur Ratios of Unaltered and Regenerated Legs of the Stumpy Mutant of the German Cockroach

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ABSTRACT—In wild-type legs of the German cockroach, the ratio of tibia length to femur length (T/F ratio) is low in the foreleg, high in the hindleg, and intermediate in the midleg throughout nymphal instars up to the adult stage. After autotomy at the trochanterofemoral articulation, regenerates from foreleg coxae show intermediate T/F ratios between the normal foreleg and midleg, and those from the hindlegs, intermediate between the normal hindleg and midleg. Those from the midlegs are unaltered in the ratios. Similar studies were made on a mutant, stumpy (*sty*), which has shorter legs than wild type. The relative length of *sty* legs to wild-type legs was around 80%, showing some fluctuation with the stage and the type of legs. The T/F ratios of unoperated *sty* fore-, mid-, and hindleg were, respectively, similar to those of regenerated wild-type. This suggests that *sty* may have primitive legs that are not fully differentiated from midleg-like nonspecific forms. The T/F ratios of regenerated *sty* legs were similar to those of the unoperated *sty* when the legs were autotomized in early instars. When the legs were autotomized in middle and late instars, the ratios of *sty* regenerates increased in all legs. In the adult regenerates, the ratios of the *sty* fore-, mid-, and hindleg almost reached those of the *sty* midleg, *sty* hindleg, and wild-type hindleg, respectively.

INTRODUCTION

The fore-, mid- and hindleg of the German cockroach, *Blattella germanica* (L.), seem to be very similar in their gross morphology. Precise measurements, however, have revealed that the three legs differ from each other in the relative lengths of their segments: coxa, trochanter, femur, tibia and five tarsomeres [1]. In particular, the ratio of tibia length to femur length (T/F ratio) is quite different in the three legs. The ratio is low in the foreleg, high in the hindleg, and intermediate in the midleg throughout nymphal instars up to the adult stage [2].

When the legs of wild-type cockroaches were regenerated after autotomy at the trochanterofemoral articulation, some interesting facts were disclosed in the T/F ratios. In each nymphal instar and the adult stage, regenerates from foreleg coxae showed intermediate T/F ratios between the

normal foreleg and midleg, and those from the hindlegs, intermediate between the normal hindleg and midleg. Those from the midlegs were unaltered in the ratios [3]. In other words the T/F ratios of regenerates from the fore- and hindlegs converged to those of the midlegs, showing incomplete homeotic regeneration toward the midleg. This tendency suggested that the fore- and hindlegs were differentiated from midleg-like primitive forms during normal embryonic development.

Stumpy (*sty*) belongs to a group of mutants on chromosome 9 of the German cockroach that appear to have been highly conserved during the course of evolution [4]. The mutant is also of interest because it alters the shape of the entire body, including that of the legs [5]. The purpose of this study is to investigate the effects of *sty* on growth and development by (i) comparing the development of leg segments, specifically the femur and tibia, to that of wild type, and (ii) determining the effects of regeneration on the development of leg segments of the *sty* mutant.

MATERIALS AND METHODS

Selection of *sty* nymphs

The *sty* mutant is a simple autosomal recessive located on chromosome 9 [5]. Newly-hatched *sty* nymphs were obtained from oothecae produced by *sty/+* females that were mated to *sty/sty* males, because *sty/sty* females are almost sterile. Consequently, each ootheca about 50% *sty* and 50% phenotypically normal embryos. A *sty* nymph could be easily distinguished with its shorter legs from a phenotypically normal nymph as early as the 1st instar by simple inspection (Fig. 1). Only *sty* nymphs were selected for the present studies: regeneration experiments and control measurements. When the first operation was performed in the 1st instar, the detached legs were examined to ascertain whether they were really *sty* legs by measuring the lengths of femur and tibia (Fig. 2).

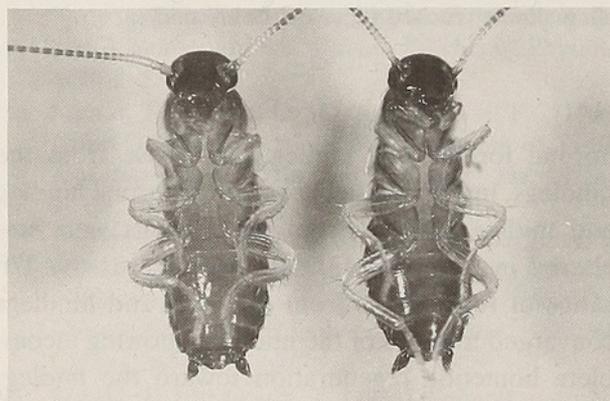


FIG. 1. First-instar nymphs: stumpy (*sty/sty*, left) and wild type (*+/+*, right). Note the short legs in the *sty* mutant.

Rearing and anesthetizing methods

Each 1st-instar *sty* nymph was isolated in a small plastic container (61 × 43 × 17 mm high, Sanplatec, No. 2), since it is known that operated nymphs are often attacked by others under group-rearing conditions. The container was transparent and the nymph could be easily inspected from the outside. A small piece of dog food and water was supplied *ad libitum*. The temperature was maintained at 25 ± 1°C throughout postembryonic development. Prior to each operation mentioned in the following

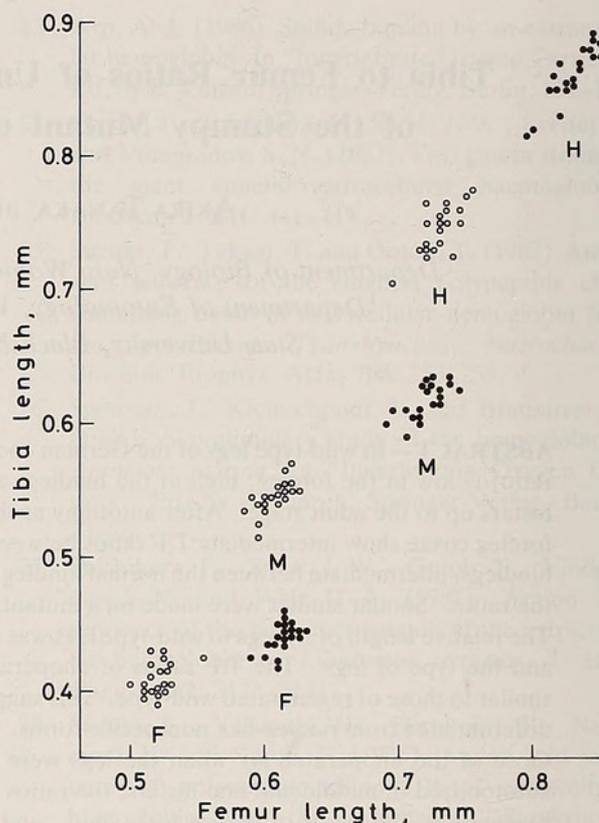


FIG. 2. Femur and tibia lengths of 1st-instar nymphs hatched from a single ootheca. A typical example of comparison between stumpy (*sty/sty*, open circles) and phenotypically normal (*sty/+*, filled circles): foreleg (F), midleg (M), and hindleg (H).

section, carbon dioxide was introduced from a cylinder to the container via a flexible tube. An anesthetized cockroach was carefully taken out, immediately subjected to the operation, and put back in the container before it recovered from anesthesia.

Trochanterofemoral autotomy

Repeated-regeneration experiments were carried out on each fore-, mid-, and hindleg of both sexes during each nymphal stadium. Only one leg was operated on in order to avoid possible complex interactions between operated legs. The leg was autotomized at the trochanterofemoral articulation by gentle pulling of the femur with fine forceps in order to minimize physiological damage. The first operation was performed on day 1 or 2 in the 1st instar before the regeneration critical period [6, 7] so that a regenerate appears in the 2nd instar. The regenerated leg was autotomized

again on day 1 or 2 in the 2nd instar. The same procedures were repeated in the following instars until the insect reached the adult stage. Operations were made always on the same leg of the same side of the body, usually the right side.

Size measurements

The maximal lengths of both the femur and tibia of all legs were measured along the median line of the segments as in previous studies [1, 3]. The measurements were carried out under a dissecting microscope equipped with a measuring apparatus (Kogaku Ltd.). The unit of measurement was 5 μm . Data that appeared in Tanaka [2] and Tanaka *et al.* [1] were used as wild-type control measurements. The lengths of unoperated *sty* legs, usually from the right side of the body, were measured in every nymphal instar and the adult stage. No less than 40 insects (20 males and 20 females) were measured in each stadium, except: 31 in the 2nd, 35 in the 3rd instar. The lengths of regenerated *sty* legs were also measured. Regeneration experiments were initiated with no less than 40 insects (20 males and 20 females) in each series of fore-, mid-, and hindlegs, totaling more than 120 nymphs. Although the absolute lengths of the segments were slightly longer in females than in males in late nymphal instars and adults, no significant differences were detected between the sexes in the relative lengths so far calculated. Therefore, the values are given as combined totals of both sexes.

RESULTS

Effects of stumpy on the development of leg segments

The relative lengths of the stumpy (*sty/sty*) legs to the wild-type (+/+) legs are shown in Figure 3. Stumpy femurs (filled circles) were shorter than wild-type femurs throughout postembryonic development in all legs. The average values of relative femur lengths ranged from 75% to 85% of those of wild type, showing some minor fluctuation with the stage and the type of legs. Stumpy tibiae (open circles) were also shorter than wild-type tibiae throughout development of all legs. The

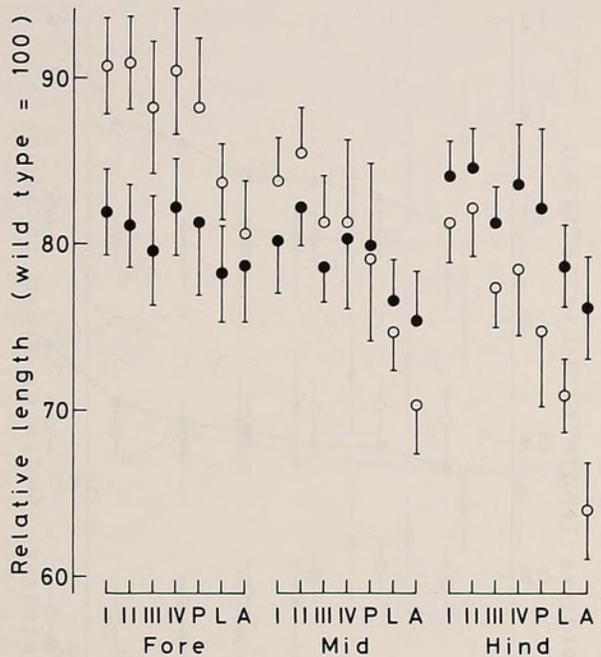


FIG. 3. Relative lengths of *sty* legs to wild-type legs: femur (filled circles) and tibia (open circles). Bars indicate standard deviation. I-IV, P, L, and A represents the 1st-4th, penultimate, last instar, and adult, respectively. No less than 40 insects were measured in each stadium, except: 31 in instar II, 35 in instar III.

relative lengths ranged from 64% to 91% to the wild type, showing more difference than the femur with the stage and the type of legs. The average values tended to decrease from foreleg to hindleg, and also from early instars to the adult stage. Figure 3 confirms that all *sty* legs are shorter than wild-type legs throughout development as far as the lengths of femur and tibia are concerned. It was also found that the variation in relative lengths is larger in the tibia than in the femur. The relative lengths of the foreleg were longer in the tibia than in the femur, and *vice versa* in those of the hindleg.

Figure 4 shows the ratios of tibia length to femur length of *sty* legs together with those of wild-type legs throughout postembryonic development. In wild-type legs, the T/F ratio is low in the foreleg, high in the hindleg, and intermediate in the midleg. The T/F ratios of the *sty* foreleg were consistently higher than those of the wild-type foreleg, showing intermediate ratios between the wild-type foreleg and wild-type midleg; 0.77-0.81 in the average ratios but varying a little with the stage.

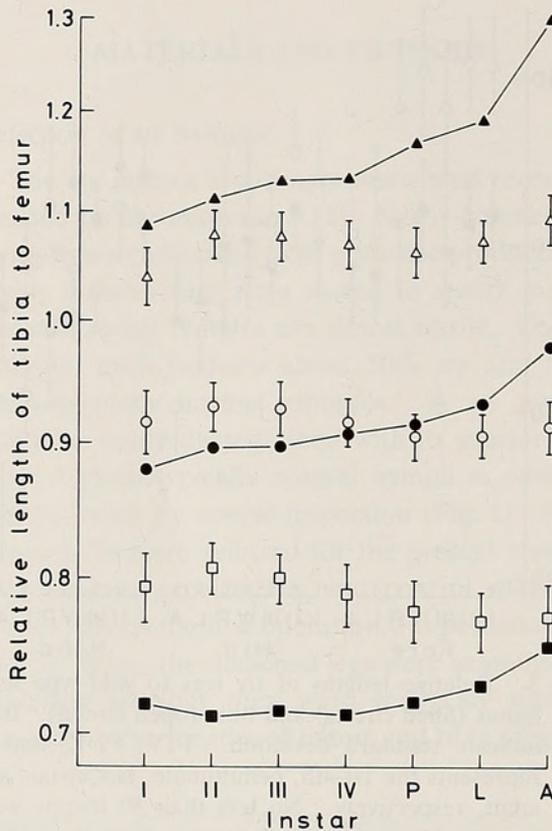


FIG. 4. Ratios of tibia length to femur length of *sty* legs: foreleg (open squares), midleg (open circles) and hindleg (open triangles). Bars indicate standard deviation. No less than 40 insects were measured in each stadium, except: 31 in instar II, 35 in instar III. The ratios of wild type are also marked: foreleg (filled squares), midleg (filled circles) and hindleg (filled triangles). Each symbol of the wild type represents the average of 40 legs; standard deviation is omitted. P, L, and A indicate penultimate, last instar, and adult, respectively.

This tendency of the ratios shifting to the midleg was stronger in early instars than in later instars. The T/F ratios of the *sty* midleg, ranging from 0.90 to 0.93 in the average, were almost unaltered from those of the wild-type midleg, showing a little higher ratios than those of wild-type in early instars and a little lower in later stages. The average ratios of the *sty* hindleg ranged from 1.04–1.09, varying a little with the stage. They were lower than those of the wild-type hindleg throughout postembryonic development, showing intermediate ratios between the wild-type midleg and wild-type hindleg. The extent of the shift toward the midleg was relatively small in early

instars and became larger as the stage advanced. The ratios of both *sty* foreleg and *sty* hindleg tended to converge toward those of the midleg in every nymphal instar and the adult stage. The ratios of all legs were closer to each other in *sty* than in wild type. The difference in the T/F ratio between the foreleg and hindleg was clearly smaller in *sty* (0.29 on the average of all stages) than in wild type (0.45).

Characteristics of regenerated *sty* leg segments

The number of instars of *sty* German cockroaches was usually six, as in the wild type. However, the number was increased to seven or eight when the leg was repeatedly autotomized. All the regenerated legs of *sty* had tetramerous tarsi, one tarsomere less as compared with normal pentamerous tarsi, as in the case of wild-type regenerates [3].

The relative lengths of regenerated *sty* legs to unoperated *sty* legs are shown in Figure 5. The relative lengths were calculated in the 2nd, 3rd, last (L) instar, and the adult stage (A). In the 4th and penultimate (P) instar, however, the lengths could not be appropriately compared with those of unoperated corresponding instars, because the extra instar(s) caused by repeated regeneration, *i.e.* instar(s) V (and VI), were inserted between the instars IV and P, which prevented accurate comparison between unaltered and regenerated *sty* during these middle stages. The average values of regenerated femur (filled circles) ranged from 73% to 87% of those of unoperated femur when compared in the 2nd, 3rd, last instar, and the adult stage. The relative lengths of regenerated tibia (open circles) also varied with the stage and the type of legs. The values were nearly equal to those of femur in the 2nd and 3rd instar, whereas they were considerably higher than those of femur in the last instar and the adult stage. Especially in the foreleg, tibia lengths showed almost full recovery after regeneration.

The relative lengths of *sty* regenerates, both femur and tibia, tended to decrease from instar II to III, and again from the last instar to the adult stage in each of the three legs. However, these tendencies are not authentic, because the number of instars increased by one or two with repeated

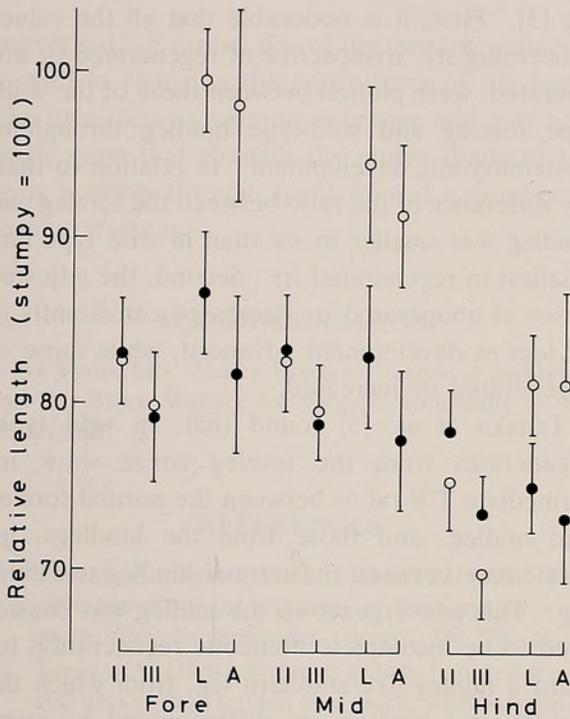


FIG. 5. Relative lengths of regenerated *sty* legs to unoperated *sty* legs: femur (filled circles) and tibia (open circles). Bars indicate standard deviation. The number of regenerates included in each symbol ranged from 31 to 41. II, III, L, and A represent the 2nd, 3rd, last instar, and adult, respectively. In the intermediate instars (IV-P), regenerated *sty* were not compared with unoperated *sty*, because the number of instars was increased with repeated regeneration and the additional instar(s), V (and VI), were inserted between IV and P, which prevented accurate comparison between regenerated and unoperated *sty* during these instars.

autotomy, and the extra instar(s) were inserted between instar IV and P, which affected the calculation of relative lengths compared to the wild type.

The T/F ratios of *sty* regenerates are shown in Figure 6 together with those of unaltered *sty* legs. The T/F ratio of the regenerated foreleg averaged about 0.80 in the 2nd instar; the value was almost equal to that of the unoperated control. The ratio, however, gradually increased and departed from that of the control in later instars. It finally reached 0.93 in the adult stage, a little higher than that of the midleg control. The ratio of the regenerated midleg in the 2nd instar was about 0.92, nearly equal to that of the uninjured control. The ratio also increased in later instars, reaching

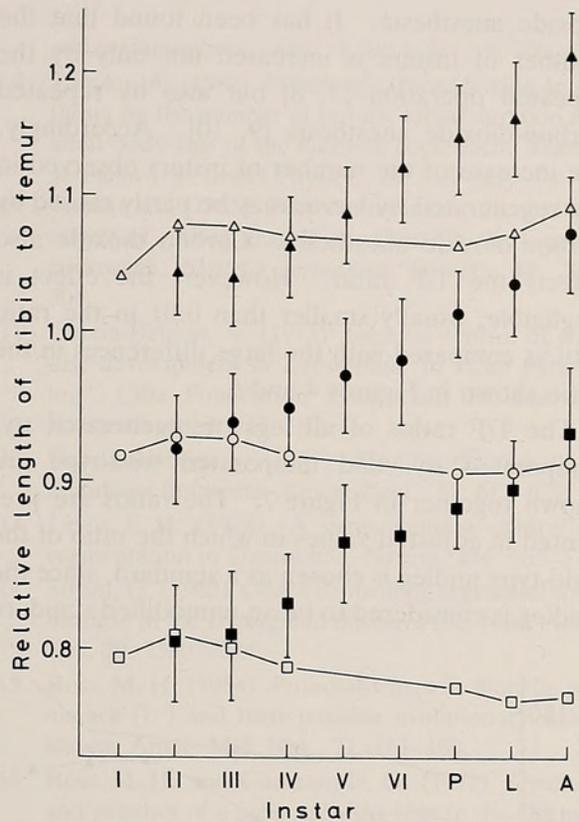


FIG. 6. Ratios of tibia length to femur length of *sty* regenerates: foreleg (filled squares), midleg (filled circles) and hindleg (filled triangles). Each symbol represents the average ratio of regenerates; the numbers ranged from 31 to 41, except: 9-15 in the instar VI. Bars indicate standard deviation. The ratios of unoperated *sty* legs are also marked: foreleg (open squares), midleg (open circles) and hindleg (open triangles); standard deviation is omitted. Instar V includes that of VII- and VIII-instar type; instar VI, that of VIII-instar type. These instar types were not found in unoperated controls. P, L, and A indicate penultimate, last instar, and adult, respectively.

1.07 in the adult stage, almost that of the unoperated hindleg. The ratio of the regenerated hindleg was 1.04 in the 2nd instar, a little lower than that of the unaltered hindleg. However, it exceeded the control in the 5th instar, and reached 1.22 in the adult stage when the autotomy was performed in the last instar. The ratio in some late instars was almost as high as that of the wild-type hindleg.

DISCUSSION

Operation by autotomy necessitates carbon-

dioxide anesthesia. It has been found that the number of instars is increased not only by the repeated operation [3, 8] but also by repeated carbon-dioxide anesthesia [9, 10]. Accordingly, the increase of the number of instars observed in the regenerated *sty* larvae may be partly caused by carbon-dioxide anesthesia. Carbon dioxide also affects the T/F ratio. However, the effect is negligible, usually smaller than 0.01 in the ratio [10] as compared with the large differences in the ratio shown in Figures 4 and 6.

The T/F ratios of all legs of regenerated *sty*, unoperated *sty*, and unoperated wild-type are shown together in Figure 7. The ratios are presented in adjusted values in which the ratio of the wild-type midleg is chosen as a standard, since the midleg is considered to be an unmodified standard

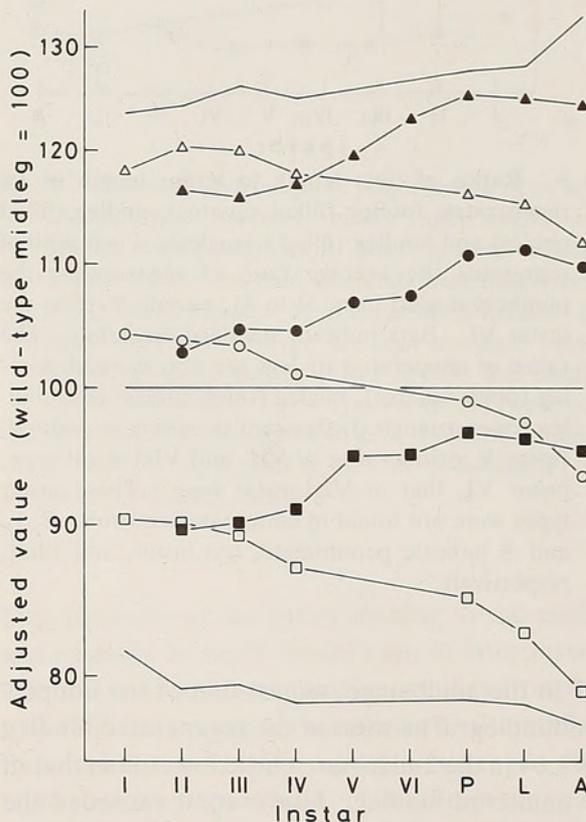


FIG. 7. Ratios of tibia length to femur length in adjusted values. The ratio of the wild-type midleg is regarded as the standard in each stage. Lowest line indicates the ratio of wild-type foreleg; highest line, that of wild-type hindleg. Unoperated *sty* foreleg (open squares), midleg (open circles) and hindleg (open triangles); regenerated *sty* foreleg (filled squares), midleg (filled circles) and hindleg (filled triangles).

leg [3]. First, it is noticeable that all the values concerning *sty*, irrespective of regenerated or unoperated, were plotted between those of the wild-type foreleg and wild-type hindleg throughout postembryonic development. In relation to that, the difference of the ratio between the foreleg and hindleg was smaller in *sty* than in wild type and smallest in regenerated *sty*. Second, the adjusted ratios of unoperated *sty* decreased consistently in all legs as development advanced, while those of regenerated *sty* increased.

Tanaka *et al.* [3] found that, in wild type, regenerates from the foreleg coxae show intermediate T/F ratios between the normal foreleg and midleg, and those from the hindlegs, intermediate between the normal hindleg and midleg. This convergence to the midleg was considered to be incomplete homeotic regeneration toward a midleg-like standard leg, from which the foreleg and hindleg are differentiated by some modifiers. The hypothesis that the midleg is a general type of leg seems comparable to the concept that a 'developmental sink' or 'ground state' is located in the mesothorax of *Drosophila* [11-14]. At the same time, it has been suggested that homeotic mutations found in *Drosophila* differ from the rather continuous alteration of characters such as the T/F ratio. Hence the analysis of the latter kind is also important to our understanding of developmental organization.

As we first expected, the T/F ratio of *sty* fore- and hindlegs showed convergence to that of the midleg. This implies that *sty* may have primitive legs that have not differentiated greatly from a midleg-like nonspecific form. This mutant also has shortened wings in adult males [5]. From a viewpoint that *sty* concerns the expression of a primitive developmental pathway, it is intriguing that the *sty* locus is included in the chromosome 9 gene cluster along with other primitive traits: prowing [15, 16], notched sternite [17, 18], miniature-wing [19], and maxillary-palp-elongate [20]. During the course of evolution, wild-type alleles of these genes seem to have suppressed primitive characters and modified the ancestral forms into the normal extant segments. It is of particular interest that *sty* has dual effects on both the dorsal (short wings in adult males) and ventral (short legs and

converged T/F ratios) side of the thoracic segments as well as changing the entire form of the body. The locus acts as a simple recessive and may be a single gene that changes the entire shape of the body possibly through regulation of a number of structural genes.

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