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# Developmental continuity between larval and adult leg patternings in *Coccinella septempunctata* (Coleoptera: Coccinellidae)

*Pengxiang Wu, Xiaofei Xiong, Zhen Li, Shuo Yan, Xiaoxia Liu* and Qingwen Zhang[1,*]*

**Abstract**

Complete metamorphosis, a key innovation in insect development, promotes diversification of species, but whether larval and adult morphologies evolve independently remains unknown. To analyze morphological continuity between the legs of the larva and the adult, the regeneration frequencies and lengths of regenerated legs of the seven-spotted lady beetle, *Coccinella septempunctata* (Coccinella: Coccinellidae), were studied in the laboratory. The results showed the regenerated legs appeared only in the adult stage rather than in the larval instars. Regeneration frequency was greater following half ablation than after complete ablation and decreased with increasing instar of the ablated larvae, but it was unaffected by the thoracic location of the ablated leg or the side of the body to which the leg was attached. The length of the regenerated leg was longer in half ablation than in complete ablation, but it had no significant relationship with the above locations and the number of the instar. Partially regenerated legs in complete ablation were shorter than in half ablation, while completely regenerated legs appeared to be slightly shorter than the contralateral legs, which had not been ablated. A critical point in time of development when the frequency of leg regeneration began to decline was found to be at day 3.5 of the fourth instar's stadium. Subsequently on day 4, the regeneration frequency became quite small. The present study indicated that adult leg patterning is inherited from the larval leg and does not develop independently of the larval leg. Thus larval morphology constrains adult morphology in *C. septempunctata.*

Key Words: *Coccinella septempunctata*; critical period for leg regeneration; developmental continuity; length of regenerated leg; regeneration frequency

**Resumen**

La metamorfosis completa, como una innovación clave, promueve la diversificación de las especies, pero si la morfología de las larvas y los adultos evolucionan de una forma independiente sigue siendo desconocida en gran parte. Para analizar la continuidad morfológica de las patas en las larvas y los adultos, la frecuencia de regeneración y la longitud de las patas regeneradas de *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) fueron estudiadas en el laboratorio. Los resultados mostraron que después de la ablación de las patas en las larvas, en todos los casos, se presentan patas regeneradas en el estadio adulto, pero nunca en el estadio larval. La frecuencia de regeneración fue mayor cuando sólo la mitad distal de la pata fue cortada (corte medio) que cuando toda la pata fue eliminada (corte completo). Además, la frecuencia de regeneración fue menor en el cuarto estadio que en los 3 estadios anteriores. Sin embargo, la frecuencia de regeneración no fue afectada por la ubicación (del segmento torácico) ni el lado del cuerpo con la pata cortada. Las patas regeneradas fueron más largas en el tratamiento de corte medio que en el tratamiento de corte completo, pero la longitud de las patas regeneradas no se relacionaron significativamente con su ubicación torácica o el estadio larval durante el cual se había producido el corte. Las patas parcialmente regeneradas en el tratamiento de corte completo fueron más cortas que en el tratamiento de corte medio, mientras que las patas completamente regeneradas parecía ser ligeramente más cortas que las del control. Se encontró que el período crítico final para la regeneración de las patas sucedio en el día 3.5 del cuarto estadio. El presente estudio indicó que el patrón de las patas de los adultos fue heredado de las patas de las larvas, y que las patas de los adultos no se desarrolla independientemente de la patas de las larvas.

Palabras Clave: ablación; período crítico; regeneración de la patas; continuidad del desarrollo; frecuencia de regeneración; mariquitas de siete manchas

Complete metamorphosis is composed of 2 morphologically discrete postembryonic phases, which has been considered as adaptive strategies allowing different developmental stages to evolve independently, and ultimately aid the diversification of insects (Moran 1994). However, whether larval morphology is completely free to evolve without influencing the adult phenotype or not remains unclear. In fruit flies, imaginal discs within the larval body develop into most part of the adult body, and larval cells make almost no contribution to the adult body (Siegel & Fristrom 1978). However, in many other insect species, adult structures do not arise from free-floating imaginal discs

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but from cells associated with larval structures (Truman & Riddiford 2002; Nagel 1934). In lepidopterans, imaginal cells embedded within the larval tissues replace many of the larva-specific cells to generate the adult structures (Tanaka & Truman 2005; Truman & Riddiford 2002), although more studies are needed for definitive conclusions. Most insects do not make their entire adult body from set-aside imaginal discs, and the larval morphology likely imposes some constraints on the development of the adult morphology. Therefore, it is essential to understand the developmental relationships between the larval and adult stages.

Regeneration is a process of regrowth or repair of lost tissues or organs in organisms (Gui & Yi 2002; Kumar et al. 2007), and in-depth understanding of regeneration may lead to technical developments to cure damaged human body parts (Marsh & Theisen 1999). Regeneration of tissues and organs is prevalent in metazoan taxa including coelenterates, annelids and, especially in arthropods (Tan et al. 2013). Limb regeneration in insects was reported in at least 36 genera of 11 orders (Bulliére & Bulliére 1985; Beauchemin et al. 1998; Tan et al. 2004), including Blattaria, *Periplaneta americana* (Bodenstein 1962), *Leucophaea maderae* (Bohn 1974), *Eupolyphaga sinensis*Walker (Tan et al. 2004), Phasmida, *Sinophasma* spp. (Chen 1999), Orthoptera, *Acheta domestica* (Maleville & Reggi 1981), Lepidoptera, *Galleria mellonella* (Madhavan & Schneiderman 1969), Odonata, *Ischnura cervula* (Parvin & Cook 1968), Dictyoptera, *Blattella germanica* (O'Farrell & Stock 1953), Triatominae, *Rhodnius prolixus* (Knobloch & Steel 1988), Heteroptera, *Oncopeltus fasciatus* (Shaw & Bryant 1974), Coleoptera, *Tribolium castaneum* (Alison et al. 2013). Given the widespread occurrence of leg regeneration in arthropods and even in vertebrates, research on this phenomenon should be advanced, in part, for its potential to help replace damaged organs and tissues in man.

Regeneration frequencies and lengths of regenerated leg were affected by several factors. Tan et al. (2004) reported that in *E. sinensis* of all instars had the ability to regenerate and legs ablated at any site could be regenerated. The regenerated legs were smaller than normal legs. In addition, leg regeneration underwent 4 general stages, which involved (i) wound healing, (ii) blastema formation, (iii) blastema proliferation, and (iv) subsequent re-patterning of the de-differentiated tissue. The first step of healing relies on the formation of the wound epithelium via the secretion of matrix metalloproteinases (Call & Tsonis 2005). The second step of leg regeneration involved formation of a blastema at the wound site, which consisted of a mass of partially de-differentiated cells that develop in response to signals emanating from the nerves and the epidermis (Endo et al. 2004; Mescher 1996). Next the blastema cells proliferated, and finally, the de-differentiated tissue repatterned itself to form the missing part of leg (Shah et al. 2011; Bergantinos et al. 2010; Bosch et al. 2005; Sustar et al. 2005). Regenerated legs were found to grow faster than normal legs (Tan et al. 2013).

To study leg regeneration, we chose the seven-spotted lady beetle, *Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae), as a model insect because it develops external larval legs, and it is easy to observe how alterations of larval leg morphology affects adult leg morphology. In this study, we addressed 3 main questions: 1) whether the factors including ablation sites, locations, sides and instars have impacts on the leg regeneration frequencies and lengths of regenerated legs, 2) whether the larval leg pattern and adult leg-patterning are linked, 3) what are the time limits of regeneration during larval development, and especially when does this critical period end? Several figures in this manuscript are displayed in color in **s**upplementary material for this article online in Florida Entomologist 98(1) (March 2015) at http://purl.fcla.edu/fcla/entomologist/browse .

## Materials and Methods

### INSECTS

The seven-spotted ladybeetles, *C. septempunctata*, were taken from our laboratory colony in China Agricultural University, Beijing, P. R. China. Larvae were kept in plastic containers (7 cm in length × 4.5cm in width × 8 cm in height), reared with fresh bean aphids (*Acyrthosiphon pisivorum*) under a 16:8 h L:D photoperiod at 28–30 °C.

### EFFECT OF ABLATION SITE, SIDE OF BODY, THORACIC LOCATION AND INSTAR ON REGENERATION FREQUENCY

To analyze the factors influencing the regeneration frequency and the length of the regenerated leg, the legs of lady beetle instars were ablated at the base of the tibia (half ablation) or at the base of the coxa (complete ablation) in 3 instars (2nd, 3rd and 4th), 2 body sides (left and right), and 3 thoracic locations (foreleg, middle leg, and hind leg) (Fig. 1). After anesthetization, the larvae were placed on double-sided tape, and the legs were ablated using a pair of micro-scissors. Then the ablated larvae were held and fed in the same conditions as before. The newly emerged adults were checked by microscope to determine whether and to what extent the ablated legs were regenerated. Each treatment was replicated 3 times, and each replication included 10 lady beetles.

### EFFECT OF ABLATION SITE, THORACIC LOCATIONS AND INSTARS ON THE LENGTHS OF REGENERATED LEGS

The relative leg length (ratio of the length of the regenerated leg to that of the control) and total leg length (sum of the lengths of all segments) were calculated and analyzed based on measurements of the length of a line between the 2 endpoints of segments of adult leg. The legs of control groups were used as the base for analyzing the lengths of regenerated coxae, femurs, tibiae and tarsi of the treated adults.

The adults were stored in a solution of 70% ethanol and 15% glycerol to measure the lengths of regenerated legs by digital microscopy (VHX-1000, Keyence, Osaka, Japan). We scored regeneration as partial regeneration [regeneration into an incomplete leg with missing or fused segments (Fig. 2)], and as complete regeneration [regeneration into a complete leg with normal segments (Fig. 3)]. The relative leg lengths (the

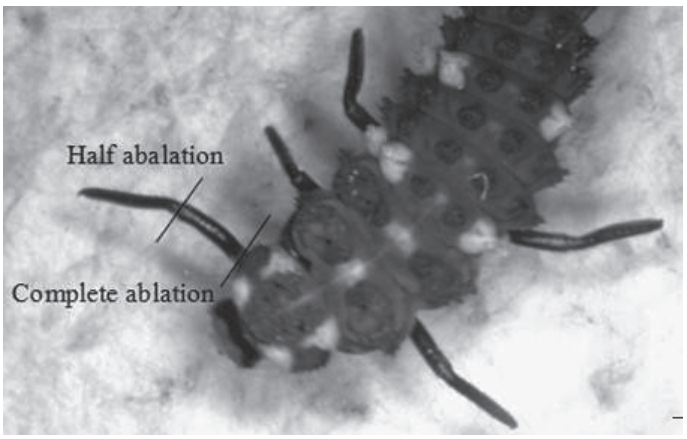



**Fig. 1.** Complete ablation: larval leg of in *C. septempunctata* was ablated at the base of the coxa. Half ablation: larval leg was ablated at the base of the tibia. Scale bars equal 500 µm. This figure is shown in color in a supplementary document online as Suppl. Fig. 1 in Florida Entomologist 98(1) (March 2015) at http://purl.fcla.edu/fcla/entomologist/browse.

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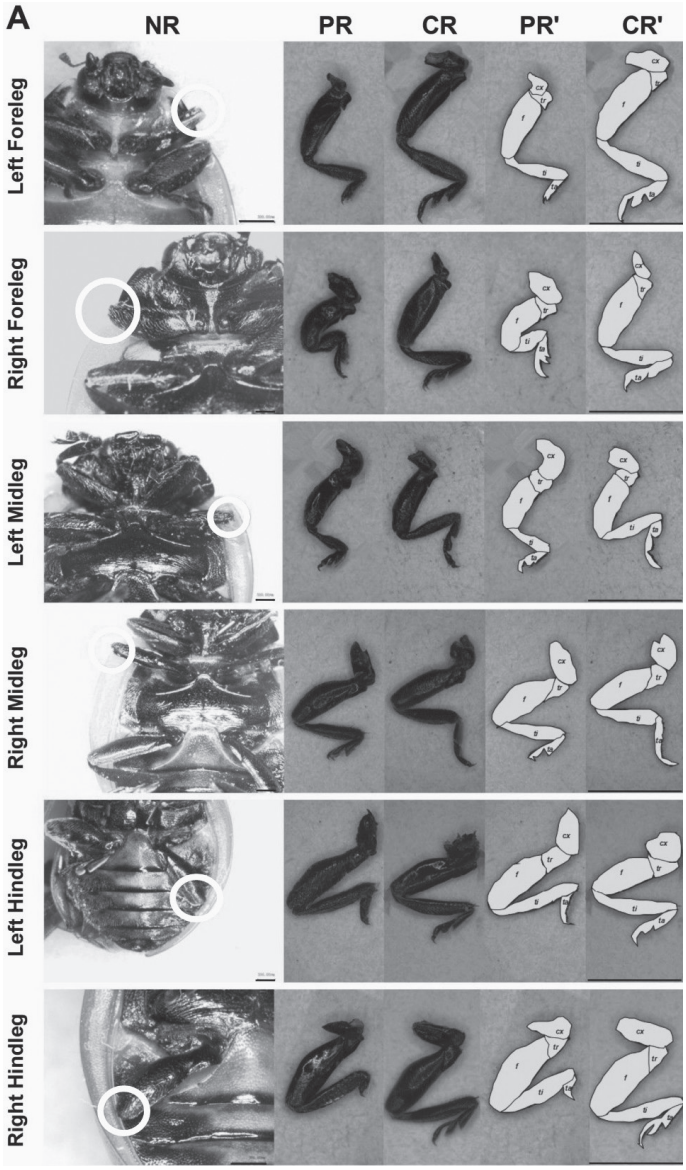



**Fig. 2.** The effects of half ablation on adult leg regeneration in *C. septempunctata*. (**Left Foreleg**): Phenotypes of the forelegs of adults derived from larvae whose left forelegs had been half ablated. No regeneration **(NR)** in the circle, partial regeneration **(PR)** and complete regeneration **(CR)** are shown. In the 2 columns on the right, **PR'** and **CR'** are color-level inversion images of the leg segments highlighted. Each scale bar equals 500 µm. This figure is shown in color in a supplementary document online as Suppl. Fig. 2 in Florida Entomologist 98(1) (March 2015) at http://purl.fcla.edu/fcla/entomologist/browse.

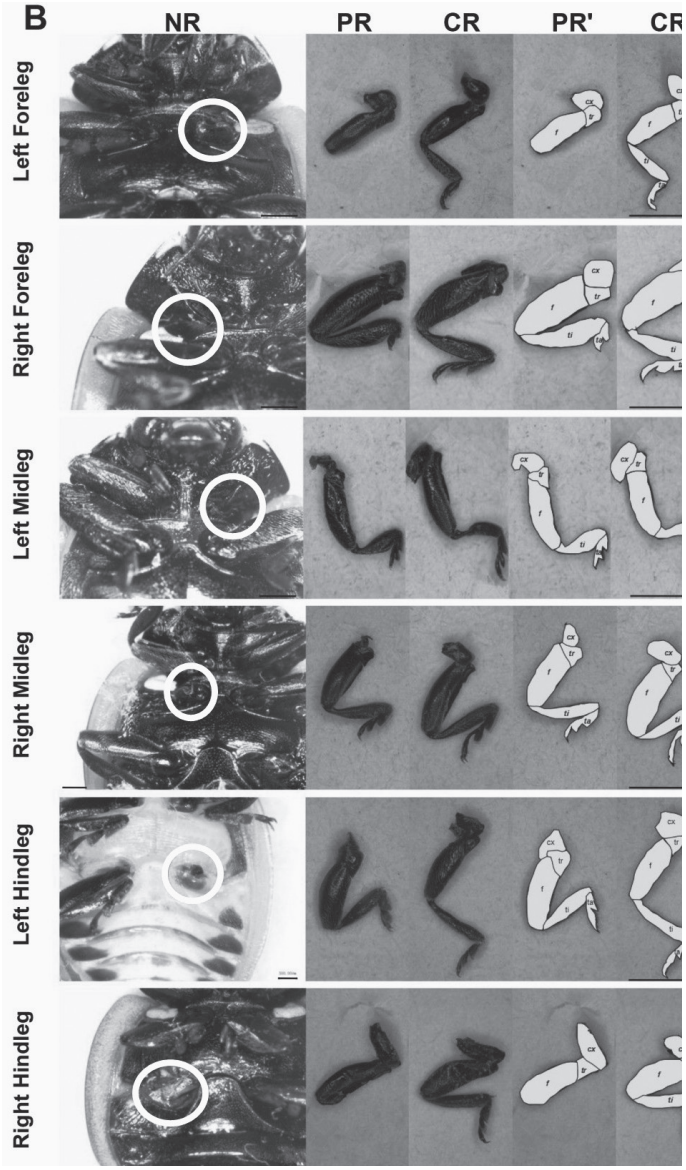



**Fig. 3.** The effects complete ablation on adult leg regeneration in *C. septempunctata*. (**Left Foreleg**): Phenotypes of the forelegs of adults derived from larvae whose left forelegs had been completely ablated. No regeneration **(NR)** in the circle, partial regeneration **(PR)** and complete regeneration **(CR)** are shown. In the 2 columns on the right, **PR'** and **CR'** are color-level inversion images of the leg segments highlighted. Each scale bar equals 500 µm. This figure is shown in color in a supplementary document online as Suppl. Fig. 3 in Florida Entomologist 98(1) (March 2015) at http://purl.fcla.edu/fcla/entomologist/browse.

ratio of regenerated leg length to leg length of the normal contralateral leg) and total leg lengths (sum of the all segments lengths) were calculated and analyzed based on measurements of the length of a line between the 2 endpoints of the segments of each adult leg. The contralateral legs were used as the base of comparison (control) for analyzing the lengths of regenerated coxa, femur, tibia and tarsus as controls.

## MEASUREMENT OF END OF THE CRITICAL PERIOD FOR REGENERATION

In order to determine the time in stadium when the critical period for leg regeneration of *C. septempunctata* comes to an end, fourth instar larvae were chosen for the experiment because the second and third stadia were too short to precisely determine the end of the critical period for leg regeneration. We ablated the right midleg of a *C. septempunctata* 4th instar at base of the tibia every 12 h. The regeneration frequencies of tested insects ablated at different time points were recorded and compared. In all 9 treatments, each treatment was replicated 3 times and each replication contained 15 lady beetles.

## STATISTICAL ANALYSIS

The effects the ablated site, and the side of the body where the ablation occurred on the frequency of regeneration were analyzed by Student's t-test. We checked the data for normal distribution, subjected the data to one-way ANOVA, and separated the means by the post hoc Tukey's honest test of significance at the 5% level of statistical

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significance. All statistical analyses were performed using SPSS analysis software (20.0 for Windows; SPSS Inc., Chicago, USA).

## Results

### MORPHOLOGY OF LEG REGENERATION

Adult legs were regenerated after larval legs had been ablated. Three phenotypes detected in both half ablation (Fig. 2) and complete ablation (Fig. 3) treatments were as follows: 1) no regeneration, i.e., no part of the leg was regenerated either during formation of adult or after adult emergence; 2) partial regeneration, i.e., at least one segment was regenerated, or some segments either were not regenerated, or they were regenerated but were fused together; 3) complete regeneration of legs with normal segments.

### EFFECTS OF ABLATION SITE, LOCATION AND LARVAL INSTAR ON REGENERATION FREQUENCY

The regeneration frequency in half ablation (mean ± SE = 88.0 ± 2.0 %, n = 457) was significantly higher than that in complete ablation (66.0 ± 3.1%, $n$ = 424; $t$ = 3.589, df = 28.334, $P$ = 0.001; Fig. 4A). No significant difference occurred in ablation between both sides (half ablation, $t$ = 0.036, df = 52, $P$ = 0.971; complete ablation, $t$ = 0.597, df = 52, $P$ = 0.553; Fig. 4B) and among 3 locations (foreleg, midleg and hindleg; half ablation, $F$ = 0.276, df = 2, 51, $P$ = 0.760; complete ablation, $F$ = 0.258, df = 2, 51, $P$ = 0.774; Fig. 4C).

For half ablation, the regeneration frequencies were significantly different when the leg ablations were performed at the second, third or the fourth instars ($F$ = 68.151, df = 2, 51, $P$ < 0.001). For complete ablation, the tendency was similar to that in half ablation, and regeneration frequencies significantly decreased when legs of 4th instars were ablated ($F$ = 92.461, df = 2, 51, $P$ < 0.001; Fig. 4D).

### LENGTH ANALYSIS OF REGENERATED LEGS

*Lengths of Regenerated Legs in Half Ablation.* The leg lengths of partial regeneration and complete regeneration were unaffected by the location of half ablation, i.e., the fore-, mid- or hind leg (partial regeneration, $F$ = 0.636, df = 2, 24, $P$ = 0.538; complete regeneration, $F$ = 0.137, df = 2, 24, $P$ = 0.872; Fig. 5A). Similarly, there were no significant differences among the 3 instars for both regeneration phenotypes

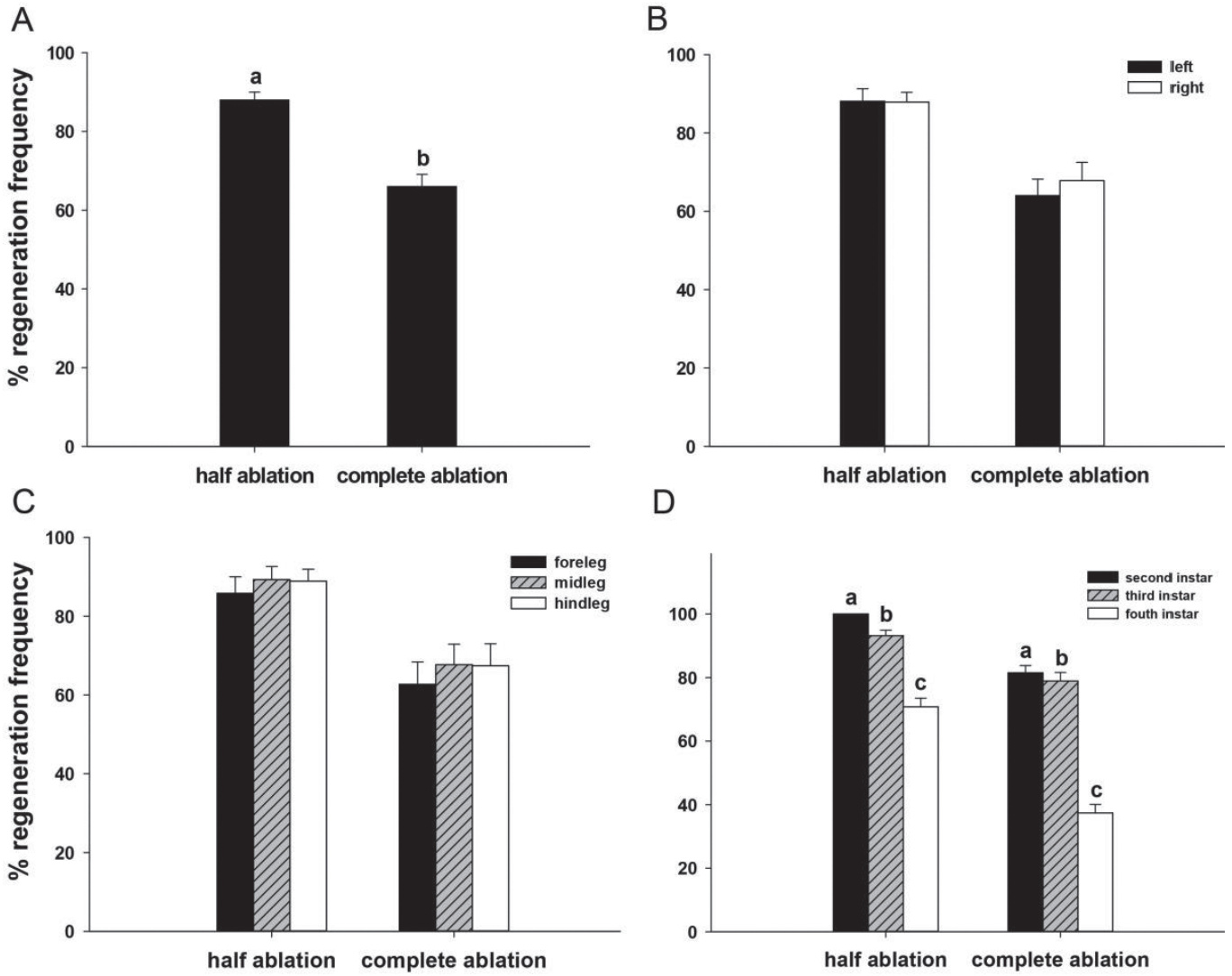



**Fig. 4.** Regeneration frequencies (%) of the adult legs after either half ablation or complete ablation of larval legs (Means ± SE). **A.** Combined results; **B.** Results on either the left or the right side of the body. **C.** Results at various thoracic locations; and **D.** Results on the 2nd, 3rd, and 4th instar. Different letters indicate significant differences among the treatments (mean separation by Tukey's HSD, $P$ < 0.05).

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(partial regeneration, $F = 0.018$, df = 2, 24, $P = 0.982$; complete regeneration, $F = 0.027$, df = 2, 24, $P = 0.973$; Fig. 5B).

Leg lengths differed significantly among partial regeneration, complete regeneration and the control (contralateral leg) phenotypes. However, leg lengths did not differ significantly between complete regeneration and the control phenotypes. Among the partial regeneration phenotypes, significantly shorter segments occurred mainly at the distal end of the tibia and the tarsus, which caused the whole leg to appear to be significantly shorter than in the control ($F = 6.091$, df = 2, 60, $P = 0.004$; Fig. 5C).

*Lengtsh of Regenerated Legs in Complete Ablation*. In the complete ablation treatments, the leg lengths of the partial and complete regeneration phenotypes were similar to those in the half ablation treatments. Ablation location (partial regeneration, $F = 1.184$, df = 2, 24, $P = 0.323$; complete regeneration, $F = 0.013$, df = 2, 24, $P = 0.987$; Fig. 5D) and larval instar (partial regeneration, $F = 0.060$, df = 2, 24, $P = 0.942$; complete regeneration, $F = 0.004$, df = 2, 24, $P = 0.996$; Fig. 5E) did not affect the lengths of regenerated legs in the complete ablation treatments. On the other hand, because of the occurrence of smaller or absent tibiae and fused tarsi in the partial regeneration phenotype, the regenerated legs were shorter than in the control and in the complete regeneration phenotype ($F = 10.247$, df = 2, 60, $P < 0.001$; Fig. 5F).

### REGENERATION AND DEVELOPMENTAL COUPLING BETWEEN LARVAL AND ADULT STAGES.

In the partial regeneration phenotype, regenerated leg lengths in the complete ablation treatments were significantly shorter than in the half ablation treatments ($F = 6.465$, df = 2, 60, $P = 0.003$). In the complete regeneration phenotype, the lengths of regenerated leg segments both in the complete ablation treatments and the half ablation treatments were slightly shorter than the control ($F = 0.071$, df = 2, 60, $P = 0.931$).

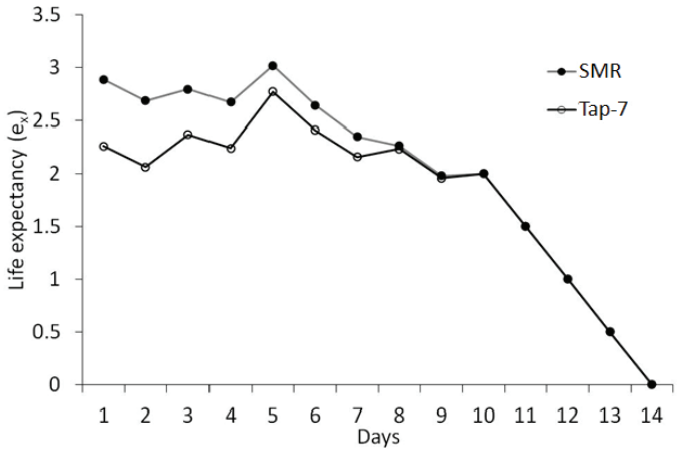



**Fig. 5.** Ratios (%) of lengths of regenerated legs to contralateral legs (means ± SE) after either partial or complete regeneration when oblation of larval legs was as follows: **A.** Half ablation at 3 thoracic locations (fore-, mid- and hind legs); and **B.** Half ablation in the 2nd, 3rd, and 4th instar. **C.** Half ablation showing lengths of regenerated coxa, femur, tibia, tarsus and entire leg. **D.** Complete ablation at 3 thoracic locations (fore-, mid- and hind leg); and **E.** Complete ablation in 3 instars (2nd, 3rd, 4th). **F.** Complete ablation showing length of regenerated coxa, femur, tibia, tarsus and entire leg. Different letters indicate significant differences (Tukey's HSD, $P < 0.05$) among the regenerated patternings within one segment or leg.

### DETERMINATION OF THE END OF THE CRITICAL PERIOD FOR REGENERATION IN THE FOURTH INSTAR

The frequency of leg regeneration by some adults was small when the leg ablations were performed on fourth instars. Therefore, we further examined the critical period of ablation for leg regeneration in fourth instar *C. septempunctata*. The results showed that the non-regeneration began to be detected on the day 3.5 (92 ± 8.3%), and regeneration progressively declined at day 4 (50 ± 14%) and at day 4.5 (21 ± 11%) of the fourth instar's stadium, while all legs of larvae ablated before day 3.5 could regenerate in adult stage. So in the fourth instar, as the regeneration frequency on the 4th day became significantly low, the critical period for regeneration occurred on about day 3.5 ($F = 13.574$, df = 3, 8, $P = 0.002$; Fig. 6).

## Discussion

### REGENERATION FREQUENCY AFFECTED BY THE DEGREE OF ABLATION AND INSTAR BUT NOT BY THORACIC LOCATION AND SIDE OF BODY

This is the first report of leg regeneration in *C. septempunctata*. Although some publications reported that larval legs of many species were able to regenerate after several molts (Bulliére & Bulliére 1985; Beauchemin et al. 1998; Tan et al. 2004), we found the initial appearance of the regenerated leg happened in prepupae and not in the larva. Our findings are inconsistent with previous studies that suggested that leg regeneration occurred just after the first molt after when larval leg had been ablated (Tan et al 2004; Bulliére 1985).

The leg phenotype suggested that the regeneration blastema possessed a memory of its position along the leg (Stocum 1984) that enabled it to retain the integrity of the leg, and that it guarantees that related functions of the mass of partially de-differentiated cells in the regeneration blastema result in the normal development of the regenerated leg (Tan et al. 2013). This is consistent with past studies of the ablation site in *Eupolyphaga sinensis* (Walker) (Blattaria: Polyphagidae) leg regeneration. Therefore we had surmised that in *C. septempunctata* leg regeneration could occur when leg ablations were made at different segments of the leg.

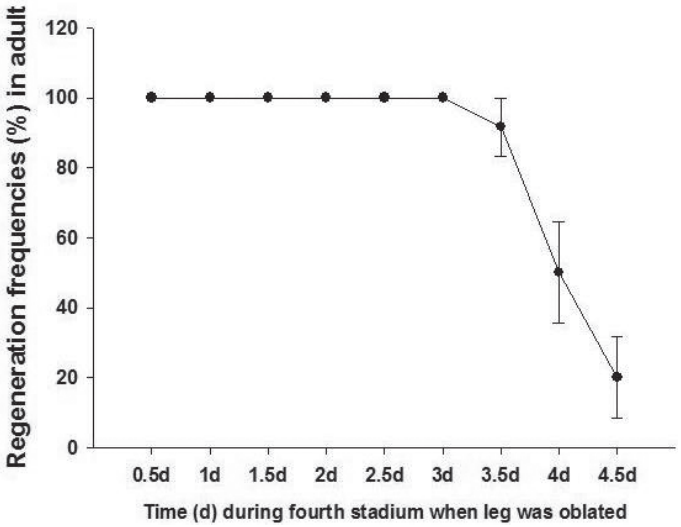



**Fig. 6.** Determination of the end of the critical period for regeneration in the fourth instar. Adult leg regeneration frequencies (%) after the right midleg was ablated at various times in the 4th stadium.

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Our study showed that the regeneration frequency of legs in half ablation was higher than in complete ablation (Fig. 4A), which suggested that leg regeneration was more difficult when greater amounts of the leg were missing. Our study also showed that there was a significantly lower regeneration frequency of the fourth instars compared to second and third instars (Fig. 4D). The result was consistent with previous studies that regenerative ability significantly decreased with increasing instar (Wang & Cai 2004; Angelini et al. 2009; Suzuki et al. 2009). These studies showed that, in each successive instar, the duration leading to non-regeneration between ablation and the end of the instar became progressively longer. However, ablation of legs at the different thoracic locations and body sides did not affect the regeneration frequency of the legs (Fig. 4B, 4C). These data indicated that thoracic location and side of the body could scarcely affect the generation rate of ablated legs.

### LENGTHS OF REGENERATED LEGS WERE MEASURED TO PROVE DEVELOPMENTAL CONTINUITY BETWEEN LARVAL AND ADULT STAGES

We found that partially regenerated legs had shorter segments than completely regenerated legs in both ablation treatments. Especially shortened were the distal ends of the tibia and tarsus (Figs. 5C and 5F), which showed that leg regeneration proceeded along the proximo-distal axis. The lengths of partially regenerated legs in half ablation treatments were remarkably longer than in complete ablation treatments. This indicated that the de-differentiated regeneration blastema was not substantially involved in the patterning of the distal segments of the developing regenerated legs.

The regenerated segment at the point of ablation appeared to be stunted. Thus in the complete regeneration phenotype, leg lengths in both ablation treatments were slightly shorter than the control (contralateral legs) as was reported previously (Bohn 1970; Tan et al 2004). The data indicated that the alterations of larval leg morphology affected the morphology of the adult leg. There appeared to be a smooth transition between the larval appendages and the regenerated adult appendages. This implied that the larval leg patterning imposes a developmental constraint on the adult leg rather than allowing the adult leg to develop independently. The lengths of the regenerated legs were unaffected by the thoracic location of the ablated leg and side of body both in the half ablation and complete ablation treatments, which indicated that thoracic location and side of body could little affect the length of the regenerated leg.

### DETERMINATION OF THE BOUNDARY OF THE CRITICAL PERIOD FOR REGENERATION

In our preliminary study, we found that there was a boundary between regeneration and non-regeneration in each instar. The terminal edge of the boundary was defined as the critical period for regeneration (Lüscher 1948). We found the critical period of fourth instar lady beetles was at day 3.5 in the fourth instar's stadium (Fig. 6). Some authors have suggested that there may be a correlation between the peak-hour of ecdysone and critical period as ecdysone had the function of regulating regeneration (Bulliére & Bulliére 1985; 0'Farrell et al. 1956; Madhavan & Schneiderman 1969; Marks et al. 1970; Maleville & Reggi 1981), and this needs to be examined by additional research with *C. septempunctata*.

### DEVELOPMENTAL CONTINUITY BETWEEN LARVAL AND ADULT LEG MORPHOLOGIES

Although the fundamental mechanism of developmental continuity between larval and adult legs in *C. septempunctata* is not clearly understood, superficially there appears to be a smooth transition between the larval and regenerated adult legs, and the latter developed with total dependence on the former. However, in lepidopterans, adult legs develop independently of larval legs and they actually arise from set-aside imaginal cells that reside in the larval legs and proliferate during the prepupal period rather than during the pupal period (Tanaka & Truman 2005). Thus developmental patterning of the leg in *C. Septempunctata* differs with that in many other arthropod species.

Our study showed that adult leg patterning of *C. septempunctata* was inherited from the larval leg, and that the patterning of the larval leg appears to have a developmental affect on the adult leg. For example, when a larval leg was ablated and the medial segments were removed, then this morphological alteration was also observed in the adult stage. Thus in the case of *C. septempunctata* 2 life-history stages (the larva and the adult) which must cope with somewhat different habitats may affect each other. The 2 distinct morphs may constrain each other not only in *C. septempunctata* but possibly also in most holometabolous insects.

Developmental features are major facilitators of evolution in that they allow the compartmentalization of the different stages of arthropods with complex life cycles (Moran 1994). Developmental continuity between larval and adult stages might be a constraint on evolutionary change. Many insect species have independently evolved imaginal discs including lepidopterans and fruit flies (Siegel & Fristrom 1978). This developmental feature has enabled the larval stage to be ecologically completely separated from the adult stage (Moran 1994; Truman 1999; Svacha 1992). However, through our anatomical experiments, we did not find imaginal discs in the larval legs of *C. septempunctata*, which cannot develop adult legs independently of the larval legs. Thus, in order to adapt rapid growth within the context of a short life cycle, the evolution of imaginal discs may play a critical role in complete dissociation of larval and adult structures in future evolutionary development.

## Conclusions

Our study indicated that there was developmental continuity between larval and adult leg patterning of *C. septempunctata*. Leg regeneration occurred in the prepupal period, and the regenerated adult legs first appeared in the newly emerged adult. Regeneration ability was higher in the earlier instars, and it was not related either to the thoracic location or the side of the body of the ablated larval leg. Lengths of completely regenerated legs were non-significantly shorter than contralateral legs. Lengths of partially regenerated legs in complete ablation treatments were significantly shorter than that in half ablation treatments. And in fourth instars, the termination of the critical period determining leg regeneration happened on day 3.5.

Our findings suggested that there was a smooth transition between larval and regenerated adult appendages. We surmise that the evolution of insects is characterized by a gradual loss of developmental constraints between larval and adult life-history stages. Thus, the appearance of free-floating imaginal discs might be a key step towards completely removing the developmental constraint that existed between larval and adult structures (Alison et al. 2013). On the other hand, in the case of *C. septempunctata* both immatures and adults are predators of various herbivore prey species on the same plant species, and there is an obvious benefit to the survival of this species to maintain continuity between the life stages to ensure success in predation, even the though the adult also must disperse, reproduce and locate hosts in complex environments.

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