THE EFFECT OF APPENDAGE AND SCALE LOSS ON INSTAR DURATION IN ADULT FIREBRATS, *THERMOBIA DOMESTICA* (THYSANURA)

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Summary

The loss of appendages immediately following a moult shortens the subsequent intermoult interval in adult *Thermobia domestica*. The loss of increasing numbers of appendages accelerates the ensuing moult incrementally. Leg amputation at sites distal to the trochanter–femoral autotomy plane has comparable effects in accelerating the moult. Functional legs regenerated at the first moult following amputation are at least 80 % of normal length, while abdominal cerci are regenerated at a slower pace, attaining about 20 % of normal length at the first post-operative moult. Scale loss also causes an acceleration of the following moult. It is proposed that changes in diffuse sensory input, rather than specific proprioceptive feedback, mediate the acceleration of the moult cycle.

Introduction

Appendage loss affects the timing of the moult in a wide range of immature arthropods. The mechanism is not well understood, but it is clear that the endocrine events of the moult cycle can be accelerated, as in crustaceans, e.g. land crabs *Gecarcinus lateralis* (Skinner, 1985), or retarded to varying degrees, as in various orthopteroid insects (Bullière and Bullière, 1985), depending on the species and the time of injury within the instar. There is evidence from the cockroach *Blattella germanica* that proprioceptive feedback may mediate the effect (Kunkel, 1981).

The phenomenon persists in mature stages of the Thysanura, where adults continue to moult repeatedly on a regular cycle. Our objective in this study was to evaluate the effects of appendage and of scale loss on the duration of the steady-state intermoult interval in adult firebrats (*Thermobia domestica*). We confirm the results of Sahrhage (1953) in demonstrating an acceleration of the moult following injury, but differ in finding an incremental effect on acceleration of the moult as additional limbs are removed.

The incremental effects of appendage loss and the effect of leg tissue loss

regardless of amputation location on the acceleration of the moult, as well as the effect of scale abrasion, where the tips of the mechanoreceptor dendrites are broken, suggest that modulation of the moult cycle in *Thermobia* is not mediated by localized mechanoreceptors but may depend on more generalized sensory feedback.

Materials and methods

Thermobia domestica cultures were maintained at $35\pm0.5\,°C$ in an incubator in which a large open surface of saturated NaCl ensured an equilibrium relative humidity of 75 %. Bulk cultures were kept in 2-l jars loosely stuffed with paper towel and were fed on dry cat food (Little Friskies, Carnation Co.) *ad libitum*. Experimental animals were transferred in pairs to 5 cm diameter Petri dishes containing pleated paper towel and dry food.

Adults moult with a regular periodicity under constant environmental conditions. It is thus possible to obtain animals at known stages within the moulting cycle by marking adults selected at random from bulk cultures. A waterproof pen (Sharpie brand) was used to mark individual females, which could be reliably recognized as adult by the length of the ovipositor. The duration of the instar of marked unoperated females did not differ significantly from that of unmarked controls, provided that care was taken to limit scale abrasion.

Marked animals were examined daily for ecdysis. *Thermobia* normally eats the newly cast cuticle from the anterior end to the posterior, but they did not eat the ink-marked cuticle. Thus, evidence for the occurrence of a moult was twofold: an unmarked animal and remnants of moulted cuticle.

All operations were performed within 24 h of moult. Both experimental and control animals were subjected to CO_2 anaesthesia for comparable periods. Animals were marked with ink immediately after surgery, and the number of days to the next moult was recorded on the basis of daily observation.

Legs were amputated at three different sites: at the trochanto-femoral joint by inducing autotomy as a response to pinching with watchmaker's forceps, at the femoro-tibial joint and at the midpoint of the tibia by cutting with iridectomy scissors. Posterior abdominal appendages were amputated by breaking them with watchmaker's forceps. Scales were removed from a 1 mm strip on the dorsal midline by lightly touching a strip of adhesive tape to the surface. Ovipositors were amputated at a point one-third of their length from the base, avoiding damage to other appendages.

Results

Effect of leg autotomy within 24 h of a moult on the duration of the ensuing instar

Two series of leg autotomy experiments were performed, under slightly different incubator conditions, in which the effects of loss of up to six legs on instar

Table 1. *Effect of leg loss on duration of adult instar in adult* Thermobia domestica

Treatment	Number of legs removed	Position of removed legs	N	Instar duration (days) mean±S.D.
Autotomy series I	0	Control	5	13.8±2.8[a]
	1	L3	9	10.9±1.5[b]
	2	L2 L3	8	9.4±2.0[c]
	3	L2 L3 R3	17	8.0±1.1[d]
	4	L2 L3 R2 R3	19	7.7±1.2[d]
Autotomy series II	0	Control	12	16.3±2.3[a]
	1	L3	10	12.9±3.3[b]
	2	L2 L3	7	11.1±3.2[c]
	3	L2 L3 R3	6	8.7±0.55[d]
	4	L2 L3 R2 R3	4	8.0±0[d]
	5	L1 L2 L3 R2 R3	11	7.5±0.8[d]
	6	L1 L2 L3 R1 R2 R3	9	7.5±0.7[d]
Asymmetric autotomy (stress)	3	L2 L3 R3	6	8.7±0.5
	3	L1 L2 L3	7	8.1±0.9
	4	L2 L3 R2 R3	4	8.0±0.0
	4	L1 L2 L3 R3	12	7.8±1.4
Amputation at femoro-tibial joint	0	Control	23	16.6±2.9
	4	L2 L3 R2 R3	32	9.3±1.3
Amputation at mid-femur	0	Control	18	15.8±2.2
	4	L2 L3 R2 R3	17	8.8±0.9

The two series of autotomy (I and II) differ in minor aspects of incubator environment.
Leg position is indicated as L=left, R=right, 1=anterior, 2=middle, and 3=posterior.
Instar durations with the same superscript letters within the same treatment are not significantly different by Fisher's test, $P<0.0001$.

duration were compared (Table 1). Mean instar duration as a function of number of legs lost is shown in Fig. 1. It is clear from these data that the ensuing moult is hastened incrementally with each additional leg loss up to three legs. The effect is additive and linear.

Despite the apparent severity of the treatment, animals with five or six legs removed were able to locomote by undulating the abdomen; these groups showed no greater mortality than those with less severe treatments.

The possibility that the effect of limb loss on instar duration might be modified directly or indirectly by degree of locomotor stress due to asymmetry in the site of limb loss was tested by varying the sites of amputation, as shown in Table 1. While further combinations will be needed to eliminate the possibility completely, it appears that the distribution of autotomy sites among the limbs does not significantly affect the duration of the shortened instar.

Effect of surgical amputation distal to autotomy point

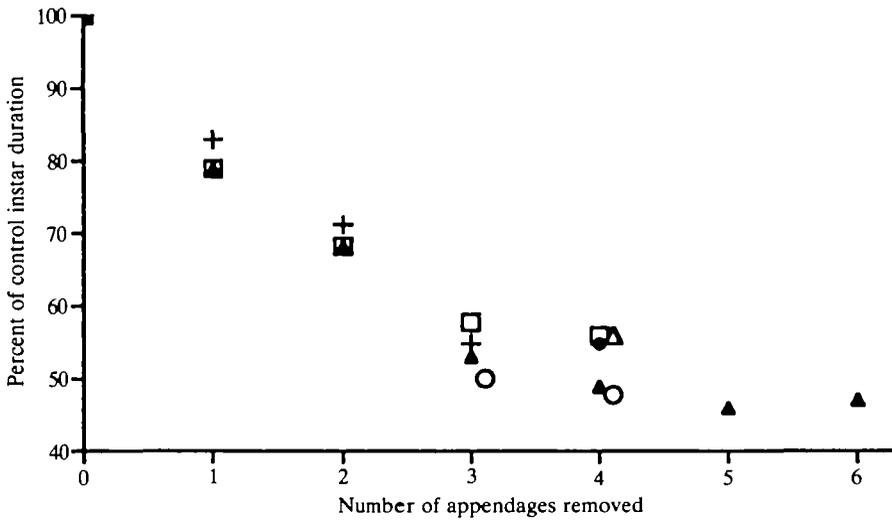Limb loss by surgical amputation at the femoro-tibial joint, i.e. at the next joint

Fig. 1. Effect of appendage loss on instar duration in adult *Thermobia domestica*. ■, control; □, autotomy series I; ▲, autotomy series II; ○, asymmetric (motor stress) autotomy;
+, sensory appendages; △, amputation at femoro-tibial joint; ●, amputation at mid-tibia.

distal to the autotomy plane, also shortens the instar. With four legs removed, the two amputation sites do not differ significantly in their effect on the duration of the instar. Similarly, severing the leg at the midpoint of the tibia shortens the instar to the same degree as does autotomy (Table 1; Fig. 1).

Effect of removal of abdominal appendages

The terminal sensory appendages, the paired cerci and the caudal filament, lack autotomy sites but are fragile and break readily at preformed lines of weakness in the integument (Edwards and Reddy, 1986). The effect of removing sensory appendages at or near their bases, summarized in Table 2, is comparable to the removal of legs in showing an incremental effect on instar duration. The loss of abdominal styli and amputation of the ovipositor also shorten the instar (Table 2).

Loss of scales

Removal of scales, as described in Materials and methods, reduces the length of the instar to 66 % of the control duration ($N=29$), and is thus equivalent to the loss of two appendages (Fig. 1; Table 2).

Quality of regenerates

Leg regeneration in *Thermobia* is remarkably vigorous under the experimental regime described above. In every experimental group where limbs were autoto-mized, an entire functional limb was regenerated by the next moult. In all cases the

Table 2. *Effect of abdominal appendage amputation and scale removal on duration of adult instar in* Thermobia domestica

Treatment	Number of appendages removed	Appendages removed	N	Instar duration (days) mean±s.d.
Amputation of terminal	0	0	12	16.3 ± 2.3^a
sensory appendages	1	LC	19	13.5 ± 3.3^b
	2	LC RC	13	11.5 ± 2.7^c
	3	LC RC CF	6	9.0 ± 1.7^d
Amputation of	0	Control	23	16.6 ± 2.9
abdominal styli	6	All	29	$14.9 \pm 3.0*$
Amputation of	0	Control	20	15.5 ± 2.7
ovipositor	1	Ovipositor	29	$11.7 \pm 1.1***$
Scale removal		Control	23	16.6 ± 2.9
		Scales	29	$11.0 \pm 2.9**$

Instar durations with the same superscript letters within each treatment are not significantly different by Fisher's test, $P < 0.0001$.

Significantly different from control by t-test within each treatment, $* P < 0.1$, $** P < 0.001$, $*** P < 0.0001$.

RC=right cercus, LC=left cercus, CF=caudal filament.

femur length of the regenerate was at least 80 % of the contralateral (normal) femur. A full set of functional legs is regenerated within one instar after removal of all six legs. Contrary to Sahrhage's (1953) claim that the first regenerate after autotomy resembles early instar legs in lacking scales, we observed scales, in reduced number and size, on all regenerates at the first postoperative moult.

Cerci regenerated less vigorously than legs. Their mean length after the first moult following amputation was 22 ± 7.5 % ($N=20$) of the normal contralateral pair. Equality was reached after several moults.

Discussion

It has been known for 100 years that limb amputation and regeneration can accelerate the onset of the subsequent moult in certain immature insects, e.g. mayfly (DeWitz, 1890). A similar acceleration also occurs in crustaceans (e.g. Skinner, 1985). In the apterygote insect orders Archaeognatha (bristletails) and Thysanura (silverfish and firebrats), in which the capacity for repeated moulting and regeneration persists throughout adult life (e.g. *Lepisma*, Przibram and Werber, 1907; *Thermobia*, Sweetman, 1938), appendage and scale loss also accelerate the onset of the subsequent moult (Sahrhage, 1953). Acceleration is not, however, a universal response in such circumstances, for limb loss or autotomy early in immature instars of orthopteroid insects generally delays the next moult (Bullière and Bullière, 1985). In other cases, where regeneration is less vigorous, as in *Carausius* (Voy, 1951; W. P. Chan and J. S. Edwards, unpublished

observations), limb loss appears to have no significant effect on the duration of the instar.

Our principal objective in this study was to assess the effect of incremental amputations on the instar duration. For this reason the time of amputation, with respect to the previous moult, was held constant to within 24 h of the moult. It seems probable that there is a critical period, related to the moulting cycle, after which autotomy or amputation does not modify instar duration, but the issue was not addressed in this study.

Contrary to Sahrhage (1953), who implies, without supporting data, that timing of the reset moult is constant and independent of the number of legs lost, we show that there is a statistically significant additive effect of incremental autotomy for the first three legs (Table 1). A comparable, though less pronounced, effect was also found with increments in the amputation of sensory appendages. Stock and O'Farrell (1954), in their classic work on *Blattella germanica*, also reported an additive effect on delay of the ensuing moult when one or both metathoracic legs were amputated.

Two mechanisms are plausible for the mediation of incremental moult acceleration. The release of a wound factor from damaged epidermal cells or neural tissue at the site of damage might give rise to an increasing titre in the haemolymph with each increment in autotomy. This hypothesis would require a central mechanism for measurement of wound factor titre and a coordinated mechanism for mediating the accelerated release of hormones of the moulting cycle. Alternatively, neural input might more or less directly influence the onset of the moult cycle. Kunkel's (1977) demonstration that proprioceptive input from mechanoreceptors located at the autotomy site mediates the delayed moult in *Blattella* supports the latter hypothesis, but the neural pathway and central integration must differ in *Thermobia* since non-autotomy locations (mid-tibia), partial loss of the ovipositor, and a diffuse loss of scales, also influence the timing of the moult. All body scales are innervated in *Thermobia* (Larink, 1976). The abrasion and loss of scales would sever the tips of sensory dendrites associated with the scales. Central mechanisms must integrate the diffuse injury input and in turn modify the neural input to the median neurosecretory cells of the brain, whence prothoracicotropic hormone is released. Larink (1976) similarly concludes that modification of sensory input caused by scale abrasion mediates the resetting of the moult cycle. While the role of modified sensory input is plausible and is consistent with all available data, it is not susceptible to disproof since it is not possible to inflict a wound without damaging peripheral nerves. Since injury presumably elicits elevated levels of spontaneous activity in sensory nerves, it may be that raised levels of mechanosensory input from diffuse sources would simulate injury responses. We therefore propose to determine whether chronic mechanosensory hyperstimulation can mimic injury in accelerating the moult cycle.

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References

BULLIÈRE, D. AND BULLIÈRE, F. (1985). Regeneration. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, vol. 2 (ed. G. A. Kerkut and L. I. Gilbert), pp. 371–424. Oxford, England: Pergamon Press.

DEWITZ, H. (1890). Einige Beobachtungen betreffend das geschlossene Tracheensystem bei Insektenlarven. *Zoologischer Anzeiger* 13, 500–525.

EDWARDS, J. S. AND REDDY, G. R. (1986). Mechanosensory appendages and giant interneurons in the firebrat (*Thermobia domestica*, Thysanura): a prototype system for terrestrial predator evasion. *J. comp. Neurol.* 243, 535–546.

KUNKEL, J. G. (1977). Cockroach molting. II. The nature of regeneration induced delay of molting hormone secretion. *Biol. Bull. mar. biol. Lab., Woods Hole* 153, 145–162.

KUNKEL, J. G. (1981). Regeneration. In *The American Cockroach* (ed. W. J. Bell and K. G. Adiyodi), pp. 425–443. London: Chapman & Hall.

LARINK, O. (1976). Entwicklung und Feinstruktur der Schuppen bei Lepismatiden und Machiliden (Insecta, Zygentoma und Archaeognatha). *Zool. Jb. (Anat.)* 95, 252–293.

PRZIBRAM, H. AND WERBER, E. I. (1907). Regenerationsveruche allgemeinerer Bedeutung bei Borstenschwanzen (Lepismatidae). *Roux Arch. Entwicklungs.* 23, 615–631.

SAHRHAGE, D. (1953). Ökologische Untersuchungen an *Thermobia domestica* (Packard) und *Lepisma saccharina*. *Z. wiss. Zool.* 157, 77–168.

SKINNER, D. M. (1985). Molting and regeneration. In *The Biology of Crustacea*, vol. 9 (ed. D. E. Bliss and L. H. Mantel), pp. 43–146. New York: Academic Press.

STOCK, A. AND O'FARRELL, A. F. (1954). Regeneration and the moulting cycle in *Blattella germanica* (L.). II. Simultaneous regeneration of both metathoracic legs. *Aust. J. Biol. Sci.* 7, 302–307.

SWEETMAN, H. L. (1938). Physical ecology of the firebrat *Thermobia domestica* (Packard). *Ecol. Monogr.* 8, 285–311.

VOY, A. (1951). La croissance des régénérats au cours des deux derniers âges de la phase larvaire chez le phasme (*Carausius morosus* Br.). *C. R. Acad. Sci. Paris* 232, 894–895.