THE EFFECTS OF LEG-REMOVAL TO THE MOLTING OF THE CRAB,
SESARMA (HOLOMETOPUS) HAEMATOCHEIR
(H. MILNE EDWARDS)

With 3 Tables and 4 Text-figures

by

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アカテガニの脱皮に及ぼす歩脚除去の影響

表 3, 挿図 4

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Removal of multiple walking legs accelerates molting in many species of crabs and regeneration of missing legs occurs during the premolt period (BLISS, 1956; PASSANO and JYSSUM, 1963; SKINNER and GRAHAM, 1972; BITTNER and KOPANDA, 1973; FINGERMAN and FINGERMAN, 1974; SUZUKI, 1978; NAKATANI and ŌTSU, 1979). Recently, it has been thought that the precocious molting caused by legloss of the crabs is presumably due to the severing of the nerve of walking legs, not to loss of body mass (SKINNER and GRAHAM, 1972; BITTNER and KOPANDA, 1973). It is also possible that the severing of the leg nerve activates the Y-organ which secretes the molting hormone of Crustacea (PASSANO, 1960; FINGERMAN and FINGERMAN, 1974).

Removal of a pair of eyestalk is also a common means of inducing precocious molting in Crustacea (BROWN and CUNNINGHAM, 1939; ABRAMOWITZ and ABRAMOWITZ, 1940; SKINNER and GRAHAM, 1972; BITTNER and KOPANDA, 1973). Eyestalk ablation causes faster precocious molting than multiple leg autotomy, injection of molting hormone (WARNER and STEVENSON, 1972; BITTNER and KOPANDA, 1973; WEIS, 1976). The precise mechanism of precocious molting caused by legloss or eyestalkloss is not yet known.

The author has performed the experiment on *Sesarma haematocheir* to compare the relative effectiveness of leg removal between the eyed and eyestalkless crabs in inducing precocious molting.

Materials and Methods

Sesarma haematocheir were collected from the Ena River of Miura Peninsula, Kanagawa Prefecture in March, 1979. They were kept in a vessel at room temperature. All animals used in this study were male, 12.0–15.0 mm in carapace width. After molting, 2 (a pair), 4 (two pairs) or 6 (three pairs) walking legs of the crabs were removed at the point of autotomy plane by compressing their exoskeleton. Eyestalks were removed at the base with scissors. These operations were performed on one day after molting. Some of the molted animals were left intact as controls. The operated and control animals were kept separately in a plastic vessel (800 ml) with 20% sea water which was changed every other day. They were fed with pellets of cat fish every other day and placed under natural light. Room temperature was $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

Results

Molting of eyed crabs:

The results are summarized in Table 1 and Figs. 1–4. Control animals, Group A-0, which had been left intact reached the next molting in 91.3 ± 13.2 (74–106) days (Table 1 and Text-fig. 1). In Group A-2 (missing 2-legs) the mean time to molt was 75.8 ± 15.3 (54–95) days, in A-4 (missing 4-legs); 39.2 ± 11.5 (25–59) days and in A-6 (missing

Table 1. Effects of leg removal on molting in eyed and eyestalkless *Sesarma haematocheir*.
アカテガニの脱皮に及ぼす歩脚除去の影響

Group	No. of animals	No. of eyestalks removed	No. of legs removed	Time to molt (days) Mean $\pm$ S. D.	Molting %	Mortality %	Increase in carapace width %
*A-0	10	0	0	91.3 ± 13.2	30.0	10.0	11.9
A-2	10	0	2(a pair)	$75.8 \pm 15.3a$	50.0	0	10.0
A-4	10	0	4(2 pairs)	$39.2 \pm 11.5^{**b}$	60.0	0	6.4
A-6	14	0	6(3 pairs)	$26.6 \pm 6.2^{***c}$	78.6	14.5	5.6
B-0	16	2	0	18.7 ± 4.2	81.3	18.8	7.1
B-2	14	2	2(a pair)	20.9 ± 3.6	71.4	21.4	8.5
B-4	14	2	4(2 pairs)	22.4 ± 2.2	78.6	21.4	7.8
B-6	14	2	6(3 pairs)	$23.3 \pm 3.7^*d$	50.0 ^{*e}	50.0	8.7

t-test

a, $P < 0.4$ vs A-0

**b, $P < 0.01$ "

***c, $P < 0.001$ "

*d, $P < 0.05$ vs B-0

X²-test

*e, $P < 0.05$ vs B-0

*, control

Carapace width was compared with previous size after molting.

Removal of walking legs and eyestalks were performed on the day after molting.

6-legs); 26.6 ± 6.2 (20-43) days. Ablation of walking legs induced precocious molting in the eyed crabs with regeneration of legs which were slightly small in length compared with nonregenerated counterparts. As shown in Table 1 and Text-fig. 1, the time to molt is directly correlation with the number of walking legs removed from the eyed crabs. The differences of the time to molt between A-O and A-2, A-O and A-4, A-O and A-6 are shown in Table 1. Still more, the significant value of the time to molt between A-2 and A-4 is $P < 0.01$, A-2 and A-6 is $P < 0.001$, A-4 and A-6 is $P < 0.05$ by t-test. The difference in the time to molt is highly significant when it is compared with different eyed groups. The molting rate of the eyed groups, as shown in Table 1 and Text-fig. 2, increased gradually in correlation with the number of removed legs. This correlation is almost same as that of the time to molt. The mortality which was probably induced by legloss was negligible during the intermolt period in this study except multiple legloss (Group A-6) (Table 1 and Text-fig. 3). Removal of 1-4 walking legs does not bring the crabs to death. The carapace width of the eyed crabs which had molted after operation was compared with the previous size (Table 1 and Text-fig. 4). The percentage of increase in carapace width gradually decreases on proportion to the number of legs removed from the eyed crabs. In the animals lost 6-legs

Text-fig. 1. Relationship between the time to molt and number of walking legs which were removed from eyed and eyestalkless *Sesarma haematocheir*. ○, eyed crabs (group A); ●, eyestalkless crabs (group B).

アカテガニの脱皮間隔と除去歩脚数との関係 ○, 有眼個体; ●, 除眼個体

Text-fig. 2. Relationship between molting and removal of walking legs of *Sesarma haematocheir*. ○, eyed crabs (group A); ●, eyestalkless crabs (group B). アカテガニの脱皮率と歩脚除去との関係。○, 有眼個体; ●, 除眼個体

Text-fig. 3. Relationship between mortality during intermolt cycle and number of legs removed from eyed and eyestalkless *Sesarma haematocheir*. ○, eyed crabs (group A); ●, eyestalkless crabs (group B).

アカテガニの死亡率と歩脚除去との関係
○, 有眼個体; ●, 除眼個体

Text-fig. 4. Relationship between increase in carapace width after molting and number of legs removed from eyed and eyestalkless *Sesarma haematocheir*. ○, eyed crabs (group A); ●, eyestalkless crabs (group B).

アカテガニにおける脱皮後の甲皮生長と歩脚除去との関係
○, 有眼個体; ●, 除眼個体

(A-6), the increased percentage of carapace width was reduced to one-half from that of the intact group (A-0).

Molting of eyestalkless crabs:

Table 1 and Text-figs. 1-4 also show the effects of leg removal on the molting of eyestalkless *Sesarma haematocheir*. The eyestalkless crabs with intact legs, Group B-0, molted in 18.7 ± 4.2 (13-28) days (Table 1 and Text-fig. 1). Group B-2 with 2-legloss molted in 20.9 ± 3.6 (14-26) days, B-4 with 4-legloss in 22.4 ± 2.2 (19-25) days and B-6 with 6-legloss in 23.3 ± 3.7 (19-30) days respectively. Ablation of a pair of eyestalks from the crabs was more accelerative in their molting effectively than in removal of legs from the eyed crabs. However, removal of legs from the eyestalkless crabs induced a slightly retarded molting in correlation with the number of missing legs. As shown in Table 1, no significant difference of retardation in the time to molt was found by t-test among eyestalkless groups except the case in which the difference is significant ($P < 0.05$) between B-0 and B-6. The molting rate of the eyestalkless crabs was presented in Table 1 and Text-fig. 2. The degree of molting rate was almost constant in B-0, B-2 and B-4, but it decreased to 50% in B-6. The difference between

B-O and B-6 was statistically significant by χ^2 -test ($P < 0.05$) (Table 1). The mortality rate of the eyestalkless groups was generally higher than that of the eyed group A (Table 1 and Text-fig. 3). The mortality of Group B-6 was particularly as high as 50%. So, ablation of a pair of eyestalks causes about 20% mortality and the removal of multiple legs from the eyestalkless crabs also brings the death to them. The increase of carapace width after molting was, as shown in Table 1 and Text-fig. 4, not statistically different among eyestalkless group B. In the eyestalkless crabs, regenerated legs were much paler in colour and smaller in length after molting, comparing with those of the eyed crabs. The new exoskeleton of eyestalkless crabs was very thin and pale in colour as that of regenerated legs. The exoskeleton had a tendency to be wrinkled.

Discussion

As shown in Table 1 and Text-figs. 1-2, removal of walking legs (2, 4 and 6) caused an acceleration of the time to molt and increased molting frequency on proportion to the number of legs removed from *Sesarma haematocheir*. In this species, removal of even only one leg could bring about precocious molting (SUZUKI, 1978). SKINNER and GRAHAM (1972) reported that in the land crabs, *Gecarcinus lateralis*, loss of 1-4 legs did not trigger precocious molting. On other some species, the multiple legloss also did not cause an acceleration of molting process, for example, adult *Ocypode macrocera* (RAO, 1966), male *Carcinus maenas* (PASSANO and JYSSUM, 1963) and *Libinia emarginata* (SKINNER and GRAHAM, 1972). The effects of legloss and eyestalkloss on the molting

Table 2. Effects of leg and eyestalk removal on molting in some decapods.
十脚類の脱皮に及ぼす歩脚と眼柄除去の影響

Animal	Time to molt days (Mean±S.D.)				Researcher
	Intact		Treatment		
	Control	6 or 8 legs removed	Eyestalks removed	Eyestalks plus 6-legs removed	
<i>Uca pugilator</i>	48		35		Abramowitz and Abramowitz 1940
<i>Sesarma reticulatum</i>	>60	36.4±7.9	17.7±0.9		Passano and Jyssum 1963
<i>Uca pugnax</i>		25± 2	22±3		Skinner and Graham 1972
<i>Gecarcinus lateralis</i>	>200	70±12			Skinner and Graham 1972
<i>Procambarus clarki</i>	84±26	56±20	18±4.5		Bittner and Kopanda 1973
<i>Uca pugilator</i>	>37	29.6	18.3	25.0	Fingerman and Fingerman 1974
<i>Gecarcinus lateralis</i>		50–55	30–35		Holland and Skinner 1976
<i>Procambarus clarki</i> (young)	19.6	14.1	6.5		Nakatani and Ōtsu 1979
<i>Sesarma haematocheir</i>	91.3±13.2	26.6±6.2	18.7±4.2	23.4±3.7	Suzuki (present study)

have been studied by many researchers in some decapods as shown in Table 2. The shortening rate of the molting cycle, when *Sesarma haematocheir* are removed of multiple legs, is 71% of the control. This shortening rate is higher than that of the other species. The precocious molting is remarkable in ablation of eyestalks in some decapods (Table 2). However, the ablation of eyestalks causes high mortality during intermolt.

In eyed *Sesarma*, the removal of walking legs induces the precocious molting, while in eyestalkless crab, it retards the coming molting. These phenomena of molting are affected in proportion to the number of missing walking legs (Table 1 and Text-fig. 2). The same effect as that of the eyestalk ablation on the molting of *Sesarma* is reported in *Uca* (FINGERMAN and FINGERMAN, 1974) and *Gecarcinus* (MCCARTHY and SKINNER, 1977a). In *Uca pugilator*, when 1-8 legs were removed from the eyestalkless crabs the rate of ecdysis decreased progressively in the crabs missing 1-3 legs, but the degree of inhibition was essentially constant in the crabs missing 3-8 legs. The same stimulus, removal of legs, will have opposite effects on the rate of ecdysis, decreasing it in eyestalkless *Uca* and increasing it in eyed crab. In *Gecarcinus lateralis* the time to molt appears to be independent of the number of limbs autotomized at the time of eyestalk ablation with slight delay of molting. This delay seems to reflect in the time required for the formation of a completed papilla. In these species; *Sesarma*, *Uca* and *Gecarcinus*, removal of legs from the eyestalkless crabs slightly delayed molting compared with molting of the eyestalkless crabs with intact legs. But the degree of delay is different among three species.

The increased rate in carapace width of eyestalkless crabs has been more obvious than that of eyed crabs in some species except *Sesarma haematocheir* shown in Table 3. In the present experiment, this increased rate of eyestalkless *Sesarma* was less than that of the eyed.

The substance which delays or stops ecdysis was traditionally known as molt inhibiting hormone (MIH) (SMISS, 1940). Recently a more complex molting mechanism has been proposed by MCCARTHY and SKINNER (1977a and b). And also some new

Table 3. Increase in carapace size after molting in some decapods.
十脚類における脱皮後の甲殻生長

Animal	Normal %	Eyestalks removed %	6-legs removed %	Researcher
<i>Uca pugilator</i>	0	3.8-20		GUYSelman 1953
<i>Carcinus maenas</i>	19.8±1.0	32.1±1.8		PASSANO and JYSSUM 1963
<i>Uca pugilator</i>		9.2	3	FINGERMAN and FINGERMAN 1974
<i>Procambarus clarki</i>	9.6	17.9	8.9	NAKATANI and ŌTSU 1979
<i>Sesarma haematobheir</i>	11.9±1.4	7.1±4.8	5.6±3.8	SUZUKI (present study)

additional factors which control molting and leg regeneration have been identified from the crustacean eyestalks, for example, limb growth promoting factor (LGPF), limb growth inhibiting factor (LGIF) and molt inhibiting factor (MIF) (SOYEZ and KLEINHOLZ, 1977; HOPKINS et al., 1979).

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摘 要

アカテガニの脱皮に及ぼす歩脚除去の影響を眼柄を切除した場合と無処理の場合とで比較した。アカテガニを脱皮直後に2(1対), 4(2対)あるいは6(3対)本の歩脚を除去すると次の脱皮は正常(歩脚を除去しない)個体よりも促進され, 脱皮率も上昇する。この脱皮の促進は除去した歩脚数に比例する(表1, 図1と2)。一方眼柄を切除した場合には今まで十脚類で報告された(表2)ようにアカテガニでも脱皮を誘発し歩脚除去よりも著しい効果を示す。眼柄と歩脚を同時に除去すると次の脱皮は眼柄だけを切除した個体よりも遅延する。この脱皮の遅れる程度は除去歩脚数と比例関係にあった(表1と図1)。FINGERMAN and FINGERMAN (1974)は *Uca pugilator* で, MCCARTHY and SKINNER (1977a)は *Gecarcinus lateralis* でそれぞれ無眼のカニから歩脚を除去すると脱皮は遅延することを認めているが, アカテガニによる結果とは多少相違している。