Chela asymmetry in a durophagous crab: predominance of right handedness, and
handedness reversal linked with chela size and closing force

Nobufumi Masunari¹, Masanori Hirooku¹, Shigeki Dan², Takahiro Nanri³, Masayoshi
Kondo¹, Masaki Goto¹, Yoshitake Takada⁴, and Masayuki Saigusa³*

1. Research Institute for Fisheries Science, Okayama Prefectural Technology Centre for
Agriculture, Forestry, and Fisheries, Ushimado 6641-6, Okayama-Setouchi 701-4303,
Japan
2. Tamano Laboratory, National Research Institute of Fisheries and Environment of
Inland Sea, Fisheries Research Agency, Chikko 5-21-1, Okayama-Tamano 706-0002,
Japan
3. Biodiversity Project for Research and Education (NPO), Tsushima-Fukui 1-8-71,
Okayama-Kitaku 700-0080, Japan
4. Japan Sea National Fisheries Research Institute, Suido-cho 1-5939-22, Niigata
951-8121, Japan

* Corresponding author: upogebiid@gmail.com
Summary

*Portunus trituberculatus* is a durophagous brachyuran. Right-handed crabs are predominant, but left-handed crabs are also found in nature. Left-handedness may arise from loss of the right crusher. We examined whether heterochely (morphology) was correlated with differences in closing force (physical property) and handedness (behaviour). The closing force was stronger in the larger chela and larger apodeme height, and handedness resided in the chela with stronger closing force. With loss of the right chela (autotomy), handedness was transitioned from the right to left chela, and all crabs were left-handed thereafter. Reversed handedness was accompanied with reduction of size and closing force in the regenerated right chela, and growth of the original left chela. After handedness reversal, dentition on the left dactylus of the newly-converted crusher was close to that of the original right crusher, but did not attain the same shape even 10 times of molt. So left-handed crabs were significantly worse at crushing hard-shelled preys than right-handed crabs. Chela formation was symmetric in the zoea; and heterochely and right handedness started in the megalopa, regardless of maternal handedness. Since the left chela is capable of being the crusher, heterochely may be caused by differences in morphogenetic velocity between the right and left chelae, under a signal discriminating right from left. Right handedness is an attribute of *P. trituberculatus*, and would be inheritable across generation. It is probable that right handedness was employed in the earliest durophagous crabs, and this trend has been succeeded to extant species.

**Key words:** crab, durophagy, handedness reversal, autotomy, linked morphogenic and functional regulation, ontogeny, evolution
List of symbols and abbreviations:


R-cr and L-cu: right crusher and left cutter. R-cu and L-cr: right cutter and left crusher.

α: tooth on the dactyle. α-R and α-L: α on the right and left dactyls.

β: tooth on the propodus. β-R and β-L: β on the right and left propodus.
Introduction

A prominent feature of the chelae of decapod crustaceans is lateral asymmetry (heterochely) in shape and size. One dimorphic chela is identified as the ‘crusher’, and is generally equipped with occlusive morphology and leverage capable of strong crushing forces, whereas the ‘cutter’ on the other side generates weaker forces and is used for manipulating and cutting food, or for grooming (Lee, 1995; Seed and Hughes, 1995). Heterochely is fully functional in the male fiddler crab *Uca*, where the major chela is used for display and combat (Levington et al., 1995; Rosenberg, 2002).

Heterochely is also well-developed in durophagous crabs (i.e. those preying on hard-shelled prey) (Seed and Hughes, 1997; Schenk and Wainwright, 2001). Durophagous brachyurans include Calappidae (Lewis, 1969), Cancridae (Warner and Jones, 1976; Vermeij, 1977a; Yamada and Boulding, 1998), Portunidae (Brown et al., 1979; Abby-Kalio and Warner, 1989; Seed and Hughes, 1995, 1997), Xanthidae (Brown et al., 1979; Blundon, 1988), and some other taxa (Vermeij, 1977a; Seed and Hughes, 1995). With the exception of superficially homochelous cancrids, most durophagous brachyuran crabs also have asymmetric chelae, usually with the right chela being the crusher and the left being the cutter (e.g., Lewis, 1969; Vermeij, 1977a; Brown et al., 1979; Abby-Kalio and Warner, 1989; Seed and Hughes, 1997; Schenk and Wainwright, 2001). Right-handedness thus may be predominant in most durophagous crabs.

The proportion of right-handedness often differs among decapod crustaceans. Herrick (1909) found 1,266 specimens of the American lobster, *Homarus americanus*, possessed left crushers and right pincers, while 1,164 possessed the opposite configuration. Yamaguchi (1977) also found that the proportion of right-handed and left-handed males of the fiddler crab (*Uca lactea*) was approximately even; out of 8,088
males, 4,071 were right-handed, and 4,017 were left-handed, i.e. the ratio was not significantly different from 1:1. These crustaceans begin post-larval development with symmetrical chelae. In the lobster, the trigger which determines handedness is chela-use at the critical juvenile stage (Govind, 1989). Once cheliped laterality is determined, the presence of a crusher on one side inhibits the other side from becoming a crusher (Govind, 1992). On the other hand, in the fiddler crab, one of the male chelipeds is spontaneously lost at the post-larval stage to regenerate as a minor one, while the remaining one develops into a major chela (Yamaguchi, 1977). Thus, heterochely and handedness in these animals arises through different developmental mechanisms, although the resulting predicted and observed proportions of right and left-handedness are equal.

The crusher is often lost by autotomy, and a new cheliped is regenerated one or two molts later. If the right crusher is lost, the left cutter is converted to the new crusher. This phenomenon is known as ‘handedness reversal’ (Emmel, 1907; Prizibram, 1931; Lewis, 1969; Govind and Pearce, 1988; Pynn, 1998). In the blue crab *Callinectes sapidus*, the proportion of right-handed crabs decreased from 100% in the smallest crabs to 74% among the largest crabs (Hamilton et al., 1976). Handedness reversal may provide a mechanism for explaining this trend. Simonson (1985) assumed that in the stone crab *Menippe mercenaria*, handedness reversal following chela loss was the most likely cause of an observed decrease from initial 100% right-handedness to 80% among crabs in natural populations (see also Abby-Kalio and Warner, 1989; Norman and Jones, 1991).
In *Callinectes sapidus*, the mechanical advantage of newly-converted crushers has been found to be greater than that of original cutters, but less than that of original crushers (Govind and Blundon, 1985). The same pattern has been reported in the red rock crab, *Cancer productus* (Brock and Smith, 1998). In *Menippe mercenaria*, a normal stridulatory pattern on the major chela was not regained on a regenerated chela (Simonson, 1985). On the other hand, in the shore crab *Carcinus maenas*, regenerated chelae may grow to the same size as original chela (Pynn, 1998). Of note is that chela growth is accelerated in the left crusher after reversal of handedness (Juanes et al., 2008). So it may be possible for the newly-converted left crusher to attain the same size as the original major chela. The relationship between the morphological and functional properties of newly-converted major chelae and those of the right chelae regenerated after autotomy is as yet unclear.

Furthermore, in the shore crab *Carcinus maenas*, all individuals are by birth destined to bear the crushing chela on the right side of the body (Przibram, 1931). In *Cancer productus*, juvenile crabs also go on to develop right-handedness (Smith and Palmer, 1994). In other brachyurans, cheliped asymmetry begins at post-larval development (Hamilton et al., 1976; Govind and Blundon, 1985; Simonson, 1985; Pynn, 1998; Ladle and Todd, 2006) similar to that of *Homarus* (Emmel, 1908; Govind and Pearce, 1989), and *Uca* (Yamaguchi, 1977). Analysis of a critical period in which handedness is determined would be essential for understanding formation of chela asymmetry.

The swimming blue crab, *Portunus trituberculatus*, is a durophagous brachyuran with heterochely. Right-handed crabs are predominant in this species, but a small number of left-handed crabs are found in nature. The frequency of left handedness
seemed to increase with growth of crabs. To determine whether this increasing frequency is due to handedness reversal, we have examined whether handedness (behaviour) is closely connected with heterochely (morphology) and differences in closing force of the chela (physical property). Next, to investigate the main factor to cause handedness reversal, we focused on morphological and physiological changes between the right and left chelae before and after autotomy.

For investigation of development of chela asymmetry, it is necessary to determine a critical period where handedness is determined. For this purpose, crabs with and without autotomy were tracked for more than one year from the larval stages to mature specimens. In addition, to determine whether right handedness is a property by birth, zoeas collected from right- and left- handed females were reared in the aquaria.

A question further arose as to the predominance of right handedness. If left handedness is caused by the loss of the right crusher chela, majority of left-handed crabs would originally be right-handed. Right handedness may be an attribute of durophagous crabs. Predominance of right handedness in durophagous species has been explained in terms of inheritability of handedness.
Materials and Methods

Morphological parameters of chelae: chelae size, dentition, and apodeme height

At the Okayama Fishery Institute, zoeas of *Portunus trituberculatus* hatched from 20 ovigerous females are reared in several big aquaria every year. When they attain the third (C3) or fourth (C4) crab developmental stage, most of these crabs are released into the Seto Inland Sea. A portion of the crabs are retained in several aquaria for experiments. More than 300 crabs, including males and females, were kept individually in plastic cages (40 cm long, 30 cm wide, and 30 cm high), each of which was placed in shallow aquaria. Juvenile crabs were also kept individually in a small aquarium made of a plastic cylinder with mesh under the bottom (7-15 cm in diameter, and 10-20 cm in height). Other experiments were done using mature crabs purchased from the Fishery Cooperation at Yorishima, Okayama prefecture, and kept in the aquaria.

*P. trituberculatus* has four zoea stages (Z1-Z4) and one megalopa stage (M) before the first crab stage (C1). Zoeas were first fixed with 3% formalin (v/v) for one day, and were then transferred to a solution of 70% ethanol and 2.5% glycerol for preservation. The propodus height was measured under the biological microscope (OPTIPHOTO, Nikon Co. Ltd., Tokyo, Japan). Chela size was represented by the propodus height (Fig. 1A). The cuticle of megalopa was easy to be broken. So megalopae were put in PBS (phosphate buffered saline), and was frozen at -80°C. Juvenile crabs were fixed with a solution of 70% ethanol and 2.5% glycerol until cheliped measurement. Propodus height of megalopa and juvenile crabs was measured under the biological microscope, the stereomicroscope (SZX 12, Olympus Co. Ltd., Tokyo, Japan), or using digital venire callipers (Fig. 1B, 1D).
The magnitude of the force exerted by chela is closely related to the area of the closer apodeme, onto which the closer muscle is inserted (Warner and Jones 1976; Seed and Hughes 1995). The closer apodeme was absent in zoea, but was found in megalopa (Fig. 1C). The closer apodeme height was measured under the biological microscope up to C3-C4 crab stages, and was measured by use of digital venire callipers thereafter.

Asymmetry was found on the shape and size of the tooth arranged on the dactylus and propodus as well as the chela size. The height of these teeth was measured under the profile projector (V-12, Nikon Co. Ltd., Tokyo, Japan), or under the stereomicroscope if the former was not possible. We determined morphogenetic stage where these teeth ($\alpha$-R and $\alpha$-L) appeared, and further examined how their shape was altered by autotomy.

*Feeding experiment*

When soft-shelled prey such as annelids, squid, small fish, and mysid shrimps were given to crabs, they were immediately captured with both chelipeds and directly carried to the mouth. Thus, soft-shelled prey was not useful for testing handedness. Handedness was clearly apparent when preying on hard-shelled items. Bivalves such as *Ruditapes philippinarum*, *Phacosoma japonicum*, or *Cyclina sinensis* were adequate for testing handedness of mature crabs (carapace width more than about 12 cm). The left cheliped was often used for secondary crushing following the first attack with the right chela. When these bivalves were partially crushed and fed to crabs, the frequency of left chela use increased. Hence, handedness of mature crabs was determined using bivalves with undamaged shells.
However, these clams (R. philippinarum, P. japonicum, and C. sinensis) were too big for small crabs (5-10 cm in carapace width). Smaller snails such as Granata lyrata and Reticunassa festiba (5-10 mm in shell diameter) were given to these immature crabs. The size and hardness of these snails were adequate for judging the handedness of immature crabs. (When these snails were fed to mature crabs, they were easily crushed by the mandibles with use of both chelipeds).

Even small snails such as G. lyrata were too big to determine the handedness of megalopae and juvenile crabs (C1 and C2). Soft-shelled prey such Artemia larvae and small mysid shrimps were broken by the mandibles without use of chelipeds. We prepared PFA tubes (300 µm external diameter, and 100 µm internal diameter) for feeding experiments. Mysid shrimps were homogenized by glass and ultrasonic homogenizers, and then injected to the glass tubes by use of a syringe. The hardness of the PFA tubes was adequate for determining handedness of megalopae and juvenile crabs (Fig. 1E, 1F). Hard-shelled prey was given to crabs according to their carapace size (Fig. 1G).

**Measurement of closing force and measurement of the apodeme height**

The crushing power of crab chelae primarily depends on the magnitude of the force that can be generated by the closer muscle. We estimated crushing power as closing force. As shown in Figure 2, closing force was estimated using a Drag Checker, (Bouz Production Co. Ltd., Tokyo, Japan). Slender, right-angled metal plates were fixed to the board at the top and the bottom. The upper, movable, metal plate could pull a strong nylon string through the Drag Checker. The space between the opposing metal plates was less than 1 cm, and was adjusted according to chela size. Each crab was
picked up from its cage in the aquaria, and the slender opposing metal plates were
pinched by the chela at the same time. Closing force was measured at around the centre
of the dactylus. One of two types of Drag Checker, i.e. DC-1005 (0-5 kgf) or DC-1015
(0-15 kgf) was used depending on crab size.

After estimation of the closing force (N) of the right and left chelae, the apodeme
height was measured for the same specimens after freezing.

*Influence of maternal handedness*

Zoeas were obtained from both right- and left-handed mature females. They were
separately reared in the aquaria, and the handedness of megalopae and the first crab
stage (C1) were determined.

*Long-term experiment to estimate handedness reversibility*

Megalopae were randomly collected from a big aquarium, and were divided into
two groups. Each group was further divided into 4-5 small aquaria. One group (control)
was kept without autotomy of the right chela. In another group, the right chela was
autotomized at the first crab stage (C1). The handedness of each crab was determined
four times, until the juvenile crabs attained stage C12 to C14. For the first test,
handedness was determined by the glass tube experiment (Fig. 1E, 1F). The second test
was made by use of the snail, *G. lyrata*. The third and fourth tests were done using glass
tubes. Crabs were fed on mysids and other animals between these experiments.
Immobilization of the right chela

Megalopae were collected from the communal aquarium, and kept in small aquaria. Two experiments were performed. In the first (adhesion 1), use of the right chela was immobilized using a quick-drying adhesive (cyanoacrylate adhesive) applied after each molt (five times from C1 to C5). Handedness was tested by the glass tube experiment (Fig. 3E). Dentition was also examined at these same stages. In the second experiment (adhesion 2), use of the right chela was immobilized using adhesive at the first (C1) and second (C2) crab stages. Dentition and behaviour were inspected at stage C10.

Results

Close relationships among chela size, closing force, and handedness

Relationships between chela size and closing force were determined for more than 300 mature crabs that were individually kept in the aquaria. Chela size was represented by propodus height. As shown in Figure 3A, when the right chela was larger than the left, the closing force of the right chela was always greater than that of the left. Conversely, for the crabs with larger left chela, the closing force of the left chela was stronger than the right (Fig. 3B).

Mature crabs were collected from the Seto Inland Sea, and were starved for more than two months in aquaria. The closing force was measured in each individual (Fig. 4). Handedness was determined by feeding experiment (Fig. 1G). Nineteen of 31 crabs possessed larger right chela, the other 12 crabs possessed larger left chela. For right-handed crabs, the right chela was always stronger than the left. Conversely, for left-handed crabs, the left chela was stronger than the right. Thus, handedness is
determined as the chela with the stronger closing force. The mean closing force of the major chela in the right-handed crabs was more than 141.3±15.6 N. (The closing force of 8 out of 31 individuals exceeded 150 N, maximum force that could be measured by the apparatus illustrated in Figure 2). The mean closing force of the major chela was 105.0±24.0 N for left-handed crabs.

**Ontogeny of chela asymmetry**

As shown in Figure 1A, chelipeds in *P. trituberculatus* first appeared as an immovable structure at the 2nd zoeal stage (Z2). Chela size was symmetrical up to the last stage (Z4) of zoea (Fig. 5A). The cheliped of zoeas was not employed for feeding; prey (rotifers and *Artemia* larvae) was caught by the maxillipeds and other appendages. Chela asymmetry was first detected in megalopae (Figs. 1B and 5A). The closer apodeme (Fig. 1C) was first present in megalopa (Fig. 5B). Size of the apodeme was close to asymmetrical. Asymmetry was more obvious in juvenile crabs (Figs. 1D and 5B).

**Influence of maternal handedness**

Ovigerous females with different handedness (7 females with right-handedness, and 5 females with left-handedness) were kept in separate aquaria, and zoeas were randomly collected from those aquaria. Handedness of megalopae and crabs in the first stage was determined by a feeding experiment. As shown in Table 1, 75 of 77 megalopae (97%) and 29 of 30 first crabs (97%) from right-handed females were right-handed. The remaining two megalopae and one crab used both chelae. Eighty-six of 88 megalopae (98%), and all 42 crabs (100%) were right-handed, with the remaining
two megalopae using both chelipeds. This indicates that megalopae start out right-handed irrespective of the handedness of maternal females. Right handedness would be an attribute of this species.

Irreversible reverse-handedness

Crabs of the first stage (C1) were randomly collected from aquaria 17 days after hatching, and were divided into two groups (each of 36 individuals): a control group (without autotomy), and experimental group in which the right chela was autotomized by pinching the basement of the cheliped with fine forceps. Both groups were separately reared through subsequent crab stages (up to C14 stage). Handedness was determined by feeding experiments (Fig. 1E, 1F, and 1G). In the control group, the right-handedness was maintained throughout the experiment (Table 2). For the autotomized group, the right chela regenerated after one or two molts. Handedness transitioned from the right to left chela, and all crabs were left-handed thereafter. Right-handedness never reappeared in crabs with loss of the right chela.

Table 1. Handedness of the larval and juvenile crabs born from females with different handedness.

<table>
<thead>
<tr>
<th>Aquarium</th>
<th>Maternal female</th>
<th>Megalopa</th>
<th>The first crab (C1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Handedness</td>
<td>Right-handed</td>
</tr>
<tr>
<td>No. 1</td>
<td>1 R</td>
<td>24 0 2</td>
<td>7 0 1</td>
</tr>
<tr>
<td>No. 2</td>
<td>2 R</td>
<td>23 0 0</td>
<td>12 0 0</td>
</tr>
<tr>
<td>No. 3</td>
<td>2 R</td>
<td>28 0 0</td>
<td>—* —* —*</td>
</tr>
<tr>
<td>No. 4</td>
<td>2 R</td>
<td>—* —* —*</td>
<td>10 0 0</td>
</tr>
<tr>
<td>No. 5</td>
<td>1 L</td>
<td>28 0 2</td>
<td>9 0 0</td>
</tr>
<tr>
<td>No. 6</td>
<td>2 L</td>
<td>36 0 0</td>
<td>18 0 0</td>
</tr>
<tr>
<td>No. 7</td>
<td>2 L</td>
<td>22 0 0</td>
<td>15 0 0</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>161 0 4</td>
<td>71 0 1</td>
</tr>
</tbody>
</table>

—*: Not inspected.
Decrease of the closing force in the regenerated right chela, and increase in the left chela

Figure 6A compares the closing force between right and left chela in the right- and left-handed crabs reared in the aquarium (Table 2). In the right-handed crabs (control), the closing force of the right chela was always stronger than that of the left chela. The mean closing force was 32.5±11.4 N on the right chela, and was 16.5±6.7 N on the left chela. In the left-handed crabs (a group in which the right chela was autotomized at stage C1), the mean closing force was 29.6±12.7 N in the left chela (crusher), and was 12.6±7.7 N on the right chela (Fig. 6B). Thus, the closing force of the right chela decreased to 39% in the autotomy group. On the other hand, the closing force of the left chela increased to 1.8 times that of the control group.

Statistically significant differences were detected between right and left chela both for crabs without autotomy (paired t-test, df=10, t=6.237, p<0.001) and crabs with autotomy of the right cheliped (paired t-test, df=9, t=5.199, p<0.001).

<table>
<thead>
<tr>
<th>Table 2. Feeding experiment to examine whether right-handedness reappear.</th>
</tr>
</thead>
<tbody>
<tr>
<td>test (Date) Day 0       Day 23-25 (15-17 Jul.) Day 140-142 (9-12 Nov.) Day 344-346 (1-3 Jun., 2013) Day 382-383 (10-11 Jul.)</td>
</tr>
<tr>
<td>Handedness</td>
</tr>
<tr>
<td>Crab stage</td>
</tr>
<tr>
<td>Total carapace width (cm)</td>
</tr>
<tr>
<td>Carapace width (cm)</td>
</tr>
<tr>
<td>no: no response to the snail; * The left cheliped was lost at ecdysis on Day 47. ** Number of molts drastically decreases in the second year.</td>
</tr>
</tbody>
</table>
Alteration of chela size and handedness reversal after autotomy

Figure 7A indicates growth of chela size in one crab without autotomy. Difference of right and left chela size seemed to be remarkable with molts. However, actually it was always within 1.10-1.19 folds in the ratio (Table 3). When the right cheliped was autotomized at C1 (two crabs), the right chela regenerated after two molts (C3), and chela size reversed between right and left (Fig. 7B). A feature of note was that the left chela (L-cr) was of 141% (mean) as much as the regenerated right chela (R-cu) (Table 3). The ratio rapidly decreased to 116-113% in C4-C6, and was of 107-111% thereafter. Thus, chela size rapidly increased in the newly-converted left crusher.

Table 3. Growth of the left chela and conversion to the crusher chela following autotomy of the right chela.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Chela height</th>
<th>Crab growth stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ratio</td>
<td>C1</td>
</tr>
<tr>
<td>Control</td>
<td>R-cr (mm)</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>L-cu (mm)</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>R/L</td>
<td>1.10</td>
</tr>
<tr>
<td>Autotomy</td>
<td>R-cu (mm)</td>
<td>Au.</td>
</tr>
<tr>
<td></td>
<td>L-cr (mm)</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>L/R</td>
<td>-</td>
</tr>
</tbody>
</table>

Au.: The right chela was autotomized at stage C1 (the first crab). Abs.: Absence of the right chela. *: A small chela regenerated at the right. Note that in the same age, the crusher chela (cr) was a little larger in the control group than in the autotomy group.

Figure 7C summarizes the chela-size relationship of the specimens with or without autotomy of the right chela. Crab stage used for measurement was C12-C14 (see Table 2). The regenerated right chela became the minor chela (R-cu), but its size was a little larger than the left chela (L-cu) of the control group with no autotomy. A statistically significant difference was detected in the intercept of each two regressions (df=11, t=2.260, p=0.0451 between R-cr and L-cr; and df=11, t=3.097, p=0.0102 between R-cu and L-cu). Thus, alteration never returned to the original relationship before autotomy.
Growth of chela dentition, and morphological changes after autotomy

Figure 8 (upper panel) shows the right and left chelae of a mature female (frontal view), and dentition on the dactylus (movable finger) and propodus (immovable finger). In the right-handed crab, a sharp canine, α-R (upper and middle panels in Fig. 8) that was observed at the base of the right dactylus significantly enhances chelae mechanical advantage. In contrast, the tooth (α-L) had a groove on the left dactylus. A paired tooth (β) was also observed on the propodus (bottom panel in Fig. 8). While the right molar (β-R) had a vertical groove (type I), that on the left propodus (β-L) had vertical and cross grooves (type II).

After the loss of the right major chela (R-cr) at C1, the tooth of the newly-converted left crusher (α-L) developed, and the shape was close to that of a canine (Fig. 9, upper panel). Nevertheless, the shape of the left chela did not attain the same shape as that of the original right chela even 10 times of molt after autotomy. In the regenerated right dactylus (R-cu), the tooth (α-R) was smaller than that of the original left cutter (L-cu). Comparison of Figure 9 (middle panel) with Figure 8 (middle panel) shows that the tooth shape (α-R) was similar to the molar of the original left cutter (L-cu) in that it was partitioned by a vertical groove (type I), even after several molts.

The molar of the right propodus (β-R) had a vertical groove in the original right crusher (Fig. 8, bottom panel). This tooth had both vertical and cross grooves after regeneration (Fig. 9, bottom panel). The molar on the left propodus (β-L) was much the same as that of the original left cutter (type II). Thus, the molar of the right propodus (β-R) was altered from type I to type II after autotomy, but that of the left propodus (β-L) was of much the same type as before (type II).
Figure 10A shows the change of the tooth size (height) between the right and left dactylus after autotomy of the right chela in C1 (Table 2). The right chela was regenerated in C3, and measurement was done from stage C5. The tooth height ($\alpha$-L) in the newly-converted left crusher (L-cr) was taller than that ($\alpha$-R) of the regenerated right cutter (R-cu), and this difference became larger with age (number of molts). As well as the chela size (Fig. 7C), tooth height of the left crusher (L-cr) was less than that of the original right crusher (R-cr) (Fig. 10B). Tooth height of the regenerated right cutter (R-cu) was smaller than that of the original left cutter (L-cu). A statistically significant difference was detected in the intercept of each two regressions ($df=11$, $t=7.349$, $p<0.001$ between R-cr and L-cr; and $df=11$, $t=3.547$, $p=0.0046$ between L-cu and R-cu).

*Allometric relationships between apodeme height and closing force*

Figure 11A shows allometric relationships between the closing force ($\log(y)$) against the closer apodeme height ($\log(x)$) in the major (R-cr) and minor (L-cu) chelae of the right-handed crabs (control group); and Figure 11B indicates those in the major (L-cr) and minor (R-cu) chelae of the left-handed crabs (autotomy group). In the control group, the mean apodeme height of the right chela was $17.1 \pm 1.5$ mm (R-cr), but was $11.1 \pm 1.6$ mm (R-cu) in the autotomy group (decrease at 65%). The mean closing force was $139 \pm 19$ N (R-cr) in the control group, but was $58 \pm 20$ N (R-cu) in the autotomy group (decrease at 42%). On the other hand, in the autotomy group, the apodeme height of the left chela was $12.5 \pm 1.6$ mm (L-cu), and was $14.7 \pm 1.0$ mm (L-cr) (increase at 1.17 folds). The closing force was $63 \pm 16$ N in the control group, and was $105 \pm 24$ N in the autotomy group (increase at 1.68 folds).
Statistically significant difference was detected among the mean apodeme heights of the four chela groups (one-way ANOVA, F(3, 63)=44.17, p<0.001; Tukey's HSD test, p<0.05). When the major right chela (R-cr) is lost, apodeme height and closing force of the regenerated new chela (R-cu) were smaller than those of the original left chela (L-cu) (Fig. 11C). Since handedness always resides in the chela with stronger closing force (Fig. 4), the original left chela (L-cu) should be converted to the major chela (L-cr) with regeneration of the right cheliped.

Crabs with two crusher chelae

Movement of the right cheliped got immovable by an adhesive several times at each molt (Table 4). Handedness was examined by feeding experiments at C11-13 stages, along with chela morphology. For the control group, all crabs showed right-handedness; and for the autotomy group, all crabs showed left-handedness. In the first chela-immobilizing experiment (Adhesion 1), six out of 13 individuals showed right-handedness (Rcr-Lcu). In the other seven individuals, the left chela became a crusher as well as right crusher (Rcr-Lcr) (see also Fig. 12, upper panel). The second experiment (Adhesion 2) was carried out on seven individuals. Four crabs possessed a right crusher and a left cutter (Rcr-Lcu), but the other three crabs had crushers on both chelae (Rcr-Lcr). When the right cheliped was released from adhesion, both chelae were employed for feeding. Frequency of right and left chelae use somewhat differed in each individual (data not shown). Nevertheless, the right crusher was never converted to the cutter.
For the crabs with both crushers (Rcr-Lcr), the tooth of the left chela (α-L) was a sharp canine as well as that on the right dactylus (α-R) (Fig. 12, middle panel). The molar on the left propodus (β-L) was of much the same type (type I) as the right molar (β-R) (Fig. 12, bottom panel). (While the canine was very sharp in the right dactylus, it was somewhat round in the left dactylus.) Type I molars were seen on the left propodus in only two out of 10 specimens (Table 5). Six had type II molars, and the molar of the other two crabs were intermediate between type I and type II.

Table 4. Experiment to examine the effect of immovalization in the right crusher chela.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Rcr-Lcu</th>
<th>Rcu-Lcr</th>
<th>Rcr-Lcr</th>
<th>No. of crabs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Autotomy</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Adhesion 1</td>
<td>6</td>
<td>0</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Adhesion 2</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>


For the crabs with both crushers (Rcr-Lcr), the tooth of the left chela (α-L) was a sharp canine as well as that on the right dactylus (α-R) (Fig. 12, middle panel). The molar on the left propodus (β-L) was of much the same type (type I) as the right molar (β-R) (Fig. 12, bottom panel). (While the canine was very sharp in the right dactylus, it was somewhat round in the left dactylus.) Type I molars were seen on the left propodus in only two out of 10 specimens (Table 5). Six had type II molars, and the molar of the other two crabs were intermediate between type I and type II.

Table 5. Dentition (β) of the crabs with the crusher on both sides.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Type I (L)</th>
<th>Intermediate</th>
<th>Type II (L+C)</th>
<th>β-L (L)</th>
<th>Intermediate</th>
<th>Type II (L+C)</th>
<th>Number of crabs inspected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Adhesion 1</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Adhesion 2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

L: longitudinal axis. C: cross axis. Intermediate: cross axis was very weak, and the molar was close to the original crusher.

Difference in shell-crushing success between the right- and left-handed crabs

The ratio of shell-crushing success was compared between right- and left-handed crabs that had been kept from the megalopa (Table 2). Feeding experiment was carried out by use of a small, hard-shelled snail, *Reticunassa festiba*. The success proportion increased with growth of crabs (Fig. 13A). It was largely different among individuals. So the success ratio at each size was normalized (Fig. 13B). Proportion of success in
shell-crushing was significantly affected by the factor handedness (logistic regression, z-test, p=0.009) and size (z-test, p<0.001). Thus, it is obvious that right-handed crabs were better at crushing hard-shelled prey than left-handed crabs.
Discussion

Left handedness caused by loss of the right chela

Bilateral cheliped asymmetry is a prominent feature of decapod crustaceans (Emmel, 1907; Herrick, 1909; Przibram, 1931; Govind, 1989, 1992; Palmer, 2004, 2012). In many durophagous crabs, crusher chelae occur on the right side of the body, and cutters occur on the left (e.g. Lewis, 1969; Hamilton et al., 1976; Vermeij, 1977a; Juanes and Hartwick, 1990; Seed and Hughes, 1995; Pynn, 1998; Schenk and Wainwright, 2001; Ladle and Todd, 2006; Juanes et al., 2008). The ratio of left handedness may be different in species and habitat (Hamilton et al., 1976). The shore crab Carcinus maenas was found to be 79% right-handed and 21% left-handed in nature, and it was suggested left-handedness may have been caused by loss of the right chela (Abby-Kalio and Warner, 1989). Handedness reversal in the velvet crab Necora puber is also thought to be due to autotomy of the major cheliped (Norman and Jones, 1991). The proportion of Callinectes sapidus possessing the crusher chela on the right decreased with age (size) from 100% in very small crabs to 74% in larger sized crabs (Hamilton et al., 1976).

In Portunus trituberculatus, among more than 300 mature individuals, 75% had a right crusher and left cutter, 25% had the opposite configuration (Fig. 3A and 3B). The chelipeds that first appeared were symmetrical (Figs. 1A and 5A), but megalopae were right-handed (Figs. 1B, 1E, 5A and 5B). As shown in Table 1, right handedness occurs in the megalopae irrespective of maternal handedness. This may indicate that right handedness is the property of P. trituberculatus by birth. When the right cheliped was autotomized, handedness was reversed with change of the chela size (Tables 2, 3 and
Fig. 7B). Handedness reversal would not occur when the minor chela is autotomized. Thus, handedness reversal always occurs from right to left chela, and left handedness is fixed with the change of chela size and closing force (Tables 2 and 3). Hence the proportion of left-handed crabs should increase with growth in nature. It is highly plausible that majority of these left-handed crabs must have experienced autotomy of the original right crusher.

_Handedness linked to greater closing force_

In crustaceans with heterochely, the crusher chela has a greater mechanical advantage and a larger closer muscle volume than the cutter chela (Elner and Campbell, 1981; Warner et al., 1982; Blundon, 1988; Schenk and Wainwright, 2001). A difference in the closer muscle composition has been suggested in the lobster, *Homarus americanus*, (e.g., Lang et al., 1977; Govind et al., 1981). In the blue crab, *Callinectes sapidus*, however, fibre composition was not different between the crusher and cutter of individual crabs (Govind and Blundon, 1985). Furthermore, there may be no bilateral asymmetry between closer muscles in terms of motor neurons or size and number of sensory axons (Govind and Blundon, 1985). This may suggest that different closing forces between the asymmetric chelae may results from differences in the volume of the closer muscle and the mechanical advantage.

Closing force is a function of the stress produced per cross-sectional area of muscle, the cross-sectional area of the closer muscle, and the mechanical advantage of the lever arm to the length of the lever arm (Warner and Jones, 1976; Vermeij, 1977a; Elner and Campbell, 1981; Schenk and Wainwright, 2001; Swanson et al., 2013). In *P. trituberculatus*, closing force was proportional to the apodeme size (Fig. 11A and 11B),
and handedness always resided in the chela with stronger closing force (Fig. 4). Larger apodeme height and chela size may be closely related to the volume of the closer muscle, and handedness would occur on the chela with stronger closing muscle.

Irreversible handedness, and remodelling of the chela operating system

As indicated in Figure 6A and 6B, the mean closing force of the right chela was 32.5 N in the control group, and decreased to 12.6 N in the autotomy group. In contrast, the mean closing force of the left chela was 16.5 N in the control group, and increased to 29.6 N in the autotomy group. In Figure 11A (control group), the apodeme height of the right chela (R-cr) was much larger than that of the left chela (L-cu). This relation was reversed in the autotomy group (Fig. 11B): i.e., the apodeme height of the left chela (L-cr) was larger than the right chela (R-cu). The chela with stronger closing force was transitioned from the right to left chela (Fig. 11C). Since handedness is linked with the chela with stronger closing force (Fig. 4), handedness should also be transitioned from the right to the left chela in the autotomy group. With transition of handedness, the left chela further grew bigger (Table 3). Chela function would be changed between right and left, possibly via the central nervous system.

Crabs with symmetrical crusher chela

In the fiddler crab *U. lactea*, the proportions of right-handed and left-handed males are nearly equal (Yamaguchi, 1977), but in the other species, e.g. *U. vacans* and *U. tetragonon*, almost all males are right-handed (Barnwell, 1982). In *U. lactea*, chelae of individual crabs are symmetrical up to the juvenile crab stage; and heterochely occurs after spontaneous loss of either chela, resulting in the equal proportions of right- and
left-handed individuals. When both chelae remained intact, or were lost at the same time, symmetry was fixed as paired major or minor chelae (Yamaguchi (1977). These results indicate that loss of either chela is one of important factors that trigger asymmetry.

In the lobster *Homarus americanus*, the critical period for chela differentiation was delimited to occur in the fifth stage; the development of chela asymmetry required at least five days in the presence of a substrate such as oyster chips (Govind and Pearce, 1989). A four-step mechanism chela asymmetry has been proposed in *H. americanus* (Govind 1992).

In *P. trituberculatus*, the left chela was not lost at metamorphosis (Fig. 1A, 1B and 1D), so it is clear that asymmetry is not triggered by loss of either chela in the larval stage. The crusher chela was also formed at the left side by immobilization of the right chela (Fig. 12 and Table 4). In the lobster, once cheliped laterality is determined, the presence of a crusher on one side inhibits the other side from becoming a crusher (Govind, 1992). Handedness and reversed handedness in *P. trituberculatus* may be explained physiologically as follows. With generation of chela asymmetry (Figs. 1B, 5A and 7A), presence of right larger chela may inhibit the opposite chela to be a crusher. When the crusher chela is lost by autotomy (Table 2), or is immobilized by the adhesive (Fig. 12), suppression on the opposite chela would be lost. Then the opposite chela grows bigger to be a crusher (Table 3). Presence of newly-converted crusher may inhibit the regenerated chela to be a crusher.

Megalopae in the brachyurans are transitional forms between zoea and crab, and morphological changes in internal anatomy and external shape are most pronounced in the postembryonic stage (Felder et al., 1985). Chela asymmetry in *P. trituberculatus* may develop through two morphogenetic steps (Fig. 14A). In the first step (*s*1), chela
shape is symmetrical (see also Figs. 1A and 5A). Asymmetry starts at the second step (s2). Differences in chela formation in the morphogenetic steps may be the primary cause of asymmetry. Asymmetry appears not only on chela size (Fig. 5A) but also on the apodeme height (Fig. 5B). The developmental stage where asymmetry in tooth morphology (α and β) appears may be different between dactylus and propodus (Fig. 14).

The first sign of chela asymmetry was seen in the megalopa stage, being a prominent tooth on the right dactylus (Fig. 1B), with difference in chela size (Fig. 5A) and closer apodeme height (Fig. 5B). Since right handedness was not influenced by maternal handedness (Table 1), morphogenetic facilitation of the right chela would occur spontaneously at metamorphic stage, under a signal distinguishing right from left.

Comparison of Figure 8 (middle panel) with Figure 9 (middle panel) indicates that in the autotomy group, the shape and size of the tooth on the left dactylus (α-L) was close to that on the original right crusher (α-R), although the molar on the propodus remained similar to type II (bottom panels in Figs. 8 and 9). These morphological differences were observed more than one year after autotomy, in C12-14 individuals (Table 2). Most crabs have two molts in the second year, and only one molt in the third year (Hamasaki, 1997). The tooth shape (especially β-R) in the newly-converted left crusher could not attain the same as that of the major chelae of the right-handed crab (Fig. 9), even if autotomy occurs in the young age.

Shell-cracking behaviour

Handedness may be advantageous for feeding on hard-shelled preys and for fighting, especially in durophagous brachyurans (Seed and Hughes, 1995, 1997). The
advantage of right handedness has been typically accounted for as an adaptation for opening dextrally coiled shells (e.g., Ng and Tan, 1985; Dietl and Hendricks, 2006). In the xanthid crab *Eriphia smithii*, left-handed crabs had more difficulty in breaking the shell aperture of large individuals of the dextrally coiled snail *Planaxix sulcatus* (Shigemiy, 2003). However, left handedness may not have any disadvantage for left-handed crabs in either feeding or agonistic behaviour (Ladle and Todd, 2006).

In *P. trituberculatus*, the success proportion of shell crushing increased with growth of crabs in both right- and left-handed crabs (Fig. 13A). Left handedness arose from loss of the right crusher, and the regenerated right chela was always smaller than the left chela (Fig. 7B and 7C). The closing force of the newly-converted left chela (L-cr) was always less strong than that of the original right crusher (R-cr) (Figs. 6B and 11C). When compared in the same carapace size, the closing force of the major chela (R-cr or L-cr) would be significantly stronger in the right-handed crab. Hard-shelled preys given to crabs were snails such as *Granata lyrata* and *Reticunassa festiba* with 5-10 mm in shell diameter. Crabs cracked the snails with the same manner regardless of handedness. Differences in the closing force between right and left chela would result in higher proportion of success in shell-crushing in the right-handed crabs (Fig. 13B). This suggests that right-handed crabs clearly have advantage in feeding and agonistic behaviour.

*Predominance of right handedness in the durophagous crab*

Handedness may be advantageous for feeding on hard-shelled prey and for fighting in durophagous brachyurans (Vermeij, 1977a; Brown et al., 1979; Govind and Blundon, 1985; Seed and Hughes, 1995, 1997; Sneddon et al., 1997; Yamada and
Boulding, 1998; Scheck and Wainwright, 2001). In most durophagous brachyuran crabs, right handedness may be predominant (e.g., Lewis, 1969; Vermeij, 1977a; Brown et al., 1979; Abby-Kalio and Warner, 1989; Seed and Hughes, 1997; Schenk and Wainwright, 2001).

In *P. trituberculatus*, left handedness is caused by autotomy (or injury and malfunction) of the original right crusher (Table 2). In addition, right handedness appeared in the megalopae regardless of maternal handedness (Table 1), suggesting that right handedness is the attribute of this species. Heterochely may be brought by differences in morphogenetic velocity between the right and the left chela (Figs. 5A, 5B, Fig. 7A and 10B), under a signal discriminating right from left. Right handedness would be inheritable across generation.

The heterochely of the durophagous fossil crab, *Megaxantho zoque*, from the Cretaceous (Vega et al., 2001; Dietl and Vega, 2008) clearly suggests right-handedness. The brachyuran fossil record indicates that most taxa had appeared by the Early Eocene (see fig. 3 in Brösing, 2008). Although *M. zoque* became extinct at the end of the Cretaceous (Vega et al., 2001), the diversity of durophagous brachyurans greatly increased from the Late Cretaceous to the Early Eocene (see figure 3 in Oji et al., 2003; and table 1 in Schweitzer and Feldmann, 2010). Most existing brachyuran groups that forage for hard-shelled prey are consisted of right-handed species. A possible explanation for predominance of right handedness is that they are the descendants of the durophagous brachyurans that appeared in the Cretaceous.
Acknowledgements

We thank Dr. Hideo Yamanoi, Director of the Research Institute for Fisheries Science for affording us every facility during our investigation. We also thank the staff of the Biodiversity Project for Research and Education for helping us rear crabs.

Funds

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.
References


Figure 1. Morphology of the cheliped and feeding behaviour of the swimming crab *Portunus trituberculatus*. A: Zoea (Z4) right (left side) and left (right side) chelae. R:
right cheliped; L: left cheliped. Note that chelipeds are shown in frontal view, so the right cheliped is presented in the left side, and the left cheliped is shown on the right side in all figures. Double-pointed arrows indicate propodus height. B: Megalopa chelipeds. The upper arrow (pointing right) indicates a canine of the right dactylus (movable finger). C: Closer apodeme a juvenile crab (C1). The apodeme height (double-pointed arrows) was measured. Diagonal arrow: a canine on the right dactylus. D: Morphology of the right and left chelae in the first crab stage (C1). The propodus height (vertical double-pointed arrows) was measured. Downward pointing small arrow: a canine on the right dactylus. E: feeding experiment on a megalopa, which repeatedly tried to crack the fine glass tube with the right chela (R). The left chela (L) was used for grasping the glass tube. F: feeding experiment on a juvenile crab (C1). Only the right cheliped was employed for cracking the glass tube. G: Shell-cracking behaviour. Hard-shelled snails or bivalves (diagonal arrow) are carried to the mouth by use of either chela, and then they are broken by the right chela.
Figure 2. Apparatus to estimate closing force of the chela. dc: drag checker. mp: right-angled metal plate. nst: nylon string. Closing force was measured at the middle of the chela.
Figure 3. Difference of closing force between right and left chelae in the crabs with right larger chela and those with left larger chela. A: Closing force (N) of right and left chela in individuals with right larger chela (open circles). B: Closing force (N) of right and left chela in individuals with left larger chela (open triangles). The dashed line indicates equal closing force for right and left chelae. Straight line indicates the regression line. $R^2$: coefficient of correlation.
Figure 4. Comparison of the closing force between right and left chela in right- and left-handed crabs. Open circles: right-handed crabs. Filled triangles: left-handed crabs. The dashed line indicates equal closing force (N) for right and left chelae. The closing force of eight individuals (right-handed crabs) exceeded the maximum level (150 N) of measurement (shown on the right edge of the figure). All crabs were collected from the field, but note that the proportion of handedness is not reflective of that in the field.
Figure 5. Ontogeny of chela asymmetry. A: The relation of size between the right and left chela. Chela size is represented by propodus height. Z4 (open circles): chela size of zoea. M (open triangles): that of megalopae. C1 (open squares): that of the first crab stage. The dashed line indicates equal chela height in the right and left chelae. Number of individuals used for each experiment: Z4=10, M=10 and C1=11. B: Generation of chela asymmetry (apodeme). M (open triangles): apodeme height of megalopae. C1 (open squares): that of the first crab stage. The apodeme was not found in zoea. Number of individuals used for each experiment: M=10 and C1=10.
Figure 6. Difference of closing force between the right and left chela in crabs with and without autotomy. A: Closing force (N) in the right-handed (control) and left-handed crabs (autotomy). Filled circles: closing force of the control group; open square: that of the autotomy group. The dashed line indicates equal chela height in the right and left chelae. B: Comparison of the closing force (mean value) between right and left chela in the right-handed crabs (control) and left-handed crabs (autotomy). Right: right chela (dark bar). Left: left chela (open bar). Vertical bar indicates standard deviation.
Figure 7. Chela size asymmetry with growth, and alteration by autotomy. A: Growth of chela size (propodus height) in crabs without autotomy (control). R-cr (dark circles): chela on the right. L-cu (dark squares): chela size on the left (left cutter). Z, M,
and C show zoea, megalopa, and crab, respectively. Data were obtained from one individual. B: Alteration of chela size relation after autotomy at C1. Open squares with dashed line indicate growth of the left chela. Note that function of the left chela changes from cutter (L-cu) to crusher (L-cr) after autotomy of the right chela. Open circles with dotted line indicate growth of the right chela before (R-cr) and after (R-cu) autotomy. Data show the mean value of two individuals. C: Relation of chela size with or without autotomy. Dark circles (R-cr) and dark squares (L-cu) indicate growth of the right crusher and left cutter without autotomy (control group). Open circles (R-cu) and open squares (L-cr) indicate growth of the right cutter and left crusher after loss of the right chela (autotomy group). Measurement was done on the crabs (Tables 2 and 3) on day 390 (C12-C14). $a$: the slope of each regression line. $R^2$: coefficient of correlation. Data were obtained from measurement of 8 (control) and 6 (autotomy) individuals.
Figure 8. Dentition on the dactylus and propodus in the control group: frontal view.

Upper panel: dentition of the right (R) and left (L) chelae of a mature crab. A female at the crab stage C13 (i.e. 13th molt). The chela consists of dactylus (upper, movable finger) and propodus (lower, immovable finger). We have focused on the teeth at the bases of the dactylus and propodus (shown by the square). α with downward arrow shows the tooth on the dactylus, and β with upward arrow shows the tooth on the propodus. Middle panel: tooth shape on the right dactylus (α-R), and that of the left
dactylus (α-L). A sketch of each tooth is shown to the right or left of the photograph. The vertical line on α-L indicates a shallow groove. Bottom panel: tooth shape on the right propodus (β-R), and that of the left propodus (β-L). A sketch of each tooth is shown at the right or left of the photograph. There was a vertical groove on β-R (type I), and vertical and cross grooves on β-L (type II).
Figure 9. Morphological changes in chela dentition after loss of the right chela: frontal view. Upper panel: dentition of the regenerated right chela (R) and the original left chela (L). The right chela was autotomized at C1, and then dentition was inspected at crab stage C13. α: the tooth on the dactylus; β: the tooth of the propodus. Middle panel: the shape of the dactyle tooth in the regenerated right cutter (α-R) and the newly-converted left crusher (α-L). Bottom panel: the shape of the tooth on the propodus. β-R: regenerated right cutter; β-L: converted left crusher.
**Figure 10. Regeneration and growth of the tooth on the dactylus.** A: Growth of the tooth on the dactylus after regeneration at C3. α-L (open square): tooth on the left. α-R (open circle): tooth on the right. B: Relationship between tooth height and carapace width, with and without autotomy (C12-C14). Dark circles (R-cr) and dark squares (L-cu) indicate growth of the right crusher and left cutter, respectively (control group). Open circles (R-cu) and open squares (L-cr) indicate growth of the right cutter and left crusher after the loss of the right chela, respectively (autotomy group). Lines are regression lines. \( a \): the slope of each group. \( R^2 \): coefficient of correlation. Measurement was done on the crabs shown in Tables 2 and 3. Data were obtained from measurement of 8 (control) and 6 (autotomy) individuals.
Figure 11. Allometric relations between the apodeme height and the closing force.

A: Right-handed crab. Filled circles (R-cr) show the right chela (open circles indicate crabs in which the closing force exceeded maximum value of the apparatus), and crosses (L-cu) show left chela. B: Left-handed crab. Filled circles (L-cr) indicate the left
chela, and crosses (R-cu) indicate the right chela. C: The mean value with standard deviations in each chela. R-cr: right crusher; R-cu: right cutter; L-cr: left crusher; L-cu: left cutter. The closing force (N) of the right and left chelae was estimated in 22 right-handed and 12 left-handed crabs that were newly collected from the field.
Figure 12. Expression of the left crusher by immobilization of the right chela.

Upper panel: a male crab with major chelae on both sides (a frontal view). Arrows (cn) indicate the tooth (canine) on each dactylus. Middle panel: photograph and sketch of the tooth (canine) on each dactylus. α-R: a canine on the right dactylus; α-L: a canine on the newly-formed left crusher. A sketch of each tooth is shown at the right or left of the photograph. Bottom panel: contrast of tooth (molar) shape between the original right crusher (β-R) and newly-formed left crusher (β-L). Inspection at C12 stage.
Figure 13. Comparison of the success proportion of shell-cracking between the right and left chelae with growth of crabs. A: Original data. Filled circles: right-handed crabs. Open squares: left-handed crabs. Number of crabs used for measurement each day: 21-27 for the right-handed crabs, and 13-15 for the left-handed crabs. Each plot is the mean of these crabs. Measurement was done for the crabs shown in Table 2. B: Normalization of the original data for statistical treatment. Right-handed: \( \text{logit}(y) = 0.052x - 4.37 \). Left-handed: \( \text{logit}(y) = 0.052x - 5.13 \).
Figure 14. Development of chela asymmetry, and alteration of handedness before and after loss of the major chela (hypothesis). A: Ontogeny and enlargement of chela
asymmetry. A crab with no history of autotomy. R-cr and L-cu indicate the right crusher and the left cutter, respectively. Chela first appears as a symmetrical structure (s_1) at the 2nd stage of zoea. Asymmetry appears at the megalopa (s_2). Difference in tooth morphology (α and β in Figure 8) appears in the megalopa for the dactylus, and in C3 for the propodus, respectively. The growth curve is actually step-like due to molting, but is drawn as a straight curve here for clarity. B: Alteration of chela size after the loss of the major chela (R-cr): the case where the right chela is lost at stage C2, and the regenerated right chela (R-cu) is regenerated at stage C4. Autotomy is shown by ‘au’ with vertical dotted line. Chela regeneration is shown by ‘re’ with diagonal dotted line. The dashed curve indicates growth in the case where the right chela is not autotomized. C: Alteration of apodeme height after the loss of the right chela (R-cr): the same case as in Fig. 14B. Note that the closer apodeme height of the regenerated right chela (R-cu) significantly becomes smaller than that of the original left cutter (L-cu).
**Movie 1.** Shell-cracking behavior of the left-handed crab.