30 to 60 min on ice. The cells were washed twice with binding buffer then incubated with a 1:500 dilution of phycoerythrin (PE)-labeled goat antibody to human IgG, goat antibody to mouse IgG, or goat antibody to rabbit IgG (Calbiochem, San Diego, CA) for 30 min. Similar concentrations of isotype-matched control antibodies or normal rabbit serum were used to determine nonspecific staining. Analyses were done with a FACScan instrument and LYSIS II software (Becton Dickinson, Mountain View, CA).
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Effects of Manipulated Diet on Size and Performance of Brachyuran Crab Claws

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Crabs grown experimentally on fully shelled prey developed larger and stronger claws than those raised on nutritionally equivalent unshelled prey. When one claw was immobilized, claws also became asymmetrical. These use-induced changes differ from skeletal remodelling in vertebrates and many invertebrates because changes in the rigid exoskeleton can occur only after molting, and claw muscle mass must be reduced substantially before the molt. Such short-term adaptive responses to environmental stimuli, if heritable, could yield long-term evolutionary changes in claw size and, if combined with behavioral biases toward one side (handedness), could also promote the evolution of claw dimorphism.

The form of biological structures can be developmentally sensitive or insensitive to environmental conditions. Plasticity, however, may be either adaptive or nonadaptive depending on whether fitness is altered in individuals whose form has changed (*1*, *2*). In addition, plasticity may either accelerate the rate of evolution by generating phenotypic variation or retard it by reducing the concordance between genotypes and phenotypes (*3*–*5*). Phenotypic plasticity can thus be significant on both ecological and evolutionary time scales. Although numerous predators induce changes that make prey more resistant (*6*–*8*), relatively little is known about how sensitive predator form is to differences in prey toughness, because previous studies have had difficulty separating nutritional effects from use-induced effects in fishes (*9*–*11*) and insects (*12*). Adaptive plasticity in predator feeding structures may have contributed to the coevolutionary arms race between durophagous predators and shelled prey (*13*).

We tested the effect of diet toughness on both claw form and performance of the brachyuran crab, *Cancer productus*. Brachyuran crabs include many ecologically important predators that use their claws to subjugate shelled prey (*14*). Claw size and leverage properties determine crushing force (*15*, *16*), and induction or reversal of claw asymmetry after autotomy in certain heterochelous species suggests substantial developmental lability (*17*–*19*). But because of constraints imposed on muscle volume by a rigid exoskeleton and because of extensive muscle atrophy before molting, we don't know whether differential use can affect subsequent form in crustaceans.

Cancer productus is a common molluscivorous crab in estuaries and bays of the northeastern Pacific Ocean (*20*). Field-collected juveniles were held in running-seawater aquaria at the Bamfield Marine Station, Bamfield, British Columbia (*21*). Roughly equal numbers of male and female crabs were assigned randomly to one of four treatments that manipulated use of the claws (Table 1). Before the first molt in captivity, all crabs had full use of both claws and were fed an excess of small, intact mussels (*Mytilus*) well below the maximum size they could crush. After the first molt, by which time crabs were nearing maturity, all crabs were placed on their respective experimental diets. Crabs on a hard diet (H) received intact, closed mussels within 20% of the maximum size they could crush (*22*). Those on a soft diet (S) received similarly sized, opened mussels, where the flesh was easily accessible. For half of the individuals on each diet type, either a right or left claw was glued closed at random with cyanoacrylate glue within 48 hours after the first and all subsequent molts in captivity. By using only *Mytilus* as food, we avoided potentially confounding nutritional differences among treatments that have complicated interpretation of diet-induced effects elsewhere (*9*, *11*, *12*). Crabs were reared through one or two entire intermolt cycles on the experimental diets, and crushing forces were measured after the second molt (*23*). Claw measurements were obtained from preserved exoskeletons (*24*).

We examined the effects of diet toughness on relative claw size by computing the approximate volume of the manus (the portion of the claw excluding the fingers) (*24*) for each molt of each crab and adjusting for differences in overall crab size (*25*). For one analysis, we also computed a standardized claw size by adjusting for differences in relative claw size among individuals in the pretreatment molt. Differences in relative claw size among the six treatments (note that glued and free claws in the one-claw glued treatments were analyzed separately) were tested with

Table 1. Sample sizes, carapace widths, and intermolt durations of *C. productus* used in the experiments. (See legend to Fig. 1 for a description of "Origin of molt" categories and experimental group labels.) nr, not relevant.

	Origin of molt			
	Field	Pre-treatment	Expt. 1	Expt. 2
	*Number of crabs in each treatment**			
Hard diet, both claws free (H2)	12	12	8	8
Soft diet, both claws free (S2)	13	13	11	5
Hard diet, one claw glued (H1)	13	13	10	8
Soft diet, one claw glued (S1)	13	13	12	7
Total	51	51	41	28
	Carapace width (mm)†			
Mean	32.8	44.0	57.3	68.6
Standard error	0.80	1.10	1.65	2.18
	Intermolt duration (days)			
Mean	nr	31.7	52.0	75.9
Standard error		1.91	2.12	5.97

*For treatments H1 and S1, the number of claws per analysis was equal to the number of crabs, but for H2 and S2, it was twice the number of crabs. †Carapace widths did not differ between sexes nor among treatments at any of the four molts ($P > 0.2$, two-way ANOVA, Sex X Treatment for each molt).

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analysis of variance (ANOVA) (26).

Among-claw variance of unstandardized deviations declined significantly ($P = 0.031$) between the field and pretreatment molts (Fig. 1A); hence manus volumes became more similar after growth on a similar diet. By the end of the experiment, crabs clustered into two groups: One group had relatively larger claws (H1 free, H2 free, and S1 free) and the other, relatively smaller ones (S2 free, H1 glued, and S1 glued) (Fig. 1A).

The foregoing 10 to 13% differences in manus volume among free claws translated into functional differences (Fig. 2) (27). Soft-diet individuals with both claws free (S2 free) produced lower crushing forces than did any others with free claws. Crushing force differences reflected a strengthening on a hard diet, rather than a weakening on a soft diet, because individuals from this latter group were comparable in strength to crabs collected fresh from the field. Crushing forces of soft-diet crabs whose other claw was glued (S1 free) were also stronger than field-collected animals. Unfortunately these claws were larger at the start of the experiment despite random assignment to treatments, and because their relative size varied little over time (Fig. 1A), crushing forces remained relatively high.

Growth trajectories of claws, after an adjustment for differences in claw size among individuals at the pretreatment molt (Fig. 1B), revealed two effects of diet toughness: (i) for free claws, manus volumes of hard-diet crabs were greater than those of soft-diet crabs by the end of the experiment (Table 2, H2 free vs. S2 free) and (ii) immobilization of one claw resulted in asymmetry of claws, regardless of diet toughness (Table 2, H1 free vs. H1 glued and S1 free vs. S1 glued). Free claws either increased in relative size (H1 free) or remained constant (S1 free), whereas glued claws decreased in relative size on both diets (Fig. 2B).

The rate of induced response to experimental diets differed depending on whether one or both claws were free (Fig. 1B). In hard-diet crabs with two free claws (H2 free), change was not evident until the second experimental molt. Such a delayed response might be expected if muscle expansion occurred in smaller increments because of equal claw use. In contrast, when one claw was glued in hard-diet crabs, the contralateral claw (H1 free) increased in size in the first experimental molt. Crabs with two free claws experienced little exercise on a soft diet (S2 free); consequently, relative manus volume declined steadily. No such decrease, however, was observed on the soft diet when one claw was free (S1 free). Once again, the free claw appeared to compensate for its constrained counterpart, but because the diet was soft, the effect was less pronounced.

Because differential use affected both the size and performance of claws, the plasticity appears to be adaptive. Plasticity in feeding structures should be favored when (i) the proportion of morphologically resistant prey in the diet varies over space or time, (ii) the phenotypic response correlates directly with feeding success, and (iii) fitness costs of plasticity are low (1). Given the extensive dispersal of many crustacean species [*Cancer magister* larval periods last for 80 to 160 days

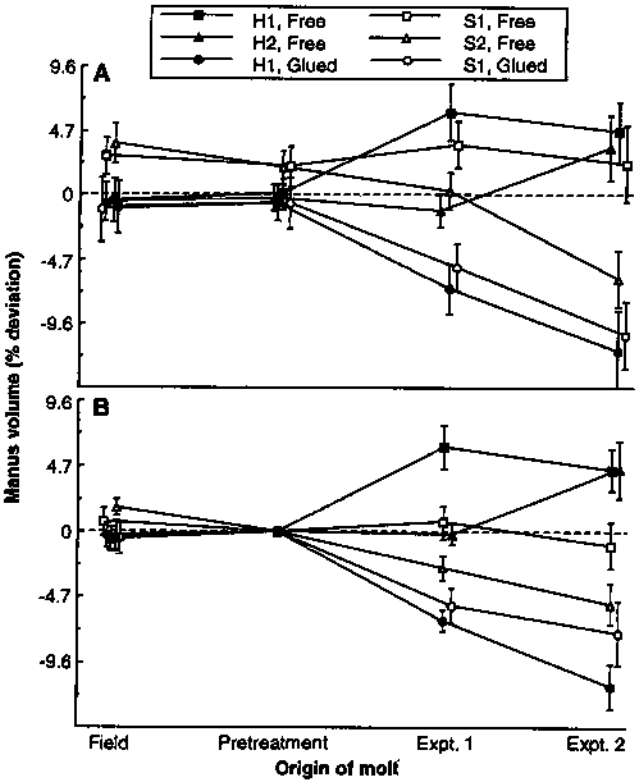

Fig. 1. Effect of diet and claw treatment on the relative manus volume of *Cancer productus* grown in the laboratory. (**A**) Data expressed as unstandardized deviations from regression. (**B**) Data expressed as deviations from regression after standardizing for statistically significant among-individual differences in initial claw size in the pretreatment molt ($P < 0.001$, ANOVA). All points are approximate manus volume (*24*) expressed as a percent deviation from that expected at a given carapace width (± standard error) (*25*). H2, hard diet, both claws free; S2, soft diet, both claws free; H1, hard diet, one claw glued closed and the other free; S1, soft diet, one claw glued closed and the other free; Free, unconstrained claw; Glued, claw glued permanently in the closed position; Field, exoskeleton at time of collection; Pretreatment, first exoskeleton produced in the laboratory after variable amounts of time on identical diets; Expt. 1 and Expt. 2, first and second exoskeletons produced after complete intermolt periods under experimental conditions. See Table 1 for sample sizes, carapace widths, and intermolt durations.

Table 2. A priori comparisons (orthogonal linear contrasts) (*39*) of the effects of differential use on relative claw size of *C. productus*. For all contrasts df = 1. MS, mean squares; *F*, f value; *P*, exact probability. Error mean squares were 0.000284 and 0.000639 for molt 1 and molt 2, respectively. Figure 1 legend explains the experimental group labels. Analyses were conducted on the log deviations shown in Fig. 1B.

Contrast	Experimental molt 1			Experimental molt 2		
	MS	*F*	*P*	MS	*F*	*P*
H2 free vs. S2 free	0.00103	3.63	0.061	0.01043	16.32	<0.001
H1 free vs. H1 glued	0.01398	49.20	<0.001	0.01752	27.42	<0.001
S1 free vs. S1 glued	0.00394	13.86	<0.001	0.00254	3.97	0.052
H1 free vs. H2 free	0.00425	14.95	<0.001	<0.00001	<0.01	0.99
S1 free vs. S2 free	0.00153	5.40	0.023	0.00135	2.11	0.15

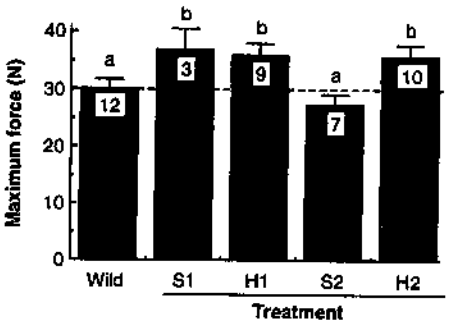

Fig. 2. Mean maximum crushing force (Newtons ± standard error) generated by the free claws of wild and laboratory-reared *Cancer productus* (*27*). Laboratory-reared crabs experienced at least two entire intermolt periods under different experimental conditions (see Fig. 1 legend for treatment abbreviations). Letters identify groups of means that differed significantly. Numbers inside each bar indicate sample sizes. The dashed line highlights the mean force generated by wild crabs.

(28)] and the variation in prey toughness or composition among habitats (29, 30), juveniles should encounter a variety of prey types. Modulation of relative claw size through use would thus seem adaptive, though we can say little about the fitness costs of plasticity. Compensation by one claw if the other were injured would also be beneficial, because dactyl breakage occurs commonly in some species [up to 22% in *C. magister* males (31)]. Foraging costs associated with dactyl breakage (31) or with the autotomy of one cheliped (32) might thus be ameliorated.

The plasticity observed in *C. productus* claws differs from the environmental induction of claw asymmetries in conspicuously heterochelous crustaceans. In lobsters, for example, differential use triggers differentiation of the crusher claw at a critical stage in ontogeny (18), but because pronounced asymmetry is the norm, the environmental cue simply triggers differentiation of one side or the other. In contrast, our results reveal that claw morphology in *C. productus* responds to differential use throughout growth.

Skeletal remodelling in crustaceans differs from that in organisms with accretionary growth. In vertebrates, bone is living tissue whose form or strength can change with amount of use (33). Mollusk shells can also be modified continuously by deposition or resorption (34). In crustaceans, however, changes in the rigid, nonliving exoskeleton occur only during brief postmolt periods while the new cuticle is still malleable. Exoskeletal morphology reflects properties of the underlying muscle tissue, and sarcomere lengths of claw muscle fibers appear to respond to exercise during the intermolt (35). Although these fibers undergo substantial atrophy preceding ecdysis, their number remains constant (36). Thus, claw inflation after ecdysis may be modulated either by properties of the muscle tissue itself or by the central nervous system in a manner similar to its regulation of claw asymmetry (37).

Our results also suggest a mechanism whereby asymmetrical claws might begin to evolve from symmetrical progenitors. Because many individuals of bilaterally symmetrical species develop a behavioral preference toward one side or the other (handedness) [see references in (38)], use-induced effects could easily translate be- havioral biases into morphological asymmetries. To the degree that such inducibility is heritable, asymmetries could then be amplified by natural selection (3, 4).

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21. Fifty-one *C. productus* juveniles (28 males and 23 females; 21- to 41-mm carapace width) were collected from the lower shore of Dixon Island (48°51'N, 125°07'W) in Barkley Sound, British Columbia, Canada, between 2 and 4 July 1992. Each crab was held in constantly flowing seawater (~32 parts per thousand salinity, 8° to 12°C) in a separate plastic freezer container (12 cm length by 10 cm width by 13 cm height) with Nitex mesh sides (602 μm). All crabs were fed five mussels per day. Exoskeletons were stored in 70% EtOH and glycerine.
22. Preliminary tests indicated that the maximum vulnerable size of *Mytilus californianus* increased with *C. productus* size: log(maximum shell length) = 0.745 log(carapace width) + 1.024, $r^2 = 0.89$, $n = 12$. Mussels were collected from well within a single, established *M. californianus* bed, but because juveniles are so difficult to identify, some *M. trossulus* (previously *M. edulis*) may have been included.
23. We measured crushing force by encouraging crabs to grasp a force gauge near the midpoint of the dactyl and squeeze for ~10 s. The fixed finger was inserted into a lower, stationary steel ring and the dactyl fit into a movable upper ring attached to a 2-mm-thick flexible steel beam by means of a set screw. The distance between the inner margins of the rings was adjusted to about 60% of the maximum gape at the midpoint of a dactyl. Forces were transduced by two strain gauges (Bean BAE-13-250BB-350TE; 350 ohm) affixed to opposite sides of the beam. The signal was amplified by a Wheatstone bridge and recorded on a Hewlett-Packard 8045A X-Y chart recorder. Crushing forces were calculated after calibration with known weights. Each crab was tested once per day for three consecutive days, and tests were repeated several times during the intermolt period. To minimize extraneous variation due to differences in crab motivational state, we included only the maximum closing force produced by an individual claw in the analysis. In March and April 1993, similar-sized *C. productus* from the field were also tested.
24. An outline of each claw was drawn approximately perpendicular to the plane of movement of the dactyl with a camera lucida fitted to a Wild M5A dissecting scope with a ×0.3 reducing lens [average propus length of the drawings was 145 ± 32.4 mm (SD), $n = 342$]. The *x,y* coordinates of claw landmarks were digitized on a Summagraphics drawing tablet (20 dots/mm resolution). Calibrated distances were computed between landmarks (mm): Manus length = from the midpoint of the line between the upper and lower condyle sockets at the proximal end of the manus to the intersection of the ventral margin of the dactyl with the manus when the dactyl was between 50 and 75% open (equivalent to the point of insertion of closer apodeme on dactyl); manus height = distance between the upper and lower margins at the midpoint of the manus; and manus depth = dimension of the manus perpendicular to the drawing at the midpoint of the manus (measured to 0.01 mm with digital calipers). Approximate manus volume was computed as length × height × depth. To estimate measurement error, we drew and measured twenty claws twice, and each drawing was digitized twice. Coefficients of variation due to error were 0.87%, 0.98%, 0.53%, and 1.38%, for length, height, depth, and volume, respectively.
25. We used carapace width (CW) to adjust for differences in overall size because it is a widely used measure of crab size and it varied isometrically relative to two other measures of overall size (carapace length and blotted wet weight). Least squares linear regressions were used to determine expected manus volume for each sex separately because males exhibited greater positive allometry than females [$P < 0.001$, analysis of covariance (ANCOVA)]. Males: log(manus volume, mm^3) = 3.134 log(CW, mm) − 2.373; $N_{claws} = 182$, $r^2 = 0.995$, slope SE = 0.01700, $P < 0.001$, and range of CW was 21.3 to 90.9 mm. Females: log(manus volume, mm^3) = 3.026 log(CW, mm) − 2.198; $N_{claws} = 160$, $r^2 = 0.996$, slope SE = 0.01607, $P < 0.001$, and range of CW was 25.4 to 90.1 mm. Each claw of each molt was expressed as a deviation from the appropriate regression, and all subsequent analyses were conducted on these log-deviations. This approach homogenized the variances among molts and allowed us to analyze the entire range of crab sizes simultaneously and thus describe the size-dependence of manus volume more accurately than if we had used a separate ANCOVA for each molt. For crabs in the H2 and S2 treatments, each claw was considered an independent datum.
26. Descriptive statistics and ANOVA were conducted with Statview II (version 1.03, Abacus Concepts). Regression analyses, ANCOVA, and contrast analyses were conducted with SuperANOVA (version 1.1, Abacus Concepts).
27. Because maximum crushing force varied with overall crab size, least square means were computed for each group from ANCOVA with CW as a covariate [log(maximum crushing force, N) = 1.7603 log(CW, mm) − 1.7369; $N_{claws} = 40$, $r^2 = 0.68$, slope SE = 0.1958, $P < 0.001$, and range of CW was 45 to 91 mm]. The crushing force for the smallest field-collected crab (CW = 44.1 mm, male) was found to be a statistically significant outlier (3.72 SD from the observed mean) and was excluded from the analysis.

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