Claw Asymmetry in Lobsters: Case Study in Developmental Neuroethology

C. K. Govind

Life Sciences Division, Scarborough Campus, University of Toronto, Scarborough, Ontario, Canada M1C 1A4

SUMMARY

An enduring debate in the study of development is the relative contribution of genetic and epigenetic factors in the genesis of an organism, that is, the nature vs. nurture debate. The behavior of the paired claws in the lobster offers promising material for pursuing this debate because of the way they develop. The paired claws and their closer muscles are initially symmetrical; both are slender in appearance and have a mixture of fast and slow fibers in their closer muscles. During a critical period of development, they become determined into a major (crusher) and minor (cutter) claw and during subsequent development acquire their final form and behavior: The crusher becomes a stout, molar-toothed claw capable of closing only slowly because its closer muscle has 100% slow fibers while the cutter becomes a slender, incisor-toothed claw capable of closing rapidly because its closer muscle has 90% fast fibers. Our initial hypothesis was that the more active claw became the crusher and its less active counterpart the cutter. Presumably, nerve activity would influence muscle transformation, which in turn would influence the exoskeleton to which they attach and hence claw morphology. Curtailing nerve activity to the claw prevented crusher development, while reflex activation of a claw promoted its development; both results support the notion that nerve activity directly regulates claw form and function. This is not, however, the case, for when both claws were reflexly exercised neither formed a crusher, signifying rather that bilateral differences in predominantly mechanoreceptive input to the paired claws somehow lateralized the claw ganglion [central nervous system (CNS)] into a crusher and cutter side. The side experiencing the greater activity becomes the crusher side while the contralateral side becomes the cutter and is also inhibited from ever becoming a crusher. This initial lateralization in the CNS is expressed, via as yet unknown pathways, at the periphery in claw morphology, muscle composition, and behavior. The critical period defines a time when the CNS is susceptible to being lateralized into a crusher and cutter side. Such lateralization is dependent upon experience of the environment in the form of mechanoreceptive input. In the absence of such experience, the CNS is not lateralized and paired cutter claws develop. Thus, while the critical period for crusher determination is genetically determined the actual trigger is influenced by experience.

Keywords: **claw dimorphism, critical period, bilateral asymmetry, lobster, muscle composition, CNS lateralization.**

INTRODUCTION

In recent years, there has been an explosive growth in the neurosciences, reflecting our desire to understand and control what has been termed the last frontier, viz., the brain. This venture encompasses studies ranging from that of individual molecules to full-blown behavior in a number of broad fronts, one of which is developmental neuroethology. Understanding the ontogeny of behavior, especially its neural substrates, will provide a useful backdrop to understanding its expression in adults. There are many examples that testify to the usefulness of this approach; brief mention of a few of the better-known examples will suffice. Imprinting in birds is a case in point (Lorenz, 1970). Newly hatched gos-

Received August 12, 1992; accepted August 12, 1992
Journal of Neurobiology, Vol. 23, No. 10, pp. 1423–1445 (1992)

CCC 0022-3034/92/101423-23

lings will imprint on the first moving object they see as their parent. Under normal circumstances, this object is usually a goose but under experimental conditions it could be a human or a dog. The role of early experience in shaping behavior is also highlighted in experiments where newly born monkeys were reared in isolation and with real or surrogate mothers (Harlow and Harlow, 1973). The effects of such rearing is profound, resulting in disturbances in both social and sexual behavior. Singing in birds is another behavior strongly influenced by early experience; young chaffinches, for instance, although possessing the basic rudiments must learn the complete song from conspecifics during a critical period in development (see Arnold, this issue). A final example to be considered is the effects of suturing shut one eye in kittens, resulting in the permanent inactivation of cortical neurons and the failure of ocular dominance columns to segregate normally (Hubel, 1982; Wiesel, 1982).

All these examples are characterized by the fact that there is a clearly defined period during development, the critical period, when the animal is most susceptible to its experience. The critical period is an innately programmed event in development while the animal's interaction with its environment, that is, its experience, is not preprogrammed. In this respect, the critical period would represent the nature part, while experience would represent the nurture part, in the age-old nature vs. nurture debate. The development of some behaviors therefore depends upon propitious timing between experience and the critical period. In the ensuing pages, we explore this theme with respect to the development of the paired claws in the lobster.

CLAW ASYMMETRY IN CRUSTACEANS

The body plan of higher animals from annelids to primates is that of bilateral symmetry in which the right half is a mirror image of the left half. Within this basic plan are occasional asymmetries manifested most dramatically by handedness in humans (Corballis and Morgan, 1978), neural mechanisms for vocalization in songbirds (Nottebohm, 1977), and claw asymmetry in crustaceans (Przibram, 1901). In decapod crustaceans such as crayfish, crabs, shrimps (Fig. 1), and lobsters (Fig. 2), the first pair of thoracic limbs are directed anteriorly and elaborated into claws that are much larger than the remaining four pairs of thoracic limbs. These chelipeds are specialized for grasping, having a well-developed thumb or dactyl that can effectively close against the palm or propus. Because of their large size and closing action, the chelipeds participate in aggression, defense, food capture, and sexual display. The four remaining pairs of thoracic limbs are considerably smaller than the claws and are used primarily in locomotion.

The differences between claws and the remaining thoracic limbs are characteristic features of crustaceans, suggesting that these structures develop according to a preprogrammed set of instructions or a genetic blueprint. There are, however, differences between the paired claws that, in some species at least, do not appear to be as rigidly preprogrammed. The differences between the paired claws are denoted by the generic terms major and minor, the former being much larger and often morphologically and functionally distinct from the latter.

The apparent lack of a fixed program in the development of asymmetry in the paired claws may be gleaned from the following observations: First, in some species such as fiddler crabs, lobsters, and snapping shrimps, claw placement is random—the major and minor claws appear with equal probability on the right or left sides of the body (Herrick, 1895). Second, in the species mentioned above there are instances where the paired claws are symmetric, both being of either the major or minor type (Herrick, 1911; Darby, 1934; Yamaguchi, 1977). Third, claw placement, while fixed in adult fiddler crabs and lobsters, may be altered in snapping shrimps (Przibram, 1901). Removal of the major claw in these shrimps results in transformation of the existing minor claw into a major while a new minor claw regenerates at the site of the lost major claw. Collectively, these observations suggest that the development of a major and minor claw and of bilateral asymmetry may be susceptible to epigenetic influences. Thus, studying their development may shed some light on the interaction between genes and the environment, that is, between nature and nurture, in shaping behavior.

CLAW ASYMMETRY IN LOBSTERS

The animal in which we studied the development of paired, homologous but asymmetric claws is the American lobster, *Homarus americanus*. As any gourmet knows, the adult lobster has two different claws; a major and a minor (Fig. 2). The major claw is stout and heavy with molar-like teeth along its biting surfaces while the minor claw is more

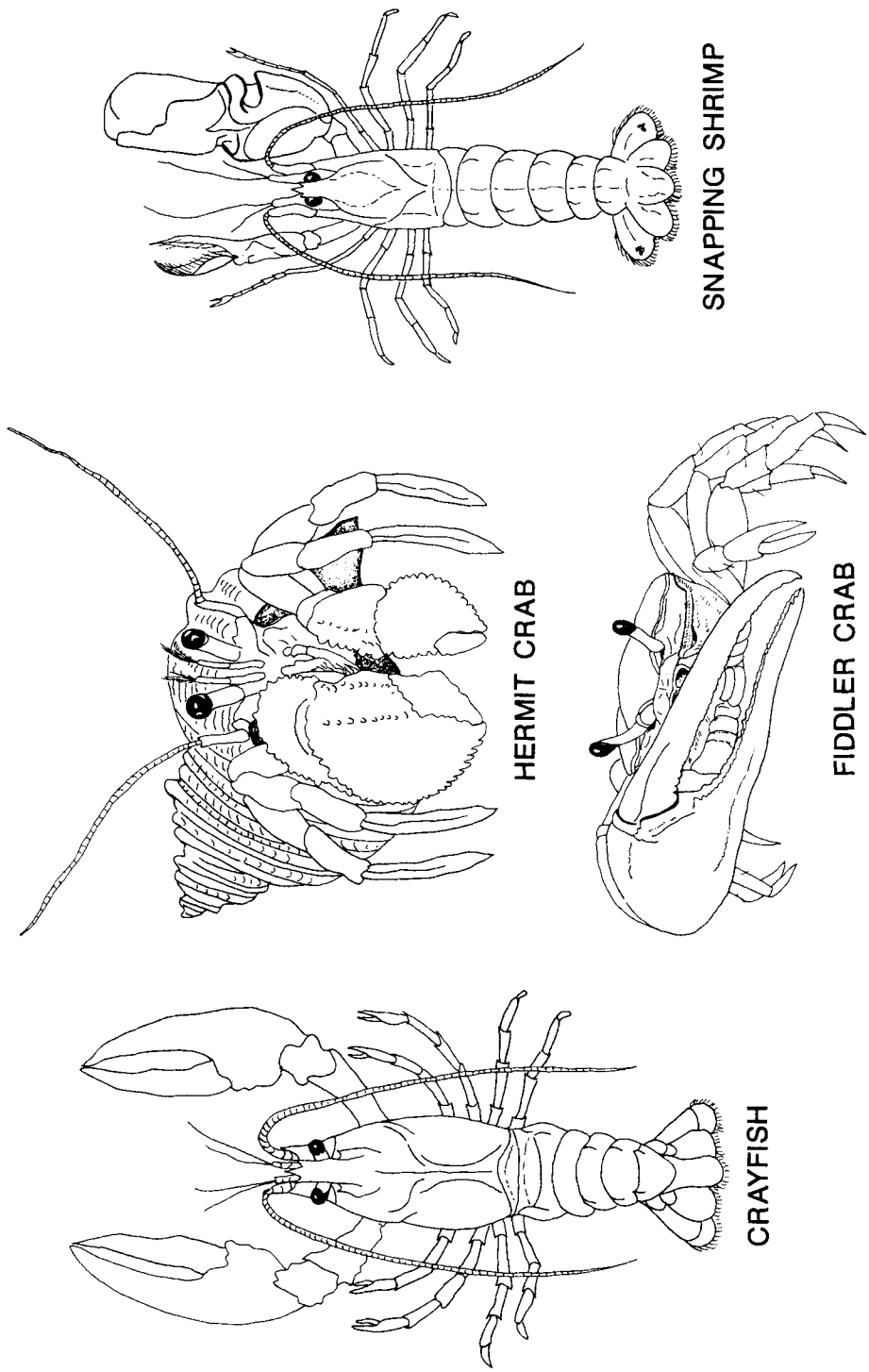

Figure 1 Representative crustaceans showing the first pair of thoracic limbs as enlarged chelipeds or claws compared to the remaining pairs of much smaller thoracic limbs that are bilaterally symmetrical in keeping with the rest of the body. The paired claws, however, may be bilaterally symmetrical as in the crayfish or asymmetrical as in fiddler crabs, hermit crabs, and snapping shrimps, where they consist of a major and a minor claw. Line drawings by Joanne Pearce.

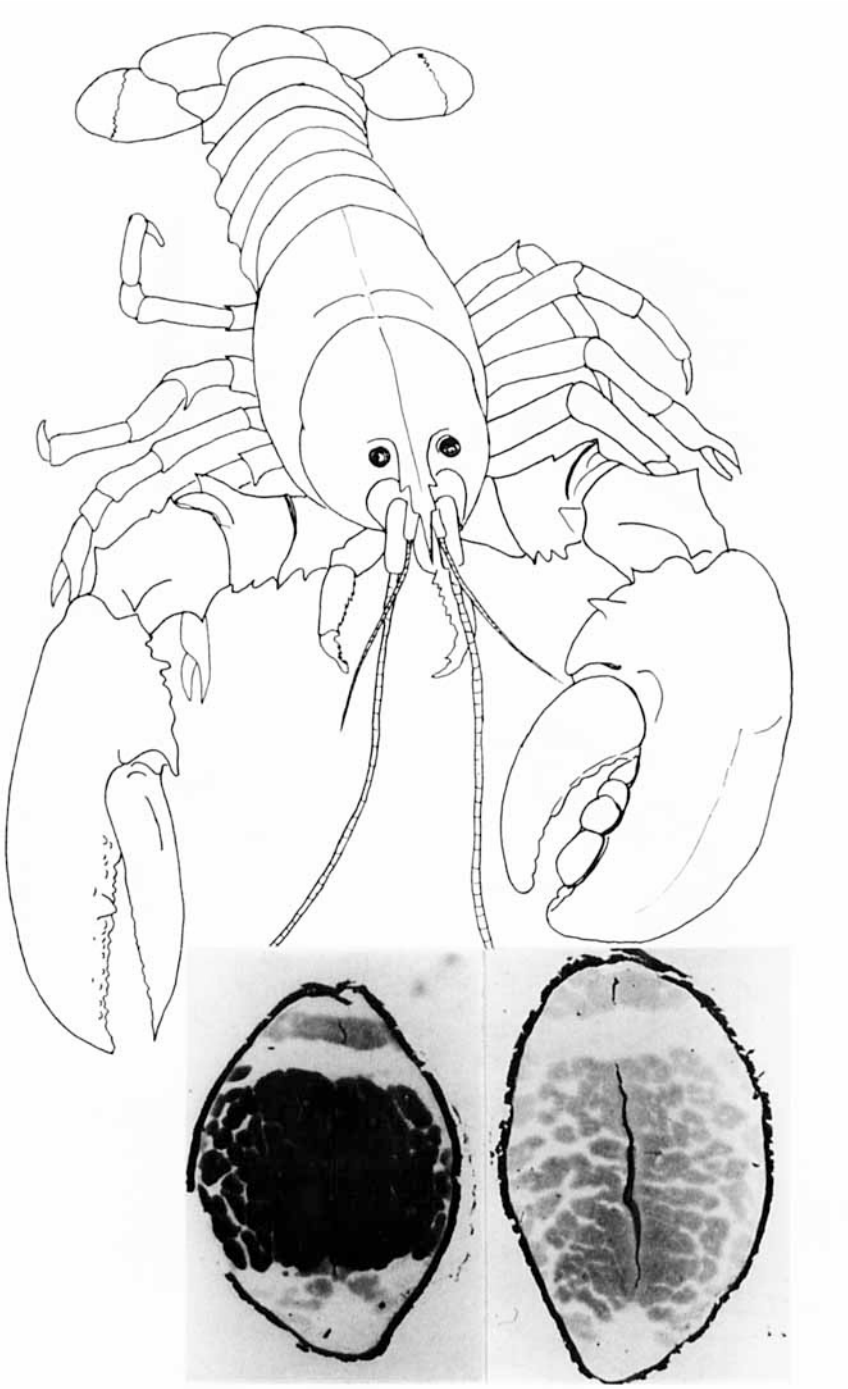

Figure 2 Adult lobster, *Homarus americanus,* with paired asymmetrical claw consisting of a stout, molar-toothed major or crusher claw and a slender, incisor-toothed minor or cutter claw. Below each claw is a representative cross-section through the propus showing the small dorsally situated opener muscle and the massive closer muscle, which occupies most of the cross-sectional area. These are frozen sections histochemically treated for myofibrillar adenosine triphosphatase (ATPase) activity, which stains intensely for fast fibers and less so for slow. In both claws, faint staining of the opener muscle confirms its slow fiber composition while the pattern for the closer muscle differs between the paired claws; in the crusher, the muscle is composed entirely of faintly staining, slow fibers while in the cutter most of the muscle is composed of intensely staining, fast fibers except for a small ventral band of faintly staining slow fibers. Line drawing by Joanne Pearce.

slender and lighter with incisor-like teeth. What the gourmet may not know is that these dimorphic claws also behave differently (Scrivener, 1971; Govind and Lang, 1974). The major claw always closes its dactyl very slowly but with tremendous force, enough to crack open the shells of oysters, mussels, and other bivalves, hence its designation as a crusher claw. The minor claw may also close slowly but also rapidly, within 20 ms, fast enough to catch fish, hence its designation as a cutter, nipper, or seizer claw. The rapid closing action of the cutter claw may only be elicited two or three times in succession before fatigue sets in (Costello, Hill, and Lang, 1984) and judging from motor firing patterns such rapid closure is infrequently used (Lnenicka, Blundon, and Govind, 1988). In contrast, slow closure of the crusher claw may be elicited many times in succession without any sign of fatigue and its firing patterns suggest more frequent use of this claw. Clearly, the paired claws of the lobster differ in their behavior.

The underlying basis for the different speeds of closing of the paired claws lies principally in the fiber composition of the closer muscle. This is a large muscle that occupies most of the propus segment and its contraction closes the claw (Fig. 2). The only other muscle in this segment is a relatively small opener muscle, contraction of which opens the claw. The opener muscle is composed of slow fibers in both claws (Govind, Stephens, and Trinkaus-Randall, 1981). The closer muscle differs dramatically in fiber composition between the paired claws (Jahromi and Atwood, 1971; Lang, Costello, and Govind, 1977a; Ogonowski, Lang, and Govind, 1980). In the cutter claw, the closer muscle is composed predominantly (90%) of fast fibers with a small ventral band of slow fibers, while in the crusher claw it is composed entirely of slow fibers (Fig. 2).

Motor innervation to the claw muscles is relatively simple (Wiersma, 1961; Wiens, 1985). The opener muscle receives a single excitor motoneuron and two inhibitor neurons, a specific and a common inhibitor, while the closer muscle receives the common inhibitor and two excitor motoneurons. The small number of motoneurons to these muscles makes it possible to characterize their individual firing patterns. Thus, for example, one of the excitor neurons to the closer muscle, the fast closer excitor, fires longer bursts of spikes that have a lower impulse frequency in the crusher claw than in its cutter counterpart (Lnenicka et al., 1988). The asymmetry in the firing patterns of the fast closer excitor axon is complemented by an asymmetry in their neuromuscular synapses: Facilitation at these synapses is greater in the cutter than the crusher claw. Consequently, the asymmetry seen in the fiber composition of the paired closer muscles and in the firing patterns and synaptic facilitation of homologous motoneurons constitute the underlying substrate for the asymmetry in claw behavior. Such bilateral asymmetry in an animal in which the right side of the body is otherwise a mirror image of the left side becomes an intriguing puzzle in development.

Development of Asymmetry

Soon after an adult female has molted, in which the entire exoskeleton is shed, it copulates with a male to receive sperm (Herrick, 1895, 1911). The fertilized eggs are subsequently extruded to the outside and carried glued to the swimmerets on the underside of the abdomen, where they undergo embryonic development. The eggs hatch into larvae, which are actively dispersed into the plankton by a fanning motion of the tail. There are three larval stages—first, second, and third—in which the paired claws are small, undifferentiated, and bilaterally symmetrical (Fig. 3). Juvenile development begins with the molt to the fourth stage, a metamorphic stage, with the paired claws being considerably larger than the other thoracic limbs and extended in front of the animal in the adult posture. The claws appear as long, slender, prehensile structures, with numerous sensory bristles and fine teeth, including a prominent central incisor-like tooth. The paired claws are symmetrical in appearance in the fourth and fifth stages but from the sixth stage onward gradually begin to differentiate into cutter and crusher types (Herrick, 1911). One of the paired claws, the putative crusher, becomes stouter and its central tooth more rounded and molar-like, while the other claw, the putative cutter, remains long and narrow and retains the incisor-like central tooth. Differentiation into a crusher and cutter claw continues throughout juvenile development, which extends over several years and includes 20–25 molts, resulting in adult lobsters with markedly asymmetrical claws.

The development of the closer muscle into cutter and crusher types appears to go hand in hand with changes in claw morphology (Govind, 1984). In the larval stages, the paired muscles are symmetrical, each characterized by a central band of fast fibers sandwiched dorsally and ventrally by slow fibers (Lang, Govind, and She, 1977b) (Fig. 4). This pattern is retained in the fourth and fifth

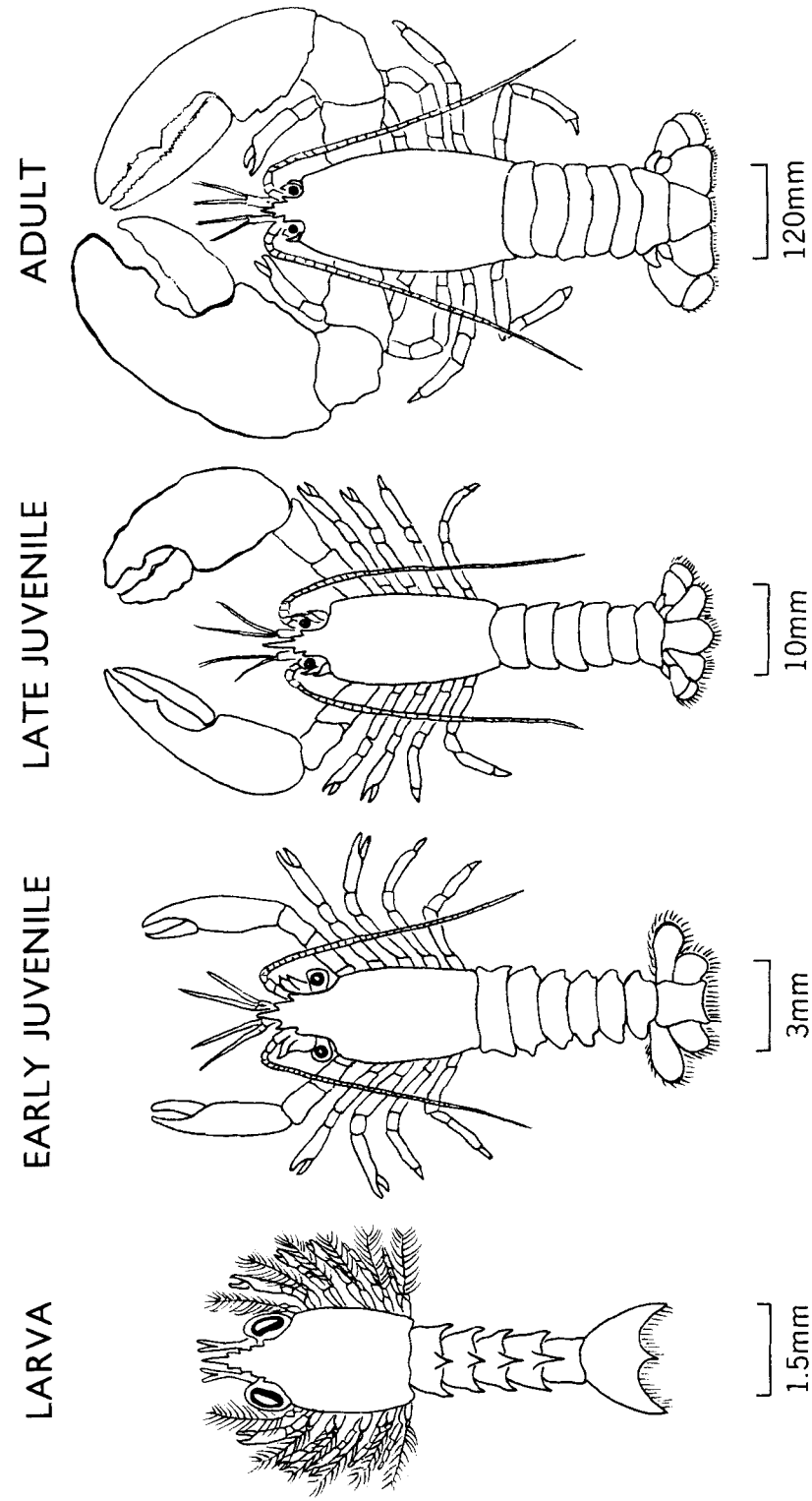

Figure 3 Selected stages in the development of the lobster showing paired claws in a bilaterally symmetrical condition in larval (first stage) and early juvenile (fourth stage) lobsters and transformed to an asymmetrical condition of a cutter and crusher claw in late juvenile and adult lobsters.

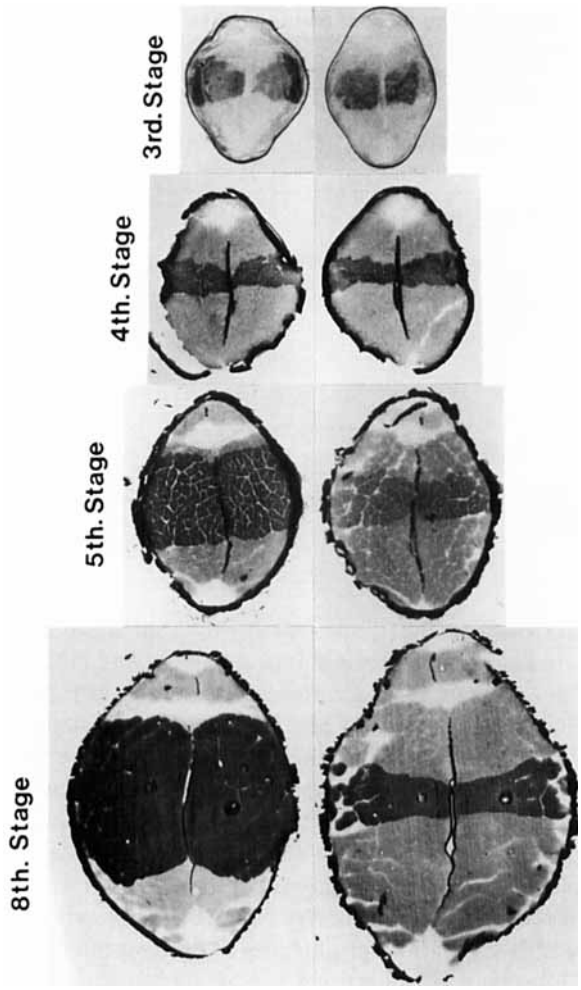

Figure 4 Development of the paired homologous closer muscles in lobster claws. The paired muscles are symmetrical in the third (larval) and fourth (juvenile) stages, each comprised of a small central band of fast (dark staining) fibers sandwiched by slow (light staining) fibers. In subsequent juvenile stages, represented here by the fifth and eighth, the closer muscle in the putative cutter claw (left side) gradually transforms its slow fibers to fast over most of the muscle except for a small ventral band while in the putative crusher the central band of fast fibers is transformed to slow. (Reprinted with permission from Ogonowski et al., *J. Exp. Zool.* **213**:359–367, © 1980.)

stages but begins to diverge thereafter (Govind and Lang, 1978). In the putative cutter muscle, the fast fiber population increases, presumably by transformation of the existing slow fibers to fast, until most of the muscle (90%) is fast, except for a narrow ventral band of slow fibers. In the putative crusher muscle, the slow fiber population gradually increases to 100% by transforming the central band of fast fibers into slow. While differentiation of the cutter muscle is complete by the 8th or 9th stage, that of the crusher muscle takes somewhat longer, extending to the 13th stage or later.

The pattern of innervation of the closer muscle by its fast and slow excitor motorneurons differs between the early juvenile lobsters (fourth, fifth, and sixth stages), where the paired claws are undifferentiated, and adult lobsters, where differentiation into cutter and crusher types is evident (Costello, Hill, and Lang, 1981). In the early juvenile muscle, the majority of fibers are innervated by both axons while a minority are innervated exclusively by each axon (Fig. 5). This pattern is essentially retained in the adult crusher muscle but not in the cutter muscle, where most fibers are supplied exclusively by the fast axon while the remaining fibers receive, in equal proportion, either the slow axon or both fast and slow axons. Therefore, the early juvenile pattern of innervation can, with some fine-tuning, give rise to the crusher pattern but must undergo dramatic changes to produce the cutter pattern either via proliferation of fast axon

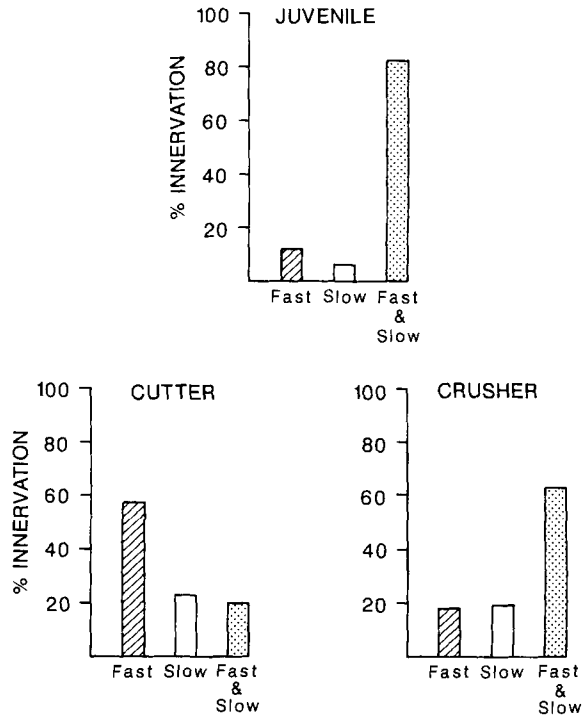

Figure 5 Innervation of the claw closer muscle by both fast and slow excitor motoneurons in early juvenile (fourth, fifth, and sixth stages) lobsters, where the claw is undifferentiated, and in adult lobsters, where the claw is differentiated into a cutter or crusher type. Data taken from Govind and Lang (1974) and Costello et al. (1981).

Table 1 Configuration of the Paired Claws in Juvenile Ninth-Stage Lobsters Reared with and without a Substrate During Selected Fourth, Fifth, and Sixth Juvenile Stages of Development

Substrate	Configuration of Paired Claws: Asymmetrical (crusher/cutter)	Configuration of Paired Claws: Symmetrical (cutter/cutter)	*n*	*p*
Substrate in all stages	21	1	22	Control
Series 1				
Substrate in 4th stage	2	20	22	<0.001
Substrate in 5th stage	20	0	20	ns
Substrate in 6th stage	1	21	22	0.001
Series 2				
No substrate in 4th stage	15	3	18	ns
No substrate in 5th stage	10	11	21	<0.001
No substrate in 6th stage	19	0	19	ns

Rearing with a substrate in all stages served as a control against which all the other conditions were compared statistically using the contingency χ^2 test; ns = not significant (from Govind and Pearce, 1989a).

synapses and or selective elimination of slow axon synapses.

In addition to changes in the excitatory innervation pattern during differentiation of the paired muscles, changes in facilitation of neuromuscular synapses of the fast axon have also been recorded (Lnenicka et al., 1988). For a select group of muscle fibers, synaptic facilitation was similar between the paired claws of fourth-stage lobsters in which claw laterality is undetermined. In subsequent juvenile stages, viz., fifth, sixth, and seventh, synaptic facilitation in the putative crusher remained at the fourth-stage level while in the putative cutter facilitation increased significantly. Consequently, differentiation of this physiological property of fast axon synapses follows a format similar to that of innervation patterns in that the undifferentiated fourth-stage pattern is retained in the crusher but altered in the cutter.

Critical Period for Determining Asymmetry

In a few simple but clever experiments, Victor Emmel (1908) found the fourth and fifth stages to be the period during development when claw type and hence asymmetry is determined. Removal of a claw in the fourth or fifth stage induced the intact claw to become a crusher. Removal in earlier or later stages did not affect the outcome, hence delimiting the critical period to the fourth and fifth stages. Each of these stages is approximately 2 weeks long and hence the critical period extends for about 1 month. To determine if this period could be narrowed still further, we reared lobsters with or without a substrate of oyster chips, at selected periods of juvenile development (Govind and Pearce, 1989a) (Table 1). The reason for using this strategy is that when reared with a substrate the paired claws differentiate into a crusher and cutter type but when reared without a substrate both develop as cutter types (Lang, Govind, and Costello, 1978). Presence of a substrate in the fifth stage alone resulted in bilateral asymmetry of the claws, whereas substrate in the fourth or sixth stage alone was not effective (Table 1, series 1). Conversely, lack of a substrate in either the fourth or sixth stage did not prevent asymmetry whereas its absence in the fifth stage alone produced a significant number of lobsters without a crusher claw (Table 1, series 2). The fifth stage therefore appears to be the most critical period for determining claw type. Moreover, during the fifth stage at least 5 days of exposure to the substrate is necessary for the development of asymmetric claws; when given substrate for less than 5 days, a crusher did not develop (Govind and Pearce, 1989a).

The data in Table 1 also shows that a few animals did not adhere strictly to the critical period being limited to the fifth stage. The determination in these animals occurred either at an earlier or later time, that is, in the fourth or sixth stage, demonstrating some amount of individual variability. The majority of juvenile lobsters, however, displayed a critical determinative period that was limited to the fifth stage. In this respect, the critical period appears to be a preprogrammed or genetically fixed event. During this period, the animal is sensitive to factors that will determine whether a crusher will develop and on which side of the animal. Providing these determinative factors are present during the critical period, a crusher claw will develop, but in their absence both claws will develop as cutters. The next step was therefore to

identify these hypothetical factors that determine the differentiation of a crusher claw and of bilateral asymmetry.

Before going on to discuss the determinative influences, it is interesting to note that the critical period is also the time when the lobster changes its habitat. The larval stages are all pelagic and live in the plankton, staying afloat by accessory swimming appendages (Neil, Macmillan, and Laverack, 1976). These accessory appendages degenerate at the molt to the fourth stage (Govind, Kirk, and Pearce, 1988a). Meanwhile, the abdominal swimmerets have developed and allow the fourth-stage juvenile to retain its pelagic habitat. During this stage, however, lobsters begin to scout the ocean floor preparatory to adopting a bottom-living or benthic habitat that is assumed in the fifth stage (Botero and Atema, 1982). The critical period for determining claw asymmetry is propitiously linked to a time when the lobster begins to be in contact with the substrate and therefore has ample opportunity to use its claws.

The relationship between the substrate and use of claws in determining whether a crusher claw develops therefore represents an epigenetic event. In this case, it is the interaction between the environment and the animal, specifically in this case between the environment and the claws (experience), that could influence the determination of asymmetry.

Role of Experience in Determining Asymmetry

The notion that activity or use of the claw itself may influence its development as a crusher may be deduced from experiments where removal of one of the paired claws in the critical period causes the intact one to invariably become a crusher (Emmel, 1908). With one of the claws missing, the remaining intact one would experience greater activity, making it become a crusher. Rather than invoking claw activity as a determinative factor, another explanation would simply be that the intact claw gains a growth advantage that allows it to develop into a crusher, while the missing claw develops as a cutter following regeneration. Although the crusher is larger than the cutter, the difference in mass is less than twofold. Much greater size differences are seen in male fiddler crabs, where the major claw is 30 times larger than the minor claw. In fiddler crabs, loss of a claw during early development, when the paired claws are symmetrical, results in the intact claw developing as the major one while the regenerate claw becomes the minor claw (Yamaguchi, 1977). Indeed, unilateral claw loss has been proposed as the mechanism that operates in nature to ensure bilateral asymmetry in fiddler crabs. This was based largely upon the finding that male fiddler crabs raised individually, so that they do not lose a claw, developed paired major claws and, conversely, when both claws were missing regenerated paired minor claws.

In lobsters, however, the common practice is to rear them individually, and under appropriate conditions they usually develop a major and a minor claw. On occasion, they may develop paired minor claws but never paired major claws (Govind, 1984, 1989). Clearly, claw loss is not the mechanism that triggers asymmetry in lobsters. It may well be that when a claw is lost whatever factors govern asymmetry are also removed but claw loss in itself is not the minimal condition. Hence, it is appealing to suggest that differences in claw activity or use may be responsible for controlling bilateral asymmetry. In its simplest form, we would argue that the claw that is more active becomes the crusher while its less active counterpart becomes the cutter. The hypothesis becomes even more appealing when we recollect that activity controls the differentiation of muscle fiber types in mammals (for reviews, see Jolesz and Sreter 1981; Pette and Vrbova, 1985). Prolonged tonic stimulation of the nerve or the muscle directly transforms fast-twitch fibers to slow-twitch type. Extrapolating this finding to the lobster, the closer muscle in the more active claw would transform its fast fibers to slow, resulting in a typical crusher muscle with 100% slow fibers. The closer muscle, via its attachment to the exoskeleton, would correspondingly change the claw into a larger, stouter, crusher-type claw. In this way, claw activity would directly influence the final form of the claw and muscle. Conversely, subthreshold levels of activity or a total lack of it would transform slow fibers to fast, producing a closer muscle with 90% fast fibers characteristic of a cutter muscle and a matching claw morphology. This view gained considerable support when it was found that the development of a crusher claw could be easily suppressed.

Suppressing the Development of a Crusher Claw. Fisherman have long known the existence of lobsters with paired cutter claws, although they were less certain about the origins of this condition. In a species where the normal condition of the paired claws is of a crusher and cutter, the paired cutter condition could arise either during primary development or in adults when a cutter claw is regenerated in place of a lost crusher claw. The latter

Table 2 Configuration of Paired Claws in Juvenile Eighth- or Ninth-Stage Lobsters Reared with a Variety of Substrates or without any Substrate in the Fourth and Fifth Stages

Substrate	Configuration of Paired Claws			n	p
	Right Crusher, Left Cutter	Left Crusher, Right Cutter	Double Cutter		
Oyster chips	15	14	3	32	Control
No substrate	1	0	33	34	<0.001
Gravel	13	7	2	22	ns
Mud	7	12	3	22	ns
Plastic chips	13	9	1	23	ns

With the oyster chip condition as control, statistical significance was assessed for each of the other conditions using the contingency χ^2 test; ns = not significant (from Govind and Kent, 1982).

scenario is unlikely as the regenerate claw faithfully resembles its predecessor (Herrick, 1911; Kent, Pearce, Gee, and Govind, 1989). This makes it likely that the occasional condition of claw symmetry in the form of paired cutter claws arose because a crusher claw was suppressed during primary development. The initial discovery of such a suppression was made casually and independently by John Hughes of the Massachusetts State Lobster Hatchery and Akella Sastry of the University of Rhode Island Institute of Oceanography, both of whom found a few animals with symmetrical cutter claws in their laboratory-reared population of juvenile lobsters. They attributed this condition to a lack of use of the claws as lobsters were individually reared in smooth-walled containers and without a substrate.

To test this observation more rigorously, individual rearing chambers were constructed from plastic trays in which all the walls were smooth (Lang, 1975). A single newly hatched fourth-stage lobster was reared in each tray until it had molted to the eighth or ninth stage, at which time the claw and closer muscles had developed sufficiently to be categorized as cutter or crusher type. This was the standard procedure followed in the experiments described below.

Substrate Manipulation. We used a few pieces of broken oyster shells (oyster chips) each approximately $6 \times 3 \times 1$ mm, on the bottom of the tray as a substrate that lobsters would grasp with their claws and move around the tray. We began by rearing lobsters with and without oyster chips (Govind and Kent, 1982). The results were unequivocal and dramatic (Table 2): Those with oyster chips developed paired asymmetrical (cutter/crusher) claws while those without the chips developed paired symmetrical cutter claws (Fig. 6). The fiber composition of the closer muscle matched the external claw morphology; the asymmetrical condition showed a distinct cutter and a crusher muscle while the symmetrical condition showed both muscles to be of the cutter type (Fig. 6). The simple expedient of omitting the oyster chips during the critical juvenile stages suppressed development of a crusher claw.

The substrate did not necessarily have to be oyster chips as other substrates, such as gravel or mud with debris in it, were equally effective in ensuring the development of a crusher claw (Table 2). Indeed, even a chemically inert substrate such as plastic buttons was sufficient. This series of experiments (Table 2) together with those reported earlier (Table 1) showed convincingly that lack of a graspable substrate during a critical period in juvenile development suppressed formation of a crusher claw.

The crucial role of the substrate is presumably to provide an opportunity for the claws to be used; in the absence of a substrate, the smooth, molded-plastic rearing trays provided little opportunity for gripping by the claws. Thus, the principle requirement for the substrate to be effective was that it could be gripped by juvenile claws. This proposal was tested in our next series of experiments, in which we reared lobsters without a substrate but painted spots resembling oyster chips on some parts of the bottom and sides of the plastic trays (Govind and Pearce, 1986) (Table 3). A significant majority developed paired cutter claws, a result similar to that from the no substrate condition but unlike that from the oyster chip condition, where asymmetry prevailed. Clearly, the presence of a physical substrate was required for the development of a crusher claw and bilateral asymmetry. Even the presence of a single oyster chip was adequate in producing claw asymmetry in a significant number of animals. However, an experiment that

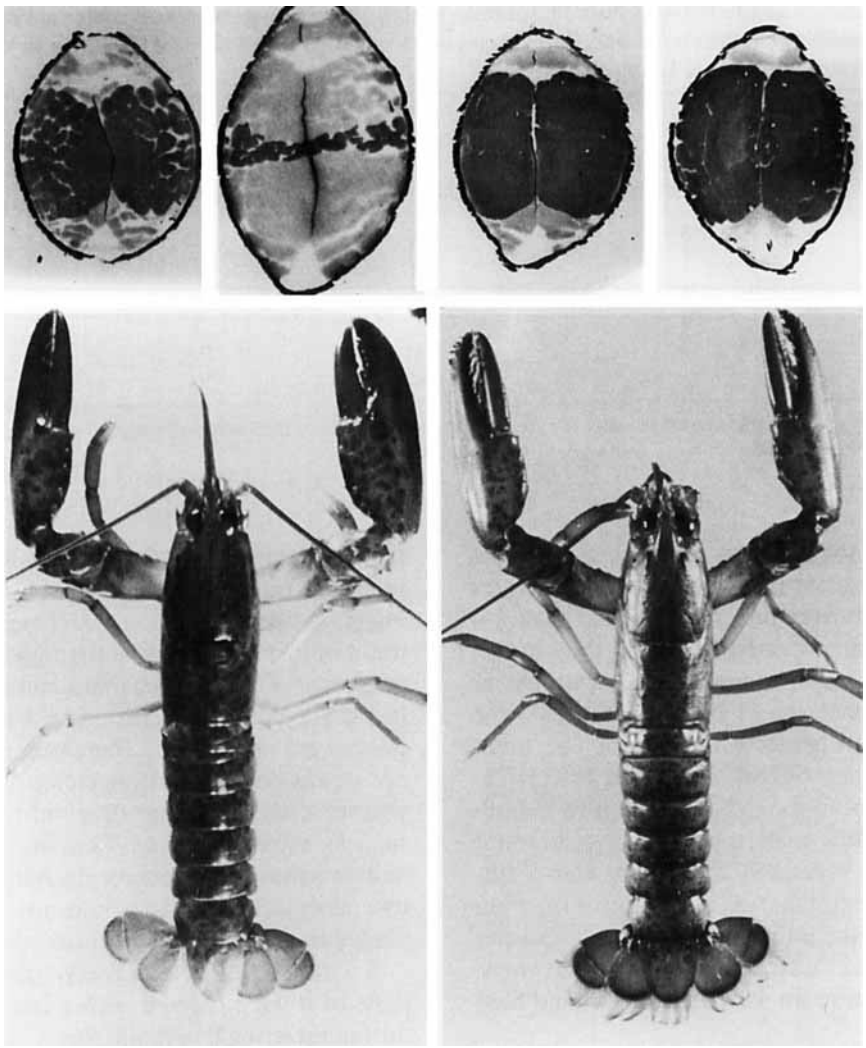

Figure 6 Two juvenile lobsters, the left with paired asymmetrical (right crusher and left cutter) claws and the right with symmetrical (cutter type) claws. Above each claw is shown a representative cross-section stained for myofibrillar ATPase activity. In these, the cutter closer muscles have mostly fast fibers (dark staining) except for a ventral slow band and the crusher closer muscles have mostly slow fibers (light staining) except for a thin central fast band that transforms to slow muscle in subsequent development. (Reprinted with permission from Govind and Pearce, *Development* **107**:547–551, © 1989b.)

truly underscored the role of claw activity was where two lobsters were reared together in a single tray without a substrate. Lobsters are solitary animals and when two are confined together they fight with their claws. Usually, one of the lobsters lost one or both claws over their period of confinement, which included the fourth and fifth stages. Configuration of the claws was assessed in those lobsters with paired intact claws and most were found to possess asymmetrical cutter and crusher claws in a random distribution. The possibility that this effect was not due to the use of the claws in antagonistic encounters but simply to the visual presence of another lobster was tested in the final experiment in this series (Table 3). In this experiment, individual lobsters were reared without a substrate but with a mirror placed along one of the long sides of the tray. Lobsters appeared to approach their reflection but did not interact further. Under these conditions, a significant number failed to develop a crusher claw and instead developed paired cutter claws. Clearly, the lack of activity via use of the claws prevented development of a crusher claw.

Claw Manipulation. The activity or use hy-

Table 3 Configuration of the Paired Claws in Juvenile Eighth-Stage Lobsters Reared under a Variety of Environmental Conditions in the Fourth and Fifth Stages and Compared to the Control Condition in Which They Were Reared with a Substrate of Oyster Chips

Substrate	Configuration of Paired Claws: Right Crusher, Left Cutter	Left Crusher, Right Cutter	Double Cutter	*n*	*p*
Oyster chips	8	9	1	18	Control
One oyster chip	7	9	7	23	ns
Painted chips	4	0	16	20	<0.005
Two animals	9	11	3	23	ns
Mirror	7	3	19	29	<0.005
No substrate	2	4	20	26	<0.005

The contingency χ^2 test was used to determine the significance between the control and experimental groups; ns = not significant (from Govind and Pearce, 1986).

pothesis would suggest that although both claws have the potential for becoming a crusher greater use of one claw during the critical period makes it become the crusher. Consequently, in the next series of experiments we reared lobsters with oyster chips but restricted use of one of the claws, the right, to varying degrees while leaving the other claw, the left, intact (Govind and Kent, 1982) (Table 4). Treatment to the right claw applied in both the fourth and fifth stages, if effective, would result in the right claw invariably developing into a cutter. But, if the treatment was not effective the right claw would develop either into a cutter or crusher type. Claw use was restricted in one of two ways, viz., immobilization, in which a rubber band held the dactyl in the closed position, and dactylotomy, in which most of the dactyl was cut off. Surprisingly, neither condition prevented the development of a crusher claw as the right claw became a crusher as often as it became a cutter; a result similar to the control condition in which the paired claws were untouched. Therefore, use of the claws *per se* was not the limiting factor in determining a crusher claw but some other minimal condition such as muscle activity. This would explain why neither immobilization nor dactylotomy was effective as muscle activity would not have been prevented in either of these conditions.

Table 4 Configuration of the Right Claw—Whether Cutter or Crusher— in Juvenile Ninth-Stage Lobsters Following Various Treatments to the Right Claw in the Fourth and Fifth Stages of Development

Treatment	Configuration of Right Claw: Cutter	Crusher	*n*	*p*
Intact	18	14	32	Control
Immobilization	9	11	20	ns
Dactylotomy	8	7	15	ns
Paint claw	15	4	19	ns
Paint pollex and dactyl	15	3	18	ns
Closer tenotomy	34	3	37	<0.001
Opener tenotomy	19	3	22	<0.02
Denervation	27	5	32	<0.02

Each of the experimentally treated groups was compared to the control group in which the right claw was intact, using the contingency χ^2 test; ns = not significant (from Govind and Kent, 1982).

To reduce muscle activity, the right claw or parts of it were painted with a fast-drying lacquer during the critical juvenile stages. The painting inactivated surface sensory receptors, resulting in functional deafferentation that reduced reflexly evoked muscle activity. In a large number of cases, the painted claw did become a cutter (Table 4), suggesting, at first glance, that deafferentation effectively inhibited development of a crusher claw. But, statistically these results did not differ significantly from those of the control condition. They do, however, suggest that deafferentation may have a suppressive effect if it more drastically reduced muscle activity.

To this end, we cut the tendon of the claw closer muscle where it attaches to the dactyl in the fourth and fifth stages. Tenotomy of the closer muscle effectively inhibited the claw from developing into a crusher (Table 4). In this type of tenotomy, not only is the muscle incapable of contracting, that is, generating active tension, but it also loses its passive tension, and in addition there is the disruption of proprioceptive feedback from this muscle to the CNS. Which of these particular effects is responsi-

ble for suppressing a crusher claw cannot be deduced from our closer tenotomy experiment. However, we can distinguish between the effects of muscle tension, both active and passive, and proprioceptive input to the CNS if the tendon to the opener muscle was cut rather than to the closer muscle. As the name suggests, the opener muscle is the antagonist to the closer. It is also a relatively small muscle, occupying less than 10% of the claw muscle mass. Hence, its tenotomy would not jeopardize active or passive tension in the closer muscle. On the other hand, opener tenotomy will reduce not only proprioceptive but other sensory input as well because cutting the opener tendon also cuts an overlying large sensory nerve from the dactyl. Cutting the opener tendon to the right claw prevented that claw from developing into a crusher as effectively as cutting the tendon to the closer muscle (Table 4). Presumably, the disruption of sensory feedback with opener tenotomy correspondingly reduces the motor output to the claw muscles and thus activity of the closer muscle is decreased. The failure to achieve some minimal level of activity of the fibers in the closer muscle prevents their transformation from fast to slow type and thus prevents the claw from developing into a crusher.

A more direct test of the above hypothesis was to denervate the claw, thereby eliminating activity of the claw muscle but otherwise leaving the claw with its muscles and receptors intact. We denervated the right claw in the fourth and fifth stages by destroying the right side of the ganglion to the claws. The CNS in lobsters consists of a series of segmentally arranged ganglia, with the most anterior ones being compressed into a brain and the more posterior ones to the thorax and abdomen remaining as discrete entities. Each of these segmental ganglia consist of two halves or hemiganglia. In the claw hemiganglion, the cell bodies of the motoneurons to the claw muscles were located in the most anterior region (Govind and Lang, 1981). By appropriate stereotaxic manipulation of a finely sharpened pin, we destroyed this part of the hemiganglion and eliminated reflex activity in this claw. Such denervation had a dramatic effect in that most lobsters failed to develop a crusher claw on the treated side (Table 4). Therefore, eliminating motor activity to the closer muscle prevents its fast fibers from transforming to slow and the claw from transforming into a crusher.

Promoting the Development of a Crusher Claw. An hypothesis emerging from the experiments described above is that the development of a crusher claw is due to nerve-mediated muscle tension. During the critical fourth and fifth juvenile stages, one of the claws, at random, is more active than the other, and the resulting contractile activity in the closer muscle transforms its fast fibers to slow, producing a crusher-type muscle. Because of its attachment to the exoskeleton, the transformation of the closer muscle influences the shape and size of the claw such that it assumes a typical crusher morphology. A similar scene is unfolding in the opposite claw, which, because it is less active, has most of its slow fibers transforming to fast, resulting in a cutter-type muscle and claw. This hypothesis is based largely upon the fact that eliminating or reducing the nerve-mediated muscle activity in a claw during the critical period suppresses it from becoming a crusher claw. As a corollary, it should therefore be possible to promote the development of a crusher claw by increasing its activity during the critical period.

Attempts were made to electrically stimulate the closer muscle via fine wires implanted in the claw, but these procedures were unsuccessful because animals tended to autotomize (discard) the treated claws. A more expedient way of enhancing activity in a claw was to exercise it (Govind and Pearce, 1986). This consisted of holding the lobster between the thumb and forefinger and stroking its claw with a paint brush so that its bristles were gripped several times in a 1-min session. These sessions were repeated thrice daily throughout the fourth and fifth stages in lobsters reared without a substrate to suppress crusher development. Despite such rearing conditions, the exercised claws usually became a crusher (Table 5, series 1). A control group of lobsters in which animals were handled but not exercised was also reared without a substrate. The majority of these developed paired cutter claws, emphasizing the fact that handling did not influence crusher development but exercise did. Thus, some minimal level of reflex activity in a claw is required to transform fast fibers to slow in its closer muscle and differentiate a crusher claw.

If a minimal level of reflex activity is the sole requirement for a crusher claw to develop, then imposing such activity in both claws of a pair ought to result in lobsters with paired crusher claws. Consequently, lobsters were reared without a substrate and now both claws were exercised. The results were initially baffling as the majority developed paired cutter claws (Table 5, series 1). Not only did these lobsters not develop paired crusher claws, they failed to develop a single crusher claw. Yet, the exercise regimen used in this experiment was

Table 5 Configuration of the Paired Claws in Juvenile Eighth- and Ninth-Stage Lobsters Following Manipulation of the Substrate and Claws in the Fourth and Fifth Stages of Development

Condition	Configuration of Paired Claws			n	p
	Right Crusher, Left Cutter	Left Crusher, Right Cutter	Double Cutter		
Series 1					
No substrate and handled	2	2	18	22	Control
No substrate and Exercise left	3	13	1	17	<0.002
No substrate and exercise both	4	3	11	18	ns
Series 2					
Substrate and handled	10	10	1	21	Control
Substrate and exercise left	10	13	0	23	ns
Substrate exercise both	10	13	0	23	ns

The control group was the one in which lobsters wee handled but not exercised and this group was compared with the exercised condition. Statistical significance was determined using the contingency χ^2 test; ns = not significant (from Govind and Pearce, 1986, 1992).

the same regimen that when applied to one claw of a pair induced it to become a crusher but when applied to both claws suppressed crusher development. How do we explain this finding?

A minimal level of reflex activity is not by itself a sufficient condition for crusher development but perhaps differences in activity levels between the two sides ensures that the side with the greater activity develops into a crusher while the opposite side develops into a cutter. In the absence of such bilateral differences in reflex activity, both claws develop as cutters. This would explain why exercising one claw induces crusher development but exercising both claws fails to do so because bilateral differences in reflexive activity would be minimized or nonexistent. According to this explanation, reflex activity does not directly induce crusher development, that is, it does not itself transform fast fibers to slow and as a result transform claw morphology. Rather, the role of reflex activity in determining claw type is most likely exerted via an indirect pathway. Such a pathway must involve a central site where the activity from the paired claws may be monitored and compared. This site, in the first instance, would be the ganglion serving this thoracic segment. Neural input arising from activity of the claws would converge into the claw ganglion from both sides. Here, a comparison would occur such that the hemiganglion receiving the greater input would become the crusher side. Determination into a crusher or cutter claw could therefore occur initially in the ganglion in the critical fourth and fifth stages of juvenile development and be expressed at the periphery in terms of claw morphology and muscle fiber composition in subsequent juvenile development. Reflex activity therefore serves to lateralize the CNS, which in turn lateralizes the claws via as yet unknown pathways. To the extent that activity of the claws is regulated by the environment in which the lobster finds itself, experience in terms of claw activity therefore plays a formative role in determining claw asymmetry.

Threshold for Determining Asymmetry. Initial lateralization of the CNS via bilateral differences in input from the claws raises questions about the nature of such input. Because the input converges from the periphery into the ganglion, it is assumed to be sensory in nature although a further definition as to the type of sense cells involved such as chemoreceptors, proprioceptors, mechanoreceptors, and so forth is not known. Some experiments, such as painting the claw with lacquer, rule out the possibility that chemoreceptors are involved. In addition to identifying the specific type of input, we were also concerned about the quantity of the input itself (Govind and Pearce, 1992). In its simplest form, the question regarding quantity of input was whether there was an absolute minimum or threshold level for the determination of asymmetry or whether any difference in input between the two sides regardless of absolute level would suffice. To test the latter possibility, we reared lobsters without a substrate to suppress development of a crusher claw and promote development of paired cutter claws (Table 6, series 1). Removing one of the claws in the critical fourth and fifth stages would create bilateral differences in input to the CNS. If the intact claw developed into a crusher and its counterpart into a cutter, the results would suggest a primary role for the differential input between the two sides in determining asymmetry. If,

Table 6 Configuration of the Paired Claws in Juvenile Eighth- or Ninth-Stage Lobsters Reared without a Substrate and with Their Claws Manipulated in the Fourth and Fifth Stages

Condition	Configuration of Paired Claws				
	Asymmetrical		Symmetrical		
	Right Crusher Left Cutter	Left Crusher Right Cutter	Right and Left Cutters	*n*	*p*
Series 1					
Paired intact claws	1	2	18	21	Control
Left claw autotomy	1	0	19	20	ns
Series 2					
Left claw autotomy and animal handled	0	0	21	21	Control
Left claw autotomy and right claw exercised	15	0	4	19	<0.001

Each of the experimental conditions was tested, using the contingency χ^2 test, against the appropriate control condition; ns = not significant (from Govind and Pearce, 1992).

however, the intact and regenerated claws developed into cutters, it would suggest that in addition to bilateral differences the input had to be of threshold value to form a crusher claw and that in the absence of a substrate this threshold was not reached by the intact claw. The fact that all but one of the lobsters in this experiment (Table 6, series 1) developed paired cutter claws favors the latter explanation, that determination of a crusher claw requires that activity exceeds a threshold value.

Presumably, this threshold value is reached with exercise, as this produced a crusher claw in lobsters reared without a substrate. This deduction was confirmed when lobsters were reared without a substrate but had one of the claws removed and the other claw exercised during the determinative juvenile stages (Table 6, series 2). The intact exercised claw developed as a crusher in a significant number of experimental animals as compared to the control condition, in which the animal was handled but the intact claw was not exercised. Clearly, the exercise regime adopted in these experiments exceeds the minimal level of reflex activity necessary for determination of asymmetry.

Is it possible to apply a minimal level of reflex activity to both sides to develop lobsters with paired crusher claws? To test this possibility, lobsters were reared with oyster chips and had their claws exercised as well. Rearing with oyster chips would ensure the development of paired asymmetrical claws with the crusher appearing either on the left or right side, while exercising would ensure that the other claw, the putative cutter, would also receive the minimal level for the development of a crusher. In the experiment in which only one of the paired claws was exercised (left one in Table 5, series 2), our intent was to stimulate minimal activity in the left putative cutter claw that would occur in 50% of lobsters reared with oyster chips. In exercising both claws, our intent was to ensure that the putative cutter on either side experienced minimal reflex activity. In both these experiments, lobsters developed bilateral asymmetry with an approximately equal distribution of the crusher on the right or left side (Table 5, series 2). In no case did a crusher develop on both sides. These results were similar to a control group in which lobsters were handled but not exercised. While a minimal level of reflex activity is required for determination of a crusher on one side, the imposition of this minimal level on the opposite side does not result in development of another crusher. Indeed, determination of a crusher on one side inhibits the opposite side from becoming another crusher. Presumably, interaction between the paired hemiganglia ensure bilateral asymmetry of the claws in the form of a crusher and a cutter type. That such interactions exist is seen in the case where sensory stimuli evokes responses in claw motoneurons on the ipsilateral as well as the contralateral side (Govind, Meiss, and Lang, 1979).

Mechanoreceptive Input for Determining Asymmetry. Differences in reflex activity between the paired claws in the critical fourth and fifth stages were instrumental in determining claw laterality in juvenile lobsters: The side with the greater activity became the crusher while the opposite side became the cutter (Govind and Pearce, 1986). Because these differences in reflex activity determine lateral-

Table 7 Configuration of the Paired Claws in Juvenile Eighth- or Ninth-Stage Lobsters Reared with or without a Substrate and with Their Claws Untouched or Manipulated in the Fourth and Fifth Stages

	Configuration of Paired Claws				
	Asymmetrical		Symmetrical		
Condition	Right Crusher, Left Cutter	Left Crusher, Right Cutter	Right and Left Cutters	*n*	*p*
Series 1					
No substrate and paired intact claws	1	2	18	21	Control
No substrate and left claw exercised	3	15	2	20	<0.001
No substrate and left claw stroked	3	2	18	23	ns
Series 2					
Oyster chips	10	12	1	23	Control
Free-standing plastic spheres	4	2	15	21	<0.001
Fixed plastic spheres	1	2	18	21	<0.001

Each of the experimental conditions was tested using the contingency χ^2 test against the appropriate control condition; ns = not significant (from Govind and Pearce, 1992).

ity, initially in the ganglion, the sensory component of the reflex activity is strongly implicated. Hence, the proximate factor in determining laterality is the sensory component and specifically that associated with movements of the claw, that is, mechanoreceptors.

A large variety of mechanoreceptors are found in crustaceans and these may be broadly classified into internal, cuticular, and supracuticular (Bush and Laverack, 1982). The supracuticular receptors with end organs or accessory structures projecting beyond the cuticle comprise seta, campaniform sensilla, and articulated pegs, while the cuticular receptors comprise those located within the cuticle either in the hypodermis or connective tissue. In contrast to these external mechanoreceptors, located wholly within the exoskelton are internal mechanoreceptors or proprioceptors comprising muscle receptor organs, apodeme receptors, chordotonal organs, and innervated strands. The occurrence and distribution of mechanoreceptors in crustacean claws is poorly understood.

In the claws of the lobster *Homarus americanus*, the morphology and distribution of setae has been described (Solon and Cobb, 1980), as well as the axon number and composition of the chordotonal organ spanning the propus–dactyl joint, that is, the PD chordotonal organ (Cooper and Govind, 1991). This organ contains the endings of movement- and position-sensitive cells embedded in an elastic strand that spans the joint by attaching to the dactyl at one end and to the apodeme of the closer muscle at the opposite end. Recordings from axons of PD organs in crabs have shown that they are sensitive to length and tension changes in the elastic strand brought about by movements of the dactyl (Wiersma and Boettiger, 1959). By monitoring movements of the dactyl brought about passively or actively by muscle contraction, the PD organ provides a major source of proprioceptive input. We examined whether external or internal mechanoreceptors were essential in determining bilateral asymmetry of the paired claws in developing lobsters by designing experiments in which predominantly one or both types could be activated (Govind and Pearce, 1992).

Hence, in one experiment one of the paired claws was stroked with a paintbrush for thrice-daily 1-min bouts. The stroking was either vigorous or gentle. Vigorous stroking elicited closing and opening reflexes, thereby exercising the claw and presumably activating both external and internal mechanoreceptors. Gentle stroking did not elicit claw closing or opening so presumably only external mechanoreceptors were stimulated (the possibility that there was some muscle contraction without visible movements of the dactyl cannot be ruled out entirely in these experiments). Exercise of the left claw (vigorous stroking) resulted in a majority of these animals developing a crusher on that side, while for controls in which the paired claws were untouched the majority developed paired cutter claws (Table 7, series 1). Gentle stroking of the left claw (without exercise) also

Table 8 Configuration of the Paired Claws in Juvenile Eighth- and Ninth-Stage Lobsters Reared with a Substrate of Oyster Chips and with Their Claws Manipulated in the Fourth and Fifth Stages

Condition	Configuration of Paired Claws			n	p
	Asymmetrical		Symmetrical		
	Right Crusher, Left Cutter	Left Crusher, Right Cutter	Right and Left Cutters		
Series 1					
Paired intact claws	10	12	1	23	Control
Right claw dactylotomy	2	11	4	17	<0.05
Series 2					
Left claw autotomy	23	0	1	24	Control
Left claw autotomy and right claw dactylotomy	3	2	17	22	<0.001
Left claw autotomy and right closer muscle tenotomy	0	1	17	18	<0.001
Left claw autotomy and right opener muscle tenotomy	2	1	14	17	<0.001

Each of the experimental conditions was tested, using the contingency χ^2 test, against the appropriate control condition (from Govind and Pearce, 1992).

failed to cause the claws to differentiate a crusher. Paired cutter claws developed much as in the control condition.

The results of another experiment in which mainly external mechanoreceptors were stimulated are given in Table 7, series 2. Here, a more indirect method was used to ensure that external mechanoreceptors, but not necessarily internal mechanoreceptors (proprioceptors), were stimulated by providing a substrate that could not be gripped by the claws. Several (6 to 8) solid plastic spheres approximately 3 mm in diameter were provided in each rearing tray during the fourth and fifth stages. The spheres were smooth and of a sufficiently large size that the claws were not able to grip them. For one group of lobsters, the plastic spheres were free-standing objects while for another group they were glued to the bottom of the tray in random positions. In both cases, the majority of lobsters failed to develop a crusher and instead developed paired cutter claws. This was significantly different from the control condition in which lobsters reared with oyster chips developed asymmetrical claws.

The above experiments suggest that stimulation of external mechanoreceptors is not a sufficient condition for determination of claw asymmetry and point to the possibility that input from internal mechanoreceptors is critical. Because one of the major sources of such proprioceptive input is the PD chordotonal organ, this receptor was eliminated in one of the paired claws in lobsters reared with a substratum (Table 8, series 1). This was done simply by cutting off the dactyl where it articulates with the propus. In this group, the majority of lobsters developed a crusher on the intact side while the treated side developed into a cutter. For comparison, in a control group of lobsters with paired intact claws the crusher appeared on either side. Therefore, sectioning of the PD organ together with removal of the dactyl appears to prevent development of a crusher claw on the treated side. In an earlier experiment, cutting off most of the dactyl but leaving the PD organ intact did not prevent that claw from developing into a crusher (Govind and Kent, 1982). Presumably, input from the PD organ is crucial in determination of asymmetry although there may well be other receptors at this joint that may contribute to the effect.

A more stringent test for the role of proprioceptive input was to eliminate this input in a claw destined to become a crusher. This can be achieved by rearing lobsters with oyster chips and removing one of the claws; the remaining one always develops into a crusher (Emmel, 1908). Using this protocol, we reduced proprioceptive input in one of several ways in the remaining claw (Table 8, series 2). The dactyl was removed at its articulation with the propus so that the PD organ would be inactivated or the claw opener and closer muscles were tenotomized in separate experiments because they bring about movements of the dactyl. Each of these treatments effectively prevented the develop-

ment of a crusher claw even though that claw was predisposed to become one. In all three experiments, the majority developed paired cutter claws. In controls, the majority developed a crusher claw on the intact side.

Experiments designed to isolate the role of external and internal mechanoreceptors suggest that input from predominantly internal mechanoreceptors or proprioceptors is necessary for the determination of claw asymmetry, in particular as its reduction suppressed crusher development in a claw otherwise predisposed to become a crusher. Presumably, input from largely mechanoreceptors serves to lateralize the first thoracic ganglion into a crusher and cutter side. Such lateralization is subsequently transmitted via unknown pathways to the periphery, resulting in differentiation of the claws into crusher and cutter types.

How mechanosensory input may lateralize the ganglion is unknown, nor is the nature of such lateralization known. However, in male fiddler crabs with markedly asymmetrical claws the hemiganglion on the side of the major claw is larger than its contralateral minor counterpart (Young and Govind, 1983). In Alpheid snapping shrimps, which also have markedly asymmetrical claws, the somata of motoneurones innervating the closer muscle of the major claw are larger than those innervating its minor counterpart (Mellon, Wilson, and Phillips, 1980). In these same animals, there are also many more axon profiles in the limb nerve to the major hemiganglion compared to its minor counterpart (Govind and Pearce, 1988). Structural asymmetries in the nervous system are also found among the vertebrates, for example, in the CNS of mammals (Galaburda, 1984) and singing birds (Nottebohm, 1984).

Loss of Target Delays and Suppresses Determination. The picture that emerges so far is that claw laterality is determined during a critical period of juvenile development, when sensory input arising from reflex activity of the claws lateralizes the claw ganglion or CNS into a crusher and cutter side (Govind and Pearce, 1986). In subsequent juvenile development, this lateralization is expressed at the periphery in the morphology of the claw and the fiber composition of the closer muscle. When a limb of a lobster is gripped by a predator, it may be discarded, or autotomized, at a preformed breaking plane at its base. A blastema forms at the stump and a new limb is regenerated. Autotomy may occur at any time in the life of lobsters but seems to be much more readily induced in juvenile stages than in adults, perhaps because regeneration occurs more rapidly in these early stages. In any event, juvenile lobsters will readily discard claws and in the wild early juvenile lobsters have been reported with missing claws. How does claw loss affect the determination of bilateral asymmetry?

Loss of a single claw in the juvenile fourth or fifth stage will induce the intact one to become a crusher (Emmel, 1908; Govind and Pearce, 1989a). In this instance, claw loss controls laterality and guarantees that asymmetry occurs. But, what happens when both claws are lost? This question was addressed in a series of experiments in which juvenile lobsters were reared with oyster chips as substrate and made to autotomize both claws by gently pinching each one (Govind and Pearce, 1989b) (Table 9). Removal of the paired claws in each of the fourth, fifth, or sixth stage resulted in the development of asymmetrical, crusher and cutter, claws in the eighth or ninth stage (Table 9). The crusher appears either on the right or left side in a random distribution typical of lobsters reared under control conditions. The loss of the paired claws as target tissue in each of these stages did not affect determination of asymmetry, presumably because the target tissue was present during part of the critical period, that is, either in the fourth, fifth, or both fourth and fifth stages.

Removing the regenerated paired claws in the fourth and again in the fifth stage also resulted in lobsters developing bilateral asymmetry (Table 9). This was surprising as the paired claws were missing for the entire critical period yet asymmetry was being expressed. Either the claws as target tissue were not essential for the determination of asymmetry or determination was being delayed to the sixth stage, when regenerate claws were present. The former explanation appears highly improbable bearing in mind that activity of the claws themselves generates the sensory feedback that lateralizes the CNS and that this input has to be of a certain minimal quantity. Moreover, the probability that the target tissue is not essential for the determinative process will be ruled out in the experiments described below.

This leaves us with the notion that absence of the target tissue in the critical fourth and fifth stages delays determination of claw identity until regenerated claws are present in the sixth stage. This view may be easily tested by making use of two previous observations, viz., (1) that rearing without a substrate during the determinative period suppressed the development of a crusher claw (Lang et al., 1978) and (2) that removal of one

Table 9 Configuration of the Paired Claws, Whether Asymmetrical or Symmetrical, in Juvenile Ninth-Stage Lobsters Reared with a Substrate and with Their Claws Removed at Different Stages of Development

Condition	Configuration of Paired Claws: Asymmetrical (crusher/cutter)	Configuration of Paired Claws: Symmetrical (cutter/cutter)	*n*	*p*
Paired intact claws	22	2	24	Control
Paired claws removed in stage				
4	17	0	17	ns
5	25	1	26	ns
6	22	2	24	ns
4 and 5	16	6	22	ns
4, 5, and 6	10	8	18	<0.001
4, 5, 6, and 7	2	13	15	<0.001

Statistical significance of the results was determined between the intact claw condition and each of the experimental conditions using the contingency χ^2 test; ns = not significant (from Govind and Pearce 1989b).

claw during the critical period induces the remaining one to develop into a crusher. If the determinative period was delayed to the sixth stage, then either of these treatments applied usually in the fourth and fifth stages ought to be just as effective when applied to the regenerate claws in the sixth stage. Consequently, lobsters reared with oyster chips had their claws removed in the fourth and fifth stages to ensure that the target tissue was missing in the usual critical period. Once the paired claws had regenerated in the sixth stage, one of the two treatments described above was performed (Table 10). Thus, in one group the substrate was removed for the duration of the sixth stage and replaced at the molt into the seventh stage. The effect of this seemingly innocuous maneuver was dramatic in that the majority of lobsters failed to develop a crusher claw and instead developed paired cutter claws (Table 10). In the second group, one of the regenerate claws was autotomized in the sixth stage with the result that the other claw became a crusher in a significant majority of lobsters. Clearly, the regenerate claws in the sixth stage were as sensitive to treatments that suppressed crusher determination as were the intact claws in the fourth and fifth stages. The differentiation of claw asymmetry is therefore delayed until the target tissue is present, underscoring the essential nature of the target tissue. This also means that the critical period normally restricted to the fourth and fifth stages is extended, in the event the claws are missing in the critical stages, to the sixth stage, when regenerate claws are present.

How much longer may determination of claw

Table 10 Configuration of Paired Claws in Juvenile Ninth-Stage Lobsters in Control Animals with Intact Claws and Reared with a Substrate and Experimental Animals with Regenerated Claws in the Sixth Stage Subjected to a Lack of Substrate or Unilateral Claw Loss

Condition	Configuration of Paired Claws: Right Crusher, Left Cutter	Configuration of Paired Claws: Left Crusher, Right Cutter	Configuration of Paired Claws: Double Cutter	*n*	*p*
Paired intact claws	11	12	0	23	Control
Paired claws removed in fourth and fifth stages; substrate removed in sixth	0	0	20	20	<0.001
Paired claws removed in fourth and fifth stages; left regenerate removed in sixth	16	2	3	21	<0.001

Statistical significance of the results was determined between the intact claw condition and each of the experimental conditions using the contingency χ^2 test (from Govind and Pearce 1989b).

asymmetry be delayed? Or is there a time beyond which such determination may not occur? These questions were answered by removing the target tissue in successive juvenile stages beyond the critical period (Table 9). Thus, removal of the paired claws successively in the fourth, fifth, and sixth stages resulted in about half the lobsters not developing a crusher, a number significantly different from the control condition in which over 90% developed a crusher claw and hence asymmetry. When the paired claws are missing from the fourth to the seventh stage, an overwhelming 85% of lobsters failed to develop a crusher claw and instead developed paired cutter claws. Clearly, determination may be delayed to the sixth stage in the event claws are missing in the earlier stages but not beyond the sixth stage. There is a limit to the time when the CNS is sensitive to input that tends to lateralize the ganglion. Normally, this time limit encompasses the fourth and fifth stages and under extraordinary circumstances, such as claw loss, may also encompass the sixth stage. The ability to extend the critical period may have evolved to compensate for claw loss, common especially among juvenile lobsters. Beyond the sixth stage, however, the CNS loses its receptivity, with the result that it fails to lateralize and the paired claws develop as symmetrical cutter claws.

Hypothesis for Determination of Asymmetry. At this stage, it would be of help to review and refine our understanding of how the paired claws that are symmetrical in larval lobsters become determined into an asymmetrical, crusher and cutter, pair during juvenile development. On the basis of the experimental evidence, the following steps are proposed:

1. Determination of claw type occurs during a critical period of juvenile development normally restricted to the fourth and fifth stages but extended to the sixth stage if the paired claws are missing in the earlier stages. In the earlier larval stages, lobsters have a free-floating, planktonic existence but change to a bottom-living, benthic habitat during the fourth and fifth stages. Contact with the substrate on the ocean floor allows ample opportunity for the claws to be used. Because activity of the claws is crucial in determining claw type, the occurrence of the critical period during the fourth and fifth stages is propitious.
2. Differences in levels of reflex activity predominantly of internal mechanoreceptors (proprioceptors) between the paired claws ensures that a crusher is determined on the more active side and a cutter on the less active side. Therefore, asymmetry is determined initially in the claw ganglia or CNS, which becomes lateralized into a crusher and cutter side. The lateralization is subsequently expressed at the periphery in the development of a crusher and cutter claw.
3. A threshold level of reflex activity is required to determine a crusher claw as subthreshold activity levels produce only paired cutter claws. Paired symmetrical cutter claws also result when threshold or suprathreshold activity level is applied to both claws. This suggests that determination of a crusher side in the CNS inhibits the opposite side from also becoming a crusher. In this way, claw bilateral asymmetry of the paired claws is assured.
4. Once claw type is determined during the critical juvenile period, it is fixed for life and loss of one or both claws in later juveniles and adults results in regeneration of the same type.

These various steps involved in determination of claw laterality may be summarized by likening the process to a child's teeter-totter (see-saw) (Govind, 1989). The two ends of the teeter-totter remain in a horizontal plane when forces are equal on both sides. This condition would be equal to the paired cutter claw configuration that would arise because of subthreshold activity on both sides or threshold activity equal on opposite sides. Differences in force applied to the two sides will lower one end and simultaneously elevate the opposite end. This is equivalent to bilateral differences in claw activity, with the greater activity promoting a crusher claw and the lesser activity a cutter claw. In this way, a double crusher configuration is highly unlikely unless the connection between the two sides is broken, that is, bilateral interactions within the ganglion are eliminated.

In the wild, the ocean floor would provide an abundance of substrate that could be manipulated by the claws, resulting in unbalancing the teeter-totter in a random fashion and producing lobsters with paired asymmetrical claws. This would be the normal situation. Only rarely would both claws experience subthreshold activity levels or bilaterally equal suprathreshold levels, resulting in paired cutter claws. This would account for the small percentage (<0.2%) of symmetrical cutter-clawed animals. Even more rarely, if at all, would there be no

cross-talk between the hemiganglia, resulting in paired crusher claws. In one of these rare individuals, although the external morphology showed two crusher claws one of the paired closer muscles had about 40% fast fibers (Govind and Lang, 1979). In over 15 years of rearing approximately 2000 lobsters, we have not encountered the double crusher configuration. Clearly, this particular configuration of the paired claws is almost impossible to generate and herein lies the reason for likening claw determination to a teeter-totter, that is, when one side is a crusher the other side must be a cutter. The teeter-totter model illustrates why most wild lobsters display paired asymmetrical claws with the crusher either on the right or left side; only a handful have paired cutter claws and almost none have paired crusher claws.

The teeter-totter model may also be useful in explaining the reversal of claw asymmetry in adult snapping shrimps (Govind, Wong, and Pearce, 1988b). In these animals, the major or snapper claw is specialized so that its enlarged propus closes with tremendous force into a socket on the propus, resulting in a jet of water and a loud popping sound, both serving as warnings to conspecifics (Hazlett and Winn, 1962). The minor or pincer claw is 5–10× smaller, does not have a hammer and socket, and is used for grooming, feeding, and excavating. Loss of the major claw transforms the existing minor into a major while a new minor regenerates at the site of the old major (Przibram, 1901). In this way, claw asymmetry is reversed but maintained. Because reversal of claw asymmetry appears to have a neural basis (Wilson, 1903; Mellon, 1981), the teeter-totter model may be useful here. Accordingly, differential input from the paired snapper and pincer claws will maintain the status quo. When the snapper is lost and correspondingly its neural input is decreased, the relatively greater input on the pincer side initiates its transformation into a snapper. When the pincer is lost, the differential in sensory input is not changed and hence a pincer regenerates. When both claws are lost simultaneously, presumably the "memory" within the CNS allows regeneration of the claws in their previous configuration. Even the rare case of a shrimp with paired crusher claws may be explained according to this hypothesis. The double snapper condition may be experimentally induced by sectioning the nerve to the snapper (Mellon and Stephens, 1978). This causes transformation of the pincer to a new snapper because the sensory input is now in its favor, while the old snapper regains its neural connections. Shrimps with paired pincer claws are rare (Darby, 1935) and usually do not persist for more than one molt, presumably because bilateral differences in sensory input prevail and trigger asymmetry. Determination of claw asymmetry is clearly more plastic in snapping shrimps than it is in the lobster, where once established in the critical juvenile stages asymmetry cannot be reversed in later stages.

The teeter-totter model for both shrimps and lobster is heuristically valuable as it allows us to formulate questions regarding: (1) the nature of the sensory input from the periphery, which lateralizes the CNS; (2) cellular changes within the paired hemiganglia, which constitute lateralization; and (3) identification of the messages from the CNS to the periphery, which brings about the expression of a particular claw phenotype.

The late Fred Lang of the Boston University Marine Program initiated studies in claw asymmetry in the lobster in summer 1973 at the Marine Biological Laboratory, Woods Hole, Massachusetts. The author was fortunate to be a member of his team and equally fortunate over the ensuing years to have been guided, in the design and execution of the experiments reported above, by Joanne Pearce, Karla Kent, Russel Hill, and Walter Costello. Among the many colleagues who have encouraged and enhanced this venture are Harold Atwood, Bob Josephson, Ron Young, Phil Stephens, Greg Lnenicka, and Jay Blundon. John Hughes, Mike Syslow, and Kevin Mann of the Massachusetts State Lobster Hatchery on Martha's Vineyard have generously given advice and supplies of larval lobsters and the staff of the Marine Biological Laboratory provided facilities to do the experiments. Institutional support was provided by the Boston University Marine Program and Scarborough Campus, University of Toronto, while financial support was forthcoming from the Grass Foundation, the National Science Foundation of the U.S.A., the National Sciences and Engineering Council, and the Muscular Dystrophy Association of Canada. To all these people and institutions, the author is deeply grateful.

REFERENCES

BOTERO, L. and ATEMA, J. (1982). Behavior and substrate selection during larval settling in the lobster, *Homarus americanus*. *J. Crust. Biol.* **2**:59–69.

BUSH, B. M. H. and LAVERACK, M. S. (1982). Mechanoreceptors. In: *The Biology of Crustacea. vol. 3. Neurobiology: Structure and Function.* D. E. Bliss, H. L. Atwood, and D. C. Sandeman, Eds. Academic Press, New York, pp. 399–468.

COOPER, R. L. and GOVIND, C. K. (1991). Axon composition of the proprioceptive PD nerve during growth

and regeneration of lobster claws. *J. Exp. Zool.* **260**:181–193.

CORBALLIS, M. C. and MORGAN, M. J. (1978). On the biological basis of human laterality: I. Evidence for a maturational left-right gradient. *Behav. Brain Sci.* **2**:261–336.

COSTELLO, W. J., HILL, R., and LANG, F. (1981). Innervation patterns of fast and slow motor neurons during development of a lobster neuromuscular system. *J. Exp. Biol.* **91**:271–284.

COSTELLO, W. J., HILL, R., and LANG, F. (1984). Firing patterns of closer motoneurons during reflex activity in the dimorphic claws of the lobster, *Homarus americanus*. *J. Exp. Zool.* **231**:167–175.

DARBY, H. H. (1934). The mechanism of asymmetry in the *Alpheidae*. *Carnegie Inst. Wash. Publ.* **435**:347–361.

DARBY, H. H. (1935). The mechanism of chela differentiation in the Crustacea. *Carnegie Inst. Wash. Publ.* **29**:153–170.

EMMEL, V. E. (1908). The experimental control of asymmetry at different stages in the development of the lobster *Homarus americanus*. *J. Exp. Zool.* **231**:167–175.

GALABURDA, A. M. (1984). Brain asymmetries in human: Anatomical asymmetries. In: *Cerebral Dominance*. N. Geschwind and A. M. Galaburda, Eds. Harvard University Press, Cambridge, MA, pp. 11–25.

GOVIND, C. K. (1984). Development of asymmetry in the neuromuscular system of lobster claws. *Biol. Bull.* **167**:94–119.

GOVIND, C. K. (1989). Asymmetry in lobster claws. *Am. Sci.* **77**:468–474.

GOVIND, C. K. and KENT, K. S. (1982). Transformation of fast fibres to slow prevented by lack of activity in developing lobster muscle. *Nature* **298**:755–757.

GOVIND, C. K., KIRK, M. D., and PEARCE, J. (1988a). Highly active neuromuscular system in developing lobsters with programmed obsolescence. *J. Comp. Neurol.* **272**:437–449.

GOVIND, C. K. and LANG, F. (1974). Neuromuscular analysis of closing in the dimorphic claws of the lobster *Homarus americanus*. *J. Exp. Zool.* **190**:281–288.

GOVIND, C. K. and LANG, F. (1978). Development of the dimorphic claw closer muscles of the lobster, *Homarus americanus*. III. Transformation of dimorphic muscles in juveniles. *Biol. Bull.* **154**:55–67.

GOVIND, C. K. and LANG, F. (1979). Physiological asymmetry in the bilateral crusher claws of a lobster. *J. Exp. Zool.* **207**:27–32.

GOVIND, C. K. and LANG, F. (1981). Physiological identification and asymmetry of lobster claw motoneurons. *J. Exp. Biol.* **94**:329–339.

GOVIND, C. K., MEISS, D. E., and LANG, F. (1979). Lobster claw motoneurons respond to contralateral sensory stimuli. *J. Neurobiol.* **10**:513–517.

GOVIND, C. K. and PEARCE, J. (1986). Differential reflex activity determines claw and closer muscle asymmetry in developing lobsters. *Science* **233**:354–356.

GOVIND, C. K. and PEARCE, J. (1988). Remodeling of nerves during claw reversal in adult snapping shrimps. *J. Comp. Neurol.* **268**:121–130.

GOVIND, C. K. and PEARCE, J. (1989a). Critical period for determining claw asymmetry in developing lobsters. *J. Exp. Zool.* **249**:31–35.

GOVIND, C. K. and PEARCE, J. (1989b). Delayed determination of claw laterality in lobsters following loss of target. *Development* **107**:547–551.

GOVIND, C. K. and PEARCE, J. (1992). Mechanoreceptors and minimal reflex activity determining claw laterality in developing lobsters. *J. Exp. Biol.* (to appear).

GOVIND, C. K., STEPHENS, P. J., and TRINKAUS-RANDALL, V. (1981). Differences in motor output and fiber composition of the opener muscle in lobster dimorphic claws. *J. Exp. Zool.* **218**:363–370.

GOVIND, C. K., WONG, A., and PEARCE, J. (1988b). Experimental induction of claw transformation in snapping shrimps. *J. Exp. Zool.* **248**:371–375.

HARLOW, H. F. and HARLOW, M. K. (1973). Social deprivation in monkeys: In: *Readings From the Scientific American. The Nature and Nurture of Behavior (1962)*. W. H. Freeman, New York, pp. 108–116.

HAZLETT, B. A. and WINN, H. E. (1962). Sound production and associated behaviour of Bermuda crustaceans (*Panulirus, Gonodactylus, Alpheus* and *Synalpheus*). *Crustaceana* **4**:25–38.

HERRICK, F. H. (1895). The American lobster: A study of its habits and development. *Fish. Bull. US* **15**:1–252.

HERRICK, F. H. (1911). Natural history of the American lobster. *Bull. US Bur. Fish.* **29**:149–408.

HUBEL, D. H. (1982). Exploration of the primary visual cortex, 1955–1978. *Nature* **299**:515–524.

JAHROMI, S. S. and ATWOOD, H. L. (1971). Structural and contractile properties of lobster leg muscle fibers. *J. Exp. Zool.* **176**:475–486.

JOLESZ, K. S. and SRETER, F. A. (1981). Development, innervation, and activity-pattern induced changes in skeletal muscle. *Annu. Rev. Physiol.* **43**:531–552.

KENT, K. S., PEARCE, J., GEE, C., and GOVIND, C. K. (1989). Regenerative fidelity in the paired claw closer muscles of lobsters. *Can. J. Zool.* **67**:1573–1577.

LANG, F. (1975). A simple culture system for juvenile lobsters. *Aquaculture* **6**:389–393.

LANG, F., COSTELLO, W. J., and GOVIND, C. K. (1977a). Development of the dimorphic claw closer muscles of the lobster *Homarus americanus*. I. Regional distribution of muscle fiber types in adults. *Biol. Bull.* **152**:75–83.

LANG, F., GOVIND, C. K., and COSTELLO, W. J. (1978). Experimental transformation of muscle fiber properties in lobsters. *Science* **201**:1037–1039.

LANG, F., GOVIND, C. K., and SHE, J. (1977b). Develop-

ment of the dimorphic claw closer muscles of the lobster, *Homarus americanus*. II. Distribution of muscle fiber types in larval forms. *Biol. Bull.* **152**:382–391.

LNENICKA, G. A., BLUNDON, J. A., and GOVIND, C. K. (1988). Early experience influences the development of bilateral asymmetry in a lobster motoneuron. *Dev. Biol.* **129**:84–90.

LORENZ, K. (1970). *Studies in Animal and Human Behaviour*. Harvard University Press, Cambridge, MA.

MELLON, DEF., JR. (1981). Nerves and the transformation of claw type in snapping shrimps. *Trends Neurosci.* **4**:245–248.

MELLON, DEF., JR. and STEPHENS, P. J. (1978). Limb morphology and function are transformed by contralateral nerve section in snapping shrimp. *Nature* **272**:246–248.

MELLON, DEF., JR., WILSON, J. A., and PHILLIPS, C. E. (1980). Modification of motoneuron size and position in the central nervous system of adult snapping shrimp. *Brain Res.* **233**:134–140.

NEIL, D. M., MACMILLAN, D. L., and LAVERACK, M. S. (1976). The structure and function of thoracic exopodites in the larvae of the lobster *Homarus americanus* (L). *Phil. Trans. Roy. Soc. Lond. B* **274**:69–85.

NOTTEBOHM, F. (1977). Asymmetries in neural control of vocalization in the canary. In: *Lateralization in the Nervous System*. S. Harnad, R. W. Doty, L. Goldstern, J. Jaynes, and G. Krauthamer, Eds. Academic Press, New York, pp. 23–44.

NOTTEBOHM, F. (1984). Brain asymmetry in other species: Learning, forgetting, and brain repair. In: *Cerebral Dominance*. N. Gerschwind and A. M. Galaburda, Eds. Harvard University Press, Cambridge, MA, pp. 93–113.

OGONOWSKI, M. M., LANG, F., and GOVIND, C. K. (1980). Histochemistry of lobster claw closer muscles during development. *J. Exp. Zool.* **213**:359–367.

PETTE, D. and VRBOVA, G. (1985). Neural control of phenotypic expression in mammalian muscle fibers. *Muscle Nerve* **8**:676–689.

PRZIBRAM, H. (1901). Experimental studien uber regeneration. *Arch. Entwckl. Mech. Org.* **11**:321–345.

SCRIVENER, J. C. E. (1971). Agonistic behavior of the American lobster, *Homarus americanus* (Milne-Edwards). *Fish. Res. Bd. Can. Tech. Rep.* **235**:1–128.

SOLON, M. H. and COBB, J. S. (1980). The external morphology and distribution of cuticular hair organs on the claws of the American lobster, *Homarus americanus* (Milne-Edwards). *J. Exp. Mar. Biol. Ecol.* **48**:205–215.

WIENS, T. H. (1985). Triple innervation of the crayfish opener muscle: The astacuran common inhibitor. *J. Neurobiol.* **16**:183–191.

WIERSMA, C. A. G. (1961). The neuromuscular system. In: *The Physiology of Crustacea*. T. H. Waterman, Ed. Academic Press, New York, pp. 191–240.

WIERSMA, C. A. G. and BOETTIGER, E. G. (1959). Unidirectional movement fibres from a proprioceptive organ of the crab, *Carcinus maenas*. *J. Exp. Biol.* **36**:102–112.

WIESEL, T. N. (1982). Postnatal development of the visual cortex and the influence of environment. *Nature* **299**:583–591.

WILSON, E. B. (1903). Notes on the reversal of asymmetry in the regeneration of the chelae in *Alpheus heterochelis*. *Biol. Bull.* **4**:197–210.

YAMAGUCHI, T. (1977). Studies on the handedness of the fiddler crab, *Uca lactea*. *Biol. Bull.* **152**:426–436.

YOUNG, R. E. and GOVIND, C. K. (1983). Neural asymmetry in male fiddler crabs. *Brain Res.* **280**:251–262.