Fig. 3. Aggregation of bedbugs (females) on filter paper scented by the odour of female or male bedbugs. The paper disc was inverted for photographing (approximately 2 × natural size).

Table I. Attraction of male and female bedbugs to the scents of male and female *C. lectularius*

Scent source			Aggregation of		
Males		Females	Males (%)[a]	Females (%)[a]	Both sexes 1:1 (%)[a]
12		–	30	68	33
25		–	68	92	63
100		–	73	93	
–		12	12	57	33
–		25	68	86	70
–		100	86	84	
6	+	6	15	50	48[b]
12	+	12	25	75	56[b]
25	+	25	60	80	82[b]
50	+	50			100[b]

[a] 60 adults of either sex or mixed sexes were tested per scent level; their aggregation under the scented papers was recorded 4 h after start of experiments. [b] Females and males were attracted in equal proportion.

Table II. Attraction of *C. lectularius* to graded levels of mixed female and male scents

Bedbug equivalents[a]	0.1	1	2.5	5	10	20	50
Females assembled under scented paper (%)	0	14	28	42	58	62	90

[a] Methanol extract of female and male scent 1:1.

were similarly attractive at 100 and 25 bedbug equivalents. It is interesting that fifth instar nymphs respond only slightly to those scents, despite the fact that in natural harborages the former are usually found in clusters with adults. The latter may be mainly due to tactile reactions among bedbugs of different ages.

Methanol extraction of papers impregnated by the odour of male and female *C. lectularius* (1:1) yields a relatively effective scent solution, of which an aliquot of 1 bedbug equivalent attracts 14% of the exposed females (Table II). However the above scent mixture was found to be insoluble in diethylether. The scent can be efficiently volatilized from living bedbugs at 32 °C and atmospheric pressure, and can then be adsorbed on filter paper suspended in the air above the insects.

Since the bedbug scent causes hetero-as well as homosexual attraction, we have classified it among the assembling scents[11]. It should be recalled however, that scents to which this term has been applied, may differ widely in their modes of action[9,12]. We assume that the above mentioned odour induces bedbugs of both sexes to move into harborages which provide the tactile stimuli necessary to maintain the aggregation of these insects. The mutually opposed effects of assembling and alerting scents may be of ecological significance[13].

Zusammenfassung. Es wird gezeigt, dass die Aggregation der Bettwanzen (*Cimex lectularius*) durch einen von beiden Geschlechtern abgegebenen Versammlungsduft eingeleitet wird. Letzterer ist in Methylalkohol, aber nicht in Diäthyläther löslich, verdampft bei 32 °C und lockt unbegattete sowie begattete Wanzen beider Geschlechter an. Die Lockreaktion ist von der Duftmenge abhängig und bleibt bei antennenlosen Wanzen aus. Die Stinkdrüsen gereizter, respektive verletzter Bettwanzen sondern Hex-2-en-1-al und Oct-2-en-1-al als Alarmduft ab, der die Zerstreuung der Wanzengruppen hervorruft.

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L-Isoleucine: An Inducer of the Feeding Response in Decapod Crustaceans

Pachygrapsus crassipes, the lined shore crab, exhibits the following feeding behavior when dead squid is presented: the animals scrape the bottom of the tank with their claws and lift their claws to their mouths. This behavior is followed by searching movements during which the crabs walk around the tank until they contact the squid. The crabs take the squid in their claws, tear it into small pieces, and place it in their mouths. Experiments were performed to determine what elicits this response.

The nature of the feeding response was determined in a preliminary study using filtered squid juice (homogenate of 10 g of frozen squid in 30 ml of seawater). A piece of Whatman No. 1 filter paper (1–3 mm²), pre-

viously immersed in squid juice and dried, was held with forceps and placed in the water directly in front of the test animals. The animals took it in their chelae within 2 min and placed it in their mouths where it was retained and swallowed. Based on this observation, the feeding response was defined as taking a piece of filter paper into the mouth and retaining it for 5 min after having first rejected a plain piece of filter paper. Animals that were molting, those lacking both chelae, and ovigerous females were not used. *Pachygrapsus crassipes* and *Cancer magister* were starved for 3 days prior to testing, and *Hemigrapsus oregonensis* and *Pagurus hemphillii*, 2 days.

Since the line shore crab, *Pachygrapsus crassipes*, is an omnivore and like most crabs exhibits a preference for decaying flesh, the chemical substance which stimulates its feeding response is likely to be one of the components of protein commonly found in most plants and animals, the release of which increases with decay.

In order to detect a specific inducer of the feeding response in decapod crustaceans, an experiment was first conducted with the branched-chain amino acids L-leucine, L-isoleucine, and L-valine. These were selected for testing because of a) their wide distribution in nature, b) their relatively low molecular weight and c) relatively high solubility in aqueous solutions.

All 15 *Pachygrapsus crassipes* tested with a saturated solution of L-isoleucine dried on filter paper exhibited the feeding response. L-leucine elicited no observable response. Of the 7 crabs tested with L-valine, 3 placed the filter papers in their mouths; however, they removed them within 10–22 sec.

L-isoleucine did not elicit the feeding response when the crabs seemed satiated. When frozen squid (400 g) was placed in an aquarium containing 28 *Pachygrapsus crassipes*, the crabs began to feed immediately. Since squid remained in the tank 18 h later, the crabs were assumed to be satiated. When these crabs were tested in isolation either with L-isoleucine or with squid juice, none of the crabs exhibited the feeding response.

Comparative studies established that L-isoleucine induced the feeding response in the following species of crustaceans: *Cancer magister* (5 individuals), *Pugettia producta* (5 individuals), and *Pagurus hemphillii* (22 individuals). When L-isoleucine was dropped from a disposable glass pipette onto the mouthparts of a *Crago nigromaculata* (a particulate feeder), the shrimp was observed to grasp the pipette with the chelae and pull it to its mouthparts, where the tips of the 3rd maxillipeds were inserted into the pipette. A pipette filled with seawater did not produce the response. All 3 caridean shrimps tested displayed the response described.

The minimal concentration of L-isoleucine required to produce the feeding response was determined with *Pagurus hemphillii* and *Hemigrapsus oregonensis*. Individual animals were placed in a beaker containing 100 ml of seawater. The beaker was set on a magnetic stirrer. The animals were separated from the stirring bar by nylon mesh. After 5 min 10 µl of $10^{-3}M$ L-isoleucine were dropped from a microsyringe into the beaker. After 1 min the animal was offered a piece of clean filter paper (1 mm²) held by forceps as bait. This procedure was repeated until the animal placed the filter paper in its mouth. The effective concentrations varied between $1.2\times10^{-6}M$ and $1.6\times10^{-6}M$ for *Pagurus hemphillii* (6 specimens), and between 1.2×10^{-6} and $2.3\times10^{-6}M$ for *Hemigrapsus oregonensis* (7 specimens).

When a solution containing L-isoleucine and D-allo-isoleucine was tested on 15 crabs, the feeding response was of the same intensity as the response to L-isoleucine alone. No competition seemed to occur between the 2 diastereomers.

In order to test the possibility that other commonly occurring amino acids would elicit the feeding response, a piece of filter paper soaked in a saturated solution of each of the following amino acids was tested on 15 *Pachygrapsus crassipes*: L-alanine, L-arginine hydrochloride, L-aspartic acid, L-cystine, L-glutamic acid, glycine, L-histidine hydrochloride, L-leucine, L-lysine hydrochloride, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, α-aminoisobutyric acid, L-α-aminobutyric acid, L-norvaline, D-valine, and D-allo-isoleucine. The feeding response was not observed in any of the 15 crabs.

The 22 amino acids previously listed were combined into one solution. Pieces of filter paper soaked in the solution and dried were tested on 15 *Pachygrapsus crassipes*, 5 *Cancer magister*, 15 *Hemigrapsus oregonensis* and 5 *Pugettia producta*. None of the animals tested showed the feeding response.

L-isoleucine was added to the solution containing the 22 other amino acids. Pieces of filter paper soaked in the solution and dried were presented to 15 *Pachygrapsus crassipes*. All 15 animals showed the feeding response.

HODGSON[1] in electrophysiological studies found that chemoreceptors on the medial branch of the antennule and the claws of the crayfish *Cambarus bartonii sciontensis* were insensitive to sodium chloride, sucrose, D-levulose, citric acid, oil of citronella, and oil of wintergreen. However, he recorded spike potentials from *Cambarus* when stimulating the chemoreceptor cells with glycine ($2.5\times10^{-1}M$) and DL-glutamic acid ($2.5\times10^{-1}M$). LAVERACK[2] using electrophysiological methods reported that L-glutamic acid (saturated solution) and L-glutamine ($1\times10^{-1}M$) produced only occasional responses when applied to the first walking legs of the crustaceans *Carcinus maenas*, *Portunus puber*, and *Homarus vulgaris* while L-aspartic acid, tryptamine, glutathione, urea, L-tyrosine, γ-aminobutyric acid, glycogen, glycine, L-cysteine, L-methionine, L-serine, L-leucine, and histamine had no effect. LAVERACK reported that both buffered and unbuffered solutions of betaine hydrochloride and trimethylamine oxide hydrochloride in concentrations of $1\times10^{-2}M$ to $1\times10^{-1}M$ consistently produced action potentials. The behavioral response of crustaceans to betaine and trimethylamine oxide has not yet been tested.

Other water soluble compounds, such as polypeptides, might induce the feeding response, but among the commonly occurring amino acids L-isoleucine appears to be the only one that is an effective inducer of the behavioral response[3].

Zusammenfassung. Es wird gezeigt, dass L-Isoleucin eine starke Fressreaktion bei 6 Arten dekapoder Krebse hervorruft. Die wirksame Konzentration dieser Substanz war unter $2{,}3\times10^{-6}M$/l.

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