a 6-hour period demonstrates the sensitivity of the beetles to this material.

The female-infested bolt and frass extract attracted beetles in a 1:1 ratio of males to females (Table 1); but the ratio of beetles attracted by *exo*-brevicomin and by *exo*-brevicomin with myrcene was 2.2 : 1. We do not know whether the high proportion of males is caused by differences in concentration or by the absence of compounds. Besides myrcene, other components of the terpene hydrocarbon fraction from the extract of female frass synergize *exo*-brevicomin in laboratory tests (*1*, *2*). These components remain to be identified and tested in the field.

Temnochila virescens chlorodia, one of the principal predators of *Dendroctonus brevicomis* larvae and adults, has been observed to aggregate on trees that were under mass attack (*6*). The synchronous arrival of this predator and the western pine beetle on the host tree is a result, at least in part, of the predator's response to one of the pheromones produced by the bark beetle, namely, *exo*-brevicomin (Table 2). The female predator apparently must feed on adult bark beetles for reproductive maturation (*7*). This response to a component of the pheromone of the bark beetle may be the mechanism whereby high prey densities can be efficiently located. The predators then oviposit, and their larvae feed on the immature stages of the bark beetle.

The possible nature of the mass attack in the western pine beetle is suggested by this study. Myrcene is a prominent constituent of the xylem oleoresin of two host species, *Pinus ponderosa* (*8*) and *P. coulteri* (*9*), of the western pine beetle in California. *Exo*-brevicomin attracts low numbers of beetles initially, but the mass attack probably depends upon the release of myrcene and other unknown compounds from the host as a result of boring activity by the female.

WILLIAM D. BEDARD
PAUL E. TILDEN
Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture, Berkeley, California 94701
DAVID L. WOOD
Department of Entomology and Parasitology, University of California, Berkeley 94720
ROBERT M. SILVERSTEIN
ROBERT G. BROWNLEE, J. OTTO RODIN
Life Sciences Division, Stanford Research Institute, Menlo Park, California 94025

References and Notes

1. R. M. Silverstein, R. G. Brownlee, T. E. Bellas, D. L. Wood, L. E. Browne, *Science* **159**, 889 (1968). *Endo*-brevicomin (*endo*-7-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]octane) was also identified in female frass, but in laboratory tests was inactive alone.
2. D. L. Woods, R. M. Silverstein, M. Nakajima, *Science* **164**, 203 (1969).
3. D. L. Wood, L. E. Browne, W. D. Bedard, P. E. Tilden, R. M. Silverstein, J. O. Rodin, *ibid.* **159**, 1373 (1968).
4. T. E. Bellas, R. G. Brownlee, R. M. Silverstein, *Tetrahedron*, in press. An additional sample was supplied by H. H. Wasserman.
5. W. D. Bedard and L. E. Browne, *J. Econ. Entomol.*, in press.
6. G. R. Struble, *Pan-Pac. Entomol.* **18**, 97 (1942).
7. T. B. O'Connell, thesis, University of California, Berkeley (1967).
8. R. H. Smith, *Science* **143**, 1337 (1964).
9. ———, *Forest Sci.* **13**, 246 (1968).
10. Supported by the Forest Service, U.S. Department of Agriculture, under contract 13-267 to Stanford Research Institute and grant 5 to University of California, Berkeley; also supported by the California Division of Forestry, T. B. Walker and Surdna Foundations, and various other forest industries. We thank L. E. Browne for assistance.

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Structural Determinant of a Ribosomal Protein: K Locus

Abstract. *One of the ribosomal proteins (30S-8B) of* Escherichia coli *B has a greater electrophoretic mobility than the homologous protein (30S-8K) in* E. coli *K. The amino acid compositions, tryptic peptides, and cyanogen bromide peptides of these two homologous proteins unambiguously indicate that the genetic locus which determines the electrophoretic mobility of protein 30S-8 is the structural gene for this protein.*

There are two genetic loci which affect the structure of ribosomal proteins. One is the locus which determines the streptomycin phenotype of the ribosomes; this is expressed in the structure of at least one of the proteins of the 30*S* ribosomal subunit (*1*). The second locus is that of "K character," which is expressed in the electrophoretic mobility of one of the 30*S* proteins (*2*). Comparison of the 30*S* proteins from K strains of *Escherichia coli* with those from other strains of *E. coli* reveals a marked difference in the electrophoretic mobility of a protein which we designate as 30*S*-8 (*3*).

The identification of a protein which is altered in some manner with a genetic locus does not necessarily establish that the locus in question is the structural gene for that protein. It is possible that the phenotypic alteration is a consequence of the action of some enzyme which modifies the structure of the protein but does not determine the primary structure of the protein (for example, by acylation). We now show that the K locus determines the primary structure of the K protein.

Protein 30*S*-8 from B strains (30*S*-8B) has a greater electrophoretic mobility on polyacrylamide gels than protein 30*S*-8 from K strains (30*S*-8K) (*2*). This suggests that 30*S*-8B is either smaller or more positively charged than 30*S*-8K. When the molecular weights of the two proteins are compared by chromatography on Sephadex or by equilibrium centrifugation, they both appear to be of approximately the same size: between 22,000 and 25,000 daltons. Therefore, 30*S*-8B must have a greater net positive charge than 30*S*-8K, and we would anticipate that this would be reflected in the amino acid compositions of the two proteins.

The data in Table 1 confirm this expectation. There are significant differences in the mole percentage of eleven amino acids for 30*S*-8B and 30*S*-8K. The numbers of amino acids per molecule of protein 30*S*-8 can be calculated from the data on the mole percentage composition and from the molecular weight of the protein. By physical measurements, the molecular weight of 30*S*-8B is 22,000 daltons (*4*), and the methionine content of this protein is almost precisely 4 moles per 22,000 daltons; therefore, all of the data on amino acid composition have been presented at moles per 22,000 daltons. Significantly, there are three additional arginines and one more lysine in 30*S*-8B, which accounts for the greater electrophoretic mobility of 30*S*-8B relative to 30*S*-8K.

Table 1. Comparison of amino acid compositions of ribosomal proteins 30*S*-8B and 30*S*-8K. The compositions were measured as described (*4*) and are expressed as moles per 22,000 daltons.

Amino acid	Composition (moles/22,000)		Difference
	30*S*-8K	30*S*-8B	
Lysine	15.8	17.2	+ 1.4
Histidine	3.2	2.8	
Arginine	19.8	23.0	+ 3.2
Aspartic	16.2	15.4	− 0.8
Threonine	7.0	7.2	
Serine	16.0	13.0	− 3.0
Glutamic	22.4	23.8	+ 1.4
Proline	7.6	8.2	+ 0.6
Glycine	15.2	12.6	− 2.6
Alanine	24.2	23.0	− 1.2
Valine	17.0	19.2	+ 2.2
Methionine	4.0	4.0	
Isoleucine	5.4	6.0	+ 0.6
Leucine	17.0	15.2	− 1.8
Tyrosine	3.8	3.8	
Phenylalanine	5.4	5.2	

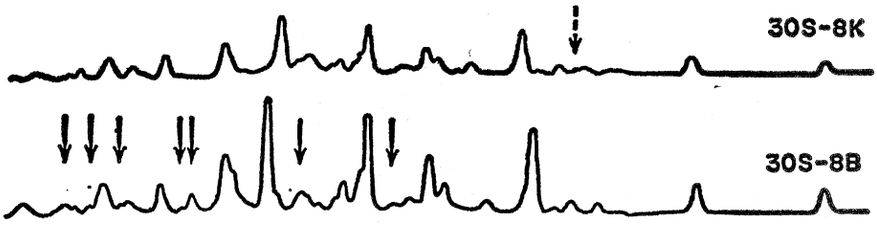

Fig. 1. Chromatography (Dowex 50) of the tryptic peptides of proteins 30*S*-8B and 30*S*-8K (*4*). The arrows mark peaks which occur in one pattern and not in the other.

The relatively greater content of arginine and lysine in 30*S*-8B is also reflected in the tryptic peptide pattern of this protein, which is compared to that of 30*S*-8K in Fig. 1. There are several more tryptic peptides present in 30*S*-8B than there are in 30*S*-8K. We have also observed a few minor peptides in 30*S*-8K which are different from those in 30*S*-8B. Finally, the tryptic peptides of a different protein, 30*S*-9, have been analyzed with samples obtained from B and K strains. These two homologous proteins are indistinguishable.

If it is assumed that the four methionines of 30*S*-8 are all internal amino acids, then cyanogen bromide digestion of both 30*S*-8B and 30*S*-8K should yield five major peptides, with one or more of those from 30*S*-8B more basic than the homolog of 30*S*-8K. This prediction has been verified.

While four of the cyanogen bromide fragments from these two proteins are indistinguishable, one of them is significantly different in the two proteins. This observation suggests that the amino acid replacements responsible for the different charges of the two homologous proteins are restricted to one region of protein 30*S*-8.

All the data indicate that proteins 30*S*-8B and 30*S*-8K have different amino acids at several positions in their primary sequences. Therefore, the K locus is the first positively identified genetic determinant for the primary structure of a ribosomal protein.

E. A. Birge, G. R. Craven
S. J. S. Hardy, C. G. Kurland
P. Voynow
Departments of Zoology and *Genetics* and *Laboratory of Molecular Biology, University of Wisconsin, Madison*

References and Notes

1. P. Traub and M. Nomura, *Science* **160**, 198 (1968)
2. P. S. Leboy, E. C. Cox, J. G. Flaks, *Proc. Nat. Acad. Sci. U.S.* **52**, 1367 (1964).
3. S. J. S. Hardy, C. G. Kurland, P. Voynow, G. Mora, *Biochemistry*, in press.
4. G. Craven, P. Voynow, S. J. S. Hardy, C. G. Kurland, *ibid.*, in press.
5. Supported by PHS grants GM-13832, GM-12411, and 5-P01-GM-15422. E.A.B. and P.V. are predoctoral fellows of NIH and S.J.S.H. is supported in part by funds from the Wisconsin Alumni Research Foundation. We thank R. Erickson, K. Mellenberger, and L. Wollangk for technical assistance.

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Vibrio parahaemolyticus from the Blue Crab Callinectes sapidus in Chesapeake Bay

Abstract. *Strains of* Vibrio parahaemolyticus, *the etiologic agent of "Shirasu" food poisoning in Japan, were isolated from moribund blue crabs* Callinectes sapidus *and identified by biochemical and serological techniques.*

Since 1950, when Fujino *et al.* (*1*) isolated a new species of bacteria during an outbreak of "Shirasu" food poisoning, a series of papers has appeared describing the taxonomy and epidemiology of the etiologic agent *Vibrio parahaemolyticus* (*2*). The first recorded isolations of *V. parahaemolyticus* in the United States reported this species in seawater, sediments, and shellfish from the Puget Sound region (*3*). From estuarine sediments collected from the Gulf of Mexico and southern Atlantic coasts, Ward (*4*) isolated organisms related serologically to *V. parahaemolyticus*.

During studies of mortality in populations of the blue crab *Callinectes sapidus*, strains of bacteria *Vibrio parahaemolyticus* were isolated from lethargic and moribund crabs being retained in commercial tanks during "shedding" of soft crabs. Mortality of crabs in some tanks was in excess of 50 percent. Dead animals did not have the signs or the etiological agent associated with the "gray crab" disease (*5*). Animals from which bacteriological samples were taken were abnormally weak, and examination of their hemolymph revealed large numbers of bacteria. Broken claws and appendages consistently contained necrotic, liquefied tissue, and large numbers of bacteria were found in smears examined with the phase microscope.

Inoculation of brain-heart infusion agar (Difco, NaCl added to adjust final salt concentration to 1.5 percent) with material from necrotic claws and with hemolymph removed aseptically from 16 animals permitted isolation of a number of strains of bacteria. Of 28 pure cultures selected from the most abundant colony types that appeared on the brain-heart infusion agar, 21 cultures were identified as *Vibrio parahaemolyticus*.

Identification procedures included taxonomic analysis by computer with 210 coded features. The 21 *V. parahaemolyticus* strains clustered at *S* equal to 82 percent with known strains (*6*). This degree of similarity is well above that usually accepted for species (*7*).

The base composition of the DNA of *V. parahaemolyticus* isolates from blue crabs ranged from 44 to 46 mole percent of guanine plus cytosine. Base compositions were determined by melting temperature measurements of purified DNA (*8*). This range is well within that determined for *V. parahaemolyticus* in a previous study (*9*). Serological confirmation of the diagnosis was made by slide agglutination tests with antiserums prepared against K antigens (*10*).

Strains of *V. parahaemolyticus* isolated from diseased blue crabs demonstrated lipase and lecithinase activity and were capable of liquefying gelatin and hydrolyzing casein. These properties may contribute to the invasiveness of the bacteria.

Previous reports on *V. parahaemolyticus* described its involvement in outbreaks of food poisoning in humans. Epidemiologic studies of "Shirasu" in Japan have suggested the existence of a nonhuman reservoir of *V. parahaemolyticus*, and Akazawa (*11*) reports frequent isolation of this bacterium from diseased marine and estuarine fishes.

This is the first isolation of *Vibrio parahaemolyticus* from diseased crabs and from the Chesapeake Bay region. *Vibrio parahaemolyticus* is very likely