utes). Heart rate was continuously monitored through two electrodes implanted subcutaneously and bilaterally on the chest and read out on an oscilloscope.

Approximately 80 percent of the reactive hemoglobin amino groups in the experimental animals had been carbamoylated after 2 weeks of ingestion of the water containing 0.5 percent NaOCN (Table 1). Simultaneously, p_{50} was reduced from 37.3 mm-Hg in the controls to 21 mm-Hg in the experimental animals (Table 1). No significant differences between the two groups were observed in hemoglobin or hematocrit value, but red cell DPG concentrations were somewhat lower in the experimental animals. Exposure of the rats to the reduced atmospheric pressure revealed highly significant differences in both heart rate (Fig. 1A) and survival (Fig. 1B). Throughout, experimental animals consistently displayed a much slower heart rate than did surviving control rats, which probably reflected less severe hypoxemia in the experimental animals. Furthermore, all of the treated animals survived the 90-minute trials, whereas eight of ten control animals died. The differences between the two groups in percent survival after 90 minutes is highly significant ($\chi^2 = 11.28$ with Yates's correction for continuity, $P < .001$). All experimental animals had an uneventful recovery from the exposure and remained alive until they were killed at a much later date. Because cyanate will react with the amino groups of many proteins, we recognize the possibility that the functional characteristics of other proteins may have been altered. However, it is most likely that the protective effect of cyanate which we have found is specifically due to a cyanate-induced alteration of hemoglobin-oxygen affinity.

Hemoglobin-oxygen affinity is decreased both in humans indigenous to high altitude areas (*7*) and in newcomers after exposure for about 12 to 24 hours (*8*). Although appropriate to most forms of hypoxia encountered by man at lower altitudes, this diminished oxygen affinity may be of no adaptive value in high altitude hypoxemia. At an altitude of 4,540 m (14,900 feet), arterial saturation in healthy human males is approximately 80 percent (arterial $po_2 = 45$ mm-Hg) and venous saturation is roughly 65 percent (*7*). In this "steep" portion of the oxygen dissociation curve, any increase in oxygen delivery to tissue gained by decreased hemoglobin-oxygen affinity will be accompanied by an almost equal loss in arterial oxygen saturation. Our results demonstrate that increased, rather than decreased, oxygen affinity is an effective mode of short-term adaptation to markedly reduced environmental oxygen pressures. This may prompt a reevaluation of the idea (*7–9*) that decreased hemoglobin-oxygen affinity is of adaptive value to humans at high altitudes.

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4. We estimated p_{50} from percentage oxyhemoglobin at $po_2 = 27.1$ mm-Hg ($pco_2 = 40$ mm-Hg), assuming no variation in Hill's parameter n. Since the average pH of whole blood following equilibration with the standard gas mixture did not differ between the two groups, values for p_{50} were corrected to pH 7.40 by using the standard Severinghaus nomogram.
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Bacteriophage Structure: Determination of Head-Tail Symmetry Mismatch for Caulobacter crescentus Phage φCbK

Abstract. *Electron micrographs of negatively stained bacteriophage φCbK have been analyzed by Fourier methods. Computer-calculated Fourier transforms that contain phase as well as magnitude information have established fivefold rotational symmetry for the head and threefold rotational symmetry for the tail. These results indicate that a symmetry match is not necessarily required between separate structural components of a bacteriophage.*

Simple viruses are constructed from multiple copies of one or a small number of protein subunits. These proteins bind noncovalently in a process analogous to crystallization to form a three-dimensional shell that functions to contain and protect the viral nucleic acid. The angles and positions of bonding contracts which any one of these proteins can make with another are quite specific and, thus, symmetry considerations are of great importance in determining the size and shape parameters of the protein coat or capsid for simple viruses and may also control the self-assembly in vivo. For isometric or "spherical" viruses, Caspar and Klug (*1*) proposed a scheme for packing of the subunits into a capsid with icosahedral point group symmetry. This scheme, which places constraints on the number of packing units making up the viral structure, has been repeatedly verified for many spherical viruses. The rod-shaped viruses, such as the plant viruses, and the filamentous bacteriophages are constructed from structural proteins organized with helical symmetry and are apparently limited as to size by the length of the nucleic acid which they encapsulate.

The tailed bacteriophages are one step further in structural complexity from the simplest viruses and contain structurally differentiated components with functionally distinct roles. Again the separate components of the structure are assembled from many copies of one or a few identical subunits, and symmetry, very likely, plays an important part in their interaction. However, it is not known what symmetry principles may be involved in the interaction of structurally distinct com-

ponents of the phage or whether a symmetry match is a requirement. Thus, for example, in the most obvious case, which is the symmetry relatedness of the bacteriophage tail and head about their point of junction, arguments have been put forward for both symmetry match (*2*) and symmetry mismatch (*3*). We report the results of structural studies on both the head and tail of bacteriophage ϕCbK which indicate that a rotational symmetry correspondence between these linked substructures is not necessarily required.

ϕCbK is a large bacteriophage, consisting of a prolate cylindrical capsid, about 200 nm in length by 60 nm in diameter, to which is attached a flexible noncontractile tail, about 290 nm in length (*4*) (Fig. 1A). Each bacteriophage contains one length (60 μm) of linear, double-stranded DNA. Clear optical transforms can be obtained from electron micrographs of both the empty capsid (*5*) and linear regions of the tail (*6*). Analysis of optical diffraction patterns enabled us to determine lattice parameters for the capsid and to restrict the helical parameters of the tail to three possible alternatives. In both instances it was impossible to ascertain unambiguously the order of rotational symmetry about the longitudinal axis of the phage. This ambiguity was resolved by utilizing Fourier phase information that is contained in the computer-calculated Fourier transform of the image, but which is not accessible in the optical transform.

The determination of the helical structure for the bacteriophage tail has been described (*6*). We summarize here the results of computer image analysis only insofar as they affect the determination of symmetry. The optical and computer Fourier transforms for the tail show Fourier amplitude distributed on layer lines as is expected for a helical structure (Fig. 1, C and D). An estimate can be made for the order of rotational symmetry about the helix axis by measuring the separation of reflections placed symmetrically about the meridian of the transform. Since this requires a value for the average radius of the helix, which is somewhat ill-defined in the electron micrographs, the rotational symmetry could not be determined unambiguously, but was estimated to be 2, 3, or 4. An unequivocal choice between these alternatives was made possible by examining the phase behavior of the layer line maxima in the computed transform. Determination of helical symmetry from the phase behavior of computer calculated transforms is a comparison of the structure of the back of a helix with that derived from the front (*7*), and can be ascertained by comparing the phases of reflections symmetrically displaced on either side of the meridian (*8*). If the rotational symmetry is even, reflections on all layer lines must have the same phases on either side of the meridian. If the rotational symmetry is odd, however, reflections on a given layer line can either differ in phase by 180° about the meridian or they can have the same phase. The phases on the layer lines $l = 8$ and $l = 15$ (Fig. 1D) are 180° out of phase, and thus the helix must have odd rotational symmetry, which from previous measurements can only be threefold.

In the earlier study of the optical diffraction patterns obtained from the images of bacteriophage capsids (*5*), analysis of the patterns suggested a value of 5 or 6 for the symmetry order about the head-tail attachment axis. This estimate was obtained by dividing the structural repeat in the equatorial

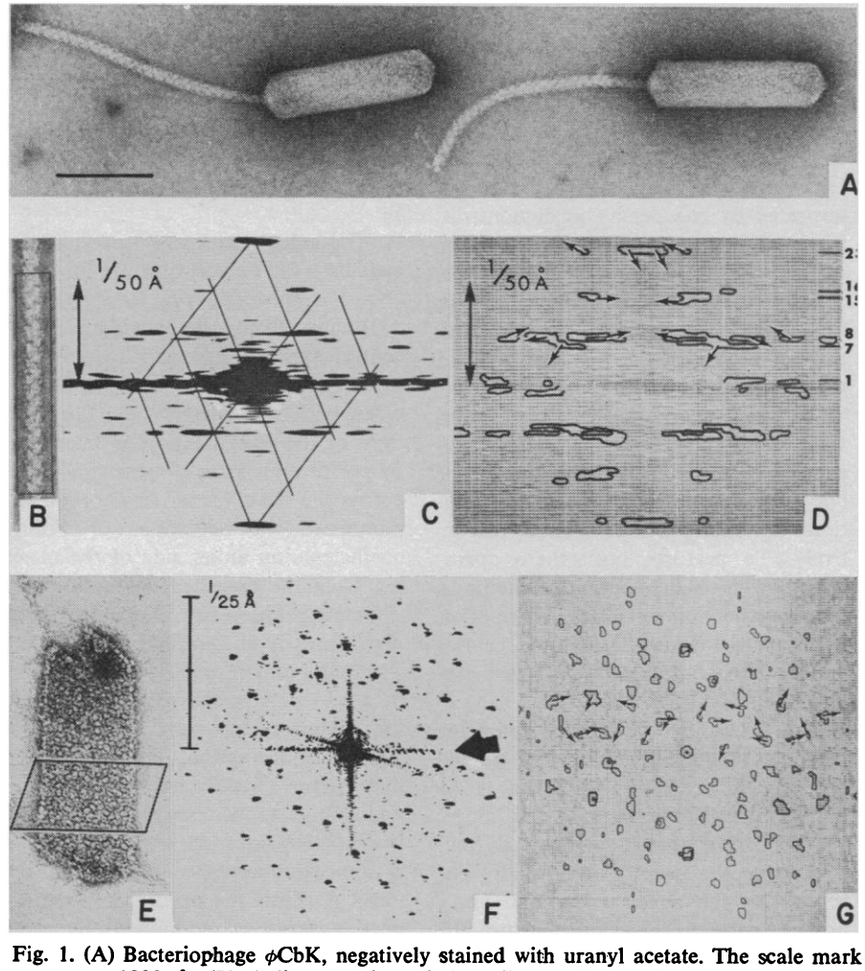

Fig. 1. (A) Bacteriophage ϕCbK, negatively stained with uranyl acetate. The scale mark represents 1000 Å. (B) A linear region of the tail (× 267,000). The rectangular area was used for optical diffraction. A length of the tail two repeats long was selected for computer Fourier transformation. (C) The optical diffraction pattern of (B). The reciprocal net corresponding to one side of the structure has been drawn in. (D) The computer-calculated Fourier transform for the tail image in (B). The numerical computer output has been contoured to show the principal maxima. The phases of key reflections are indicated by the directions of the small arrows. (An arrow pointing to the right indicates a phase angle of zero, a vertical arrow corresponds to a phase of $\pi/2$, and so forth.) The layer lines are numbered at the right of the transform. (E) An empty capsid, negatively stained with uranyl acetate (× 193,000). The area included within the parallelogram was used for computer Fourier transformation. (F) The optical transform of the image in (E). The large arrow indicates the equatorial diffraction arising from the parallel edges of the capsid. The masking aperture has been set obliquely to the capsid axis so that its diffraction spike is inclined to the equator. (G) The computer-calculated Fourier transform of (E) contoured to show the principal Fourier maxima. Phase angles are represented by the direction of the arrows.

direction of the capsid into the circumference of the particle measured from the electron micrographs both of phages that contain DNA and of empty phages. The uncertainty in the circumference again introduced an ambiguity into the determination of symmetry order. To resolve this ambiguity, Leonard *et al.* (*5*) examined the moiré patterns produced by overlap of the two sides of flattened capsids and concluded that the superposition patterns possess glide planes of symmetry along the axis of the heads. This is only possible (*9*) if the cylindrical lattice present in the uncollapsed head has odd (fivefold) rotational symmetry. Although the images were interpreted correctly, direct visual assessment of symmetry in this way has two shortcomings. First, visual verification of a glide line is relatively subjective, with the result that features in the image which can be interpreted by one person as demonstrating a glide line may be looked upon by another as showing a mirror line. Second, the choice of center line for the image, corresponding to the position in projection of the long axis of the head, must be established exactly. Since the superposition pattern contains both mirror and glide lines, whichever of these coincides with the center line will determine whether the rotational symmetry is even or odd, respectively. To perform both these operations, we devised quantitative methods, utilizing the Fourier phase information to fix both the position of the central axis and the position of the symmetry lines.

The determination of the center-line position for the capsid was first carried out with the use of the equatorial terms in the Fourier transform of the image. In the optical transforms, this diffraction along the equator is normally obscured by the strong equatorial spike arising from the masking aperture. If, however, the mask is tilted slightly, the strong equatorial diffraction that arises from the meniscus of stain along the edge of the capsid becomes apparent (Fig. 1B). Because this meniscus of negative stain defines the edges of the capsid (*10*), it is symmetrical about the capsid center line. By choosing an origin for the computer Fourier transform which most nearly (in a least-squares sense) makes the equatorial diffraction terms real, we have found it possible to define the center line to within a distance of approximately 1 Å. The calculated root-mean-square residual Q (*11*) as a function of origin choice is shown in Fig. 2A.

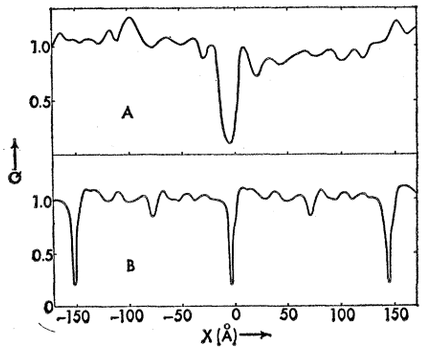

Fig. 2. (A) Computer search for the position of the center line of the capsid. The center line is about 7 Å to the left of that selected initially by eye. (B) Computer search for the position of lines of glide symmetry in the equatorial direction of the capsid. The position of the central glide coincides almost exactly with that determined for the head center line, thus confirming that the capsid has fivefold rotational symmetry.

Having fixed the position of the central axis, we had only to determine whether a glide or a mirror line coincides with the center line. The positions of the glide lines were determined by performing a one-dimensional search across the head for a phase origin that minimized the deviations of Fourier coefficients on either side of the meridian from the expected values for glide symmetry. The result of the search for glide lines is shown in Fig. 2B (*12*). The minimum residual Q was obtained at $x = -7$ Å, which is within 1 Å of the center-line position determined by equatorial diffraction. The positions of the mirror lines were also searched and found to lie exactly halfway between glide lines, as was expected, or at approximately $x = -82$ Å and $x = +68$ Å. Thus the rotational symmetry of the capsid is unambiguously established as being odd, and therefore is fivefold. This method was accurate and objective and appears to have general applicability to this type of image where symmetry elements of a finite structure have to be determined.

Our results thus indicate that symmetry correspondence is not required in the case of head-tail attachment in this bacteriophage. The capsid has fivefold rotational symmetry and can be readily capped at each end by a pentagonal pyramid. This structure is a modified $T = 7$ icosahedral shell (*1*) which has been elongated in one direction. The tail structure is a three-start helix with threefold rotational symmetry about its axis. We are thus faced with the question of the manner in which structural integrity can be maintained at the point where one vertex of the head (fivefold symmetric) joins the end of the tail (threefold symmetric) which is proximal to it. In considering the same problem for *Escherichia coli* T-even phages, where a definite structure for the head has not so far been established but where the tail is known to have sixfold rotational symmetry (*9, 13*), Moody (*3*) has proposed that the hypothetical symmetry mismatch could be overcome by interposing an adapter of composite symmetry between the two structures.

A specialized collar structure is frequently observed at the head-tail junction point and on isolated tails in images of ϕCbK, which may serve as such an adapter. Alternatively, if it is part of the tail structure, this collar may simply provide a nonspecific mechanical linkage to the head. This would be the case if it were located within the capsid and if it were too large to pass through the opening in the wall at the position of tail attachment. Minor proteins have been found by sodium dodecyl sulfate gel electrophoretic analysis (*5*), which seem to be consistent with either of these two hypotheses. To answer this question, it will be necessary to study the head-tail attachment region in more detail and to attempt to determine the source of the minor proteins more exactly.

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11. The center line of the capsid was determined by minimizing the function

$$Q = A\Sigma(F - F_{real})^2/\Sigma(F + F_{real})^2$$

where F is the computer-calculated, equatorial Fourier transform; F_{real} is the real part of F; and A is a normalization factor such that a value of $Q = 1.0$ corresponds to random agreement between F and F_{real}.

12. The position of the glide line was determined by minimizing the function $Q = A\Sigma[F_{left} - F_{right} \cdot \exp(i\pi kb)]^2/\Sigma[F_{left} + F_{right} \cdot \exp(i\pi kb)]^2$, where F_{left} and F_{right} are the Fourier coefficients symmetrically related to the left and right sides of the meridian; b is the repeat distance in the direction of the glide, and A is the normalization factor. The position of the mirror line was determined by minimizing $Q = A\Sigma(F_{left} - F_{right})^2/\Sigma(F_{left} + F_{right})^2$ where all symbols are defined as above.

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14. We thank M. Pendergast for assistance with the electron microscopy. Supported by NSF grant GB-38354X to J.A.L. Samples of bacteriophage ϕCbK were provided by Drs. Lucille Shapiro and Nina Agabian-Keshishian.

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Social Facilitation and Development in Ephestia kühniella Z.

Abstract. *Total time required for larval and pupal development in* Ephestia kühniella *Z. was significantly modified when habitable space of the food mass was increased by dilution with a nontoxic sawdust. Doubling the living space resulted in an increased developmental rate, presumably due to a reduction in the number of larval interactions. Tripling the living space, however, produced a somewhat unexpected delay in development, accompanied by a marked increase in variance about the means for both males and females.*

Studies of the development of *Ephestia kühniella* Z. (Mediterranean flour moth) from egg deposition to adult eclosion have shown that, within limits, an inverse proportionality exists between available food and the average time required for complete development. Other effects that have been related directly to an increase in population density in a food mass are an increase in mortality and decreases in weight, length of body, and wing length (*1*). Moreover, crowding is accompanied by a reduction in egg (or progeny) production (*2*). Still, these phenomena are only a partial function of the size of the food mass; some cornmeal remains in excess after all larval feeding has been completed in all cultures, regardless of the degree of crowding.

It has been suggested (*3*) that by-products of development (such as excreta) that accumulate in the food mass are contacted increasingly through ingestion or chemoreception in crowded cultures, and thus indirectly influence the growth of individual larvae. A similar phenomenon has been observed in *Kalotermes flavicollis*; the maintenance of the polymorphism of this social termite is dependent on direct contact, and pheromone transfer, between individuals (*4*). Direct observations of crowded meal moth cultures have shown that physical encounters between larvae increase as a result of crowding (*5*). Resulting from each contact is a burst of activity that may include biting, butting, or rapid movement through the food tunnel. These behavioral responses interfere with quiet feeding and food storage.

Hence, the thesis was developed that increasing habitable space while holding the amount of food (cornmeal) per animal constant would decrease the likelihood of chance encounters, and thus decrease both first-order effects (direct physical contact) and second-order effects (chemical perception of metabolites) between the individual larvae. A reduction in the avoidance or aggressive responses to other feeding larvae could then result in a reduction of the time required for the larval phase of development, and thus for total developmental time from egg deposition to eclosion of the adult moth.

A nontoxic substance, basswood sawdust, was used to dilute the cornmeal, either one or two volumes of sawdust per volume of cornmeal, and thus double or triple the living space. The test animals were F_1 hybrids of the *aa0* (red eye) and *wawa0* (white eye) stocks. Newly hatched larvae (less than 4 hours after emergence) were isolated, and 360 were transferred in groups of ten to three series of plastic bowls, 9.0 cm in diameter. The sawdust for the 1 : 1 or 1 : 2 dilutions was mixed thoroughly with the standardized volume of food before being poured into the bowl containing the larvae. The food was commercially available enriched and degerminated cornmeal; each volume contained 9.7 g, or approximately 1.0 g per larva. Vented soft plastic snap lids were fitted to each bowl before shelving. An experimental set consisted of three bowls that were filled in sequence with food only (control), food-sawdust 1 : 1, and food-sawdust 1 : 2. Twelve replicate sets were established on the same day and were maintained on the same shelf in a darkened room at 20 ± 1°C. After the onset of pupation, the cultures were surveyed daily at 0900, and all adults were collected, sexed, and scored. The data of the daily collections, which were separated by sex and pooled, are presented in Fig. 1.

For the determination of the eclosion distributions, day 1 was taken as the first day when adults appeared in any of the 36 culture bowls; the first adults in some cultures were not recorded until day 2 or 3. In the control (food only) cultures, the mean time required for eclosion of all adults was shorter for females (group A) than for males (group D) (Fig. 1), but the difference was not significant (*t*-test, $.10 < P < .50$).

The sawdust used to dilute the food mass apparently had minimal toxicity. Of 240 larvae introduced into bowls containing cornmeal diluted with sawdust, 239 were obtained as adult moths (< 1 percent mortality), whereas only 112 adults of the initial 120 first-instar larvae completed eclosion in the control cultures with undiluted cornmeal

Table 1. Values of t and P for t-test comparisons of the mean times of eclosion for males and females in the control (groups A and D) and experimental sets of cultures of *Ephestia kühniella*. The cornmeal-sawdust ratio was 1 : 1 for groups B and E and 1 : 2 for groups C and F.

Group and sex	Group and sex									
	A♀		B♀		C♀		D♂		E♂	
	t	P	t	P	t	P	t	P	t	P
B♀	1.33	.10–.50								
C♀	3.78	< .01	5.33	< .01						
D♂	1.12	.10–.50								
E♂			0.22	.50			3.32	< .01		
F♂					1.36	.10–.50	1.48	.10–.50	3.68	< .01