confines the adjustment process to one quadrant of directions.

The influence of vision on auditory localization apparently decreases with age. Before an owl is 50 to 60 days old, it can adjust its auditory error in any direction, presumably guided by vision (*1*). In the experiments presented here, in which the owls were between 75 and 220 days old, younger birds made larger and more rapid adjustments than did older birds (for example, compare Fig. 2B with 2C), but in no case could an owl be induced to change the sign of its auditory error. Finally, adult owls (more than 7 months old) maintain auditory localization errors indefinitely, even when they experience normal vision (*1*). Thus, the ability of vision to generate a corrective force, or the ability of the auditory system to respond to it, or both, diminish with age. This corrective force exerted by the visual system on the auditory system provides a mechanism for fine-tuning the associations that underlie auditory localization during development.

References and Notes

1. E. I. Knudsen, S. D. Esterly, P. F. Knudsen, *J. Neurosci.* **4**, 1001 (1984); E. I. Knudsen, P. F. Knudsen, S. D. Esterly, *ibid.*, p. 1012.
2. J. D. Pettigrew, *Proc. R. Soc. London Ser. B 204*, 435 (1979); E. I. Knudsen, G. C. Blasdel, M. Konishi, *J. Comp. Physiol.* **133**, 1 (1979).
3. J. R. Lackner, *Neuropsychologia* **11**, 29 (1973); B. R. Shelton and C. L. Searle, *Percept. Psychophys.* **28**, 589 (1980); D. H. Warren, R. B. Welch, T. J. McCarthy, *ibid.* **30**, 557 (1982).
4. Long-term monaural occlusion was accomplished by suturing a dense, foam-rubber plug (E.A.R. Corporation) into the external meatus while the animal was anesthetized with halothane and nitrous oxide.
5. The method used in this study has been described in detail (*1*). Briefly, we measured auditory localization by comparing the accuracy with which an owl oriented its head to auditory and to visual stimuli. The stimuli were presented from a remotely controlled, movable speaker and light source, which were positioned at a new, random location before every trial. Auditory stimuli consisted of repeated noise bursts presented at 10- to 50-dB sound-pressure level (re 20μPa). Visual stimuli consisted of a continuous glow from a light-emitting diode. A trial consisted of a presentation of either the auditory or the visual stimulus; the final head orientation (as indicated by an infrared beam reflected from a mirror mounted on the owl's head) relative to the true location of the stimulus was recorded. The stimulus continued until the animal settled on a particular direction. A test session included 15 to 25 responses to auditory stimuli and an equivalent number to visual stimuli. When an owl responded to a stimulus with a quick movement of the head followed by a steady fixation, it was given a food reward. Because the reward was not contingent on the location to which the owl oriented, the reward did not bias the localization response.
6. Owls were anesthetized with halothane and nitrous oxide, and a metal plate was secured to the skull with screws and dental cement. The spectacle frames were bolted to this plate. Each frame was 10 mm in diameter and permitted approximately a 70° field of view as determined by retinoscopy. The frames did not physically obstruct or interfere with the external ears.
7. The auditory localization error was defined as the difference between the owl's mean response to the acoustic stimulus, based on 15 to 25 responses measured that day, and the reference value, which was based on more than 100 visual responses recorded before prisms or occluders were installed. The vertical component of the barn owl's auditory error results from the fact that, due to an asymmetry in its external ears, interaural intensity differences indicate the vertical location of the sound source.
8. The prisms were 20-diopter Fresnel (Optical Sciences Group). They were oriented in the spectacle frames through the use of an optical bench. Their orientation on the animal was determined by comparing the visual responses of the bird before and after mounting the spectacles.

9. We thank J. Middlebrooks, S. du Lac, and S. Esterly for reviewing the manuscript. Supported by grants from the March of Dimes (1-863), Sloan Foundation, the National Institutes of Health (RO1 NS 16099-05), and a neuroscience development award from the McKnight Foundation.

29 April 1985; accepted 24 July 1985

Evolution of Bunyaviruses by Genome Reassortment in Dually Infected Mosquitoes (*Aedes triseriatus*)

Abstract. Aedes triseriatus *mosquitoes became dually infected after ingesting two mutants of LaCrosse (LAC) virus simultaneously or after ingesting, by interrupted feeding, the two viruses sequentially within a 2-day period. After 2 weeks of incubation, approximately 25 percent of the vectors contained new virus genotypes as the result of RNA segment reassortment. New viruses were transmitted when the mosquitoes fed on mice. Viruses ingested more than 2 days after the initial infecting virus did not cause superinfection of the mosquito vectors.*

BARRY J. BEATY
DANIEL R. SUNDIN
LAURA J. CHANDLER
Department of Microbiology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins 80523
DAVID H. L. BISHOP
Natural Environmental Research Council, Institute of Virology, Oxford, OX1 3SR, United Kingdom

The bunyavirus genome consists of three negative-strand RNA segments, designated L (large), M (medium), and S (small) (*1*). The M RNA segment codes for the two virion glycoproteins (G1 and G2) and a nonstructural protein (NS_M). The S RNA codes for the nucleocapsid protein and a nonstructural protein (NS_S). The L RNA presumably codes for a polymerase (*1*). The segmentation of the genome provides a mechanism for natural evolution of bunyaviruses by reassortment of RNA segments as described for influenza viruses (*2*). Bunyavirus segment reassortment has been reported in vitro, in vivo in mosquitoes, and in nature (*1, 3*). Either the vertebrate host or the vector could be the site for reassortment. Studies to demonstrate bunyavirus genome reassortment in vertebrates have been unsuccessful. In contrast, high-frequency reassortment was detected when mosquitoes (*Aedes triseriatus*) were intrathoracically inoculated with La Crosse (LAC) virus and snowshoe hare (SSH) bunyaviruses (*3*). Thus, it seemed that the vector would be the site of segment reassortment in nature. However, subsequent studies complicated this hypothesis; interference to superinfection was demonstrated between related bunyaviruses in intrathoracically inoculated mosquitoes (*4*). Intrathoracic inoculation is not a natural route of infection. However, if mosquitoes became resistant to superinfection by natural routes, opportunities for dual infection in nature would be limited. Many mosquitoes exhibit a behavior called interrupted feeding, which could preclude interference (*5*). If defensive behavior of the host interrupts the mosquito during engorgement, the vector may finish engorgement on an alternate host. Thus, a mosquito could ingest a blood meal from two different vertebrate hosts viremic with two different viruses in a period of time short enough to preclude interference. A series of experiments was conducted to

Table 1. Phenotype of stock viruses after passage in mosquitoes.

Virus	Meal titer*	33°C		40°C	
		Infection frequency†	Mean titer‡	Infection frequency	Mean titer
LAC wt	7.0–7.3	5/5	1.6×10^5	5/5	1.1×10^5
LAC-II-5§	6.8–7.1	5/5	1.7×10^5	0/5	<10
LAC-I-16	6.5–6.6	5/5	9.9×10^4	0/5	<10

*Log_{10} [tissue culture infective dose $(TCID)_{50}$ per milliliter]. Range of titers of viruses used to infect mosquitoes in three experiments. †Number infected/number tested. ‡Plaque-forming units per mosquito. §The ts mutant viruses have been assigned to complementation-recombination groups. Group II mutants contain a ts lesion in the L RNA segment; group I mutants contain the ts lesion in the M RNA segment (*1*). The permissive temperature is 33°C; the nonpermissive temperature is 40°C.

determine (i) if and when interference to oral superinfection occurs in mosquitoes, (ii) if dually infected mosquitoes could serve as a site for RNA segment reassortment, and (iii) if newly generated reassortant viruses could be transmitted to vertebrates.

To determine if mosquitoes could become dually infected subsequent to ingestion of two viruses and to determine possible temporal limitations of the phenomenon, *A. triseriatus* were permitted to ingest a partial blood meal containing a temperature-sensitive (ts) mutant virus, LAC-I-16 (Table 1). At predetermined times, the mosquitoes were permitted to engorge on a meal containing wild-type (wt) LAC virus (Table 2). One group of mosquitoes ingested LAC-I-16 and wt virus in the same meal. For controls, mosquitoes ingested either a ts mutant virus (LAC-I-16 or LAC-II-5) or wt virus. After 14 days' incubation, mosquitoes were triturated and assayed at permissive (33°C) and nonpermissive (40°C) temperatures. The titers of the meal preparations used to infect the mosquitoes are shown in Table 1. All virus preparations resulted in infection of 100 percent of the mosquitoes ingesting the respective meal. The viruses were phenotypically stable; mosquitoes infected with ts virus contained no demonstrable wt virus when assayed at the nonpermissive temperature (Table 1). Mosquitoes that ingested the two viruses simultaneously or within 24 hours of each other consistently became dually infected; 100 percent (49/49) of the mosquitoes that ingested wt LAC within 4 hours of LAC-I-16 contained wt virus (Table 2). In contrast, only 27 percent (3/11) of the mosquitoes that ingested wt LAC virus 48 hours after LAC-I-16 contained wt virus. A greater elapsed time between the two meals resulted in all mosquitoes becoming refractory to superinfection.

To determine if mosquitoes dually infected orally could serve as a site for RNA segment reassortment, we conducted two basic experiments: simultaneous dual infection and dual infection via interrupted feeding. In the simultaneous infection experiments, *A. triseriatus* engorged either LAC-I-16 and wt LAC or LAC-I-16 and LAC-II-5 viruses in the same meal. In the interrupted feeding experiments, the mosquitoes were permitted to engorge partially on a blood meal containing LAC-I-16 virus. Approximately 2 hours later, the mosquitoes were permitted to engorge to repletion on a meal containing wt LAC or LAC-II-5 virus. Mosquitoes were retained for 14 days and then assayed. At this time, some of the mosquitoes were permitted to engorge upon suckling mice to determine if newly generated virus genotypes could be transmitted.

Table 2. Interference to oral superinfection of *A. triseriatus*. Data were compiled from three experiments. Mosquitoes first ingested LAC-I-16 virus (titers ranged from 6.5 to 7.8 $\log_{10}$ ($TCID_{50}$/ml). Mosquitoes then ingested either a partial meal to repletion or a full meal containing wt LAC virus [titers ranged from 7.0 to 7.8 $\log_{10}$ ($TCID_{50}$/ml)]. Mosquitoes were then kept for 14 days before assay. All mosquitoes assayed at 33°C were infected.

Time until ingestion of challenge virus	Infection frequency*	
	33°C	40°C
Simultaneous	15/15	15/15
30 minutes	8/8	8/8
2 hours	19/19	18/19
4 hours	7/7	7/7
1 day	18/18	11/18
2 days	11/11	3/11
7 days	6/6	0/6
14 days	5/5	0/5
21 days	3/3	0/3
28 days	4/4	0/4

*Number infected/number tested.

In the simultaneous infection studies, all mosquitoes that engorged a meal containing LAC-I-16 and LAC wt virus contained wt virus after 14 days incubation (Table 3). It should be noted that 25 percent of those engorging two ts mutant viruses contained wt virus (Table 3). Thus, segment reassortment between the two ts viruses had occurred when these mosquitoes were simultaneously infected with the two viruses.

In the interrupted feeding experiments, virus was detected at the nonpermissive temperature in all but one mosquito challenged with wt LAC (Table 3). Thus, there was virtually no interference to superinfection at 2 hours post-engorgement. When mosquitoes first ingested LAC-I-16 and then ingested LAC-II-5 ts mutant viruses, 20 percent contained wt virus (Table 3). Thus, reassortment had occurred after dual infection by interrupted feeding.

Since segment reassortment would be epidemiologically significant only if virus was transmitted, a study was conducted to determine if new viruses could be orally transmitted. After 14 days of incubation, mosquitoes from the experiments described previously were permitted to feed in groups of five to seven on baby mice. Brains were extracted from moribund and dead mice and assayed for virus at permissive and nonpermissive temperatures. Wild-type virus was isolated from mice that had been fed upon by mosquitoes that had ingested LAC-I-16 followed by wt LAC virus. Thus, prior infection with one virus did not inhibit transmission of the second virus. Wild-type virus was also isolated from mice that had been fed upon by mosquitoes that had ingested LAC-I-16 then LAC-II-5 viruses. Therefore, a new virus had been generated in the dually infected mosquitoes and the new genotype was transmitted. As related previously, attempts to generate reassortant viruses in suckling mice after dual infection have been unsuccessful. Thus, reassortment most likely occurred in the dually infected mosquito. As in the mosquitoes themselves, no wt virus was detected in mice fed upon by vectors that had ingested one of the ts mutant viruses.

We have demonstrated that mosquitoes can become dually infected when ingesting two bunyaviruses simultaneously or by interrupted feeding. After dual oral infection, the mosquito can serve as a site for reassortment of bunyavirus genomes. These new viruses can be transmitted by bite to vertebrates. Should two different bunyaviruses be able to replicate in the same mosquito host, there is a real possibility that a new reassortant virus could be generated, transmitted, and established in the original or in a new niche in nature. The M RNA segment of LAC virus is a major determinant of LAC virus infection and transmission by *A. triseriatus*, viremia in vertebrate hosts, and neurovirulence in mice (*6*). LAC virus is a major cause of encephalitis in children in the United States, but other bunyaviruses, which

Table 3. Dual infection of *A. triseriatus* and generation of reassortant bunyaviruses.

Dual-infection procedure	33°C		40°C	
	Infection frequency*	Mean titer†	Infection frequency	Mean titer
Simultaneous feeding				
LAC-I-16 and LAC wt	15/15	4.7×10^4	15/15	1.9×10^4
LAC-I-16 and LAC-II-5	8/8	3.1×10^4	2/8	4.9×10^3
Interrupted feeding				
LAC-I-16 then LAC wt	19/19	2.8×10^4	18/19	1.6×10^4
LAC-I-16 then LAC-II-5	20/20	3.3×10^4	4/20	6.3×10^2

*Number infected/number tested. †Plaque-forming units per mosquito.

could potentially reassort with LAC virus, infect humans much more frequently (*7*). Since LAC virus is sympatric in portions of its distribution with certain of these bunyaviruses, it is possible that reassortment of genomes could occur, resulting in serious epidemiologic consequences.

Dual infection of mosquitoes by interrupted feeding may enhance possibilities for evolution of bunyaviruses in vectors. Although *A. triseriatus* is a relatively long-lived mosquito, the number of gonadotrophic cycles is limited (*8*). Thus, chances for dual infection, segment reassortment, and transmission of new virus genotypes would be more likely if dual infection occurred during the first gonadotrophic cycle. Opportunities for generation of new virus genotypes in mosquitoes infected with the first virus in one gonadotrophic cycle and the second virus during a subsequent cycle would be limited both by interference and by the life history of the mosquito. Most females would not live long enough to successfully transmit newly generated reassortant viruses to susceptible vertebrates. Virtually all mosquitoes became dually infected in the interrupted feeding studies (Table 3). In only about 25 percent of the specimens did both viruses infect the same cell, yielding reassortant viruses. Perhaps different virus combinations, different vector mosquitoes, or different experimental conditions, such as a longer incubation period or additional gonadotrophic cycles, would yield more reassortant viruses. Alternatively, there may be genetic constraints on the generation of reassortant viruses in mosquitoes.

These studies demonstrate the potential for RNA segment reassortment of bunyaviruses in dually infected vectors. The frequency of these events in more biologically relevant systems (such as heterologous wt virus crosses) and the significance of these events in nature remain to be determined.

References and Notes

1. D. Bishop and R. Shope, in *Comprehensive Virology 14*, H. Fraenkel-Conrat and R. Wagner, Eds. (Plenum, New York, 1979), pp. 1–156; F. Fuller, A. Bhown, D. Bishop, *J. Gen. Virol.* **64**, 1705 (1983); F. Fuller and D. Bishop, *J. Virol.* **41**, 643 (1982); D. Bishop *et al.*, in *Segmented Negative Strand Viruses, Arenaviruses, Bunyaviruses, and Orthomyxoviruses*, R. Compans and D. Bishop, Eds. (Academic Press, New York, 1984), pp. 3–11.
2. P. Palese and J. F. Young, *Science* **215**, 1468 (1982); W. Laver, R. Webster, C. Chu, *J. Infect. Dis.* **149**, 108 (1984).
3. B. Beaty, E. Rozhon, P. Gensemer, D. Bishop, *Virology* **111**, 662 (1981); R. Klimas *et al.*, *Am. J. Epidemiol.* **114**, 112 (1981); C. Pringle, J. Lees, W. Clark, R. Elliot, *Virology* **135**, 244 (1984).
4. B. Beaty, D. Bishop, M. Gay, F. Fuller, *Virology* **127**, 83 (1983).
5. J. Edman and A. Downe, *Mosq. News* **24**, 154 (1964); M. Klowden and A. Lea, *Am. J. Trop. Med. Hyg.* **27**, 827 (1978); L. Magnarelli, *J. Med. Entomol.* **13**, 445 (1977); C. Mitchell and K. Millan, *ibid.* **18**, 249 (1981).
6. B. J. Beaty *et al.*, *Science* **211**, 1433 (1981); B. Beaty, B. Miller, R. Shope, E. Rozhon, D. Bishop, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1295 (1982); R. Shope, G. Tignor, E. Rozhon, D. Bishop, in *Replication of Negative Strand Viruses*, D. Bishop and R. Compans, Eds. (Elsevier, New York, 1981), pp. 147–152; G. Tignor, T. Burrage, A. Smith, R. Shope, in *California Serogroup Viruses*, C. Calisher and W. Thompson, Eds. (Liss, New York, 1983), pp. 129–138; C. Seymour, T. Amundson, T. Yuill, D. Bishop, *Am. J. Trop. Med. Hyg.* **32**, 1147 (1983); R. Janssen, F. Gonzalez-Scarano, N. Nathanson, *Lab. Invest.* **50**, 447 (1984).
7. J. LeDuc, *J. Med. Entomol.* **16**, 1 (1979); T. Monath, *Bull. WHO* **57**, 513 (1979); P. Grimstad, *Adv. Virus Res.* **28**, 357 (1983).
8. M. Sinsko and G. Craig, *J. Med. Entomol.* **15**, 89 (1979); L. Haramis and A. Woodbridge, *ibid.* **20**, 391 (1983); G. DeFoliart, in *California Serogroup Viruses*, C. Calisher and W. Thompson, Eds. (Liss, New York, 1983), pp. 89–104.

9. Supported by U.S. Public Health Service Research grants AI 19688 and AI 15400 from the National Institutes of Health.

29 March 1985; accepted 26 July 1985

Cyanobacterial Light-Harvesting Complex Subunits Encoded in Two Red Light–Induced Transcripts

Abstract. *The major light-harvesting complex in cyanobacteria and red algae, the phycobilisome, is composed of chromophoric and nonchromophoric polypeptides. Two linked genes encoding major chromophoric components, the polypeptide subunits of phycocyanin, were isolated from the cyanobacterium* Fremyella diplosiphon. *Transcripts from this phycocyanin subunit gene cluster were present as major species in the cyanobacterium grown in red light, but not in cultures maintained in green light. The genes for the subunits of the red light–induced phycocyanin were transcribed together (β-phycocyanin followed by α-phycocyanin) on two messenger RNA species; one contained 1600 bases while the other had 3800 bases. The latter, which encompassed the smaller transcript, contained additional sequences extending from the 3′ end of the coding region of the α-phycocyanin gene. It may encode other light-induced components of the phycobilisome. Since phycocyanin, which effectively absorbs red light, becomes a dominant constituent of the phycobilisome in red light, these different levels may reflect an important adaptive mechanism of these organisms to their environment.*

PAMELA B. CONLEY
PEGGY G. LEMAUX
ARTHUR R. GROSSMAN
Department of Plant Biology, Carnegie Institution of Washington, Stanford, California 94305

Phycobilisomes are major light-harvesting complexes in prokaryotic cyanobacteria and eukaryotic red algae and may constitute 50 percent of soluble cellular protein (*1*). Chromophoric proteins (phycobiliproteins) present in the two domains of this complex, the core which is directly anchored to the photosynthetic membranes and the rods which are attached to the core (*2, 3*), serve to absorb light energy and transfer this energy to the photosynthetic reaction center (*4*). The three major chromophoric proteins are phycocyanin (PC), allophycocyanin (APC), and phycoerythrin (PE), each of which is composed of two related subunits, α and β. In addition to these polypeptides, specific nonchromophoric linker polypeptides mediate the association between the different phycobiliproteins. The constituents of the phycobilisome in many cyanobacteria vary to optimize the absorption of prevalent wavelengths of light (*1, 5*), a phenomenon termed complementary chromatic adaptation (*1*). Evidence obtained with inhibitors of transcription (*6*) suggests that a change in light quality triggers an alteration in the transcription of phycobiliprotein genes. However, little is known about the genes encoding phycobilisome polypeptides and how their transcription is controlled.

We recently isolated and characterized the genes for four phycobiliprotein subunits (α- and β-PC and α- and β-APC) from the plastid genome of the eukaryotic alga *Cyanophora paradoxa* (*7, 8*). Others have isolated PC genes from the cyanobacterium *Agmenellum quadruplicatum* (*9, 10*). In eukaryotic algae, the subunit genes for PC and APC are each transcribed on a dicistronic messenger RNA (mRNA) (*8*), as is the case for the PC subunit genes in *A. quadruplicatum* (*9*). The PC subunit genes of *C. paradoxa* (*7*) were used in heterologous hybridizations to isolate analogous genes in the cyanobacterium *Fremyella diplosiphon*. Figure 1 shows a Southern hybridization in which two fragments generated from the plastid-encoded PC subunits were hybridized to genomic DNA from *F. diplosiphon* digested with various restriction enzymes. One fragment used was a 0.8-kilobase (kb) sequence (*8*) encoding almost all of β-PC and the amino terminal third of α-PC. A second fragment, generated by Sau3A digestion of the insert from pCPC2368 (*7*), contained 63 base pairs (bp) encoding the con-