(i) both species face a common enemy at high frequency, (ii) the mechanism by which they compete displays density-dependent effectiveness, and (iii) the two species harm each other less than either is harmed by the common enemy. That this suggestion is sufficiently general to accommodate cooperation between species is supported by observations of plant defense guilds (*9*), aggregations of tube-building polychaetes (*10*), associations of hydroids and bryozoans (*11*), and my own observation that *B. simplex*, a treelike congener of *B. turrita*, settles preferentially into dense stands of young *B. turrita* colonies (*12*).

I have shown here that a density dependence in interference competition can lead to group living and that the formation of groups in a sessile organism necessitates intraspecific cooperation. Yet cooperation and competition are generally viewed as being virtually opposite extremes of organism interactions. My results suggest the reverse. In certain cases, interspecific competition may provide the very selective pressures that lead to the evolution of cooperation.

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3. Fertile colonies were collected from Eel Pond, Woods Hole, Mass., and induced to release their larvae by light shock. A minimum of 100 larvae were released into clean Nalgene containers. Each container was covered with a piece of plastic mesh (Vexar) divided into six equalized (100 cm^2) regions. In each region colonies of *B. turrita* were attached in different densities in a modified Latin Square by looping the stalks through the holes in the mesh. After a period of 6 to 8 hours, the mesh was removed and the number of larvae settled in each region was counted.
4. Colonies that had previously settled on Vexar mesh were attached to a new (10 by 10 cm) Vexar substratum with cable ties and monofilament line. Attachment by monofilament failed in some instances, resulting in the loss of some colonies. Only one loss, however, could not be unambiguously attributed to a failure in the attachment method. All weight gains are expressed as averages of those colonies that remained attached.
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6. Clearly the evolution of group living will be dependent upon the frequency at which interference competition occurs.
7. Cooperation may be defined as "a dynamic ecological state of organisms living in aggregation characterized by sufficient mutual benefit to outweigh disadvantages associated with crowding" (Webster's Collegiate, ed. 7). Allee (*1*) was the first to use the word in this general sense. Recent workers have restricted the use of the term cooperation to apply only when there is a risk of fitness loss if others do not participate (*8*). Allee was aware of such risks although he did not incorporate them in his definition of cooperation; for example, see "the costs of undercrowding" [W. C. Allee, *The Social Life of Animals* (Norton, New York, 1939), p. 293, figure 2]. The gregariousness of *B. turrita* is a cooperative act under either definition.
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12. An experiment designed exactly like that described in the text was attempted with *B. simplex* larvae and *B. turrita* residents. All *B. turrita* colonies were 0.05 g or less in weight. Results are presented as *B. turrita* density (colonies per square centimeter), and the percentage of *B. simplex* larvae settling: 0.01 colonies, 15 percent; 0.05, 10; 0.08, 35; 0.13, 28; 0.17, 8; 0.22, 2.
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Milk of Dairy Cows Frequently Contains a Leukemogenic Virus

Abstract. *Milk or viable milk cells collected from 24 dairy cattle naturally infected with bovine leukemia virus were inoculated into lambs, which were subsequently examined for the development of infection. With this bioassay, infectious virus was demonstrated in the milk of 17 of the cows. Bovine leukemia virus is leukemogenic in at least two mammalian species, is widespread in commercial dairy herds, and can infect a wide range of hosts in vivo and cells, including human cells, in vitro.*

Bovine leukemia virus (BLV) is a horizontally transmitted, probably insect-borne oncornavirus that differs from the leukemia viruses of other species in several important ways (*1, 2*). In vivo, BLV is usually present in the lymphocytes in a covert, nonproductive state; the infected lymphocytes do not show viral RNA, viral antigens, or viral particles unless they are grown in vitro for a few hours (*2–4*). BLV is universally regarded as the causative agent of the adult enzootic form of bovine leukemia (lymphosarcoma), the most common fatal malignancy of dairy cattle (*1*). Under experimental conditions BLV infects sheep (*5, 6*), goats (*7*), and apparently chimpanzees (*8*). BLV-infected sheep frequently develop leukemia (*5, 6*). There is evidence that BLV can cross species barriers under natural conditions and infect both sheep and capybaras (*9*). In vitro, BLV infects cells of various origins, including human and simian cells (*10, 11*).

More than 20 percent of the dairy cows and approximately 60 percent of the herds surveyed in the United States were infected with BLV (*1*). Bovine leukemia virus infection is also prevalent among cattle of most other countries studied. Thus the question of whether dairy cows naturally infected with BLV release infectious virus into milk is an important public health consideration.

Several in vitro infectivity assays for BLV have been developed, but they are not suitable for detecting the virus in milk, mainly because of the composition and frequent bacterial content of this secretion. Sheep provide an alternative means for determining the presence of BLV in milk because they are highly susceptible to BLV infection and seem to resist the bacteria present in various bovine secretions and excretions (*12*). In a preliminary experiment, BLV was demonstrated in one sheep injected with the milk of one naturally infected cow (*12*).

We used the sheep bioassay to determine the frequency with which infectious BLV is released into the milk of cows naturally infected with BLV. In our first experiment, BLV-free lambs

Table 1. Detection of BLV in lambs inoculated with whole milk from naturally infected cows. Each milk sample was injected intraperitoneally into one or, in most cases, two lambs less than 7 days old. The lambs were subsequently examined for the presence of BLV antibodies by means of the immunodiffusion test with BLV glycoprotein antigen (*23*) and, in most cases, for the presence of infectious BLV by means of the syncytia induction assay (*14*). Symbols: +, one or both sheep positive for BLV 12 or more months after injection; −, sheep negative for BLV.

Milk donor	Day of lactation on which milk was collected		
	10	30	50
G-245	+	−	
BF-157	+	−	
BF-138	+	−	
G-142	−	+	
G-266	−		
BF-269	−		
BF-291		+	
G-255		−	
G-189		−	
G-43		−	+
G-257		−	−
G-256			+
G-263			−
G-24			+
G-265			−
G-198			−

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were injected with fresh milk (100 ml) collected from 16 BLV-infected cows belonging to a dairy herd (*13*) maintained at the University of Pennsylvania (New Bolton Center) and to a commercial dairy herd. The milk from six cows was assayed on two different lactation days, but, owing to the short lambing season, recipient sheep of appropriate age were not available for use in assaying milk from any one cow more frequently. Seven lambs injected with milk samples from a BLV-free cow and seven lambs that were not inoculated served as controls. All lambs were obtained from the same BLV-free flock and were raised in the same facilities. Transmission of BLV from sheep to sheep has not been observed (*5, 6*).

As shown in Table 1, the injection of milk from 8 of the 16 infected cows induced BLV antibodies in one or both recipient lambs. These antibodies were usually detected 8 months after inoculation and persisted at least until the sheep were 12 to 16 months old. In no instance was the milk from a cow positive for BLV on more than one of the lactation days. In several instances only one of a pair of sheep inoculated with a milk sample developed BLV antibodies. It appears, therefore, that the quantity of infectious BLV in 100 ml of milk is close to the sheep bioassay's limit of sensitivity. If this is the case, it cannot be determined whether the failure to detect BLV in milk of an infected cow at successive lactation dates is due to intermittent release of the virus or to a constant release at low levels.

The persistence of the antibody response of the recipient sheep indicated that the response was due to infection rather than to immunization by virion antigens in the milk inoculum. Furthermore, infectious virus was detected in the blood lymphocytes of six of nine antibody-positive sheep examined with the syncytia induction assay for BLV (*14*). None of the control sheep showed BLV antibodies or infectious BLV during the 16 months of observation.

In cattle, BLV seems to infect only the lymphocytes, and BLV-infected lymphocytes do not usually synthesize BLV particles in vivo (*2–4*). We therefore assumed that the infections in milk-injected sheep are due mainly to the presence in milk of BLV-infected lymphocytes rather than to free extracellular virus. Thus, in an attempt to increase the sensitivity of the sheep bioassay, we conducted a second experiment in which lambs were injected with viable cells isolated from milk rather than with whole milk. Of the nine infected dairy cows used as donors, only one (BF-138) had been used in the first experiment. As shown in Table 2, milk cells collected from the cows at one or more of three sampling times induced BLV infection or BLV antibodies in the recipient lambs. Most of the sheep developed antibodies before their lymphocytes became positive for BLV. However, by 8 to 10 months after inoculation there was a close correlation between the presence of antibodies and the presence of BLV. There were no significant differences among lactation days in the frequency with which BLV-infected cells were present in the cows' milk. Nine sheep injected with lymphocytes from BLV-free cows and eight uninjected sheep housed with the infected sheep remained negative for BLV for at least 16 months.

In most instances, BLV was detected in both sheep inoculated with milk cells from a BLV-positive cow (Table 2). In contrast, only half of the infected whole milk samples in the first experiment induced BLV infection in both recipient lambs. Moreover, whereas most of the sheep inoculated with milk cells became infected within 3 months, BLV was usually detected only after 8 months in the sheep inoculated with whole milk. Thus the sensitivity of the sheep bioassay was indeed greatly increased when milk cells were used as the inoculum. It is likely that in the first experiment the percentage of cows identified as having infectious BLV in their milk would have been greater had milk cells rather than whole milk been assayed.

The fact that infectious BLV or BLV-infected cells are present in the milk of most naturally infected cows does not necessarily mean that calves become infected with BLV by the oral route. Some

Table 2. Detection of BLV in lambs inoculated with milk cells from naturally infected cows. The cells were isolated by centrifugation, washed twice, and injected subcutaneously (10^8 viable cells per lamb); N.D., not determined.

Milk donor	Day of lactation	Recipient sheep	3 to 4 months after inoculation		8 to 10 months after inoculation	
			BLV antibodies*	BLV†	BLV antibodies	BLV
G-4182	4	14	+	+	+	+
G-149	1 to 2	23	+	−	N.D.	N.D.
		24	+	+	+	+
	4 to 6	3	+	−	+	+
		60	+	−	+	−
	10 to 13	54	+	−	+	+
		62	+	−	+	+
G-312	1 to 2	64	+	−	+	+
		18	+	−	+	+
	4 to 6	55	+	−	+	+
	10 to 13	59	+	−	+	+
		65	+	+	+	+
G-314	1 to 2	52	−	−	+	+
		67	−	−	−	−
G-281	1 to 2	57	−	−	−	−
		66	−	−	−	−
	4 to 6	53	+	−	+	+
		63	+	−	+	+
BF-138	1 to 2	8	+	+	+	+
		15	+	+	+	+
	4 to 6	19	+	−	+	+
	10 to 13	7	+	+	+	+
		9	−	+	+	+
BF-283	1 to 2	11	+	+	+	+
		16	+	+	+	−
	4 to 6	12	+	+	+	+
		22	+	−	+	+
	10 to 13	51	+	−	+	−
		58	+	−	+	+
BF-306	1 to 2	4	+	N.D.	N.D.	N.D.
	4 to 6	2	+	+	N.D.	N.D.
	10 to 13	1	+	+	N.D.	N.D.
BF-316	4	10	+	+	+	+
	10 to 13	6	+	+	+	+

*As determined by the immunodiffusion test with BLV glycoprotein antigen. †As determined by the competitive radioimmunoassay for BLV p25 (*24*). Peripheral blood lymphocytes were cultured for 48 hours with phytohemagglutinin (*3*), washed by centrifugation, resuspended in buffer [0.02*M* tris, 0.1*M* NaCl, 0.001*M* EDTA (*p*H 7.5), 0.5 percent Nonidet P40, 0.2 percent sodium deoxycholate, and 2-phenylmethylsulfanilyl fluoride] to a final concentration of 3×10^7 cells per milliliter, and frozen and thawed three times. After incubation at 37°C for 15 minutes, the extract was clarified by centrifugation and tested with the competitive radioimmunoassay. An extract was considered to be positive for BLV when 40 μl (equivalent to 10^6 lymphocytes) displaced more than 20 percent of the labeled antigen.

researchers in Europe have concluded that bovine leukemia, and therefore BLV, is transmitted via milk (*15, 16*). However, owing to the experimental design and methodology used, the data on which this conclusion was based do not indicate whether the calves became infected prenatally, by milk ingestion, or by contact. Studies conducted under appropriate experimental conditions have shown that milk-borne transmission of BLV, as compared with contact transmission, is rare if it occurs at all (*17, 18*). The resistance of calves to milk-borne infection with BLV is probably due mainly to the maternal virus–neutralizing antibodies that all calves nursed on BLV-positive dams acquire through colostrum (*17–19*).

The present study and the data on the prevalence of BLV infection in dairy herds indicate that humans are often orally exposed to BLV. Although the infectivity of BLV is apparently destroyed by pasteurization (*20*), people in many countries consume unpasteurized milk. Moreover, it is not known whether pasteurization destroys the biological activity of the proviral BLV DNA in the infected cells of milk. There is no evidence that BLV can infect humans, but neither do the data exclude this possibility. Although attempts to demonstrate BLV antibodies, particles, and antigens in humans have been negative (*1*), the findings are not conclusive because of the limited sensitivity of the assays used and because some BLV-infected cells do not synthesize virus particles or viral antigens (*2–4, 10, 11*). Molecular hybridization studies have failed to demonstrate BLV-related sequences in human tumors (*21*), but only a few tumors were examined and the probe used was only partially representative of the viral genome. While earlier epidemiological surveys showed no association between human and bovine leukemia (*1*), the most recent survey, involving a large number of cases, showed a statistically significant increase in human acute lymphoid leukemia in areas with a high incidence of bovine leukemia and BLV infection (*22*). Clearly, the question of whether BLV poses a public health hazard deserves thorough investigation with the most sensitive virological and immunological techniques available, particularly highly representative molecular probes.

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Emergence of Posttetanic Potentiation as a Distinct Phase in the Differentation of an Identified Synapse in *Aplysia*

Abstract. *The developmental time course of posttetanic potentiation was studied at an identified chemical synapse. In stage 11 juveniles (3 weeks after metamorphosis), the synaptic connections made by cholinergic neuron L_{10} onto postsynaptic neurons L_2 to L_6 were present but showed no posttetanic potentiation. In stage 13 adults (12 weeks after metamorphosis), the same tetanus resulted in an increase of 300 percent in the synaptic potential. A similar pattern was observed at two other identified synapses in the abdominal ganglion. Thus, the initial steps in synapse formation do not include the expression of this plastic capability. Rather, at least 10 weeks is required between the onset of synaptic function and the final expression of mature synaptic properties.*

Many chemical synapses in the adult animal can be altered for long periods as a result of previous stimulation. It is important in the study of neural differentiation to determine whether the capability for plastic change is part of the machinery that is present in the initial establishment of a functioning synapse, or whether these capabilities represent separate regulatory processes that emerge independently later in development.

Although the initial development and maintenance of synapses have been studied extensively (*1–4*), little is known about how neurons acquire plastic capabilities. Knowledge about the development of synaptic plasticity may help to elucidate the mechanisms of normal behavioral maturation, as well as the mechanisms that contribute to disorders in perception and motor coordination that occur when animals are reared in abnormal perceptual or motor environments (*5*). We examined posttetanic potentiation (PTP), a common form of plasticity, at an identified chemical synapse in the abdominal ganglion of the marine mollusk *Aplysia californica*. We found that PTP develops as a late step in the maturation of the synapse and is independent of transmission. The gradual emergence of PTP at an identifiable synapse provides an opportunity for studying the detailed mechanisms underlying this form of synaptic plasticity.

We used laboratory-reared animals at various stages of development, from stage 11 juveniles weighing 3 mg at 3 weeks after metamorphosis to reproductively mature, stage 13 adults weighing 100 g or more at 12 weeks after metamorphosis (*6*). We examined a specific, identified inhibitory synapse between presynaptic neuron L_{10} and one of its five identified follower cells (L_2 to L_6) located in the rostral quadrant of the left abdominal ganglion (*7*). Even in the youngest animals studied, both the pre- and postsynaptic neurons were clearly identifiable and large enough (15 μm in diameter in animals weighing 3 mg) to permit successful intracellular recordings. To obtain reliable postsynaptic potentials, we hyperpolarized the follower cells to at least 50 mV below the reversal potential of the inhibitory postsynaptic

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