access to such receptors should exist endogenously. A morphine-like, nonpeptide compound with immunological cross-reactivity to morphine has been reported in mammalian brain (*19*) and in samples of human cerebrospinal fluid (*20*). This compound exhibited specific binding to opiate receptors (*21*) and was active in both guinea pig ileum and mouse vas deferens, but its activity was not reversed by naloxone or naltrexone (*19*). Nevertheless, immunocytochemical studies (*22*) with specific morphine antibodies indicated its localization within neurons. To date, the chemical identity of this substance has not been elucidated. Killian *et al.* (*23*) confirmed the existence in brain of an immunologically morphine-like substance, whose pharmacological activity could be readily reversed by naloxone. These results have been confirmed in our laboratory, where similar activity has also been identified in extracts of the intestinal tract of rodents (*24*). It is possible that this substance is morphine, that it is derived from dietary sources, and that it is a natural ligand for the specific μ receptors of the brain and gastrointestinal tract. Under certain circumstances (for example, during breast feeding) other exogenous compounds, such as morphiceptin (*9*), may also interact physiologically with μ receptors. Other precedents exist for the occurrence of receptors or enzymes for substances elaborated exclusively outside the body, such as vitamins, essential fatty acids, and essential amino acids.

ELI HAZUM
JULIE J. SABATKA
KWEN-JEN CHANG
DAVID A. BRENT
JOHN W. A. FINDLAY
PEDRO CUATRECASAS

Wellcome Research Laboratories,
Research Triangle Park,
North Carolina 27709

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11. The column was 30 cm by 1 inch, containing C-18/Porasil B (particle size, 37 to 75 mm); mobile phases, 480 ml of H_2O, 320 ml of H_2O and acetonitrile (9:1 by volume), 320 ml of H_2O and acetonitrile (1:1 by volume), 400 ml of H_2O and acetonitrile (1:1 by volume), and 400 ml of acetonitrile; flow rate, 100 ml/min.
12. The active compound (Fig. 1C) was lyophilized and, after the addition of 1 ml of 10 m*M* sodium carbonate (*p*H 9), extracted three times with 1 ml of chloroform containing 5 percent ethanol. After the solution was evaporated under a nitrogen stream, mass spectrometry was performed. A Varian MAT 731 double focusing mass spectrometer coupled to a Varian 1024 time-averaging computer was tuned to 8000 resolution with 10 percent valley definition. The sample was introduced by direct probe and a glass crucible was used; the sample evaporated over a temperature range of ambient to 350°C. The accurate mass of the molecular ion of morphine was monitored according to the procedures described by G. L. Peele and D. A. Brent [*Biomed. Mass Spectrom.* **5**, 180 (1978)].
13. For example, B. Sandmann, D. Szylczewski, J. Windheuser, and T. Higuchi [*J. Pharm. Sci.* **59**, 427 (1970)], used thin-layer chromatography, nuclear magnetic resonance, optical rotatory dispersion, and infrared spectroscopy to characterize an unknown, but the unknown was misassigned [D. A. Brent, P. Chandrasurin, A. Ragouzeos, B. S. Hurlbert, J. T. Burke, *J. Pharm. Sci.* **69**, 906 (1980)]. J. Sphon [*J. Assoc. Off. Anal. Chem.* **61**, 1247 (1978)] suggests that a retention time for a compound and three ions in the mass spectra of a gas chromatography and mass spectrometry experiment are satisfactory for the confirmation of a drug. Such evidence is more than sufficient for the Food and Drug Administration to prosecute cases. D. A. Brent and D. A. Yeowell [*Chem. Ind. (London)* (1973), p. 190] used one or two ions in the high-resolution mass spectrum of a mixture to characterize impurities in that mixture, and this evidence was used in the prosecution of a patent infringement case.
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1 December 1980; revised 6 April 1981

Group Living, Competition, and the Evolution of Cooperation in a Sessile Invertebrate

Abstract. *Competition and cooperation are thought to represent the opposite extremes of organism interactions. I here show that the formation of aggregations in a sessile organism requires cooperation between individuals and that the gregarious pattern of habitat selection generating these aggregations is a response to a density dependence in the outcome of interference competition.*

One of the oldest concerns of ecologists, evolutionary biologists, and ethologists is why organisms live in groups (*1*). As group living often intensifies competition among members of the group, its occurrence appears anomalous. If group living is adaptive, its benefit to members of the group must outweigh its cost (*1*). These benefits have been thought to be threefold: an increased protection from inclement weather, a reduced risk of predation, or an increased feeding efficiency (or some combination thereof) (*1, 2*). I now report the existence of a fourth type of benefit—increased interspecific interference competitive ability—and show that this benefit accounts for an observed pattern of gregarious settlement in a sessile, colonial invertebrate.

Virtually all cost-benefit analyses of group living involve the study of behaviorally sophisticated mobile organisms (*1, 2*). In mobile organisms, the formation of groups often varies in a complex fashion with environmental conditions, age, sex, and other factors. Hence, quantification of the costs and benefits of group living is difficult. Sessile invertebrates, in contrast, have only one opportunity to choose whether or not to live in a group. Thus, direct field measurements of the relative costs and benefits of group living are not only feasible, but also lead to new insights into the conditions favoring the evolution of group living.

The bryozoan *Bugula turrita* commonly occurs in dense stands on pilings and rocks at shallow depths along North American coastlines. It grows as a single arborescent colony with a flexible stalk, lightly calcified skeleton, and loosely articulated zooids. By mid- to late summer at Woods Hole, Massachusetts, virtually all colonies (> 90 percent) of *B. turrita* on pilings below a depth of 1 m are fertile and are found in contact with colonies of another bryozoan, *Schizoporella errata*. Unlike *B. turrita*, *S. errata* is an encrusting organism composed of heavily calcified zooids. This rigid skeleton enables colonies of *S. errata* to push down and overgrow the more flexible colonies of *B. turrita*. As such overgrowth inevitably leads to the death of the *B. turrita* colony, this form of interference compe-

0036-8075/81/0828-1012$01.00/0

tition is potentially quite severe. Accordingly, I initiated experiments to determine the conditions under which *B. turrita* larvae form groups, the relative costs of group living in terms of intraspecific competition, and the relative benefits in terms of release from interspecific competition.

A series of four laboratory settlement experiments was performed to survey the conditions under which larvae of *B. turrita* establish groups. I provided larvae with an array of substrata to which live *B. turrita* colonies had been attached, and monitored the patterns of subsequent habitat selection (*3*). In each experiment, the size of the resident colonies was different, but, in a given experiment, size was held constant and the population density of the residents varied (Fig. 1A). When resident colonies were small, larvae preferentially selected locations where resident density was high or intermediate. When residents were large, larvae chose locations where resident density was low. Thus, larvae settle preferentially in locations rich in small colonies.

To assess the cost incurred by colonies of *B. turrita* which settle gregariously, I performed the following experiment. I attached colonies of *B. turrita* of known weight to substrata in differing densities and submerged them in the Eel Pond for two months. The cost of group living due to intraspecific competition was assessed in terms of weight gain (Fig. 1B) (*4*). The results are clear; *B. turrita* suffers significant intraspecific competition, even at densities far below that at which larvae choose to settle.

This pattern of settlement cannot be construed as adaptive unless some selection pressure favors *B. turrita* colonies occurring in groups. I made two sets of observations to determine whether the interference competitive ability of *B. turrita* is density-dependent. The first required locating in situ interspecific contacts between *S. errata* and *B. turrita* and then to observe whether overgrowth had occurred; and the second was a simultaneous measure of *S. errata* growth rate as a function of *B. turrita* density. The growing margins of *S. errata* are characterized by lateral projections of calcium carbonate destined to become the interior wall of new zooids. The length of these giant buds is an accurate measure of instantaneous growth rate (*5*). Results are presented in Fig. 1C. At low densities, *B. turrita* colonies were overgrown by *S. errata* and *S. errata* growth rates were high, whereas at high densities, *B. turrita* was rarely overgrown and *S. errata* was either growing at a low rate or not growing at all. Hence the interspecific competitive ability of *B. turrita* is density-dependent.

Group living can evolve if the benefits of living in a group exceed the costs of not doing so (*1, 2*). In *B. turrita*, the costs incurred by intraspecific competition involve a loss of proliferative potential (Fig. 1B), whereas the benefits accrued in interference competition with *S. errata* involve whole colony mortality (Fig. 1C). Since mortality always results in a greater loss in fitness than does a reduction of growth rate, the density-dependent competitive ability of *B. turrita* is sufficient to account for its gregarious pattern of settlement (*6*).

Discussion of group living has traditionally led to the topic of intra- and interspecific cooperation (*1, 7*). The manner in which cooperation may arise has been a difficult problem in evolution because an individual that initially cooperates, in a population of individuals that does not, may often be at a selective disadvantage. This disadvantage arises from (i) the possibility that any two individuals will interact only once and (ii) the possibility that one individual will "defect" or fail to cooperate reciprocally (*8*). These conditions reveal a fundamental difference in the evolution of cooperation in sessile and mobile organisms. Once a sessile individual has settled and permanently attached adjacent to another, continuous interaction between the two individuals is unavoidable and "defection" simply cannot occur. This is not necessarily the case with a mobile organism. A mobile organism that joins a group can also leave that group either to live a solitary life or to join a better group if it becomes available. Hence, the difficulties in evolving cooperation from a previously asocial state in mobile forms are less restrictive for sessile forms.

Although these data represent cooperation between colonies of the same species, the argument relating density-dependent competition and evolution of cooperation need not be restricted to this level of biological organization. For example, different species may cooperate if

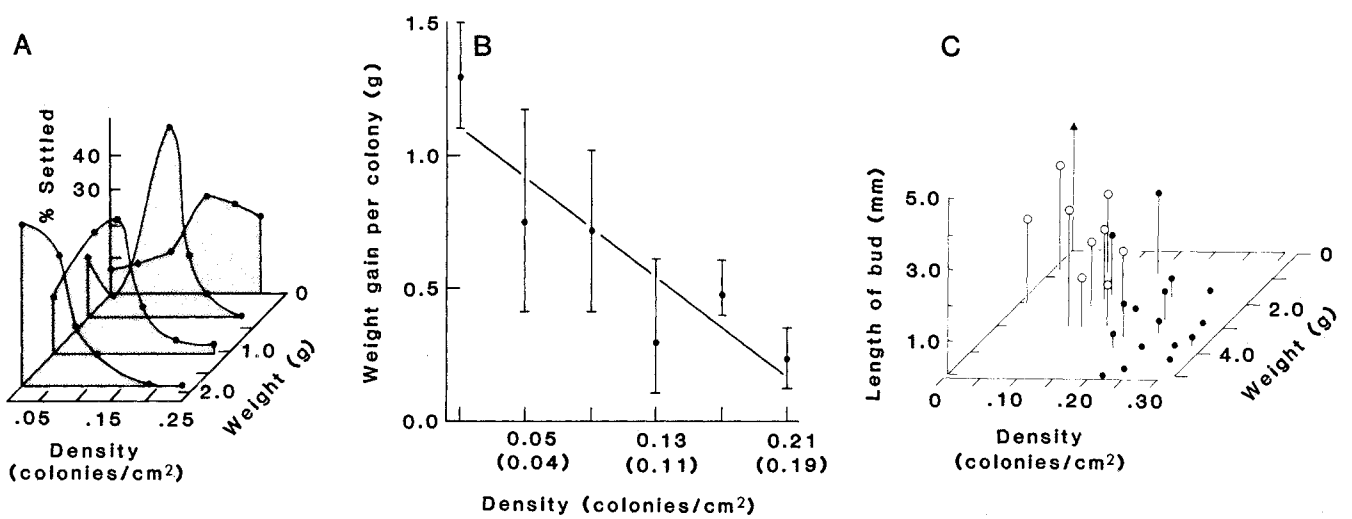

Fig. 1. (A) Habitat selection experiment. Each curve is the result of a single experiment in which the weights of resident colonies were held constant and the density of residents varied. (B) Intraspecific competition. The gradations along the abscissa are in original density (final density) (*4*). Data points are medians for five replicates and bars enclose the range. The weight gain in the lowest density treatment is significantly greater than those in all other treatments at the $P < 0.05$ level ($r_s = -.90$, Spearman rank correlation). (C) Interspecific competition. Open circles are interspecific contacts with overgrowth apparent and closed circles, with no overgrowth apparent. The triangle denotes the mean of 20 observations of the length of giant buds of *S. errata* colonies not in competition (standard deviation, 0.6 mm). The minimum length of an *S. errata* zooid is 0.4 mm, the maximum, 0.7 mm. The occurrence of overgrowth at high densities but at low total weights indicates that competitive ability is dependent on density rather than biomass.

(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.

LEO W. BUSS

Department of Biology, Yale University, New Haven, Connecticut 06511

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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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13. I thank R. Grosberg, G. E. Hutchinson, J. B. C. Jackson, B. Keller, N. Knowlton, J. Moore, C. Wahle, and J. Wulff for technical assistance or comments (or both). Supported by a Steps Toward Independence fellowship from the Marine Biological Laboratory, Woods Hole, Mass.

3 February 1981; revised 5 May 1981

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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