Reports

Disturbance and Ecologic Succession in an Upper Ordovician Cobble-Dwelling Hardground Fauna

Abstract. *An Ordovician hardground fauna in northern Kentucky provides an example from the fossil record of the maintenance of species diversity by periodic disturbance of an autogenic ecologic succession. The marine invertebrates found encrusting limestone cobbles show an order of community development from a low-diversity pioneer assemblage through a high-diversity association to a monospecific stable fauna. All species, including the late successional dominants, were present in the early stages of colonization. Periodic overturning of the cobbles killed the encrusters and opened up new spaces on which succession was reinitiated. These disturbances maintained high diversity within the encrusting community by limiting the spatial distribution of the most efficient space competitors.*

Disturbance has been recognized by ecologists as a primary force in the maintenance of species diversity in many ecosystems (*1*). The disturbances, whether biotic (for example, influxes of predators, disease, or loss of a primary nutrient) or abiotic (for example, wind, fire, or storm waves), usually create open patches within a community, providing temporary escapes in space and time for opportunistic early successional organisms and preventing the monopolization of space (or another limiting resource) by competitively dominant later successional forms.

Eighty-four flattened irregular limestone cobbles (*2*) were found in a single bed of the shaly Upper Ordovician Kope Formation exposed in an outcrop on the southern bank of Gunpowder Creek at its confluence with the Ohio River in Boone County, Kentucky. Sixty-eight percent of these cobbles are encrusted on tops and bottoms with a variety of organisms (Table 1). Thin sections of the cobbles show a continuous oxidized rim, approximately 3 to 5 cm thick, upon which the encrusting organisms are attached.

To map the encrusted surface of each cobble in the original sample of 16, a Nikon dissecting microscope equipped with a micrometer and a drawing tube was used. Where possible, the surface areas of the various encrusting species on the top and bottom of each cobble were recorded separately, along with the unoccupied ("blank") space. A total of 1052.26 cm^2 were mapped and measured, including blank space; 481.79 cm^2 of encrusting organisms were identified and measured. A second sample of 68 cobbles was used only for overgrowth and total percentage of encrustation measurements of each surface.

A modified Shannon-Weiner measure was developed to assess the diversity of encrusting organisms on both sides of each cobble

$$ED = -\sum_{s=1}^{n} P(s)[\log P(s)]$$

where ED is encrusting diversity, P is the proportion of total area of the cobble surface (top or bottom) occupied by species s, and n is the total number of species in the sample. This measure differs from the standard Shannon-Weiner formula in which p is the proportion of individuals of a species; the standard formula cannot be applied to the colonial organisms in this study. This modified Shannon-Weiner measure also gives a better assessment of the use of space, which was apparently the primary limiting factor in this hardground ecosystem.

A strong curvilinear correlation between encrusting diversity on the cobbles and the total percentage of the cobble surfaces encrusted can be seen in Fig. 1. Diversity increases with encrustation at a nearly constant rate to a peak measure of 0.43, and then it declines to 0. No surfaces (in the entire sample of 84 cobbles) were encrusted with more than 50 but less than 100 percent cover; those cobble surfaces completely (100 percent) encrusted had a monospecific fauna, producing a diversity measure of 0. No statistically significant relationships were found between encrusting diversity and total cobble surface area, and cobble size had no apparent effect on species occurrence (*3*).

All 84 cobbles were analyzed for overgrowth relationships between the encrusting species. (An overgrowth consisted of one species superimposed in living position over any portion of another.) A total of 81 overgrowths between 14 species pairs were recorded (Table 1).

The cobbles in this study are often encrusted on both top and bottom, indicating that many were overturned at least once and probably several times while the encrusting organisms were alive. The fossiliferous shaly matrix in which the cobbles are found indicates that they originally rested on a muddy and shallow marine substrate. Overturning a cobble in this mud would certainly have killed the organisms encrusting the top surface, and it would have exposed a fresh surface to new colonizers. If there were an order to community development on these cobbles, and a significant number of overturning events occurred before the community reached a stable configuration (*4*), then the stages of community development should still be preserved on the cobble surfaces. It should be possible to discern the successional order of the various encrusters because many had hard skeletal elements that would survive many overturns.

The overgrowth data in Table 1 reveal the framework of the ecological succession on these cobbles. The most promi-

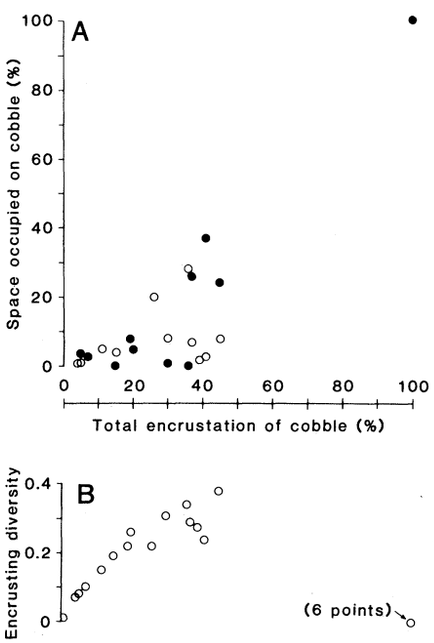

Fig. 1. Relation between total encrustation of each cobble surface and (A) percentage of space occupied by the tabular bryozoan *Amplexopora* sp. indet. (●) and the vinelike bryozoan *Corynotrypa* sp. indet. (○) and (B) encrusting diversity. Sample size, 16 cobbles (tops or bottoms or both) over 1052.26 cm^2.

Table 1. Fossils of encrusting organisms and their percentage overgrowth on the Ordovician cobbles. Encounters (one encruster superimposed on another) represent the total number involving each species. Overgrowth represents the percentage of encounters in which each species was on top.

Encrusting species	Total encounters (No.)	Overgrowths (%)
Cystaster stellatus (edrioasteroid)	13	100
Amplexopora sp. indet. (tabular bryozoan)	21	67
Ceramoporid gen. and sp. indet. (tabular bryozoan)	43	49
Crinoid bases	29	45
Cornulites sp. indet. (tube dweller)	7	43
Corynotrypa sp. indet. (vinelike Bryozoan)	49	35

nent early colonizers should on average be overgrown much more frequently than the later forms, which are often attaching themselves to earlier skeletal remains (*5*). There was no skeletal evidence of direct interference competition (*6*). The species in Table 1 are ranked in order of successful overgrowths, which is apparently the reverse order of their position in the community succession, with one exception. The edrioasteroid *Cystaster stellatus* was never found overgrown, but its skeleton of calcareous plates held together with organic material was probably not stable enough after death to support an overgrowth. It was evidently among the late successional organisms because it has been found overgrowing almost all other species.

The top competitor for space in this cobble ecosystem was the trepostome bryozoan *Amplexopora* sp. indet. (*7*). This is shown by the overgrowth data (Table 1, after accounting for the position of *C. stellatus*) and the fact that all cobbles that are encrusted completely are occupied entirely by *Amplexopora* sp. indet. If we assume that a cobble left undisturbed would be encrusted through time from 0 to 100 percent, the percentage of the cobble surface encrusted is correlative, in a general sense, with ecological time or time since last disturbance. Those cobbles with low encrustation values are representative of the early stages of community development, and those with high values are from the latter stages. This successional model is supported by the correlation between the total percentage of cobble surface encrusted and the percentage of space occupied by *Amplexopora* sp. indet. Figure 1A shows the curve of *Amplexopora* sp. indet. colonization from a minor (but present) constituent in the early stages of community development to the dominant form in the later (*8*). Conversely, the early successional *Corynotrypa* sp. indet. peaks in abundance at 35 percent total encrustation and declines from there (Fig. 1A). Overall the community developed from a low-diversity pioneer assemblage to a high-diversity association to approximately 50 percent total encrustation (Fig. 1B). If the cobble was left undisturbed, *Amplexopora* sp. indet. monopolized the entire surface, and the diversity index consequently dropped to 0 at 100 percent encrustation. *Amplexopora* sp. indet. continued to develop until it produced a large moundlike colony that may have protected the cobble from future overturning.

As Fig. 2 suggests, all cobbles would have been dominated by *Amplexopora* sp. indet. in the absence of overturning or other disturbances. However, since only 7.1 percent of the cobbles are entirely encrusted, storm currents probably overturned most of the cobbles frequently enough to prevent complete domination. Disturbances thus maintained a high diversity within the cobble-dwelling community and on the majority of individual cobbles. This process of disturbance-induced diversity has been reported in contemporary ecosystems (*1*) but not in the fossil record (*9*).

MARK A. WILSON
Department of Geology,
College of Wooster,
Wooster, Ohio 44691

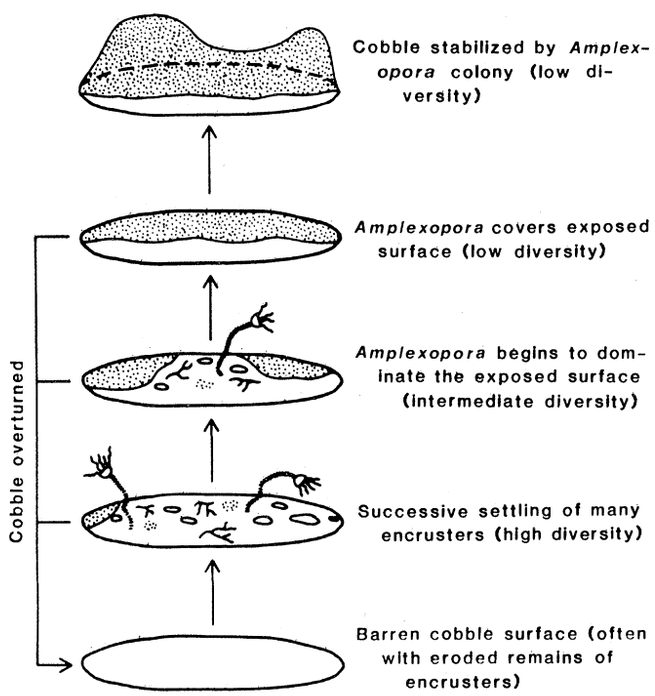

Fig. 2. Community development on the Ordovician cobbles. Note that the overturning events maintain high diversity unless *Amplexopora* sp. indet. produces a massive colony sufficient to stabilize the cobble.

References and Notes

1. See, for examples, J. Lubchenco and B. A. Menge, *Ecol. Monogr.* **48**, 67 (1978); J. H. Connell, *Science* **199**, 1302 (1978); F. H. Bormann and G. E. Likens, *Am. Sci.* **67**, 660 (1979); R. T. Paine and S. A. Levin, *Ecol. Monogr.* **51**, 145 (1981); D. G. Sprugel and F. H. Bormann, *Science* **211**, 390 (1981); M. N. Dethier, *Ecol. Monogr.* **54**, 99 (1984); L. G. Harris *et al.*, *Science* **224**, 1336 (1984). Studies specifically concerned with shallow marine epifaunal community dynamics on cobbles and boulders include R. W. Osman, *Ecol. Monogr.* **47**, 37 (1977); W. P. Sousa, *ibid.* **49**, 227 (1979); *Ecology* **60**, 1225 (1979); M. Lieberman *et al.*, *ibid.*, p. 1151; J. R. McAuliffe, *Ecology* **65**, 894 (1984).
2. The cobbles, the longest dimensions of which are between 4.0 and 20.0 cm, are composed of a pyrite-rich unfossiliferous micrite that is laminated in portions. These cobbles apparently represent an earlier laterally continuous hardground that was broken up and partially eroded.
3. In a study of algally encrusted cobbles off the Ghana coast, M. Lieberman *et al.* [*Ecology* **60**, 1151 (1979)] found a relation between cobble moment of inertia (resistance to tumbling) and species richness. The cobbles rested on sandy beds and were disturbed mainly by storms associated with the rainy season. The cobbles described here rested on a muddy substrate, and the mechanism for their overturning is not yet clear. Small cobbles may have rested in the mud within the boundary layer of water created by the larger cobbles and would thus have been overturned only by current that affected all the cobbles.
4. J. B. C. Jackson, *Am. Nat.* **111**, 743 (1977); M. J. Keough, *Ecology* **65**, 423 (1984).
5. Petrographic thin sections of these cobbles show several generations of encrustation that are not visible on the cobble surfaces.
6. J. B. C. Jackson [*Univ. Tenn. Stud. Geol.* **5**, 22 (1981)] noted several morphological responses to interference competition displayed by encrusting bryozoans.
7. This competitive ability is predicted by the models for solitary and colonial encrusters outlined by Jackson in (*4*).

8. Because of the initial presence of late successional organisms, the ecological succession was not a case of facilitation. It therefore would be classified under the neutral (tolerance) or inhibition models of J. H. Connell and R. O. Slatyer [*Am. Nat.* **111**, 1119 (1977)]. M. J. Keough [*Ecology* **65**, 423 (1984)] showed that recruitment was a much more important process than interference competition on small isolated patches, so that the neutral model of succession is most plausible here.
9. T. J. Palmer and C. D. Palmer [*Lethaia* **10**, 197 (1977)] discussed the morphological and evolutionary effects of scour on a Middle Ordovician hardground community. M. A. Wilson [*ibid.* **15**, 263 (1982)] described a Pennsylvanian brachiopod-bryozoan ecosystem in which diversity was gradually enhanced by the accumulation of biogenic debris.
10. I thank G. M. Wilson, W. P. Sousa, M. J. James, and G. G. Whitney for reviewing this manuscript, and J. W. Bartley for bryozoan identification. Supported by grants from Sigma Xi and College of Wooster.

15 October 1984; accepted 14 February 1985

Expression of a Microinjected Porcine Class I Major Histocompatibility Complex Gene in Transgenic Mice

Abstract. *A porcine class I major histocompatibility complex (SLA) gene has been introduced into the genome of a C57BL/10 mouse. This transgenic mouse expressed SLA antigen on its cell surfaces and transmitted the gene to offspring, in which the gene is also expressed. Skin grafts of such transgenic mice were rejected by normal C57BL/10 mice, suggesting that the foreign SLA antigen expressed in the transgenic mice is recognized as a functional transplantation antigen.*

The introduction of isolated class I major histocompatibility complex (MHC) genes into cultured mammalian cells has been used to study their expression, antigenicity, and antigen-presenting capacity (*1*). This approach allows the introduction of genes not normally expressed in the recipient cell, such as foreign, mutant, or hybrid MHC genes constructed in vitro (*2, 3*). In addition, the expression of individual members of a multigene family can be studied in isolation.

However, such studies have been generally limited to a small number of cell types, such as L cells, and the questions addressed have been limited to those related to the expression of the introduced gene in that particular cellular environment. It has not been possible to address developmental issues such as the patterns of tissue-specific expression of individual class I MHC genes or the influence of newly introduced genes on the induction of self tolerance and the maturation of the immune response. To study these unanswered questions, we microinjected a cloned xenogeneic class I MHC gene into the fertilized eggs of mice. We now report the identification of a transgenic mouse that expresses a porcine class I MHC gene that can be transmitted to progeny, in which it is also expressed.

Earlier we showed that the introduction of PD1, a porcine genomic clone containing a class I MHC (SLA) gene, into mouse L cells by DNA-mediated gene transfer resulted in the cell-surface expression of SLA antigen (*3*), as detected by antibody-mediated, complement-dependent cytotoxicity, cell-surface immunofluorescence, and immunoprecipitation. The latter studies also revealed that the SLA heavy chain associates with the endogenous murine β_2-microglobulin of the L cell. Further studies indicated that the expression of the swine MHC gene was actively regulated (*4*). In addition, the swine DNA and RNA sequences as well as the protein products were readily distinguishable from the endogenous murine sequences. Therefore, this gene seemed to be an ideal candidate for microinjection experiments.

Male pronuclei of one-cell C57BL/10SCN (B10) fertilized eggs were injected either with approximately 500 copies of a 9-kilobase (kb) Hind III fragment derived directly from the PD1 clone or with about 2000 copies of a 5.6-kb Hind III–Bam HI fragment, subcloned into pBR322 and linearized with either Hind III or Eco RI (Fig. 1). These fragments of swine DNA each contain the entire SLA gene and have been shown to direct the synthesis of SLA antigen in mouse L cells (*5*). Of 171 eggs injected with the 5.6-kb Hind III–Bam HI DNA fragment, 100 were transferred to pseudopregnant Sim:SW females, and there were 63 offspring. Twenty-seven eggs were injected with the 9-kb Hind III DNA fragment, and 20 were transferred to a single foster mother; there were five offspring.

In testing for the swine DNA sequences, DNA was extracted from a segment of tail of each of the mice and hybridized with an SLA DNA probe (Fig. 1) under conditions in which only SLA DNA sequences, but not endogenous H-2 sequences, would hybridize. A single SLA-positive mouse (3931), which had been injected as an embryo with the swine 9-kb Hind III DNA fragment, was identified by dot blot analysis. For further verification of the SLA gene in animal 3931, total tail DNA was digested with the restriction enzyme Bgl II. This enzyme releases three SLA coding-sequence DNA fragments of 2.9, 1.8, and 0.9 kb from the intact gene (Fig. 1). Therefore, it was expected that all three of these bands would be present in the DNA of animal 3931 but not in the DNA of its negative littermates. The SLA probe hybridized specifically to appropriately sized DNA fragments from animal 3931 and pig liver but not to those from negative littermates or normal B10 animals. This confirms the presence of the SLA gene in animal 3931.

The ability of the SLA gene to be expressed in the transgenic mouse was assessed by cell-surface labeling of peripheral blood lymphocytes (PBL's) with a monoclonal antibody specific for SLA determinants (*6*), which does not cross-react with murine class I MHC antigens. This monoclonal antibody has been shown to react specifically with L cells transfected with either the whole PD1 DNA or the Hind III 9-kb DNA fragment but not with control L cells (*4, 5*). PBL's from animal 3931 and normal B10 mice were stained with biotin-conjugated antibody to SLA. Binding of the monoclonal antibody was assessed with fluoresceinated avidin, which binds to biotinylated antibody on the cell surface. The antibody to SLA reacted specifically with PBL from animal 3931 (Fig. 2a). In contrast, lymphocytes from normal B10 animals were not stained above background levels by this reagent (Fig. 2b).

In efforts to examine the stability of expression and heritability of the SLA

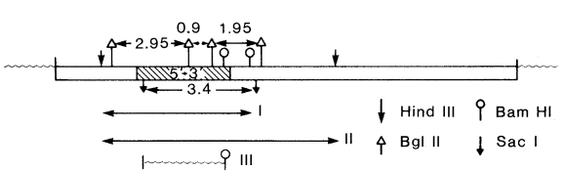

Fig. 1. Partial restriction map of PD1, which contains a functional SLA gene (shaded area) (*3*). The 5.6-kb swine DNA fragment subcloned into pBR322 and used in microinjection (I), the 9-kb Hind III swine DNA fragment used in microinjection (II), the 2.5-kb Sac I–Bam HI DNA fragment used as an SLA-specific probe in hybridization experiments, and the origins of the hybridizing 3.4-kb Sac I fragment and of the 2.95-, 1.95-, and 0.9-kb Bgl II fragments are noted (see Fig. 3).