organism is probably involved. In addition, the presence of dikaryotic hyphae (Fig. 1C) and internally born sporangia containing four and eight spores (Fig. 1, B and D) suggests that the body (Fig. 1A) is a sexual reproductive structure and not a sclerotium as previously reported (*7*).

Sterile mycelial remains, with septa, of higher fungi are occasionally encountered in excellent states of preservation in Carboniferous strata (*8*); however, only on rare occasions do specimens exhibit reproductive stages of the types that are diagnostic of various extant groups (*6*). The discovery of such reproductive structures that characterize the advanced fungi in the Paleozoic provides a time scale on which various concepts of phylogeny of the Ascomycetes and Basidiomycetes may be judged. *Palaeosclerotium pusillum* may represent a fungus having basidiomycete affinities but retaining reproductive structures similar to those of ascomycete progenitors rather than having a basidium and basidiocarp. Dikaryotic vegetative hyphae, clamp connections, and complex septal pores occur ubiquitously throughout taxa of extant Basidiomycetes; therefore, the immediate ancestors of the Basidiomycetes might be expected to have these features. Alternatively, *Palaeosclerotium* might represent an early occurrence of an ascomycete having clamp connections and complex septa. Some workers have suggested that the ascomycete crosier is homologous to the clamp connections of basidiomycetes, and some species of *Tuber* are reported to have a dikaryophase and true clamp connections (*9*). In addition, some ascomycetes apparently produce complex septa in their ascogenous hyphae (*5*). The combination of ascomycete reproductive features and complex vegetative features characteristic of basidiomycetes suggests that a group of fungi intermediate to Ascomycetes and Basidiomycetes existed early enough in the fossil record to have been ancestral to extant fungi.

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Acid Precipitation and Embryonic Mortality of Spotted Salamanders, *Ambystoma maculatum*

Abstract. *Spotted salamanders breed in temporary pools formed in early spring by melted snow and rain. Many of these pools reflect the low* pH *of precipitation in the northeastern United States. Egg mortality is low (< 1 percent) in pools near neutrality, but high (> 60 percent) in pools more acid than* pH *6. Developmental anomalies and the embryonic stage at which death occurs are the same in field situations as at corresponding* pH*'s in laboratory experiments.*

The deleterious effects of acid precipitation on forests and fisheries have been a source of concern for two decades in Scandinavia and more recently in North America (*1*). Acid precipitation has lowered the *p*H of lakes and streams, but temporary ponds that form by accumulation of melted snow or rain may be still more vulnerable to alteration of *p*H because precipitation entering them has little contact with buffer systems and is not diluted by mixing with standing water (*2*). Temporary ponds are important breeding sites for a number of species of frogs, toads, and salamanders, and these amphibians may be the vertebrate animals most immediately and directly affected by acid precipitation.

Laboratory studies (*2*) showed that embryos of Jefferson and spotted salamanders (*Ambystoma jeffersonianum* and *A. maculatum*) are sensitive to *p*H. Hatching success of 90 percent or more was achieved by Jefferson salamanders only at *p*H 5 and 6, and by spotted salamanders only at *p*H 7, 8, and 9. Beyond those limits, mortality rose sharply and was associated with distinctive embryonic malformations. Many of the natural breeding sites of spotted salamanders in the Ithaca, New York, region are acidic. The *p*H of 17 temporary pools was measured during April and May 1975, the period of egg deposition and development (*3*). Fourteen pools had a *p*H of 6 or less, only one had a *p*H of 7. In contrast, only one of 13 permanent ponds in the same area had a *p*H below 6, and ten were *p*H 7 or higher (*4*).

Laboratory data indicate that acidity measured in some breeding ponds should cause embryonic mortality at specific stages of embryonic development in spotted salamander eggs. This prediction was tested under field conditions by following development of spotted salamander eggs in five ponds on Connecticut Hill, Tompkins County, New York. The ponds are within a circle 1 km in diameter and span an altitudinal range from 460 to 625 m. At the time eggs were laid, pond *p*H's ranged from 4.5 to 7.0; by hatching, the *p*H had increased by 0.25 to 0.5 *p*H units in each pond. Water temperatures rose from 8° to 23°C during development (*5*). Temperature did not differ significantly among the ponds ($F_{4,33} = 0.07$, $P > .05$).

Every egg that could be found in each pond was examined at intervals of 3 to 7 days. The stage of development was determined and the number of live and dead eggs in each clutch was recorded (*6, 7*). Most clutches were counted several times during the study, but no clutch was counted twice at the same embryonic stage. Dead eggs decay and disappear over a period of days; thus, this index is an estimate of the minimum cumulative mortality that had occurred by a given stage of development.

An abrupt transition from low to high embryonic mortality occurred below *p*H 6 (Fig. 1). Mortality reached a maximum of 0.66 percent at *p*H 7 (pond A) and 0.91 percent at *p*H 6 (pond B). At *p*H 5.5 (pond C) maximum mortality rose to 43.7 percent and at *p*H 5.0 and 4.5 (ponds D and E) exceeded 65 percent. Mortality was low in all ponds during early embryonic development. Even at *p*H 4.5 (pond E) mortality did not exceed 1.3 percent through late gastrulation. In all ponds mortality increased at neurulation and again at late stages of gill development and at hatching. These observations are strikingly similar to laboratory observations (*2*), both in the levels of mortality at different *p*H's and in the embryonic stages at which death occurred. This similarity strongly suggests that the acidity of the ponds was responsible for embryonic mortality.

Further evidence that acidity of the ponds was the cause of death is provided by the similarity of developmental anomalies observed in field and laboratory studies. Pough and Wilson (*2*) associated characteristic embryonic malformations with different *p*H's. Moderate stress (*p*H 5 to 6) was associated with abnormalities that appeared late in development—swelling of the chest near the heart and stunting and asymmetry of the gills. More severe stress (*p*H 4 to 5) led to failure to retract the yolk plug and consequent deformation of the posterior trunk region. At *p*H 4 the egg membranes of spotted salamanders shrank, forcing the embryos into tight coils. These deformities appear to be responses to acidity (*8*). They are distinct from the deformities produced by temperature-induced chromosomal abnormalities (*9*), low oxygen and high carbon dioxide levels (*10*), or metal salts (*11*).

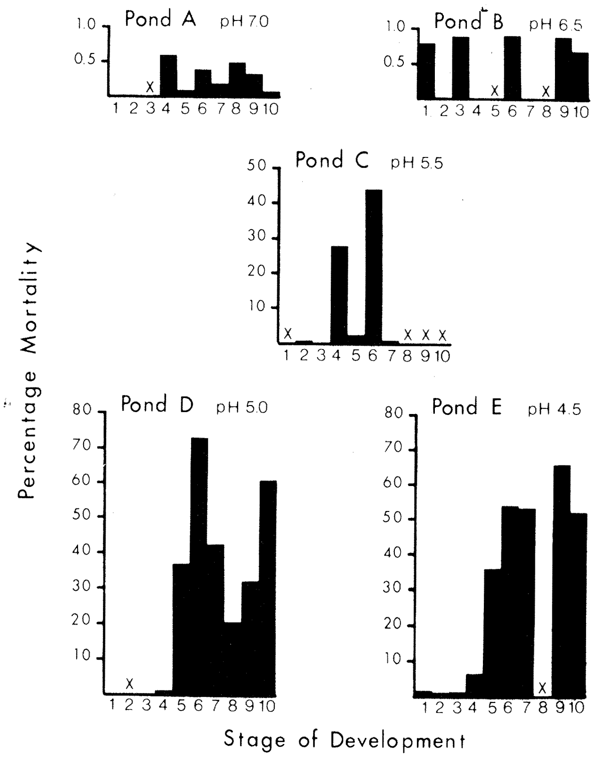

Fig. 1. Cumulative embryonic mortality of spotted salamander eggs in five pools. The horizontal axis shows developmental stages; see (*7*) for explanation. The average number of eggs examined in each pond at each stage was 712, range 100 to 2708. An x indicates that no eggs were examined at that developmental stage. Eggs were washed out of pond C by a flood before development was completed. Note the × 20 expansion of the vertical scale for ponds A and B.

The same abnormalities were observed in the field study, and they occurred at the same *p*H's as in the laboratory. Stunted gills and swelling near the heart were seen in embryos from pond D (*p*H 5.0), and those two features plus failure of yolk plug retraction were seen in pond E (*p*H 4.5). Embryos from pond E were more tightly coiled than those in other ponds, and eggs from pond E had significantly ($F_{1,58}$ = 10.87, $P < .01$) smaller volumes ($\bar{x}$ = 221 mm^3, N = 30) than those from pond B ($\bar{x}$ = 312 mm^3, N = 30).

While field studies of this sort do not lend themselves to unequivocal "proof" of the effect of a particular stress, the controlled experiments in the laboratory plus the fieldwork strongly support the inference that acidity is an important factor for the following reasons: (i) the percentage of eggs surviving was approximately the same at a given *p*H in the laboratory and field; (ii) deaths occurred at the same embryonic stages at a given *p*H in the laboratory and field; and (iii) characteristic embryonic deformities were observed at specific *p*H's in the laboratory and field. A synergistic effect of several stresses is possible, but results to date suggest that *p*H is the critical variable (*12*).

Large differences in sensitivity to acid conditions between clutches were observed in the field as they had been in laboratory studies (*2*). Even in ponds D and E, where few eggs survived, there were a few clutches with low mortality. These differences in sensitivity may reflect genetic differences within the species; larvae from high- and low-mortality clutches were raised in the laboratory to confirm species identification. Considering the high levels of embryonic mortality observed, selection for acid tolerance must be strong, and if specific alleles are involved (*12*) it could be effective.

Eggs are laid in groups of 5 to more than 200. They are surrounded by and enveloped in a gelatinous material secreted by the female. This material absorbs water for several hours after the eggs are laid, swelling to many times its original volume (*13*). The final composition is 99.5 percent water (range 99.3 to 99.6 percent, N = 10). The fully hydrated gel has little buffer capacity ($\bar{x}$ = 1.9 milliequivalents of H^+ per gram dry weight, N = 8), but there may be significant ion exchange as water is absorbed. In acid ponds, mortality is usually greatest among embryos on the outside of egg masses; if there are any surviving embryos they are in the center of the mass. If ion exchange occurs as the mass swells, eggs in the center would be best protected from acid surroundings. This reasoning is consistent with the observed pattern of mortality, but does not rule out other environmental stresses, including predators or extremes of temperature, from which eggs in the center of the mass would be sheltered. Predation has been proposed as an important cause of mortality of *Ambystoma* eggs (*14*), but the very low embryonic mortality in ponds A and B suggests it was not significant in this study. The similarity of pond temperatures makes it unlikely that they were responsible for the pattern of mortality.

Precipitation in the Ithaca region is acidic with an annual average *p*H below 4 (*15*) and is probably the major source of acidity in temporary ponds. The gradual increase in *p*H in the pools over the course of the study suggests an initial acid inflow of water from melted snow followed by gradual neutralization by windblown debris and contact with the pond basin (*16*). Heavy rainfall lowered the *p*H of some ponds by as much as 0.5 *p*H unit; one pond, which had a *p*H of 7.5 when it dried, refilled 2 weeks later after a rainstorm at *p*H 6.0. Sulfate is the predominant anion in the pools, as it is in precipitation (*17*). Differences in soil buffer capacity in drainage areas and the amount of vegetation in a pond probably account for diferences in acidity among the five ponds. The two most acidic are on acid soil (*p*H 5), while the others receive drainage predominantly from more basic soil (*p*H 6.8 to > 7.2). Alkaline dust (*p*H > 7.2) raised by cars on nearby roads may have an important buffering influence on ponds A and C.

The future of spotted salamanders appears bleak, and their problems with acid precipitation may well be shared by other species of amphibians that are specialized for reproduction in temporary ponds where they do not face competition and predation by fishes (*18*). Acidification of breeding sites may be a factor contributing to the recent decline in British frog populations (*19*). Even if the few egg clutches that exhibited low mortality in the current study indicate evolution of some tolerance of acidity, it is not at all likely that the process will proceed fast enough to keep pace with the cumulative effects of acid precipitation on salamander breeding sites (*2*). The significance of widespread failure of salamander reproduetion will extend beyond the salamanders themselves; salamanders are important predators on dipteran larvae in temporary pools (*20*) and an important source of energy for higher trophic levels in an ecosystem (*21*). Temporary ponds are also important breeding sites for many invertebrates, and changes in those ponds could have far-reaching effects.

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4. Seventeen breeding sites of spotted salamanders were measured, the average *p*H was 5.2, range 4.5 to 8.0. Six of these sites were permanent ponds, their average *p*H was 6.1, range 5.5 to 8.0. Eleven breeding sites in temporary pools had an average *p*H of 5.0, range 4.5 to 7.0.
5. Temperature was measured beside an egg mass each time a pond was visited. Amphibian eggs absorb solar radiation and may be several degrees warmer than water temperatu [D. D. Hassinger, *Herpetologica* **26**, 49 (1970); F. H. Pough, in preparation].
6. To reduce stress eggs were not lifted from the water or removed from the supports to which they were attached. Masses containing fewer than 50 eggs were counted individually; where there were more than 50 a portion (one-quarter to one-half) of the clutch was counted and the total estimated. Preliminary trials indicated that this method produced values within 10 percent of actual egg numbers.
7. Ten developmental stages could be identified under field conditions. They correspond to the Harrison stages [R. Rugh, *Experimental Embryology* (Burgess, Minneapolis, Minn., 1962), pp. 82–87] as follows.

Field stage	Harrison stage	Field stage	Harrison stage
1	1–3	6	23–29
2	4–9	7	30–32
3	10–11	8	33–34
4	12–13	9	35–39
5	14–22	10	40

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16. Water in puddles of melted ice on the surface of ponds in April was usually 0.5 *p*H unit more acidic than water drawn from below the ice. In one pond melted snow and rain had collected in a small bay separated from the main pond by a dam of ice. Water in the bay was *p*H 4.0, in the main pond *p*H 5.5. A day later the ice dam had melted, allowing some pond water to flow into the bay, and its *p*H had risen to 5.0.
17. Analyses of water from five ponds with *p*H's between 4.0 and 5.5, stored for 2 weeks in the laboratory, were made by courtesy of G. E. Likens and the Hubbard Brook Ecosystem Study. The SO_4^{2-} concentration averaged 3.9 mg/liter (range 2.9 to 4.9).
18. *Rana sylvatica* breeds simultaneously and sympatrically with *Ambystoma* in this region, but it breeds in some pools from which *Ambystoma* is absent, possibly because they are too acidic (*p*H 3.5 to 4.0). Laboratory experiments show that *R. sylvatica* embryos can tolerate *p*H's at least as low as *p*H 4 [F. H. Pough and R. E. Wilson, unpublished data; Gosner and Black (*8*)].
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Tool Use in a Social Insect and Its Implications for Competitive Interactions

Abstract. *Four species of myrmicine ants,* Aphaenogaster rudis, A. treatae, A. tennesseensis, *and* A. fulva, *use pieces of leaf, mud, and sand grains as tools to carry soft foods from distant sources to the colony. Tools are tended on the food and removed by colony members without regard to which individual brought the tool. Food is gathered more efficiently by tool use than by internal transport. Tool-using behavior may increase the competitive ability of* A. rudis *in an interspecific dominance hierarchy.*

We report here what we believe to be the first case of tool use in a social insect and discuss the importance of this behavior in terms of competitive efficiency within an interspecific dominance hierarchy. Tool use is defined (*1*) as the manipulation of an inanimate object, not internally manufactured, with the effect of improving the animal's efficiency in altering the position or form of some separate object. Only four genera of invertebrates, solitary wasps (*Ammophila*), ant lions (*Myrmeleon*), and worm lions (*Vermilio, Lampromyia*) are known to use tools (*1, 2*). We have observed tool use in four species of myrmicine ants, *Aphaenogaster rudis*, *A. treatae*, *A. tennesseensis*, and *A. fulva*; individuals of these species use pieces of leaves, dry mud, and small sand grains to transport soft food from a distant source to the colony.

We first observed this behavior in *A. rudis* located in a woodlot in College Park, Prince George's County, Maryland. We placed small portions of jelly on index cards (7.6 by 12.7 cm) on the ground to attract ants. When individuals of *A. rudis* reached a sample of bait, they would leave after 5 to 60 seconds and return with pieces of leaves which they then placed on the jelly. As leaf fragments accumulated, ants from the same colony tended them, adjusting the positions of the leaves or sometimes pulling the leaves off completely and repositioning them. By individually marking ants with small spots of paint, we were able to determine that a given individual may bring several leaves and that ants tend leaves brought by other ants.

After 30 to 60 minutes, ants began to remove leaves from the bait and carried them directly back to the colony, once as far as 152 cm. These leaves were visibly covered with jelly, and we suggest that the ants are using these leaves as tools to transport large quantities of food (see Fig. 1). This behavior may have evolved from the tendency shown by many ant species to cover and protect distant food sources (*3*) or to cover immovable, disagreeable objects near the nest with dirt or debris (*4*).

To determine what happens to tools taken into a colony, we set up a small colony of *A. fulva* in a transparent ant house. Ants placed tools on the jelly provided, and we observed an individual carry a tool from the jelly to the chamber containing the queen, eggs, and other workers. Several workers fed from this