Evolutionary ecology, antibiosis, and all that rot

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Pity members of the Animal Kingdom. They must compete for prey that hide, defend themselves, or run away. This competition only intensifies when such prey die uneaten. The carcasses and leaves strewn across the landscape are now available to microbial competitors that are everywhere, grow rapidly, and know how to poison. So how can an animal scavenger make a living? In a study in this issue of PNAS (1) *Nicrophorus* beetles that raise their grubs on mouse carcasses are shown to use a 2-pronged strategy: avoid the nastiest carcasses and apply the antibiotics.

Ecologists have typically studied competition among suites of closely related species (e.g., 5 species of *Dendroica* warblers) (2). However, in 1977, evolutionary ecologist Dan Janzen (3) pointed out that a large fraction of potential food—fruits, seeds, and carcasses—may be lost to animals through competition with bacteria and fungi. Microbes have a huge advantage, after all, because they are abundant both in the soil and the gut of the carcass. In warm environments, they can also reproductively overrun a carcass before it can be discovered by a hungry animal. Janzen posited that the resulting malodorous hydrogen sulfide and fatty acids that say "rotten" in any language may in fact be microbial messages proclaiming "this food is taken." Few options were considered open to scavengers, other than to specialize on efficient discovery (i.e., be a vulture) or only eat a carcass when it is "still warm"(i.e., be an opportunist) (4). In this view, spoilage, not ingestion, should be the common fate of the dead.

Some animals, it turns out, may have additional options. Rozen and colleagues (1), in a clever set of experiments, suggest animals may also employ antibiotics—small molecules like peptides, glycopeptides, terpenes, and alkaloids that are effective at low densities in suppressing microbial competitors (5). They studied the burying beetle, *Nicrophorus vespilloides*, which uses small mammal carcasses as eat-in nurseries (Fig. 1). *Nicrophorus* parent their young and tend the carcass in which they burrow. This was doubly fortuitous. First, Rozen and colleagues had an animal that was heavily invested in keeping a carcass's microbe titer in check. Second, the scientists could measure beetle fitness: the survival, growth rate, and size at maturity of the larval offspring, something often lacking in studies of competition. They found that female *Nicrophorus* preferred to raise young on freshly thawed mouse carcasses compared with those that sat at room temperature for 7 days (with the expected olfactory result). Furthermore, larvae grown on rotten carcasses were 10% smaller (size is a good predictor of future reproductive success in insects). Moreover, if stuck with a rotten carcass, the mothers could still do something about it. Before their eggs hatched, mothers deposited anal and oral secretions. Brood on old carcasses who were deprived of this prenatal care were smaller and took longer to develop than those raised on fresh carcasses. The authors conclude that there is something in the parental care, especially those secretions, which kept decomposer microbes at bay [carcasses treated with *Nicrophorus* secretions have been shown to grow less fungi (6) and bacteria (7)]. They acknowledge that how, exactly, the microbes decrease the fitness of beetle larvae and what, precisely, is in the secretions produced by the mothers, are both open questions.

Fig. 1. Most animals do not eat carcasses because microbes get there first, spreading rapidly and poisoning the food with antibiotics (blue dots). Burying beetles fight back by regurgitating antibiotics (pink puddles) that lyse bacteria and preserve the carcass for their larvae. Illustration by Deborah Kaspari.

Despite the importance of understanding antibiosis in a world of infectious diseases, we know surprisingly little about its evolutionary ecology. Most ecologists study macrobes and have devoted little attention to antibiosis, a form of interference competition—the allocation of resources, not to grow yourself, but to suppress the growth of your competitors. Early models found it hard to conjure scenarios in which an animal engaging in costly, spiteful behavior could invade a new community (8, 9). As it turns out, the secret to successful antibiosis is staying put. When you give antibiotic-producing bacteria a structured medium, they affix to substrate, grow clonally, and produce a "no mans land," absent competitors, where the antibiotics diffuse outward. Carrion and soil are full of such structure. Moving microbes out of the chemostat and into a more structured, realistic world [as Rozen and colleagues (1) do] has been a dependable recipe for insight

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into microbial evolutionary ecology (10, 11).

One surprising result of this study: mother beetles raised their young on fresh carcasses, even as they preferred to consume ones that had been mouldering for a week. One reason may be the way that different microbes, with differing tactics, accumulate and replace each other as the carcass decomposes. Decomposers that arrive early often act like fast-growing (and palatable) weeds (12). As microbes accumulate, resource quality declines, and decomposer competition heats up. There may be increasing selection for resource defense, with existing clones up-regulating antimicrobial genes, or competitive replacement favoring strains and species that constitutively generate antibiotics.

Indeed, models of optimal interference already exist from the study of animal territoriality (13). Here's the translation. When resources are abundant (a fresh carcass), maximal uptake is achieved without the help of antibiotics; when resources are scarce (nothing but bones), basal requirements go unmet when antibiotics are built. Antibiosis should thus be the optimal behavior somewhere in the middle of the resource spectrum. In fact, antibiosis is rare on poor resources: the mycelia of soil fungi commingle when grown on water, but generate "no man's lands" when raised on richer malt agar (14). At the high end, readily available sources of carbon, energy, nitrogen, and phosphate can inhibit the production of a wide array of antibiotics (15). *Penicillium* fungi, which must be kept at intermediate levels of glucose to maximize antibiotic yield, behave like territorial honeycreepers that defend trees with intermediate numbers of flowers (13). Microbiologists will find much inspiration in the literature of behavioral ecology, which represents some of the biggest successes in evolutionary biology.

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If antibiotic warfare increases over the early life of the carcass, this may shed light on the dietary divergence between mother and grub. Other animals, like sowbugs and millipedes (isopods and diplopods), have consortia of bacteria and fungi in their guts (16); similar consortia in the foreguts of ruminants help detoxify plant secondary metabolites (17). As when our mothers breastfed us, *Nicrophorus* mothers may very well be inoculating their young with competent gut flora. Furthermore, if it takes time to develop the consortia and enzymes necessary to thrive on a well-decomposed carcass, then larvae may best be raised on fresh carrion. This is especially so if developmental pathways are sensitive to various chemical insults (18). It is interesting, in this regard, that larvae feeding on old carcasses begged more for food from their mother's crop.

New chemical and computational tools continue to bring the microbial world into clearer focus. As they do so, ecologists are finding new ways that microbes impact macrobial niches. Microbiologists, in turn, should look to ecological theory, developed initially with macrobes in mind, for insights into the way microbes behave and microbial communities are organized. With only a handful of steps from gene → developmental pathway → enzymes, antibiotics, and structural products (i.e., the microbial niche), the next model systems in evolutionary ecology may well be microbial.

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