THINK SMALL

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"The key to taking the measure of biodiversity lies in a downward adjustment of scale. The smaller the organism, the broader the frontier and the deeper the unmapped terrain. Bacteria, protistans, nematodes, mites, and other minute creatures swarm around us, an animate matrix that binds Earth's surface. They are objects of potentially endless study and admiration."—E. O. Wilson

DUMBACHER (1999) and Mills, Lombardo, and Thorpe (1999) lead us into the unmapped terrain described above by Wilson (1994). Each focuses on small organisms, Dumbacher on feather-chewing lice and toxins in the feathers of the New Guinea shrike-thrushes (*Pitohui* spp.), and Mills et al. on bacteria that affect development and survival in nestling Tree Swallows (*Tachycineta bicolor*). In so doing, each opens a new frontier to scientific exploration.

In 1992, Dumbacher et al. stunned the ornithological world by demonstrating that the feathers and skin of three species of *Pitohui* contained homobatrachotoxin, a potent alkaloid that binds sodium channels and depolarizes electrogenic membranes, thereby disabling the nerves and muscles of most vertebrates and invertebrates. Merely holding a pitohui near one's face could cause nasal irritation and tearing, which led Dumbacher and Pruett-Jones (1996) to hypothesize that homobatrachotoxin was a defense against predators. Later, Mouritsen and Madsen (1994) and Poulsen (1994) suggested that the toxin was a defense against ectoparasites. Few data addressed either possibility, until now. In an elegantly simple set of experiments, Dumbacher (1999) has shown that given a choice of two feathers, chewing lice avoid the feather that's more toxic. Furthermore, lice confined to feathers containing homobatrachotoxin have shortened life spans compared with those munching on nontoxic feathers from close relatives of pitohuis.

Lice used in the experiments were removed by fumigation with CO_2 from birds captured in mist nets in Papua, New Guinea. Because identification of lice was not possible in the field prior to experiments, all of which began within an hour of capture, lice were grouped by body

plan and size, and members of each group were assigned to experimental conditions based on a randomized design that facilitated statistical analysis. One louse was placed in a petri dish on one of two feathers differing in toxicity and its position monitored hourly until its death. Natural variation in the concentration of homobatrachotoxin among pitohuis provided the variation needed to test the effect of toxicity on choice of toxic, less-toxic, or nontoxic feathers by lice. The latter were plucked from closely related species. To facilitate movement of the louse, the two feathers were placed one on top of the other. Lice are negatively phototactic, so Dumbacher controlled for placement of the louse on a toxic, less-toxic, or nontoxic feather and position of the feather. Choice of feathers by lice may be influenced by structural details of the feather (Tompkins and Clayton 1999), which could also affect the life span of the louse. Experimental design might have been improved by using only lice from one of the species from which feathers were taken. However, this would have greatly reduced the number of replicates and compromised the statistically balanced design and careful control of confounding variables. Dumbacher (1999) superbly illustrates how natural history observations can lead to simple, definitive experiments. His work also shows how amenable arthropods are to quantitative analysis of important evolutionary relationships.

Feather lice reduce host fitness by (1) destroying the insulative properties of feathers, thereby increasing basal metabolic rate and reducing survival of the host (Booth et al. 1993); (2) decreasing the number of eggs laid and reducing hatching success (Derylo 1974); (3) providing a visible measure of condition on which potential mates can base their choice of partners (Clayton and Tompkins 1995); and (4)

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transmitting pathogens (Marshall 1981). There-fore, lice select for defensive adaptations among birds. Clayton and Cotgreave (1994) have shown that preening behavior and bill morphology are adapted to removal of lice, but because lice feed on feathers, skin, and subder-mal blood, toxins in the feathers and skin could provide another formidable adaptation. Toxins could (1) reduce fecundity of lice; (2) reduce their survival; (3) reduce the effect of lice on host fitness by delaying maturation, suppress-ing appetite, etc.; and (4) reduce immigration or increase emigration. Dumbacher (1999) has shown that survival is reduced. His observa-tions suggest that lice on toxic feathers are less active, which would reduce damage to the feather. Measurements are needed. Also, less-active lice may be easier to remove during preening, an untested possibility. His unequiv-ocal demonstration that lice prefer less-toxic feathers suggests that emigration may occur, but under what conditions would lice, which cannot live apart from the bird, emigrate? Leg-ge and Heinsohn (1996) describe cooperative breeding by *Pitohui dichrous*, the most toxic of the pitohuis. Because the amount of homoba-trachotoxin varies among individuals (Dum-bacher 1999), contact among members of a co-operatively breeding unit would offer lice a choice of individuals that vary in toxicity. If lice chose the least-toxic individuals, selection for toxic feathers and skin would be maintained. Data correlating the number of lice and the tox-icity of feathers and skin of avian hosts are needed, particularly within cooperatively breeding groups of *P. dichrous*. Data on the tox-icity of nestlings and fledglings and on patterns of colonization by lice could provide a perspec-tive on the ontogeny and evolution of avian chemical defense.

Despite possessing toxic feathers, pitohuis have lice (Dumbacher 1999), and lice taken from *P. ferrugineus* had the longest captive life span in the presence of toxic feathers of all lice tested. These observations raise the possibility that pitohui lice have evolved some degree of insensitivity or immunity to homobatrachotox-in during their evolutionary relationship with pitohuis. What are the adaptations of the lice? Do they have the physiological ability to detox-ify homobatrachotoxin? If so, can pitohuis counter by increasing the toxicity of their feath-ers? Should lice with the ability to detoxify

homobatrachotoxin be considered a new spe-cies?

Behavior offers another exciting line for fu-ture research. The maintenance behavior of pi-tohuis is unstudied. Is it different in form or frequency among individuals with different concentrations of toxin, or between pitohuis and species that lack anti-louse toxins? Is pho-resis, in which lice hitch a ride on a more mo-bile ectoparsite (e.g. hippoboscid fly or mos-quito), more common among pitohui lice than among lice on nontoxic birds? Do lice migrate to regions of the body that are less toxic and, if so, what is the distribution of toxin on the body and in the feathers? What is the energetic cost of producing the toxin? Are other chemicals found in feathers effective in reducing the in-fluence of lice, and do lice avoid these chemicals (e.g. melanin)? Are pitohuis the only birds to have evolved such a defense against lice? They are not the only toxic species of birds (Dum-bacher and Pruett-Jones 1996), but other toxins have not been tested on lice.

Other effects of homobatrachotoxin are pos-sible. Dumbacher and Pruett-Jones (1996) sug-gest that it deters predators. Circumstantial ev-idence supports this possibility, but experi-mentation is needed. Homobatrachotoxin may be antibacterial. Degradation of feather keratin by chewing lice depends on *Rickettsia* (Marshall 1981) housed in fat bodies in the louse, and one might extend Dumbacher's study to the effects of the toxin on these symbionts in an effort to understand the mechanism of the chemical's action. Perhaps the toxin prevents *Bacillus li-cheniformis* from degrading feathers as de-scribed recently by Burtt and Ichida (1999). Dumbacher (1999) has lead us to a new frontier where intriguing questions abound.

A second paper blazes a different and equal-ly exciting trail along the "frontier of the small." Articles on avian microbiology are few, scattered, and mostly in journals outside the or-nithological mainstream. Important authors in-clude Pugh (1965), Hubálek (1994), and Pi-nowski et al. (1995). Most studies are of birds associated with human agriculture and focus on transmission of human pathogens or those of domestic livestock. We are ignorant of the extent to which these and most microorgan-isms affect birds. We do not even know whether birds are vectors or victims. Mills, Lombardo, and Thorpe (1999) are the first to quantify the

effect of enteric bacteria on nestling development and evolutionary fitness.

Interest in avian microbiology has increased recently (Brittingham et al. 1988, Sheldon 1993, Lombardo et al. 1996, Nuttall 1997, Burtt and Ichida 1999). Mills et al. (1999) offer compelling reasons for the increased interest: (1) numerous microbial species have been isolated from wild birds (Nuttall 1997, Burtt and Ichida 1999); (2) infectious diseases and toxic byproducts are important sources of mortality and reduced fitness (Robinson and Bolen 1988); and (3) transmission between individuals has potentially important fitness and evolutionary consequences (Lombardo et al. 1999). Gut bacteria can compete with the host for nutrients, a point not mentioned by the authors that further strengthens their case. However, wild birds are difficult to monitor, and little is known about how microorganisms influence reproductive success of birds.

Tree Swallow nestlings were sampled 2, 3, 5, 7, 12, 16, and 19 days after hatching by inserting a dacron swab into the cloaca for 10 seconds and then inoculating multiple cultures, each of which selected for different microorganisms. The number of broods sampled at each age was small, and the analysis, which treats nestlings as independent samples, may have been confounded by the similarity of microbial communities in siblings. Nonetheless, the breadth of microorganisms sampled, the rigor of the sampling procedure, and the number of ages sampled were truly impressive. No longitudinal study of comparable breadth has been attempted. Furthermore, the authors made innovative use of fluctuating asymmetry, which measures nondirectional deviations from bilateral symmetry, to measure subtle but potentially important developmental anomalies associated with the presence of pathogenic enteric bacteria.

Enteric bacteria had colonized two-day-old nestlings, which raises the possibility that colonization began prior to hatching. This possibility should be studied. The number of enteric microorganisms increased as the nestlings became older. One wonders if the diversity of bacteria and the complexity of the bacterial community also increased. That large numbers of *Esherischia coli*, *Salmonella* spp., and *Shigella* spp. were strongly correlated with increased wing asymmetry and less so with tarsal asymmetry

suggests that developmental processes were stressed by heavy loads of pathogenic bacteria. Mills et al. argue convincingly that such asymmetry, particularly of the wing, is potentially important to the survival of an aerial insectivore. Data are lacking, however, pointing up an opportunity for definitive field research.

The authors emphasize correctly that nestling development is the product of many factors (among them microorganisms) that can affect nestling development either positively or negatively. Furthermore, minor changes in a nestling's internal or external environment can alter its microbial diversity with important consequences for the nestling's future fitness. This important point cannot be overemphasized. That "normal" juvenile growth and development of proper immune functioning depend on early colonization of the gut by "normal" intestinal microflora is well established in both human clinical medicine and domestic animal production (Tannock 1995). We know that gut bacteria can compete with their hosts for nutrients. For example, human babies fed formula milk products need iron supplements because *Escherichia coli*, the predominant gut bacterium in such babies, uses large quantities of iron. *Bifidobacterium infantis* in the gut of breast-fed infants do not compete for iron and so the lower amounts of iron in human milk are sufficient. Why have ornithologists, except the eastern Europeans, not previously considered the effect of microorganisms on nestling growth and survival?

The work of Mills et al. (1999) raises many interesting questions. How different are the enteric microbial communities of species with different diets? How different are the communities in species with similar diets (e.g. seeds, insects, etc.), but different habitats? We know that plumage bacteria vary with habitat of the bird (Burtt and Ichida 1999). Does departure from the nest and its microbial community alter the diversity of microorganisms in the digestive track of young birds? Does the microbial community change with seasonal changes in diet or with migration to new environments? And what about brood parasites? Do they have a characteristic intestinal microbial community, or is the community characteristic of the host species? The more one thinks about the potential influences of microorganisms living in or on birds, the more one sees them as an impor-

tant selective force shaping the evolution of many aspects of avian behavior, ecology, and development (e.g. parental care, social behavior, growth, molt patterns, etc.).

Dumbacher (1999) and Mills et al. (1999) have adjusted the investigative scale downward and explored an uncharted land populated by lice and microorganisms, a frontier characterized by chemical warfare and communities of cells that affect the development and survival of hosts many times their size. It is, in E. O. Wilson's words, a frontier of "potentially endless study and admiration."

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