Evolutionary Biology of Plant Defenses against Herbivory and Their Predictive Implications for Endocrine Disruptor Susceptibility in Vertebrates

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Hormone disruption is a major, underappreciated component of the plant chemical arsenal, and the historical coevolution between hormone-disrupting plants and herbivores will have both increased the susceptibility of carnivores and diversified the sensitivities of herbivores to man-made endocrine disruptors. Here I review diverse evidence of the influence of plant secondary compounds on vertebrate reproduction, including human reproduction. Three of the testable hypotheses about the evolutionary responses of vertebrate herbivores to hormone-disrupting challenges from their diet are developed. Specifically, the hypotheses are that *a*) vertebrate herbivores will express steroid hormone receptors in the buccal cavity and/or the vomeronasal organ; *b*) absolute sex steroid concentrations will be lower in carnivores than in herbivores; and *c*) herbivore steroid receptors should be more diverse in their binding affinities than carnivore lineages. The argument developed in this review, if empirically validated by support for the specific hypotheses, suggests that *a*) carnivores will be more susceptible than herbivores to endocrine-disrupting compounds of anthropogenic origin entering their bodies, and *b*) diverse herbivore lineages will be variably susceptible to any given natural or synthetic contaminant. As screening methods for hormone-disrupting potential are compared and adopted, comparative endocrine physiology research is urgently needed to develop models that predict the broad applicability of those screening results in diverse vertebrate species. *Key words:* endocrine disruptors, estrogen receptor, evolution, diet, fertility, plant–herbivore coevolution, sex steroids.*Environ Health Perspect* 109:443–448 (2001). [Online 24 April 2001]
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Developing a coherent science and policy agenda to define the reproductive threat posed by man-made environmental contaminants is an immense task (*1*). A key component of any scientific and policy response to the threat of endocrine disruptors will be an ability to predict the effects of diverse chemical compounds on wildlife, as well as on humans (*2*,*3*). Hormone disruption is an underappreciated component of plant chemical arsenals. The ability to disrupt vertebrate reproduction is clearly present in plants, the relevant biochemical pathways are available to plants, and the risks to plant reproduction are minimal. An evolutionary history of hormone-disruptor activity in plants and the coevolutionary responses of herbivores will manifest itself in variable responses of herbivores to the same contaminant, overall increases in steroid concentration in herbivores relative to carnivores, and sensitive abilities to adjust contaminant ingestion in herbivores. If the hypotheses developed in this analysis are empirically validated, then ecology and diet will predict susceptibility to anthropogenic hormone disruptors across a wide range of vertebrate species.

Plants Do Not Want to Be Eaten

Plants do not benefit from having their foliage and structural support (e.g., shoots, roots, tubers) eaten. They respond to damage (or pruning) by regrowth, altered patterns of growth, and flowering, but losses to herbivory are expensive in terms of lost photosynthetic capacity, lost mechanical strength, lost moisture (through wounds), lost investment in the foliage that must be replaced, and lost energetic reserves to devote to reproduction and survival (*4*–*6*).

Plants have evolved a broad array of defenses to reduce the damage caused by herbivory. Some of those defenses are physical, from thorns to fiber bulk that defies vertebrate digestive capacity (*4*), but the majority are chemical (*5*,*6*). The array of chemical defenses is staggeringly large (*7*). For example, plants can release volatile turpenes that relieve herbivory pressure on the plant by attracting carnivores to eat the insect herbivores (*8*,*9*). Similarly, bitter-tasting repellents (*10*), mammalian cardiac glycosides (*11*), severe skin irritants (*12*), and cyanides or oxygen radicals released upon mastication (*13*,*14*) are well-characterized components of the plant chemical arsenal.

Although the plants we eat are predominantly domesticated, we are all familiar with secondary compounds in plants and often use them to our economic and health advantage. Three types of plant compounds relevant to humans illustrate the diversity: First, fungi and bacteria attack plants, and plants have evolved suites of responses to those attacks which include fungicides and antibacterial compounds. When humans are faced with fungal or bacterial attacks, we have successfully coopted those plant compounds for our own defense or for the defense of our plant and animal agricultural crops. Second, in our diet, we include fruits for their vitamins, but also for their enhancing effect on the motility of our digestive tract. Both their coloration and their sugar content confirm that fruits are adapted for animal consumption. Nevertheless, enhanced motility of the vertebrate digestive tract has survival value for the plant, and only incidental value for us. When motility is increased, the seed is deposited sooner after ingestion (and therefore not too far from the habitat which was suitable for the parent) and simultaneously buried, fertilized, and watered. Third, plants also contain compounds that interfere with cognitive and motor function in vertebrates. Opium from poppies is an effective defense of the poppy as well as a mind-altering pain killer in us. A herbivore under the influence of opium is likely to wander into the open, fail to remain alert, and thus to become food for a carnivore. The secondary plant compound, opium, results in the death of the herbivore and effectively protects the plant by reducing the chance that another meal will be taken. These three examples represent a tiny fraction of the plant chemical arsenal. That arsenal is unequivocally diverse and effective in improving the survival of the plant, but faces continual challenges from herbivore counterstrategies.

Herbivores Fight Back

Herbivores need to gain nutrients from plant tissue for their growth and survival. Therefore, counterstrategies to circumvent or minimize the damage from plant defenses

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are also diverse. Herbivores can learn to avoid well-defended plants, but there may be no plants that are undefended. Instead, mammalian herbivores often consume a diverse diet composed of a variety of sublethal doses of chemical defenses (*15–19*) or carefully consume only the tissues that are least toxic to them (*6*). Herbivores can also evolve detoxification mechanisms that allow them to consume specific plants in spite of their chemical defenses (*20*), or they can consume therapeutic antidotes to the ingested toxins (*21,22*). Plant tannins are a well-characterized example. If ingested, tannins can inactivate digestive enzymes and impair nutritional uptake (*23,24*), reducing growth and survivorship (*25*). Herbivores, as expected, prefer to avoid ingesting tannins if alternative foods are available. However, they will consume tannins in response to increasing hunger or increasing food value (*18,19*). Rodents and rabbits which routinely prefer and consume high-tannin plant foods, on the other hand, secrete salivary proteins which are proline-rich and bind the tannins so that they do not interfere with digestion (*25*). Defenses and counterdefenses set the stage for the coevolution of plants and herbivores.

Coevolution of Plant Secondary Compounds and Vertebrate Herbivores Is Widespread

Evidence of coevolution of plants and herbivores is abundant (*26*). For example, the present-day distribution of cytochrome P450 enzymes is closely linked to plant–herbivore relationships (*7*). Extant P450 genes, numbering in the thousands, originally evolved in bacteria to detoxify the free oxygen appearing in the atmosphere as a result of photosynthesis. They still function in a metabolic detoxification role but now vary widely among species (*27*). Explosive diversification occurred as the Silurian to Devonian conquest of land by terrestrial plants was followed by the colonization of land by arthropods and vertebrates. That diversification is attributed to plant defenses (phytoalexins) against herbivory being countered by new P450 genes to detoxify them (*28*).

These elaborate coevolutionary relationships are facilitated by a clear difference between predation and herbivory (*29*). A victim of predation is dead, whereas a victim of herbivory has received damage that adversely affects its potential for survival and reproduction but it is not usually killed. Thus, plant defenses against herbivory immediately benefit the individual plant [compared with predation where the evolution of chemical defense is typically selected for by the indirect benefits to surviving kin (*30,31*)], and those benefits include reduced predation during the remainder of its life, which can be very long with asexual reproductive/propagation strategies.

The majority of research into plant–herbivore coevolution has been directed at insect herbivores rather than vertebrate herbivores. This is because vertebrate biomass is currently much lower than insect biomass and because vertebrates pose less of a threat to agriculture and forestry. However, large-bodied vertebrate herbivores have been diverse and abundant over the last 10,000 to many million years, when these coevolutionary attacks and defenses originated (*32,33*). In addition, chemical defense strategies that work against insects can also be broadly effective in vertebrates. A clear example, and the focus of this argument, are compounds that disrupt reproduction through their effects on steroid hormones and receptors.

Steroids Are Cheap, Low-Risk Weapons for Plants

In arthropods, the molting hormone, ecdysone, is a steroid that is mimicked in some plants (*34*). In vertebrates, estrogens and androgens are essential sex steroids (*35*). The reproductive life cycle of plants does not involve sex steroid hormones the way it does in arthropods and vertebrates. Nevertheless, the biosynthetic pathways for the sterol precursors are ubiquitous and essential for cell membrane synthesis in all living cells (primarily stigmasterol in plants and cholesterol in animals) (*36*). Thus, interference with herbivore reproduction through effects on steroid hormones does not risk interference with plant reproduction and uses minor modifications in existing biosynthetic pathways. In addition, hormone disruption can be caused by low levels of plant secondary compounds (*37*). Vertebrate sex steroid concentrations in peripheral plasma typically fall in the picogram to low nanogram range (between 1×10^{-12} and 1×10^{-9} g/mL) (*35*). Chemical castration or reproductive impairment through disruption of herbivore endocrine systems would therefore be an effective strategy to reduce local populations of the herbivore or induce herbivore avoidance of the plant.

From Curiosity to Homeopathy

Our current understanding of plant chemicals that alter vertebrate reproductive function spans more than 60 years, but it is limited by the specific challenges scientists were tackling. Chemicals that influence the fertility and fecundity of vertebrate herbivores are already known in the plant chemical arsenal. Some are identical or very similar to vertebrate sex steroids (*36*). Other plant compounds bear little structural homology to endogenous sex steroids but interact either with the native steroid receptors as agonists or antagonists or bind to enzymes involved in the metabolism of steroid hormones (*38*). The latter compounds are probably derived from ancestral genes that are homologous in plants and animals (*39–41*).

Guided by exploration of structural homology as a key to biological function, it was only a few years from the isolation of the first vertebrate sex steroid to the isolation of plant compounds with similar biological activity in castrated animals but not in the plant (*42*). These compounds were known as secondary plant compounds because they did not play a role in primary metabolism. Soon, however, plant compounds that influenced vertebrate reproduction became an economic challenge in agriculture.

Fifty years ago, the Australian clover, *Trifolium subterranean* L., was identified as the source of a feminizing compound responsible for impaired sexual performance in rams and a 70% reduction in lambing (*43*). The active compounds turned out to be isoflavonoids, which are widely distributed in legumes, where they play a role in establishing nitrogen-fixing bacterial symbioses, and in flowers, where they contribute to color. Although their estrogenic potencies are low relative to endogenous estrogens, they are still a hazard to domestic mammalian herbivores. In spite of selective breeding to reduce isoflavone content in the clovers, more than 1 million Australian ewes annually fail to lamb as a direct result of "clover disease" (*44,45*).

It soon became clear that structural homology with sex steroids was not a prerequisite for effective disruption of vertebrate reproduction. For example, a depilatory for sheep used about 40 years ago, L-mimosine, which is a heterocyclic, nonprotein amino acid found in two legume genera, caused irregular estrous cycling, reduced litter size, and increased embryo fatality in mammals as well as reduced egg production in poultry (*46*). Thus, chemicals of plant origin that had applications in agriculture had the potential to adversely affect reproduction as well. However, this research was concerned with protecting livestock, not with understanding the possible biological activities of those plant compounds in ecological settings. Instead, the next wave of attention to plant–herbivore reproductive chemistry was a celebration of adaptive exploitation by the herbivore.

6-Methoxybenzoxazolinone (MBOA) was identified as a plant secondary compound which was used by montane voles, *Microtus montanus*, to stimulate reproduction at exactly the appropriate time for food availability to support pup rearing (*47–49*). Physiologists quickly detected MBOA in lettuce, spinach, and a host of other plants (*50*),

whereas ecologists recognized the broad adaptive value of coopting such environmentally obtained signals for timing reproductive activity in small mammalian herbivores with seasonal breeding (*37*,*51*–*53*). The value of MBOA to the plant remains unknown.

Soon, however, the economic needs of agriculture reemerged as researchers, particularly in Australia, sought biological pest control methods to reduce rodent populations. Chemosterilants (hormone disruptors) could directly target survivors and thereby reduce the probability of accidentally selecting for pest resistance to the poisoning agents (*54*). Unfortunately, rodents reduced their intake when breeding and avoided the chemosterilizing effects. Although there is still interest in the role of isoflavonoids in determining plant resistance to arthropod herbivory (*55*), the focus is no longer on adverse reproductive effects in vertebrates.

Most recently, the pendulum has swung completely back again, and considerable research effort is being devoted to the potential health benefits of phytoestrogens in women (*56*,*57*). Public enthusiasm for natural herbal remedies (*58*) includes championing soybean (*Glycine max*) consumption as an alternative to estrogen replacement during and following menopause (*59*) and promises protection against breast cancer (*60*). Of course, humans are animals with herbivory in our family tree (*16*), and we would expect plant secondary compounds to influence human reproduction.

Plant Compounds That Alter Human Fertility

Whereas phytoestrogens in soy products might be therapeutic in postreproductive women, the effects of plants on human reproduction are also felt during the peak reproductive years. For example, cottonseed oil containing gossypol is such a potent inhibitor of sperm formation that entire regions of the province of Jiangsu in China produced no children while they were using the oil for cooking (*61*,*62*). Although that inadvertent experiment was disastrous for those families, the fertility of those men recovered when the gossypol was removed from their diet. Thus, recent attention to gossypol focuses on its potential as a reversible male contraceptive (*61*,*62*).

Commonly ingested human food products such as beans, nuts, legumes, grains, licorice, thyme, oregano, turmeric, hops, and verbena have high biological activity as phytoestrogens and retain that activity after ingestion (*63*). For example, soybean products are a potent source of isoflavonoids, particularly genistein and daidzein, which are potently estrogenic in mammals (*44*). Given the positive spin of soy products and women's health, it is perhaps not surprising that there is little discussion of the effects on men of simultaneously increasing the phytoestrogen content of their diet (*64*). Indeed, the potential for adverse developmental effects in infants raised on soy milk formulations, which are exceptionally bioactive as estrogens, is only now becoming an important health issue (*56*,*65*,*66*).

Ancestral human cultures were probably well aware of the potential for some plants to stimulate ovulation in women while interfering with men's fertility. Women are primarily responsible for cultivation of food crops in indigenous societies of Papua New Guinea, but yams are traditionally grown and harvested by men, who then present them to women as ceremonial gifts (*67*). Diosgenin, a steroidal sapogenin from the tubers of yams, is such an abundant source of vertebrate sex steroids that it remains a commercial source for hormones used in oral contraceptives (*68*).

Domestication Might Have Enhanced Hormonal Bioactivity in Our Diet

Much of human population growth has arisen from winning the battle against plant secondary compounds by changing them. Humans cook and process foods to reduce toxicity. For example, cassava (*Manihot esculenta*) is the major starchy food for more than 300 million people living in the tropics, yet most cultivars contain sufficient cyanide to be highly toxic, and elaborate processing is essential before manioc can be safely consumed (*69*). Humans have also artificially bred and selected all of our major food crops to decrease toxicity and increase nutritional value (*67*,*70*,*71*). There is no equivalent cultural or historical record of selection against reproductive effects.

This is not because cooking solves all of the problems. Cooking and processing have little effect on the biological activity of flavones and isoflavones (*63*). Thus, although our artificially selected food supply has fewer defenses against being eaten than our ancestral diet, it may be exceptionally rich in secondary plant compounds that influence human reproduction. In fact, plants chosen for domestication may be more likely to contain phytoestrogens than other plants because they might have been the ancestral foods that, in small doses, enhanced ovulatory cyclicity in women.

Ancestral human cultures were unavoidably aware of the need to maintain a diverse diet because food abundances varied seasonally. Even now, Machiguenga Indians from the Amazon cultivate at least 80 food crops, with about 30 of them in the household garden of a typical family (*67*). This generalist strategy, where the adverse effects of any given plant defense are minimized by limiting consumption, is remarkably similar to the advice of health professionals. After decades of testing by the U.S. Food and Drug Administration, the recommendations can be reduced to a simple edict: A well-balanced diet with plenty of exercise is the best prescription for health (*72*). After all, in herbivory as well as in pharmacology, it is the dose that makes the poison.

Predictions

The potential to disrupt vertebrate reproduction is clearly present in plants. The biochemical pathways are available to them and the risks to their own reproduction are minimal. Thus, hormone disruption is a major, underappreciated component of the plant chemical arsenal. Following is a series of three hypotheses that emerge directly from that argument. Specifically, if plants use hormone disruption as a defense against herbivory, then: *a*) herbivores should be able to detect hormone-disrupting plant allelochemicals orally; *b*) carnivores should have lower circulating sex steroid concentrations than herbivores; and *c*) herbivore steroid receptors should be more diverse in their binding affinities than carnivore lineages. Other predictions are likely and are welcomed.

Hypothesis 1: Vertebrate herbivores will express steroid hormone receptors in the buccal cavity and/or the vomeronasal organ. If hormone-disrupting compounds in their diet have been important in herbivore evolution, then herbivores should be able to detect those compounds in their mouths and adjust food intake accordingly. There is evidence supporting buccal/vomeronasal organ receptors for native steroids and phytochemical compounds that interact with those receptors. Discrete subpopulations of neurons in the vomeronasal organ respond to discrete pheromonal signals (*73*), many of which are steroid metabolites (*74*,*75*). Animals also avoid feed that contains steroid hormones. In a study evaluating synthetic steroids as chemosterilants for rodent control, rats refused to ingest ethinyl estradiol and developed a specific aversion for methyl testosterone when pregnant or lactating (*54*). Although taste aversions generalize to other foods, mammalian herbivores continue to taste aversive foods in small quantities and readily resume consumption when the aversive stimulus is reduced or removed (*76*). Therefore, both plants and herbivores will always be under dynamic selection relative to the production and ingestion of plant secondary compounds.

As a test of this prediction, the presence or absence of steroid hormone receptors in the buccal cavity and/or vomeronasal organ

could be empirically confirmed in a range of vertebrate species. One possible outcome is that the presence of buccal or vomeronasal steroid receptors in herbivores, but not carnivores, will support the hypothesis. The second possible outcome is that steroid receptors in both herbivores and carnivores will be consistent with the hypothesis but not exclude alternative interpretations. For example, selection for increased ability to detect the hormonal status of potential mates might also have favored expression of these receptors. Saliva contains an accurate measure of biologically available hormone (free of bound steroid) which reflects serum and plasma concentrations (*77–80*) and is not restricted to humans (*81*). Therefore, facial licking (*82*), as well as urine sniffing (*83,84*), might provide accurate information about the current hormonal status of the donor (*85,86*). The third possible outcome is that the absence of steroid hormone receptors in the buccal cavity of herbivores consuming a diverse diet will fail to support the argument that hormone disruption has been widespread in the coevolution of plants and herbivores.

Hypothesis 2: Absolute sex steroid concentrations will be lower in carnivores than herbivores. One obvious mechanism by which a herbivore could successfully consume limited quantities of hormone-disrupting plant material over evolutionary time would be to alter the signal-to-noise ratio by increasing the concentrations of endogenous sex steroid that evoke physiological responses. Whereas a dietary intake of 10 µg/kg/day might disrupt reproduction in a species with 30 µg/kg circulating in plasma, it would be unlikely to disrupt reproduction against a background of 300 µg/kg in circulation. Since the development of sensitive assays for steroid hormones, comparative endocrinologists have appreciated that both the concentrations of steroid hormones necessary to elicit biological activity (*87*) and the absolute concentrations of steroid hormones in peripheral circulation (*88*) differ substantially from species to species. The same is true of intraspecific variation in steroid hormone concentrations. For example, the normal adult male salivary testosterone concentration ranges from 30 to 160 ng/mL (*89*). No satisfying explanation for this variability has previously been offered. If the dietary threat from hormone-disrupting compounds has been important in the evolution of vertebrate herbivores, then, on average, herbivores should have higher steroid hormone concentrations in peripheral plasma than carnivores.

As a test of this prediction, a survey of estrogen and testosterone concentrations in a wide range of vertebrate species representing carnivory, omnivory, specialist herbivory, and generalist herbivory should reveal a general pattern of increasing sex steroid concentrations. One possible outcome is that a robust pattern would be observed within reptiles, within amphibians, within birds, and within mammals. This would support the hypothesis. A second possible outcome is that, if plant defense strategies are different in unicellular plants or aquatic macrophytes than in higher plants, the pattern might prevail in terrestrial ecosystems, but not aquatic ecosystems. Results would support the hypothesis but require further investigation. A third possible outcome is that the absence of the predicted pattern in terrestrial vertebrates would fail to support the hypothesis.

Hypothesis 3: Herbivore steroid receptors should be more diverse in their binding affinities than carnivore lineages. Another successful evolutionary strategy that would allow a herbivore to continue to eat a plant food containing hormone-disrupting compounds would be a mutation in the steroid receptor that allowed continued binding with the endogenous steroid but greatly decreased the biological activity of the exogenous compound. Binding affinities are strongly affected by the stereochemistry of interaction with the target molecule and differ by orders of magnitude for different phytoestrogens interacting with the same and with different estrogen receptors (*38,39*). If the dietary threat from hormone-disrupting compounds has been important in the evolution of vertebrate herbivores, then diversification of steroid hormone receptors should be higher in herbivores than in carnivores.

Genetic identification, sequencing, and characterization of sex steroid receptors in a wide range of vertebrate species would test this prediction. This test may have to wait until a sufficient number of species have been studied in detail. Present evidence is minimal either way (*90,91*). The discovery of estrogen receptor β (ERβ) caused great excitement in the field of endocrinology (*92*). Since then, researchers have documented different localization and biological functions for the two estrogen receptors (*93,94*) and different isoforms of ERβ (*95,96*) with different binding sensitivities for 17β-estradiol (*97*) and the ability to form heterodimers with other estrogen receptors (*98*). If hormone-disrupting compounds in plants have selected for diversification of steroid hormone receptors, particularly in herbivores, then the pace of discovery is likely to increase in the future.

Implications for Herbivore versus Carnivore Susceptibility to Endocrine Disruptors of Anthropogenic Origin

The argument developed in this review suggests that *a*) carnivores will be more susceptible than herbivores to endocrine-disrupting compounds of anthropogenic origin entering their bodies, and *b*) diverse herbivore lineages will not be equally vulnerable to any given environmental contaminant.

The prediction that vertebrate carnivores are at greatest risk from environmental contaminants that disrupt reproduction is particularly troubling. The array of chemical compounds in our environment that are of anthropogenic origin is huge (*3*). Many of those compounds are soluble in lipid and not biologically degraded (*99*). Therefore, they tend to accumulate through generations and through food chains. In mammalian species the challenges are further increased by the potential for transgenerational transfer of lipid-soluble contaminants from mother to infant through lactational ejection of maternal lipid into milk. If this argument is upheld, then carnivores will also have lower endogenous steroid concentrations, which will increase their susceptibility to low doses of man-made endocrine disruptors. On a more positive note, they may also have less diverse receptor structure and function than herbivores, improving the potential for screening to generalize effectively across species.

The opposite is unfortunately true for vertebrate herbivores. If this argument is upheld, then diverse species of vertebrate herbivores may not have similar susceptibilities to contaminants. For any given man-made compound, there may be species with low susceptibilities and species with high susceptibilities. Screening will be more difficult, although higher endogenous steroid concentrations in vertebrate herbivores may be broadly prophylactic against man-made endocrine disruptors.

Summary

The hypothesis that the coevolutionary relationship between plants and herbivores will clarify patterns of susceptibility to environmental contaminants is easily tested. If empirical validation of the hypothesis emerges from tests such as those proposed here, then there are immediate practical applications for policy, research focus, and preservation of human health.

First, research in reproductive endocrinology has been almost exclusively focused on a small group of domesticated species. The rat, mouse, and rhesus monkey account for the overwhelming majority of basic health research in reproductive endocrinology. Likewise, the sheep and, to a lesser extent, the cow, have been the primary focus of agricultural reproduction research. If ecological variables, such as dietary burden of hormone-disrupting compounds, have altered susceptibility to anthropogenic contaminants, then a more diverse research base is urgently needed.

Second, a more diverse research base will also improve our ability to generalize hormone disruptor effects to human health. Common laboratory and agricultural species are all domesticated. In spite of the domestic work humans perform, we are not domesticated. Domestication decreases the sensitivity of the reproductive axis to environmental cues including social odors and season. Domestication also disrupts ancestral abilities to avoid consumption of toxic plant secondary compounds (*76*). The richness of our potential interactions with our environment will never be accurately represented by a domesticated animal model.

Third, identifying patterns in susceptibility to anthropogenic contaminants with hormone-disrupting potential will allow us to design screening protocols that protect wildlife as well as human welfare.

Facing environmental health challenges and developing sensible policy responses needs all the informed advice we can muster. The process can only benefit from including the perspectives of diverse scientific disciplines. In this particular example, insights from ecology, plant biology, evolutionary biology, and zoology offer integrative as well as prescriptive solutions for dealing with endocrine disruptor susceptibility in vertebrates, including ourselves.

References and Notes

1. U.S. EPA. Research Plan for Endocrine Disruptors. Washington, DC:U.S. Environmental Protection Agency, Office of Research and Development, 1998.
2. Munkittrick KR, McMaster ME, McCarthy LH, Servos MR, VanDerKraak GJ. An overview of recent studies on the potential of pulp-mill effluents to alter reproductive parameters in fish. J Toxicol Environ Health B Crit Rev 1:347–371 (1998).
3. Ankley G, Mihaich E, Stahl R, Tillitt D, Colborn T, McMaster S, Miller R, Bantle J, Campbell P, Denslow N, et al. Overview of a workshop on screening methods for detecting potential (anti-) estrogenic/androgenic chemicals in wildlife. Environ Toxicol Chem 17:68–87 (1998).
4. Iason GR, Van Wieren SE. Digestive and ingestive adaptations of mammalian herbivores to low-quality forage. In: Herbivores: between Plants and Predators (Olff H, Brown VK, Drent RH, eds). Malden, MA:Blackwell Science, 1999;337–369.
5. Seigler D, Price PW. Secondary compounds in plants: primary functions. Am Nat 110:101–105 (1976).
6. Karban R, Baldwin IT. Induced Responses to Herbivory. Chicago:University of Chicago Press, 1997.
7. Berenbaum MR, Zangerl AR. Genetic variation in cytochrome P450-based resistance to plant allelochemicals and insecticides. In: Herbivores: between Plants and Predators (Olff H, Brown VK, Drent RH, eds). Malden, MA:Blackwell Science, 1999;55–84.
8. Turlings TCJ, Loughrin JH, McCall PJ, Roese USR, Lewis WJ, Tumlinson JH. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. Proc Natl Acad Sci USA 92:4169–4174 (1995).
9. Vet LEM. Evolutionary aspects of plant-carnivore interactions. In: Insect-plant Interactions and Induced Plant Defense (Chadwick DJ, Goode JA, eds). New York:John Wiley & Sons Ltd., 1999;3–20.
10. Picman AK. Biological activities of sesquiterpene lactones. Biochem Syst Ecol 14:255–281 (1986).
11. Malcolm SB. Cardenolide-mediated interactions between plants and herbivores. In: Herbivores: Their Interactions with Secondary Plant Metabolites (Rosenthal GA, Berenbaum MR, eds). New York:Academic Press, 1991;251–296.
12. Berenbaum MR, Zangerl AR. Phytochemical diversity: adaptation or random variation. In: Phytochemical Diversity and Redundancy in Ecological Iinteractions, (Romeo JT, Saunders JA, Barbosa P, eds). New York:Plenum Press, 1996;1–24.
13. Poulton JE. Cyanogenesis in plants. Plant Physiol 94:401–405 (1990).
14. Lamb C, Dixon RA. The oxidative burst in plant disease resistance. Ann Rev Plant Physiol Plant Mol Biol 48:251–275 (1997).
15. Oates JF, Waterman PG, Choo GM. Food selection by the South Indian leaf-monkey, *Presbytis johnii*, in relation to leaf chemistry. Oecologia 45:45–56 (1980).
16. Popovich DG, Jenkins DJA, Kendall CWC, Dierenfeld ES, Carroll RW, Tariq N, Vidgen E. The western lowland gorilla diet has implications for the health of humans and other hominoids. J Nutr 127:2000–2005 (1977).
17. Pfister JA, Provenza FD, Manners GD, Gardner DR, Ralphs MH. Tall larkspur ingestion: can cattle regulate intake below toxic levels? J Chem Ecol 23:759–777 (1997).
18. Simmen B, Josseaume B, Atramentowicz M. Frugivory and taste responses to fructose and tannic acid in a prosimian primate and a didelphid marsupial. J Chem Ecol 25:331–346 (1999).
19. Burritt EA, Provenza FD. Role of toxins in intake of varied diets by sheep. J Chem Ecol 26:1991–2005 (2000).
20. Boyle R, McLean S, Foley WJ, Moore BD, Davies NW, Brandon S. Fate of the dietary terpene, p-cymene, in the male koala. J Chem Ecol 26:1095–1111 (2000).
21. Gilardi JD, Duffey SS, Munn CA, Tell LA. Biochemical functions of geophagy in parrots: detoxification of dietary toxins and cytoprotective effects. J Chem Ecol 25:897–922 (1999).
22. Mahaney WC, Milner MW, Sanmugadas K, Hancock RGV, Aufreiter S, Wrangham R, Pier HW. Analysis of geophagy soils in Kibale Forest, Uganda. Primates 38:159–176 (1997).
23. Felton GW, Donato K, Del Vecchio RJ, Duffey SS, Activation of plant foliar oxidases by insect feeding reduces nutritive quality of foliage for noctuid herbivores. J Chem Ecol 15:2667–2694 (1989).
24. Peumans WJ, Van Damme EJM. Lectins as plant defense proteins. Plant Physiol 109:347–352 (1995).
25. Butler LG. Effects of condensed tannin on animal nutrition. In: Chemistry and Significance of Condensed Tannins (Hemingway RW, Karchesy JJ, eds). New York:Plenum Press, 1989;391–402.
26. Janzen DH. New horizons in the biology of plant defenses. In: Herbivores: Their Interactions with Secondary Plant Metabolites (Rosenthal GA, Janzen DH, eds). New York:Academic Press, 1979;331–350.
27. Nebert DW, Nelson DR, Feyereisen R. Evolution of the cytochrome P450 genes. Xenobiotica 19:1149–1160 (1989).
28. Gonzalez FJ, Nebert DW. Evolution of the P450 gene superfamily: animal-plant "warfare," molecular drive and human genetic differences in drug oxidation. Trends Genet 6:182–186 (1990).
29. Ehrlich PR, Raven PH. Butterflies and plants: a study in coevolution. Evolution 18:586–608 (1964).
30. Hamilton WD. The evolution of altruistic behavior. Am Nat 97:354–356 (1967).
31. Brower LP, Calvert WH, Hedrick LE, Christian J. Ecological chemistry and the palatability spectrum. Science 161:1349–1351 (1968).
32. Howe HF, Westley LC. Ecological Relationships of Plants and Animals. New York:Oxford University Press, 1988.
33. Palo RT, Robbins CT. Plant Defenses Against Mammalian Herbivory. Boca Raton, FL:CRC Press, 1991.
34. Schmelz EA, Grebenok RJ, Galbraith DW, Bowers WS. Damage-induced accumulation of phytoecdysteroids in spinach: a rapid root response involving the octadecanoic acid pathway. J Chem Ecol 24:339–360 (1998).
35. Hadley M. Endocrinology. Upper Saddle River, NJ:Prentice Hall, 2000.
36. Taiz L, Zeiger E. Plant Physiology. Sunderland, MA:Sinauer Association, 1998.
37. Labov JB. Phytoestrogens and mammalian reproduction. Comp Biochem Physiol 57A:3–9 (1977).
38. Kao Y-C, Zhou C, Sherman M, Laughton CA, Chen S. Molecular basis of the inhibition of human aromatase (estrogen synthetase) by flavone and isoflavone phytoestrogens: a site-directed mutagenesis study. Environ Health Perspect 106:85–92 (1998).
39. Martin ME, Haourigui M, Pelissero C, Benassayag C, Nunez EA. Interactions between phytoestrogens and human sex steroid binding protein. Life Sci 58:429–436 (1996).
40. Patisaul HB, Whitten PL, Young LJ. Regulation of estrogen receptor β mRNA in the brain: opposite effects of 17β-estradiol and the phytoestrogen, coumestrol. Brain Res Mol Brain Res 67:165–171 (1999).
41. Baker ME. Endocrine activity of plant-derived compounds: an evolutionary perspective. Proc Soc Exp Biol Med 208:131–138 (1995).
42. Fieser LF, Fieser M. Natural products related to phenanthrene. New York:Reinhold Publishing Corp., 1949.
43. Bradbury RB, White DE. Estrogens and related substances in plants. Vitam Horm 12:207–233 (1954).
44. Harborne JB. Flavonoid pigments. In: Herbivores: Their Interactions with Secondary Plant Metabolites (Rosenthal GA, Berenbaum MR, eds). New York:Academic Press, 1991;389–429.
45. Chamley WA, Clarke IJ, Moran AR. Seasonal changes in LH secretion in normal ewes which grazed oestrogenic clover. Aust J Biol Sci 38:109–113 (1985).
46. Rosenthal GA. Nonprotein amino acids as protective allelochemicals. In: Herbivores: Their Interactions with Secondary Plant Metabolites (Rosenthal GA, Berenbaum MR, eds). New York:Academic Press, 1991;1–34.
47. Sanders EH, Gardner PD, Berger PJ, Negus NC. 6-Methoxybenzoxazolinone: a plant derivatative that stimulates reproduction in *Microtus montanus*. Science 214:67–69 (1981).
48. Berger PJ, Sanders EH, Gardner PD, Negus NC. Phenolic plant compounds functioning as reproductive inhibitors in *Microtus montanus*. Science 195:575–577 (1977).
49. Berger PJ, Negus NC, Sanders EH, Gardner PD. Chemical triggering of reproduction in *Microtus montanus*. Science 214:69–70 (1981).
50. Korn H. A feeding experiment with 6-methoxybenzoxazolinone and a wild population of the deer mouse (*Peromyscus maniculatus*). Can J Zool 67:2220–2224 (1989).
51. Frandsen TC, Boyd SS, Berger PJ. Maternal transfer of the 6-MBOA chemical signal in *Microtus montanus* during gestation and lactation. Can J Zool 71:1799–1803 (1993).
52. Nelson RJ, Blom JMC. 6-Methoxy-2-benzoxazolinone and photoperiod: prenatal and postnatal influences on reproductive development in prairie voles (*Microtus ochrogaster*). Can J Zool 71:776–789 (1993).
53. White RM, Bernard RTF. Lack of a response of the subtropical rodent (*Saccostomus campestris*) to a secondary plant compound, 6-methoxybenzoxazolinone - consequences for reproductive strategy. S Afr J Zool 34:108–113 (1999).
54. Gao Y, Short RV. Use of an oestrogen, androgen or gestagen as a potential chemosterilant for control of rat and mouse populations. J Reprod Fertil 97:39–49 (1993).
55. Wang SF, Ridsdill-Smith TJ, Ghisalberti EL. Role of isoflavonoids in resistance of subterranean clover trifoliates to the redlegged earth mite *Halotydeus destructor*. J Chem Ecol 24:2089–2100 (1998).
56. Sheehan DM. Herbal medicines, phytoestrogens and toxicity: risk:benefit considerations. Proc Soc Exp Biol Med 217:379–385 (1998).
57. Albertazzi P, Pansini F, Bottazzi M, Bonaccorsi G, De Aloysio D, Morton MS. Dietary soy supplementation and phytoestrogen levels. Obstet Gynecol 94:229–231 (1999).
58. Johns T. Behavioral determinants for the ingestion of food phytochemicals. In: Functionality of Food Phytochemicals (Johns T, Romeo JT, eds). New York:Plenum Press, 1997;133–154.
59. Jensen K. Menopause: A Naturopathic Approach to the Transitional Years. Toronto:Prentice Hall, 1999.
60. Bouker KB, Hilakivi-Clarke L. Genistein: does it prevent or promote breast cancer? Environ Health Perspect 108:701–708 (2000).
61. Yu ZH, Chan HC. Gossypol as a male antifertility agent—why studies should have been continued. Int J Androl 21:2–7 (1998).
62. Waites GMH, Wang C, Griffin PD. Gossypol: reasons for its failure to be accepted as a safe, reversible male antifertility drug. Int J Androl 21:8–12 (1998).
63. Zava DT, Dollbaum CM, Blen M. Estrogen and progestin bioactivity of foods, herbs, and spices. Proc Soc Exp Biol Med 217:369–378 (1998).
64. Santti R, Mäkelä S, Strauss L, Korkman J, Kostian ML.

Phytoestrogens: potential endocrine disruptors in males. Toxicol Ind Health 14:223–237 (1998).
65. Irvine CH, Shand N, Fitzpatrick MG, Alexander SL. Daily intake and urinary excretion of genistein and daidzein by infants fed soy- or dairy-based infant formulas. Am J Clin Nutr 68:1462S–1465S (1998).
66. Irvine CH, Fitzpatrick MG, Alexander SL. Phytoestrogens in soy-based infant foods: concentrations, daily intake, and possible biological effects. Proc Soc Exp Biol Med 217:247–253 (1998).
67. Lewington A. Plants for People. New York:Oxford University Press, 1990.
68. Alizadeh S, Mantell SH, Viana AM. In vitro shoot culture and microtuber induction in the steroid yam *Dioscorea composita* Hemsl. Plant Cell Tissue Organ Cult 53:107–112 (1998).
69. Seigler DS. Cyanide and cyanogenic glycosides. In: Herbivores: Their Interactions with Secondary Plant Metabolites (Rosenthal GA, Berenbaum MR, eds). New York:Academic Press, 1991;35–77.
70. Sotelo A. Constituents of wild food plants. In: Functionality of Food Phytochemicals (Johns T, Romeo JT, eds). New York:Plenum Press, 1997;89–111.
71. Liener IE. Toxic Constituents of Plant Foodstuffs. New York:Academic Press, 1980.
72. Abelson PH. Flaws in risk assessments [Editorial]. Science 270:215 (1995).
73. Dudley CA, Moss RL. Activation of an anatomically distinct subpopulation of accessory olfactory bulb neurons by chemosensory stimulation. Neuroscience 91:1549–1556 (1999).
74. Novotny M, Jemiolo B, Harvey S. Chemistry of rodent pheromones: molecular insights into chemical signalling in mammals. In: Chemical Signals in Vertebrates 5 (Macdonald DW, Muller-Schwarze D, Natynczuk SE, eds). Oxford, UK:Oxford University Press, 1990;1–22.
75. Eggert F, Holler C, Luszyk D, Muller-Ruchholtz W, Ferstl R. MHC-associated and MHC-independent urinary chemosignals in mice. Physiol Behav 59:57–62 (1996).
76. Bryant JP, Reichart PB, Clausen TP, Provenza FD, Kuropat PJ. Woody-plant-mammal interactions. In: Herbivores: Their Interactions with Secondary Plant Metabolites (Rosenthal GA, Berenbaum MR, eds). New York:Academic Press, 1992;343–370.
77. Dabbs JM Jr. Salivary testosterone measurements: collecting, storing, and mailing saliva samples. Physiol Behav 49:815–817 (1991).
78. Meulenberg PM, Hofman JA. Salivary progesterone excellently reflects free and total progesterone in plasma during pregnancy. Clin Chem 35:168–172 (1989).
79. Vittek J, L'Hommedieu DG, Gordon GG, Rappaport SC, Southren AL. Direct radioimmunoassay (RIA) of salivary testosterone: correlation with free and total serum testosterone. Life Sci 37:711–716 (1985).
80. Choe JK, Khan-Dawood FS, Dawood MY. Progesterone and estradiol in the saliva and plasma during the menstrual cycle. Am J Obstet Gynecol 147:557–562 (1983).
81. Beerda B, Schilder MBH, Janssen NSCRM, Mol JA. The use of saliva cortisol, urinary cortisol, and catecholamine measurements for a noninvasive assessment of stress responses in dogs. Horm Behav 30:272–279 (1996).
82. Smith BA, Block ML. Preference of Mongolian gerbils for salivary cues: a developmental analysis. Anim Behav 39:512–521 (1990).
83. Brown RE, Hauschild M, Holman SD, Hutchison JB. Mate recognition by urine odors in the Mongolian gerbil (*Meriones unguiculatus*). Behav Neur Biol 49:174–183 (1988).
84. Perret M, Schilling A. Sexual responses to urinary chemosignals depend on photoperiod in a male primate. Physiol Behav 58:633–639 (1995).
85. Sobel N, Prabhakaran V, Hartley CA, Desmond JE, Glover GH, Sullivan EV, Gabrieli JD. Blind smell: brain activation induced by an undetected airborne chemical. Brain 122:209–217 (1999).
86. Stern K, McClintock MK. Regulation of ovulation by human pheromones. Nature 392:177–179 (1998).
87. Young WC. The hormones and mating behavior. In: Sex and Internal Secretions (Young WC, ed). Baltimore, MD:The Williams and Wilkins Co., 1961;1173–1239.
88. Heap RB, Flint APF. Pregnancy. In: Hormonal Control of Reproduction (Austin CR, Short RV, eds). New York:Cambridge University Press, 1984;153–194.
89. Dabbs JM Jr. Salivary testosterone measurements: reliability across hours, days, and weeks. Physiol Behav 48:83–86 (1990).
90. McEwen BS, Alves SE. Estrogen actions in the central nervous system. Endocr Rev 20:279–307 (1999).
91. Couse JF, Korach KS. Estrogen receptor null mice: what have we learned and where will they lead us? Endocr Rev 20:358–417 (1999).
92. Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson J. Cloning of a novel estrogen receptor expressed in rat prostate and ovary. Proc Natl Acad Sci 93:5925–5930 (1996).
93. Laflamme N, Nappi RE, Drolet G, Labrie C, Rivest S. Expression and neuropeptidergic characterization of estrogen receptors (ER alpha and ERβ) throughout the rat brain: anatomic evidence of distinct roles of each subtype. J Neurobiol 36:357–378 (1998).
94. Krege JH, Hodgin JB, Couse JF, Enmark E, Warner M, Mahler JF, Sar M, Korach KS, Gustafsson JA, Smithies O. Generation and reproductive phenotypes of mice lacking estrogen receptor β. Proc Natl Acad Sci 95:15677–15682 (1998).
95. Petersen DN, Tkalcevic GT, Koza-Taylor PH, Turi TG, Brown TA. Identification of estrogen receptor β2, a functional variant of estrogen receptor β expressed in normal rat tissues. Endocrinology 139:1082–1092 (1998).
96. Hanstein B, Liu H, Yancisin M, Brown M. Functional analysis of a novel estrogen receptor β isoform. Mol Endocrinol 13:129–137 (1999).
97. Kuiper GGJM, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson J-A. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and β. Endocrinology 138:863–870 (1997).
98. Pettersson K, Grandien K, Kuiper GGJM, Gustafsson J. Mouse estrogen receptor β forms estrogen response element-binding heterodimers with estrogen receptor alpha. Mol Endocrinol 11:1486–1496 (1997).
99. Colborn T, Dumanoski D, Myers JP. Our Stolen Future. New York:Penguin Books, 1996.