Chemical Communication Threatened by Endocrine-Disrupting Chemicals

***Jennifer E. Fox*[1,2]**

[1]Environmental Endocrinology Laboratory, Center for Bioenvironmental Research at Tulane and Xavier Universities, New Orleans, Louisiana, USA; [2]Center for Ecology and Evolutionary Biology and Department of Biology, University of Oregon, Eugene, Oregon, USA

Communication on a cellular level—defined as chemical signaling, sensing, and response—is an essential and universal component of all living organisms and the framework that unites all ecosystems. Evolutionarily conserved signaling "webs," existing both within an organism and between organisms, rely on efficient and accurate interpretation of chemical signals by receptors. Therefore, endocrine-disrupting chemicals (EDCs), which have been shown to disrupt hormone signaling in laboratory animals and exposed wildlife, may have broader implications for disrupting signaling webs that have yet to be identified as possible targets. In this article, I explore common evolutionary themes of chemical signaling (e.g., estrogen signaling in vertebrates and phytoestrogen signaling from plants to symbiotic soil bacteria) and show that such signaling systems are targets of disruption by EDCs. Recent evolutionary phylogenetic data have shown that the estrogen receptor (ER) is the ancestral receptor from which all other steroid receptors have evolved. In addition to binding endogenous estrogens, ERs also bind phytoestrogens, an ability shared in common with nodulation D protein (NodD) receptors found in *Rhizobium* soil bacteria. Recent data have shown that many of the same synthetic and natural environmental chemicals that disrupt endocrine signaling in vertebrates also disrupt phytoestrogen–NodD receptor signaling in soil bacteria, which is necessary for nitrogen-fixing symbiosis. Bacteria–plant symbiosis is an unexpected target of EDCs, and other unexpected nontarget species may also be vulnerable to EDCs found in the environment. *Key words:* convergent evolution, ecosystem, endocrine-disrupting chemicals, endocrine disruption, environmental signaling, estrogen receptor, nitrogen fixation, *Rhizobium*, symbiosis. *Environ Health Perspect* 112:648–653 (2004). doi:10.1289/ehp.6455 available via *http://dx.doi.org/* [Online 29 January 2004]

Chemical Communication via Signaling

Chemical communication is a common means of endogenous and exogenous signaling for countless species. The endocrine system of vertebrates consists of an intricate web of agonistic as well as antagonistic hormone signals, which control sexual development and reproduction (McLachlan 2001). For example, circulating hormones such as 17β-estradiol (E_2) control a variety of cellular processes, including developmental cues, differentiation events, and growth in organs such as breast, ovary, and uterus. The timing and concentration of estrogen signaling determine sexual maturity, ovulation, and pregnancy. In much the same way, a multitude of organisms rely on chemical cues for development and differentiation. For example, some insects and crustaceans rely on ecdysteroids to signal molting and growth (Oberdorster et al. 2001), and the slime mold *Dictyostelium* relies on a chlorinated alkyl phenone called DIF-1 to signal individual cells to differentiate into a multicellular sporulating stalk (Kay 1998; Town et al. 1976).

Plants produce versatile chemical signals, called phytochemicals or phytoestrogens, which serve both as endogenous signals, triggering color and scent production within the plant, and exogenous signals secreted for communication with other organisms, such as to inhibit sexual reproduction of predatory herbivores (Wynne-Edwards 2001). Leguminous plants (soybeans, clover, and alfalfa) secrete phytoestrogens into the soil as recruitment signals for symbiotic mycorrhizal fungi and *Rhizobium* soil bacteria, which both provide selective growth advantages to the host plant, including increased water/phosphate availability and nitrogenous fertilizer, respectively (Baker 1998; Kuiper et al. 1997; Peters et al. 1986). Although phytoestrogens serve specific signaling functions between the plants that produce them and insects, fungi, and bacteria, many chemical signals, including the fungal agent zearalenone and the phytoestrogens genistein and luteolin, are often "misinterpreted" as estrogenic signals in nontarget organisms such as vertebrates.

For chemical communication to occur within or between organisms, a receptor must have affinity for specific chemical ligands or signals, and this recognition must initiate a response. In fact, a wide variety of natural and synthetic chemicals exist in the environment that mimic hormones and disrupt endocrine signaling in vertebrates through interaction with various nuclear receptors and signal transducer proteins, including the estrogen receptor (ER), orphan receptors, and the thyroid receptor (Cheek et al. 1998; Crump et al. 2002; Ishihara et al. 2003; McLachlan 2001; Moriyama et al. 2002; Takeshita et al. 2001). Some flavonoid phytoestrogens are able to bind ER-α and ER-β and act as weak agonists (Collins-Burow et al. 2000) that compete with endogenous E_2 for ER binding and activation of estrogen-responsive genes (Blair et al. 2000; Kuiper et al. 1997). Despite their ability to bind these receptors, phytoestrogens exhibit only a fraction (10^{-2}–10^{-3}) of the estrogenic activity of E_2 (Collins-Burow et al. 2000). Nevertheless, the most active phytoestrogens found in plants have been shown to induce breast cancer cell proliferation *in vitro* as well as influence the *in vivo* endocrine function of experimental animals and livestock that consume high quantities of these phytoestrogen-laden plants (Bennetts et al. 1946; Facemire et al. 1995; Whitten and Patisaul 2001; Zava et al. 1997). The mechanism of action is defined by vertebrate ERs, which bind phytoestrogen ligands, endogenous ligands, and endocrine-disrupting chemicals (EDCs) with specific affinity. Similarly, nodulation D protein (NodD) receptors in *Rhizobium* soil bacteria recognize phytoestrogens as recruitment signals to initiate nitrogen-fixing symbiosis. The specific affinity for and recognition of similar natural and synthetic ligands by receptors such as ER and NodD provide an example of shared or analogous functionality (Fox et al. 2001, 2004).

In an evolutionary context, it may seem odd that phytochemical signals, produced by plants as recruiting signals for symbiotic soil bacteria, are intercepted by humans and affect estrogenic signaling by binding to ERs and influencing estrogen-responsive gene expression. Both plants and humans have the ability to synthesize steroids, and plants express proteins that are homologous in sequence and identical in function to human 5α-reductase enzymes, in that they both catalyze the reduction of steroid substrates (Li et al. 1997). Nevertheless, how is it that plants and humans, two organisms known to be derived from separate lineages (Meyerowitz 2002), both share the ability to produce and recognize steroid signals? After all, there are no ERs in plants or in their most common partners, insects. However, one intended target of phytoestrogen signaling, *Rhizobium* soil

Address correspondence to J.E. Fox, University of Oregon, 335 Pacific Hall, Eugene, OR 97403 USA. Telephone: (541) 346-1537. Fax (541) 346-2364. E-mail: jenfox@uoregon.edu

J.E.F. was supported by a National Science Foundation graduate fellowship. All work was supported by U.S. Department of Energy grant 540841.

The author declares she has no competing financial interests.

Received 14 May 2003; accepted 27 January 2004.

bacteria, does express ligand-dependent transcriptional activator proteins/receptors, called NodD proteins, which have been reported to share genetic homology with the human ER (Gyorgypal and Kondorosi 1991; Long 1989). In addition, some Nod proteins have been shown to share significant sequence homology with human steroid biochemical intermediates (Baker 1989, 1991).

Recent genetic sequence analysis has shown no significant nucleotide level homology between rhizobial NodD and human ER genes. Nevertheless, these two evolutionarily distant receptors both recognize and respond to a shared group of chemical signals and ligands, including both agonists and antagonists. A lack of homology at the nucleotide level does not preclude the possibility that NodD and ER are receptors with analogous signaling functions. In fact, recent advances in X-ray crystallography have helped uncover many examples of proteins that share little or no nucleotide level homology and yet, when crystallized, have been shown to share significant homology in three-dimensional protein folding, domains, and structural characteristics (Benner et al. 2000; Lai et al. 2000; Rives and Galitski 2003; Suel et al. 2003). The emerging proteomics field is based on the principle that the structural characteristics of proteins are more telling determinants of a protein's function and evolutionary origin than is simple nucleotide-level homology (Koonin et al. 2002; Meyerowitz 2002; Todd et al. 2001). Although the crystal structure of the NodD protein has not yet been solved, based on the similar ligand-binding profiles (both natural and synthetic ligands) and DNA-binding ability of NodD and ER, these two proteins may share some degree of structural identity.

Although they share no common evolutionary ancestor, NodD and ER recognize and respond to a similar profile of chemical signals found in the environment. Convergent evolution may explain the shared ligand recognition properties common to both ER and NodD proteins. Convergent evolution is demonstrated when two species that do not share a common ancestor exhibit similar traits that have arisen, through natural selection, as adaptations to similar ecologic and environmental conditions or signals (Thompson 1999). I contend that NodD and ER may have separately evolved, in lineages leading to *Rhizobium* bacteria and vertebrates, to adapt to the presence of natural estrogenic ligands, such as those produced by vertebrates, fungi, and plants (phytoestrogens). Recent evolutionary analysis has found that some invertebrates express an ER, and phylogenetic analysis of these sequences has demonstrated that the ER is the earliest ancestral receptor of the entire steroid receptor family (Thornton 2001; Thornton et al. 2003). Conversely, the endogenous natural

Table 1. EDCs disrupt NodD signaling and ER signaling.

Chemicals tested	Percent inhibition of *nod* expression (I_{max})	Relative effect on signaling		
		NodD	ER-α	ER-β
Insecticides				
Pentachlorophenol	90	++++	+	
Methyl parathion	89	++++		
Kepone	42	++		
p,p´-DDT	45	++	+	+
p,p´-DDE	44	++	++	
o,p´-DDT	43	++	++	+
o,p´-DDE	42	++	+	
p,p´-DDD	35	++		
o,p´-DDD	34	++		
Hexachlorocyclohexane	24	+		
Dicofol	22	+		
Malathion	20	+		
Lindane	13	+	++	
Toxaphene	7	+		
Methoprene	5	+		
Endosulfan	None	–		
Endosulfan sulfate	None	–	+	+
Methoxychlor	None	–	+	+
Aldrin	None	–		
Dieldrin	None	–	+	
Carbofuran	None	–	+	
EPTC	None	–	+	
Diazinon	None	–		
Dursban	None	–		
Herbicides				
2,4,5-T	37	++		
2,4-D	32	++	+	
Pendimethalin	16	+		
Trifluralin	12	+	+	
Atrazine	10	+		
Metolachlor	10	+		
Alachlor	None	–	+	
trans-Nonachlor	None	–		
Acetochlor	None	–		
Fungicide				
Vinclozolin	None	–		
Plasticizers				
Bisphenol A	66	+++	+++	++
tert-Octylphenol	25	++	+++	+++
4-Nonylphenol	20	+	+++	++
Benzyl butylphthatlate	19	+	+	
PCBs				
4-OH-2´,3´,4´,5´-PCB	60	+++		
4-OH-2´,4´,6´-PCB	56	+++		
Arochlor	27	++		
3,3´,4,5-PCB	23	+		
2,3,4,5-PCB	15	+		
2,4,6-PCB	None	–		
PAHs				
6-OH-chrysene	29	++		
cis-Nonachlor	12	+		
Hormone-active compounds				
DES	55	+++	+++++	+++
4-OH-stilbene	53	+++	+++	
Zearalenone (fungal)	33	++	++++	++
Progesterone	17	+	+	
ICI 182,780	15	+	+	+
Testosterone	10	+	–	
Estriol	7	+	++++	
E_2	None	–	++++	++++
Phytochemicals				
Genistein	86	++++	++++	+++++
Chrysin	85	++++	+++	+
Coumestrol	76	++++	++++	++++
Chalcone	60	+++		
Kaempferol	59	+++	+++	+++
Daidzein	None	–	+	
Apigenin	None	–	+++	++
Luteolin	None	++++	+	

Abbreviations: –, no significant effect; 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; DDD, dichlorodiphenyldichloroethane; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; EPTC, *S*-ethyl dipropylthiocarbamate; I_{max}, maximum inhibition. Luteolin was set as 100% potency (++++) for NodD signaling. EDCs were ranked in order of the most potent disruptor of *nod* gene activation (relative percent potency): 1–25% (+), 25–50% (++), 50–75% (+++), 75–100% (++++), and > 100% (+++++). E_2 was set as the measure of 100% potency for ER signaling, and EDCs were ranked in order of most potent disruption of E_2–ER signaling. Potency data for each EDC in the NodD and ER signaling systems are also reported in Figure 1. Adapted from Fox et al. (2004).

ligand for ER, E_2, is the terminal product of the steroid biochemical pathway. Therefore, when compared on an evolutionary time scale, the ER may have arisen long before its endogenous ligand, E_2, was produced. In this absence of E_2, ancestral ERs may yet have functioned as receptors for exogenous/environmental signals. At the time of the evolutionary emergence of the ER, organisms such as insects, fungi, bacteria, and plants existed and may have been actively producing chemical signals that served, then as they do today, as potent ER ligands. These environmental signals may have included a wide variety of phytoestrogens, including those that signal through rhizobial NodD receptors to initiate symbiosis.

In addition to having specific affinity for and being activated by many of the same ligands and phytoestrogens, vertebrate ER proteins and rhizobial NodD proteins can also be affected by many of the same environmental cues and ligands. ER and NodD both require chaperone proteins, hsp70 and GroESL, respectively, for proper folding and full activation of transcription (Cheung and Smith 2000; Nair et al. 1996; Takayama and Reed 2001; Yeh et al. 2002). Specific ligand binding to ER and NodD results in either activation or inhibition of responsive gene transcription; therefore, both receptors exhibit ligand-concentration–dependent activity. Moreover, ligand binding to ER and NodD results in both receptors binding to highly conserved consensus sequences of DNA, the estrogen response element and the Nod box, respectively, in the promoter regions of responsive genes. ER- or NodD-induced transcription of responsive genes is responsible for growth and differentiation events (Fisher and Long 1993; Katzenellenbogen et al. 2000; McLachlan 2001; van Rhijn and Vanderleyden 1995). ER can also be activated in a ligand-independent manner via cross-talk with growth factor signaling pathways (Bjornstrom and Sjoberg 2002; Frigo et al. 2002; Klotz et al. 2002). For example, ER can be activated via mitogen-activated protein kinase (MAPK) phosphorylation cascade members (Weinstein-Oppenheimer et al. 2002; Weldon et al. 2002). Interestingly, a member of the MAPK eukaryotic signal transduction pathway, Raf (MAPK kinase) protein, has a homologous protein in plants that also functions in signal transduction of the plant hormone ethylene (Clark et al. 1998). ER's ability to signal in the absence of ligand and be influenced by multiple signal transduction pathways, as well as ER's promiscuous binding of an array of environmental compounds, has led to hypotheses that the original signaling function of ER may have been as a receiver and translator of many varied environmental signals and cues.

On the basis of the functional similarities listed above and their shared affinity for similar chemical signals, I propose that the evolutionarily distinct ER and NodD receptors are functionally analogous in their response to and mediation of chemical signaling. Therefore, it follows that both of these signaling systems are vulnerable to disruption by EDCs present in their shared environment.

Disruption of Chemical Communication by Natural and Synthetic EDCs

Initial studies (Fox et al. 2001), as well as the expanded studies (Fox et al. 2004), have shown that signaling via both ER and NodD receptors is adversely affected by a similar profile of EDCs at environmentally relevant concentrations. The original hypothesis—that phytoestrogens and EDCs that disrupt E_2–ER signaling in mammalian cells (Blair et al. 2000; Danzo 1997) will similarly disrupt symbiotic phytoestrogen–NodD signaling—has led to testing of more than 80 natural and synthetic environmental compounds, at a wide range of concentrations, for agonist and antagonist effects on phytoestrogen–NodD signaling. Environmental compounds, including phytoestrogens, fungal chemicals, insecticides, herbicides, plasticizers, polyaromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs), were chosen because of their ability to disrupt endocrine signaling in vertebrates, humans, and exposed wildlife as reported in numerous scientific reports over the past 60 years (Bennetts et al. 1946; Bitman et al. 1968; Guillette 2000; Korach et al. 1979; McLachlan et al. 1984; Nelson 1974). EDCs were tested for disruption of NodD signaling by measuring effects of EDCs on phytoestrogen-induced NodD-responsive reporter gene expression (Table 1).

In the presence of 1 μM luteolin inducer, NodD activates the transcription of a number of nodulation (*nod*) genes. To measure antagonist effects of EDCs, I added increasing concentrations of each EDC (insecticides, herbicides, plasticizers, PCBs, PAHs, synthetic and natural hormones, and phytochemicals) in the presence of 1 μM luteolin and quantified the effects of EDCs on expression of a responsive *nod* gene fused to a *lacZ* reporter gene. The maximum inhibition (I_{max}) of *nod* gene expression was quantified for each EDC tested (Table 1). The natural NodD ligand luteolin was set as 100% potency (++++). Each EDC tested was ranked in order of the percent inhibition of *nod* gene activation (relative percent potency): 1–25% (+), 25–50% (++), 50–75% (+++), 75–100% (++++), and above 100% (+++++). Similarly, potency data were pooled from the literature for many of the EDCs tested in this study regarding potency with which each EDC caused disruption (either agonist or antagonist) of signaling through ER (Coldham et al. 1997; Collins-Burow et al. 2000; Kuiper et al. 1997; Petit et al. 1997; Sheeler et al. 2000; Zava et al. 1997). The natural ER ligand E_2 was set as 100% potency for ER signaling, and EDCs were ranked in order of most potent disruption of E_2–ER signaling. Potency data for each EDC in the NodD and ER signaling systems are reported in Table 1 and Figure 1.

For comparative analysis of NodD-disruption data (Fox et al. 2001, 2003), I ranked the potency with which each EDC disrupted phytoestrogen-NodD signaling. This EDC "potency profile" was used to construct the *x*-axis of Figure 1. In order to create a comparable "potency profile" of EDCs that

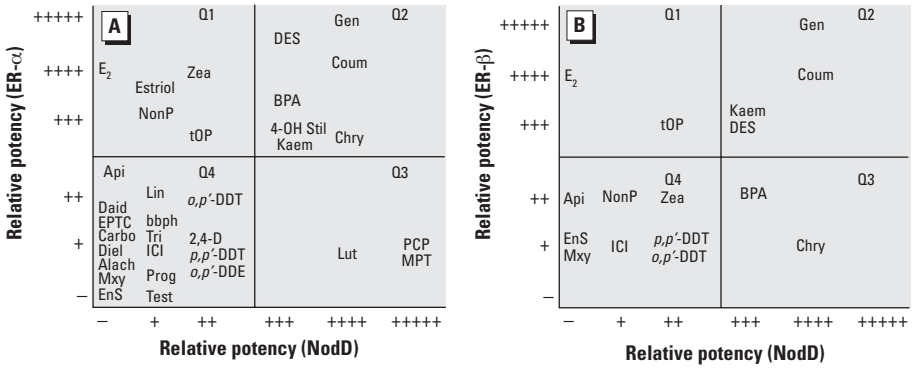

Figure 1. EDC disruption of vertebrate ER and *Rhizobium* NodD signaling shown as the relative potency of each EDC for ER-α (*A*) and ER-β (*B*) compared with NodD. Relative potencies in disrupting signaling were taken from the literature for each EDC for ER (Coldham et al. 1997; Collins-Burow et al. 2000; Kuiper et al. 1997; Petit et al. 1997; Sheeler et al. 2000; Zava et al. 1997) and NodD (Fox et al. 2001, 2004). The potency profile of each EDC is expressed as a relative value compared with the natural ligand (set as 100% potency), either E_2 for ER or luteolin for NodD. Abbreviations: 2,4-D, 2;4-dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; 4-OH Stil, 4-OH-stilbene; Alach, alachlor; Api, apigenin; bbph, benzylbutylphthalate; BPA, bisphenol A; Carbo, carbofuran; Chry, chrysin; Coum, coumestrol; Daid, daidzein; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; Diel, dieldrin; EnS, endosulfan sulfate; EPTC, *S*-ethyl dipropylthiocarbamate; Gen, genistein; ICI, ICI 182,780; kaem, kaempferol; Lin, lindane; Lut, luteolin; MPT, methyl parathion; Mxy, methyoxychlor; PCP, pentachlorophenol; Prog, progesterone; Q, quadrant; Test, testosterone; tOP, *tert*-octylphenol; Tri, trifluralin; Zea, zearalenone.

affect ER signaling, I pooled data from the existing body of scientific literature concerning the effects of phytoestrogens and EDCs on ER signaling (Coldham et al. 1997; Collins-Burow et al. 2000; Kuiper et al. 1997; Petit et al. 1997; Sheeler et al. 2000; Zava et al. 1997) and used these data to create the *y*-axis of Figure 1. This allowed me to compose a "potency profile" for each EDC, ranking the relative amount of disruption to both ER-mediated and NodD-mediated signaling.

By graphing the potency data for all EDCs tested, ranking relative effects on either estrogenic (*y*-axis) or symbiotic (*x*-axis) signaling, I determined four different categories of EDCs (Figure 1). The largest category in number consists of EDCs that are not potent effectors of either ER or NodD signaling (quadrant 4), whereas the two smallest categories consist of EDCs that are potent signal disruptors of either only ER signaling (quadrant 1) or only NodD signaling (quadrant 3) (Figure 1). Finally, quadrant 2 contains EDCs that are potent disruptors of both ER and NodD signaling; this quadrant illustrates that ligand affinity and signaling properties are often shared in common between estrogenic and symbiotic signaling (Figure 1). The category of shared disruptors was primarily composed of phytoestrogens or plant-derived compounds such as stilbenes. Interestingly, all of the stilbenes and bisphenolic compounds tested fall into this shared disruptor category. The predominance of phytoestrogens as potent agents for ER and NodD signaling further supports the theory that an evolutionarily ancient or ancestral ER may have recognized and responded to phytoestrogens even before the emergence of the natural endogenous ligand E_2.

The few synthetic EDCs found to be potent disruptors of both ER and NodD signaling include the estrogenic plastics by-product and surfactant bisphenol A and the pharmaceutical estrogen diethylstilbestrol (DES). Both bisphenol A and DES are among the earliest synthesized pharmaceutical estrogens (Dodds and Lawson 1936; Dodds et al. 1938). In fact, DES is a stilbestrol derivative whose core structure is that of the plant product stilbene, of which 4-OH-stilbene was also found to be an active disruptor of ER and NodD signaling. Other studies have indicated that this very same group of compounds—stilbenes, bisphenol A, and DES—are not only potent estrogens but also inhibitors of microtubule polymerization (Metzler and Pfeiffer 1995). Many of the phytochemicals found to be disruptors of ER and NodD signaling are themselves signaling molecules produced by soybeans and clover to signal to and recruit their own specific symbiotic strains of rhizobia and mycorrhiza. One may hypothesize that these related phytochemical–symbiont signaling webs may also be potential targets of disruption by EDCs found in the environment. I have identified fundamental similarities in the profile of EDCs that disrupt phytochemical–NodD symbiotic signaling and E_2–ER hormone signaling, further supporting the theory of an evolutionary convergence of chemical communication pathways (Baker 1992b; Wynne-Edwards 2001). Therefore, it is probable that EDCs and other phenolic ring substitution compounds, which disrupt NodD symbiotic signal and ER signaling, may be capable of disrupting a much broader web of signaling than had previously been considered (Firmin et al. 1986; Djordjevic et al. 1987; Peters and Long 1988). I have used the same reasoning that has been applied to studying the effects of EDCs in vertebrates and have found parallels between the profile of EDCs and the effects on endogenous signaling in the plant–rhizobial system. In addition, the chemicals tested are commonly found in the soil environment in concentrations comparable with those used in other assays, and a lower level of *nod* gene expression may result in reduced symbiotic signaling.

Although endocrine disruption studies in vertebrates have been criticized for exceeding the range of environmentally relevant concentrations of EDCs, there is no question that EDCs are abundantly used and found in the same environment as NodD–phytoestrogen signaling. *Rhizobium* are ubiquitous in the soil environment of agricultural regions in the first 10 in. below ground. EDCs, such as organochlorine pesticides, are applied in amounts measured in tons to the very agricultural fields in which this new "target" of endocrine disruption exists. Significant residual levels of EDCs, such as DDT, can be measured 20 years after spraying in micromolar or parts-per-billion concentrations (Aigner et al. 1998; Mitra and Raghu 1998). Therefore, depending on the timing of pesticide application, it is probable that phytochemicals produced by host plants are competing with micromolar or millimolar concentrations of active endocrine disruptors for signaling to NodD receptors in soil bacteria. Recent studies of crops that rely on nitrogen-fixing symbiosis in agricultural areas with heavy pesticide use have reported stunted plant growth and reduced mycorrhiza and rhizobia symbiosis (Abd-Alla et al. 2000), mirroring the effects predicted by *in vitro* laboratory studies (Fox et al. 2001, 2004). These data identify a novel target of EDCs, the chemical communication between two organisms, which mediates symbiosis. Furthermore, *in vitro* laboratory results showing EDC inhibition of the symbiotic signaling system necessary for recruitment of nitrogen-fixing bacteria to plants for symbiosis (Fox et al. 2001, 2004) may indicate the mechanism behind deleterious effects reported in the field.

Implications

Phenotypic evidence of endocrine disruption commonly observed when vertebrates are exposed to high concentrations of organochlorine pesticides and pollutants include feminization of fish, reduced phallus size of reptiles, decreased mating ability in mammals, reduced fertility and viability in a variety of species, and disruption of E_2 signaling to ERs (Bennetts et al. 1946; Danzo 1997; Facemire et al. 1995; McLachlan 2001; Tyler et al. 1998). ER homologues have been found in species as evolutionarily distant as invertebrates and humans (Thornton 2001; Thornton et al. 2003). In addition, many homologues of human signaling pathway members (Bolduc et al. 2003; Stracke et al. 2002), including estrogen-like receptors, have been identified in plants (Milanesi et al. 2001) and, as discussed here, may have analogous receptors in *Rhizobium* bacteria. Meanwhile, most research on endocrine disruption has focused on what effects EDCs and phytoestrogens have on hormone signaling in humans and wildlife, but the evolutionary targets of phytoestrogen signaling, namely, symbiotic soil bacteria, had been overlooked as a target of EDCs. Communication via hormone signaling is subject to cross-talk and disruption by both natural and synthetic environmental chemical signals, which are communicated to all organisms residing in a shared ecosystem.

Phytoestrogen signaling is a prime example of signal cross-talk found in the environment. Phytoestrogens, produced as recruitment signals for symbiotic soil bacteria, are recognized as hormone signals by some herbivores resulting in reduced fecundity (Wynne-Edwards 2001). In addition, these same phytoestrogens may be responsible for hormone alterations in humans that lead to reduced risk of hormone-dependent cancers (Zava and Duwe 1997). This example illustrates how a hormone signal, released into the environment for the sole purpose of communicating with a receptor in a target organism, may have unintentional consequences as a hormone signal that communicates to any and all nontarget organisms with analogous signal receptors. In most studies involving environmental estrogens, the receptor of endocrine-disrupting signals has been identified as the ER. The limitation to operating under the assumption that EDCs are only a threat to organisms that express a recognizable ER is that many invertebrates have been overlooked as targets of endocrine disruption. Only recently have laboratories begun looking for endocrine disruption phenotypes in organisms such as coral, *Daphnia*, and *Caenorhabditis elegans*, which do not have classic ERs (Custodia et al. 2001; Tarrant et al. 1999; Wu et al. 2001). Additionally, by focusing on extreme endocrine disruption phenotypes, such as sex reversal in fish or

amphibians (Hayes et al. 2002; Jobling et al. 2002), more subtle but important phenotypes of endocrine disruption may be overlooked. After all, EDC-inhibition of one member of a signaling or regulatory cascade (NodD in rhizobia) results in reduced symbiotic gene activation leading to population-level effects on nitrogen-fixing symbiosis (Fox et al. 2004). On the basis of these emerging examples of endocrine disruption in a variety of unexpected target species, I propose a more mechanism-based approach for assessing the effects of EDCs on ecosystem-wide signaling webs.

By studying the intraorganismal and interorganismal communication mediated by hormone signaling in a multitude of species, we can begin to identify analogous signaling systems based on how EDCs and environmental estrogens may disrupt these signaling webs. An example of a signaling web, or a microcosm of hormone signals mediating communication, may be found in 1 $in.^2$ of soil, where signals are produced by plants to communicate with other plants, to recruit symbiotic soil bacteria and fungi, and to ward off nematodes and aphid pests (Dunn and Handelsman 2002; Guerrieri et al. 2002; Stracke et al. 2002). Just as soil bacteria must receive and integrate each of these hormone signals in the soil microenvironment and find the one recruitment phytochemical signal among so much chemical signaling "noise," so must humans exist in an environment awash in hormone chemical signals and yet still recognize appropriate endogenous hormone signals. As if this task of signal communication and interpretation were not daunting enough, each organism must contend not only with natural hormone signals but also with an ever-increasing number of "unintentional" hormonally active synthetic chemicals in the environment (Seiler 2002). By identifying and monitoring analogous signaling systems, we could more easily understand how the release of an abundance of synthetic chemicals into the environment, such as organochlorine pesticides intended to antagonize insects and unwanted pests, could inadvertently disrupt hormone signaling in a wide variety of organisms from mammals to symbiotic bacteria, all of whom rely on signaling webs for communication. After all, an assault on one form of signaling-based communication is likely an assault on many. As we begin to better understand the mechanisms by which organisms signal or communicate information, both endogenously and within their environment, we may discover an unlimited number of new targets for EDCs and environmental estrogens.

Because signaling communication webs are not unidirectional, we must consider not only how synthetic chemicals produced by humans may adversely affect biota in the environment but also how signals originating in bacteria or plants may affect signaling within the human body (McFall-Ngai 2002). Studies of analogous signaling methods used by a number of organisms may shed light on endocrine signaling and human health. For instance, researchers have shown that genes necessary for mammalian pathogenic bacteria to establish chronic infections in humans are homologous to genes in *Rhizobium* responsible for infecting plant hosts and establishing symbiosis (LeVier et al. 2000). As more evidence of homologous and analogous hormone signals and receptors emerges, we must consider how the inadvertent hormone signaling of EDCs, which is known to disrupt signaling cascades, gene activation, and hormonal homeostasis, may not only affect individuals (fecundity, reproduction) but also confound ecosystem-wide communication webs leading to unpredictable population-level effects.

The evolution of communication via hormone signaling may be one of the oldest and most crucial links shared between all organisms. To determine what is most important for the viability of an organism or an ecosystem, we must determine either what has been conserved over evolutionary time or what has been reinvented and selected for by convergent evolution (Baker 1992a, 2002; McLachlan 2001; Whiting et al. 2003). Communication via chemical signaling is a shared characteristic of all organisms and, thus, affects all members of an ecosystem. Therefore, I propose that chemical communication is a crucial component of ecosystem health and the primary target of EDCs found in the environment. The characterization of the disruption of an evolutionarily ancient symbiotic signaling system by EDCs (Fox et al. 2004) may provide insights into the mechanisms by which these same EDCs disrupt endocrine signaling in vertebrates. The study of analogous communication systems, such as mammalian endocrine signaling and plant–*Rhizobium* symbiotic signaling, will yield new insights into hormone signaling and disruption of hormone signaling by environmental estrogens and EDCs and may provide information on the evolution of nuclear receptors.

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