Int J Primatol (2013) 34:861–878
DOI 10.1007/s10764-013-9699-3

The Roles of Phytoestrogens in Primate Ecology and Evolution

Michael D. Wasserman · Katharine Milton · Colin A. Chapman

Received: 7 June 2013 / Accepted: 17 June 2013 / Published online: 24 July 2013

Abstract Most primates depend heavily on plant foods; thus their chemical composition is key to understanding primate ecology and evolution. One class of plant compounds of strong current interest are phytoestrogens, which have the potential to alter fertility, fecundity, and survival. These plant compounds mimic the activity of vertebrate estrogens, resulting in altered physiology and behavior. Here, we review what is known about phytoestrogens from an ecological and evolutionary perspective. Much of what is known about the effects of phytoestrogens on the endocrine system comes from research on human foods, especially soybeans (*Glycine max*). Two opposing perspectives have resulted from this research: 1) phytoestrogens provide health benefits, such as cancer prevention, or 2) phytoestrogens act as endocrine disruptors and threaten reproductive health. Studies of wild primates have only recently begun examining the presence of estrogenic plants in the primate diet and the effects of their consumption. Evidence that a number of primate species eat plants containing phytoestrogens and research documenting behavioral and hormonal effects of estrogenic plant consumption for red colobus monkeys (*Procolobus rufomitratus*) augment captive and laboratory studies to suggest that these compounds promote differential survival and reproduction. Although much debate is currently taking place over the role of phytoestrogens and other endocrine disruptors in human health issues and in threatening biodiversity, we argue that an ecological and evolutionary approach is needed to reach appropriate conclusions.

M. D. Wasserman · K. Milton
Department of Environmental Science, Policy, and Management, University of California, Berkeley, Berkeley, California 94720, USA

C. A. Chapman
Department of Anthropology, McGill University, Montreal, Quebec, Canada H3A 2T7

Present Address:
M. D. Wasserman (✉)
Department of Anthropology, McGill University, Montreal, Quebec, Canada H3A 2T7
e-mail: michael.wasserman@mail.mcgill.ca

Keywords Endocrine disruption · Environmental endocrinology · Estrogenic plants · Plant–animal interactions · Self-medication · Reproductive ecology

Incorporating Phytoestrogens into Primate Nutritional Ecology

Most primates depend heavily on the leaves, fruits, and flowers of tropical plants to meet their nutritional demands (Fashing *et al.* 2007; Milton 1979, 2000; Rothman *et al.* 2012). As a result, the chemical composition of these plant parts is critical to understanding primate ecology and evolution. For example, research on leaf-eating primates has shown that the availability of high-protein, low-fiber leaves is related to food choice and biomass (Chapman *et al.* 2002; Fashing *et al.* 2007; Ganzhorn 1992; Milton 1979; Oates *et al.* 1990; Wasserman and Chapman 2003). However, the significance of plant secondary metabolites to primates is less clear. Plants produce certain compounds, e.g., alkaloids and tannins, as a defense mechanism against herbivory (Coley and Barone 1996), but evidence that such compounds function to deter mammalian herbivores is not strong (Burgess and Chapman 2005; Milton 1979, 1998) and their efficacy depends on the specific plant compound and physiology of the mammal (Robbins *et al.* 1991). Mammalian herbivores have the ability to deal with many disruptive compounds either by avoiding them through selective feeding or by evolving detoxification mechanisms (Foley and Moore 2005; Freeland and Janzen 1974). This is especially true for primates as some taxa have specialized symbiotic microbes that can likely detoxify many defensive compounds, e.g., the forestomach fermenting colobines) (Bauchop and Martucci 1968; Milton 1980, 1998). However, a recent study demonstrated that tannins did reduce mammalian reproductive success in the common brushtail possum (*Trichosurus vulpecula*) through negative effects on nitrogen availability (DeGabriel *et al.* 2009). Alternatively, some animals may select certain plants to benefit from their secondary metabolites through self-medication (Huffman, 1997). For example, chimpanzees (*Pan troglodytes*) chew the pith of *Vernonia amygdalina* to treat gastrointestinal parasites (Huffman, 2001).

It is thought that primates, along with other herbivores, must minimize consumption of plant secondary metabolites, such as tannins, when selecting their foods (Feeny 1970; Glander 1978; Mckey *et al.* 1981). This is particularly relevant for more folivorous primates as compared to more frugivorous species. Because leaves are photosynthetic organs essential to a plant's energy production, they are commonly defended from herbivory through the production of chemicals (Coley and Barone 1996). In contrast, fruits exist mainly as a means for seed dispersal, and thus, ripe fruits are not expected to contain chemical defenses. Milton (1998) discussed two types of plant compounds that primates (especially folivores) are faced with: 1) those that are toxic to the feeder or the feeder's gut microbes and 2) those that inhibit digestion or absorption of nutrients. An overlooked third type of plant secondary metabolite also exists, and it can be defined by its ability to alter long-term internal processes of the feeder, such as endocrine functioning and fertility (Wang *et al.* 2006; Wynne-Edwards 2001). Phytosteroids are an example of this third type of secondary metabolite. Because such compounds affect the production or activity of endogenous vertebrate steroid hormones, their consumption may have important repercussions for primates that are both beneficial and costly. For example, phytoestrogens, or plant-produced estrogenic compounds, are known to disrupt mammalian fertility (Hughes 1988; Wynne-Edwards 2001). More than 160 plant compounds found in >300 plant species from 32 plant families have thus far been identified as estrogenic (Dixon 2004; Reynaud *et al.* 2005). These phytoestrogens are divided into two main groups of phenolic compounds based on chemical structure: isoflavonoids, e.g., isoflavones, coumestans, and stilbenes, e.g., resveratrol (Cornwell *et al.* 2004; Reynaud *et al.* 2005). Lignans are also considered phytoestrogens, but must first be converted by gut microbes to mammalian lignans to show estrogenic activity (Cornwell *et al.* 2004). The isoflavonoids appear to be the most abundant phytoestrogens and are found predominately in the subfamily Papilionoideae of the legume family (Fabaceae) (Dixon 2004; Reynaud *et al.* 2005), which is an important plant family providing many foods of primates (Chapman *et al.* 2002; Wasserman and Després-Einspenner *unpubl. data*).

Here we provide a review of what is known about phytosteroids from an ecological and evolutionary perspective, including a summary of how phytoestrogens interfere with the roles of sex steroid hormones in primate physiology and behavior. We suggest that for primates that consume significant amounts of phytoestrogens, their physiological and behavioral effects likely promote differential survival and reproduction. We conclude by discussing how to improve our understanding of how steroidal plant compounds affect the ecological and evolutionary relationships between primates and plants.

Ecological and Evolutionary Interest in Phytoestrogens

Plant compounds that affect endogenous vertebrate androgens, e.g., testosterone; progestins, e.g., progesterone; and glucocorticoids, e.g., cortisol have been the focus of a number of studies (Beck *et al.* 2003; Heftmann 1977; Iino *et al.* 2007; Janeczko and Skoczowski 2005). For example, studies have investigated the *in vitro* effects of licorice (*Glycyrrhiza glabra*) on cortisol metabolism (Whorwood *et al.* 1993), rooibos (*Aspalathus linearis*) on androgen and glucocorticoid synthesis in the adrenal gland (Schloms *et al.* 2012), and gossypol from cotton seeds (*Gossypium* spp.) on testosterone production in the testes (Ye *et al.* 2011). Most research on phytosteroids has focused on phytoestrogens. A quick search on the Web of Knowledge supports this conclusion: “phytosteroid*” = 76 articles, “phytoandrogen*” = 7, “phytoprogestin*” = 4, “phytoglucocorticoid*” = 0, and “phytoestrogen*” = 10,824 (search conducted on June 4, 2013).

Interest in phytoestrogens stems mainly from their presence in human foods, including soybeans, chickpeas, flaxseed, peanuts, barley, and broccoli (Mazur 1998). As a result, many *in vitro* and *in vivo* laboratory studies using human cell assays and captive rodents and monkeys have been conducted, along with human clinical and epidemiological studies, on the physiological and behavioral effects of phytoestrogen consumption to determine their health effects (Whitten and Patisaul 2001). Two opposing perspectives have resulted from this research: a) phytoestrogens provide health benefits, such as cancer prevention (Leitman *et al.* 2010), or b) phytoestrogens act as endocrine disruptors and threaten reproductive health (Cederroth *et al.* 2010a). An ecological and evolutionary perspective that considers both why a plant produces such compounds and why primates consume estrogenic plants would advance our understanding of the consequences of phytoestrogen consumption more than simply framing the issue in dichotomous terms. Yet, little is known about the ecological, e.g., 32 articles for "phytoestrogen* ecolog*" search, or evolutionary, e.g., 33 articles for "phytoestrogen* evolution*," context of phytoestrogen consumption for humans, primates, or any vertebrate (Wynne-Edwards 2001).

Despite this lack of data, numerous hypotheses about the ecological and evolutionary significance of phytoestrogens have been postulated. Here we summarize these hypotheses in three main categories and set them up as mutually exclusive scenarios based on the cost–benefit ratio for herbivores: 1) The plant defense hypothesis suggests that plants benefit from producing phytoestrogens by reducing vertebrate herbivory through suppression of fertility, i.e., consuming phytoestrogens is costly for herbivores (Harborne 1993; Hughes 1988; Wynne-Edwards 2001). 2) The self-medication hypothesis suggests that vertebrate herbivores benefit from consuming phytoestrogens through increased survival, i.e., health benefits, or reproductive success, while the plant produces such compounds for reasons other than herbivore deterrence (Fidler *et al.* 2008; Forbey *et al.* 2009; Glander 1980; Huffman 1997; Leopold *et al.* 1976; Strier 1993). 3) The biochemical coincidence hypothesis suggests that there are no significant benefits or costs of consuming phytoestrogens for vertebrates. A lack of relevant, concurrent data on feeding behavior, estrogenic activity of the plant, and physiological or behavioral effects on the feeder has prevented a critical evaluation of these hypotheses.

Herbivores and Phytosteroids: The Current Evidence

One early example of an ecological study on phytoestrogens examined the possibility that increased phytoestrogen consumption by California quail (*Callipepla californica*) during drier years inhibited their reproduction. It was postulated that the phytoestrogen content of the quail's foods limited production of offspring to wetter years when food was more available (Leopold *et al.* 1976); however, variation in other nutrients that could have also affected reproduction was not considered. This chemical cue hypothesis was reiterated for mountain voles (*Microtus montanus*: Berger *et al.* 1981, 1977). Soon after, it was suggested that howlers (*Alouatta palliata*) were consuming certain plant species because of their effects on reproductive timing (Glander 1980). Later, seasonality in chimpanzee (*Pan troglodytes*) reproduction was hypothesized to result from annual variations in rainfall and diet, with possible mediation by phytoestrogens (Wallis 1997). These suggestions concerning the potential role of phytoestrogens in reproductive seasonality were pioneering in that they began incorporating environmental endocrinology into ecological and primatological studies, but they were also based on little supporting data owing to the lack of methodology for examining endocrinological questions in a field setting. Although the ecological relationship between estrogenic plants and wild vertebrates is unclear, it is well established that certain phytoestrogens can have dramatic physiological effects. This was first documented in Western Australia, where it was discovered that phytoestrogens found in clover (*Trifolium subterranean*) consumed by domesticated sheep caused widespread infertility that led to greatly reduced numbers of lambs and considerable economic loss (Bennetts and Underwood 1951; Cornwell *et al.* 2004).

Recently, researchers have again become interested in this phenomenon, likely owing to methodological advances that allow questions about the steroidal properties of plants and their effects on wild animals to be addressed using a mix of field and laboratory approaches. For birds, Fidler *et al.* (2008) proposed a hypothetical mechanism for how phytoestrogens could mediate reproductive timing of the kakapo (*Strigops habroptilus*), a New Zealand parrot that produces offspring in a supra-annual pattern that correlates with mast fruiting cycles, i.e., every 3–5 yr. Three recent primate field studies have examined phytosteroids in the diets of Phayre's leaf monkeys (*Trachypithecus phayrei*: Lu *et al.* 2011), common chimpanzees (*Pan troglodytes*: Emery Thompson *et al.* 2008), and olive baboons (*Papio anubis*: Higham *et al.* 2007) (Table I). These studies suggested that consumption of *Vitex* affected female reproduction through altered hormone levels, cycle length, probability of conception, and/or receptivity. All three studies documented an increase in fecal or urinary progestin levels when the primates were feeding on *Vitex*; however, whether this plant genus contains phytoestrogens, phytoprogestins, or other types of phytochemicals that affect reproductive physiology is unclear. Research on a human-used species (*Vitex agnus castus*) suggests a complex mechanism of action where estrogenic and dopaminergic compounds contribute to physiological changes, including reduction in prolactin levels

Table I Summary of field studies that either examined or suggested the possibility of phytosteroid-containing plants to influence primate ecology

Primate species	Plant species	Evidence
Modern human (*Homo sapiens*) (Whitten and Patisaul 2001; Wynne-Edwards 2001)	Many, e.g., soy (*Glycine max*) (Fabaceae)	Ethnobotanical, hormonal (serum), *in vitro* and *in vivo* assays, phytochemical, epidemiological, clinical
Common chimpanzee (*Pan troglodytes*) (Emery Thompson *et al.* 2008; Wallis 1997)	*Vitex fisheri* fruit (Lamiaceae)	Hormonal (urinary), behavioral, ethnobotanical for phytoprogesterones
Mountain gorilla (*Gorilla beringei*) (Wasserman *et al.* 2012b)	*Ipomoea involucrata* leaves (Convolvulaceae)	Behavioral, transfection assay for plant estrogenic activity
Olive baboon (*Papio anubis*) (Higham *et al.* 2007)	*Vitex doniana* ripe fruit and young leaves (Lamiaceae)	Hormonal (fecal), behavioral, morphological, immunoassay for phytoprogesterones, ethnobotanical
Vervet (*Chlorocebus aethiops*) (Garey 1993; Whitten 1983)	*Acacia elatior* flowers (Fabaceae)	Behavioral, *in vitro* bioassay for plant estrogenic activity
Red colobus (*Procolobus rufomitratus*) (Wasserman *et al.* 2012a,b)	Many, but *Millettia dura* young leaves most important (Fabaceae)	Hormonal (fecal), behavioral, transfection assay for plant estrogenic activity, ethnobotanical
Phayre's leaf monkey (*Trachypithecus phayrei*) (Lu *et al.* 2011)	*Vitex spp.* fruit and leaves (Lamiaceae)	Hormonal (fecal), behavioral, ethnobotanical for phytoprogesterones
Muriquis (*Brachyteles arachnoides*) (Strier 1993)	*Enterolobium contortisiliquum* fruit (Fabaceae)	Behavioral, phytochemical for presence of stigmasterol
Kenyan galago (*Galago senegalensis*) (Nash and Whitten 1989)	*Acacia drepanolobium* gum (Fabaceae)	Behavioral, phytochemical for presence of flavonoids with weak estrogenic activity and antiestrogenic activity

(Wuttke *et al.* 2003). We recently documented the prevalence of estrogenic plants in the diets of red colobus monkeys (*Procolobus rufomitratus*) and mountain gorillas (*Gorilla beringei*), with 10.6% and 8.8% of the diet containing phytoestrogens, respectively (Wasserman *et al.* 2012b). We found that the more adult male red colobus monkeys living in Kibale National Park, Uganda, ate estrogenic *Millettia dura* young leaves the higher their fecal estradiol and cortisol levels, while their rates of aggression and mating increased and grooming decreased (Wasserman *et al.* 2012a). Combined, these primate studies suggest that phytosteroids are influencing primate physiology and behavior in ways that have not yet been fully appreciated.

Phytoestrogens as Plant Adaptation

Although phytoestrogens may play a role in defending plants against vertebrate herbivory through interference with the vertebrate endocrine system, there have yet to be any studies that have addressed and demonstrated such benefits in a field setting for wild animals. Steroids, i.e., estrogens, androgens, progestins, glucocorticoids, and steroid-like compounds, e.g., isoflavonoids, are found in many plants as secondary metabolites in biosynthetic pathways (Janeczko and Skoczowski 2005), but their similarities to endogenous vertebrate steroids and biological effects in these animals may simply be a coincidence of chemical pathways used for other functions. Cholesterol is widely distributed in plants and plants produce steroids from it using metabolic pathways similar to those of vertebrates (Heftmann 1977). These plant steroids may have primary roles in plant physiology, affecting plant germination, growth, flowering, and sex expression (Heftmann 1977; Janeczko and Skoczowski 2005). For example, experiments that applied vertebrate steroid hormones to plants showed that these plants exhibited biological responses (Janeczko and Skoczowski 2005).

The strongest support for a plant defense explanation of steroid production is found with insect herbivory (Harborne 1993; Kubo *et al.* 1983). Some plants convert cholesterol to phytoecdysteroids, i.e., insect-molting hormone mimics, which disrupt the development of herbivorous insects and can even lead to death (Kubo *et al.* 1983). Phytoestrogens do not appear to play a similar role in plant interactions with some insects, as a recent experimental study demonstrated that a diet containing natural levels of phytoestrogens had no effect on survivorship or growth of gypsy moths (*Lymantria dispar*: Karowe and Radi, 2011). But, without more evidence, it is not possible to rule out the possibility that phytoestrogens play a role in plant–insect interactions.

We have found that plant steroids or steroid-like compounds may be fairly common, as all 13 plant items we tested from Kibale National Park, Uganda, contained compounds that bound to various steroid hormone antibodies, with most showing progesterone-like structures (Table II; Wasserman, *unpubl. data*). These plant samples were collected fresh from the forest, homogenized in a blender, and stored frozen for 5 d. Then, potential steroidal compounds were extracted from 0.5 g of each sample using 10 ml of a 1:1 95% ethanol/pH 5.0 citrate buffer solution. The extracts were stored in solid phase extraction cartridges until immunoassay, i.e., enzyme immunoassay (EIA) and radioimmunoassay (RIA) analyses were conducted to quantify binding to steroid antibodies. However, binding to an antibody only infers structure and does not indicate

Table II Amount of plant compounds that bound to four steroid hormone antibodies in immunoassays for various plant species collected in Kibale National Park, Uganda

Plant species	Plant part	Estradiol	Progesterone	Androgen	Cortisol	Total Binding
Celtis durandii	YL	10.9	1054	207.3	81.1	1353.3
Celtis durandii	ML	10.2	675	145.1	62.2	892.5
Spathodea campanulata	ML	15.1	526	193.5	54.4	789.0
Balanites wilsoniana	BA	6.1	503	39.5	35.9	584.5
Celtis Africana	YL	9.7	295	106.6	47.9	459.1
Newtonia buchananii	ML	7.8	270	52.5	29.2	359.6
Albizia grandibracteata	YL	7.4	208	70.6	36.2	322.2
Millettia dura	YL	18.8	110	68.3	40.7	237.8
Erythrina abyssinica	FL	21.8	160	9.2	0.0	191.0
Prunus Africana	ML	7.6	112	22.1	18.1	159.8
Prunus Africana	YL	11.4	50	48.1	29.5	139.0
Eucalyptus grandis	BA	2.9	89	14.4	2.8	109.1
Spathodea campanulata	BA	5.4	67	15.5	5.7	93.6

Samples were collected fresh from the forest, homogenized in a blender, and stored frozen for 5 d. Then, potential steroidal compounds were extracted from 0.5 g of each sample using 10 ml of a 1:1 95% ethanol/5.0 pH citrate buffer solution. The extracts were stored in solid phase extraction cartridges until analyses were conducted. Phytosteroid levels are listed as ng/g of dry plant material. YL = young leaves; ML = mature leaves; BA = bark; FL = flowers.

activity. Thus, many of these steroidal plant compounds may show little to no biological effects in the plants themselves or in animals consuming them. *In vitro* and *in vivo* studies are needed to show steroidal activity of such plant compounds.

Although the endogenous role of phytoestrogens for plants remains unclear, they do appear to aid in recruiting soil microbes, thus promoting symbiosis between plants and nitrogen-fixing bacteria (Fox *et al.* 2004). This interspecific communication role likely explains the abundance of isoflavonoids in legumes, which are known for their mutualistic relationships with nitrogen-fixing bacteria. Phytoestrogens are also known to protect plants against fungal and bacterial pathogens (Fox *et al.* 2004). In addition, flavonoids (a broader group of secondary metabolites that includes the estrogenic isoflavonoids) play a role in protecting plants against harmful ultraviolet light and in altering the wavelength of light to appropriate physiological levels (Mazur and Adlercreutz 1998). We are only beginning to understand the role of phytoestrogens from the plant's perspective, but it is becoming clear that their occurrence is due to a complex set of factors and they appear to provide important benefits to plants.

Despite evidence for other functions of steroids and steroid mimics in plants and the possibility of their activity in vertebrates being simply a biochemical coincidence, the costs and benefits of such compounds arising from plant–animal interactions cannot be discounted. If a secondary benefit to the plant from negative effects on vertebrate herbivores were to occur, increased production of such compounds could be selected for, even though such compounds were originally or are mainly produced for other functions. Therefore, the various roles of phytoestrogens for the plant are not mutually exclusive and potential mediation of plant–animal interactions could be one very important consequence of plant steroids and steroid mimics.

Molecular Mechanism of Action: How Phytoestrogens Interfere with Sex Steroids

Before addressing how phytoestrogens could influence the ecology and evolution of primates, an understanding of how endogenous vertebrate steroid hormones regulate physiology and behavior is needed. It is through the disruption of the physiological and behavioral endpoints of steroid hormones that phytoestrogens can alter survival and reproduction.

Endogenous estrogens operate mainly through binding to estrogen receptors (ERs) found inside certain cell types, e.g., brain, urogenital, bone, gonadal, which results in an estrogen receptor–estrogen complex (Heldring *et al.* 2007; Leitman *et al.* 2010). This complex binds to the estrogen response element on the intranuclear chromatin and regulates the transcription and translation of estrogen-dependent genes (Heldring *et al.* 2007; Leitman *et al.* 2010). By directly regulating the transcription of certain genes and consequently the synthesis of specific proteins, estrogens alter the physiology and behavior of an organism starting *in utero* and continuing throughout life (Hadley 1999).

There are two main forms of estrogen receptors in vertebrates, ERα and ERβ. The original steroid receptor evolved long ago (*ca.* 400–500 mya), and this ancestral receptor evolved into the five current types of steroid receptors found in vertebrates today, i.e., estrogen receptors, progesterone receptors, androgen receptors, glucocorticoid receptors, and mineralocorticoid receptors (Thornton *et al.* 2003). The estrogen receptor arose first and is conserved across all vertebrates (Thornton 2001; Thornton *et al.* 2003). However, the ancestral ER evolved into two different forms, ERα and ERβ, at two points; unique sets are found in the teleosts and the tetrapods (Thornton 2001). Owing to the conservative nature of the endocrine system across tetrapods, studies within this group can inform us about how estrogens function in primates.

From studies of knockout mice that lack either ERα or ERβ, we know that each ER has different roles in the nervous, immune, cardiovascular, and skeletal systems, as well as opposing actions on cell proliferation across numerous tissues, including the uterus, ovary, and brain (Heldring *et al.* 2007; Leitman *et al.* 2010). Generally, it is ERβ that arrests cell growth, whereas ERα promotes cell proliferation (Heldring *et al.* 2007). The distribution of ERα and ERβ varies across tissues and organs, as well as within them. For example, various parts of the brain differ in ER distribution, with each regulating different aspects of behavior (Patisaul *et al.* 2009). Specifically, ERα appears to mediate sexual behavior in males and females (Patisaul *et al.* 2004), whereas ERβ mediates levels of aggression and anxiety (Patisaul and Bateman 2008).

Although phytoestrogens can disrupt the activity of endogenous estrogens by interacting with either ER (Krishnan *et al.* 1993), interfering with enzymes responsible for hormone metabolism, e.g., aromatase (Hayes *et al.* 2002), or binding to the sex-hormone binding globulin (SHBG) responsible for transporting sex hormones throughout the body (Whitten and Patisaul 2001), most phytoestrogens operate through their selective activity at ERβ (Kuiper *et al.* 1998). Through their interaction with ERβ and competition with endogenous estrogens for binding to these receptors, phytoestrogens can act as either estrogen agonists, i.e., promoting estrogenic activity, or antagonists, i.e., blocking estrogenic activity, depending on the dose ingested, strength of the specific compounds, and endogenous hormonal state of the feeder (Almstrup *et al.* 2002; Leitman *et al.* 2010). At low doses, phytoestrogens tend to decrease estrogenic activity, whereas at high doses they increase it (Almstrup *et al.* 2002).

For a phytoestrogen to bind to ERβ and alter gene activity, an estrogenic plant must first be ingested by a primate. This plant is digested, and nutrients and other plant chemicals are absorbed at various points along the gastrointestinal (GI) tract. Once phytoestrogens pass from the GI tract into the bloodstream, with the amount and type of compound absorbed dependent on both the primate species and individual owing to differences in gut morphology and microbial community, which can alter metabolism of phytoestrogens (Gultekin and Yildiz 2006; Setchell and Clerici 2010), they travel throughout the body. This interspecific and interindividual variation in phytoestrogen metabolism alters the downstream physiological and behavioral effects due to changes in bioactivity (Setchell and Clerici 2010). Nonetheless, from studies of rats we know that phytoestrogens are highly bioavailable and pass from the GI tract into the bloodstream (Sfakianos *et al.* 1997). As they pass into cell types containing ERβ (Whitten and Patisaul, 2001), they form a ligand–ERβ complex (Leitman *et al.* 2010). This complex travels to the nucleus of the cell and binds to the estrogen response element (ERE) on intranuclear chromatin or causes cellular changes through nongenomic mechanisms (Leitman *et al.* 2010), which alters biological functioning at the molecular level. More importantly for the ecology and evolution of primates is whether these molecular effects are significant enough to manifest themselves in physiological and behavioral changes.

Physiological and Behavioral Endpoints: How Phytoestrogens Interfere with Reproduction

Not all phytoestrogens binding to and activating ERβ produce the same endpoints, so generalizations about likely physiological and behavioral effects are difficult (Whitten and Patisaul, 2001). To complicate matters further, such compounds have tissue-specific effects and their concentration in the blood, the concentration of endogenous estrogens that will compete for binding to the ERs, and the concentration of ERs and SHBG will all affect the outcome; thus the biological effects of phytoestrogen consumption are complex and context dependent (Coldham and Sauer, 2000; Whitten and Patisaul 2001). Further, examining the physiological and behavioral endpoints of phytoestrogen consumption is an active area of research, so new discoveries are being made continually. Thus, here we highlight a few studies that have documented effects, as well as several that have not.

Hormonal Changes

In utero exposure to phytoestrogens has been found to influence directly both maternal and fetal estradiol levels in pregnant rhesus macaques (*Macaca mulatta*), with individuals fed fruit dosed with an estrogenic isoflavone found in soy having substantially higher estradiol levels than those fed a control (Harrison *et al*. 1999). Further, newborn male captive-bred common marmoset monkeys (*Callithrix jacchus*) fed soy milk had lower testosterone levels than their twins that were fed standard cow milk (Sharpe *et al*. 2002). For adult humans eating their usual diet, consumption of phytoestrogens was related to an increase in SHBG levels and a decrease in plasma estradiol levels (Adlercreutz *et al*. 1987). In captive adult female cynomolgus monkeys (*Macaca fascicularis*) fed estrogenic *Pueraria mirifica*, urinary follicle-stimulating hormone (FSH) and estradiol levels were suppressed when they consumed this plant daily at the highest dose given, whereas no effect was detected when fed only a single dose (Trisomboon *et al*. 2007). This reduction in FSH and estradiol was thought to occur through the action of phytoestrogen on ERs in the hypothalamus and pituitary, thus triggering the negative feedback mechanism of hypothalamic–pituitary–gonadal (HPG).

Effects of phytoestrogens on endogenous hormone levels are likely to occur through disruption of the negative feedback loop of the HPG axis. It is not only estradiol production that can be suppressed, but also testosterone production, which can be reduced with increasing levels of estrogens (Hadley 1999). Exemplifying this, soy phytoestrogens have been shown to suppress the HPG axis, with testosterone and luteinizing hormone (LH) levels reduced in adult male mice exposed to this compound (Whitten and Patisaul 2001). In contrast, no effects of isoflavone consumption on testosterone, estradiol, or cortisol levels were found in captive cynomolgus monkeys (Simon *et al*. 2004; Wood *et al*. 2004), but soy isoflavone consumption was related to a decrease in adrenal weight in these monkeys (Wood *et al*. 2004). Nonetheless, it is generally accepted that consumption of isoflavones relates to changes in estrogen levels (Cline and Wood 2009). There are a number of factors that influence steroid hormone levels in wild primates, including social factors, e.g., dominance hierarchies (Abbott *et al*. 2003) and ecological factors, e.g., parasitism (Chapman *et al*. 2006; Fig. 1), and evidence from captive studies suggests that phytoestrogens are one important but overlooked factor.

Fertility

If phytoestrogens act as agonists and increase estrogenic activity, adult male fertility (through sperm production) can decrease and feminization can occur (Cederroth *et al.* 2010a; Guillette 2000; Hayes 2005). For example, Cederroth *et al.* (2010b) found a 25%

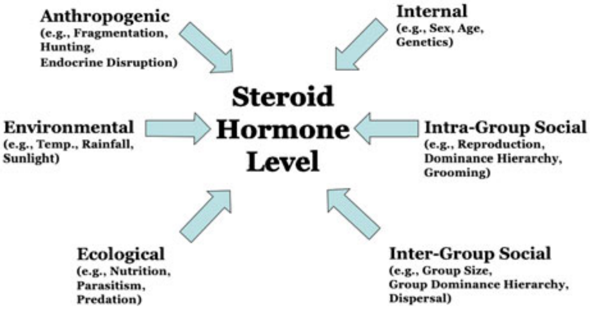

Fig. 1 Factors that can influence the steroid hormone levels of wild primates.

reduction in sperm counts in male mice (*Mus musculus*) fed a high soy diet starting *in utero* and a 21% reduction in litter size compared to mice fed a soy-free diet. In addition, growth and reproductive development, i.e., decreased anogenital distance, of male rats (*Rattus norvegicus*) were affected by exposure to soy phytoestrogens through the maternal diet during gestation and lactation as compared to a phytoestrogen-free maternal diet, suggesting an irreversible organizational effect from phytoestrogens on morphology important to reproduction (Ball *et al*. 2010). However, the estrogenic *Pueraria mirifica* did not alter male mice fertility, although moderate impairment of sperm motility and viability was documented at the highest phytoestrogen dose (Jaroenporn *et al*. 2006).

For females exposed to phytoestrogens, changes in the timing of ovulation, and even failure to ovulate, have been documented, likely mediated by disruption of negative feedback of HPG axis. This is a mechanism similar to birth control, where exogenous estrogens and progestins block the LH/FSH surge necessary for ovulation through the negative feedback mechanism (Hadley 1999). More specifically, soy phytoestrogens were found to affect reproductive development, i.e., earlier vaginal opening and smaller ovaries, and cause longer estrous cycles in female rats (Kouki *et al*. 2003). In addition, resveratrol, a phytoestrogen found in grapes that binds to both ER subtypes, reduced body weight, disrupted the estrous cycle, and increased ovarian weight in adult female rats (Henry and Witt 2002). In female cynomolgus monkeys fed the estrogenic *Pueraria mirifica*, the estrous cycle length increased at the lowest and moderate dose and ceased at the highest dose given (Trisomboon *et al*. 2005).

Behavioral Changes

One of the most exciting and promising areas of research related to phytoestrogens for primatologists is identifying their effects on the brain and behavior. Although behavioral effects have been studied less than other phytoestrogen effects, evidence is building that the brain may be an important target for these compounds (Patisaul 2005). Effects in the brain include changes in reproductive, stress-related, and social behaviors, as well as cognitive functioning (Patisaul 2005), all of which are likely linked to the phytoestrogen-caused changes in hormone levels and/or fertility mentioned previously. Phytoestrogens, including those found in soy, have been found to decrease mating behavior in adult female rats (Hartley *et al*. 2003; Kouki *et al*. 2003; Patisaul *et al*. 2004). In captive adult male cynomolgus monkeys, a 15-mo soy-based high-isoflavone diet resulted in an increase in aggressive behaviors, i.e., 67% more frequent compared to individuals fed an isoflavone-free diet; an increase in submissive behaviors, i.e., 203% more frequent; and a decrease in affiliative behaviors, i.e., 68% less time in body contact and 30% more time alone (Simon *et al*. 2004). It was postulated that these effects were likely due to the weaker action of isoflavones on ERβ than estradiol, thus reducing the inhibition of the aggression-promoting action of ERα (Simon *et al*. 2004). Male rats fed a high-isoflavone diet spent less time in social interactions and had higher corticosterone response to stress than rats fed an isoflavone-free diet (Hartley *et al*. 2003). Overall, the isoflavone-fed rats had greater anxiety-related physiological and behavioral measures than isoflavone-free rats. Similarly, the ERβ agonist equol increased aggression and anxiety in male rats that were exposed neonatally (Patisaul and Bateman 2008). However, other studies found no effect of equol on anxiety-related behaviors in adult male rats (Patisaul *et al*.

2009). Behavioral effects may be most prominent when exposure occurs early in life or greatly influenced by dose or timing of exposure (Patisaul 2005).

Phytoestrogens as a Selective Pressure on Primates?

The physiological and behavioral effects discussed in the previous section have the ability to influence survival and reproduction, and thus phytoestrogens may exert a negative selective pressure on primates, as suggested by the plant defense hypothesis. Alternatively, the consumption of phytoestrogens could increase fertility, fecundity, or survival as suggested by the chemical cue/self-medication hypotheses. Either way, effects of phytoestrogen ingestion on differential survival and reproduction may play an important, thus far neglected, role in primate ecology and evolution. However, ecological and evolutionary interest in phytoestrogens may be misguided if the biochemical coincidence hypothesis best explains the presence of these estrogenic plant compounds in the diets of herbivores.

To test among these three hypotheses, field research that examines variation in the presence and amount of phytoestrogens in the diets of various primate groups, populations, and species is needed, along with behavioral and physiological effects of their presence and consumption, e.g., do primates prefer or avoid such plants; does physiology, behavior, or fitness change in a positive or negative way with consumption? Further, results gathered from such research should be incorporated into what is currently known about primate nutritional ecology. For example, are there tradeoffs between the high protein content and phytoestrogen presence when deciding to consume estrogenic legumes? Such tradeoffs, along with differential effects based on dose, will make field studies of diet selection difficult for testing among our three hypotheses. Other confounding factors, such as the effects of variation in energy consumption (and other nutrients) versus phytoestrogen consumption on hormone levels, fertility, and fitness (Lu *et al.* 2011), will need to be considered and likely be difficult to tease apart using a field approach.

One solution to the complexity of interactions between phytoestrogens and nutrients is to use an experimental approach in a captive setting by conducting feeding trials where all other nutritional factors are held constant while the phytoestrogen content of a particular food is manipulated. Support for the plant defense hypothesis is found if primates avoid foods with phytoestrogens, while support for the self-medication hypothesis is found if they select or prefer such foods. If no effect of the presence of phytoestrogens in their foods is found, then this supports the biochemical coincidence hypothesis.

To test among these hypotheses further at an evolutionary scale that incorporates field studies, data on how much various primate species consume estrogenic plants can be collected and analyzed using phylogenetic methods (Nunn 2011). Owing to differences in dietary niche, primate species differ in their exposure to plant secondary metabolites, with leaves containing more and ripe fruit less. This may also be true of phytoestrogens, especially if they are a plant adaptation for deterring herbivory. Basically, if phytoestrogens are produced as a plant defense, then it is expected that they are more prevalent in leaves than fruits, and thus more prevalent in the diets of folivores than frugivores. In this case, finding dietary niche to relate to the presence of estrogenic plants in the diets of various primate species would provide support for the plant defense hypothesis. On the other hand, if dietary niche and estrogenic plant consumption are not related and instead phytoestrogen consumption relates only to phylogeny, then the self-medication hypothesis would be supported, e.g., groups with larger brains may be more likely to regulate their physiology or behavior via plant use. Finally, if there is no relationship between either phylogeny or dietary niche and phytoestrogen consumption, then the biochemical coincidence hypothesis is supported.

The best test of these three hypotheses would be an examination of the relationship between fitness and phytoestrogen consumption. A negative relationship would support the plant defense hypothesis, a positive relationship would support the self-medication hypothesis, and no relationship would support the biochemical coincidence hypothesis. Such data would depend on long-term field studies of multiple groups or populations occurring in similar environments where only the amount of estrogenic plants consumed differs. Such data are likely inaccessible unless long-term field studies are supported.

Although we cannot yet test among these three hypotheses, it is probable that most primate species encounter estrogenic plants to varying degrees in their diets because primates are generally a very strongly herbivorous taxon. Supporting this assumption, phytoestrogens are most prevalent in legumes, i.e., Fabaceae (Reynaud *et al.* 2005) and leguminous plant foods often compose an impressive percentage of the diet for many primates, particularly folivores, because of their high protein content (Chapman *et al.* 2002). Phytoestrogens have also been found in another important primate plant food family, Moraceae (Wasserman *et al.* 2012b). Regardless of whether it is the plant or the primate that is obtaining an evolutionary advantage from phytoestrogens, the heavily plant-dependent diet of primates suggests that consumption of estrogenic plants does occur to some degree for most primate species.

Conclusion

We have summarized what is known about phytoestrogens from a range of perspectives, including physiological and behavioral effects and ecological and evolutionary roles. In addition, we have provided a summary of how steroid hormones function at the molecular, physiological, and behavioral levels to demonstrate how plant chemicals can interact with these processes. Our ultimate objective has been to demonstrate that estrogenic plants have the potential to play important roles in primate ecology and evolution, especially because most primates depend heavily on plant-based diets that likely contain such hormone-mimicking compounds. This is an exciting area of inquiry with many ecological and evolutionary questions in need of study. Questions at the ecological level include the following: Which primate species consume estrogenic plants in their diets? Do primates actively select for estrogenic plants, avoid them, or are they simply indifferent to their presence? How are various phytoestrogens metabolized by gut microorganisms and to what extent do inter- and intraspecific differences in gut microbial communities alter the bioactivity of these compounds? What are the physiological and behavioral effects of consuming estrogenic plants for wild primates? Do phytoestrogens result in less cell growth, suboptimal fertility, increased aggression, or decreased sexual behavior as seen in captive studies? Questions at the evolutionary level include the following: If altered physiology and behavior are seen in wild primates, are the magnitudes of these changes great enough to affect survival and reproduction? Which of the three hypotheses, the plant defense, self-medication, or biochemical coincidence hypothesis, best explains the relationship between primates and estrogenic plants? Do phytoestrogens in wild plant foods act as important selective forces and therefore play a role in the evolution of primates? Comparative studies of primates and their plant foods are needed to address these questions and will add new context to the current phytoestrogen and endocrine disruption debate in public and environmental health issues.

Acknowledgments Approvals for plant collections were received from the Uganda Wildlife Authority, Uganda National Council for Science and Technology, and U.S. Department of Agriculture. This publication was made possible in part by grant no. P51 RR000167 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), to the Wisconsin National Primate Research Center, University of Wisconsin–Madison, where measures of plant steroidal properties were conducted. This research was conducted in part at a facility constructed with support from Research Facilities Improvement Program grant nos. RR15459-01 and RR020141-01. This publication's contents are solely the responsibility of the authors and do not necessarily represent the official views of NCRR or NIH. M. Wasserman received funding from the National Science Foundation (Doctoral Dissertation Improvement Grant (DDIG) no. 0823651 and Graduate Research Fellowship Program); the International Primatological Society; the University of California, Berkeley (UCB) Department of Environmental Science, Policy, and Management; UCB Center for African Studies; the UCB Chang-Lin Tien Scholars Program (funded by the Philomathia Foundation); and McGill University's Tomlinson Postdoctoral Fellowship to support this research. Dale Leitman, Isao Kubo, Len Bjeldanes, Toni Ziegler, Dan Wittwer, Tyrone Hayes, Paul Falso, John Fleagle, Andrew Ritchie, Alexandra Taylor-Gutt, Julie Kearney Wasserman, the anonymous reviewers, Joanna Setchell, and Jessica Rothman provided very helpful comments in the preparation of this review.

References

Abbott, D. H., Keverne, E. B., Bercovitch, F. B., Shively, C. A., Medoza, S. P., Saltzman, W., Snowdon, C. T., Ziegler, T. E., Banjevic, M., Garland, T., & Sapolsky, R. M. (2003). Are subordinants always stressed? A comparative analysis of rank differences in cortisol levels among primates. *Hormones and Behavior, 43*, 67–82.

Adlercreutz, H., Höckerstedt, K., Bannwart, C., Bloigu, S., Hämäläinen, E., Fotsis, T., & Ollus, A. (1987). Effect of dietary components, including lignans and phytoestrogens, on enterohepatic circulation and liver metabolism of estrogens and on sex hormone binding globulin (SHBG). *The Journal of Steroid Biochemistry and Molecular Biology, 27*, 1135–1144.

Almstrup, K., Fernandez, M. F., Petersen, J. H., Olea, N., Skakkebaek, N. E., & Leffers, H. (2002). Dual effects of phytoestrogens result in u-shaped dose-response curves. *Environmental Health Perspectives, 110*, 743–748.

Ball, E., Caniglia, M., Wilcox, J., Overton, K., Burr, M., Wolfe, B., Sanders, B., Wisniewski, A., & Wrenn, C. (2010). Effects of genistein in the maternal diet on reproductive development and spatial learning in male rats. *Hormones and Behavior, 57*, 313–322.

Bauchop, T., & Martucci, R. W. (1968). Ruminant-like digestion of the langur monkey. *Science, 161*, 698–700.

Beck, V., Unterrieder, E., Krenn, L., Kubelka, W., & Jungbauer, A. (2003). Comparison of hormonal activity (estrogen, androgen and progestin) of standardized plant extracts for large scale use in hormone replacement therapy. *The Journal of Steroid Biochemistry and Molecular Biology, 84*, 259–268.

Bennetts, H., & Underwood, E. (1951). The oestrogenic effects of subterranean clover (*Trifolium subterraneum*): uterine maintenance in the ovariectomised ewe on clover grazing. *The Australian Journal of Experimental Biology and Medical Science, 29*, 249–253.

Berger, P., Negus, N., Sanders, E., & Gardner, P. (1981). Chemical triggering of reproduction in *Microtus montanus*. *Science, 214*, 69–70.

Berger, P., Sanders, E., Gardner, P., & Negus, N. (1977). Phenolic plant compounds functioning as reproductive inhibitors in *Microtus montanus*. *Science, 195*, 575–577.

Burgess, M. A., & Chapman, C. A. (2005). Tree leaf chemical characters: selective pressures by folivorous primates and invertebrates. *African Journal of Ecology, 43*, 242–250.

Cederroth, C. R., Auger, J., Zimmermann, C., Eustache, F., & Nef, S. (2010a). Soy, phyto-oestrogens and male reproductive function: a review. *International Journal of Andrology, 33*, 304–316.

Cederroth, C. R., Zimmermann, C., Beny, J. L., Schaad, O., Combepine, C., Descombes, P., Doerge, D. R., Pralong, F. P., Vassalli, J. D., & Nef, S. (2010b). Potential detrimental effects of a phytoestrogen-rich diet on male fertility in mice. *Molecular and Cellular Endocrinology, 321*, 152–160.

Chapman, C. A., Chapman, L. J., Bjorndal, K. A., & Onderdonk, D. A. (2002). Application of protein-to-fiber ratios to predict colobine abundance on different spatial scales. *International Journal of Primatology, 23*, 283–310.

Chapman, C. A., Wasserman, M. D., Gillespie, T. R., Speirs, M. L., Lawes, M. J., Saj, T. L., & Ziegler, T. E. (2006). Do food availability, parasitism, and stress have synergistic effects on red colobus populations living in forest fragments? *American Journal of Physical Anthropology, 131*, 525–534.

Cline, J., & Wood, C. (2009). Estrogen/isoflavone interactions in cynomolgus macaques (*Macaca fascicularis*). *American Journal of Primatology, 71*, 722–731.

Coldham, N. G., & Sauer, M. J. (2000). Pharmacokinetics of [C-14]genistein in the rat: gender-related differences, potential mechanisms of biological action, and implications for human health. *Toxicology and Applied Pharmacology, 164*, 206–215.

Coley, P. D., & Barone, J. A. (1996). Herbivory and plant defenses in tropical forests. *Annual Review of Ecology and Systematics, 27*, 305–335.

Cornwell, T., Cohick, W., & Raskin, I. (2004). Dietary phytoestrogens and health. *Phytochemistry, 65*, 995–1016.

DeGabriel, J. L., Moore, B. D., Foley, W. J., & Johnson, C. N. (2009). The effects of plant defensive chemistry on nutrient availability predict reproductive success in a mammal. *Ecology, 90*, 711–719.

Dixon, R. (2004). Phytoestrogens. *Annual Review of Plant Biology, 55*, 225–261.

Emery Thompson, M., Wilson, M. L., Gobbo, G., Muller, M. N., & Pusey, A. E. (2008). Hyperprogesteronemia in response to *Vitex fischeri* consumption in wild chimpanzees (*Pan troglodytes schweinfurthii*). *American Journal of Primatology, 70*, 1064–1071.

Fashing, P. J., Dierenfeld, E. S., & Mowry, C. B. (2007). Influence of plant and soil chemistry on food selection, ranging patterns, and biomass of *Colobus guereza* in Kakamega Forest, Kenya. *International Journal of Primatology, 28*, 673–703.

Feeny, P. (1970). Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology, 51*, 565–581.

Fidler, A. E., Lawrence, S. B., & Mcnatty, K. P. (2008). An hypothesis to explain the linkage between kakapo (*Strigops habroptilus*) breeding and the mast fruiting of their food trees. *Wildlife Research, 35*, 1–7.

Foley, W. J., & Moore, B. D. (2005). Plant secondary metabolites and vertebrate herbivores - from physiological regulation to ecosystem function. *Current Opinion in Plant Biology, 8*, 430–435.

Forbey, J. S., Harvey, A. L., Huffman, M. A., Provenza, F. D., Sullivan, R., & Tasdemir, D. (2009). Exploitation of secondary metabolites by animals: a response to homeostatic challenges. *Integrative and Comparative Biology, 49*, 314–328.

Fox, J., Starcevic, M., Jones, P., Burow, M., & McLachlan, J. (2004). Phytoestrogen signaling and symbiotic gene activation are disrupted by endocrine-disrupting chemicals. *Environmental Health Perspectives, 112*, 672–677.

Freeland, W., & Janzen, D. (1974). Strategies in herbivory by mammals: the role of plant secondary compounds. *American Naturalist, 108*, 269–289.

Ganzhorn, J. U. (1992). Leaf chemistry and the biomass of folivorous primates in tropical forests: test of a hypothesis. *Oecologia, 91*, 540–547.

Garey, J. (1993). Dietary estrogens as a cue for primate breeding seasons. *American Journal of Physical Anthropology, S16*, 92.

Glander, K. (1978). Howling monkey feeding behavior and plant secondary compounds: A study of strategies. In G. Montgomery (Ed.), *The ecology of arboreal folivores* (pp. 561–573). Washington, DC: Smithsonian Institution Press.

Glander, K. E. (1980). Reproduction and population growth in free-ranging mantled howling monkeys. *American Journal of Physical Anthropology, 53*, 25–36.

Guillette, L. J., Jr. (2000). Contaminant-induced endocrine disruption in wildlife. *Growth Hormone & IGF Research, 10*(Suppl B), S45–S50.

Gultekin, E., & Yildiz, F. (2006). Introduction to phytoestrogens. In F. Yildiz (Ed.), *Phytoestrogens in functional foods* (pp. 3–18). Boca Raton: CRC Press.

Hadley, M. (1999). *Endocrinology* (5th ed.). Upper Saddle River: Prentice Hall.

Harborne, J. (1993). *Introduction to ecological biochemistry* (4th ed.). San Francisco: Elsevier Academic Press.

Harrison, R., Phillippi, P., Swan, K., & Henson, M. (1999). Effect of genistein on steroid hormone production in the pregnant rhesus monkey. *Proceedings of the Society for Experimental Biology and Medicine, 222*, 78–84.

Hartley, D., Edwards, J., Spiller, C., Alom, N., Tucci, S., Seth, P., Forsling, M., & File, S. (2003). The soya isoflavone content of rat diet can increase anxiety and stress hormone release in the male rat. *Psychopharmacology, 167*, 46–53.

Hayes, T., Collins, A., Lee, M., Mendoza, M., Noriega, N., Stuart, A., & Vonk, A. (2002). Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 5476–5480.

Hayes, T. B. (2005). Welcome to the revolution: integrative biology and assessing the impact of endocrine disruptors on environmental and public health. *Integrative and Comparative Biology, 45*, 321–329.

Heftmann, E. (1977). Functions of steroids in plants. *Phytochemistry, 14*, 891–901.

Heldring, N., Pike, A., Andersson, S., Matthews, J., Cheng, G., Hartman, J., Tujague, M., Strom, A., Treuter, E., Warner, M., & Gustafsson, J. A. (2007). Estrogen receptors: how do they signal and what are their targets. *Physiological Reviews, 87*, 905–931.

Henry, L., & Witt, D. (2002). Resveratrol: phytoestrogen effects on reproductive physiology and behavior in female rats. *Hormones and Behavior, 41*, 220–228.

Higham, J. P., Ross, C., Warren, Y., Heistermann, M., & MacLarnon, A. M. (2007). Reduced reproductive function in wild baboons (*Papio hamadryas anubis*) related to natural consumption of the African black plum (*Vitex doniana*). *Hormones and Behavior, 52*, 384–390.

Huffman, M. A. (1997). Current evidence for self-medication in primates: a multidisciplinary perspective. *Yearbook of Physical Anthropology, 40*, 171–200.

Huffman, M. A. (2001). Self-medicative behavior in the African great apes: an evolutionary perspective into the origins of human traditional medicine. *Bioscience, 51*, 651–661.

Hughes, C. L., Jr. (1988). Phytochemical mimicry of reproductive hormones and modulation of herbivore fertility by phytoestrogens. *Environmental Health Perspectives, 78*, 171–174.

Iino, M., Nomura, T., Tamaki, Y., Yamada, Y., Yoneyama, K., Takeuchi, Y., Mori, M., Asami, T., Nakano, T., & Yokota, T. (2007). Progesterone: its occurrence in plants and involvement in plant growth. *Phytochemistry, 68*, 1664–1673.

Janeczko, A., & Skoczowski, A. (2005). Mammalian sex hormones in plants. *Folia Histochemica Et Cytobiologica, 43*, 71–79.

Jaroenporn, S., Malaivijitnond, S., Wattanasirmkit, K., Trisomboon, H., Watanabe, G., Taya, K., & Cherdshewasart, W. (2006). Effects of *Pueraria mirifica*, an herb containing phytoestrogens, on reproductive organs and fertility of adult male mice. *Endocrine, 30*, 93–101.

Karowe, D. N., & Radi, J. K. (2011). Are the phytoestrogens genistein and daidzein anti-herbivore defenses? A test using the gypsy moth (*Lymantria dispar*). *Journal of Chemical Ecology, 37*, 830–837.

Kouki, T., Kishitake, M., Okamoto, M., Oosuka, I., Takebe, M., & Yamanouchi, K. (2003). Effects of neonatal treatment with phytoestrogens, genistein and daidzein, on sex difference in female rat brain function: estrous cycle and lordosis. *Hormones and Behavior, 44*, 140–145.

Krishnan, A., Stathis, P., Permuth, S., Tokes, L., & Feldman, D. (1993). Bisphenol-a: an estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinology, 132*, 2279–2286.

Kubo, I., Klocke, J., & Asano, S. (1983). Effects of ingested phytoecdysteroids on the growth and development of two lepidopterous larvae. *Journal of Insect Physiology, 29*, 307–316.

Kuiper, G., Lemmen, J., Carlsson, B., Corton, J., Safe, S., van der Saag, P., van der Burg, B., & Gustafsson, J. (1998). Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. *Endocrinology, 139*, 4252–4263.

Leitman, D. C., Paruthiyil, S., Vivar, O. I., Saunier, E. F., Herber, C. B., Cohen, I., Tagliaferri, M., & Speed, T. P. (2010). Regulation of specific target genes and biological responses by estrogen receptor subtype agonists. *Current Opinion in Pharmacology, 10*, 629–636.

Leopold, A. S., Erwin, M., Oh, J., & Browning, B. (1976). Phytoestrogens: adverse effects on reproduction in California quail. *Science, 191*, 98–100.

Lu, A., Beehner, J. C., Czekala, N. M., Koenig, A., Larney, E., & Borries, C. (2011). Phytochemicals and reproductive function in wild female phayre's leaf monkeys (*Trachypithecus phayrei crepusculus*). *Hormones and Behavior, 59*, 28–36.

Mazur, W. (1998). Phytoestrogen content in foods. *Baillière's Clinical Endocrinology and Metabolism, 12*, 729–742.

Mazur, W., & Adlercreutz, H. (1998). Naturally occurring oestrogens in food. *Pure and Applied Chemistry, 70*, 1759–1776.

Mckey, D. B., Gartlan, J. S., Waterman, P. G., & Choo, G. M. (1981). Food selection by black colobus monkeys (*Colobus satanas*) in relation to plant chemistry. *Biological Journal of the Linnean Society, 16*, 115–146.

Milton, K. (1979). Factors influencing leaf choice by howler monkeys: a test of some hypotheses of food selection by generalist herbivores. *American Naturalist, 114*, 362–378.

Milton, K. (1980). *The foraging strategy of howler monkeys: A study in primate economics*. New York: Columbia University Press.

Milton, K. (1998). Physiological ecology of howlers (*Alouatta*): energetic and digestive considerations and comparison with the colobinae. *International Journal of Primatology, 19*, 513–548.

Milton, K. (2000). Back to basics: why foods of wild primates have relevance for modern human health. *Nutrition, 16*, 480–483.

Nash, L., & Whitten, P. (1989). Preliminary observations on the role of *Acacia* gum chemistry in *Acacia* utilization by *Galago senegalensis* in Kenya. *American Journal of Primatology, 17*, 27–39.

Nunn, C. (2011). *The comparative approach in evolutionary anthropology and biology*. Chicago: University of Chicago Press.

Oates, J. F., Whitesides, G. H., Davies, A. G., Waterman, P. G., Green, S. M., Dasilva, G. L., & Mole, S. (1990). Determinants of variation in tropical forest primate biomass—new evidence from West Africa. *Ecology, 71*, 328–343.

Patisaul, H. (2005). Phytoestrogen action in the adult and developing brain. *Journal of Neuroendocrinology, 17*, 57–64.

Patisaul, H., & Bateman, H. (2008). Neonatal exposure to endocrine active compounds or an ERβ agonist increases adult anxiety and aggression in gonadally intact male rats. *Hormones and Behavior, 53*, 580–588.

Patisaul, H., Burke, K., Hinkle, R., Adewale, H., & Shea, D. (2009). Systemic administration of diarylpropionitrile (DPN) or phytoestrogens does not affect anxiety-related behaviors in gonadally intact male rats. *Hormones and Behavior, 55*, 319–328.

Patisaul, H., Luskin, J., & Wilson, M. (2004). A soy supplement and tamoxifen inhibit sexual behavior in female rats. *Hormones and Behavior, 45*, 270–277.

Reynaud, J., Guilet, D., Terreux, R., Lussignol, M., & Walchshofer, N. (2005). Isoflavonoids in non-leguminous families: an update. *Natural Product Reports, 22*, 504–515.

Robbins, C. T., Hagerman, A. E., Austin, P. J., Mcarthur, C., & Hanley, T. A. (1991). Variation in mammalian physiological-responses to a condensed tannin and its ecological implications. *Journal of Mammalogy, 72*, 480–486.

Rothman, J. M., Chapman, C. A., & Van Soest, P. J. (2012). Methods in primate nutritional ecology: a user's guide. *International Journal of Primatology, 33*, 542–566.

Schloms, L., Storbeck, K. H., Swart, P., Gelderblom, W. C., & Swart, A. C. (2012). The influence of *Aspalathus linearis* (rooibos) and dihydrochalcones on adrenal steroidogenesis: quantification of steroid intermediates and end products in h295r cells. *The Journal of Steroid Biochemistry and Molecular Biology, 128*, 128–138.

Setchell, K. D., & Clerici, C. (2010). Equol: history, chemistry, and formation. *Journal of Nutrition, 140*, 1355S–1362S.

Sfakianos, J., Coward, L., Kirk, M., & Barnes, S. (1997). Intestinal uptake and biliary excretion of the isoflavone genistein in rats. *Journal of Nutrition, 127*, 1260–1268.

Sharpe, R., Martin, B., Morris, K., Greig, I., McKinnell, C., McNeilly, A., & Walker, M. (2002). Infant feeding with soy formula milk: effects on the testis and on blood testosterone levels in marmoset monkeys during the period of neonatal testicular activity. *Human Reproduction, 17*, 1692–1703.

Simon, N., Kaplan, J., Hu, S., Register, T., & Adams, M. (2004). Increased aggressive behavior and decreased affiliative behavior in adult male monkeys after long-term consumption of diets rich in soy protein and isoflavones. *Hormones and Behavior, 45*, 278–284.

Strier, K. B. (1993). Menu for a monkey. *Natural History, 102*, 34–43.

Thornton, J. (2001). Evolution of vertebrate steroid receptors from an ancestral estrogen receptor by ligand exploitation and serial genome expansions. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 5671–5676.

Thornton, J., Need, E., & Crews, D. (2003). Resurrecting the ancestral steroid receptor: ancient origin of estrogen signaling. *Science, 301*, 1714–1717.

Trisomboon, H., Malaivijitnond, S., Cherdshewasart, W., Watanabe, G., & Taya, K. (2007). Assessment of urinary gonadotropin and steroid hormone profiles of female cynomolgus monkeys after treatment with *Pueraria mirifica*. *Journal of Reproduction and Development, 53*, 395–403.

Trisomboon, H., Malaivijitnond, S., Watanabe, G., & Taya, K. (2005). Ovulation block by *Pueraria mirifica*. *Endocrine, 26*, 33–39.

Wallis, J. (1997). A survey of reproductive parameters in the free-ranging chimpanzees of Gombe National Park. *Journal of Reproduction and Fertility, 109*, 297–307.

Wang, H. B., Dey, S. K., & Maccarrone, M. (2006). Jekyll and Hyde: two faces of cannabinoid signaling in male and female fertility. *Endocrine Reviews, 27*, 427–448.

Wasserman, M. D., & Chapman, C. A. (2003). Determinants of colobine monkey abundance: the importance of food energy, protein and fibre content. *Journal of Animal Ecology, 72*, 650–659.

Wasserman, M. D., Chapman, C. A., Milton, K., Gogarten, J. F., Wittwer, D. J., & Ziegler, T. E. (2012a). Estrogenic plant consumption predicts red colobus monkey (*Procolobus rufomitratus*) hormonal state and behavior. *Hormones and Behavior, 62*, 553–562.

Wasserman, M. D., Taylor-Gutt, A., Rothman, J. M., Chapman, C. A., Milton, K., & Leitman, D. C. (2012b). Estrogenic plant foods of red colobus monkeys and mountain gorillas in Uganda. *American Journal of Physical Anthropology, 148*, 88–97.

Whitten, P. (1983). Flowers, fertility, and females. *American Journal of Physical Anthropology, 60*, 269–270.

Whitten, P. L., & Patisaul, H. B. (2001). Cross-species and interassay comparisons of phytoestrogen action. *Environmental Health Perspectives, 109*(Suppl 1), 5–20.

Whorwood, C. B., Sheppard, M. C., & Stewart, P. M. (1993). Licorice inhibits 11-beta-hydroxysteroid dehydrogenase messenger-ribonucleic-acid levels and potentiates glucocorticoid hormone action. *Endocrinology, 132*, 2287–2292.

Wood, C., Cline, J., Anthony, M., Register, T., & Kaplan, J. (2004). Adrenocortical effects of oral estrogens and soy isoflavones in female monkeys. *Journal of Clinical Endocrinology and Metabolism, 89*, 2319–2325.

Wuttke, W., Jarry, H., Christoffel, V., Spengler, B., & Seidlova-Wuttke, D. (2003). Chaste tree (*Vitex agnus-castus*) pharmacology and clinical indications. *Phytomedicine, 10*, 348–357.

Wynne-Edwards, K. E. (2001). Evolutionary biology of plant defenses against herbivory and their predictive implications for endocrine disruptor susceptibility in vertebrates. *Environmental Health Perspectives, 109*, 443–448.

Ye, L., Su, Z. J., & Ge, R. S. (2011). Inhibitors of testosterone biosynthetic and metabolic activation enzymes. *Molecules, 16*, 9983–10001.