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Title:

Plant Sexual Reproduction: Perhaps the current plant two-sex model should be replaced with three- and four-sex models?

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Abstract:

The current plant two-sex model makes the assumption that there are only two sexual reproductive states: male and female. However, the application of this model to the plant alternation of generations requires the subtle redefinition of several common terms related to sexual reproduction, which also seems to obscure aspects of one or the other plant generation: For instance, the homosporous sporophytic plant is treated as being "asexual," and the gametophytes of angiosperms treated like mere gametes. In contrast, the proposal is made that the sporophytes of homosporous plants are indeed sexual reproductive organisms, as are the gametophytes of heterosporous plants. This view requires the expansion of the number of sexual reproductive states we accept for plants, therefore a three-sex model for homosporous plants and a four-sex model for heterosporous plants are described and then contrasted with the current two-sex model. These new models allow the use of sexual reproductive terms in a manner largely similar to that seen in animals, and may better accommodate the plant alternation of generations life cycle than does the current plant two-sex model. These new three-sex and four-sex models may also help stimulate new lines of research, and examples of how they might alter our view of the flower, and may lead to new perspectives in terms of sexual determination, are presented. Thus it is suggested that plants have more than merely two sexual reproductive states, and that recognition of this may promote our study and understanding of plants.

Key Words: alternation of generations; fertilization; gametophyte; mating; meiosis; plant sexual reproduction; sporophyte

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INTRODUCTION

In describing the history of the study of plant sexual reproduction (Bennett, 1875; von Sachs, 1906; Browne, 1989; Žárský and Tupý, 1995) the evidence is noted that led to the broad acceptance of what Taiz and Taiz (2017) refer to as the "two-sex model" in which plants were recognized as having distinct male and female sexual reproductive states. This was unquestionably an advance over the previous view that plants were largely asexual, and this advance led to the opening up of new areas for study. But it may be noted that to fit the plant life cycle into this two-sex model some distinctive judgements had to be made as to which aspects of the plant life cycle were sexual, or not, so that the assumption could be met that plants only had just two sexual states and no more. This review will question that assumption, and two new models of plant sexual reproduction (three-sex and four-sex models for homosporous and heterosporous plants respectively) will be presented for consideration. Each of these new models will then be compared to how the current plant two-sex model accounts for features of the plant life cycle.

The models that we use can summarize exisiting knowledge and can also set a context for our thinking. It will be suggested that the way the current plant two-sex model summarizes our knowledge fits poorly with the plant alternation of generations. The new three-sex and four-sex models may be better able to account for the reality of plant sexual reproduction, in a manner more consistent with the plant life cycle and with the terminology used with regard to animal sexuality, than does the current plant two-sex model. Furthermore, these new models may offer new ways to view plant sexual reproduction, perhaps suggesting new lines of research, and two

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examples of this will be offered. Thus plants are suggested to have more than just two sexual reproductive states, and full acceptance of this might help to further advance our studies of plants.

WHAT PROCESSES ARE SEXUAL AND REPRODUCTIVE?

To assist an examination of plant sexual reproduction the meaning of the terms "sexual" and "reproduction" will first be considered. A sexual process may be taken to be one which has the capacity to produce new genetic combinations (Schwander et al., 2014; Haig, 2015). These genetic shifts can be at the genotype level producing new combinations of alleles, or at the genome level as seen with changes in ploidy (Tchórzewska, 2017). Both meiosis and fertilization (*i.e.*, syngamy) clearly meet this definition (Raven et al., 1992; Nelms and Walbot, 2019), and here will be accepted as sexual processes in plant life cycles. In terms of reproduction, the definition given by Kondrashov (1997, pg. 393) would seem relevant: "When a new organism appears from the single cell, each process that produces this cell (mitosis, meiosis, or syngamy) is also a mode of reproduction." Obviously reproduction by mitotic divisions would be an asexual form of reproduction. However, for either meiosis or fertilization to be both sexual and reproductive the single cells they produce must have the capacity to grow into new multicellular organisms. Since in plants both the zygotes made through fertilization and the spores made by meiosis have this ability, both fertilization and meiosis as done in plants will be considered to be both reproductive and sexual processes.

Next, let us consider how the above views of sexual reproduction apply to the animal life cycle, as well as review some of the related concepts from animals which are often used in

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discussions of plant life cycles and sexuality. Most animal species fit within a two-sex model. Here gametic meiosis is done, followed immediately in the life cycle by fertilization to create a zygote (Fig. 1A). Since the animal's gametes do not normally individually divide, and so do not become multicellular organisms, animal meiosis, while sexual, is clearly not a reproductive process for animals. In contrast, since the zygote has the ability to divide mitotically, and so form a new multicellular individual, for animals fertilization is their sole process which is both sexual and reproductive. It might also be noted that post-zygotic events, such as growth and development of the offspring, its interactions with nurturing parents, competition with siblings for food, *etc.*, while critical to ensure survival to achieve eventual reproduction, need not be considered to be sexually reproductive processes themselves as they neither alter the genetic combination nor directly produce new organisms. Thus animal sexual reproduction is often just defined in terms of events closely associated with fertilization. The two adult sexual states are distinguished by the type of gametes made; with organisms that make sperm being called male, and those making eggs being called female. These two sexual states, while typically seen in separate individuals, can exist in one bisexual individual in some animal species (*i.e.*, a hermaphrodite), which may then be capable of self-fertilization (Jarne and Auld, 2006). Sexually mature males and females can engage in mating under a variety of animal mating systems (Klug, 2011).

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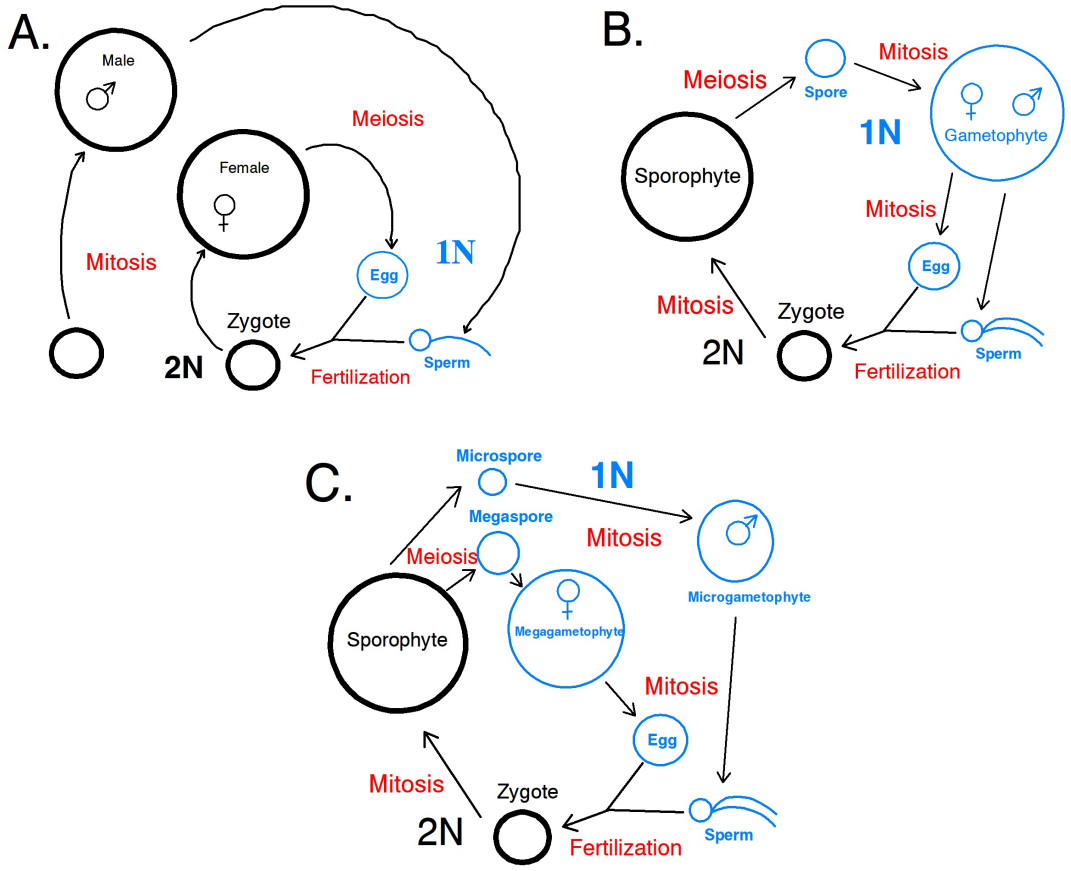

Figure 1. Life cycles of (A.) typical animals; (B.) homosporous plants, such as a fern, with a bisexual gametophyte and a monosexual sporophyte, and; (C.) heterosporous plants, such as *Selaginella* sp., with a bisexual sporophyte and two monosexual gametophytes. In each life cycle, the diploid items (2N) are shown in black, the haploid items (1N) are in blue, and processes are colored red. Notice that animals use gametic meiosis, while the plants have a distinct gametophytic stage which eventually makes the actual gametes by mitosis and a sporophytic stage which does sporic meiosis.

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HOMOSPOROUS PLANTS: THE THREE-SEX MODEL.

Now consider the case of homosporous plants (*i.e.*, bryophytes, many ferns and lycophytes) and how their sexual reproduction may be described under a three-sex model. For instance, in a homosporous fern only one type of spore is made, so only one type of sporangium is present in the sporophytic organism. This sporophyte is sexual in that it has sporangia as its sexual organs in which it does meiosis to make these sexual spores (Moore et al., 2016b; Watkins et al., 2016). However, unlike in animals, here meiosis is reproductive as well as sexual since the spores created typically undergo mitosis to create new multicellular gametophytes (Fig. 1B). We can refer to this homosporous fern sporophyte as being monosexual, or simply homosporous, and easily indicate this sexual reproductive state, which, notice, is neither male nor female as it makes no gametes. The fern gametophytes be said to have a sexual state, as they make the gametes, in their organs of antheridia and archegonia (Raven et al., 1992), and these gametes may eventually engage in the sexual process of fertilization. Here fertilization is also clearly reproductive as the resulting zygotes are able to grow into new sporophytic individuals. Thus, as Haig and Wilczek (2006) note, here the haploid gametophytes would have the attributes of male and female. In addition, the gametophyte generation of many homosporous plants, including many ferns, is often bisexual (*i.e.*, hermaphroditic), with the same organism making both sperm and egg gametes. Self-fertilization may occur in such a situation (Klekowski, 1973). This shows a sexual reproductive pattern somewhat similar to that seen in hermaphroditic animals, except that these sexual gametophytic plants are haploid and their gametes are made by mitosis. Thus for this example of a homosporous fern species there are three sexual reproductive states (*i.e.*, a monosexual sporophyte, male, and female) displayed in just two individuals in the life cycle (*i.e.*,

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a sporophyte, and a bisexual gametophyte). Of course some homosporous plants have monosexual gametophytes (Lott et al., 2003; Glime, 2007; Haig, 2016), in which case the three sexual reproductive states would be divided among three distinct individuals. Thus homosporous plants as viewed under this three-sex model have more sexual reproductive states than do typical animal species.

HOMOSPOROUS PLANTS: THE CURRENT TWO-SEX MODEL.

Let us now consider how the current two-sex model is often applied to a homosporous plant species (Fig. 1B). A critical assumption of the plant two-sex model is that there are only two actual sexual reproductive states. Similar to the above three-sex model, the homosporous plant two-sex model assigns to the gametophyte generation the attributes of maleness and femaleness (Haig and Wilczek, 2006; Taylor et al., 2007; Haig, 2013), and in many homosporous plants this gametophyte is a bisexual (*i.e.*, hermaphroditic) individual. So if it is bisexual then this individual gametophytic organism may often self-mate, and so undergo self-fertilization, to make a zygote. However, with the two sexual states of male and female already assigned, how to refer to the homosporous plant sporophytic generation under the two-sex model becomes an issue. Since spore production is not seen in animals, and perhaps because the future sexual state that arises later in the gametophyte can not be identified at the spore stage, or because with self-fertilization the sporophyte would be presumed to be genetically fully homozygous and so might not contain new genetic combinations, some refer to the spores made by meiosis in the sporangia of homosporous sporophytes as “asexual” in nature (Glime, 2007; Haufler, 2014; Petersen and Burd, 2018). Indeed some assign to homosporous plant spores only a role in asexual

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reproduction (Devos et al., 2011; Mauseth, 2017), and the sporangia of homosporous plants are also sometimes said to be "asexual" structures (Taiz and Taiz, 2017), even though meiosis is done within them. Thus, the homosporous sporophyte itself may well have no sexual designation assigned to it under the two-sex model (Raven et al., 1992).

Thus it is evident that while the plant two-sex model accepts fertilization as a sexually reproductive process, it does not consider plant meiosis to be a sexually reproductive process. Indeed some early descriptions of the homosporous plant "alternation of generations" life cycle describe it as an alternation between a sexual generation (*i.e.*, gametophytes) and an asexual generation (*i.e.*, sporophytes) (Harper, 1907; Strasburger et al., 1908; Coulter et al., 1910). In this way the two-sex model manages to view homosporous plants as having only two sexes, places an emphasis on fertilization, but ignores any sexual reproductive aspects of meiosis and of the sporophyte generation. However, the justification for the two-sex model's denial of the sexuality of the sporophyte generation in homosporous plants has largely not been made clear.

HETEROSPOROUS PLANTS: THE FOUR-SEX MODEL.

Next let us consider the four-sex model. In a heterosporous non-seed plant (Fig 1C), such as *Selaginella* sp., there is a bisexual sporophyte. Its sexual organs are the microsporangia and megasporangia, in which sporic meiosis is done to make sexual microspores and megaspores. These spores lead to distinct monosexual male and female gametophytic individuals (Kondrashov, 1997). Therefore we have here four sexual reproductive states (megasporangiate, microsporangiate, male, and female) distributed between three organisms (bisexual sporophyte,

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male gametophyte, and female gametophyte). This would be a four-sex condition, and shows how heterosporous plants can be argued to have more sexual reproductive states than is accepted under the current plant two-sex model, and certainly more than is seen in most animal species.

Next consider the situation in a typical heterosporous seed plant. Haig and Wilczek (2006) notes that in angiosperms all the gametophytes are dioicious, and that the sporophytes may be bisexual or dioecious. Consider the pea plant (*Pisum sativum*), here the sporophyte is bisexual, having two types of sporangia (anther sac/microsporangia in stamen, and ovules/megasporangia in pistils) in which different types of spores are made by meiosis. The two distinct gametophytes which grow from these two spore types create the gametes, and so these gametophytes may be referred to as male (pollen/microgametophyte) and female (embryo sac/megagametophyte). It should be noted that the male and female gametophytes of peas eventually engage in mating interactions to achieve fertilization (Friedman and Williams, 2004; Zhong et al., 2019), but while there may be inbreeding of a lineage if brother and sister gametophytes mate there can not be any self-fertilization as these gametophytes are two distinct monosexual organisms. In contrast the bisexual sporophytic pea plant does not make gametes; so while it is sexual it is neither male nor female, therefore this sporophyte can not be considered to be hermaphroditic, and so it can not engage in mating or in self-fertilization. This bisexual sporophyte might be referred to as being both staminate (or antherate), as well as carpellate/pistillate (or ovulate) (as in Pelser et al., 2017), or we may say that its flowers are perfect, or complete (Raven et al., 1992), which also conveys this sporophytic bisexual combination clearly. Thus, pea plants have four sexual reproductive states (staminate, pistillate, male, and female) divided among three individuals (a bisexual sporophyte, a male gametophyte, and a female gametophyte).

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It should also be noted that dioecious angiosperm species have the staminate and carpellate sexual states divided between two monosexual sporophytic individuals (Haig and Wilczek, 2006). Here, under the four-sex model, the four sexual reproductive states are divided between four distinct individual organisms. In contrast, a monoecious angiosperm species would have a bisexual sporophyte, but with distinct staminate and carpellate flowers made in different locations on the body of the same individual plant.

This four-sex model requires that we recognize meiosis as a fully sexually reproductive process, and requires that the sporophytes and the micro- and mega-gametophytes be each accepted as individual sexually reproductive organisms. In other words, we have to accept that the plant alternation of generations life cycle is an alternation of sexually reproductive generations. Based on the three-sex and four-sex models, therefore, in the life cycle of various species of plants there are typically either three to four distinct sexual reproductive states distributed between from two to four individual organisms. This is rather different from the limit of just two sexually reproductive states which the current plant two-sex model imposes.

ON POLLINATION.

The astute reader may have noticed that in the above outline of the four-sex model as applied to angiosperms there was no mention made of pollination. That was because pollination, according to the definitions presented earlier, is neither a sexual process nor a reproductive process: Pollination alone does not produce a new organism, nor does it alter the genetic combination or ploidy in any manner, so it is not a sexually reproductive process. A brief

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description of how angiosperm pollination would be viewed under the four-sex model follows.

The male gametophyte (pollen) has an early stage of development within the anther sac, and a later stage of development on the stigmatic surface and in the stylar tissues of the pistil. In between these two developmental stages there is a period of relative dormancy during which, by various means, this male gametophyte is moved from the anther sac to the stigmatic surface, and it is this movement which we call pollination (Raven et al., 1992). An analogous process is perhaps seen in marsupial animals where, after a period of development in the womb, the immature offspring must move to the marsupial pouch to continue its development (Edwards and Deakin, 2013). Notice that in some angiosperm species pollination is sometimes done before the pollen grain has made its sperm, and even if the sperm are present during pollination they are often not yet sexually mature (Snell, 2012; Sprunck et al., 2012). After pollination the male gametophyte must grow towards the female gametophyte, maturing as it does so, and then engage in mating interactions before fertilization can be attempted between these two gametophytic organisms (Escobar-Restrepo et al., 2007; Ge et al., 2007; Sprunck et al., 2012; Ge et al., 2017; Stegmann and Zipfel, 2017). Thus under the four-sex model pollination is not viewed as being mating since it precedes the eventually mating events, and in any event an immature male gametophyte can not "mate" with sporophytic tissues of the pistil, not just because it is immature but more tellingly because sporophytes never make gametes and so can not engage in mating. Nor is pollination considered to be fertilization as no zygote results, rather pollination happens well before fertilization. This requires that we adopt the view that self-pollination is different from self-fertilization, in at least that self-pollination typically involves two individuals of different generations (a bisexual sporophyte and a male gametophyte), while self-fertilization would be

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done by just one individual (a bisexual gametophyte, which angiosperms lack but many homosporous plant species do exhibit). Thus, pollination is a process that interrupts distinct periods of development of the male gametophyte, and has no direct analogy in many animal species, other than perhaps with a few animal species such as marsupials as already mentioned. Therefore, while pollination is clearly important in seed plants, and its study of great interest, whatever selective pressures operate to ensure that pollination occurs should perhaps not be considered to be sexual selection?

HETEROSPOROUS PLANTS: THE TWO-SEX MODEL.

Now consider how the plant two-sex model is applied to heterosporous plants, especially angiosperms. As stated previously, the plant two-sex model focuses mainly on fertilization as the sole sexual reproductive process (Crawford and Yanofsky, 2008), and largely ignores meiosis. But in order to meet its key assumption that there are only two plant sexual reproductive states, the two-sex model as applied to angiosperms and to other seed plants implicitly redefines several major concepts, including: "male," "female," "fertilization," "mating" (Table 1), as well as obscuring the gametophytes as individuals so that only the sporophytes are considered as "self." A brief description of each of these redefinitions follows.

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Table 1. A comparison of the meanings of terms and processes used to describe aspects of sexual reproduction for both angiosperms under the plant four-sex model and typical animal species, contrasted with their meaning when applied to angiosperms under the plant two-sex model.

Terms under each model:	Description:
Male:	
Animals and Plant 4-sex model	An individual organism that at sexual maturity makes sperm.
Plant 2-sex model	An individual organism that makes pollen, with the concept covering events and structures from microsporogenesis through microgametogenesis.
Female:	
Animals and Plant 4-sex model	An individual organism that at sexual maturity makes eggs.
Plant 2-sex model	An individual organism that creates ovules/seeds/fruits, with the concept covering events and structures from megasporogenesis through megagametogenesis and beyond to the setting of fruit.
Mating:	
Animals and Plant 4-sex model	Interactions between egg- and sperm-forming individuals, which directly precede and promote fertilization.
Plant 2-sex model	Often focuses mainly on achievement of pollination and the interactions between an immature microgametophyte and stigmatic tissue of a sporophyte.
Fertilization:	
Animals and Plant 4-sex model	Union of an egg and sperm to make a zygote.
Plant 2-sex model	Often viewed as starting with pollination, and includes pollen tube growth towards the embryo sac and sperm delivery to achieve zygote formation.

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Under the plant two-sex model the attributes of "male" and "female" are altered so that they no longer apply just to the gamete-forming individuals (Table 1), but extend also to the sporophytes so that stamen are considered to be "male" organs, and pistils "female" organs (Dellaporta and Calderon-Urrea, 1993; Boavida et al., 2005; Nores et al., 2015; Charlesworth, 2016; Glick et al., 2016; Lankinen et al., 2016; Matsuhisa and Ushimaru, 2019; Placette, 2020); this is done even though sporophytes never make gametes (Mauseth, 2017). Thus the concepts of "male" and "female" used under the two-sex model are broadened so that both microsporangiate sporophytes and microgametophytes fall under this broader redefined term "male," and so that megasporangiate sporophytes and megagametophytes are covered by this new redefined term "female." In addition there are new functions assigned to these new concepts of "males" and "females" which are no longer limited to just gamete formation. The new "male function" is often described as the production of pollen, while the "female function" is the production of ovules, or seeds, or fruits, and typically both of these redefined functions are attributed to the sporophytic individuals (Carr, 2013; Carper et al., 2016; Harth et al., 2016; Kamath et al., 2017; Christopher et al., 2019). Also under the two-sex model, pollination is often described as a "mating system" (Willson and Burley, 1983; Etterson and Mazer, 2016; Lankinen et al., 2016; Malagon et al., 2019), even though it seems unlikely for a sexually immature microgametophyte to "mate" with the tissues of a sporophyte which itself never makes gametes. Thus, under the two-sex model, "mating" no longer requires the involvement of just gamete-forming individuals, nor does it have to lead to the immediate production of a zygote. Furthermore, the process of pollination is often regarded as being a part of "fertilization," thus extending the concept of "fertilization" to include events from pollination through actual zygote formation (Till-Bottraud et al., 2005; Castilla et al., 2016; Taiz and Taiz, 2017). Also under the two sex model there often is consideration of how

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some angiosperms are said to carry out "self-fertilization," by which it is implied that a so-called "hermaphroditic" sporophyte "mates" with itself in some fashion (Brewbaker and Natarajan, 1960; Barrett, 2002; Cruzan and Barrett, 2016; Grossenbacher et al., 2016), and so this one individual organism achieves zygote formation.

The sum of these redefinitions (Table 1) acts to largely obscure the angiosperm gametophytic individuals and their roles. They are subsumed under the new broader definitions of "male" and "female," they are not acknowledged to be independent "selves," rather "mating" and "fertilization" become processes the sporophytes carry out by themselves. Further, by referring to pollen in anthers and to the embryo sacs in ovules as the products of "male" and "female" sporophytes, and to how the pollen "fertilizes" the ovules (Jarne and Auld, 1993; Moore and Pannell, 2011; LoPresti et al., 2018), these gametophytes are often treated as mere gametes. This seems to deny these gametophytes a role as distinct organisms in the angiosperm alternation of generations? Thus the two-sex model obscures the angiosperm gametophyte generation, and redefines common terms in significant ways. Oddly, all of these terminological alterations seem to act to make the sexual reproduction done by angiosperms appear to be similar to that seen in animals, at least by placing emphasis on fertilization as the sole sexual reproductive process. Thus, under the two-sex model, the elegant and sophisticated plant alternation of generations life cycle is treated, in effect, as if it is just like the mundane and simplistic animal life cycle. Whether that is the intent or not is unclear, as seldom are these redefinitions acknowledged, let alone justified, in the literature.

Taiz and Taiz (2017) do note that while the plant two-sex model is technically incorrect as

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applied to angiosperms, they consider it to have the advantage of being simpler. However the simplification produced by these subtle redefinitions of terms under the two-sex model can also create some confusion when applied to angiosperms. For instance, when reference is made to a "male" angiosperm, this redefined "male" concept includes two organisms under that label. So is the intent to refer to the gamete-producing organism (*i.e.*, the microgametophyte), or to the sporophyte, or both? Is an angiosperm sporophytic "male," which never makes sperm itself, truly comparable to an animal male which does make sperm? When angiosperm sporophytes are described as carrying out "self-fertilization" does that mean we are to ignore any role of the micro- and mega-gametophytes in the process of fertilization; are they not their own "selves"? Is the "self-fertilization" said to be done by some angiosperm sporophytes supposed to be considered to be similar to the self-fertilization done by a bisexual fern gametophyte, or by a hermaphroditic animal individual? These sorts of reconceptualizations can place heavy burdens on those attempting to enter this area of study as it can require that each of these term's contrasting meanings has to be considered, and often a very careful examination of the context of their use has to be made to discern the intended meaning. However, those who are unaware that the two-sex model imposes these redefinitions may be unable to carry out such an examination. This dichotomy of terminology may also lead to the creation of potentially misleading comparisons based on the use of common terms which have very different meanings for animals versus plants under this plant two-sex model. Consider, for instance, the work of Till-Bottraud et al. (2005) in which plant male gametophytes are compared to animal sperm, which seems to assume that pollen are just gametes? Or, consider the comparison of "self-fertilization" said to be done by "hermaphroditic" angiosperm sporophytes, to the self-fertilization of truly hermaphroditic animals (Jarne and Charlesworth, 1993). If our goal is to make valid comparisons about aspects of sexual

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reproduction across taxa, the question must be asked as to whether this simple plant two-sex model is helping or hindering that effort?

In contrast the proposed plant four-sex model would define these terms in a manner that is largely similar to how these terms are used for animal sexual reproduction (Table 1), perhaps clarifying matters somewhat. Notice however that the four-sex model would consider only the angiosperm gametophytes to be male and female, and so directly comparable to male and female animals. The four-sex model would require us to regard the angiosperm sporophytes as having a type of sexual reproduction not seen in animals, and so we would have to recognize the angiosperm sporophyte as having a new pair of sexual reproductive states. Thus the four-sex model challenges us to alter our thinking. Which ever of these models best describes the reality of angiosperm sexual reproduction is a matter, therefore, for biologists to consider carefully.

POSSIBLE NEW PERSPECTIVES FROM THE PLANT FOUR-SEX MODEL.

A good model not only summarizes existing knowledge, but it also sets the framework for future questions. There have been many studies that have examined aspects of angiosperm sexuality and reproduction from within the context of the plant two-sex model. However many of these areas of study may benefit from the new perspective offered by the new four-sex model. Next just two examples of areas of study will be presented which, if put into the framework of an angiosperm four-sex model might open up some conceptual space for new thinking.

The current two-sex model portrays the angiosperm sporophyte as the parent of its

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sporophytic offspring (the embryo in the seed), which suggests we should view the flower as involving just these two generations. Under the four-sex model an angiosperm flower is a larger multigenerational community. As Mauseth (2017) notes, the parent sporophyte has the male and female gametophytes as its offspring, and these in turn have the next sporophyte which arises from the zygote they form as their offspring: Thus the new embryonic sporophyte in the seed is the grandchild of the initial sporophyte which bears the flower in which these two subsequent generations arise. The way in which the four-sex model permits us to view the gametophyte generation as individuals thus may alter our view of the individuals present over time in a flower into a three generational pageant. How the original sporophyte nurtures and supports in turn both their gametophytic offspring, as well as the way the gametophytes produce the endosperm, which their parent sporophyte supplies with nutrients, for the gametophytes' child (which is the original sporophyte's grandchild) becomes a very interesting set of issues. Many of these interactions may be seen as altruistic and may fall under kin selection (Nowak, 2006; Boomsma, 2007; Bourke, 2014; Dudley, 2015; Bawa, 2016; Placette, 2020), as they occur between related individuals of different generations. This view of multigenerational cooperation and selection in the flower would seem to be one that the four-sex model promotes, but which the current two-sex model seems to obscure as it does not fully recognize the gametophytes as players in this pageant.

As another example of how the four-sex model may contribute to new lines of investigation, consider the issue of genetic controls leading to sex cell determination. Under the two-sex model in angiosperms once the type of sporogenesis is determined the "male" or "female" sex is considered to also be established. Thus some studies of angiosperm sex determination focus mainly on the sporophytic stage (Moore et al., 2016a; Sandler et al., 2018; Nelms and

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Walbot, 2019). But under a four-sex model the issue may be shifted from determination of "maleness" and "femaleness" to what gene expressions are essential in the sporophytic tissues to achieve proper microsporogenesis and megasporogenesis, versus what gene expressions are needed in the gametophytes to ensure gamete formation which might well make use of different genetic controls compared to those used in the sporophyte. Indeed, some studies have noted that the development of the angiosperm gametophytes may use distinct systems of differentiation leading to gamete formation relative to the controls used for entry of some sporophytic cells into meiosis (Ranganath, 2003; Zhao et al., 2017). Hoffmann and Palmgren (2013) note that there are many pollen-specific gene expressions which are repressed in the sporophytic individual. In the context of the four-sex model we might wish to consider the different types of selection on the sporophytic and gametophytic organisms with regard to their production of sexual spores and gametes respectively. The current two-sex model, by obscuring the angiosperm gametophyte generation, seems to inhibit consideration of this sort of investigation. Furthermore, Pannell (2017), working under the two-sex model, suggests that the control of sexual determination seems oddly to be variable across plant groups as it shifts from being done by the gametophytes in many homosporous plants, to being done by sporophytes in many heterosporous plants including the seed plants. When viewed under the three- and four-sex models this apparent shifting may well vanish: In both homosporous and heterosporous plants the sporophyte has sexual determination in terms of which tissues will form its sporangia and so make sexual spores, while the gametophytes may have another determination process involved in their formation of gametes. Thus it would seem more proper under the three- and four-sex models to compare sexual determination in sporophytes to sporophytes, and sexual determination in gametophytes to gametophytes. While there may well be different details found between species, any selective constraints on sexual

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determination in gametophytes may well differ from selective constraints operating on sex cell determination in sporophytes. The two-sex model, by its tendency to combine sporogenesis and gametogenesis, may obscure these sorts of issues, while the four-sex model may promote such thinking. There are other issues relating to plant sexual reproduction for which the three- and four-sex models may provide new perspectives, and so may promote new lines of research.

IN CONCLUSION.

The key assumption of the current plant two-sex model is that there are indeed only two sexual reproductive states: male and female. Here that assumption has been questioned. The proposal is made that the sporophyte generation, by carrying out sporic meiosis, should be considered as an additional plant sexual reproductive state, or states, while the male and female reproductive states are assigned to the gametophytic individuals. This leads to there being three sexual reproductive states in homosporous plants, and four in heterosporous plants. Whether these new models of plant sexual reproduction account better and more clearly for the reality of the plant alternation of generations, and whether these new models provide productive new avenues for future investigation, is for the community of biologists to determine. But in doing so, we would be wise to be careful to accept plants as they are and avoid making them seem to be like animals; if our goal is to get to the truth then simplifications which obscure reality should be avoided. Adopting the three- and four-sex models of plant sexuality may also give us a wonderful insight to a view of life in which many eukaryotic species may have more sexual states than the mere two that we poor animals typically display. If these new models are accepted, we should share this wonderful view of expanded sexual reproduction with our students so that when they

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walk under a forest canopy, and reach out to touch the trees, they can realize that they are encountering beings truly distinct from animals, not just by being different physiologically and structurally, but also by being different sexually.

In closing, a quote from Emerson (1924; pg. 182) would seem appropriate:

"Finally, let me observe that, even though this missionary epistle to the brethren who dwell in darkness fail to convert them, it should at least afford them a somewhat unfamiliar point of attack. And, if their subsequent efforts result in my own conversion, I, at least, shall feel that I have not labored in vain."

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STM conceived and wrote this full article in all its parts, and is sole author.

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