Sex and Rosaceae apomicts

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Abstract This paper updates earlier reviews of polyploidy and reproductive biology in the Rosaceae, and does so with a focus on hybridization in relation to polyploidy and (facultative) gametophytic apomixis. Taking data mainly from tribe Maleae, it also seeks to point out evidence for a potential role for fertilization of infrequent unreduced gametes in diploid-diploid crosses in producing autoploids. Apomixis may originate in these autoploids, and spread as they cross with diploids and other polyploids.

Keywords Amygdaloideae; apomixis; hybridization; Maleae; polyploid; Rosaceae; Rosoideae

INTRODUCTION

Asexual reproduction doesn’t find a place in accounts of the panoply of plant reproductive diversity (e.g., Barrett, 2010), and on one level that’s as it should be, since key features of plant sexuality like self-incompatibility, dioecy, and wind pollination all serve to enhance outcrossing, and hence the genetic variability of offspring. Specializations achieving genetic clonality such as the various forms of vegetative reproduction tend to do just the opposite (Vallejo-Marín & al., 2010; Meloni & al., 2013). Nevertheless, there are many forms of asexuality (as apomixis in the wide sense of the early literature; Nygren, 1967; Asker, 1979), including not only vegetative reproduction but also agamospermy. Agamospermy is the asexual production of seeds (that is, the enclosed embryos are formed asexually), and this is the restricted sense of apomixis now current (Nogler, 1984; Asker & Jerling, 1992). Apomixis is used in this restricted sense of agamospermy in what follows. Apomixis can, in turn, be either sporophytic (embryos develop directly from somatic cells, as in Citrus L.) or gametophytic (meiotically unreduced female gametes develop parthenogenetically into embryos). In the latter case, unreduced female gametophytes develop either from the megaspore mother cell (diplospory), bypassing or cancelling the effects of a first, reductional meiotic division, or else from one or more somatic cells of the nucellus (apospory). In both kinds of gametophytic apomixis the critical factor is that the apparatus of sexual reproduction is modified, but not eliminated (Fig. 1A). Moreover, pollination may still be required if successful seed-set depends on endosperm fertilization (pseudogamy; cf. box 1 in Hörandl, 2018). The modifications of gamete development and gamete fate just described are themselves subject to variation. For example, many Rosaceae and most Maleae produce very large numbers of flowers on an individual. As discussed below, developmental accidents occur, and there may be predispositions for certain such departures from patterns typical for a given taxon. For example, a tetraploid gametophytic apomict may produce both unreduced and reduced gametophytes, in the same or different ovules of the same flower, or in different flowers (similarly, in octoploid Fragaria ×ananassa (Weston) Duchesne ex Rozier; Leszczuk & al., 2018). Among the hundreds or thousands of flowers on a single individual these gametes, reduced or unreduced, in turn, may or may not develop parthenogenetically into an embryo. Each aspect of gametophytic apomixis, meiotic reduction in chromosome number and parthenogenesis, may thus be facultative (Fig. 1).

In pseudogamous gametophytic apomixis, fusion of a sperm nucleus may occur not only with polar nuclei (to form endosperm) but also with an egg nucleus, either reduced or unreduced, resulting in a biparental embryo (BII or BIII hybrid), often with a euploid increase in ploidy (Fig. 1B, C). This is what has been called facultative, or “leaky” apomixis (cf. Baker & Cox, 1984; Ludwig & al., 2013; Vallejo-Marín & Hiscock, 2016). If parthenogenesis in gametophytic apomixis is actually facultative, and syngamy is not completely eliminated, then asexuality ceases to be an evolutionary dead end (Marshall & Weir, 1979). The tempo of genetic variation is merely reduced, while potentially successful genotypes are mass-produced and dispersed across the landscape; Clausen’s “Model-T” analogy for partial apomixis (Clausen, 1954). Where these genotypes arise from wide crosses they may benefit from the resulting heterozygosity. In the extreme case, such wide crosses may involve different species, or even genera, in which case they are interpreted as representing hybridization. In such cases meiosis may be disturbed, resulting in sexual sterility. Sterility may, in turn, select for the expression of gametophytic apomixis.

Similarly, the Rosaceae (for the tribal classification used here, see Potter & al., 2007; Reveal, 2012) appear to be less well represented in recent discussions of the relationships between hybridization, polyploidy, and the occurrence of gametophytic apomixis than might be warranted, given the frequency of these phenomena, the size of the family, the number of economically important genera the family contains, and the research done on some of these genera, whether economically
important or not. In what follows, we draw upon earlier reviews (Dickinson & al., 2007; Talent & Dickinson, 2007c; cf. Majeský & al., 2017), augmented by data from the online Apomixis Database (Højsgaard & al., 2014), in order to focus particularly on the possible role of hybridization in the origin and spread of apomixis, and the process of polyploidization. In their survey of plant families exhibiting frequent hybridization, Ellstrand & al. (1996) found that in five Floras with biological data the Rosaceae were among the six families in which hybrids were concentrated. Rosaceae exhibit all three of the traits, one or more of which are found in each of these families: predominant perenniality, outcrossing, and a form of reproduction capable of transmitting hybrid genotypes intact (e.g., gametophytic apomixis, vegetative reproduction, heterogametic meiosis). In the Rosaceae, fertilization of unreduced female gametes produced as part of the syndrome of gametophytic apomixis, and as a result of crosses between species and ploidy levels, appears to be the key component in the formation of agamic complexes in this family (Campbell & al., 1991).

**Hybridization in the non-Maleae apomicts.** — In Rosaceae subfamily Rosoideae the combination of gametophytic apomixis and polyploidy has been well known as an explanation for the taxonomic complexity of large genera like Alchemilla L. (summarized by Majeský & al., 2017) and Potentilla L. (tribe Potentilleae; Johri & al., 1992; Dobeš & al., 2015), and Rubus L. (tribe Rubaeae; Kollmann & al., 2000). In Alchemilla, molecular phylogenetic studies (Gehrke & al., 2008) suggest that past hybridization is largely responsible for the incongruence between data from plastome and nrITS loci. Present-day hybridization, however, appears to be rare, because reproduction is by autonomous (rather than pseudogamous) gametophytic apomixis, and diploids are absent (Gehrke & al., 2008). Gametophytic apomixis in Alchemilla appears to be responsible for the perpetuation of hybrid, polyploid microspecies (Gehrke & al., 2008). Sepp & al. (1998) used multivariate methods to test the distinctness of 23 of these microspecies that are widespread in Estonia, and concluded that only three were morphologically distinct. One of their ordinations provided Coyne and Orr with support (fig. 1.2 in Coyne & Orr, 2004) for their caution that claims for the distinctness of agamic species may be statistically untenable. Possibly a segregate genus, Aphanes (Gehrke & al., 2008, contra; McNeill & Ertter, 2015, pro) has been shown to produce occasional unreduced female gametophytes (Izmailow, 1999). In other Potentilleae, hybridization is similarly suspected.

Fig. 1. Aspects of facultative gametophytic apomixis, illustrated with examples from crosses between Rubus taxa made by M. Rozanova (1934, 1938, 1946; cited in Mavrodiev & Solts, 2001). A, Normal gametophytic apomixis comprises formation of unreduced female gametes and their parthenogenetic development into progeny genetically identical to their mother. B, Facultative gametophytic apomixis may occur when an apomictic mother also produces, within the same or different flowers, reduced as well as unreduced megagametophytes (*). Meiotically reduced female gametes (from diploid R. idaeus L., JJ; 2n = 14) and male gametes (from the autotetraploid hybrid R. idaeus × R. caesius var. turkestanica Regel, JICCCC; 2n = 42) fuse to yield the tetraploid BIII hybrid (JICC; 2n = 28). C, Facultative gametophytic apomixis may also occur when, in ovules of one or more flowers, an unreduced female gamete from an apomictic mother is fertilized instead of developing parthenogenetically (**). In this case, the mother is the triploid hybrid R. idaeus × R. caesius var. turkestanica (JCC; 2n = 21). The unreduced female gamete (JCC) was fertilized by a reduced male gamete (J) from R. idaeus to yield the tetraploid BIII hybrid (JICC; 2n = 28). Note that the JCC allotetraploid was also formed in Rozanova’s crosses by unreduced female gametes (JJ) of R. idaeus having been, Rozanova inferred, fertilized by reduced male gametes (CC) from the Permian race of R. caesius L.; in other words failure of meiotic reduction of female gametes is rare in diploid R. idaeus, but evidently may occur (*; Mavrodiev & Solts, 2001).
Gametophytic apomixis in *Fragaria* L. (also Potentilleae), however, appears to be evident only following hybridization (Asker & Jerling, 1992; Dziadczyk & al., 2011; Leszczuk & al., 2018), although even then evidence for its occurrence has been seen as equivocal (Nosrati & al., 2010; Dobeš & al., 2015). Confirmation of the hybrid status of several *Fragaria* species by Kamneva & al. (2017) using hyb-seq data did not involve checking the occurrence of gametophytic apomixis. In tribe Corulieae polyploidy is widespread, and allopolyploidization events have been inferred from molecular data in the *Geum* L. group (Snedmark & al., 2003, 2005). Gametophytic apomixis is asserted to be present in *Geum montanum* L. (Krch, 2002).

*Rubus* is possibly the genus most notorious in the Rosaceae for taxonomic complexity resulting from reticulate evolution involving gametophytic apomixis and polyploidization. In part this is because of the difficulties attendant on its frequently biseriate habit, but also because of the accumulation of synonyms for an already vast number of species (just European *R.* subg. *Rubus* comprises four extant diploids, and 744 named polyploid, apomictic species; Sochor & al., 2015). As described by Mavrodiev & Soltis (2001), hybridization studies in *Rubus* date back to the demonstration by Lidforss (1905, 1907, 1914; cited in Mavrodiev & Soltis, 2001) of extensive morphological variation in the offspring of hybrids. Mavrodiev & Soltis (2001) then recount in detail the hitherto little known work of the Russian researcher, Maria Rozanova (1934, 1938, 1946; cited in Mavrodiev & Soltis, 2001). Rozanova’s crossing experiments using diploid *R. idaeus* L. and two races of tetraploid *R. caesius* L., and her interpretation of their results, demonstrated both aspects of facultative gametophytic apomixis: the occasional formation of unreduced female gametophytes by the diploid species (Rozanova, 1934, 1938, cited in Mavrodiev & Soltis, 2001), and the formation and syngamy of reduced gametes from both the diploid and the hexaploid hybrid (B1b hybrid) between the two species (Fig. 1B). More recently, apomictic diversification in *R. subg. Rubus* has been shown (by comparing plastome and nuclear loci) to be driven by pollen flowing from tetraploid, apomictic *Rubus* species to species that are sexual tetraploids (Sochor & al., 2015; Šarhanová & al., 2017). Flow cytometric analyses of seeds (i.e., gametophytic apomixis and polyploidization) have been demonstrated (Kerr, 2004), but nothing is known about the breeding system in *Cliffortia*, apart from its monocoe and wind-dispersed pollen (Fellingham & Linder, 2003). The occurrence of gametophytic apomixis is nevertheless suggested by the wide distribution, abundance, and documented establishment from seed of species in which male flowers are observed only rarely (Whitehouse, 2002). The South American genus *Polylepis* Ruiz & Pav. (ca. 30 spp.) is similar in that polyploidy occurs, sequence data document hybridization, and gametophytic apomixis is suspected but has not been demonstrated (Kerr, 2004; Kessler & al., 2014; Segovia-Salcedo, 2014). In both *Cliffortia* and *Polylepis* the small size of the flowers and fruits, as well as the indehiscence of the latter, appear to have impeded both embryological and flow cytometric investigations of the breeding system.

Rosaceae subfamily Amygdaloideae comprises seven tribes, of which three contain genera with only one to a few species, and in which polyploidy does not appear to be present (Dickinson & al., 2007; Mabberley, 2008). Three more tribes each contain only one genus with 20 or more species, these genera being ones in which hybridization or polyploidy is present. The most striking example of these is *Prunus* L. (tribe Amygdaleae; cherries, plums, peaches, apricots, almonds; Chin & al., 2014). Sweet cherries (*Prunus avium* (L.) L.) are diploids while sour cherries (*Prunus cerasus* L.) are allotetraploids that resulted from natural hybridization involving unreduced female gametes of *P. avium* in crosses with the autotetraploid *P. fruticosa* Pall. (Dirlewanger & al., 2009). Unreduced gametes have apparently also played a part in forming at least one other allotetraploid, *P. ×gondouinii* (Poit. & Turpin) Rehder (Tavaud & al., 2004; Dirlewanger & al., 2009). Gametophytic apomixis does not, however, appear to be a factor in the cherries. Plums are somewhat similar in comprising both diploids and hexaploids (Old World cultivated plums), but without gametophytic apomixis being known; almonds, apricots, and peaches are all diploid (Robertson, 1974). Zhao & al. (2016) have hypothesized an allopolyploid origin for the *Prunus* species with racemose inflorescences (a group of mainly tetraploids that does not include any of the domesticated species) that posits four independent fertilizations of unreduced female gametes in an extinct ancestor. Some other, dry-fruited Amygdaloid genera in other tribes are relatively large (e.g.,...
Hybridization in the Maleae apomicts. — The presence of gametophytic apomixis and the formation and fertilization of unreduced gametes in the non-Maleae Amygdaloideae and in the Rosoideae suggest that these processes were central to the origin of the Malus Mill. genome (and, implicitly, the origin of all Maleae with $x = 17$) from a whole-genome duplication in an $x = 9$ ancestor resembling Gillenia Steud. (Evans & Campbell, 2002; Velasco & al., 2010 – their fig. 3 explains loss of one chromosome). Well-supported phylogenies of the Rosaceae based on next-generation sequence data from plastome (Zhang & al., 2017) and nuclear (Xiang & al., 2017) loci support the phylogenetic relationships central to this hypothesis, falsifying earlier hypotheses that explained the origin of the Malineae by allopolyploidization (Sax, 1931; Stebbins, 1950; Phipps & al., 1991). These phylogenies also document the transition, early in the evolution of the tribe, from dry, dehiscent fruits with winged seeds (Gillenia, Kagenecka Ruiz & Pav., Lindleya Kunth, Vauquelinia Corrêa ex Bonpl.) to fleshy ones derived from hypanthial ovaries (the remaining Maleae, apart from Dichotomanthes Kurz; Robertson & al., 1991; Potter & al., 2007; Xiang & al., 2017). This transition is, equivalently, one from wind dispersal to vertebrate frugivore-mediated dispersal.

Sorbus L. sensu lato. — Hybridization in the Maleae has been recognized, if not widely, since the pioneering work of Hedlund (1901; in German) on Scandinavian Sorbus s.l. Hedlund inferred hybridity from the combination of morphological intermediacy (notably between parents with either simple or pinnate compound leaves), the quality (stainability) of pollen, and the results of pollination experiments (Hedlund, 1901; Gustafsson, 1947). Subsequently, chromosome counts demonstrated that species with good pollen were diploids, whereas the hybrids with poor pollen were apomictic polyploids (Liljefors, 1934, 1953, 1955). Liljefors's work established the utility of genomic formulae for understanding hybridization in European Sorbus s.l.; this is especially evident in the work on Sorbus s.l. in the British Isles and Europe (McAllister, 2005; Rich & al., 2010).

Until recently, Sorbus has been interpreted as a large and morphologically diverse genus, Sorbus s.l. However, both morphological data (Robertson & al., 1991; Zheng & Zhang, 2007) and molecular data (Lo & Donoghue, 2012) support dismembering a polyphyletic Sorbus s.l. into two clades, Cormus Spach and Sorbus, and Aria (Pers.) Host, Torminalis Medik., and Chamaemespilus Medik., plus an Aria × Sorbus hybrid, Micromeles Deene. (Lo & Donoghue, 2012). Interpreted in this way, each of the first five genera consists of a single diploid species (Sennikov & Kurotto, 2017). Only Aria also comprises numerous polyploid species with gametophytic apomixis. The rest of Sorbus s.l. consists of 144 bi- or tri-generic hybrids almost all of them polyploid, exhibiting gametophytic apomixis, and all involving Aria (table 1 in Sennikov & Kurotto, 2017). The Aria complex thus includes diploids, allotriploids and -tetraploids, and at least two autotetraploid species (Liljefors, 1955). Recent studies in the Balkans (Hajrudinović & al., 2015) demonstrate the key role, in mixed local populations of cytotypes, of pollen flow from apomictic, tetraploid Aria and Aria × Sorbus hybrids to Aria sexual diploids. Comparable work remains to be done on the Aria and Sorbus s.str. of eastern Asia and (Sorbus s.str. only) North America. It is noteworthy that the Aria × Torminalis hybrid, Karpatiosorbus eximia (Kovanda) Sennikov & Kurotto (syn. S. eximia Kovanda) that was previously thought to be a diploid apomict is in fact triploid (Vit & al., 2012). This removes a possible objection to the argument that pseudogamous gametophytic apomixis, polyploidy, and self-fertility are intimately linked (Talent, 2009).

Amelanchier Medik. — This genus has a North Temperate distribution, but exhibits the greatest species diversity in North America so that almost all recent systematic studies have focused on this continent. Early studies of Amelanchier noted the association between anthropogenic disturbed habitats and the occurrence of intermediate forms inferred to be of hybrid origin (Wiegand, 1935). Since then, flow cytometric surveys of ploidy level have demonstrated that there are only 10 New and 3 Old World diploid taxa (Burgess & al., 2015). Tetraploids are considerably more frequent, while triploids are comparatively rare (Burgess & al., 2014). Molecular studies have demonstrated that hybridization in Amelanchier principally involves tetraploids crossing with diploids by means of their pollen wherever the cytotypes co-occur; tetraploids produce seed by gametophytic apomixis (Burgess & al., 2014; Cushman & al., 2017). By comparison, instances of diploid-diploid hybridization are rare (Burgess & al., 2015). These authors used quoted, informal names for microspecies (Burgess & al., 2014).

Aronia Medik. — The molecular phylogenies obtained by Guo & al. (2011) and Lo & Donoghue (2012) suggest that this small North American genus is distinct from mainly Asian Photinia Lindl. and warrants generic recognition. Triploid hybrids between diploid A. melanocarpa (Michx.) Elliott (pollen parent) and tetraploid A. arbutifolia (L.) Pers. (seed parent) have been made (Brand, 2010). Gametophytic apomixis in tetraploid A. melanocarpa and A. arbutifolia has been inferred from RAPD data by (Persson Hovmalm & al., 2004) as well as from examination of batches of seedlings and the fertility of triploids (Brand, 2010).

Chaenomeles Lindl. — Five species are known from eastern Asia, and in cultivation (Gu & Spongberg, 2003). Three species are known to be diploid, as are most cultivars; one cultivar is tetraploid (Weber, 1964; Rumpunen & al., 1998). Interspecific and intergeneric hybrids are known. Embryological evidence for gametophytic apomixis (and nucellar embryo) has been reported for cultivated C. japonica (Thunb.) Lindl. ex Spach (Daskalova, 2005).

Cotoneaster Medik. — Data from phylogenetic and flow cytometric analyses are accumulating for at least some of the approximately 400 species in this genus (Li & al., 2014; Ma & al., 2015; Mansour & Sliwinska, 2017). Species of Cotoneaster for which data are available are predominantly (approximately 70%) tetraploid, 15% triploid, 10% diploid, and 4%–5% penta–hexaploid (Fryer & Hylmö, 2009: 25; Rothleutner & al., 2016). Only the relatively few diploid species are widely known to hybridize. McAllister has identified five diploid species where their phenetic similarity to one or
more tetraploid species is so great as to suggest that the latter are autotetraploids in which gametophytic apomixis has arisen independently (H. McAllister, pers. comm. December 2017). Crosses between ploidy levels evidently occur. Diploid C. glaucophylloides Franch. has been shown to be the maternal parent of an unnamed tetraploid hybrid, while tetraploid C. dielsianus E.Pritz ex Diels was shown to be the pollen parent (Li & al., 2017). Although flow cytometry was used to obtain the ploidy levels of individual plants in the study, seeds were apparently not studied, so that it is impossible to tell at this point whether the hybrid was formed by means of a triploid bridge, or whether it arose directly by fertilization of an unreduced female gamete of the diploid parent by a reduced sperm from the tetraploid one. Flow cytometric analyses of plants from five single-cytotype and five mixed-cytotype populations of C. integerrimus Medik. in Bosnia and Herzegovina showed tetraploids predominated (85%); diploid (10%), triploid (3%), and pentaploid (2%) individuals were found only in the mixed-cytotype populations (Mahmutović-Dizdarević & al., 2015). Only the diploids produced seeds sexually. Gametophytic apomixis was responsible for almost all seed production by polyploids.

**Crataegus L.**—In hawthorns the occurrence of hybridization has been controversial and was either dismissed as infrequent or described as being at the root of all taxonomic problems in the genus. Ample evidence of this, as it relates to North American workers during the first half of the 20th century, is provided elsewhere (Dickinson, 1999). In Europe, as with Hedlund’s work on Sorbus s.l. (Hedlund, 1901), conspicuous morphological intermediacy between two relatively divergent European Crataegus species was sufficient for Raunkiær (1925) to describe (in Danish) hybridization between C. monogyna Jacq. and C. laevigata (Poir.) DC. Not surprisingly, this publication had little impact in North America. Instead, data on pollen infertility (Standish, 1916) and polyploidy (Longley, 1924; Moffett, 1931) were obtained; gametophytic apomixis was only inferred from its occurrence in other taxa, and the patterns of variation documented in two studies by H.W. Rickett (1936, 1937; compare fig. 3 in Dickinson & Campbell, 1991).

Crataegus hybridization was not studied quantitatively until work in Britain on hybrids between C. monogyna and C. laevigata by Bradshaw (1953, 1971) and Byatt (1975). In western North America, studies were made of naturalized C. monogyna, native C. suksdorfii (Sarg.) Kruschke, and their hybrid by Love & Feigen (1978). Data on ploidy level variation in several European and North American species, including C. monogyna and C. laevigata (both shown to be diploid), came from studies by Gladkova (1968), Byatt (Byatt & Murray, 1977), and Muniyamma & Phipps (1979b). Wells & Phipps (1989) documented the diploidy of the eastern North American hybrid between naturalized C. monogyna and native C. pumila Jacq., using morphometric and flavonoid data to establish its intermediacy. Both North American hybrids have since been confirmed using DNA sequence data from bipaternally and maternally inherited loci, as well as having been shown to be diploid using flow cytometry (Christensen & al., 2014).

In North America, these two hybrids are the only ones known that are diploids, having formed as the result of fusions of reduced gametes from two diploid parents. As noted earlier by Longley (1924) and Moffett (1931), however, polyploids are abundant in North America (Muniyamma & Phipps, 1979b; Talent & Dickinson, 2005). When gametophytic apomixis was finally documented for the first time in Crataegus, it was in triploid C. pruinosa (H.L.Wend.) K.Koch (Muniyamma & Phipps, 1979a). Since then, additional European and North American species (references in Dickinson & al., 1996; Dickinson, 1999) have been shown to exhibit gametophytic apomixis as well as being polyploid. Flow cytometry increased the number of species for which ploidy level data are available (Talent & Dickinson, 2005), and made possible comparison of embryo and endosperm ploidy levels (Talent & Dickinson, 2007a, b; Lo & al., 2013) as a means of documenting whether seed had been produced sexually or by apomixis (Matz & al., 2000; Talent & Dickinson, 2007b; Kolarčík & al., 2018). The seed scan data reported by Lo & al. (2013: fig. 3) demonstrated that allotriploid and allotetraploid individuals in C. sect. Douglasia Loudon set seed apomictically, evidently employing either one or both reduced sperm nuclei from self pollen to do so. Pollen stainability in tetraploid C. douglasii Lindl. mostly exceeds 80% (Dickinson & al., unpub. data).

The extent to which these results informed subsequent work, however, was uneven. On the one hand, models by means of which hybridization might be inferred from phenetic data were developed (Phipps, 1984) but have not been pursued except as noted. On the other hand, however, hard evidence of hybridization involving apomictic polyploids was lacking, so that it remained easy to dismiss it as infrequent (Phipps, 2005; Haines, 2011). New species from hitherto poorly explored areas of western North America were described without reference to the possibility they could be either apomictic or of hybrid origin (Phipps, 1990; Phipps & O’Kennon, 1998, 2002). A notable exception, however, is the analysis of Crataegus ser. Aestivales (Sarg.) Rehder, where ample morphometric evidence demonstrates the intermediacy of triploid C. rufula Sarg. relative to the diploid species, C. aestivalis (Walter) Torr. & A.Gray and C. opaca Hook. & Arn. (Phipps, 1988; Talent & Dickinson, 2005). In this case, the hypothesis of a hybrid origin for C. rufula, possibly involving apomixis, was clearly advanced, even in the absence, at that time, of data on ploidy-level variation within the series. Another is the description of C. tenior J.B.Phipps (Phipps, 2013), where the need for molecular data with which to evaluate this possibility was recognized.

Molecular evidence for hybridization, and for the occurrence of both autoploidy and allopolyploidy, came first from microsatellite data (Lo & al., 2009). Subsequently, data on persistent introindependent ITS2 ribotype diversity in polyploid (but not diploid) hawthorns from western North America have been used to demonstrate hybridity by revealing the co-occurrence of ribotypes from eastern and western North American clades (Zarrei & al., 2014; cf. Feng & al., 2007; Sochor & al., 2015). These ITS2 results also corroborated the earlier evidence for the occurrence of autoploids in Crataegus. Our studies of North American species so far have shown little involvement between the diploid hybrids and the polyploids. Rather, they suggest that some widespread tetraploids with facultative
gametophytic apomixis hybridize with diploids, including diploids from other taxonomic sections of the genus, to produce B_{11} triploid hybrids also with gametophytic apomixis that may themselves participate in further crosses between cytotypes, to form both B_{22} and B_{11}B_{22} hybrids (fig. 5 in Talent et al., 2009). Hybridization in which both parents are polyploids with facultative gametophytic apomixis also appears to occur. New data from European species are more limited, and document the occurrence of gametophytic apomixis, and both diploid-diploid and diploid-tetraploid hybridization as well as apparently more complex situations (Dickinson et al., unpub. data).

The population-level sampling in the Lo et al. (2009) study demonstrated a clear contrast between diploids and tetraploids with respect to the geographic extent of their ranges. Subsequently, wider geographic distributions in allopolyploids relative to diploids were found to be correlated with greater ecological amplitude in the polyploids (Lo et al., 2013; McGee et al., 2014; Coughlan et al., 2017). Coughlan et al. (2014) showed that these features of the polyploids also correlated with increased relative investment in fruit biomass compared to seed biomass, suggesting a contrast between features favoring dispersal (in polyploids) versus establishment (in diploids). What we now know about hybridization in these hawthorns (Zarrei et al., 2014) suggests that this contrast in fruit composition in the black-fruited taxa arose from apomixis and polyploidy having been introduced by crosses with widespread eastern North American apomictic tetraploids with much larger, red fruits (Coughlan et al., 2014, 2017).

The contrasting patterns of morphological variation associated with gametophytic apomixis and sexuality have been documented (Dickinson & Phipps, 1985; Dickinson, 1986; Dickinson & Campbell, 1991; Lo et al., 2010a, b). Crataegus (like some other large Malae genera, e.g., Amelanchier and Sorbus) may flower and fruit abundantly, especially in alternate (or more widely spaced) years (Phipps & Muniyamma, 1980; Dickinson, 1985). In Crataegus this is arguably related to internal, physiological responses to environmental factors (water, nutrient availability) in habitats where soil fertility and precipitation may be limited, or herbivory is an issue. Escape from seed predators (Kelly & Sork, 2002) seems a less likely explanation, given that Crataegus seeds are enclosed in massive pyrenes and can remain viable for years in the soil (Brinkman, 1974; Dickinson, 1985; Young & Young, 1994). When frugivore-mediated seed shadows intersect with suitable sites (erosion surfaces, openings on forest margins, abandoned or poorly managed agricultural land; Phipps & Muniyamma, 1980; Dickinson, 1985; Phipps & al., 2003) the result may be large, local, more or less even-aged colonies (topodemes) of genetically similar individuals (Lo et al., 2010b). Such colonies, if they comprise individuals potentially all derived from selfed or apomictic seeds of a single polyploid mother may give the appearance of populations of interbreeding individuals. When these individuals also share some morphological novelty because their parent was an apomict or selfing hybrid this impression may be even stronger, especially if more than one such genotype occupies a single site. Depending on the extent to which sampling occurs within such topodemes, the individuals sampled may appear as phenetic clusters, albeit clusters representing only single geographic localities (fig. 3 in Dickinson & Phipps, 1985; Dickinson, 1986). Because both apomixis and selfing restrict opportunities for crossing, even though topodemes like these may be more or less even-aged, when more than one distinct genotype is present it might appear that two or more distinct taxa are sympatric. It can be argued that many of the narrowly circumscribed, often tetraploid species with restricted geographic ranges that were described by earlier workers and some modern ones may represent situations like these (Dickinson & Phipps, 1985; Dickinson, 1986, 1999; Dickinson & al., 1996, 2007, 2008).

The extent to which Crataegus species correspond to phenetic clusters (cf. Coyne & Orr, 2004; Rieseberg et al., 2006) varies. The first phenetic analysis of hawthorns found three clusters in data from Ontario C. ser. Pruinosae (Sarg.) Rehder that each comprised several taxa that had been identified a priori (Sinnott & Phipps, 1983). However, a subsequent floristic treatment (Phipps, 2015) recognized two of these clusters (or their subclusters) as accepted taxa (species or varieties). The third cluster proved to comprise several taxa recognized in the later treatment. As described above, topodeme samples (including sympatric pairs) of triploid and tetraploid Ontario C. crus-galli L. s.l. formed distinct phenetic clusters (Dickinson & Phipps, 1985; Dickinson, 1986), but this work also made it clear that more or less arbitrary choices about sampling (frame, method), choice of descriptors and their measurement scales, resemblance function, and sorting algorithm can have huge effects on clustering results (Podani & Dickinson, 1984), and hence the interpretation of phenetic clusters as part of the evidence for or against species status. Nevertheless, the results from phenetic clustering can be instructive. Some analyses of C. sect. Douglasia make use of samples emphasizing taxonomic and geographic coverage, rather than population structure (fig. 9b in Dickinson et al., 2008; Dickinson et al., unpub. data). In these, the contrast between individuals with 10- and 20-stamen flowers dominates the analysis, and phenetic clusters may comprise individuals and taxa that are readily distinguishable by other criteria (other morphological descriptors, geographic distribution, ploidy level, etc.). Some of these analyses (Dickinson et al., unpub. data) include several species segregated from the widespread C. douglasii Lindl. that have been shown to be apomictic, polyploid intersectional hybrids (Zarrei et al., 2014). These entities fail to form distinct phenetic clusters and instead make up part of the large clusters that include C. douglasii evidently because they are all tetraploid and share the 10-stamen trait, while at the same time they are differentiated from C. douglasii in only minor ways (Dickinson et al., unpub. data).

Eriobotrya Lindl. — Loquat hybrids are being investigated for their horticultural value (Wang et al., 2017). Chromosome counts from root tips of open-pollinated seedlings of 21 cultivars demonstrated triploids, tetraploids, and pentaploids at a cumulative rate of 0.68% in this otherwise diploid genus of 15–20 species (Guo et al., 2007; Badenes et al., 2009). These results suggest that unreduced gametes are formed occasionally, and are fertilized, even if gametophytic apomixis is not known to occur.
**Malus Mill.** — Much of the recent research on this genus has focused on the origin of the cultivated apple, *M. pumila* Mill., a self-incompatible sexual diploid that originated in Central Asia (Nikiforova & al., 2013). Hybridizations at the diploid level have played a major role in the evolution of this species and elsewhere in the genus (the crabapples; Volk & al., 2015). In North America the taxonomy of the native crabapples has been a challenge (Dickson, 1995, 2015), as has concern about introgression between the exotic crop species and native species (Dickson & al., 1991; Kron & Husband, 2009). The North American crabapples in *M*. sect. *Chloromeles* (Rehder) Likhnos form an agamic complex comprising diploids, triploids, and tetraploids (Dickson, 2015), and Kron & Husband have shown that at their study site (a natural population of *M. coronaria* (L.) Mill. with abundant feral *M. pumila*) tetraploid *M. coronaria* mothers produced as many seed with *M. pumila* pollen as they did with conspecific pollen. Not only that, open-pollinated seeds of the *M. coronaria* mothers, analyzed by flow cytometry, comprised tetraploids (57.4%, sexual or apomictic), diploids (7%, apomictic), hexaploid or octaploid (7.4%, sexual), and triploid or pentaploid hybrids (27.5%). A flow cytometric survey of a wide range of flowering crabapples (Ranney & al., 2004) demonstrated a similar diversity of reproductive pathways in open-pollinated seeds. Another outcome of the economic importance of the crop species and awareness of the role of unreduced female gametes in the origin of the Malineae is the study by Considine & al. (2012) in which the authors carried out crosses between seven diploid *Malus* populations, four of *M. pumila*, one of *M. prunifolia* (Willd.) Borkh., and one each of two hybrids, *M. baccata* (L.) Borkh. × *M. pumila*, and *M. baccata × M. prunifolia*. These authors analyzed a total of 27,542 viable F1 seedlings, and found that one percent of the seedlings were not diploid, but rather triploid (0.199%), tetraploid (0.052%), or aneuploid (0.778%). Microsatellite genotyping of the seedlings enabled the authors to determine that unreduced eggs contributed only to triploid and tetraploid offspring, whereas unreduced sperms were responsible for triploid, tetraploid, and aneuploid offspring. Considine & al. proposed an alternative model for the origin of the x = 17 Malineae by aneuploidization involving formation of a first meiotic division restitution nucleus, as in diplospory, but do not appear to have considered that, in the Rosaceae, apospory is the predominant means by which unreduced eggs develop. Nevertheless, their data show that, in diploid apples, unreduced gametes are produced by whatever means at a (very) low frequency, and this could be the origin of the original autotetraploids that appear to be implicated in the origin of agamic complexes in the Rosaceae, as in *Aria*, *Cotoneaster*, *Prunus*, *Sorbus* s.str., and possibly *Crateagus*.

**Pyrus L. and Pyraria A.Chev.** — Species of *Pyrus* are diploid (Zielinski & Thompson, 1967), but cultivated varieties of *P. communis* L. include triploids and tetraploids (including the material in which the characteristic Malaece shift from self-incompatibility (in diploids) to -compatibility (in spontaneous autotetraploids) was demonstrated; Lewis, 1947). The introduction and widespread use of *P. calleryana* DeCne. cultivars as ornamental street trees in North America and elsewhere has led to recognition of their invasiveness (Culley & Hardiman, 2007). Responses to this problem include developing triploid cultivars that have proven to be largely sterile; even some of these cultivars have, however, shown that at very low frequencies they nevertheless produce viable offspring, some of them either by fertilization of unreduced female gametes, or by parthenogenetic development of an unreduced female gamete (Phillips & al., 2016). Elsewhere, efforts are underway to obtain *Pyrus* haploids for use in breeding programs by inducing apomixis by pollination from other Malaece genera such as *Chaenomeles* (Dolmatov & al., 2013). The triploid hybrid between *P. communis* and *Aria* is called *Pyraria ×irregula-ris* (Münchh.) C.A.Wimm. (Postman, 2011; syn. *Sorbarpyrus auricularis* (Knoop) C.K.Schneid.; Sennikov & Kurtto, 2017). Its seeds are sterile, and propagation is exclusively by means of grafting, usually onto *P. communis* (Hoff, 2007), so the reason for discussing it here lies in the evidence it provides for fertilization of unreduced *Pyrus* female gametes by reduced sperm of diploid *Aria* (Sax, 1929). Others (e.g., Grevtzoa, 1978) have similarly associated the production of unreduced female gametophytes with hybridization. Recent phylogenetic analyses of *Pyrus* (Zheng & al., 2014; Korotkova & al., 2017; Wu & al., 2018) refer to variation in ploidy level, and especially hybridization, as underlying the complexity of species-level taxonomy in this genus. Zheng & al. (2014) included a triploid accession of *P. ussuriensis* Maxim. in their sample, suggesting again that unreduced female gametes are also produced and can be fertilized at least occasionally in species of *Pyrus* other than *P. communis*, even if gametophytic apomixis itself is unknown.

DISCUSSION

Recent discussions of hybridization and polyploidy (Renny Byfield & Wendel, 2014; Soltis & al., 2014; Yakimowski & Rieseberg, 2014) have explored the great biological significance of these two phenomena in plants. However, study of these phenomena in the Rosaceae suggests that it is important that we not neglect the way they are frequently linked causally with gametophytic apomixis and the fertilization of unreduced female gametes. The importance of unreduced gametes and their fertilization for the origin of polyploidy has been known for some time (reviewed by Harlan & De Wet, 1975; cf. De Storme & Mason, 2014), notably but by no means exclusively in the literature related to crop plants (Carpuito & al., 2003). Despite the existence of the literature on gametophytic apomixis, some explorations of the role of unreduced gametes in the genesis of polyploidy resolutely ignored the way in which apomicts regularly produce unreduced gametes that may be capable of being fertilized by sperm from self or non-self pollen (conspecific or not) (Bretagnolle & Thompson, 1995; Ramsey & Schemske, 1998, 2002; Ramsey & Ramsey, 2014). A notable exception is the recognition that both polyploidization and gametophytic apomixis involve production of unreduced gametes by Husband & al. (2013), but these authors fail to make the connection between fertilization of unreduced female gametes and polyploidy in apomorphic Asteraceae, Poaceae, and Rosaceae.
Harlan & De Wet (1975) characterized three possible origins of polyploidy (their classes I–III, respectively) as due to (a) the fusion of an unreduced gamete with another gamete that may be reduced or unreduced, meiotic non-reduction having occurred without necessarily involving hybridization; (b) gamete fusion involving an unreduced gamete formed in a hybrid in which meiosis is disturbed (cf. Winge, 1917); and (c) increases in chromosome number in somatic tissues as a result of disturbances of mitosis. There is abundant evidence for the first two processes from species in many different families. The third process, corresponding to what has been referred to as “somatic doubling” (Meurman, 1933; Stebbins, 1947; Grant, 1963), is much less well known in nature (e.g., Primula × kewensis W. Watson; table 4 in Harlan & De Wet, 1975). Doubling of the somatic chromosome number by means of experimental treatments such as colchicine (Blakeslee & Avery, 1937), however, is well known and is responsible for the persistence of “somatic doubling” as an explanation for polyploidy (G.L. Stebbins, Jr. pers. comm. 1980).

There seem to be three aspects of gametophytic apomixis in the Rosaceae. First, gametophytic apomixis is relatively common in the Rosales, occurring in approximately 45% of the families, and 10% of the genera in the order (Hojsgaard et al., 2014). Within the Rosaceae, gametophytic apomixis occurs throughout the two major clades (subfamilies Rosoideae and Amygdaloideae; Dickinson & al., 2007; Potter & al., 2007). The family is crassinucellate, and characterized by a multicellular archesporium. Normally, only a single megasporeocyte differentiates within the archesporium, to give rise to a reduced megagametophyte, and hence reduced gametes (Johri & al., 1992). Gametophytic apomixis in the Rosaceae consists almost exclusively of apospory, in which one or more cells of the archesporium become competent to develop into unreduced megagametophytes (Johri & al., 1992). Gametophytic apomixis in the Rosaceae is also pseudogamous, meaning that pollination is required for endosperm development and successful seed-set regardless whether seeds are produced asexually or sexually (Dickinson & al., 2007). In diploids the Rosaceae also exhibit gametophytic self-incompatibility (GSI; Raspé & Kohn, 2002) that has been shown to break down with euploid increases in ploidy level (Lewis, 1947; Dickinson & Phipps, 1986; Campbell & al., 1991; Dickinson & al., 1996; De Nettancourt, 2001).

Second, autopolyploids may arise at a very low frequency from diploid × diploid crosses (Lewis, 1980), as in Malus (Considine & al., 2012), and probably also Aria (Liljefors, 1955). Asker & Jerling (1992) and Asker (2009) summarized the occurrence of similar events in apomictic Rosoideae, noting the obligate sexuality of autotetraploid Potentilla argentea. Presumptive autotriploids and autotetraploids are known in Crataegus (Dickinson & al., 1996; Lo & al., 2009; Zarrei & al., 2014), but their wider evolutionary significance is not yet understood. Autopolyploids are important in this model, because they represent low frequency results of the routine gamete fusion in plants with GSI that is responsible for virtually every diploid apple tree (or Hawthorn, or whitebeam, etc.) that has ever existed. Genera in the Maleae evidently have a predisposition for cells of the multicellular archesporium to develop into female gametophytes without meiosis (apospory). If fewer than half a percent of the female gametes involved in successful seed set are unreduced, that is still a great many potentially autotriploid offspring produced by a tree over its reproductive lifetime. Once there are autotriploids in a population, back-crosses to the diploids are inevitable, and potentially self-fertile autotetraploids will be produced, if they were not already formed (at whatever very low frequency) in the diploid × diploid crosses.

Third, apomixis arises, and agamic complexes develop, because of selection (in triploids) for, and the acquisition of, parthenogenetic development of eggs into embryos (both conditions for gametophytic apomixis will then be present; Talent & Dickinson, 2007c; Talent, 2009). All things being equal, apomictic self-fertile pseudogamous autopolyploids will proliferate (Nio rot & al., 1997; Talent, 2009). Reduced pollen from apomictic tetraploids is implicated in gene flow from apomictic tetraploids to sexual tetraploids in Rubus (Sochor & al., 2015; Šarhanová & al., 2017), from sympatric, tetraploid Aria × Aria × Sorbus hybrids to Aria diploids (Hajrudinović & al., 2015), and in intersectional crosses to yield presumptive BII hybrid microspecies in western North American Crataegus (Zarrei & al., 2014, 2015).

Majeský & al. (2017) review the ways in which apomictic taxa have been treated in the current taxonomy of several genera in the Asteraceae and Rosaceae. Their text and table 1 should be consulted for details, but these authors summarize the criteria that, collectively, they deem critical for the taxonomic recognition of apomicts as follows. (1) Genetic origin; do apomicts arise as allopolyploids (recognize as separate species) or autopolyploids (treat as conspecific)? (2) Repeated origin; is there evidence of multiple, possibly polytopic origins of the apomict, as might be inferred from greater than expected genetic variation, in which case separate origins should confer status as separate species? (3) Diagnosability; apomixis should occur with sufficient consistency that morphological variation is not so great that recognition of the species is made ambiguous. (4) Distribution; Majeský & al. depurate recognizing species that are known from only a single locality especially where apomicts and sexuals (or simply, facultative apomicts?) are sympatric, citing the problem of a potentially unending array of new entities claiming recognition as species. (5) Diagnosability (again); for recognition of an apomictic species, meeting the first four criteria must be correlated with “observed stability of morphological traits” (Majeský & al., 2017; cf. Coyne & Orr, 2004). These criteria impose a substantial burden of work on taxonomists seeking to describe species in groups where apomixis occurs. Majeský & al. propose two answers to this problem. For purposes of communication, notably with non-specialists, they note the value of infrageneric groups, among them the sections, series, and nothotaxa that have been employed with Crataegus. This approach can be extended to informal infraspecific names for apomictic microspecies, following Burtt (1970; Dickinson, 1999; McAllister, 2005; Burgess & al., 2014). Such an approach recognizes diagnosable variants observed in the field, but defers formal taxonomic recognition until the data are available to evaluate all of the
criteria suggested by Majeský & al. (2017). For the purpose of understanding observed phenomena (diagnosable variants observed in the field) in the context of a group in which (facultative) gametophytic apomixis, hybridization, and polyploidy occur, Majeský & al. (2017) recommend the approach taken by Mayden (1997) in distinguishing the evolutionary species concept (ESC, “… a lineage … evolving separately from others and with its own unitary evolutionary role and tendencies”, Simpson, 1961) as the primary species concept. Mayden argues that the alternatives to the ESC are secondary concepts providing operational tools for discovering evolutionary units (Mayden, 1997).

Conclusions and recommendations for future work. — The intimate linkage between asexuality in the form of producing unreduced gametes (gametophytic apomixis), hybridization, and polyploidy may be clearer when looking at the Rosaceae and a few other families than it is in others. Wider awareness of how this linkage involves the unreduced gametes becoming fertilized, rather than developing parthenogenetically, may alert some workers to the value of studying apomicts, on the one hand and, on the other, prompt discovery of gametophytic apomixis operating where it had not been suspected. In either case, the results may help clarify how production of unreduced gametes at low frequencies, possibly in relation to environmental insult or merely chance, is connected with the acquisition of gametophytic apomixis.

We need molecular methods with which to document reticulate evolution in diploid-polyploid complexes. Next-generation sequencing can provide the raw data, but new strategies are needed such as the one suggested by Rothfels & al. (2016). Progeny analyses should be used to quantify the relative frequencies of apomixis, selfing, and outcrossing especially now that a method is available whereby tetraploids can be compared with diploids (Lo & al., 2010b). Advantage needs to be taken of flow cytometric methods for dried tissues and pollen (Roberts, 2007; Kron & Husband, 2012), ideally in tandem with promulgation of information about inexpensive, reliable cytometers. In the meantime, some of the papers cited above remind us of the value of “traditional” cytogenetic methods, and also their use with in situ hybridization techniques of visualization.

**AUTHOR CONTRIBUTIONS**

The author declares that he alone conceived and wrote the manuscript, and in doing so has credited all relevant resources. — ORCID: TAD, https://orcid.org/0000-0003-1366-145X

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**LITERATURE CITED**


Dickinson • Sex and Rosaceae apomicts


