

The classification of asexual organisms: Old myths, new facts, and a novel pluralistic approach

Elvira Hörandl

Department of Systematics, Biodiversity and Evolution of Plants (with Herbarium), Albrecht-von-Haller Institute for Plant Sciences, University of Goettingen, 37073 Göttingen, Germany; elvira.hoerandl@biologie.uni-goettingen.de

DOI <https://doi.org/10.12705/676.5>

Abstract Organisms reproducing via asexuality harbor a great diversity of lineages, morphotypes and ecotypes. However, classification of asexual taxa does not fit into contemporary species concepts, and hence the diversity of apomictic plant complexes is not well reflected in taxonomy. Plants reproducing via apomixis (i.e., asexual seed formation = agamospermy) exemplify the theoretical and practical problems of classification. Obligately asexual organisms do not form reproductive communities, but they do constitute ancestor-descendant lineages. From the conceptual side, evolutionary lineage concepts would fit best for species delimitation. Recent research showed that these lineages are not necessarily threatened by rapid extinction and do have persistence in time and space. Facultative sexuality and low levels of residual recombination counteract the accumulation of deleterious mutations due to the lack of recombination (Muller's ratchet). Apomictic lineages do have adaptive potential, which is demonstrated by the ability to occupy large distribution areas and to experience ecological niche shifts. The challenge for classification of asexual lineages, however, is to find operational criteria for species delimitation. Current practices of species delimitation can be grouped into four main principles: (1) the sexuals-first principle means that obligate sexual progenitor species are classified separately from their apomictic derivatives. (2) The all-in-one principle merges sexual progenitors and highly facultative apomictic derivatives into one species, whereby the apomicts often represent autopolyploids without differentiated phenotypes. (3) The cluster concept applies to allopolyploid complexes with facultative apomixis and a huge diversity of genotypes and morphotypes; here the main genetic clusters are treated as species. (4) Almost obligate apomictic lineages are being classified as agamospecies. The four principles reflect quite well evolutionary traits and diversity of lineages. Finally, a recommendation for a workflow is given, following this gradient from obligate sexuality to obligate apomixis.

Keywords apomixis; hybridization; lineages; polyploidy; species concepts; species delimitation

■ INTRODUCTION

The classification of asexual organisms is still a major challenge for biodiversity research. Asexuality is prevalent in prokaryotes and occurs in all eukaryotic kingdoms (Burt, 2000; Heitman, 2015). The classification of asexual organisms has inherent theoretical problems because most species concepts for eukaryotes are designed for sexual organisms (Coyne & Orr, 2004). Flowering plants with apomixis, defined as asexual reproduction via seed (= agamospermy), are a good example of this problem (Asker & Jerling, 1992; Mogie, 1992; Richards, 1997). Apomictic plants occur in all major clades of angiosperms, in more than 50% of all orders, and in 293 genera (Hojsgaard & al., 2014b); see also online database at <http://www.apomixis.uni-goettingen.de>. Abundance of apomictic plants at the species level relative to the rest of the regional flora appears to be greater at higher latitudes and at higher altitudes (Bierzychudek, 1985; Asker & Jerling, 1992; Gregor, 2013). However, this tendency is not apparent on higher taxonomic levels, as genera with apomictic taxa are found in all geographical zones (Hojsgaard & al., 2014b). Apomixis occurs mostly in diverse, species-rich plant families and genera, and might even be a factor promoting diversification (Hörandl &

Hojsgaard, 2012; Hojsgaard & al., 2014b). However, it is often difficult to disentangle the effects of polyploidy and apomixis on biodiversity. Regarding phenotypic diversity, most apomictic plant complexes do have much higher numbers of distinguishable morphotypes and ecotypes than congeneric sexual taxa (Stace, 1998).

The definition of species as basic units of biodiversity and evolution remains a persistent challenge for classification (Sukumaran & Knowles, 2017). From the conceptual side, most modern authors agree that species are lineages in the sense of ancestor-descendant series (De Queiroz, 2007; Sukumaran & Knowles, 2017). Lineages should have an evolutionary role to be accepted as species (Freudenstein & al., 2017). Lineages per se can be maintained both by sexual and asexual reproduction. In both cases the challenge is on the one hand the circumscription of a lineage, and on the other hand to define an evolutionary role – is it persistence in time and space, or is it a certain ecological niche, or a shared phenotype?

At the dawn of plant evolutionary biology many authors denied any evolutionary potential for asexual plants (Babcock & Stebbins, 1938; Darlington, 1939; Stebbins, 1950). This pessimistic view was based on theoretical assumptions that asexuals would be short-lived and would lack adaptive potential. Later

Article history: Received: 23 Jan 2018 | returned for (first) revision: 20 Mar 2018 | (last) revision received: 16 Oct 2018 | accepted: 18 Oct 2018 | published: online fast track, 26 Nov 2018; in print and online issues, 13 Dec 2018 || **Associate Editor:** Timothy A. Dickinson || Published online “open access” under the terms of the Creative Commons CC-BY-SA 4.0 License || © International Association for Plant Taxonomy (IAPT) 2018, all rights reserved

on, early extinction of asexual lineages was postulated because of the accumulation of deleterious mutations in the absence of recombination (Muller, 1964; Kondrashov, 1988; Kouyos & al., 2007). Research during the past two to three decades, and the application of *-omics* methods in evolutionary research, has questioned or even falsified many of the predictions for asexual lineages. In the first part of my review I will discuss recent research on evolutionary persistence in space and time and adaptive potential of apomictic plants. In this context, the applicability of other prominent species concepts beside the biological species concept will be revisited. This discussion of species concepts and their applicability is needed to meet the fundamental requirement that species classifications have theoretical background, predictivity, practicability, and informational content (De Queiroz, 2007; Mishler, 2009).

Conceptualization of species should be kept separate from species delimitation (De Queiroz, 2007). The second part of my review is focused on operational criteria, methodology and a workflow for classification. Modern *-omics* methods and coalescent approaches offer powerful tools for recognizing evolutionary lineages, but do not provide criteria for discriminating population structure from species structure (Sukumaran & Knowles, 2017). This was also a major problem for traditional morphology-based approaches. For sexual species it is easier to find additional operational criteria for species delimitation than for asexual lineages: the strong genetic cohesion within sexual lineages and reproductive barriers between them result in phenotypic cohesion, because of inheritance and reciprocal exchange of alleles/epialleles controlling the phenotype. Hence, individual members of sexual species can be easily recognized by their shared phenotypic features or “morphotype” with only minor individual variation. Quite often individuals of a sexual species share also distinct ecological niches and/or geographical distributions. Therefore, sex indirectly provides obvious operational criteria for species delimitation. Humans used these morphotypes for delimiting and describing species as biological entities long before they thought about evolutionary origins. Sexual species thus not only have a biological and evolutionary reality, but they also have a “mental reality” (Stuessy, 1989). Under this view, the practicability of classification also needs to be considered (Mayden, 1999; Mishler, 2009).

Asexual lineages can be recognized by morphological characters as well (Stace, 1998) and so they also have a strong “mental reality” in the sense of Stuessy (1989). Traditional taxonomists tried to recognize the enormous phenotypic diversity of asexual lineages by describing hundreds and thousands of distinguishable morphotypes as species. The need to recognize phenotypic diversity – in the sense of an evolutionary role of a lineage – was just recently emphasized (Freudenstein & al., 2017). However, as I will discuss below, a distinguishable phenotype is not necessarily a reliable indicator for an asexual lineage. Moreover, without the cohesive effect of sexuality and the lack of crossing barriers, operational phenotypic criteria for the “degree of distinctness” of morphotypes are missing or at least difficult to define consistently. Morphometric analyses and statistical tests are needed to recognize objectively “distinctness” of phenotypes. Otherwise, the purely pragmatic

approach of classifying similar phenotypes without any other criteria will lead to many differences of opinion and treatments, and in some cases the result is thousands of described species (Stace, 1998).

In the last few decades, the practice for taxonomists working with apomictic plants has been to follow the rather pragmatic views of genus-wise classifications, and quite often genus-specific concepts exist (Haveman, 2013; Majesky & al., 2017). The disadvantage of genus-specific concepts for assessment of overall biodiversity is that the resulting species are not readily comparable. For a better overview, I will group the various current approaches to the taxonomy of apomictic plants as follows: sexuals-first, all-in-one, cluster, and agamospecies. Basically these categories follow a gradient from obligate sexuality to obligate asexuality. Other than Majesky & al. (2017) I will not provide detailed literature reviews of plant genera with gametophytic apomixis, but rather show how presently known apomictic plants would fit into these four approaches to species delimitation. I will discuss how operational criteria can work quite well to delimit species according to an evolutionary lineage concept, without the need of any additional taxonomic categories. This pluralistic approach envisions a more unified classification scheme, in which sexual and asexual taxa would represent comparable units of classification. Finally, a recommendation for an operational workflow is given, which will hopefully stimulate further research.

■ THE EVOLUTIONARY BACKGROUND: OLD MYTHS AND NEW FACTS ABOUT ASEQUAL TAXA

The biological species concept is nice, but not universal.

— The widely used biological species concept (BSC, Mayr, 1942) requires sexual reproduction to define a species. For eukaryotes, the underlying biological feature – sex – is a highly conservative mechanism. Meiosis-fertilization cycles vary little among eukaryotes, the genes controlling sexuality are highly conserved, and meiotic sex is probably an ancestral feature of all eukaryotes (Ramesh & al., 2005; Cavalier-Smith, 2010; Speijer & al., 2015; Hörandl & Speijer, 2018). Many arguments support the hypothesis that meiotic sex is an indispensable tool for DNA repair and mutation elimination in the germline, one that ensures genomic integrity over generations (Bernstein, 1991; Michod, 1995; Hörandl, 2009a; Speijer, 2016; Hörandl & Speijer, 2018). If this hypothesis is correct, then sex is simply a physiological necessity. Meiosis and mixis require mating compatibility to be functional and hence the formation of reproductive communities. Hence, sex makes species (in the sense of the BSC). As a consequence, meiotic sex has in eukaryotes a strong intrinsic constraining effect via gene flow and establishes vertical inheritance of lineages, in contrast to prokaryotic pangenomes (Ku & al., 2015). The intrinsic connection within a sexual species is also reflected in the lineage concept of a “metapopulation” (De Queiroz, 2007).

Asexual reproduction also establishes ancestor-descendant lineages (Hörandl, 1998; Majesky & al., 2017). However,

asexuality does not have such an ancient unified mode of reproduction, but rather many different developmental pathways exist (see Box 1 and Fig. 1 for apomixis in flowering plants). Altogether, these represent modifications of the sexual pathway (Koltunow & Grossniklaus, 2003; Mirzaghaderi & Hörandl, 2016), whereby in most cases the key repair mechanisms and residual sexuality are retained (Mirzaghaderi & Hörandl, 2016). Whenever meiosis-mixis cycles are not acting, the functional constraints of mating compatibility simply become meaningless. Reproductive barriers between lineages do not evolve as there is no selective pressure for homology and mating compatibility. Hence, species concepts based on sexuality only, as the BSC, simply become inapplicable for obligately asexual lineages (Hörandl, 1998). Without gene flow and the intrinsic coherence mechanism of sex, asexual lineages neither form meta-populations in the sense of De Queiroz (2007), nor do populations cluster together as demanded by cohesion or cluster species concepts. In conclusion, the biological species concept is nice, but not universal and hence insufficient to describe biodiversity.

To complicate matters, plants often reproduce via facultative asexuality. The male function is usually maintained, with formation of meiotically reduced, recombined and partly fertile pollen. Selection for pollen fertility is probably driven by the need for fertilization of polar nuclei for proper endosperm formation (Mogie, 1992; Mogie & al., 2007). This means that

not only fertilized unreduced, apomeiotic egg cells (resulting in so-called B_{III} hybrids in the offspring, see Box 1, Fig. 1), but also fertilized sexually formed, reduced egg cells (resulting in B_{II} hybrids) can arise. Hence apomictic lineages can produce recombined offspring, they can intercross with other lineages and backcross to sexual species. Facultative apomixis can slow down the tempo of meiosis-mixis cycles (Clausen, 1954). A facultatively apomictic plant complex (Fig. 2) may have rather a “pangenome”-like structure as typical for prokaryotes (Ku & al., 2015). The resulting apomictic complexes match neither concept of obligate sexual nor obligate apomictic lineages. Under a strict BSC the whole complex would have to be classified as one “big” species, resulting in a very heterogeneous entity of lineages of different age, morphology and ecology.

Evolutionary origin and phylogenetic concepts. — Phylogenetic species concepts (PSCs) appear to be independent from sexuality, as they are based on shared ancestry, and a pattern of ancestry and descent (Coyne & Orr, 2004). Theoretically, PSCs might be applicable to asexual lineages as well (Hörandl, 1998), and were successfully applied to obligately asexual animals (Fontaneto, 2014). However, it has long been recognized that most asexual plants and animals are hybrids, and/or polyploids (Ernst, 1918; Babcock & Stebbins, 1938; Stebbins, 1950; Asker & Jerling, 1992; Simon & al., 2003). Hybridization and/or polyploidy are important speciation processes in sexual plants as

Box 1. Overview of developmental pathways and terminology for apomixis in plants (see also Fig. 1).

Adventitious embryony: See *Sporophytic apomixis*.

Agamospecies: An asexual lineage which has been classified as species.

Agamospermy: Reproduction via asexually formed seed. Synonym of apomixis.

Apomeiosis: Development of an unreduced embryo sac. Can be used as umbrella term for apospory and diplospory.

Apospory: Development of an unreduced female gametophyte out of an unreduced initially somatic cell of the nucellus.

B_{II} offspring: Sexually formed offspring of a facultative apomict, i.e., the embryo sac is formed meiotically, and the reduced egg cell is fertilized ($n + n$).

B_{III} offspring: The embryo sac is formed apomictically (either via *Diplospory* or *Apospory*). The unreduced egg cell is fertilized, resulting in a ploidy increase in the embryo ($2n + n$).

Diplospory: Development of an unreduced female gametophyte out of an unreduced megaspore that resulted from restitutional meiosis.

Facultative apomixis: A single plant can produce sexual and apomictic seeds within the same generation. The resulting offspring have the same ploidy as the mother plant, but are partly clonal ($2n + 0$), and partly recombined ($n + n$). Often this term also includes cases of *Partial apomixis* (see there).

Gametophytic apomixis: Development of an unreduced female gametophyte out of an unreduced initial cell. This can happen via *Diplospory* or *Apospory* (see there). The unreduced egg cell develops without fertilization ($2n + 0$).

Haploid parthenogenesis: The embryo sac is formed sexually (i.e., after meiosis). The reduced egg cell is not fertilized, but develops parthenogenetically. Hence the embryo has half the ploidy of the mother plant.

Parthenogenesis: The development of an egg cell into an embryo without fertilization.

Partial apomixis: Occurs when embryo sac formation is not coupled to parthenogenesis and results in ploidy shifts in the embryo. Sometimes subsumed under *Facultative apomixis*. For the increase of ploidy ($2n + n$) see *B_{III} offspring*, for the decrease of ploidy ($n + 0$) see *Haploid parthenogenesis*.

Polyembryony: Two or more embryos develop within one seed; this can result from *Sporophytic apomixis* (see there), when both sexual and apomictic embryos are formed.

Pseudogamy: Pollen-dependent apomixis; the egg cell develops without fertilization, but the polar nuclei are fertilized by one or two sperm nuclei. This is often essential for endosperm development and formation of functional seed.

Sporophytic apomixis: Development of an embryo directly from a somatic cell of the ovule (= adventitious embryony). The embryo is a clone of the mother plant ($2n + 0$), and may also develop in parallel with the formation of sexual embryos ($n + n$).

well (Grant, 1981; Coyne & Orr, 2004). Also for sexual plant species the PSC is conceptually problematic as hybrid speciation and polyploidy will often result in paraphyly (Hörandl, 2006b). The classification of paraphyletic taxa is still controversial (Hörandl & Stuessy, 2010; Podani, 2010). Here I just want to point out that for a strict phylogenetic species concept, paraphyletic groups would not be acceptable for classification of species. In principle the same problems apply to apomictic lineages.

From the phylogenetic perspective, apomictic plant lineages have a progenitor-derivative relationship to their sexual parents rather than an ancestor-descendant relationship; multiple origins are common. Hence, apomicts may be inherently non-monophyletic. Ancient hybridization may even shape the reticulate phylogenetic relationships of almost obligate asexual plant lineages, e.g., in *Hieracium* s.str. (Krak & al., 2013). Hybridization events may occur in different time levels, e.g., in *Rubus* (Šarhanová & al., 2017). Polyphyletic origins of apomixis

are documented for all levels of the taxonomic hierarchy in ferns and flowering plants (Liu, 2012; Hojsgaard & al., 2014b). Similarly, in animals multiple pathways to asexuality exist, and the trait is mostly polyphyletic within the animal phyla (Simon & al., 2003). Diversity of evolutionary origins and polyphyly make it difficult to apply strict phylogenetic concepts to the classification of asexuals.

Genetic and morphological cohesion versus divergence. —

Sexual reproduction, with gene flow and reshuffling of alleles in populations, has a constraining effect on diversification (Felsenstein, 1981). Sexual reproduction can establish genetic cohesion via gene flow, which keeps members of a genetic cluster together, which is the basis of cohesion species concepts (Templeton, 1989). Genetic cohesion is also the background for morphological clustering. Cluster species concepts are based on distinguishable clusters with few or no intermediates (Mallet, 1995). However, although these concepts use similarity as criterion for species delimitation, they are indirectly dependent

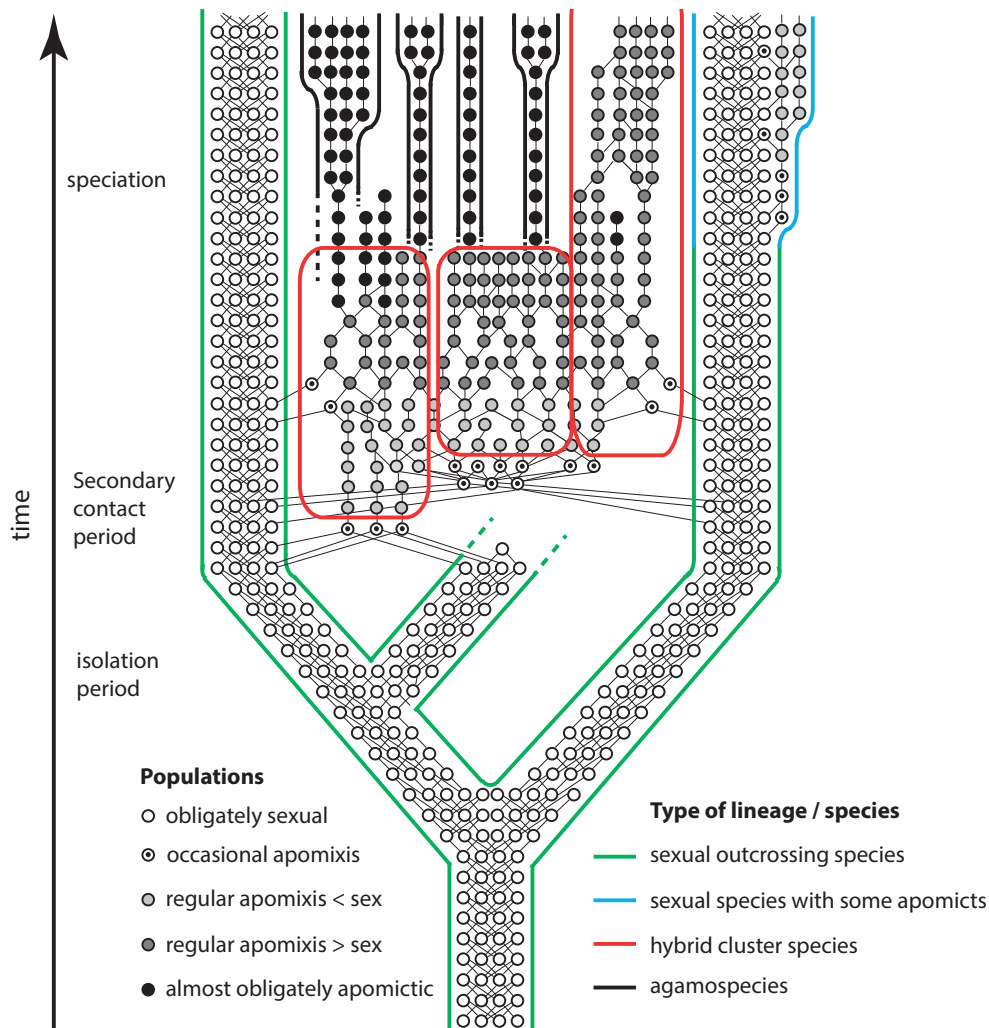


Fig. 2. Scheme of an apomictic plant complex, depicting the four main types of sexual and asexual lineages that might be classified as species. Primary hybrids are excluded from species circumscriptions. Note that new genotypes can be formed via facultative apomixis (see Box 1) within lineages, and also via crossing of apomictic pollen donors with sexual mother plants.

on sexuality, because gene flow remains the major cohesion mechanism (Coyne & Orr, 2004). Cohesion species concepts put emphasis on factors keeping individuals together rather than on reproductive barriers keeping them apart (Coyne & Orr, 2004). For asexuals, the emphasis lies on the ability to produce its own offspring and to keep the lineage stable over generations (Hörandl, 1998). Most asexual plants, however, maintain functional pollen and can act as male parent of biparental offspring as well so that cohesion extends more widely and wide-meshed genetic clusters may be distinguishable (see Box 1; Fig. 2).

Most traditional taxonomists used morphological characters for species, which usually works well for sexual organisms: “A species is a morphologically or genetically distinguishable group of individuals that has few or no intermediates when in contact with other such clusters” (Mallet, 1995). This concept fits many sexual plant species well as long as they have occasional, but negligible hybrid formation. For asexual taxa, the purely similarity-based morphological concept is problematic because of the reduced intrinsic cohesion. Habitat-mediated selection does not result in morphological clustering: obligate or nearly obligate asexual lineages of, e.g., dandelions or of *Alchemilla* spp. do not cluster morphologically, even if they grow in the same meadow (Fröhner, 1995).

The great phenotypic diversity is usually due to the hybrid nature of such a complex: Mendelian segregation of morphological characters in hybrid offspring – already observed by Gregor Mendel himself in his crossing experiments in *Hieracium* subg. *Pilosella* (Nogler, 2006) – will result in a huge diversity of morphotypes, with a unique morphotype for each genotype and each new generation. As long as facultative sexuality with both male and female functions is present, new morphotypes will be continuously produced by intercrossing and backcrossing and add to the diversity of persisting genotypes. Hence, intermediate morphotypes between main clusters will be present (Fig. 2). Other than in sexual species, morphology may be too variable to be a good indicator of a genetic cluster, and hence lacks predictivity for circumscription of a species. Such a complex evolves via multiple, small divergence events with new lineages spinning off that are maintained for some generations, then merging again with other lineages (Fig. 2). Such short-term lineages stand between the “deep forking” lineages of obligate sexual species (see Fig. 2) (De Queiroz, 2007).

Once asexuality has established in an ancestor-descendant lineage, divergence patterns of lineages follow a different pathway than in sexual taxa. Without the homogenizing and bundling effect of sexuality, lineages diverge over time more rapidly from each other than sexual species, without forming clusters. Transcriptomic data in the *Ranunculus auricomus* complex indeed suggest that two hexaploid apomictic lineages originated from the same hybrid cross ca. 80,000 years ago, but in two different locations, and diverged genetically in this short time period in the same amount as their sexual progenitor species which separated ca. 900,000 years ago (Pellino & al., 2013). Genomic studies on other apomictic genera would be highly desirable to confirm the speed of divergence processes of apomictic lineages.

Evolutionary fate and persistence in time. — The evolutionary species concept demands “a lineage with its own evolutionary fate” (Wiley, 1978). Asexual lineages with ancestor-descendant relationships would fulfil this criterion if they would persist over time scales that are comparable to sexual species, and could fill certain ecological niches. A common, traditional view of obligate asexual lineages is the threat of rapid extinction, mainly by accumulation of slightly deleterious mutations. Although this constraint, known as Muller’s ratchet (Muller, 1964; Kondrashov, 1988), was proved in theoretical models for small populations, it becomes questionable as a general constraint in the light of more recent research. Asexual lineages appear to have a sufficient persistence in time to be considered as a species in the sense of an evolutionary lineage concept. This is exemplified in animals.

The existence of some ancient asexual animals (Bdelloid rotifers, Darwinulid ostracods) that had no sex for millions of years (Butlin, 2002) are evidence that long-term persistence of lineages without obligate sex is in principle possible. Genome sequencing of the Bdelloid *Adineta vaga* suggests that this ancient asexual has four collinear chromosome sets, and does not undergo any conventional meiosis, but rather exhibits gene conversion as a mutation-elimination mechanism (Flot & al., 2013). This way the lineage can persist over long time periods without sex. Moreover, obligate asexuality is no obstacle to speciation. Among ancient asexual animals the Bdelloids diversified into 19 genera and ca. 460 species within ca. 50 million years, likely just by selection on adaptive traits in the mouthparts (Fontaneto, 2014; Fontaneto & Barraclough, 2015). The speciation or net diversification interval of Bdelloids (8.2 million years) is well within the range of other animal families (Coyne & Orr, 2004).

For plants, ages of asexual lineages cannot be determined directly, as apomixis cannot be identified in fossils. In general, “twiggy” distributions of asexuality on phylogenetic trees, i.e., of splits of sexual/asexual lineages on terminal nodes, are not necessarily indicative of a low mean age (Schwander & Crespi, 2009). Since apomixis in plants is facultative and reversible to obligate sexuality (Hörandl & Hojsgaard, 2012) one cannot readily use phylogenetic methods to date the age of apomictic plants. Recent transcriptome studies of 6x hybrid apomictic *Ranunculus auricomus* tackled the problem by analyzing lineage-specific SNPs and calculating age by using plant-specific substitution rates of the nuclear genome (Pellino & al., 2013). Results suggest that lineages were ca. 80,000 years old, supporting previous hypotheses of hybrid origin during range shifts in the Pleistocene (Paun & al., 2006b). However, transcriptome data indicated no significant accumulation of deleterious mutations in asexual lineages compared to sexual species (Pellino & al., 2013). Facultative sexuality within a lineage, even at low levels of ca. 6% recombinants per population, is sufficient to eliminate mutations in hexaploids via purifying selection (Hojsgaard & Hörandl, 2015; Hodac & al., subm.). Likewise, in apomictic *Boechera*, a more detailed genomic analysis revealed mutation accumulation in non-coding regions only, but not in conserved coding regions (Lovell & al., 2017). Selection for “a little bit of sex”, especially in high

polyploids with their high mutation rates, might act against the establishment of long-term obligate asexual lineages (Hojsgaard & Hörandl, 2015). Hence, the theoretical assumption of rapid extinction of asexual lineages appears to have no generality, and each taxon probably needs to be examined carefully before any conclusions on age and longevity can be drawn. This also makes predictions for the evolvability of apomictic plants quite speculative. Stebbins (1950) noted that apomictic plants failed to evolve any new genera. However, the monophyletic genus *Alchemilla* s.str. (Gehrke & al., 2016) with high-polyploid, almost obligately apomictic species could be a candidate.

Evolutionary species concepts require that no gene flow occurs between lineages, because even minor gene flow could change the evolutionary fate (Coyne & Orr, 2004). Hence, the evolutionary species concept is not readily applicable for apomictic lineages with nearly obligate sexuality. The clonal lineage would persist only for a few generations and would be then replaced by a new one (Fig. 2). Only taxa with a more stable to obligate apomixis would fall into the category of an evolutionary species. A second problem is the lack of criteria for species delimitation and any kind of subdivisions – a problem that arises already for sexual species, as each and every population can be defined as a lineage (Freudenstein & al., 2017). In asexual taxa each and every clone or genotype can establish an ancestor-descendant lineage.

Persistence in space and adaptive potential. — Freudenstein & al. (2017) requested a unique role of a lineage, as manifested by the phenotype, to regard a lineage as a species. These authors see the role of a lineage as the way it interacts with the environment and occupies an ecological niche. Successful niche occupation would be reflected in a geographical distribution where this niche is available. One of the most popular old myths about asexual taxa is the assumption of a lack of adaptive potential and the need for a stable environment. Under changing conditions “it will be unable to meet any new changes” (Babcock & Stebbins, 1938). Contrary to expectations, most comparative ecological studies suggest that apomicts can adapt to novel environments at least as well as, or in some cases better than their sexual relatives.

Apomictic plants can be quite successful in occupying geographical space. It was long recognized that asexual plants and animals expand over much larger geographical distribution areas than their sexual progenitor, a phenomenon called geographical parthenogenesis (Vandel, 1928; Bierzychudek, 1985; Hörandl, 2006a, 2009b). Most case studies identified niche shifts of asexual taxa as an important factor contributing to their widespread distribution. The classical “Frozen Niche variation model” by Vrijenhoek (Vrijenhoek, 1979), which suggests that broad arrays of clones can use the resource space via niche differentiation among clones better than related sexual species, was supported by many case studies of animals and plants (Vrijenhoek & Parker, 2009). Niche shifts of apomicts have been identified the main causal factor for geographical parthenogenesis in *Crataegus* (Lo & al., 2013; Coughlan & al., 2014, 2017), in cytotypes of *Paspalum intermedium* (Karunarathne & al., 2018), and in *Ranunculus kuepferi* (Cosendai & Hörandl, 2010; Kirchheimer & al., 2016, 2018). In *Crataegus*, hybridity

plays a major role for range expansions (Coughlan & al., 2014). However, the two examples from *Paspalum* and *Ranunculus* are autopolyploid apomictic cytotypes, demonstrating that niche shifts can occur without involving hybrid genomes. Modelling of the colonization process in *R. kuepferi* suggests combinatorial effects of niche shifts and mode of reproduction (Kirchheimer & al., 2018). Microniche differentiation was observed in populations of sympatric apomictic and sexual *Taraxacum* species (Verduijn & al., 2004). Analysis of niche dynamics in invasive sexual/apomictic species pairs revealed no differences between modes of reproduction – during invasions apomicts can experience climatic niche shifts, and range expansions and reductions, just as much as sexuals do (Dellinger & al., 2016).

In most of the examples cited above, the observed niche shift is typical for a cytotype rather than for a single clone. Hence, the cytotype would represent the relevant ecological unit as a candidate for an ecological species. For apomictic plants, polyploidy rather than mode of reproduction may be the actual driver of niche differentiation (Bierzychudek, 1985; Mau & al., 2015). Polyploidy can increase physiological stress tolerance (Comai, 2005; Ramsey & Ramsey, 2014; Schoenfelder & Fox, 2015), which might be an important factor for acclimation to more extreme climatic conditions. Furthermore, the adaptive ability of apomictic plants to environmental stress situations might be based on epigenetic mechanisms rather than on genetic factors (Verhoeven & Preite, 2014). Epigenetic mechanisms, in turn, are strongly influenced by polyploidy (Verhoeven & al., 2010). Epigenetic variability in apomictic *Limonium* species was the mechanism for phenotypic differentiation (Róis & al., 2013). In plants, epigenetic control factors are at least partly heritable (Paszkowski & Grossniklaus, 2011), which means that selection can act on epigenetic variation and favor adaptation over successive generations (Bossdorf & al., 2008). These findings imply that lowered genotypic variation and even clonality of asexuals would be much less relevant for adaptive potential than previously thought. However, these findings also shed a new light on the relevance of phenotypes. The above-cited niche shifts in apomictic plants mostly represent adaptations to abiotic conditions, like temperature, precipitation, soil chemistry, etc. Such adaptations may often have a physiological background that is not apparent in macro-morphological features. Specifically, pollinator-specific adaptations related to flower morphology are unlikely to evolve in apomicts because there is weak selection on outcrossing pollination or pollinator attraction. Even for pseudogamous apomictic plants, pollinator interactions may be less important as most of them can use self-pollen for fertilization of polar nuclei and endosperm formation (Hörandl, 2010). Flower morphology within most apomictic complexes is quite uniform compared to pollinator-specific floral displays in sexual genera. Variation in leaf shape, although apparent and highly diverse in apomictic complexes, may not necessarily be adaptive. Fruit characters are differentiated in some genera, e.g., in *Crataegus* (Christensen, 1992; Coughlan & al., 2014), in others it is not. Is it necessary to redefine our concept of the “phenotype”, and to look at physiological features related to

primary metabolism (e.g., photosynthesis efficiency, temperature adaptation), secondary metabolites etc., and functional traits (e.g., stomata size and density, McGoey & al., 2014)? Would these features be acceptable for our “mental reality” to define a species instead of using morphology? So far, case studies on the physiology of asexual plants are largely missing to answer these questions.

Agamospecies. — Proponents of an agamospecies concept take mode of reproduction as the criterion for a species definition, as a counterpart to the biological species concept. However, this concept can only work if the main criterion, being either sexual or apomictic, would be highly obligate without intermediates, to stabilize a lineage. Unfortunately, asexuality hardly ever becomes obligate in animals and plants; clonality, as it is predicted by theory, has also been recognized to be a myth with the rise of population genetics (Loxdale & Lushai, 2003). Population genetic studies have revealed even with conservative molecular markers (isozymes) that uniclinality in apomicts is rare, whereas gene diversity (heterozygosity) is usually higher than in sexual relatives (Hörandl & Paun, 2007). The broad application of more sensitive markers like AFLPs and SSRs confirmed a considerable genetic diversity and high heterozygosity in apomictic plants (Paun & al., 2006a; Lo & al., 2009; Paule & al., 2011; Cosendai & al., 2013; Šarhanová & al., 2017). Strict clonality in apomictic plants is extremely rare and is known, e.g., from invasive populations of *Chromolaena odorata* in southeastern Asia (Yu & al., 2014).

Facultative apomixis in flowering plants allows for the parallel occurrence of both sexual and apomictic seed formation. Flow cytometric seed screening methods allow for a direct quantitative assessment of proportions (Matzk & al., 2000). Strikingly, proportions of sexual/apomictic seed formation vary not only between species and mode of apomixis, but are often also sensitive to environmental conditions (Aliyu & al., 2010; Šarhanová & al., 2012; Klatt & al., 2016, 2018; Schinkel & al., 2016). Since almost all apomictic plants are perennials, variation is to be expected not only within the same seed generation, but also among seed generations of different growing seasons – a variability which was hitherto mostly neglected. Hence, sex and apomixis do not represent a black-and-white contrast that can be easily reflected in a species-agamospecies system. There are many shades of grey in between.

Conclusion of this part. — To summarize, each of the traditional species concepts has its problems for apomictic plants. The BSC is not applicable at all; phylogenetic concepts (PSCs) will fail because of non-monophyletic origins; genetic cohesions and cluster concepts (CSC) are problematic because morphological and genetic diversification may be much higher in apomictic plants than in sexuals and less bundled; this makes also the unified species concept of a “metapopulation lineage” by De Queiroz (2007) problematic. Evolutionary species concepts (ESCs) concepts and the traditional agamospecies concept require a rather obligate asexuality to maintain the lineage over a relevant time. Ecological species concepts can be useful to recognize establishment of an evolutionary lineage in space and within a certain niche, but often apply to cytotypes because of side effects of polyploidy.

■ FROM THEORY TO PRACTICE: SPECIES DELIMITATION

Traditional taxonomic systems were usually morphology-based, which is broadly applicable for sexual species. For asexuals, pure morphological concepts will suffer from the above-mentioned lack of predictivity of a morphotype for a stable evolutionary lineage. The lack of a species concept led traditional plant taxonomists to adopt two extreme solutions: either lump all lineages under an aggregate name, or split almost every population into agamospecies. Neither approach is satisfactory – the former creates highly heterogeneous “aggregates” without any information on evolution and internal diversification; their high internal morphological diversity contradicts our mental experience of species recognition. Moreover, the progenitors of the complex, the extant sexual species, are sunk into this conglomerate. The agamospecies concept exaggerates morphological distinctness at the expense of theoretical background of whether such a “morphotype” represents an established lineage. In practice, classification of morphotypes becomes highly subjective. Both concepts are by no means comparable to sexual species (Stace, 1998). Attempts to use informal additional categories such as “microspecies” versus “macrospecies” failed because of the lack of theoretical background and definition. Most modern authors make case-by-case decisions to circumscribe agamic taxa, whereby these decisions tend to illustrate four main principles:

(1) The sexual species-first model. — The biggest problems for classification are posed by allopolyploid complexes. Usually they show a basic structure similar to the classical scheme of Babcock & Stebbins (1938) based on the North American *Crepis* agamic complex: a few sexual progenitor species hybridized and formed a huge mass of allopolyploid, apomictic derivatives. Several authors have proposed to separate out the sexual progenitor species that are usually found at the diploid or low polyploid level (Grant, 1981; Dickinson & al., 2008; Hörandl & al., 2009; Burgess & al., 2015). These diploid taxa are usually self-sterile, outcrossing and their primary hybrids often exhibit a low female and male fertility. Different ploidy levels create efficient reproductive barriers against crossing and limit gene flow between sexual progenitors and their agamic derivatives (Lo & al., 2009). In many cases sexual species fit well to a relaxed biological species concept. In plants, a strict application of the BSC is anyway not feasible because of the occurrence of sexual hybridization and of autogamy (Stace, 1998). Also, the application of genetic cohesion/clustering concepts (i.e., allowing low levels of hybrid formation, with high hybrid sterility), is feasible, and these species are usually morphologically clearly distinct.

The delimitation of sexual progenitor species is important for understanding the origin, evolution, age and phylogenetic relationships of the derivative complexes within their respective genera, and to place apomictic complexes in the framework of the classification of the entire genus (Hörandl & Emadzade, 2012; Burgess & al., 2015; Kirschner & al., 2015; Sears & Whitton, 2016). The sexuals-first concept should not be confused with the purely morphological “main” species concept of, e.g., some traditional *Hieracium* researchers (see Majesky & al., 2017).

(2) The all-in-one model. — Not all agamic plants form morphologically and genetically diverse complexes; several apomictic taxa are known where taxonomists recognized just a single species, even if this taxon comprised facultative apomictic and sexual populations. To this group we can count most species of *Paspalum* (Ortiz & al., 2013), *Poa pratensis* and other *Poa* species (Kelley & al., 2009), *Ranunculus kuepferi* (Schinkel & al., 2016), *Antennaria friesiana* and *A. monocephala* (Bayer & Chandler, 2007), among others. Also, species with adventitious embryony in tropical Melastomataceae (Renner, 1989) and in the Celastraceae (*Euonymus*; Naumova, 1992) may belong here. Quite often the above-mentioned taxa with gametophytic apomixis do have more than one cytotype, whereby diploids and tetraploids are usually sexual, while odd-ploidy levels (3x, 5x) and higher ones (6x and higher) are apomictic. As far as their evolutionary origin is known, polyploids in these taxa tend to be autopolyploids: e.g., in *Paspalum*, 72 species were analyzed with respect to their type of polyploidy and mode of reproduction, and 39 of them show multivalent formation at meiosis as is typical for autopolyploids (Ortiz & al., 2013). These taxa appear scattered over the phylogeny of *Paspalum* (Scataglini & al., 2014), suggesting multiple independent shifts to apomixis via autopolyploidy. Similarly, in diploid-autopolyploid *Ranunculus kuepferi* (Cosendai & al., 2011), apomixis evolved independently from other apomictic complexes within the *Ranunculus* phylogeny (Hörandl, 2009b). Likewise in *Antennaria monocephala* (Bayer & Chandler, 2007). Also the many cytotypes of *Poa pratensis* that originated from auto-segregation at meiosis (Grant, 1981) can be included in this category. In a flow cytometric survey of the genus *Poa*, 20 of the 33 species analyzed were determined to be apomictic; facultative apomixis was identified in all species for which more than three samples were analyzed (Kelley & al., 2009). The species with apomixis appear polyphyletically within the phylogeny of the genus (Hoffmann & al., 2013). Likewise, the species of *Euonymus* with adventitious embryony appear scattered over the phylogeny (Naumova, 1992; Li & al., 2014). All these examples exhibit (i) a high level of facultative sexuality, (ii) a low degree of morphological differentiation of sexual and apomictic cytotypes, and (iii) mostly non-hybrid, multiple origins. This is similar to many autopolyploid sexuals that usually show a low degree of morphological differentiation, and for which species status is only accepted if they represent evolutionarily diverged, reproductively isolated and diagnosable lineages (Soltis & al., 2007). Autopolyploids simply lack genomic novelty. When autopolyploid apomicts are evolutionarily young and maintain facultative sexuality, then the cohesion effect of intraspecific gene flow will be strong enough to inhibit strong divergence of lineages. This was observed, e.g., in 4x facultative *R. kuepferi*, where population genetic analyses demonstrated that a single gene pool with three genetic partitions is distributed all over its range (Cosendai & al., 2013). Because of the scarcity of macroscopic differential characters, traditional taxonomists usually classified cytotypes under one species, or sometimes differentiated subspecies, e.g., in *Ranunculus kuepferi* (Huber, 1988) and in *Antennaria friesiana* and *A. monocephala* (Bayer & Chandler, 2007).

(3) The genetic cluster concept. — The problem remains – after sexual taxa were separated out, what to do with the big nasty mass of facultative apomictic lineages? At lower polyploid levels (4x, 6x), intercrossing and backcrossing may result in new genotypes and lineages. The diversity of such complexes is illustrated in Fig. 2. Eventually some lineages may become more isolated at higher ploidy levels. Genera with such complexes in North America include the *Crepis occidentalis* complex, *Amelanchier* and *Crataegus*; in Europe *Rubus fruticosus*, *Ranunculus auricomus* agg., the *Potentilla argentea* complex, *Hieracium* subg. *Pilosella* (= *Pilosella*) (Grant, 1981), among others. The formation of such big hybrid complexes is not restricted to taxa with gametophytic apomixis, but also found in genera with adventitious embryony, e.g., in *Citrus* (Curk & al., 2016) or in the orchid genus *Nigritella* (Hedren & al., 2000). Other than assumed by the iconic scheme of Babcock & Stebbins (1938: 57; reprinted, e.g., by Grant, 1981; Coyne & Orr, 2004), there is no positive correlation of higher ploidy levels with a higher degree of apomixis. Many triploids of *Taraxacum* (dandelions) are almost obligately apomictic due to diplospory with restitutional meiosis (Van Dijk, 2003). Likewise, some triploid apomictic *Boechera* species showed 100% apomictic seed formation (Aliyu & al., 2010). On the other hand, the degree of facultative sexual seed formation varied in hexaploid *Ranunculus auricomus* accessions under different environmental conditions between 0% and 100% (Klatt & al., 2016). Likewise a great variation was observed in *Rubus fruticosus* under different environmental conditions (Šarhanová & al., 2012). Apomixis in *Poa pratensis* is facultative even at high ploidy levels (7x–12x) (Kelley & al., 2009). In *Pilosella*, some tetraploid species are fully sexual (Mráz & al., 2008), and facultative sexuality in polyploids continuously produces novel genotypes and cytotypes (Krahulcova & al., 2004, 2009; Krahulec & al., 2011). In general euploid cytotypes exhibit more residual sexuality than aneuploids, likely because pairing of homologous chromosomes at meiosis is more regular, which is essential for successful sexual development. Degree of apomixis/facultative sexuality will shape the internal genetic structure of a cluster. The problem is whether genetic coherence via residual sexuality is strong enough to keep lineages together as clusters, or whether divergence of lineages would result in a huge “cloud” of genotypes. Most apomictic complexes show the greatest variability at the tetraploid level. Cushman & al. (2017) classified morphological clusters of 4x *Amelanchier* populations and clones under species names, but rejected classification of local microspecies. Likewise, Bayer & Chandler (2007) proposed five main agamic complexes in *Antennaria*, each with one species name. These five complexes may have originated from more than two sexual progenitor species, as many diploid sexual taxa still exist. A formal addition to such an approach could be the designation of allopolyploid clusters as hybrids, either as hybrid formula or hybrid binomial. The use of hybrid formulas would help to reduce the inflation of binomials in big complexes (Hörandl & al., 2009). This of course, becomes complicated for hybrids between allopolyploid clusters.

Novel molecular methods, especially genomic tools, will help to recognize such genetic clusters and to reconstruct their

evolutionary origin. Whether morphological clustering is congruent to genetic groups requires detailed investigation. A big problem is Mendelian segregation of phenotypic traits in a hybrid if it still retains residual sexuality. This phenomenon was already shown by Gregor Mendel's experiments on crosses of *Hieracium auricula* × *H. aurantiacum*, in which the paternal hybrid parent caused a strong morphological segregation of the F₁ generation (Nogler, 2006). In the *Ranunculus auricomus* complex, three or more morphologically quite divergent sexual progenitor species (*R. carpaticola*, *R. cassubicifolius*, *R. notabilis*), have hybridized, resulting in a segregating hybrid progeny with a huge morphological diversity (Hörandl & al., 2009). Experimental crossings of these sexual progenitor species (Hojsgaard & al., 2014a) formed a rather uniform F₁ progeny with facultative apospory, which resembled altogether more the paternal parent rather than exhibiting strict intermediacy (Hodač & al., 2014). In the F₂ generation (created from F₁ × F₁), apomictic seed formation appeared in low frequencies (Barke & al., 2018), and an enormous segregation of characters and diversity of morphotypes emerged. In such a case the resulting morphospace within such a cluster may be more diverse and heterogeneous than between clusters (Hodač & al., 2018 [this issue]). As a long-term consequence, a certain hybrid morphotype may fall into a genetic cluster of the opposite morphotype of the progenitor species as it was shown with *R. ×hungaricus*, a species with undivided leaves which belonged genetically to the clade with deeply divided leaves (Hörandl & al., 2009). Recent RAD Seq studies on the complex, including more samples of *R. ×hungaricus*, rendered this species as highly polyphyletic (Karbstein & al., 2018). In the polyploids of *Crataegus*, phenetic clusters showed a great and often overlapping morphological variability (Dickinson & al., 2008). If such clusters were defined by morphology only, they may reflect an artificial assemblage of similar morphotypes of different evolutionary origins. In *Crepis*, multiple origins of polyploid cytotypes, but also repeated colonization of sites contributed to genetic heterogeneity of morphologically defined “species” (Sears & Whitton, 2016). If clusters were defined genetically, they might be good evolutionary entities, but poorly diagnosable by lacking shared macroscopic characters and hence contradict the “mental reality” of species. Other than in obligate sexual and obligate apomictic species, the morphotype is in this case not necessarily an indicator of a distinct evolutionary “role” of a lineage. Whether minute differences between morphotypes, e.g., in leaf shape, are ecologically relevant, needs to be studied. In the worst case, each local population may have its own morphotype, resulting from countless combinations of segregating characters.

Another approach attempted to use the size of distribution areas as criterion for delimitation of species (Weber, 1996). A minimum distribution area of 50 km diameter of a certain morphotype is required for species definition in brambles (*Rubus fruticosus* complex). The main argument was that a lineage can be regarded as an established species only with a significant establishment in space, which is basically an ecological concept. This pragmatic approach allows the exemption of many locally distributed primary hybrids from species definition. Although

this concept has been criticized by setting an arbitrary, genus-specific threshold, the basic idea behind it is congruent with the notion that a species should have a relevant ecological “role” (Freudenstein & al., 2017). The Weberian concept was not readily accepted by the apomixis community, as the distribution area is of course dependent on many other ecological factors (e.g., seed dispersal mechanisms) and differs between genera. An important caveat for application of ecological criteria is the relative rapid change of environments due to anthropogenic influence (Dickinson & al., 2008). Man-made habitats are under rapid change, which may either favor expansion or restriction of distribution areas of apomictic lineages within very short time scales. Dickinson (1998, 1999) further pointed out that there is a metapopulation-like structure in agamic complexes, i.e., a balance of colonization and extinction keeps a certain area occupied by different clones. Hence a geographical criterion such as absolute size of distribution area will be probably too simplistic.

(4) The agamospecies model (obligate asexuals). — Taxonomists working with more obligate apomictic taxa have argued for agamospecies concepts, i.e., classification of recognizable ancestor-descendant lineages composed of one or a few clones (Kirschner & al., 2016). Transgenerational fixation of phenotypes and inheritance of complex traits in apomictic lineages was proved experimentally (Sailer & al., 2016). Quite often, evolutionary origin and parents of these lineages are unknown and probably extinct. Usually a good training is needed to identify distinct lineages by means of combinations of many minute characters, and hundreds to thousands of such agamospecies exist in genera like *Taraxacum* (Kirschner & al., 2016), *Hieracium* s.str. (Fehrer & al., 2009), and *Alchemilla* (Fröhner, 1995). This approach was often regarded as impracticable because of the high species numbers. However, one should keep in mind that species numbers per se are not a scientific argument. Also, sexual species, e.g., beetles or butterflies, can show such a high diversity that only specialists can identify them. Classification must not be limited by species numbers that anyone can identify and remember, but rather must be fit into a concept, and must be recognized as distinct with reproducible criteria and confirmed by appropriate test statistics. Ideally agamospecies would be a monophyletic group of individuals with a discrete morphotype, as in the case of Bdelloids (Barraclough & al., 2003; Fontaneto, 2014). Genetic divergence between members of such a clade would be just due to mutation, and morphological divergence would be due to selection on adaptive features. The use of coalescence-based methods may be helpful for recognition of lineages. Candidates for such obligate apomixis might be found in the above-mentioned angiosperm taxa and in apogamous ferns, where the developmental pathway does not allow for facultative sex (Liu & al., 2012).

Conclusion of this part. — These four models may be found within a genus and even within one allopolyploid complex (Fig. 2). (i) Sexual progenitor species can be defined according to a BSC or a cluster species concept according to the sexual-first-principle. (ii) Following the all-in-one model, autopolyploid derivatives of these can be formally included in these basic species, regardless of whether they remain sexual or shift towards

facultative apomixis. These species can include occasional local backcrosses between cytotypes, and/or the triploid individuals (B_{III} hybrids) that occur during a recurrent polyploidization process (Schinkel & al., 2017). Autopolyploid cytotypes may be classified as subspecies if it appears to be useful to recognize ecological or geographical differentiation. (iii) Polyploid hybrids with high levels of facultative sexuality may be grouped according to clustering or cohesion concepts, if the cohesion of residual sexuality is strong enough. Here the challenge may be to find congruence between clusters of phenotypic and genotypic diversity. (iv) Obligately apomictic lineages with distinct phenotypes can be classified within an agamospecies concept even if information on evolutionary origin is scarce.

Formally, all four cases can be treated as one taxonomic category, namely species. No other formal or informal categories are needed, and vague discriminations of “microspecies” or “macrospecies” can be abandoned.

■ A WORKFLOW FOR DELIMITATION OF ASEQUAL TAXA

Identification of mode of reproduction and degree of residual sexuality. — The species concepts described above basically follow a gradient of declining degrees of sexuality. Hence determination of ploidy level and mode of reproduction is crucial for any practical application. For gametophytic apomixis, this is easily accomplished with flow cytometric methods, especially the flow cytometric seed screening (FCSS) method (Matzk & al., 2000). The FCSS method is further able to identify pathways of partial apomixis, which results either in B_{III} offspring or in haploid parthenogenesis (see Box 1; Fig. 1). These pathways result in ploidy shifts in the embryo and contribute substantially to cytotype diversity within agamic complexes. Potential difficulties will arise when FCSS fails, as in taxa without endosperm or in taxa with 4-nucleate embryo sacs (some grasses). In cases of adventitious or nucellar embryony the method is similarly uninformative, as embryo-to-endosperm ratios are the same in sexual and apomictic pathways. Here histological examination of developmental pathways (e.g., clearing techniques and microscopic investigation after Herr, 1971) is essential. Observations of polyembryony and/or progeny arrays using highly variable molecular markers will be required to ascertain clonal offspring production.

Circumscription of the obligate sexual progenitor species.

— The sexual lineages and their phylogenetic relationships can be reconstructed by using molecular methods accompanied by morphological studies. Here one can adopt in principle the same methodology as for any other sexual species. Crossing experiments and determination of self-compatibility systems are useful additions.

Circumscription of clusters of facultative apomictic plants.

— These will be mostly of hybrid and/or polyploid origin. The genomic composition of these clusters may on the one hand comprise the partitions from their original parents, or on the other hand partitions from secondary hybridization and introgression events after their origin. Recognition of genetic

clusters might be the most difficult step as the cohesive effect of sexuality is only weakly operating; relationships might be complicated by intercrossing of lineages in sympatry, by ploidy shifts due to partial apomixis, and by backcrossing to sexual species. It might happen in many cases that some populations or individuals fall between clusters, as also illustrated by Fig. 2. These might be primary, recently formed, local hybrids. Genomic tools will help to recognize such clusters and to estimate the age of the cluster, while geographical and ecological information may tell us about their persistence in space. Niche modelling approaches may help to detect ecologically relevant roles. To what extent morphological characters are informative, needs to be studied case by case. In any event, it would be critical to use morphological information as the primary criterion for delimitation of such clusters, as the internal morphological diversity will be higher than the internal coherence of phenotypes. An integrative taxonomic approach as proposed by Dayrat (2005) is wanted, and a balance between recognition of many evolutionary lineages and pragmatism of classifying larger taxonomic units is needed.

An open question remains, whether each and every individual needs to be assigned to a species or hybrid category. In *Rubus* research, quite a large number of plants in the field are left unclassified. This might be the most pragmatic approach for early-generation hybrids between “cluster” species, where the further evolutionary fate cannot be foreseen. However these individuals would not invalidate the cluster species concepts, as long they represent a minority. Such individual hybrid genotypes could be also designated with informal names, as suggested by Dickinson (1999).

Classification of obligate asexual lineages. — If obligate or almost obligate apomictic lineages were identified by step one, classification of lineages as agamospecies is feasible, regardless if these would reach high numbers. However, recognition of such lineages is not without complications. In nuclear genes, allelic sequence divergence can occur (Meselson effect, Mark Welch & Meselson, 2001), which can result in erroneous tree reconstructions with simple tree-building methods (Birky, 1996, 2004). Especially in high-polyploid and hybridogenetic plants with their many gene copies, allelic sequence divergence is to be expected; Meselson-like changes occurred already in facultative apomictic $6x$ *Ranunculus* lineages which are less than 100,000 years old (Pellino & al., 2013).

Coalescence methods of many molecular markers may help to overcome the problem. Coalescence-based species delimitation has become popular for recognizing divergence patterns. However, as pointed out by Sukumaran & Knowles (2017), coalescent-based methods detect mainly genetic structure and divergence of lineages, but not necessarily species. In sexual species, there is no clear distinction between population-level structure and species-level structure (Sukumaran & Knowles, 2017). In agamospecies, there often might be no clear distinction between clonal structure and species structure. Here the use of morphological characters and ecological features will be useful as they would be stable and predictive, similar as an obligate sexual species.

■ CONCLUSION

Apomictic plants harbor a great diversity of kinds of lineages that does not fit into a classical scheme of species definition. Apomicts originate and diversify in a different way than sexual species do, basically following a gradient from obligate sex to obligate apomixis. The pluralistic approach outlined above differentiates four classification schemes of species within this gradient. Accepting this approach will result in applying different species delimitation concepts within the same genus and complex (Fig. 2), but allows classification of sexual and apomictic taxa within a genus according to comparable criteria. This means that no separate formal taxonomic categories are needed, and also informal use of the vague terms of “microspecies” or “macrospecies” can be abandoned. This approach might help to overcome genus-specific schemes which made classification of asexuals so far a task for “specialists”. Pluralistic approaches reflect best the diversity of evolutionary processes, lineage formation, and ecological roles of lineages. For biodiversity research, the pluralistic approach will be more informative than singular species concepts based on just one or few criteria.

■ AUTHOR CONTRIBUTIONS

The author confirms that design, elaboration and writing of the manuscript was in her responsibility alone. — ORCID: EH, <https://orcid.org/0000-0002-7600-1128>

■ ACKNOWLEDGEMENTS

I thank Timothy Dickinson, two anonymous referees, and Ladislav Hodač for valuable comments. Basic research for this review article was funded by the German Research Foundation, projects DFG Ho4395 4-1, Ho4395/1-2 and Ho4395/10-1 to E.H.

■ LITERATURE CITED

- Aliyu, O.M., Schranz, M.E. & Sharbel, T.F. 2010. Quantitative variation for apomictic reproduction in the genus *Boechera* (Brassicaceae). *Amer. J. Bot.* 97: 1719–1731. <https://doi.org/10.3732/ajb.1000188>
- Asker, S.E. & Jerling, L. 1992. *Apomixis in plants*. Boca Raton: CRC Press.
- Babcock, G.T. & Stebbins, G.L. 1938. *The American species of Crepis: Their interrelationships and distribution as affected by polyploidy and apomixis*. Carnegie Institution of Washington Publication 504. Washington, D.C.: Carnegie Institution of Washington.
- Barke, B.H., Daubert M. & Hörandl, E. 2018. Establishment of apomixis in diploid F2 hybrids and inheritance of apospory from F1 to F2 hybrids of the *Ranunculus auricomus* complex. *Frontiers Pl. Sci.* 9: 1111. <https://doi.org/10.3389/fpls.2018.01111>
- Barracough, T.G., Birky, C.W. & Burt, A. 2003. Diversification in sexual and asexual organisms. *Evolution* 57: 2166–2172. <https://doi.org/10.1111/j.0014-3820.2003.tb00394.x>
- Bayer, R.J. & Chandler, G.T. 2007. Evolution of polyploid agamic complexes: A case study using the *Catipes* group of *Antennaria*, including the *A. rosea* complex (Asteraceae: Gnaphalieae). Pp. 317–336 in: Hörandl, E., Grossniklaus, U., Van Dijk, P.J., Sharbel, T. (eds.), *Apomixis: Evolution, mechanisms and perspectives*. Ruggell: Gantner.
- Bernstein, C. & Bernstein, H. 1991. *Aging, sex and DNA repair*. San Diego: Academic Press.
- Bierzuchudek, P. 1985. Patterns in plant parthenogenesis. *Experientia* 41: 1255–1264. <https://doi.org/10.1007/BF01952068>
- Birky, C.W. 1996. Heterozygosity, heteromorphy, and phylogenetic trees in asexual eukaryotes. *Genetics* 144: 427–437.
- Birky, C.W. 2004. Bdelloid rotifers revisited. *Proc. Natl. Acad. Sci. U.S.A.* 101: 2651–2652. <https://doi.org/10.1073/pnas.0308453101>
- Bossdorf, O., Richards, C.L. & Pigliucci, M. 2008. Epigenetics for ecologists. *Ecol. Letters* 11: 106–115. <https://doi.org/10.1111/j.1461-0248.2007.01130.x>
- Burgess, M.B., Cushman, K.R., Doucette, E.T., Frye, C.T. & Campbell, C.S. 2015. Understanding diploid diversity: A first step in unraveling polyploid, apomictic complexity in *Amelanchier*. *Amer. J. Bot.* 102: 2041–2057. <https://doi.org/10.3732/ajb.1500330>
- Burt, A. 2000. Perspective: Sex, recombination, and the efficacy of selection: Was Weismann right? *Evolution* 54: 337–351.
- Butlin, R. 2002. The costs and benefits of sex: New insights from old asexual lineages. *Nat. Rev. Genet.* 3: 311–317. <https://doi.org/10.1038/nrg749>
- Cavalier-Smith, T. 2010. Origin of the cell nucleus, mitosis and sex: Roles of intracellular coevolution. *Biol. Direct* 5: 7. <https://doi.org/10.1186/1745-6150-5-7>
- Christensen, K.I. 1992. Revision of *Crataegus* sect. *Crataegus* and nothosect. *Crataeguinae* (Rosaceae-Maloideae) in the Old World. *Syst. Bot. Monogr.* 35: 1–199.
- Clausen, J. 1954. Partial apomixis as an equilibrium system in evolution. *Caryologia* Suppl. 6: 469–479
- Comai, L. 2005. The advantages and disadvantages of being polyploid. *Nat. Rev. Genet.* 6: 836–846. <https://doi.org/10.1038/nrg1711>
- Cosendai, A.C. & Hörandl, E. 2010. Cytotype stability, facultative apomixis and geographical parthenogenesis in *Ranunculus kuepferi* (Ranunculaceae). *Ann. Bot. (Oxford)* 105: 457–470. <https://doi.org/10.1093/aob/mcp304>
- Cosendai, A.C., Rodewald, J. & Hörandl, E. 2011. Origin and distribution of autopolyploids via apomixis in the alpine species *Ranunculus kuepferi* (Ranunculaceae). *Taxon* 60: 355–364.
- Cosendai, A.C., Wagner, J., Ladinig, U., Rosche, C. & Hörandl, E. 2013. Geographical parthenogenesis and population genetic structure in the alpine species *Ranunculus kuepferi* (Ranunculaceae). *Heredity* 110: 560–569. <https://doi.org/10.1038/hdy.2013.1>
- Coughlan, J.M., Stefanović, S. & Dickinson, T.A. 2014. Relative resource allocation to dispersal and competition demonstrates the putative role of hybridity in geographical parthenogenesis. *J. Biogeogr.* 41: 1603–1613. <https://doi.org/10.1111/jbi.12316>
- Coughlan, J.M., Han, S., Stefanovic, S. & Dickinson, T.A. 2017. Widespread generalist clones are associated with range and niche expansion in allopolyploids of Pacific Northwest hawthorns (*Crataegus* L.). *Molec. Ecol.* 26: 5484–5499. <https://doi.org/10.1111/mec.14331>
- Coyne, J.A. & Orr, H.A. 2004. *Speciation*. Sunderland, Mass.: Sinauer.
- Curk, F., Ollitrault, F., Garcia-Lor, A., Luro, F., Navarro, L. & Ollitrault, P. 2016. Phylogenetic origin of limes and lemons revealed by cytoplasmic and nuclear markers. *Ann. Bot. (Oxford)* 117: 565–583. <https://doi.org/10.1093/aob/mcw005>
- Cushman, K.R., Burgess, M.B., Doucette, E.T., Nelson, G.A. & Campbell, C.S. 2017. Species delimitation in tetraploid, apomictic *Amelanchier* (Rosaceae). *Syst. Bot.* 42: 234–256. <https://doi.org/10.1600/036364417x695529>
- Darlington, C.D. 1939. *The evolution of genetic systems*. Cambridge: Cambridge University Press.
- Dayrat, B. 2005. Towards integrative taxonomy. *Biol. J. Linn. Soc.* 85: 407–415. <https://doi.org/10.1111/j.1095-8312.2005.00503.x>

- De Queiroz, K.** 2007. Species concepts and species delimitation. *Syst. Biol.* 56: 879–886. <https://doi.org/10.1080/10635150701701083>
- Dellinger, A.S., Essl, F., Hojsgaard, D., Kirchheimer, B., Klatt, S., Dawson, W., Pergl, J., Pysek, P., Van Kleunen, M., Weber, E., Winter, M., Hörandl, E. & Dullinger, S.** 2016. Niche dynamics of alien species do not differ among sexual and apomictic flowering plants. *New Phytol.* 209: 1313–1323. <https://doi.org/10.1111/nph.13694>
- Dickinson, T.A.** 1998. Taxonomy of agamic complexes in plants: A role for metapopulation thinking. *Folia Geobot.* 33: 327–332. <https://doi.org/10.1007/BF03216208>
- Dickinson, T.** 1999. Species concepts in agamic complexes. Pp. 319–339 in: Van Raamsdonk, L.W.D. & Den Nijs, J.C.M. (eds.), *Evolution in man-made habitats*. Amsterdam: Institute for Systematics & Ecology.
- Dickinson, T.A., Lo, E.Y.Y., Talent, N. & Love, R.M.** 2008. Black-fruited hawthorns of western North America – One or more agamic complexes? *Botany* 86: 846–865. <https://doi.org/10.1139/b08-072>
- Ernst, A.** 1918. *Bastardierung als Ursache der Apogamie im Pflanzenreich*. Jena: Fischer. <https://doi.org/10.5962/bhl.title.8212>
- Fehr, J., Krak, K. & Chrtek, J., Jr.** 2009. Intra-individual polymorphism in diploid and apomictic polyploid hawkweeds (*Hieracium*, Lactuceae, Asteraceae): Disentangling phylogenetic signal, reticulation, and noise. *B. M. C. Evol. Biol.* 9: 239. <https://doi.org/10.1186/1471-2148-9-239>
- Felsenstein, J.** 1981. Skepticism towards Santa Rosalia, or why are there so few kinds of animals. *Evolution* 35: 124–138. <https://doi.org/10.2307/2407946>
- Flot, J.-F., Hespeels, B., Li, X., Noel, B., Arkhipova, I., Danchin, E.G., Hejnol, A., Henrissat, B., Koszul, R. & Aury, J.-M.** 2013. Genomic evidence for ameiotic evolution in the bdelloid rotifer *Adineta vaga*. *Nature* 500: 453–457. <https://doi.org/10.1038/nature12326>
- Fontaneto, D.** 2014. Molecular phylogenies as a tool to understand diversity in rotifers. *Int. Rev. Hydrobiol.* 99: 178–187. <https://doi.org/10.1002/iroh.201301719>
- Fontaneto, D. & Barraclough, T.G.** 2015. Do species exist in asexuals? Theory and evidence from Bdelloid Rotifers. *Integr. Comp. Biol.* 55: 253–263. <https://doi.org/10.1093/icb/icv024>
- Freudenstein, J.V., Broe, M.B., Folk, R.A. & Sinn, B.T.** 2017. Biodiversity and the species concept: Lineages are not enough. *Syst. Biol.* 66: 644–656. <https://doi.org/10.1093/sysbio/syw098>
- Fröhner, S.** 1995. *Alchemilla*. Pp. 1–236 in: Conert, J. (ed.), *Gustav Hegi. Illustrierte Flora von Mitteleuropa*, Band 4, Teil 2B, *Spermatophyta: Angiospermae; Dicotyledones, 2 (3), Rosaceae, 2*. Berlin: Blackwell.
- Gehrke, B., Kandziora, M. & Pirie, M.D.** 2016. The evolution of dwarf shrubs in alpine environments: A case study of *Alchemilla* in Africa. *Ann. Bot. (Oxford)* 117: 121–131. <https://doi.org/10.1093/aob/mcv159>
- Grant, V.** 1981. *Plant speciation*. New York: Columbia University Press.
- Gregor, T.** 2013. Apomicts in the vegetation of Central Europe. *Tuexenia* 33: 233–257.
- Haveman, R.** 2013. Freakish patterns: Species and species concepts in apomicts. *Nordic J. Bot.* 31: 257–269. <https://doi.org/10.1111/j.1756-1051.2013.00158.x>
- Hedren, M., Klein, E. & Teppner, H.** 2000. Evolution of polyploids in the European orchid genus *Nigritella*: Evidence from allozyme data. *Phyton (Horn)* 40: 239–275.
- Heitman, J.** 2015. Evolution of sexual reproduction: A view from the fungal kingdom supports an evolutionary epoch with sex before sexes. *Fungal Biol. Rev.* 29: 108–117. <https://doi.org/10.1016/j.fbr.2015.08.002>
- Herr, J.M.** 1971. A new clearing-squash technique for study of ovule development in angiosperms. *Amer. J. Bot.* 58: 785–790. <https://doi.org/10.2307/2441475>
- Hodač, L., Scheben, A.P., Hojsgaard, D., Paun, O. & Hörandl, E.** 2014. ITS polymorphisms shed light on hybrid evolution in apomictic plants: A case study on the *Ranunculus auricomus* complex. *PLoS ONE* 9(7): e103003. <https://doi.org/10.1371/journal.pone.0103003>
- Hodač, L., Barke, B.H. & Hörandl, E.** 2018. Mendelian segregation of leaf phenotypes in experimental F2 hybrids elucidates origin of morphological diversity of the apomictic *Ranunculus auricomus* complex. *Taxon* 67: 1082–1092. <https://doi.org/10.12705/676.6>
- Hoffmann, M.H., Schneider, J., Hase, P. & Roser, M.** 2013. Rapid and recent world-wide diversification of bluegrasses (Poa, Poaceae) and related genera. *PLoS ONE* 8(3): e60061. <https://doi.org/10.1371/journal.pone.0060061>
- Hojsgaard, D. & Hörandl, E.** 2015. A little bit of sex matters for genome evolution in asexual plants. *Frontiers Pl. Sci.* 6: 82. <https://doi.org/10.3389/fpls.2015.00082>
- Hojsgaard, D., Greilhuber, J., Pellino, M., Paun, O., Sharbel, T.F. & Hörandl, E.** 2014a. Emergence of apospory and bypass of meiosis via apomixis after sexual hybridisation and polyploidisation. *New Phytol.* 204: 1000–1012. <https://doi.org/10.1111/nph.12954>
- Hojsgaard, D., Klatt, S., Baier, R., Carman, J.G. & Hörandl, E.** 2014b. Taxonomy and biogeography of apomixis in angiosperms and associated biodiversity characteristics. *Crit. Rev. Pl. Sci.* 33: 414–427. <https://doi.org/10.1080/07352689.2014.898488>
- Hörandl, E.** 1998. Species concepts in agamic complexes: Applications in the *Ranunculus auricomus* complex and general perspectives. *Folia Geobot.* 33: 335–348. <https://doi.org/10.1007/BF03216210>
- Hörandl, E.** 2006a. The complex causality of geographical parthenogenesis. *New Phytol.* 171: 525–538. <https://doi.org/10.1111/j.1469-8137.2006.01769.x>
- Hörandl, E.** 2006b. Paraphyletic versus monophyletic taxa: Evolutionary versus cladistic classifications. *Taxon* 55: 564–570. <https://doi.org/10.2307/25065631>
- Hörandl, E.** 2009a. A combinational theory for maintenance of sex. *Heredity* 103: 445–457. <https://doi.org/10.1038/hdy.2009.85>
- Hörandl, E.** 2009b. Geographical parthenogenesis: Opportunities for asexuality. Pp. 161–186 in: Schön, I., Martens, K. & Van Dijk, P.J. (eds.), *Lost sex*. Heidelberg: Springer.
- Hörandl, E.** 2010. The evolution of self-fertility in apomictic plants. *Sexual Pl. Reprod.* 23: 73–86. <https://doi.org/10.1007/s00497-009-0122-3>
- Hörandl, E. & Emadzade, K.** 2012. Evolutionary classification: A case study on the diverse plant genus *Ranunculus* L. (Ranunculaceae). *Perspect. Pl. Ecol. Evol. Syst.* 14: 310–324. <https://doi.org/10.1016/j.ppees.2012.04.001>
- Hörandl, E. & Hojsgaard, D.** 2012. The evolution of apomixis in angiosperms: A reappraisal. *Pl. Biosyst.* 146: 681–693. <https://doi.org/10.1080/11263504.2012.716795>
- Hörandl, E. & Paun, O.** 2007. Patterns and sources of genetic diversity in apomictic plants: Implications for evolutionary potentials. Pp. 169–194 in: Hörandl, E., Grossniklaus, U., Van Dijk, P.J., Sharbel, T. (eds.), *Apomixis: Evolution, mechanisms and perspectives*. Ruggell: Gantner.
- Hörandl, E. & Speijer, D.** 2018. How oxygen gave rise to eukaryotic sex. *Proc. Roy. Soc. London, Ser. B, Biol. Sci.* 285: 20172706. <https://doi.org/10.1098/rspb.2017.2706>
- Hörandl, E. & Stuessy, T.F.** 2010. Paraphyletic groups as natural units of biological classification. *Taxon* 59: 1641–1653.
- Hörandl, E., Greilhuber, J., Klimova, K., Paun, O., Tensch, E., Emadzade, K. & Hodalova, I.** 2009. Reticulate evolution and taxonomic concepts in the *Ranunculus auricomus* complex (Ranunculaceae): Insights from analysis of morphological, karyological and molecular data. *Taxon* 58: 1194–1215.
- Huber, W.** 1988. Natürliche Bastardierungen zwischen weissblühenden *Ranunculus*-Arten in den Alpen. *Veröff. Geobot. Inst. E. T. H. Stiftung Rübel Zürich* 100: 1–160. <https://doi.org/10.3929/ethz-a-000478752>

- Karbstein, K., Hodač, L., Tomasello S., Daubert M., Eilerts M., Hörandl E. 2018. Unraveling biodiversity in the large apomictic polyploid *Ranunculus auricomus* plant complex with genomic, karyological and morphometric data. P-1112 at Evolution Montpellier 2018. <https://oral-and-poster-abstracts.europa-group.com/index/slide/abstract/1871>
- Karunaratne, P., Schedler, M., Martinez, E.J., Honfi, A.I., Novikova, A. & Hojsgaard, D. 2018. Intraspecific ecological niche divergence and reproductive shifts foster cytotype displacement and provide ecological opportunity to polyploids. *Ann. Bot. (Oxford)* 121: 1183–1196. <https://doi.org/10.1093/aob/mcy004>
- Kelley, A.M., Johnson, P.G., Waldron, B.L. & Peel, M.D. 2009. A survey of apomixis and ploidy levels among *Poa* L. (Poaceae) using flow cytometry. *Crop Sci.* 49: 1395–1402. <https://doi.org/10.2135/cropsci2008.09.0553>
- Kirchheimer, B., Schinkel, C.C.F., Dellinger, A.S., Klatt, S., Moser, D., Winkler, M., Lenoir, J., Caccianiga, M., Guisan, A., Nieto-Lugilde, D., Svenning, J.C., Thuiller, W., Vittoz, P., Willner, W., Zimmermann, N.E., Hörandl, E. & Dullinger, S. 2016. A matter of scale: Apparent niche differentiation of diploid and tetraploid plants may depend on extent and grain of analysis. *J. Biogeogr.* 43: 716–726. <https://doi.org/10.1111/jbi.12663>
- Kirchheimer, B., Wessely, J., Gatringer, G., Hülber, K., Moser, D., Schinkel, C.C.F., Appelhans, M., Klatt, S., Caccianiga, M., Dellinger, A.S., Guisan, A., Kuttner, M., Lenoir, J., Maiorano, L., Nieto-Lugilde, D., Plutzar, C., Svenning, J.C., Willner, W., Hörandl, E. & Dullinger, S. 2018. Reconstructing geographical parthenogenesis: Effects of niche differentiation and reproductive mode on Holocene range expansion of an alpine plant. *Ecol. Letters* 21: 392–401. <https://doi.org/10.1111/ele.12908>
- Kirschner, J., Zaveska Drabkova, L., Stepanek, J. & Uhlemann, I. 2015. Towards a better understanding of the *Taraxacum* evolution (Compositae-Cichorieae) on the basis of nrDNA of sexually reproducing species. *Pl. Syst. Evol.* 301: 1135–1156. <https://doi.org/10.1007/s00606-014-1139-0>
- Kirschner, J., Oplaat, C., Verhoeven, K.J.F., Zeisek, V., Uhlemann, I., Travnicek, B., Rasanen, J., Wilschut, R.A. & Stepanek, J. 2016. Identification of oligoclonal agamosperous microspecies: Taxonomic specialists versus microsattellites. *Preslia* 88: 1–17.
- Klatt, S., Hadaček, F., Hodač, L., Brinkmann, G., Eilerts, M., Hojsgaard, D. & Hörandl, E. 2016. Photoperiod extension enhances sexual megaspore formation and triggers metabolic reprogramming in facultative apomictic *Ranunculus auricomus*. *Frontiers Pl. Sci.* 7: 278. <https://doi.org/10.3389/fpls.2016.00278>
- Klatt, S., Schinkel, C.C.F., Kirchheimer, B., Dullinger, S. & Hörandl, E. 2018. Effects of cold treatments on fitness and mode of reproduction in the diploid and polyploid alpine plant *Ranunculus kuepferi* (Ranunculaceae). *Ann. Bot. (Oxford)* 121: 1287–1298. <https://doi.org/10.1093/aob/mcy017>
- Koltunow, A.M. & Grossniklaus, U. 2003. Apomixis: A developmental perspective. *Annual Rev. Pl. Biol.* 54: 547–574. <https://doi.org/10.1146/annurev.arplant.54.110901.160842>
- Kondrashov, A.S. 1988. Deleterious mutations and the evolution of sexual reproduction. *Nature* 336: 435–440. <https://doi.org/10.1038/336435a0>
- Kouyos, R.D., Silander, O.K. & Bonhoeffer, S. 2007. Epistasis between deleterious mutations and the evolution of recombination. *Trends Ecol. Evol.* 22: 308–315. <https://doi.org/10.1016/j.tree.2007.02.014>
- Krahulcová, A., Papoušková, S. & Krahulec, F. 2004. Reproduction mode in the allopolyploid facultatively apomictic hawkweed *Hieracium rubrum* (Asteraceae, *H.* subgen. *Pilosella*). *Hereditas* 141: 19–30. <https://doi.org/10.1111/j.1601-5223.2004.01845.x>
- Krahulcová, A., Rotreklová, O., Krahulec, F., Rosenbaumová, R. & Plačková, I. 2009. Enriching ploidy level diversity: The role of apomictic and sexual biotypes of *Hieracium* subgen. *Pilosella* (Asteraceae) that coexist in polyploid populations. *Folia Geobot.* 44: 281–306. <https://doi.org/10.1007/s12224-009-9041-1>
- Krahulec, F., Krahulcová, A., Rosenbaumová, R. & Plačková, I. 2011. Production of polyhaploids by facultatively apomictic *Pilosella* can result in the formation of new genotypes via genome doubling. *Preslia* 83: 471–490.
- Krak, K., Caklova, P., Chrtek, J. & Fehrer, J. 2013. Reconstruction of phylogenetic relationships in a highly reticulate group with deep coalescence and recent speciation (*Hieracium*, Asteraceae). *Heredity* 110: 138–151. <https://doi.org/10.1038/hdy.2012.100>
- Ku, C., Nelson-Sathi, S., Roettger, M., Garg, S., Hazkani-Covo, E. & Martin, W.F. 2015. Endosymbiotic gene transfer from prokaryotic pangenomes: Inherited chimerism in eukaryotes. *Proc. Natl. Acad. Sci. U.S.A.* 112: 10139–10146. <https://doi.org/10.1073/pnas.1421385112>
- Li, Y.N., Xie, L., Li, J.Y. & Zhang, Z.X. 2014. Phylogeny of *Euonymus* inferred from molecular and morphological data. *J. Syst. Evol.* 52: 149–160. <https://doi.org/10.1111/jse.12068>
- Liu, H.M., Dyer, R.J., Guo, Z.Y., Meng, Z., Li, J.H., Schneider, H. 2012. The evolutionary dynamics of apomixis in ferns: A case study from Polystichoid ferns. *J. Bot.* 2012: 510478. <https://doi.org/10.1155/2012/510478>
- Lo, E.Y.Y., Stefanovic, S. & Dickinson, T.A. 2009. Population genetic structure of diploid sexual and polyploid apomictic hawthorns (*Crataegus*; Rosaceae) in the Pacific Northwest. *Molec. Ecol.* 18: 1145–1160. <https://doi.org/10.1111/j.1365-294X.2009.04091.x>
- Lo, E.Y.Y., Stefanovic, S. & Dickinson, T.A. 2013. Geographical parthenogenesis in Pacific Northwest hawthorns (*Crataegus*; Rosaceae). *Botany* 91: 107–116. <https://doi.org/10.1139/cjb-2012-0073>
- Lovell, J.T., Williamson, R.J., Wright, S.I., McKay, J.K. & Sharbel, T.F. 2017. Mutation accumulation in an asexual relative of *Arabidopsis*. *PLoS Genet.* 13(1): e1006550. <https://doi.org/10.1371/journal.pgen.1006550>
- Loxdale, H.D. & Lushai, G. 2003. Rapid changes in clonal lines: The death of a ‘sacred cow’. *Biol. J. Linn. Soc.* 79: 3–16. <https://doi.org/10.1046/j.1095-8312.2003.00177.x>
- Majeský, L., Krahulec, F. & Vasut, R.J. 2017. How apomictic taxa are treated in current taxonomy: A review. *Taxon* 66: 1017–1040. <https://doi.org/10.12705/665.3>
- Mallet, J. 1995. A species definition for the modern synthesis. *Trends Ecol. Evol.* 10: 294–299. [https://doi.org/10.1016/0169-5347\(95\)90031-4](https://doi.org/10.1016/0169-5347(95)90031-4)
- Mark Welch, D.B. & Meselson, M.S. 2001. Rates of nucleotide substitution in sexual and asexually reproducing rotifers. *Proc. Natl. Acad. Sci. U.S.A.* 98: 6720–6724. <https://doi.org/10.1073/pnas.111144598>
- Matzk, F., Meister, A. & Schubert, I. 2000. An efficient screen for reproductive pathways using mature seeds of monocots and dicots. *Plant J.* 21: 97–108. <https://doi.org/10.1046/j.1365-3113.2000.00647.x>
- Mau, M., Lovell, J.T., Corral, J.M., Kiefer, C., Koch, M.A., Aliyu, O.M. & Sharbel, T.F. 2015. Hybrid apomicts trapped in the ecological niches of their sexual ancestors. *Proc. Natl. Acad. Sci. U.S.A.* 112: E2357–E2365. <https://doi.org/10.1073/pnas.1423447112>
- Mayden, R.L. 1999. Consilience and a hierarchy of species concepts: Advances toward closure on the species puzzle. *J. Nematol.* 31: 95–116.
- Mayr, E. 1942. *Systematics and the origin of species*. Cambridge, U.K.: Columbia University Press.
- McGoey, B.V., Chau, K. & Dickinson, T.A. 2014. Stomata size in relation to ploidy level in North American hawthorns (*Crataegus*, Rosaceae). *Madroño* 61: 177–193. <https://doi.org/10.3120/0024-9637-61.2.177>
- Michod, R.E. 1995. *Eros and evolution: A natural philosophy of sex*. Reading, Massachusetts: Addison-Wesley.
- Mirzaghaderi, G. & Hörandl, E. 2016. The evolution of meiotic sex and its alternatives. *Proc. Roy. Soc. London, Ser. B, Biol. Sci.* 283. <https://doi.org/10.1098/rspb.2016.1221>
- Mishler, B.D. 2009. Three centuries of paradigm changes in biological classification: Is the end in sight? *Taxon* 58: 61–67.
- Mogie, M. 1992. *The evolution of asexual reproduction in plants*. London: Chapman and Hall.

- Mogie, M., Britton, N.F. & Stewart-Cox, A.J. 2007. Asexuality, polyploidy and the male function. Pp. 169–194 in: Hörandl, E., Grossniklaus, U., Van Dijk, P. & Sharbel, T.F. (eds.), *Apomixis: Evolution, mechanisms and perspectives*. Ruggell: Gantner.
- Mráz, P., Šingliarová, B., Urfus, T. & Krahulec, F. 2008. Cytogeography of *Pilosella officinarum* (Compositae): Altitudinal and longitudinal differences in ploidy level distribution in the Czech Republic and Slovakia and the general pattern in Europe. *Ann. Bot. (Oxford)* 101: 59–71. <https://doi.org/10.1093/aob/mcm282>
- Muller, H.J. 1964. The relation of recombination to mutational advance. *Mutat. Res.* 106: 2–9. [https://doi.org/10.1016/0027-5107\(64\)90047-8](https://doi.org/10.1016/0027-5107(64)90047-8)
- Naumova, T.N. 1992. *Apomixis in angiosperms: Nucellar and integumentary embryony*. Boca Raton: CRC Press.
- Nogler, G.A. 2006. The lesser-known Mendel: His experiments on *Hieracium*. *Genetics* 172: 1–6.
- Ortiz, J.P.A., Quarin, C.L., Pessino, S.C., Acuna, C., Martinez, E.J., Espinoza, F., Hojsgaard, D.H., Sartor, M.E., Caceres, M.E. & Pupilli, F. 2013. Harnessing apomictic reproduction in grasses: What we have learned from *Paspalum*. *Ann. Bot. (Oxford)* 112: 767–787. <https://doi.org/10.1093/aob/mct152>
- Paszkowski, J. & Grossniklaus, U. 2011. Selected aspects of transgenerational epigenetic inheritance and resetting in plants. *Curr. Opin. Pl. Biol.* 14: 195–203. <https://doi.org/10.1016/j.pbi.2011.01.002>
- Paule, J., Sharbel, T.F. & Dobes, C. 2011. Apomictic and sexual lineages of the *Potentilla argentea* L. group (Rosaceae): Cytotype and molecular genetic differentiation. *Taxon* 60: 721–732.
- Paun, O., Greilhuber, J., Tensch, E.M. & Hörandl, E. 2006a. Patterns, sources and ecological implications of clonal diversity in apomictic *Ranunculus carpaticola* (*Ranunculus auricomus* complex, Ranunculaceae). *Molec. Ecol.* 15: 897–910. <https://doi.org/10.1111/j.1365-294X.2006.02800.x>
- Paun, O., Stuessy, T.F. & Hörandl, E. 2006b. The role of hybridization, polyploidization and glaciation in the origin and evolution of the apomictic *Ranunculus cassubicus* complex. *New Phytol.* 171: 223–236. <https://doi.org/10.1111/j.1469-8137.2006.01738.x>
- Pellino, M., Hojsgaard, D., Schmutzer, T., Scholz, U., Hörandl, E., Vogel, H. & Sharbel, T.F. 2013. Asexual genome evolution in the apomictic *Ranunculus auricomus* complex: Examining the effects of hybridization and mutation accumulation. *Molec. Ecol.* 22: 5908–5921. <https://doi.org/10.1111/mec.12533>
- Podani, J. 2010. Monophyly and paraphyly: A discourse without end? *Taxon* 59: 1011–1015.
- Ramesh, M.A., Malik, S.B. & Logsdon, J.M. 2005. A phylogenomic inventory of meiotic genes: Evidence for sex in *Giardia* and an early eukaryotic origin of meiosis. *Curr. Biol.* 15: 185–191.
- Ramsey, J. & Ramsey, T.S. 2014. Ecological studies of polyploidy in the 100 years following its discovery. *Philos. Trans., Ser. B* 369: 20130352. <https://doi.org/10.1098/rstb.2013.0352>
- Renner, S.S. 1989. A survey of reproductive biology in Neotropical Melastomataceae and Memecylaceae. *Ann. Missouri Bot. Gard.* 76: 496–518. <https://doi.org/10.2307/2399497>
- Richards, J.A. 1997. *Plant breeding systems*, ed. 2. London: Chapman and Hall.
- Róis, A.S., López, C.M.R., Cortinhas, A., Erben, M., Espírito-Santo, D., Wilkinson, M.J. & Caperta, A.D. 2013. Epigenetic rather than genetic factors may explain phenotypic divergence between coastal populations of diploid and tetraploid *Limonium* spp. (Plumbaginaceae) in Portugal. *B. M. C. Pl. Biol.* 13: 205. <https://doi.org/10.1186/1471-2229-13-205>
- Sailer, C., Schmid, B. & Grossniklaus, U. 2016. Apomixis allows the transgenerational fixation of phenotypes in hybrid plants. *Curr. Biol.* 26, 331–337. <https://doi.org/10.1016/j.cub.2015.12.045>
- Šarhanová, P., Vasut, R.J., Dancak, M., Bures, P. & Travnicek, B. 2012. New insights into the variability of reproduction modes in European populations of *Rubus* subgen. *Rubus*: How sexual are polyploid brambles? *Sexual Pl. Reprod.* 25: 319–335. <https://doi.org/10.1007/s00497-012-0200-9>
- Šarhanová, P., Sharbel, T.F., Sochor, M., Vašut, R.J., Dančák, M. & Travníček, B. 2017. Hybridization drives evolution of apomicts in *Rubus* subgenus *Rubus*: Evidence from microsatellite markers. *Ann. Bot. (Oxford)* 120: 317–328. <https://doi.org/10.1093/aob/mcx033>
- Scatagliini, M.A., Zuloaga, F.O., Giussani, L.M., Denham, S.S. & Morrone, O. 2014. Phylogeny of New World *Paspalum* (Poaceae, Panicoideae, Paspaleae) based on plastid and nuclear markers. *Pl. Syst. Evol.* 300: 1051–1070. <https://doi.org/10.1007/s00606-013-0944-1>
- Schinkel, C.C.F., Kirchheimer, B., Dellinger, A.S., Klatt, S., Winkler, M., Dullinger, S. & Hörandl, E. 2016. Correlations of polyploidy and apomixis with elevation and associated environmental gradients in an alpine plant. *AoB Plants* 8: plw064. <https://doi.org/10.1093/aobpla/plw064>
- Schinkel, C.C.F., Kirchheimer, B., Dullinger, S., Geelen, D., De Storme, N. & Hörandl, E. 2017. Pathways to polyploidy: Indications of a female triploid bridge in the alpine species *Ranunculus kuepferi* (Ranunculaceae). *Pl. Syst. Evol.* 303: 1093–1108. <https://doi.org/10.1007/s00606-017-1435-6>
- Schoenfelder, K.P. & Fox, D.T. 2015. The expanding implications of polyploidy. *J. Cell Biol.* 209: 485–491. <https://doi.org/10.1083/jcb.201502016>
- Schwander, T. & Crespi, B.J. 2009. Twigs on the tree of life? Neutral and selective models for integrating macroevolutionary patterns with microevolutionary processes in the analysis of asexuality. *Molec. Ecol.* 18: 28–42. <https://doi.org/10.1111/j.1365-294X.2008.03992.x>
- Sears, C.J. & Whitton, J. 2016. A reexamination of the North American *Crepis* agamic complex and comparison with the findings of Babcock and Stebbins' classic biosystematic monograph. *Amer. J. Bot.* 103: 1289–1299. <https://doi.org/10.3732/ajb.1600057>
- Simon, J.C., Delmotte, F., Rispé, C. & Crease, T. 2003. Phylogenetic relationships between parthenogens and their sexual relatives: The possible routes to parthenogenesis in animals. *Biol. J. Linn. Soc.* 79: 151–163. <https://doi.org/10.1046/j.1095-8312.2003.00175.x>
- Soltis, D.E., Soltis, P.S., Schemske, D.W., Hancock, J.F., Thompson, J.N., Husband, B.C. & Judd, W.S. 2007. Autopolyploidy in angiosperms: Have we grossly underestimated the number of species? *Taxon* 56: 13–30.
- Speijer, D. 2016. What can we infer about the origin of sex in early eukaryotes? *Philos. Trans., Ser. B* 371: 20150530. <https://doi.org/10.1098/rstb.2015.0530>
- Speijer, D., Lukeš, J. & Eliáš, M. 2015. Sex is a ubiquitous, ancient, and inherent attribute of eukaryotic life. *Proc. Natl. Acad. Sci. U.S.A.* 112: 8827–8834. <https://doi.org/10.1073/pnas.1501725112>
- Stace, C.A. 1998. Species recognition in agamosperms: The need for a pragmatic approach. *Folia Geobot.* 33: 319–326. <https://doi.org/10.1007/BF03216207>
- Stebbins, G. 1950. *Variation and evolution in plants*. New York: Columbia University Press.
- Stuessy, T.F. 1989. Comments on specific categories in flowering plants. *Pl. Syst. Evol.* 167: 69–74. <https://doi.org/10.1007/bf00936548>
- Sukumaran, J. & Knowles, L.L. 2017. Multispecies coalescent delimits structure, not species. *Proc. Natl. Acad. Sci. U.S.A.* 114: 1607–1612. <https://doi.org/10.1073/pnas.1607921114>
- Templeton, A.R. 1989. The meaning of species and speciation: A genetic perspective. Pp. 3–27 in: Otte, D. & Endler, J.A. (eds.), *Speciation and its consequences*. Sunderland, Massachusetts: Sinauer.
- van Dijk, P.J. 2003. Ecological and evolutionary opportunities of apomixis: Insights from *Taraxacum* and *Chondrilla*. *Philos. Trans., Ser. B* 358: 1113–1120. <https://doi.org/10.1098/rstb.2003.1302>
- Vandel, A. 1928. La parthenogenese géographique: Contribution à l'étude biologique et cytologique de la parthenogenese naturelle. *Bull. Biol. France Belgique* 62: 164–281.
- Verduijn, M.H., Van Dijk, P.J. & Van Damme, J.M.M. 2004. Distribution, phenology and demography of sympatric sexual and asexual dandelions (*Taraxacum officinale* s.l.): Geographic parthenogenesis on a small scale. *Biol. J. Linn. Soc.* 82: 205–218. <https://doi.org/10.1111/j.1095-8312.2004.00325.x>

- Verhoeven, K.J.F. & Preite, V.** 2014. Epigenetic variation in asexually reproducing organisms. *Evolution* 68: 644–655. <https://doi.org/10.1111/evo.12320>
- Verhoeven, K.J.F., Van Dijk, P.J. & Biere, A.** 2010. Changes in genomic methylation patterns during the formation of triploid asexual dandelion lineages. *Molec. Ecol.* 19: 315–324. <https://doi.org/10.1111/j.1365-294X.2009.04460.x>
- Vrijenhoek, R.C.** 1979. Factors affecting clonal diversity and coexistence. *Amer. Zool.* 19: 787–797.
- Vrijenhoek, R.C. & Parker, J.E.D.** 2009. Geographical parthenogenesis: General purpose genotypes and frozen niche variation. Pp. 99–131 in: Schön, I., Martens, K. & Van Dijk, P. (eds.), *Lost sex*. Heidelberg: Springer.
- Weber, H.E.** 1996. Former and modern taxonomic treatment of the apomictic *Rubus* complex. *Folia Geobot. Phytotax.* 31: 373–380. <https://doi.org/10.1007/bf02815381>
- Wiley, E.O.** 1978. Evolutionary species concept reconsidered. *Syst. Zool.* 27: 17–26. <https://doi.org/10.2307/2412809>
- Yu, X.Q., He, T.H., Zhao, J.L. & Li, Q.M.** 2014. Invasion genetics of *Chromolaena odorata* (Asteraceae): Extremely low diversity across Asia. *Biol. Invas.* 16: 2351–2366. <https://doi.org/10.1007/s10530-014-0669-2>