

Review

The Role of Hybridisation in the Making of the Species-Rich Arctic-Alpine Genus *Saxifraga* (Saxifragaceae)

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Received: 25 September 2020; Accepted: 20 November 2020; Published: 23 November 2020



Abstract: Evolutionary processes fuelling rapid species diversification are not yet fully understood, although their major contribution to overall patterns of plant biodiversity is well established. Hybridisation is among the least understood of these processes, despite its multifaceted role in speciation processes being widely accepted. Species of the large arctic-alpine genus *Saxifraga* are notorious for their ability to hybridise; however, the overall role of hybridisation and polyploidisation for the diversification of this genus remains unknown. Here, we provide a comprehensive genus-wide review of hybridisation accounts and ploidy levels. We find that the sections of *Saxifraga* vary greatly in their propensity to hybridise. The majority of natural hybridisation accounts are from recent localised events ($n = 71$). Hybridisation hotspots were located in the Pyrenees and the European Alps, thus contrasting with the overall distribution of species richness in the genus. Hybrids or hybrid populations are often short-lived in *Saxifraga* due to a multitude of reproductive barriers, most commonly low F_1 hybrid fertility. However, these barriers are not always fully effective, allowing for backcrossing and the formation of hybrid swarms. In addition, we find that the incidence of polyploidy varies widely across different sections of *Saxifraga*, with species-rich sections *Porphyrium* and *Saxifraga* showing divergent polyploidy proportions. Overall, we show that hybridisation and polyploidisation played differential roles in the diversification of this large genus. Nevertheless, a significant proportion of species are yet to be scrutinised, particularly among the Asian *Saxifraga* species, illustrating the need for systematic further study to fully unravel the role of hybridisation during the evolution of *Saxifraga*.

Keywords: hybridisation; polyploidy; evolutionary radiation; *Saxifraga*; speciation; F_1 hybrid sterility

1. Introduction

Hybridisation, defined as the mating of members of genetically distinct populations [1,2], has historically been largely viewed as evolutionary noise (e.g., [3,4]) since F_1 hybrids are generally less viable and fertile than their parental species. However, the vast body of evidence from model systems involving hybridising species pairs clearly points to a multifaceted role of hybridisation in the process of speciation [4–6]. On the one hand, gene flow between divergent lineages (e.g., populations) may slow down, stop or reverse their ongoing differentiation. On the other hand, speciation may be accelerated by the transfer of adaptive traits via introgression; generation of new, favourable allele

combinations; or the process of reinforcement [6,7]. The ultimate outcome of hybridisation strongly depends on the physiological, ecological and morphological attributes of hybrids (i.e., the extent of prezygotic and postzygotic reproductive isolation [8–11]).

In any case, hybridisation generates new combinations of parental alleles and may lead not only to intermediates of parental traits but also to transgressive ones. When fixed in a hybrid population, these traits may foster reproductive isolation from parental species, and hybrid genotypes may embark on their own evolutionary track, potentially leading up to hybrid speciation [6,12,13]. This process is now well-accepted to be ubiquitous among plants, with ca. 25% of plant species known to hybridise [14]. Many plant species are believed to have arisen from hybridisation (examples from 27 plant genera and 16 plant families were cited by [5]), thus demonstrating the critical role of hybridisation in speciation and evolution [6,15,16]. However, the frequency and significance of hybridisation as an evolutionary driver varies among taxonomic groups. An example of taxa prone to hybridisation is the tribe Poeae and relatives (temperate grasses; Poaceae Barnhart). There, patterns of cytonuclear discordance were best explained by ancient hybridisation as revealed for approximately 63% of the ca. 115 genera [17], thus supporting hybrid origin not only of genera but also of higher taxa such as tribes or subtribes.

Furthermore, hybridisation and its genomic consequences can drastically impact the ecological niches of hybrid offspring compared to their progenitors. Occupancy of a different ecological niche is expected to be important for the offspring to escape parental competition and thus for long-term survival. For example, in *Helianthus* L., a classic model system for interspecific hybridisation, two parental species gave rise to a salt-resistant hybrid species, which was able to conquer a new adaptive landscape compared to the parental species [18]. This system appears to be a rare instance of homoploid hybrid speciation (parents and hybrid offspring having the same ploidy level), for which only ca. 20 cases are considered to be reliably documented [19,20]. Whereas this mode of speciation may unfold relatively slowly (e.g., [21]), hybrid speciation may be almost instantaneous when polyploidisation occurs [22]. In fact, it is likely due to the almost immediate reproductive isolation of the offspring from their progenitors that polyploid hybrid speciation appears to be far more frequent than homoploid hybrid speciation [23–25].

Hybridisation and polyploidisation may occur anywhere, yet some habitats appear more prone to generating or sustaining hybrids. Examples include disturbed habitats in *Silene* L. [26] and *Banksia* L.f. [27] or areas characterised by ecological gradients where hybrids are more likely to find an environmental niche matching their genetic makeup (e.g., in *Iris* L. [28,29]). Similarly, polyploids have been shown to occur most frequently in habitats that have undergone rapid environmental changes. This is particularly evident in areas that have undergone deglaciation, such as the Arctic and some mountain systems [30–33]. For example, 78.3% of the native Svalbard flora is made up of polyploid species [30]. However, only a few studies to date have explicitly compared the degree of polyploidy between arctic-alpine taxa and their non-arctic-alpine sister lineages. For example, in *Artemisia* L., a genus relatively well-represented in the Arctic, no higher proportion of polyploids was found in the arctic relatives of non-arctic lineages when using phylogenetic correction [34]. Thus, the link between glaciation and other historical factors, and polyploidy remains to be resolved. Additionally, hybridisation and polyploidisation appear to be more frequent in species-rich and rapidly diversifying groups, but the exact relationship between these processes and species richness is yet to be fully understood [7,35–37]. Unravelling the complex roles of hybridisation and polyploidisation as catalysts of diversification will require the use of a variety of integrative approaches across evolutionary scales, including in-depth investigations of individual species-rich plant groups such as *Saxifraga* L.

Saxifraga is considered one of the most characteristic floristic elements of the Arctic and the alpine life zones of the Northern Hemisphere [31]. The genus harbours many species that are adapted to extreme conditions, growing as high as 4507 m a.s.l. (above sea level) (*S. oppositifolia* L. [38]) and 5800 m a.s.l. (*S. punctulatoidea* J.T.Pan and *S. nangxianensis* J.T.Pan [39]) in the European Alps in the Tibeto-Himalayan region, respectively, and as far North as 81° latitude in northern Canada and Greenland (*S. oppositifolia* and *S. hyperborean* R.Brown [40]). The genus also exhibits striking levels of

species diversity, in particular, in the southern European mountain ranges, the Caucasus, the Himalayas, and the Hengduan Mountains [39,41]. It thus lends itself well to the study of species diversification in alpine and arctic habitats. *Saxifraga* comprises ca. 400 to 500 species, mostly perennial and herbaceous, currently divided into at least 13 sections [42]. The genus has experienced two episodes of rapid radiations in its two largest sections: *Ciliatae* Haw. (ca. 175 species) and *Porphyryon* Tausch (ca. 90 to 112 species) [43]. Even though these independent radiations unfolded roughly around the same time (majority of speciation events <5 million years ago, Ma), they were driven by various combinations of factors, including different geographic (Hengduan Mountains vs. Himalayas vs. European Mountains) and biotic contexts (niche evolution vs. key innovations [43,44]).

The precise evolutionary mechanisms behind these episodes of accelerated diversification have remained unclear. For example, polyploidisation was found to be positively correlated with species richness in section *Ciliatae* [44]. However, whether this pattern is also true for other sections of *Saxifraga* has not been systematically assessed. In addition, despite having long been known to be a widespread process in *Saxifraga*, the role of hybridisation for the overall diversification of this large genus and its many infrageneric groups has remained unclear. Here, we provide a comprehensive review of the available evidence regarding hybridisation and polyploidy in *Saxifraga*, framing this information in the context of *Saxifraga*'s evolutionary history, in order to elucidate the contribution of these processes to the species diversity of this large arctic-alpine genus.

2. Available Studies and the Extent of Hybridisation

Saxifraga is notoriously prone to hybridisation and accounts of hybridising *Saxifraga* species abound in the literature. In total, we identified more than 40 scientific articles and books dealing to a variable extent with hybrids and hybridisation in *Saxifraga*. However, the basis of observation and the confidence in the actual occurrence of hybridisation vary widely, with some hybrids having been recorded only a handful of times. However, in this review, we included all accounts available to us for which no reasonable doubt has been expressed (e.g., by [45–47]) other than artificial hybrids bred for the sake of horticulture. We base our phylogenetic interpretations of hybridisation processes on the most recent taxonomic treatment, developed upon a robust phylogenetic framework [42]. Even though this phylogenetic reconstruction is based on only two nuclear and one chloroplast marker(s) and not all relationships are fully resolved, this still represents the most comprehensive treatment of the genus to date. As one caveat, we refrain from drawing conclusions about infrageneric series and present all results on the level of sections and subsections.

Altogether, we recorded descriptions of and references to 84 natural *Saxifraga* hybrids (Table S1). Whereas the majority of hybridising species are known to cross with only a single congener, there are a handful of extremely interfertile species, potentially hybridising with up to six (*S. geranioides* L.), seven (*S. paniculata* Mill.) or even ten separate species (*S. exarata* Vill.). In addition, hybridisation was most often observed as currently ongoing or very recent ($n = 73$), whereas the number of reports of established hybrid species is much lower ($n = 11$). This is likely because recent hybrid populations of intermediate phenotypes can be recognised relatively easily in the wild (accounts thoroughly reviewed by [47]) whereas the detection of past hybridisation requires molecular tools (e.g., for *S. osloensis* Knaben [48,49] and for *S. wahlenbergii* Ball [50]). Overall, this cornucopia of available studies clearly renders *Saxifraga* highly suitable to investigate the mechanisms of hybridisation and its evolutionary role.

3. Taxonomic, Geographic and Temporal Distribution of Hybridisation in *Saxifraga*

The frequency of hybridisation appears to vary greatly among clades and throughout the distribution range of *Saxifraga*. Based on our current understanding of the infrageneric phylogenetic relationships (Figures 1 and 2), taxa of the European section *Saxifraga* (ca. 70 to 85 species) appear to be particularly prone to hybridisation, with at least 40 cross-fertile species pairs. Similarly, the six sections forming the sister clade to section *Saxifraga* (sections *Cotylea* Tausch, *Gymnopera* D. Don,

Ligulatae Haw., *Mesogyne* Sternb., *Porphyron* and *Trachyphyllum* (Gaudin) W.D.J.Koch), include many hybridising species pairs. For example, section *Porphyron* boasts at least 16 recorded natural hybrids. In addition, most of the species of the European sections *Gymnopera* (4 species) and *Ligulatae* (8 species) are known to hybridise with at least one other species. In contrast, we could only find isolated, circumstantial accounts of hybridisation in sections *Bronchiales* DeChaine and *Ciliatae* (see below) and none for sections *Irregulares* Haw. (including *Heterisia* (Raf. ex Small) A.M.Johnson), *Pseudocymbalaria* Zhmylev, and *Trachyphyllum* (see below) or with monotypic section *Saxifragella* (Engl.) Gornall and Zhou-Xin Zhang.

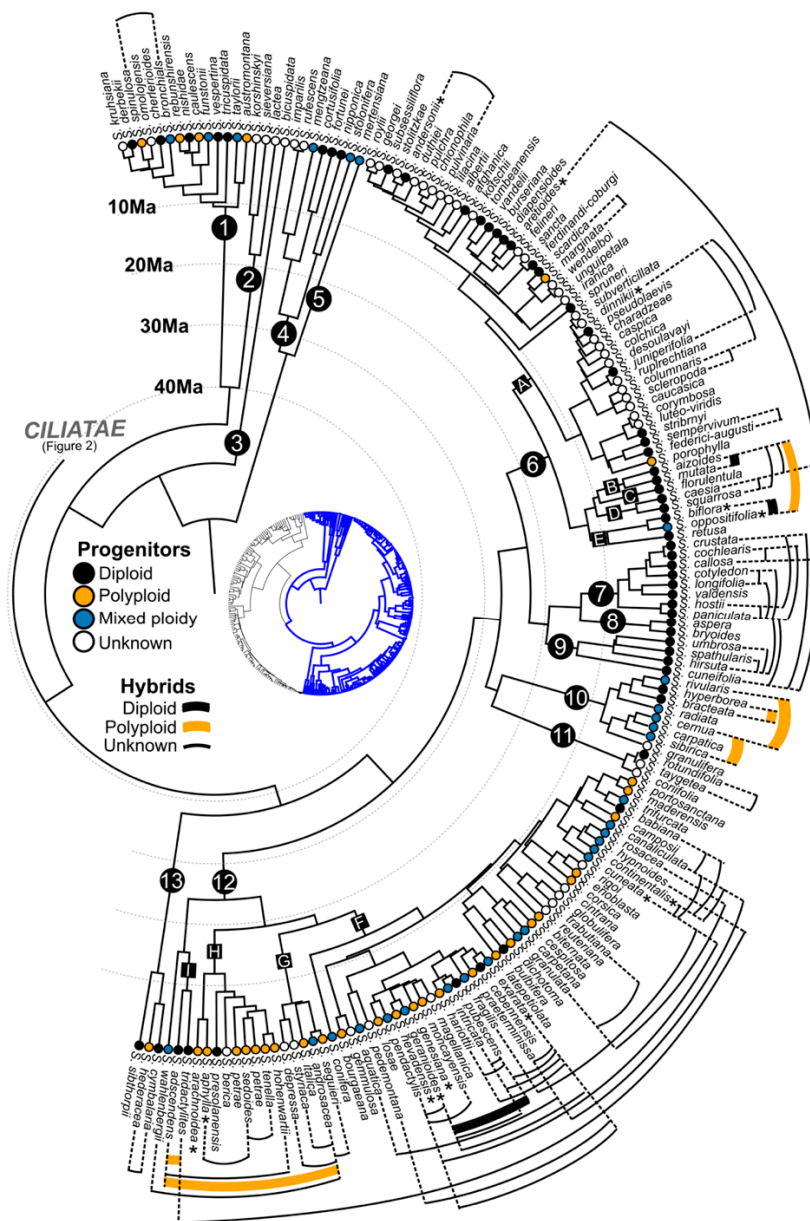


Figure 1. Time-calibrated phylogenetic tree of the genus *Saxifraga* (modified from [51]) excluding section *Ciliatae* (detailed in Figure 2): sections are indicated with black circles on branches leading to their respective crown nodes and are numbered as follows: 1. *Bronchiales*, 2. *Pseudocymbalaria*, 3. *Saxifragella*, 4. *Irregulares*, 5. *Heterisia*, 6. *Porphyron*, 7. *Ligulatae*, 8. *Trachyphyllum*, 9. *Gymnopera*, 10. *Mesogyne*, 11. *Cotylea*, 12. *Saxifraga* and 13. *Cymbalaria*. Subsections are indicated by black squares and white letters on branches as follows for sections *Porphyron* (A. *Kabschia*, B. *Mutatae*, C. *Florulentae*,

D. *Squarrosae* and E. *Oppositifoliae*) and *Saxifraga* (F. *Saxifraga*, G. *Androsaceae*, H. *Arachnoideae* and I. *Tridactylites*). The ploidy level of each species is indicated by coloured circles at the terminal nodes (black: diploids, orange: polyploids, blue: several ploidy levels whether or not including diploids, and white: unknown ploidy). Arcs connecting terminal nodes represent possible ancient or current hybridisation events described in the literature. The ploidy level of hybrids, if known, is shown as coloured arcs (thick black: diploids and thick orange: polyploids). Asterisks next to species names indicate hybridisation with a species not included in our tree.

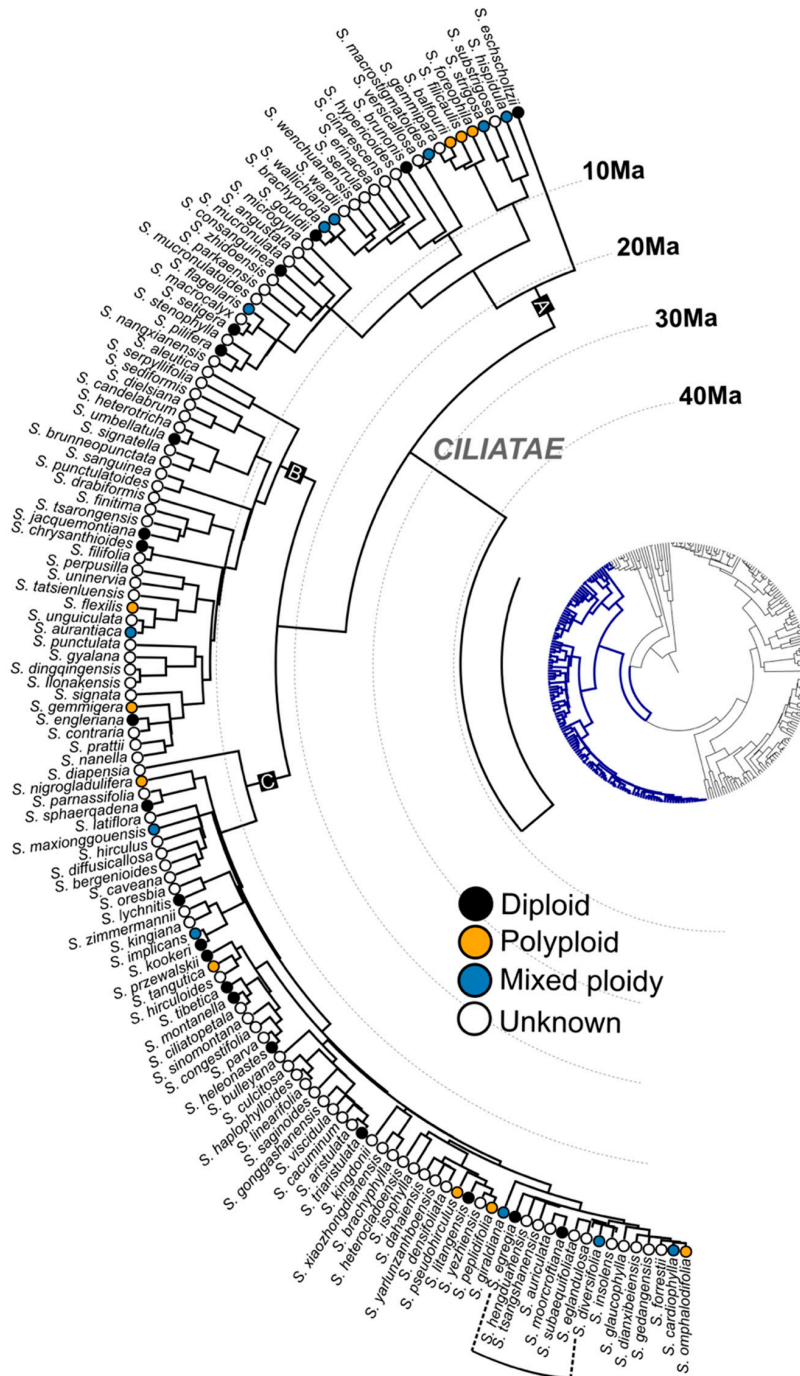


Figure 2. Time-calibrated phylogenetic tree of *Saxifraga* section *Ciliatae* continued from Figure 1 (modified from [51]): this section comprises three main clades indicated by white squares and letters on branches which include the following subsections: A. *Cinerascentes*, *Flagellares*, *Gemmiparae* and *Hemisphaericae*; B. *Rosulares* and *Serpyllifoliae*; and C. *Hirculoideae*. Colour coding is as in Figure 1.

Many of these natural hybrids are restricted to extremely narrow distribution ranges, such as single mountain tops. Most recent *Saxifraga* hybridisation zones were reported from major European mountain systems, for example, the Pyrenees and the European Alps (24 and 19 hybridising species pairs, respectively; Figure 3). Furthermore, hybridisation events among *Saxifraga* species were particularly commonly detected in Spain (N Spain excluding the Pyrenees: 11 hybridising species pairs). We also found evidence of hybridisation events for the British Isles, the Balkan Peninsula, the Caucasus Mountains and the Himalayas, albeit with much lower frequency (<5 hybridising species pairs each). Finally, relatively isolated hybridisation events have been reported from the Scandes, central Germany (Harz), the Carpathians, Morocco, Turkey, the Ural Mountains and Siberia. Strikingly, relatively few recent hybridisation events were recorded from Scandinavia and/or the Arctic ($n = 1$), but six out of 11 established hybrid species occur there. Even though it is almost impossible to know precisely where more ancient hybrid speciation took place, some of these hybrid species have extremely narrow distribution ranges (e.g., *S. opdalensis* Blytt and *S. svalbardensis* Øvstedal), which also likely represent their place of origin [30].

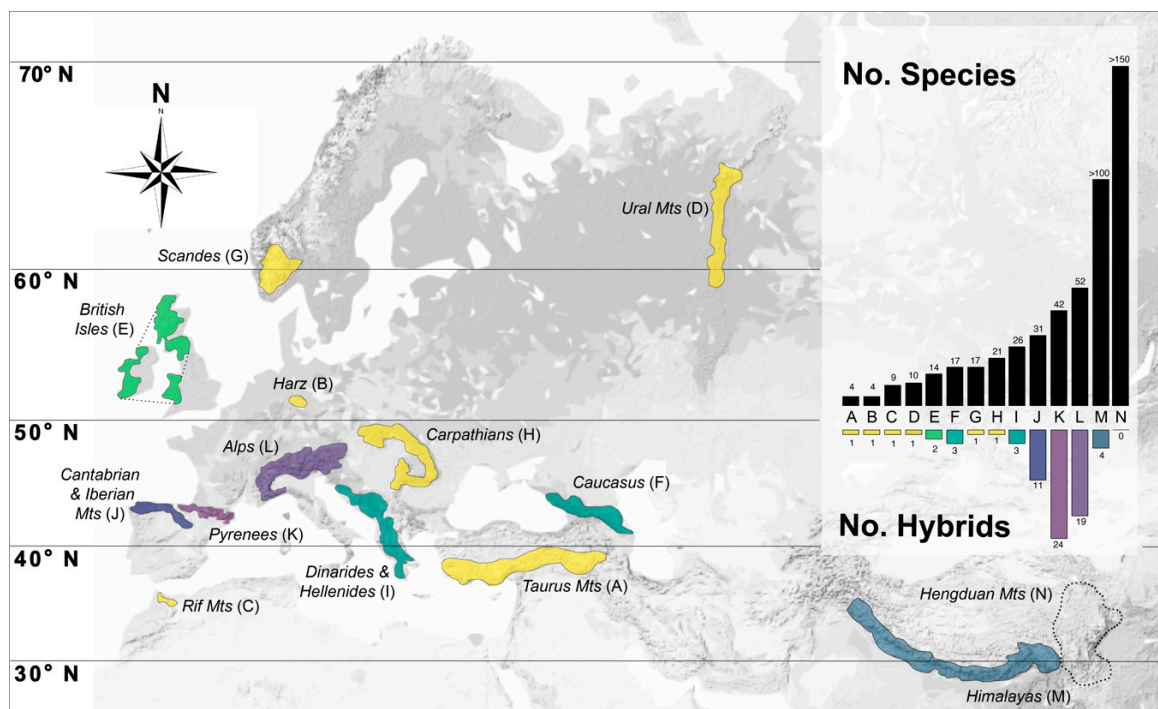


Figure 3. Hotspots of recent and/or ongoing hybridisation in *Saxifraga* in 14 mostly mountainous geographical regions: coloured areas represent approximate distribution of hybrid zones, and colours represent the number of recorded hybridising species pairs. The bar chart shows the approximate number of species in each of these regions based on the Global Biodiversity Information Facility (GBIF.org; DOIs of individual occurrence data download URLs are listed in Table S2). One hybridisation event from Siberia is not depicted because the available locality information was not accurate enough. The distribution of established hybrid species is not included, as it is uncertain where these species originated. This map is based on Google Maps Terrain Map (<https://maps.googleapis.com/maps>) created using the ggmap package [52] in R [53].

Surprisingly, hybridisation appears to be relatively rare in the mountains of Asia which house two fast-evolving clades, taxonomically treated as section *Ciliatae* subsection *Hirculoideae* and section *Porphyron* subsection *Kabschia* (Engl.) Rouy and Camus [44]. Despite thorough morphological work [39,41,45], no reliable reports of hybridisation or introgression exist for *Ciliatae*, the largest *Saxifraga* section, which has its centre of diversity in the Tibeto-Himalayan region. We could only find two circumstantial accounts of potential gene flow between species of this section. First, higher

than expected heterozygosity in microsatellite loci could be due to gene flow between *S. egregia* Engl. (subsection *Hirculoideae*) and a close relative such as *S. diversifolia* Wall. ex Ser. [54]. Second, triploid individuals discovered in four otherwise diploid *Ciliatae* species were suggested to be hybrids [55]; however, this has not yet been tested using molecular genetic methods and it remains unclear whether intra- or interspecific hybridisation was involved. In contrast, little to no evidence for hybridisation was found in *Ciliatae* subsection *Rosulares* using molecular methods [56]. Thus, hybridisation in the large section *Ciliatae* and its distribution across its subsections clearly need to be assessed more thoroughly, particularly as populations from distinct species can occur in close proximity of each other (<10 cm [57]). In section *Porphyrium*, which is also species-rich in Asian mountain systems, only four species pairs were reported to hybridise in the Himalayas [58] and only three species pairs are known to hybridise in the Caucasus Mountains [45]. Several additional species pairs were suggested to hybridise on account of herbarium specimen studies [59], but this has not been confirmed so far. Hence, based on our literature review, hybridisation in *Saxifraga* seems to overwhelmingly occur in Europe, whereas it appears to be almost absent from or not documented in other parts of the world.

As might be expected, hybridisation events most frequently occur among species which have diverged within the last few million years [6]. Hybridisation is usually encountered within sections, subsections, and series, for example, between members of the section *Mesogyne* with crown age ca. 7 Ma (Figure 1). However, some distantly related species or species which have diverged more anciently have also been shown to hybridise or to have hybridised. For example, in section *Saxifraga*, the likely progenitors of the hybrid species *S. wahlenbergii* belong to separate subsections (subsections *Tridactylites* (Haw.) Gornall and *Androsaceae* (Engl. and Irmsch.) Tkach, Röser and M.H.Hoffm.), which diverged at least 20 Ma (Figure 1) [50]. However, hybridisation is difficult to date precisely and may have occurred anytime during the evolution of these lineages.

Historically, presumed hybridisation between more distantly related *Saxifraga* species, for example, between *S. paniculata* (section *Ligulatae*) and *S. hirsuta* L. or *S. cuneifolia* L. (both section *Gymnopera*) [47] (Figure 1), has sparked particular interest to several authors. These observations are particularly striking as several of these intersectional crosses were reported between non-sister groups, which have diverged relatively anciently (e.g., sections *Ligulatae* and *Gymnopera* estimated to have diverged ca. 19 Ma [51]). However, according to the most recent molecular phylogenetic reconstruction of *Saxifraga* [42], intersectional hybridisation may not be as common in the genus as previously thought. For example, the hybridising species pairs *S. mutata* L. and *S. aizoides* L. as well as *S. squarrosa* Sieber and *S. caesia* L. [47] are now all considered to belong to the same section (*Porphyrium*), albeit to different subsections, and thus no longer constitute intersectional crosses. In addition, several older reports of intersectional hybrids, for example, between sections *Saxifraga* and *Porphyrium*, or *Saxifraga* and *Trachyphyllum*, still await confirmation and are currently considered unreliable [45,47]. This challenges the view of the perceived ease with which *Saxifraga* species are able to hybridise with rather distantly related congeners. Reliable accounts of intersectional hybrids seem to be restricted to crosses between sections *Ligulatae* and *Gymnopera* (Table S1). This observation is mirrored in topological incongruence and/or lack of node support with regard to these sections in plastid and nuclear DNA phylogenetic trees, which was previously hypothesised to be the result of ancient hybridisation and chloroplast capture [42,60].

In contrast, some closely related and broadly co-occurring species seem to be unable to hybridise, e.g., *S. aspera* L. and *S. bryoides* L. [47,61], which belong to section *Trachyphyllum* and have diverged only 2.5 Ma (Figure 1). This pattern in section *Trachyphyllum* clearly deviates from that of its closest relatives, sections *Ligulatae* and *Gymnopera*. Thus, whereas young and sympatric *Saxifraga* species appear to be predisposed to hybridising, this is far from being the rule for the genus.

4. Contemporary Hybrid Zones and Reproductive Isolation

The numerous reports of contemporary hybridisation in *Saxifraga* ($n = 73$) are almost exclusively based on morphological observations of wild populations displaying intermediate phenotypes of

two nominal species. In fact, these kinds of hybrid accounts are so common in *Saxifraga* that some authors have suggested that the identification and publication of intermediate hybrid populations had become almost like “a sport” at one point in time [47]. The sheer number of these reports clearly supports that natural hybridisation is a widespread phenomenon among *Saxifraga* species that live at least in partial sympatry, although parental species may no longer co-occur when their hybrids are found. These recent hybrids are usually short-lived [47], likely because their distribution is frequently narrowly restricted and reproductive barriers often seem to be in place to preserve the genetic integrity of parental species in the face of hybridisation.

Reproductive barriers may be either pre- or postzygotic and may act alone or sequentially. Both types contribute to varying degrees to reproductive isolation and, thus, preserve the uniqueness of the gene pool of each species [8,10,62–65]. In *Saxifraga*, several distinct reproductive barriers have been detected in some infrageneric groups, with low F_1 fertility, an intrinsic postzygotic barrier, being one of the most commonly identified. F_1 hybrid sterility was repeatedly reported to play a major role in populations of interspecific hybrids in some subsections of the section *Saxifraga* [66–68]. In addition to complete F_1 hybrid sterility, several instances of reduced F_1 hybrid fertility have been reported for some intraspecific *Saxifraga* hybrids. For example, in *S. hyperborea* (section *Mesogyne*), hybrids between populations from different arctic regions had drastically reduced pollen fertility and seed fertility [69], suggesting that divergent and locally adapted populations of the same species may not be interfertile. Interestingly, reduced F_1 fertility and sterility appear to be mediated through different mechanisms. These include reduced male fertility, in particular, due to the triploid block (for section *Ciliatae*, see [55]; for section *Mesogyne*, see [70,71]; and for section *Saxifraga*, see [72]), low seed set [73] you jump the ref 73 seed sterility (for section *Saxifraga* subsections *Saxifraga*, *Androsaceae* and formerly recognised subsection *Triplinervium*, see [67,68,74]) or a combination thereof (for section *Mesogyne*, see [69]). These properties seem to be widespread among *Saxifraga* hybrids, as pollen from hybrid herbarium specimens is often inviable and seeds are rarely included in original hybrid descriptions [47]. This pattern is similar to that of other plant systems, in which reduced F_1 fertility has commonly been established; examples include reduced seed set and pollen viability in *Helianthus annuus* L. and *H. petiolaris* Nutt. [75], reduced fruit and seed set in *Castilleja minata* Douglas and *C. rhexifolia* Rydb. [76], and reduced seed viability in *Antirrhinum valentinum* Font Quer [77].

Although F_1 hybrid sterility occurs in distant corners of *Saxifraga*'s evolution, it appears to be neither ubiquitous nor fully effective. For example, intraspecific hybrids of the section *Saxifraga* subsection *Saxifraga* (series *Ceratophyllae* (Haw.) S. Pawł.) produced predominantly weak, nonviable F_1 [78]. This suggests low F_1 viability or hybrid fitness, another commonly detected isolating mechanism [79], as the main barrier to gene flow. Importantly, some of these crosses did survive the F_1 stage and were able to produce viable F_2 offspring, suggesting that these kinds of crosses have the potential to give rise to long-lived lineages. Similar results were reported for artificial crosses between *S. adscendens* L. and *S. tridactylites* L. (section *Saxifraga* subsection *Tridactylites*), which showed low fertility but were able to give rise to few F_3 individuals under favourable greenhouse conditions [80]. This is reflective of a general pattern of postzygotic isolating barriers rarely causing complete reproductive isolation [9]. The reproductive success of such hybrids may be strongly environmentally mediated (extrinsic postzygotic barrier [65]). Such is the case for hybrids between *Silene latifolia* Poir. and *S. dioica* (L.) Clairv., which produce a normal seed set under benign conditions but have drastically reduced reproductive success under stressful conditions [81]. A similar pattern was observed for *Saxifraga* × *alpigena* Harry Sm. hybrids (section *Porphyron*), which showed more or less uniform, morphologically intermediate populations in several localities, characterised by low seed set and/or sterile seeds [58]. However, one of the surveyed populations exhibited the characteristics of a hybrid swarm, suggesting that localised environmental conditions such as environmental stability, microclimate or habitat heterogeneity could affect the long-term outcome of hybridisation. This also underlines the need for a comprehensive population sampling for studies tackling hybrid species not always prioritised in the past (e.g., [73]).

As evident from *S. × alpigena*, some *Saxifraga* hybrids seem to be subject only to weak fertility limitations or none at all. This allows the formation of hybrid swarms that are characterised by a morphological spectrum between the parental species, indicative of backcrossing afforded by interfertility. This seems to be a comparatively rare outcome of hybridisation in *Saxifraga*, with only 12 out of 73 contemporarily hybridising species pairs exhibiting this pattern, mainly in sections *Porphyrium* (5 out of 16 hybridising species reported to form hybrid swarms) and *Gymnopera* (2/2). However, some occasional hybrid swarms have also been reported for sections *Mesogyne* (1/2), *Ligulatae* (1/8) and *Saxifraga* subsection *Saxifraga* (3/40).

Hybrid swarms and parental backcrossing are of particular interest for the evolutionary implication of hybridisation. Introgression from or into the parental gene pools can result in long-term genomic consequences, including the transfer of favourable alleles (adaptive introgression [23]) or the eventual breakdown of genetic correlations (linkage groups [35]). This process has been observed in southwest Ireland, where introgressive hybridisation between *S. hirsuta* and *S. spathularis* is so common that pure populations of *S. hirsuta* are becoming rare [82]. This observation was confirmed by a recent study revealing density-dependent, asymmetric introgression into *S. hirsuta* [83]. Gene flow and introgression are often asymmetric because reproductive barriers frequently act asymmetrically (as for example, pollen competition in *Silene* [84]). In *Saxifraga*, this is exemplified by differential reproductive success (seed set and germination rate) of *S. biflora* All. × *S. oppositifolia* hybrids (section *Porphyrium*) depending on the maternal parent [73] as well as by observations of unidirectional introgression, for example, from *S. cochlearis* Rchb. into *S. callosa* Sm. ex Dicks. (section *Ligulatae* [85]) and from *S. sibirica* L. to *S. cernua* L. at their contact zone in the Ural Mountains (section *Mesogyne* [86]). These few cases are particularly important when investigating the role of hybridisation for the diversification of *Saxifraga* diversification. Introgression, in particular when it is adaptive, can be a major driving force in population differentiation, speciation and biodiversity [6,87]. This is seen, for example, in monkeyflowers (*Mimulus* L. [88]) and European white oaks (*Quercus* L. section *Quercus* [89]), and hybrid swarms have been suggested play an important role in originating adaptive radiation [7].

Unidirectional backcrossing and introgression, albeit at low levels, were also demonstrated for *Saxifraga × hausmannii* Kerner and its parental species, *S. aizoides* and *S. mutata* (section *Porphyrium* [90]). In this case, additional prezygotic reproductive barriers including phenological isolation and ecological isolation were shown to play an important role in counteracting the gene flow. It has thus become clear that the hybridising species of distinct infrageneric groups of *Saxifraga* are subject to a wide range of reproductive barriers and mechanisms counteracting gene flow. However, many questions remain regarding the role of these mechanisms in *Saxifraga*'s evolutionary history. For example, although several barriers may act in concert and thus increase the strength of reproductive isolation [9,91], studies investigating the gene flow among and within *Saxifraga* species usually concentrate on single mechanisms (e.g., [66,72,73,76,78]). Additionally, only a handful of studies have tackled gene flow and hybridisation on a genetic level (e.g., [48,50,68,70]), and genomic analyses are yet to be conducted. The roles of introgression and adaptive introgression are therefore far from being fully explained in *Saxifraga*.

5. Hybrid Speciation

In addition to numerous reports of recent or ongoing hybrid zones (Figure 3), ancient hybridisation has also given rise to at least 11 established *Saxifraga* species [50,67,71,92–94], either via autopolyploid; allopolyploid; or more rarely, homoploid speciation. Hybrid speciation events involving polyploidisation are common in *Saxifraga*, with a total of seven recognised allopolyploid or autopolyploid hybrid species. This is in line with findings regarding the role of polyploidy in the overall diversification of plants. A large proportion of angiosperm species are recent polyploids (ca. 35%), and polyploidisation played a substantial role in the process of speciation for 15% of them [95]. The process of speciation via polyploidisation is not only relatively frequent but also rapid, as it confers almost instantaneous reproductive isolation [22]. For example, it appears that some allopolyploid

species may be just a few hundred years old in *Mimulus* [96] and *Cardamine* [97]. In *Saxifraga*, examples of allopolyploid speciation include *S. nathorstii* Hayek ($2n = 52$), a cross between *S. aizoides* and *S. oppositifolia* (both $2n = 26$, section *Porphyrium*), and *S. osloensis* ($2n = 44$), via a hybridisation between *S. adscendens* and *S. tridactylites* (both $2n = 22$, section *Saxifraga*).

Similarly, autopolyploid speciation has demonstrably contributed to the diversification of some parts of the genus, for example, in *S. cernua* of section *Mesogyne* (tetraploid), which most likely is the result of an autopolyploid crossing event in *S. sibirica* (diploid [98]). Also, two independent rounds of autopolyploidisation, associated with ecological differentiation, likely were involved in the formation of the Madeiran *Saxifraga* taxa [67]. In addition to these speciation events, autopolyploidy has probably also been involved in the generation of species and populations with mixed ploidy levels as in *S. moschata* D. Don [67]. Species and populations displaying varying ploidy levels are abundant in *Saxifraga*, particularly in sections *Ciliatae*, *Saxifraga*, *Mesogyne*, *Bronchiales* and *Irregulares* (Figures 2 and 4). Although varying intraspecific ploidy levels are not necessarily associated with phenotypic differentiation [55], morphological and ecological differentiation between diploids and autopolyploids may occur in some cases [67]. This is particularly striking in the tetraploid individuals of *S. oppositifolia* (section *Porphyrium*), which have a narrower ecological niche than the diploid individuals and are characterised by varied growth forms including cushions [99]. Importantly, population structure was strongly driven by ploidy level rather than by geographical distance and only low amounts of gene flow were detected between diploid and tetraploid individuals, despite their co-occurrence in the same populations [100], suggesting that individuals of varying ploidy levels might be on a trajectory towards speciation.

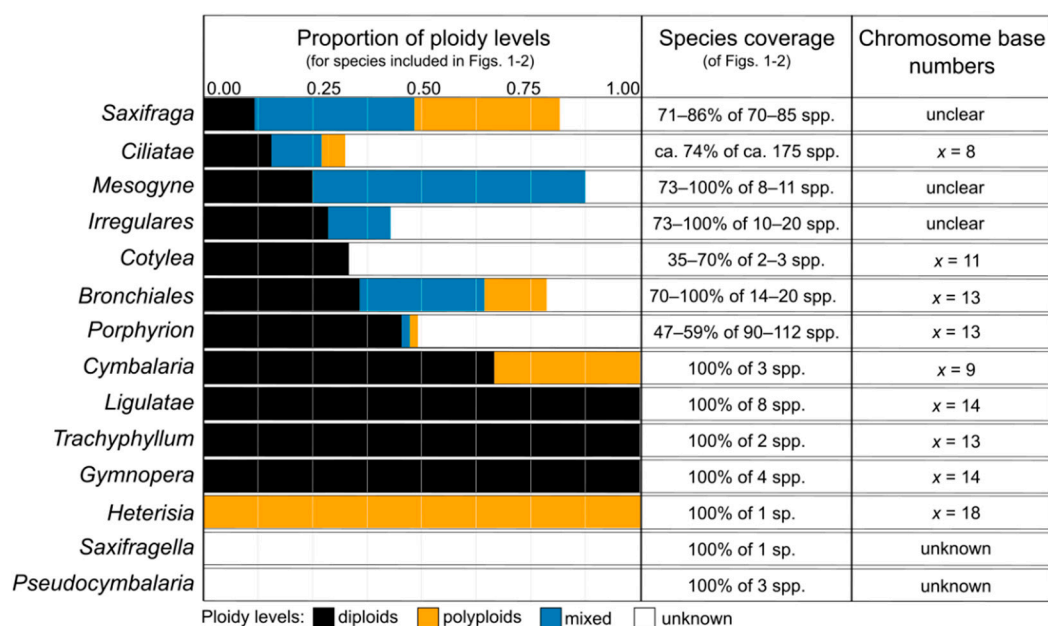


Figure 4. Ploidy levels within sections of *Saxifraga*, based upon the species included in our phylogenetic tree (see Figures 1 and 2; ca. 66% of accepted species): bars show the proportion of diploid (black) and polyplod (orange) species as well as species with unknown (white) or mixed (blue) ploidy levels. Information on species coverage refers to the percentage of species included in our study (Figures 1 and 2) with regards to the accepted number of species per section. Ranges are provided whenever the total number of species is still debated. Likely, chromosome base numbers were inferred from available chromosome number data (Table S3).

Homoploid hybrid speciation is also hypothesised for some taxa of the section *Saxifraga* (formerly recognised subsection *Triplinervium*) [67]. However, if a narrow view of homoploid hybrid speciation is adopted (e.g., see [24]), additional analyses on the *S. moschata* species complex are needed

to confirm whether hybrid speciation did in fact occur in homoploidy. Nonetheless, since uneven ploidy levels (e.g., triploidy) generally bring about sterility, parental backcrossing and hybrid swarms as described above are likely indicative of hybridisation in homoploidy. Thus, even though hybrid swarms generally point to current hybrid zones, it is likely that this process might also have been involved in past speciation events in *Saxifraga*. In general, homoploid hybrid speciation, although considered much less common than allopolyploid speciation, is increasingly acknowledged as a mechanism of speciation [20] such as in several species of *Helianthus* [101,102] and *Pinus* L. [103,104].

6. Phylogenetic and Geographic Distribution of Ploidy Levels

As exemplified by *S. oppositifolia* and *S. moschata*, a comprehensive analysis of ploidy levels and chromosome numbers will likely be critical in furthering our understanding of the diversification among species and populations, including the ongoing processes. *Saxifraga* is notorious for its cytological complexity including aneuploidy, dysploidy, occurrence of supernumerary chromosomes, homoploidy, and large differences in chromosome sizes among closely related species [44,66,67,105]. In addition, the genus displays a remarkable diversity in terms of chromosome numbers (e.g., chromosome base numbers $x = 8, 9, 11, 13, 14$ and 18 depending on the section), large infrageneric chromosome number variation (Table S3) and ploidy levels, even within sections and among closely related species. However, our knowledge of chromosome numbers and the relative frequency of diploid vs. polyploid (or mixed ploidy species) varies strongly across the genus (Figure 4). Here, we will only report on ploidy levels of species present in our phylogenetic tree ($n = 296$, ca. 66% of known species diversity), of which sampling was designed to optimise the morphological, taxonomic and geographic coverage of the genus [51].

The relative frequency of known ploidy levels varies strongly among sections (Figure 4). Overall, ploidy levels are only known for about half of the species in our tree, and information is disproportionately more complete for the European than for the Asian sections. For example, the European section *Saxifraga* (sampled $n = 59$) only includes ca. 18% of species of unknown ploidy level, whereas 72% of species in the Asian section *Ciliatae* ($n = 130$) lack any information on their chromosome number or ploidy level. With ca. 51% species of unknown ploidy level, section *Porphyryon* ($n = 53$) appears intermediate, although information is particularly scant in the Asian representatives of the section, such as in subsection *Kabschia*. This lack of available data for the Tibeto-Himalayan region makes it difficult to assess the role of polyploidisation for diversification in this region or to compare the speciation processes in distant mountain regions or between infrageneric taxonomic groups.

Despite a high proportion of missing data (no ploidy information available for 140 of 296 taxa in our tree, 47.3%), some patterns still emerge when ploidy levels are plotted onto the phylogeny of *Saxifraga* (Figures 1 and 2). Sections show varying proportions of exclusively diploid versus polyploid species, including those of variable ploidy. This is particularly interesting when combined with our results regarding hybridisation. For example, the section *Saxifraga*, which contains the highest number of hybridising species pairs, is dominated by poly- or mixoploid species (69.5%). In contrast, *Porphyryon*, *Saxifraga*'s second largest section with likewise widespread hybridisation (see above), contains only three species identified as polyploids in our sampling. Thus, diversification is not necessarily associated with polyploidy in this section. The sections *Trachyphyllum*, *Gymnopera* and *Ligulatae*, all closely related to *Porphyryon*, are also made up exclusively of diploids, despite displaying high to moderate degrees of hybridisation (except for section *Trachyphyllum*). This suggests that these groups are generally not especially prone to auto- or allopolyploidisation. Other sections, such as *Bronchiales* and *Mesogyne*, exhibit a different pattern with higher proportions of polyploid and mixed ploidy species, respectively, suggesting a greater role of polyploidisation in their evolutionary history. Indeed, the circumpolar species *S. rivularis* L. [93,106,107] and *S. cernua* [86] of the section *Mesogyne* show extremely complex reticulate evolution involving allopolyploidisation and interspecific gene flow, a pattern commonly observed in arctic taxa [30]. These processes could therefore have also been relevant to the evolution of other arctic *Saxifraga* species, for example, of the sections *Bronchiales* and *Pseudocymbalaria*.

Mixed ploidy levels can be indicative of inter- or intraspecific hybridisation. For example, all known hybridising species of the section *Mesogyne* display varying ploidy levels (Figures 1 and 4) but not all species displaying mixed ploidy levels are known to hybridise. The extent of species having mixed ploidy levels might therefore give us a better idea of gene flow in this section, even though we cannot yet distinguish between intraspecific and interspecific events. This reasoning could also be helpful for the section *Ciliatae*, for which very few chromosome counts and ploidy assessments are available. Out of a total of 43 species for which we could find information, seven were polyploid and 15 had mixed ploidy levels. This suggests that polyploidisation, associated with either intra- or interspecific gene flow, was likely an important process in the diversification of this section. However, the available cytological data are not sufficient to conclusively understand the role of polyploidy for the accelerated diversification observed in section *Ciliatae* subsection *Hirculoideae* [44].

7. Hybridisation and Polyploidisation as Drivers for *Saxifraga* Diversity

Our review of hybridisation and genome ploidy within *Saxifraga* shows that some extent of interspecific gene flow may have been an evolutionary companion of saxifrages for at least several million years. The distinct sections of this large genus vary widely in both their propensity to hybridise and the state of research on this subject. In particular, the three largest sections of *Saxifraga*, namely *Ciliatae*, *Porphyron* and *Saxifraga*, show widely contrasting patterns concerning the proportion of hybridising species, polyploidy and fates of their hybrids. Ultimately, this suggests that hybridisation and polyploidisation have played varying roles during diversification of the genus.

Section *Saxifraga* is certainly the best-studied section concerning hybridisation and cytology. This is likely due to its high proportion of hybridising species and its distribution in the major European mountain ranges, a combination which has led to a longstanding interest in the species of this section among European botanists. The large amount of empirical evidence reveals that many of the currently hybridising species pairs of this section are subject to postzygotic isolating barriers, which often lead to short-lived hybrid populations. However, extreme cytological complexity is observed in this section, including aneuploidy and dysploidy. Along with widespread topological incongruences produced by nuclear and plastid DNA data in the phylogenetic reconstructions [42], this complexity is suggestive of interspecific gene flow that produced long-term genomic signatures [108]. Largely overlapping distribution ranges in the Alps and the Pyrenees as well as incomplete reproductive isolation have likely enabled extensive hybridisation and polyploidisation, which appear to have contributed to the diversity in this section. Furthermore, this section exhibits a high degree of tolerance to chromosomal changes such as centric fusion or fission, which has likely further contributed to speciation [66,67]. Previous analyses, however, did not reveal increased diversification rates in this section [43], suggesting that the processes contributing to species differentiation were acting sequentially and not necessarily rapidly.

In contrast, the sections *Ciliatae* and *Porphyron* exhibited accelerated diversification compared to the remainder of the genus [43]. Our review showed that hybridisation is a common process in natural populations of the section *Porphyron* and that reproductive barriers seem to be ineffective at preventing backcrossing and introgression, often leading to hybrid swarms. This circumstance is also mirrored in the sheer number of artificial crosses (>100 [45]). At the same time, polyploidisation was likely not the rule in this section, which could have contributed to the apparent ease of hybrid swarm formation through the avoidance of uneven ploidy levels. Since hybrid swarms are assumed to be at the basis of rapid, adaptive radiations [7], this observation is particularly striking as it strengthens the view that rapid radiation in this group might have been adaptive, i.e., associated with significant eco-morphological differentiation and colonisation of novel adaptive niches. The rapid diversification in this section, especially in the subsection *Kabschia*, was shown to have been associated to the evolution of two key traits (“key innovations”), the cushion-habit and lime-secreting hydathodes, which facilitated the colonisation of new adaptive zones [43]. Taken together, these results could indicate a role of adaptive processes in the diversification of this group. However, differentiating the adaptive and the nonadaptive radiations is not trivial, since eco-morphological differentiation

can be cryptic or relate to nonobvious niche differentiation. Thus, even though hybridisation was not confined to the rapidly diversifying subsection *Kabschia*, it is very probable that interspecific gene flow contributed to accelerated adaptive diversification in this section. This has likely played an additional role in shaping the diversity of the section *Porphyron*. Finally, although the section *Ciliatae* is insufficiently investigated, the available evidence indicates that auto- and possibly allopolyploidy likely contributed to diversification. Still, we cannot draw any inferences on the relative contribution of this process compared to other scenarios, such as rapid allopatric speciation driven by habitat heterogeneity, soil properties and/or changing climatic conditions, as recently proposed [57], to overall species richness in this section. We had originally hoped to use the results of this review to deepen our understanding of the section *Ciliatae*. However, hybridisation and introgression are also not well-studied in its closest sister sections, *Bronchiales* and *Pseudocymbalaria*. Due to the large variation in frequency and outcome of interspecific hybridisation displayed by the rest of the genus, extrapolation of any of these insights to this section is impossible.

8. Perspectives in the Context of Mountain Biodiversity

Mountain systems have experienced varying degrees of glaciation and differ greatly in their overlap between present-day and last glacial maximum temperature profiles [109]. These differences are likely to have affected *in situ* diversification, including processes such as hybridisation and polyploidisation [32]. In theory, the observed differences in diversity, hybridisation and frequency of polyploids within *Saxifraga* (for instance, among sections *Saxifraga* and *Porphyron*) would therefore be highly suited to investigate the effect of the geographic context and differences among mountain systems, such as altitudinal ranges, habitat heterogeneity, and historical processes such as uplift or glaciation history. This requires a more detailed assessment of mountain attributes such as analysing the Himalayas, the Qinghai-Tibet Plateau and the Hengduan Mountains individually instead of as one contiguous mountainous region, as is often done. First and foremost, however, more comprehensive knowledge of ploidy levels, chromosome numbers and incidence of hybridisation as well as the mechanisms of reproductive isolation and their genomic basis is direly needed to assemble the full picture of their role in the diversification of *Saxifraga*, particularly of the Asian species. In addition, population genomics analyses and population ecological investigations will be required to fully unravel the intricacies of these processes in the distinct sections and regions of *Saxifraga*'s distribution and to compare their role to that in other arctic-alpine groups.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1424-2818/12/11/440/s1>, Table S1: *Saxifraga* hybrids included in this study and their geographic distribution, Table S2: List of GBIF occurrence download DOI URLs used to create Figure 3, Table S3: Chromosome number reports for taxa of *Saxifraga* used in this study.

Author Contributions: J.E. initiated the project and obtained the support of other authors to design it. All authors were involved with the review process. The manuscript and figures were elaborated by J.E. and A.F. with the support of N.T. All authors contributed to the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: Financial support for this study was provided by the German Science Foundation (Deutsche Forschungsgemeinschaft), project no. FA1117/1-2 to AF. We further acknowledge the financial support of the Open Access Publication Fund of the Martin Luther University Halle-Wittenberg. In addition, we thank four anonymous reviewers for their constructive feedback and are grateful to Joanne Ashnest for proofreading and language editing.

Conflicts of Interest: The authors declare no conflict of interest.

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