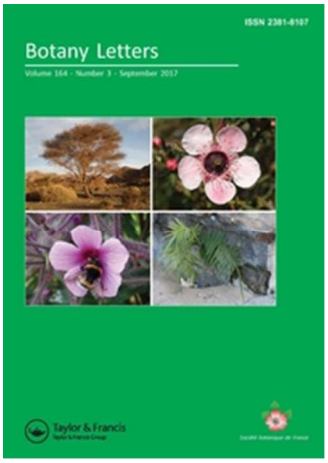

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Implications and future prospects for evolutionary analyses of DNA in historic herbarium collections

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Contribution: Co-wrote the paper.

Implications and future prospects for evolutionary analyses of DNA in historic herbarium collections

Abstract

Global herbarium collections house a vast number of plant specimens stretching back centuries. They include rare and extinct species, important historical collections, and valuable type specimens that could help to resolve long-standing taxonomic issues. Historical plant collections also provide a unique window into past evolutionary processes and population histories that does not exist with modern data alone. However, because the DNA in such specimens is often degraded and scarce, manipulation and analysis of their genetic material can be challenging. Recent developments in non-destructive genetic sampling and working with very small quantities of genomic DNA, especially in next-generation sequencing and bioinformatic analysis of ancient DNA, now make the majority of herbarium specimens potentially accessible to phylogenetic, population genetic, and barcoding studies. For example, studies of DNA from long time series of *Ipomoea batatas* and *Anacamptis palustri* collections revealed linkages between anthropogenic activity and changes in haplotype distribution. These time series also enable the identification of genes under recent selection in the genomes of both the plants and their pathogens, as was shown for *Phytophthora infestans*, a microbial pathogen of *Solanum tuberosum*. Here, we summarize the major challenges in using historical plant DNA in evolutionary studies and review genetic studies integrating herbarium specimens. We expect future genetic studies of historic herbarium specimens to use genomic, metagenomic, and population genetic approaches to: investigate how plant populations respond to environmental change; infer temporal changes in genetic diversity; identify genes under recent selection; and investigate past plant pathogens epidemics.

Keywords: ancient DNA, aDNA, historic herbarium collections, evolution, NGS, plants, genetics, genomics

Introduction

As invaluable botanical records of global plant diversity reaching into the 16th century (Sprague *et al*. 1931), the combined collections of the world's 3400 herbaria contain approximately 350M samples spanning near 400 years (Soltis, 2017) (**Figure 1**). Within these collections, the specimens themselves can be thought of as direct records of plant species' range and occurrences, their phenotypic variation and symptoms of disease, as well as time series of the phenological, demographic, and evolutionary changes and responses of populations to climatic variation, anthropogenic activity, and other biotic effects (Lister, 2011). The digitization of herbarium specimen metadata has accelerated in recent years (Tulig *et al*., 2012), and the availability of this information through online repositories like the Global Biodiversity Information Facility (GBIF) has improved natural history collections' accessibility to a diverse and general audience. This led to a remarkable expansion in the number and diversity of potential scientific applications for these records (Graham *et al*., 2004; Pyke and Ehrlich, 2010; Soltis, 2017).

Comparing modern genetic data with historic data obtained from herbarium specimens can offer unique insights into fundamental evolutionary questions. Although the utility of these collections for plant genetic studies were recognized early on (Rogers and Bendich, 1985), researchers found PCR amplification yields were extremely variable between specimens (Savolainen *et al*., 1995). Fortunately, in recent decades there has been an increase in the accessibility of the genetic information stored in these specimens, mostly due to advances in DNA extraction and sequencing approaches tailored for ancient DNA (aDNA), especially next-generation sequencing (NGS) (Pääbo *et al.*, 2004; Yoshida *et al.*, 2014). The evolution of genomes can now be directly observed without the need of inferring the past from modern samples. This is especially

relevant for endangered and extinct species, but also for invasive species (Staats *et al.*, 2013) as the analysis of these species can lead to a better understanding of past biodiversity and adaptation processes.

In this article, the challenges of using old, degraded DNA from herbarium specimens are addressed along with examples illustrating how such analysis can be applied in evolutionary studies. We offer a brief review of novel usage cases of genetics and genomic sequencing in herbarium collections (**Table 1**), and discuss the prospects for these approaches in future work. Throughout this article, we will refer to degraded DNA from historical herbarium specimens as aDNA, based on the presence of a detectable aDNA damage pattern, although we acknowledge that truly ancient plant specimens must be treated with even more stringency.

Challenges and recent progress for using herbarium specimens in genetic studies

As is generally recognized for all natural history collections (Pyke and Ehrlich, 2010), the use of herbarium specimens for ecological and evolutionary studies is limited by biases in the records themselves, including non-random and uneven collection over time and space (e.g. individual collectors contribute different taxa at different times), absences, errors and inaccuracies in associated metadata (e.g. label contains incorrect information on taxonomic identification or collection location), losses to flood and fire, or because particular specimens have been preferentially discarded by curators. Any study relying on genetic analysis of herbarium specimens should take care to assure that the study questions can be answered despite these unavoidable biases.

The particular preservation methods used can influence the quality and accessibility of genetic information in herbarium specimens (Pyle and Adams, 1989; Adams and Sharma 2010; Staats *et al*., 2011), although this general observation has not

been subjected to systematic study, likely because the voucher preservation method is not always obvious. More recent collections are often deposited after a gentle drying process using warm air, paper, and a wooden press, albeit historically harsher methods (e.g. treatment of specimens with formalin, ethanol, and/or mercuric chloride) were more popular (Doyle and Dickson, 1987; Nickrent, 1994; Srinivansan *et al*., 2002; Bakker, 2015). In tropical fieldwork scenarios, high-temperature drying and treatment with ethanol and formaldehyde are often the only way to achieve dry, fungus-free plant specimens for mounting (Bakker, 2015; Nickrent, 1994).

It follows that the age of a specimen can strongly influence the accessibility of its genetic information. Weiß *et al*. (2016) showed this is particularly true for preserved plants via their bioinformatic estimation that DNA in mounted herbarium specimens decays six times faster than in bone. Indeed, in comparison to more 'ancient' samples, DNA extracted from historical herbarium specimens can be extremely fragmented even considering their relatively young age (Adams and Sharma, 2010; Staats *et al*., 2011). Therefore, precautions used for truly ancient samples have been called for even in the use of historical plant specimens (Shepherd and Perrie, 2014). Since ancient specimens are often characterized by low DNA concentrations, attempts to amplify the endogenous DNA in PCR are prone to modern-day contamination. Thus special care should be applied during sample handling, and pre-PCR steps should be performed in dedicated clean labs (Pääbo *et al*., 2004). Although some specimens are simply too degraded to be used for sequencing, new methods for enriching DNA extracts with extremely short (40-100 bp) DNA fragments (e.g. Gutaker *et al*., 2017) push the boundaries of what can be sequenced to very low-quality specimens that contain mostly short fragments that are usually not recoverable with standard extraction protocols. For specimens too precious to be used for destructive sampling (e.g. holotypes), new non-destructive sampling

approaches (e.g. Shepherd, 2017) promise to make even these specimens available for genetic analysis.

Shortly after an organism's death, the endogenous DNA begins to decay due to exposure to intracellular nucleases and microorganisms as well as hydrolytic and oxidative processes. These processes lead to a substantial reduction in mean DNA fragment length as well as characteristic aDNA damage patterns. Most prominently at the single-stranded overhanging ends of double-stranded DNA fragments, the deamination of cytosine to uracil plays a major role, as uracil pairs with adenine while cytosine pairs with guanine during DNA-amplification. During PCR, this leads to C to T transitions (or G to A, depending on the strand sequenced) due to nucleotide misincorporation by DNA polymerase (Staats *et al*., 2011; Dabney *et al*., 2013). This characteristic damage pattern can be used to authenticate aDNA and has successfully been used to separate aDNA from modern contamination in studies of ancient hominins (Skoglund *et al*., 2014). However, damage patterns can also lead to problems, especially in direct PCR approaches, as primers might not bind to fragments with a high rate of C to T misincorporations. In addition, many of the short fragments found in the DNA extracts might not even cover the whole region of interest. Therefore, recovering the region of interest from aDNA is not always possible with direct PCR approaches. Moreover, the characteristic damage pattern is often masked by the primers during PCR as it occurs only at fragment ends. So the damage pattern cannot be used to authenticate the recovered sequences (Gutaker and Burbano, 2017).

In contrast, NGS approaches are better suited for aDNA as they are based on short fragments (modern samples are usually physically or enzymatically fragmented before NGS library preparation) (Yoshida *et al*., 2015; Gutaker and Burbano, 2017). Adapters are ligated to the ends of the fragments, increasing the number of fragments

used for sequencing in comparison with PCR approaches. Moreover, the fragment ends are sequenced, and the characteristic damage pattern can be used for authentication (Gutaker and Burbano, 2017). However, DNA misincorporation can lead to problems in the downstream analysis as some identified single-nucleotide-polymorphisms (SNPs) might only be present due to post-mortem DNA damage. To account for this problem, software tools like mapDamage (Jónsson *et al*., 2013) can be used in the bioinformatic analysis of the sequencing reads. Base qualities are then recalibrated based on the damage pattern, reducing the probability of falsely called SNPs.

Mining the diversity of species genomes stored in herbaria

Herbarium collections make available a wealth of specimens that otherwise could not be obtained or only via costly or difficult fieldwork, including rare specimens (e.g. types), endangered or extinct species, and geographically restricted populations (Shepherd and Perrie, 2014). Destructive sampling of such specimens to provide DNA for use in molecular systematics studies is a well established and now frequently used method (e.g. Savolainen *et al*., 1995; Wurdack and Davis 2009; Lehtonen *et al*., 2010; Koch *et al*., 2017; Martin *et al*. 2018). It is therefore possible to include herbarium specimens with rare morphotypes or from a wide range of sampling locations that would not easily be obtained through fieldwork (Olofsson *et al.* 2016).

Historic collections can also be used to study now-extinct species in a phylogenetic context. For example, Zedane *et al.* (2016) used genome 'skimming' data (low-depth shotgun sequencing) from an extinct, monotypic genus (*Hesperelaea* A. Gray, Oleaceae) that was only known from a single, 140-year-old collection to estimate the phylogenetic position of the genus.

Newly developed spatial phylogenetics analysis methods (Mishler *et al*., 2014) can draw surprising insights about the spatial distribution of plant genetic diversity that can be used to objectively determine where to focus biodiversity conservation resources (e.g. Thornhill *et al*., 2016; Baldwin *et al*., 2017). These methods require a reference library of conserved DNA barcode sequences for every taxon above a particular taxonomic level occurring within a continent-scale region. To complete the initial sequence alignment, authors of these studies utilize traditional PCR and Sanger sequencing of herbarium specimens to generate the missing DNA barcode data required to complement massive data collections already available on public repositories like GenBank (Benson *et al*. 2005).

High-throughput sequencing (NGS) approaches have already been shown to improve the accessibility of conserved genetic markers in degraded samples. For example, Prosser *et al*. (2016) used a nested PCR approach and Ion Torrent PGM (Life Technologies) NGS to recover some full-length DNA sequences of the COI barcode from degraded type specimens of Geometridae (Lepidoptera) collected up to 120 years ago, with per-sample costs near that of traditional Sanger sequencing. Although the authors utilized insect specimens, this approach can certainly be implemented with herbarium specimens as well. Hart *et al*. (2016) used a target enrichment approach to retrieve hundreds of transcriptome-associated nuclear loci from herbarium specimens of the Neotropical genus *Inga*, reporting success even with extractions yielding extremely low masses (16 ng) of DNA. Such approaches could become particularly valuable for connecting type specimens to genetic sequences, and therefore help to resolve long-standing taxonomic issues (Liimatainen *et al*., 2014). Sánchez Barreiro *et al.* (2017) used a modern genotyping-by-sequencing (GBS) dataset to design RNA baits that were used to enrich for restriction enzyme associated loci in historic herbarium specimens

collected up to 180 years ago. The targeted loci were enriched at 19- to 151-fold compared to shotgun sequencing. This approach is useful to integrate historic, degraded samples into existing reduced representation library datasets for population genetic studies (Sánchez Barreiro *et al*., 2017).

It has also been shown that *de novo* assembly of genome skimming data is a relatively straightforward approach to generate sequences of high-copy regions like whole plastome sequences and nuclear ribosomal units (Staats *et al*., 2013; Besnard *et al.* 2014; Zedane *et al.*, 2016; Bakker, 2017). The ongoing PhyloNorway project uses a genome-skimming approach to assemble plastome sequences and aims to complete a reference database for all Norwegian vascular plants, with the majority of the material coming from herbarium collections (Taberlet *et al*. 2018). If a reference sequence is already available, even low-copy genes can be investigated using NGS data, enabling comparative genomics (Besnard *et al.* 2014). Moreover, 'skimming' data from herbarium specimens can also be used to extract a large number of nuclear SNPs, which can be used to infer genetic structure (Olofsson *et al.* 2016).

In addition to improvements in recovering genetic data from historic herbarium specimens, new computational pipelines like PALEOMIX (Schubert *et al*., 2014), EAGER (Peltzer *et al.*, 2016) and ATLAS (Link, 2017) were developed specifically for genomic data from degraded specimens. Those software tools automate the bioinformatic analyses (e.g. DNA damage base quality recalibration, target capture enrichment efficiency calculation) that are required before evolutionary inference can begin.

Using herbarium specimens to study evolution, population history, and invasion

When drawing conclusions about the evolutionary history of species, inferences are

generally based on present-day patterns of variation. However, this approach may give an incomplete picture of population history. Major demographic events like population turnovers or bottlenecks can erase most of the genetic variation that existed in the past (Palkopoulou *et al.*, 2016; Leonardi *et al.*, 2017). Modern distribution of alleles and genotypes can therefore differ significantly from the past distribution. Simulation studies showed that this is especially the case when alleles are surfing on a migration wave (Edmonds *et al.*, 2004; Klopfstein *et al.*, 2006), as may be the case in, for example, expanding populations of an invasive plant. Sequence data from historical specimens provide direct insights into a genome's past states and offer the power to observe recent evolution in action. This direct genetic evidence can help reveal the true history of potentially beneficial alleles and therefore increase our understanding about why they experienced selection, especially when this is combined with other available data like climate records (Lister, 2011). For example, historic genetic data from *Ipomoea batatas* (Roullier *et al.*, 2012) and *Anacamptis palustris* (Cozzolino *et al.*, 2007) and metagenomic data from the plant pathogen *Phytophthora infestans* derived from 19th-century *Solanum tuberosum* collections (Martin *et al.*, 2013; Yoshida *et al.*, 2013) showed changes in the distribution of haplotypes that were linked to anthropogenic activity.

For plant population geneticists, herbarium specimens offer a unique opportunity to directly observe allele frequencies and population structure in historical times, as well as changes in these metrics in relation to the present day. Since the specimens are usually well preserved and contain metadata such as the exact sampling location, the collection date and information about the habitat, herbaria also provide information about historical geographic distributions (Chauvel *et al.*, 2006). Including historic samples in studies of genetic diversity often leads to surprising results. For

example, Saltonstall (2002) sequenced chloroplast markers from historic herbarium specimens of the reed *Phragmites australis*, finding that a non-native genotype had completed a 'cryptic invasion' of the Northeastern USA, with pre-1900 native genotypes being completely replaced by an invasive haplotype over a period of just 20 years. Roullier *et al.* (2012) used both modern and historic herbarium samples (collected between the 17th century and the early 20th century) of sweet potato (*Ipomoea batatas* [L.] Lam.) and found that most modern samples from eastern Polynesia carry a chloroplast haplogroup not found in the historic dataset from that region. Their results support the hypothesis that later introductions of sweet potato have replaced the initial pre-Columbian introduced population. Martin *et al.* (2014b) performed an analysis of common ragweed (*Ambrosia artemisiifolia*) from North American herbarium specimens and showed that the geographic boundaries between two genetic clusters had changed substantially during the last 140 years in association with the expansion of European agriculture. Cozzolino *et al.* (2007) compared plastid DNA intron haplotype frequencies in nearly 100 historic herbarium specimens of a rare orchid (*Anacamptis palustris*) to present-day populations from Italy, finding that human-mediated habitat loss precipitated the extinction of particular haplotypes of this endangered plant over the last century. Combining historic genetic data with records of human activity can therefore increase our understanding of how and why plant species spread or become extinct.

Inferring the history of alleles known to have been influenced by natural selection can help elucidate why they are advantageous. A scenario in which this could be especially useful is in the study of exotic plants that underwent rapid adaptation either before (Vandepitte *et al.*, 2014) or after (Hodgins and Rieseberg, 2011) invasion, specifically with regard to the genes underlying traits like improved growth and reproduction. As each introduction event can be seen as an independent natural local

adaptation experiment, historical genetic information can provide insight into fundamental questions regarding the evolution of introduced plants. In new results published as an online pre-print, Exposito-Alonso *et al*. (2018) report sequences of 27 historic genomes from herbarium specimens of *Arabidopsis thaliana* specimens belonging to a specific haplogroup (HPG1) that is the most abundant linage since the 1860s and possibly one of the first linages of *A. thaliana* that colonized North America. They discovered alleles that had risen to high frequency in modern populations and are connected to growth and life history traits. This may provide a path for future studies that directly investigate the rate and processes of adaptation in colonizing plants.

Just as the fossil record can be used to calibrate branch lengths and estimate divergence times in a Bayesian phylogenetic framework (Forest, 2009), historic sequences from herbarium specimens can be advantageous in phylogenetic studies. This approach was validated using historic herbarium samples with known collection dates as tip calibration points to probe the evolutionary timescale of the potato pathogen *Phytophthora infestans*, and relating the diversification of the species to the Spanish Conquest of Central and S. America (Yoshida *et al.*, 2013; Martin *et al.*, 2014a). The same approach was used in a study of *Arabidopsis thaliana* to estimate the substitution rate and the time of the most recent common ancestor, which relates to the colonization time of the plant in North America (Exposito-Alonso *et al*. 2018).

Studying plant pathogens and plant-pathogen interactions

Aside from the specimen's endogenous DNA, extractions from herbarium samples also contain metagenomic DNA from microorganisms that populated the sample (Yoshida *et al.*, 2015). This is especially useful in plant pathogen research as it enables directly probing the genetics of past disease epidemics and to reconstruct population dynamics

of the microorganisms (Yoshida *et al.*, 2014). Raw reads can be mapped against a reference for the pathogen as well as the host plant. Therefore, plant-pathogen interactions can be studied through time (Yoshida *et al.*, 2015).

Characterizing microbial communities contained within conspecific samples collected from various locations and times can enable studying the origins and introduction pathways of plant pathogens. Moreover, the evolution and population dynamics of the pathogens can be inferred when found in different time periods. Herbarium studies of the oomycete *Phytophthora infestans,* which causes late potato blight, showed that the genetic structure of the pathogen changed in Europe since its introduction during the 19th century, which triggered the Irish potato famine (Yoshida *et al.*, 2014). These collection-based studies also demonstrated that although the 19th-century outbreak was caused by the HERB-1 lineage, the distinct but closely related US-1 lineage was responsible for the early 20th-century global outbreak of late potato blight disease, completely replacing HERB-1 in Europe (Yoshida *et al*., 2013; Saville *et al*., 2016).

In an exploratory study that remains the only work of its kind, Schubert *et al*. (2014) showed that the metagenomic communities can differ substantially among herbarium vouchers. In their comparison of three historic, diseased domestic potato (*Solanum tuberosum*) specimens, one from Belgium collected in 1845 contained mostly gram-positive Actinobacteria, while two collected from Germany in the 1880s consisted almost entirely of gram-negative Gammaproteobacteria. The microbial communities that are preserved in historic plant samples remain an underexplored area, especially as they relate to past disease epidemics.

Malmstrom *et al*. (2007) used a reverse-transcriptase PCR approach to amplify and Sanger sequence barley yellow dwarf viruses from historic herbarium specimens of

various California grasses up to 100 years old. They detected phylogenetically diverse viruses were present on wild grasses back to 1917, with a strong association between California/Australia and France/Morocco. Their results suggested intercontinental virus dispersal mediated by humans in historic times and relate to the aforementioned studies of *P. infestans*, pointing to the importance of human trade and early colonial activities in facilitating the movement of plant pathogens between diverse localities.

Conclusion and future prospects

Methodological improvements in aDNA extraction and analysis now make it possible to access the vast number of plant genomes preserved in the world's herbaria. The genomic data can be used for diverse evolutionary analyses, including but not limited to estimating phylogenetic relationships and reconstructing population history. Comparison of historical and modern genomic data can be used to identify genomic regions under selection, and further functional analysis of those regions make it possible to identify pathways that contribute most to adaptation, especially in introduced and invasive plants.

Elucidating the mechanisms underlying adaptation of recently introduced plant species will be a fundamental step in understanding how species respond to environmental change and anthropogenic disturbance. This is of particular interest considering the pressing threat of global climate change, and as large numbers of species are introduced to new habitats. In order to understand how species have responded to these processes in the past, future studies should combine genetic and climatic data with metadata from voucher labels (e.g. information about the habitat) and morphological metrics taken directly from the pressed plants. For species represented by sufficiently abundant collections, it will be possible to infer temporal changes in

genetic diversity by grouping conspecific specimens (e.g. by time and/or sampling location) and then applying population genetic approaches. Thus predictions about future responses to environmental change can be based on direct measurements from the historic collections.

Finally, studying the history of interactions of plant pathogens with their hosts will increase the understanding of epidemics and inform the management of future outbreaks. Novel insights might be gained from metagenomic analysis of herbarium specimen DNA, which can reveal the presence and genotypes of pathogens associated with specimens collected in the past, perhaps even unrelated to known disease epidemics.

Disclosure statement

No potential conflict of interest was reported by the authors.

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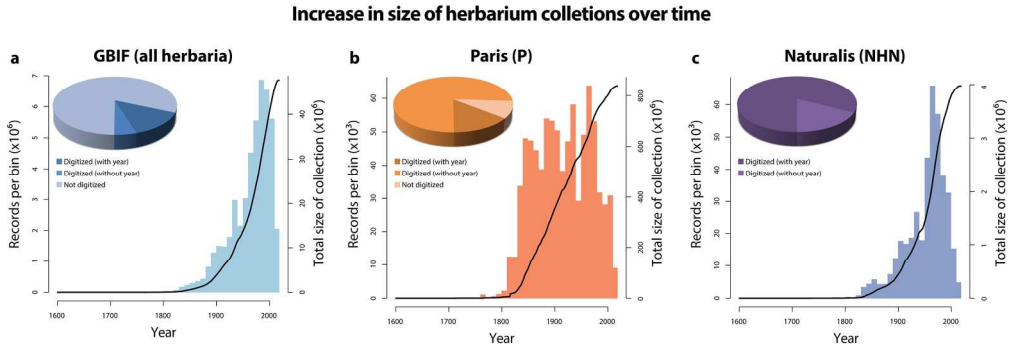

Figure 1: Trends in the number of digitized plant specimens available in global herbaria. (a) Growth of specimen collections in global herbaria over time. All data on all 'preserved specimens' in kingdom Plantae were obtained from GBIF yielding 64.7M records, of which 48.5M (75%) contained metadata about the collection year. (b) Growth of the vascular plants collection (P) at the herbarium of the Muséum National d'Histoire Naturelle (MNHN, Paris) over time. 5.4M (90%) of the estimated 6M specimens from P are databased in GBIF, of which 0.838M (15%) contain information about the collection year. (c) Growth of the Naturalis Biodiversity Center (NHN) collections over time. All 4.8M specimens from Naturalis are databased in GBIF, and 0.832M (17%) of these contain no information about the collection year. In each panel, the bars show the number of specimens collected in each 10-year period, while the black line indicates the cumulative total number of collected specimens. The general trend shows an increasing rate of global specimen deposition starting from 1800, with very few specimens collected before. The growth rate of herbarium collections dropped markedly during the periods of World War I and World War II. During the last 30-40 years, the rate of specimen deposition has decreased, although it is not clear if this reflects a real effect or a time lag or bias in digitization efforts. It is important to note that for many historic herbarium specimens, the precise collection date may be unknown, although often an approximate collection can be gleaned from associated historical records (e.g. plants collected during a botanical expedition 1804-1806). Therefore although this information is missing in the GBIF database, those specimens could still be valuable in a study of temporal genetic variation.

187x64mm (300 x 300 DPI)

Table 1. Overview of genetic studies using herbarium samples. *N*, number of herbarium samples used in the study. If marked with *, other sources (fresh material and silica-dried tissue) were used in addition to herbarium samples. Date specifies the collection date of the oldest and youngest herbarium samples used in the study. E, extraction method used for herbarium samples with "1": use of an extraction kit (e.g. DNEasy Plant Mini Kit (Qiagen)), "2": use of CTAB, "3": use of PTB/DTT, "4 ": use of Phenolchloroform. L indicates the laboratory method used. R: "1" indicates that a reference genome was used, "0" indicates no reference genome was used. Marker(s) used in the studies, with "n": nuclear markers, "p": plastid markers, and "m": mitochondrial markers.

Taxa	Study system	*N*	Date	E	L	R	Marker(s)	Nature of study	Reference
Juniperus (Cupressaceae)	Plant	50	1930 - 2009	1	PCR	0	n: ITS; p: *petN-psbM*	Study of DNA degration	Adams & Sharma 2010
Solanum tuberosum L. (Solanaceae)	Plant	64	1600 - 1910	2	PCR	0	p: *trn*V- UAC/ *ndh*C	Analysis of introduction scenarios	Ames & Spooner 2008
12 Angiosperm families	Plant	73*	1870 - 2016	2	NGS	1	genome skimming	Chloroplast genome assembly	Bakker *et al.* 2016
Sartidia (Poaceae)	Plant	9	1914 - 1998	1	PCR, NGS	0	p: *rbcL*, *ndhF*, *matK;* assembly of chloroplast and nuclear ribosomal sequences; n: 8 low-copy genes	Genome assembly, phylogenetic analysis, adaptive changes	Besnard *et al*. 2014
Anacamptis palustris (Orchidaceae)	Plant	58*	1832 - 1948	2	PCR	0	p: *tRNA-Leu*	Temporal changes in genetic variation	Cozzolino *et al.* 2007
Arabidopsis thaliana (Brassicaceae)	Plant	36*	1863 - 2006	3	NGS	1	SNPs (genome-wide)	Estimate substitution rate, split between lineages and selection	Exposito-Alonso *et a*l. 2018
Arabidopsis thaliana (Brassicaceae)	Plant	20	1839 - 1898	2, 3	NGS	0	-	Tested extraction and library prep. methods to retrieve short fragments	Gutaker *et al.* 2017
Inga umbellifera (Fabaceae)	Plant	6*	1835 - 2009	1	Capture, NGS	1	Genome wide	Phylogenetic analysis	Hart *et al*. 2016
3 Bangiaceae species	Red algae	15	1874 - 2013	2	NGS	1	p, m: whole genomes	Phylogenetic analysis	Hughey *et al.* 2014

Taxa	Study system	*N*	Date	E	L	R	Marker(s)	Nature of study	Reference
Bunias orientalis (Brassicaceae)	Plant	149*	1953 - 2015	1,2	PCR	0	p: *trn*L-UAA, *trn*L-UAA-*trn*F-GAA , *trn*G-UCC ; n: AFLP	Population genetics, study of range expansion	Koch *et al.* 2017
Lindsaeaceae	Plant	158*	?	1	PCR	0	p: *rpoC1*, *rps4*, *trnL-F*, *rps4-trnS*, *trnH-psbA*	Phylogenetic analysis	Lehtonen *et al*. 2010
Phlegmacium (Cortinariaceae)	Fungus	236	1907 - 2009	1, 2	PCR	0	n: ITS	Phylogenetic analysis	Liimatainen *et al*. 2014
BYDVs, CYDVs (Luteoviridae) on grasses	Virus	54	1894 - 1958	1	RT-PCR	0	virus coat proteins	Phylogenetic analysis, (geographic) distribution of viruses	Malmstrom *et al.* 2007
Phytophthora infestans (Pythiaceae)	Oomycete (plant pathogen)	7*	1845 - 1955	2,4	NGS	1	complete mitogenomes	Phlogenetic analysis of mitogenomes	Martin *et al.* 2014a
Ambrosia artemisiifolia (Asteraceae)	Plant	473*	1873 - 1939	1	PCR	0	p: *psbA-trnH*, *atpH-atpF*, *psbK-psbI*; n: 6 microsatellites	Temporal changes in spatial genetic structure	Martin *et al.* 2014b
Ambrosia (Asteraceae)	Plant	48*	1925 – 2008	1	PCR	0	p: *atpH-atpF*, *matK, trnH-psbA, psbK-psbI, rpl16, rpoC1,* n: ETS, ITS	Phylogenetic analysis	Martin *et al.* 2018
508 *Acacia* (Fabaceae) species	Plant	*?*	*?*	1	PCR	0	p: *psbA-trnH* , *trnL-F*, *rpl32-trnL*, *matK* ; n: ETS and ITS	Phylogenetic analysis, biodiversity analysis, identification of areas of endemism	Mishler *et al.* 2014
Alloteropsis (Poaceae)	Plant	21	1953 - 2014	1	NGS, PCR	1	SNPs (genome-wide), complete chloroplast assembly; *ppc* and *pck* genes	Genetic structure, phylogenetic analysis, analysis of selected genes	Olofsson *et al.* 2016
Ipomoea batatas (Convolvulaceae)	Plant	57*	around 1600 - 1990	1	PCR	0	p: 6 microsatellites; n: 11 microsatellites	Temporal changes in genetic variation, testing introduction hypothesis	Roullier *et al.* 2013
Phragmites australis (Poaceae)	Plant	62*	before 1910	2	PCR	0	*rbc*L-*psa*I, *trn*L	Temporal changes in genetic structure	Saltonstall 2002

Taxa	Study system	*N*	Date	E	L	R	Marker(s)	Nature of study	Reference
Ambrosia artemisiifolia (Asteraceae)	Plant	38	1835 - 1913	1	Capture, NGS	0	RRL loci	Population structure, capture experiment design	Sánchez Barreiro *et al.* 2017
Phytophthora infestans (Pythiaceae)	Oomycete (plant pathogen)	66*	1846 - 1970	1, 2	PCR	0	n: *ras*, *PiAVR2*,SSR loci, m: P3 (containes *rpl*14, *rpl*5 and tRNAs)	Population structure, phlogeographic anaysis, migration pathways	Saville *et al*. 2016
2 Cucurbitaceaes species	Plant	11*	?	1	PCR	0	p*: rbcL*, *matK*, *trnL*, *trnL- trnF*, *trnH-psbA, rpl20-rps12*; n: ITS1-5.8S-ITS2	Phylogenetic analysis	Schaefer & Renner 2010
Cucurbitaceae	Plant	76	?	1	PCR	0	p: *rpl20-rps12*, *trn*L/*trn*L-F; n: ITS1-5.8S-ITS2	Phylogenetic analysis	Sebastian *et al*. 2010
6 species covering 6 families	Plants, fungi,	6*	1897 - 1990	2	NGS	1	whole genome	Test of *de-novo* assembly and comparision with sequences aligned to a reference genome	Staats *et al.* 2013
Sisymbrium austriacum (Brassicaceae)	Plant	42*	1829 - 1955	1	SNP assay	0	SNPs	Temporal changes in genetic variation, test for selection	Vandepitte *et al.* 2014
3 species covering 2 genera and 2 families	Plant	29	1737 - 2014	2, 3	NGS	1	-	Analysis of DNA damage pattern	Weiß *et al*. 2016
190 taxa from *Malpighiales* order	Plant	?	?	1	PCR	0	p: *atpB*, *matK*, *ndhF*, *rbcL*, m: *ccmB*, *cob*, *matR*, *nad1B–C*, *nad6*, *rps3*, n: *18S*, *EMB2765*, *PHYC*	Phylogenetic analysis	Wurdack & Davis 2009
Phytophthora infestans (Pythiaceae)	Oomycete (plant pathogen)	11*	1845 - 1896	3	NGS	1	SNPs (genome-wide)	Phylogenetic analysis, selection test, effector analysis, ploidy analysis	Yoshida *et al*. 2013
Hesperelaea palmeri (Oleaceae)	Plant	1	1875	1	NGS	1	p: complete genome, n: ETS, ITS1, ITS2, 18S, 5.8S and 26S, 5 low-copy genes	Phylogenetic analysis	Zedane *et al*. 2016