Advances in Conservation Research on Wild Plants with Extremely Small Populations and Considerations for Future Work: A Postprint

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Abstract

Biodiversity conservation represents a focal issue of global concern. Plant species with extremely small populations (PSESP) constitutes a novel concept in conservation biology proposed to "rescue and protect" China's wild plant species facing imminent extinction risk, guide national biodiversity conservation initiatives, and serve the nation's ecological civilization construction. This concept has attracted widespread attention within the international conservation biology community. During the 13th Five-Year Plan period, China launched the Rescue and Protection Project for Wild Plants with Extremely Small Populations, and in the Outline of the 14th Five-Year Plan for National Economic and Social Development of the People's Republic of China and the Long-Range Objectives for 2035, explicitly incorporated special rescue efforts for PSESP into major ecosystem protection and restoration projects. The rescue and protection of PSESP constitutes a systematic undertaking characterized by strong scientific rigor, high technical and professional requirements, and long-term duration. Emphasizing both "rescue protection" and "systematic research" represents the scientific approach to conserving PSESP. Conducting systematic research on PSESP protection constitutes essential work for guiding and supporting effective conservation. This paper systematically reviews recent research on PSESP protection, focusing on survey and assessment, eco-biological characteristics, propagation techniques, and genetic diversity, with the aim of establishing new theoretical foundations and perspectives for PSESP rescue and protection. Furthermore, based on the current state of research and future conservation needs, this paper proposes three considerations for prioritizing future PSESP protection research in China. This review may serve as a reference for systematic research on PSESP rescue and protection.

Full Text

Preamble

Conservation Research of Plant Species with Extremely Small Populations (PSESP): Progress and Future Directions

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Abstract: Biodiversity conservation is a global priority. The concept of Plant Species with Extremely Small Populations (PSESP) was proposed in China as a new conservation biology framework to guide urgent, science-based rescue efforts for the nation's most threatened wild plants and to support ecological civilization development. This concept has attracted widespread international attention. During China's 13th Five-Year Plan period, a comprehensive rescue and conservation program for PSESP was launched, and the 14th Five-Year Plan and Long-Range Objectives through 2035 explicitly incorporate PSESP rescue into key ecosystem protection and restoration projects. PSESP conservation is a long-term systematic endeavor requiring strong scientific, technical, and professional expertise. Balancing "rescue protection" with "systematic research" constitutes the scientific pathway for effective PSESP conservation, with systematic research serving as the foundation for guiding and supporting effective protection efforts.

This review systematically synthesizes recent research on PSESP conservation, focusing on population surveys and assessments, eco-biological characteristics, propagation techniques, and genetic diversity, aiming to establish new theoretical foundations and perspectives for rescue efforts. Based on current research status and future conservation needs, we propose three priority areas for future PSESP conservation research in China. This synthesis provides a reference for systematic research on PSESP conservation.

Keywords: plant species with extremely small populations (PSESP), surveys and assessment, eco-biological characteristics, propagation techniques, genetic diversity

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Wild species are crucial components of natural ecosystems. Yunnan Province, renowned as the "Kingdom of Plants," "Kingdom of Animals," and "World Garden," boasts China's richest biological resources yet also faces the most severe threats to its wild species. To rescue species facing imminent extinction, the Yunnan Provincial Forestry Department organized experts in 2005 to compile the "Project Proposal for Conservation of Extremely Small Populations of Endemic Wild Fauna and Flora in Yunnan," which introduced the concept of "extremely small populations of wild fauna and flora" without providing a formal definition. In December 2009, the Yunnan Provincial Forestry Department and Department of Science and Technology jointly developed the "Outline and Emergency Action Plan for Conservation of Extremely Small Populations in Yunnan (2010-2020 and 2010-2015)," defining "extremely small population species" (including both animals and plants) but omitting the "wild" designation.

In August 2010, the "National Implementation Plan for Rescuing and Conserving Extremely Small Populations of Wild Plants (2011-2015)" (draft) first used the term "extremely small populations of wild plants" without elaboration (State Forestry Administration, 2010). Commissioned by the State Forestry Administration, researcher SUN Weibang from Kunming Institute of Botany, Chinese Academy of Sciences, revised and supplemented this implementation plan and formally defined the concept based on the existing definition of "extremely small population species" (Sun et al., 2019). On March 23, 2012, the State Forestry Administration and National Development and Reform Commission jointly issued the "Notice on Printing and Distributing the National Engineering Plan for Rescuing and Conserving Extremely Small Populations of Wild Plants (2011-2015)" (Lin Gui Fa [2012] No. 59), which included the concept and definition of PSESP as "Special Column 2" within four special columns (State Forestry Administration, 2011). In 2013, the concept, definition, and characteristics of PSESP were formally published in the book *Conservation Practice and Exploration of Extremely Small Populations of Wild Plants in Yunnan* (Sun, 2013).

PSESP exhibit several key characteristics (Ma et al., 2013; Sun, 2013): (1) **Priority**, requiring urgent conservation attention; (2) **Emergency**, demanding immediate protection action; (3) **Rescue**, emphasizing rescue protection measures; (4) **Population-level focus**, grounded in conservation biology theories such as effective population size (Ne) (Frankham, 1995) and minimum viable population (MVP) (Shaffer, 1981); (5) **Human interference**, excluding naturally rare species; and (6) **Quantitative criteria** proposing that species with fewer than 5,000 mature individuals and populations with no more than 500 mature individuals qualify, with priority given to species having fewer than 1,000 mature individuals, especially those with $ $100 individuals (Sun, 2016; Sun et al., 2019; Sun et al., 2019).

Threat factors for PSESP include both intrinsic and extrinsic elements. Intrinsic factors encompass reproductive barriers, pollen limitation, inbreeding depression, low fruit set, low germination rates, high seedling mortality, low genetic diversity, and poor adaptive capacity. Extrinsic factors involve geological history, glacial periods, climate warming, natural disasters, interspecific interactions, herbivory, human collection, habitat fragmentation, and degradation. Many PSESP are relict species millions of years old—a gift from nature that could vanish due to sudden catastrophic events stemming from both direct habitat destruction and indirect human impacts such as fire, disease, and climate change (Crane, 2020). Each PSESP possesses unique characteristics and threat mechanisms requiring tailored conservation measures. Overall, PSESP conservation emphasizes integrated approaches combining in situ protection (particularly through establishing conservation plots or sites for populations outside protected areas) with ex situ conservation, near situ conservation, population reinforcement, and reintroduction for population restoration (Sun, 2013; Sun and Han, 2015; Sun et al., 2019).

The PSESP concept and conservation practice have significantly advanced plant biodiversity protection in China and influenced integrated research and conservation efforts globally. Since its inception, close collaboration between administrative authorities and academia has created opportunities for plant diversity conservation (Yang et al., 2015). Several reviews have summarized PSESP conservation progress through special issues, monographs, and publications. Ren et al. (2012) reviewed the status and challenges of PSESP conservation and reintroduction, offering future recommendations. The 2013 book *Conservation Practice and Exploration of Extremely Small Populations of Wild Plants in Yunnan* detailed the concept and characteristics while exploring conservation methods through case studies (Sun, 2013). In 2016, *Plant Diversity* published a special issue on PSESP research, presenting studies on seed/spore preservation, genetic diversity, reproductive biology, pollination, and seed dispersal, identifying threat mechanisms and proposing conservation measures (Sun, 2016). In June 2019, Science Press published *Research and Conservation of Extremely Small Populations of Wild Plants in Yunnan*, comprehensively summarizing the concept's evolution, policy history, and conservation outcomes, with detailed case studies on *Cycas* spp., *Manglietiastrum sinicum*, *Manglietia ventii*, *Craigia yunnanensis*, *Acer yangbiense*, *Rhododendron protistum* var. *giganteum*, *Hibiscus aridicola*, and *Aristolochia delavayi* (Sun et al., 2019). Yang et al. (2020) systematically reviewed China's PSESP conservation progress in *Biological Conservation*, identifying threat mechanism clarification as key and highlighting climate change, genomics applications, and conservation effectiveness assessment as future directions requiring interdisciplinary collaboration. In 2020, *Biodiversity Science* published a special conservation issue presenting 14 case studies on community surveys, viability analysis, reproductive biology, and molecular markers, emphasizing the need for research on population decline mechanisms and population reinforcement techniques (Ma, 2020a). Additionally, *Yunnan Forestry* issue 242 featured a special report on "14 Years of PSESP Rescue and Conservation" (excerpted from *Research and Conservation of Extremely Small Populations of Wild Plants in Yunnan*) (Ma, 2020b).

China's 13th Five-Year Plan PSESP rescue project achieved internationally recognized results (Sun et al., 2019; Yang et al., 2020; Crane, 2020). The 14th Five-Year Plan and Long-Range Objectives through 2035 explicitly incorporate PSESP rescue into key ecosystem protection and restoration projects (http://www.gov.cn/xinwen/2021-03/13/content_{5592681}.htm). PSESP habitats are included in national and provincial ecological redline zoning, and germplasm resources receive legal protection under Yunnan's Biodiversity Conservation Regulations (Sun et al., 2019; Yang et al., 2020). Yunnan has also integrated PSESP rescue into its provincial development plan (http://www.yn.gov.cn/zwgk/zcwj/zxwj/202102/t20210209_{217052}.html). China's engineering approach to ensuring long-term survival of rare plants offers a meaningful model for global conservation (Crane, 2020).

PSESP integrated conservation is vital for sustainable development and improving livelihoods but remains challenging due to limited successful cases and species-specific threat factors and eco-biological characteristics that require comprehensive methodological considerations (Ren et al., 2012). To better guide PSESP conservation, this paper systematically summarizes relevant research and discusses future priorities.

1.1.1 Clarifying Taxonomic Status as a Prerequisite for Prioritizing Conservation

Conservation resources are limited. Confirming species identity, particularly for taxonomically problematic groups such as species complexes, hybrid individuals, and ecotypes, prevents resource waste and unscientific management decisions. *Christensenia assamica* is known from only one site with 10 individuals in China, listed as a nationally protected species and among Yunnan's 62 priority PSESP (Cai et al., 2018). However, Liu et al. (2019a) re-evaluated the species globally using IUCN criteria based on herbarium and distribution records, suggesting it should be classified as Least Concern or Near Threatened. Cytological and systematic studies indicate Chinese populations may represent a distinct species from Southeast Asian populations, raising the question of whether to conserve it as a full species or a "special population" requiring further research. The giant tree rhododendron (*Rhododendron protistum* var. *giganteum*) exhibits variation in flower color and leaf pubescence among individuals within the same population. Recent research found these "key traits" cannot distinguish it from its progenitor *R. protistum*, with inter-population genetic differentiation correlating primarily with geographic distance, suggesting they represent a single species. Since all Chinese populations of both taxa occur within Gaoligongshan National Nature Reserve, allocating additional resources to prioritize "giant tree rhododendron" is unnecessary (Li et al., 2018a, 2018b). Field surveys of *R. pubicostatum* revealed it co-occurs with *R. bureavii* and *R. sikangense* var. *exquisitum* at three small sites in Yunnan. Comparative morphological, reproductive, and population genetic studies indicate *R. pubicostatum* originated from natural hybridization between the latter two species at an early stage, with ongoing introgression, meaning it represents a natural hybrid lineage rather than a taxonomically independent species, and thus does not require priority conservation (Zhang et al., 2020; Zhang, 2020).

1.1.2 Investigating Population Status as the Foundation for Rescue Conservation

Supplementary surveys and verification of PSESP population status and habitat characteristics are fundamental for implementing conservation and formulating scientific rescue measures (Sun and Han, 2015). Many PSESP may become extinct before detection, while some declared extinct species may be rediscovered through field surveys. Additionally, new distribution sites or populations have been found through intensive fieldwork. Although species richness, endemism, genetic variation, and distribution modeling are important for conservation, they all require field surveys as a prerequisite—even surveying a single population provides essential data for understanding population status and developing conservation plans (Volis and Deng, 2020).

Liu et al. (2020) re-evaluated China's critically endangered rhododendrons based on recent surveys, IUCN Red List criteria, and PSESP standards, concluding that among 12 critically endangered species, four require downlisting, one is Data Deficient (DD), and one may be extinct. Numerous DD rhododendron species urgently need field surveys, with some critically endangered species meeting PSESP criteria and requiring immediate rescue action. *Rhododendron adenosum*, previously considered extinct in the wild (Qin et al., 2017), was recently rediscovered near its type locality in Muli County (Yao et al., 2020). *Firmiana major*, thought possibly extinct, was rediscovered through systematic surveys in the Jinsha River basin (Yang et al., 2018). All Yunnan populations occur outside protected areas, and studies of population structure and regeneration dynamics show human disturbance has caused dramatic declines. While sprouting promotes regeneration, it cannot reverse population decline, necessitating PSESP status and urgent conservation (Li et al., 2020). *Orchidantha yunnanensis* was long mistaken for *O. chinensis* and considered possibly extinct until field surveys discovered 15 wild clumps (Cai et al., 2019). *Acer yangbiense*, listed in Yunnan's 2010 priority PSESP catalog with only five recorded individuals and assessed as Critically Endangered (Douglas and Chen, 2009), was later found to have 703 individuals across 12 sites, providing crucial data for threat reassessment and conservation optimization (Tao et al., 2020). *Manglietia ovoidea*, assessed as Critically Endangered, had unclear population size until Han et al. (2020) systematically surveyed only 80 individuals across six sites, all outside protected areas in heavily human-disturbed regions, indicating extreme extinction risk requiring immediate action. Similarly, *Michelia lacei*, estimated to have 50-60 mature individuals in fewer than five Yunnan sites, was found through eight field surveys (2014-2016) to have only 10 individuals at three sites, warranting emergency protection (Cai et al., 2017).

1.2 Integrated Biological and Ecological Research on Threat Mechanisms

Species' biological and ecological characteristics—including pollen and seed dispersal mechanisms, seed dormancy types, interspecific interactions, competitive ability, and habitat conditions such as soil physicochemical properties and microhabitat features—are critical for PSESP distribution, colonization, environmental adaptation, and survival. Theoretically, scientific and effective rescue measures for endangered PSESP can only be developed through comprehensive understanding of these traits. Seed and pollen represent primary dispersal vectors. European yew (*Taxus baccata*) shows strong inter-population differentiation, with limited seed and pollen dispersal and weak gene flow potentially constraining adaptation (Chybicki and Oleksa, 2018). Pollen limitation affects seed germination variably across species, and supplemental pollination may influence population structure and growth models (Baskin and Baskin, 2018). When conducting inter-population pollination, effects on seed germination must be considered.

Manglietia ventii and *Manglietiastrum sinicum* require pollinators, but low fruit set due to few fertile individuals and insufficient pollen limits population regeneration, suggesting conservation should focus on increasing fertile individual numbers or density (Wang et al., 2017). *Paphiopedilum spicerianum* relies on pollinators, yet despite severe population fragmentation maintains stable natural reproduction by attracting pollinators through co-flowering *Polygonum pubescens*, indicating conservation strategies should consider plant-plant interactions and pollinator-food resource relationships (Liu et al., 2020). Chen et al. (2016) studied pollination and seed dispersal in *Aquilaria sinensis*, identifying farnesene, trans-ocimene, and benzyl salicylate as major floral scent components, with noctuid and pyralid moths as effective pollinators and wasps as key long-distance seed dispersers, highlighting the need to protect these insects in conservation efforts. Tang et al. (2020) compared pollination of four PSESP under ex situ conservation at Kunming Botanical Garden with their native habitats, emphasizing the importance of understanding reproductive strategies when developing conservation plans. Community competition studies show *Magnolia wufengensis* primarily competes with *Castanea mollissima*, suggesting conservation could involve establishing protected plots with selective removal of surrounding chestnut trees (Liu, 2019). Interspecific competition is the main factor limiting *Styrax zhejiangensis* numbers under resource constraints (Wu et al., 2020).

Habitat conditions critically affect seedling survival. *Satureja thymbra* seedlings in shrub habitats are protected from human disturbance, indicating shrubland habitat should be conserved alongside the species (Pinna et al., 2021). Denney et al. (2020) demonstrated that microhabitats influence genetic variation, phenotypic plasticity, and adaptive capacity, with habitat fragmentation affecting pollen quality and pollinator availability, suggesting future conservation research should prioritize protection of specialized habitats or landscapes. *Malania oleifera* exhibits root hemiparasitism (Li et al., 2019). Since hemiparasitic plants differ substantially from typical autotrophic plants in ecophysiological characteristics, scientific conservation planning and industrial development guidance require systematic research on parasitic critical periods, haustorium development, and host plant contributions to nutrient uptake and growth (Li et al., 2019).

Climate change can cause significant evolutionary shifts and generate new genetic variation (adaptive or maladaptive), posing potential threats and increasing extinction risk for PSESP. Conservation planning and ex situ conservation should account for climate change (Liu et al., 2019b). Qu et al. (2018) used ecological niche modeling to examine current and future habitat distributions of six Chinese PSESP, identifying priority conservation areas (PCAs) using conservation planning software. They concluded that existing nature reserves or botanical gardens within PCAs are suitable for PSESP conservation, with future resources prioritized for PCAs, such as establishing additional botanical gardens for ex situ and near situ conservation outside current reserves. They recommended integrating climate change monitoring into China's PSESP conservation planning to mitigate negative impacts. Wang et al. (2019) assessed potential suitable habitats for *Firmiana danxiaensis* using Maxent models, suggesting that reintroduction sites for range expansion should be selected based on suitability conditions. Over the past 50 years, climate warming has altered phenology and compressed suitable habitat for *Metasequoia glyptostroboides* (Zhao et al., 2020). For PSESP in human-disturbed areas, ex situ conservation is the most economical, practical, and manageable approach.

Plant ecophysiology examines relationships between ecological factors and physiological processes, exploring plant-environment interactions, material metabolism, energy flow, and adaptation mechanisms. Long-term monitoring and life history studies of PSESP growth and development reveal threat mechanisms. Research on physiological responses to environmental stressors—including light, temperature, water, humidity, acidity, and alkalinity—on seed germination, seedling regeneration, photosynthesis, flowering, and fruiting elucidates responses and adaptive capacities to habitat changes, revealing ecophysiological mechanisms underlying endangerment and providing theoretical guidance. Currently, PSESP ecophysiological research remains relatively limited. Shi et al. (2020) used a Li-6400 portable photosynthesis system to measure light response curves and diurnal photosynthetic patterns in *Annamocarya sinensis*, revealing sun-plant photosynthetic characteristics. Since extant populations occur in shaded broadleaf forests and valleys, near situ and ex situ conservation and population restoration should be conducted in sunny habitats. Plant environmental adaptation depends on water availability, with most threatened species showing poor drought tolerance. Kang et al. (2016) cultivated *Pinus wangii* seedlings in soils of seven pH levels, finding weakly alkaline soils (optimal pH 7.69–8.42) most suitable. Since this species occurs in limestone areas of southeastern Yunnan with alkaline, nutrient-poor soils, reintroduction and population restoration should select weakly alkaline environments.

1.3 Research on Propagation Techniques

Reproduction and regeneration are critical life history stages for PSESP. Ex situ conservation, reintroduction, and population restoration depend on propagation techniques including seed storage, germination, seedling cultivation, and tissue culture. Efficient artificial propagation can rapidly increase population numbers and safeguard germplasm resources for sustainable use. Sexual propagation through seed cultivation maintains genetic diversity, while vegetative methods such as layering, cutting, grafting, or tissue culture are used for species difficult to propagate sexually (Deng et al., 2020).

Studying annual growth dynamics from seed to seedling—including germination rates, seedling survival, diameter, height, root length, growth increments, and growth rates—provides theoretical foundations for artificial propagation and breeding while guiding natural recovery. Yuan et al. (2019) found that mature old shoots of *Aquilaria sinensis* had higher cutting survival than young or medium shoots, with autumn cuttings and grafting outperforming spring methods. Grafting success was highest when rootstock height exceeded 100 cm, with grafting showing higher survival than cutting. Cao et al. (2020) reported that *Bretschneidera sinensis* seeds sown in river sand achieved 46.67% germination versus only 3.33% in red soil. Studies on *Illicium difengpi* seed germination and seedling characteristics showed seeds germinate in mid-March at ~20°C with high germination rates but low germination potential and index and long average germination duration, indicating nursery management should emphasize water and fertilizer management to promote coordinated above- and below-ground growth, with shading to improve seedling survival (Liu et al., 2021).

1.4 Genetic Diversity and Integrated Conservation Research

The core of PSESP conservation is preserving genetic variation levels (genetic diversity and structure) to maintain species' genetic integrity (Sun and Han, 2015). Genetic diversity research is crucial for developing conservation strategies, directly guiding sampling strategies and protection optimization. By 2020, 44 PSESP had undergone varying levels of genetic diversity research (Yang et al., 2020). Zhao (2011) compared genetic diversity between five wild *Acer yangbiense* individuals and over 1,600 artificially cultivated seedlings, proposing minimum sampling strategies for ex situ and reintroduction conservation. Parentage analysis of cultivated seedlings revealed pollen flow from outside the source population, suggesting undiscovered individuals or closely related species (Zhao, 2011; Yang et al., 2015), later confirmed by Tao et al. (2020). Chen (2017) analyzed genetic diversity across six wild populations (40 individuals), two ex situ populations (38 individuals), and four reintroduced populations (74 individuals) of *Manglietiastrum sinicum*, finding Kunming and South China Botanical Gardens preserved 70.27% and 32.43% of wild genetic diversity, respectively, while reintroduced populations maintained 46.85%–54.05%, providing direct guidance for future conservation.

Genetic diversity research reveals evolutionary history, potential, and environmental adaptability, informing conservation strategies. High genetic diversity is generally considered essential for population survival and stress resistance (Markert et al., 2010), with diversity enhancement being the best method for long-term viability (Ralls et al., 2020). However, not all PSESP have low genetic diversity; examples include *Manglietiastrum sinicum* (Chen, 2017), *Manglietia ventii* (Wang, 2017), *Acer yangbiense* (Yang et al., 2015; Chen, 2020), and *Craigia yunnanensis* (Yang et al., 2016). Soil seed banks, sex ratios, balancing selection, mutation rates, population history (bottlenecks, migration), and recent human disturbance all influence genetic diversity. No simple negative correlation exists between genetic diversity and extinction risk; effective conservation genetic strategies must integrate genetic diversity, deleterious mutations, and ecological factors affecting fitness (Teixeira and Huber, 2021). Studies show that severe population declines do not immediately reduce genetic diversity or fitness, possibly because soil seed banks serve as genetic diversity reservoirs (Munzbergova et al., 2018), explaining why some declared-extinct species reappear. For dioecious species, increasing female individuals enhances sexual reproduction and genetic diversity (Rosche et al., 2018). Research on *Eucalyptus caesia* shows older individuals harbor greater genetic variation, making them conservation priorities (Bezemer et al., 2019). Woody and herbaceous plants require different approaches: long-generation woody species are less sensitive to fragmentation and climate change, allowing seed collection from multiple populations for transplantation to increase effective population size, whereas herbaceous species require primary habitat protection (Chung et al., 2020).

Mutation is the source of genetic variation and can increase diversity, though not all mutations are beneficial. Plants accumulate few deleterious mutations during evolution, which can persist through linkage with beneficial variants or genetic hitchhiking (Zhang et al., 2016). Small PSESP populations often suffer inbreeding-related genetic diversity loss, reducing adaptive capacity. Limited distribution and small population size may correlate with accumulated deleterious mutations reducing fitness and limiting expansion (Willi et al., 2018). Deleterious mutation accumulation can cause mutational meltdown—population size and fitness decline leading to extinction (Zhang et al., 2016). Inbreeding reduces fitness by increasing homozygosity and expressing recessive deleterious mutations (Kyriazis et al., 2021).

Genetic rescue can increase diversity, improve fitness, and enhance adaptability. However, deleterious mutation effects must be considered. Genomic advances enable detection of deleterious mutations per species or individual to identify more threatened populations for refined ex situ conservation and genetic rescue. Kyriazis et al. (2021) argue large populations harbor more recessive deleterious mutations that become expressed during contraction, increasing extinction risk. Therefore, genetic rescue should introduce genetic material (pollen, seeds, or individuals) from small to large populations, as small populations have undergone purifying selection with fewer recessive deleterious mutations. Conversely, Del Vecchio et al. (2019) and Kim et al. (2018) suggest using genetic material from large populations to rescue small ones, as this reduces genetic load and increases fitness, especially when donor populations have large effective sizes. Typically, a minimum viable population of 1,000 individuals is required to prevent deleterious mutation accumulation (Harrison et al., 2019). If outbreeding depression is low, gene flow should be default in conservation planning for fragmented PSESP (Ralls et al., 2018), as it increases offspring genetic diversity and introduces adaptive variation, enhancing fitness (Fitzpatrick et al., 2020). James et al. (2018) found strong gene flow among fragmented *Cycas megacarpa* populations within 36 km, recommending supplementation planting to create potential metapopulations.

Declining whole-genome sequencing costs now enable deep analysis of PSESP extinction mechanisms through population history, long-term adaptive evolution, and rapid environmental adaptation. Yang et al. (2019) completed the first genome sequencing and assembly for *Acer yangbiense*, providing a high-quality chromosome-level genome foundation for threat mechanism research. Recent whole-genome resequencing of 105 wild individuals from 10 sites (unpublished data) revealed seven genetic components with inter-population admixture related to geographic distance and mating systems. Stairway plot and fastsimcoal2 analyses indicate an ancestral bottleneck 0.7–0.9 million years ago, with one population diverging ~60,000 years ago before experiencing separate bottlenecks. Deleterious mutation accumulation sites occur at low frequency, unevenly distributed across 13 chromosomes, with homozygous deleterious sites varying among populations and correlating positively with inbreeding depression. *Ostrya rehderiana*, with only five remaining individuals, persists because it purges severe deleterious mutations more effectively than congener *O. chinensis*, reducing inbreeding depression (Yang et al., 2018). Genome sequencing of *Malania oleifera* (Xu et al., 2019) and *Nyssa yunnanensis* (Mu et al., 2020) similarly provides foundations for threat mechanism research and integrated conservation.

2.1 Dynamic Updating of PSESP Conservation Lists

PSESP are characterized by severe human interference and priority, urgent, and rescue-based conservation at the population level. Developing conservation lists is prerequisite for implementing "rescue conservation" and forms the basis for action plans, with conservation actions serving as the means to prevent extinction or severe threat. China's rich species resources include many severely threatened species requiring rescue conservation. Therefore, based on assessment of conservation effectiveness for listed species, lists must be adjusted, revised, updated, and improved according to latest field surveys and research, using established selection principles and criteria to remove species no longer qualifying (e.g., those with large populations, taxonomic disputes, or confirmed natural hybrid origin) and species that have achieved conservation goals or reduced extinction risk, thereby freeing resources for species at the brink of extinction. The first five-year plan of the National PSESP Conservation Engineering Plan (2011-2015) has been completed with significant results (Yang et al., 2020; Sun et al., 2019) and high international praise (Crane, 2020). We recommend national-level revision and publication of updated PSESP conservation lists and corresponding rescue plans to guide the next 5–10 years of conservation and research. Provinces should also adjust existing lists; Yunnan recently released a draft updated list (2021 version) for public comment, containing 101 species with adjustment rationales and selection principles that can serve as a reference.

2.2 Establishing Conservation Effectiveness Evaluation Indicator Systems

Monitoring and evaluation are crucial for biodiversity conservation and environmental management decision-making. Scientific assessment enables precise conservation, avoiding waste of time, labor, and financial resources while buying time for critically endangered species. Despite the importance of long-term monitoring, studies exceeding 10 years remain rare (Van Rossum and Hardy, 2020). How many PSESP have been reintroduced or conserved ex situ? What are the costs and benefits? Which species have reduced extinction risk? Which require continued investment? Exploring and establishing scientific evaluation indicator systems is essential. What quantitative assessment methods, evaluation systems, indicators, standards, or norms define PSESP conservation success? Can survival rate, plant size, growth rate, flowering/fruiting, protected status, protected area size, genetic diversity proportion, or growth/reproduction under human intervention serve as success indicators? What do these metrics reflect? Most PSESP are woody plants with long generation times (seed-to-seed) requiring years to decades. "Flowering and fruiting" may be the most basic success criterion, yet even this is a lengthy process for many woody species. *Manglietiastrum sinicum* at Kunming Botanical Garden required nearly 30 years of conservation from seed to first flowering, while *Acer yangbiense* needs about seven years (Sun et al., 2017). Theoretically, successful reintroduction requires natural regeneration, self-maintenance, harmless habitat integration, and ecosystem participation—a standard no plant species has yet achieved. Griffith et al. (2021) demonstrated that ex situ conservation of a single *Pseudophoenix sargentii* population met the Global Strategy for Plant Conservation target of preserving >70% genetic diversity, indicating species-level genetic diversity alone is insufficient for evaluating effectiveness. Future PSESP conservation should emphasize population-level genetic diversity preservation ratios rather than species-level diversity alone. Population viability analysis may effectively assess threats, extinction risk, and recovery potential (Chen and Li, 2020).

PSESP represent national strategic biological resources. How to scientifically protect these resources while enabling sustainable use is a key challenge. Substantial resources (time, funding, projects, teams) have been invested, with

Yunnan alone allocating 21.58 million RMB for 92 projects during 2009–2017 (Sun et al., 2019). Are these investments worthwhile? What value do these species provide? How do they contribute to socioeconomic development and ecological civilization? Answering these questions requires deep evaluation of genetic characteristics, chemical biology, economic value, ecological adaptability, ecosystem services (soil conservation, restoration, sand fixation), and carbon neutrality value based on collected germplasm resources (seeds, DNA, in vitro cultures, living plants). Breakthroughs in key evaluation technologies and establishment of systematic evaluation systems and databases will provide scientific foundations for resource utilization.

Biodiversity conservation success is inseparable from economic structure and development (Wang et al., 2020). How can we better develop and utilize PSESP? How can use promote conservation? For PSESP with market demand and development potential, government support for enterprises or cooperatives could explore new models of nature conservation and resource utilization, developing ecological-industrial systems to improve livelihoods while promoting conservation. Breakthroughs in rapid propagation and standardized cultivation research for valuable PSESP can resolve market supply-demand conflicts and advance sustainable resource utilization.

2.3.1 Utilizing Monitoring and Research Big Data to Build Predictive Models

Most research addresses current conditions of single or few species, lacking systematic, comprehensive, long-term studies. Due to resource constraints, few species have clear life histories from seed to seedling. While new tools and methods continuously emerge and extensive field data accumulate, integrated studies remain limited. Zizka et al. (2021) used deep neural networks for automated conservation assessment of global orchids, achieving >80% accuracy and demonstrating robustness even with erroneous or limited distribution data, showing significantly lower geographic bias than IUCN methods. Computer-based distribution prediction and ecological niche modeling using specimen or population data are valuable, but small population sizes hinder accurate modeling for PSESP.

From a metapopulation perspective, habitat heterogeneity, stochasticity, biological invasion, dispersal, and species interactions all relate to conservation, yet integrated studies addressing these factors together are rare, and even rarer are those applying integration to solve conservation problems (Chase et al., 2020). Building predictive models based on PSESP distribution, abundance, population, associated species, community, habitat, phenology, climate, and genetic diversity data to study “formation-maintenance” and “extinction-endangerment-recovery” mechanisms and develop new theories represents a challenging but urgent frontier.

2.3.2 Conducting Minimum Viable Population Research to Better Guide Conservation

An MVP of 5,000 mature individuals is widely considered a species-independent guideline crucial for conservation planning. However, Flather et al. (2011) argue that true MVP varies from hundreds to tens of thousands depending on species, environment, density dependence, life history, and population growth rate, with no universal rule. Brook et al. (2011) contend MVP is necessary for conservation, and dismissing it due to imperfections or misuse concerns is reckless—like rejecting weather forecasts because they're sometimes inaccurate. Jamieson and Allendorf (2012) note the "50/500 rule" for effective population size (Ne)—Ne = 50 to prevent inbreeding depression, Ne = 500 for long-term maintenance—can mislead managers into thinking 500 individuals constitute MVP. Since census population size (Nc) is typically 10× Ne, long-term maintenance requires Nc $\geq$ 5,000, consistent with the MVP = 5,000 guideline. Frankham et al. (2014) argue genetic factors have been underestimated, proposing the "100/1000 rule" that requires MVP $\geq$ 10,000 and Nc/Ne ratios of 0.1–0.2 (Nc = 10–20× Ne).

Most MVP research focuses on animals, with limited plant studies. Lochran et al. (2007) analyzed MVP literature from the previous 30 years, finding only 22 plant species (10.58%) among 212 studied species, with an average plant MVP of 4,824 (range: 2,512–15,992). To advance China's PSESP conservation, we previously proposed guiding criteria of <5,000 mature individuals species-wide and \$ \$500 per population, prioritizing species with <1,000 mature individuals, especially those with \$ \$100 (Sun, 2016; Sun et al., 2019; Sun et al., 2019), providing selection guidance for national and provincial lists. Since MVP varies with species, life history, regeneration capacity, and generation time, future research must determine species-specific MVP levels through multi-species systematic studies to develop more scientifically robust guiding standards for global PSESP conservation.

2.3.3 Filling Research Gaps and "Research for Application" to Translate Theory into Practice

Ottewell et al. (2016) proposed a conservation framework integrating breeding systems, genetic diversity, differentiation, and gene flow, requiring comprehensive understanding of these aspects for PSESP conservation planning. Among European, North American, and Australian conservation plans, only North America extensively considers genetic factors (Pierson et al., 2016). Many conservation actions precede research or remain disconnected from it, with researchers and practitioners having limited interaction. Research results, threat mechanisms, and "perfect" conservation recommendations often remain in publications rather than being applied. How to translate population/community ecology, reproductive biology, and genetic research into conservation practice requires urgent attention.

PSESP physiological-ecological adaptation research is extremely limited, yet understanding adaptation can predict population trends and environmental responses to guide in situ and ex situ conservation, giving rise to the discipline of conservation physiology (Wikelski and Cooke, 2006). To better guide conservation, Cooke et al. (2021) proposed 100 conservation physiology research questions across ten areas including adaptation, environmental change, invasion, monitoring, species interactions, policy, pollution, restoration, endangered species, and urbanization. Research on soil microorganism-PSESP interactions is also lacking. While plant-microbe adaptation has been demonstrated experimentally, field studies are needed, particularly on free-living microbes (Kraemer and Boynton, 2017). Unlocking the "black box" of belowground processes could transform PSESP conservation.

Future research must strengthen integrated studies in comparative conservation genomics, ecophysiology, and rhizosphere ecology to deeply reveal evolutionary history, potential, and adaptive mechanisms, and to parse biotic and abiotic interactions, thereby constructing a scientific PSESP conservation theoretical system to better guide rescue efforts.

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Note: Figure translations are in progress. See original paper for figures.