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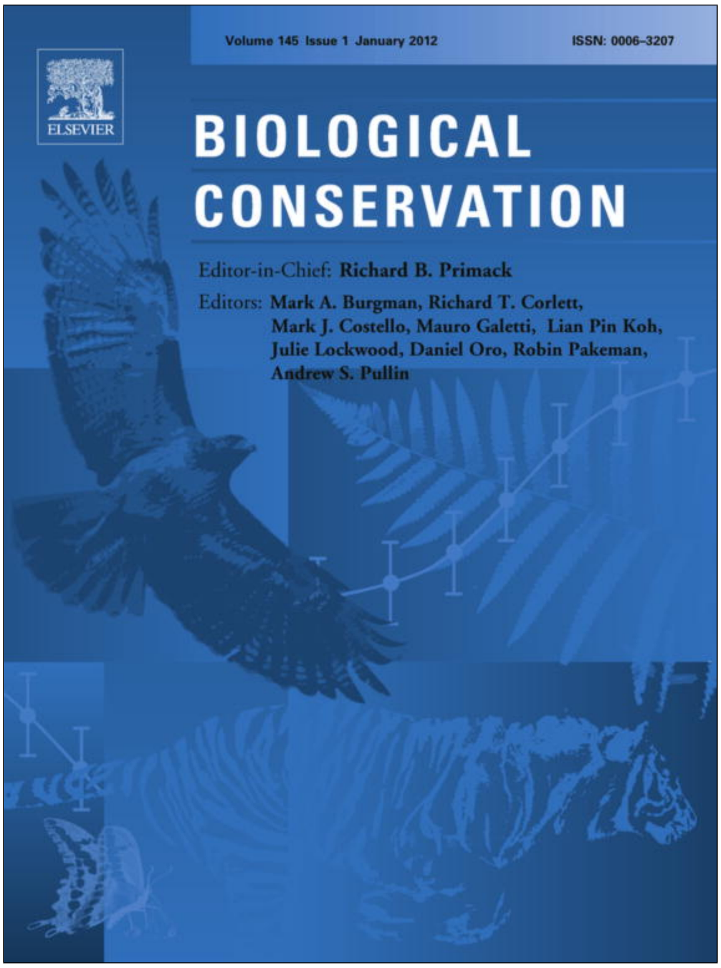

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Biological Conservation 150 (2012) 1–4

Contents lists available at SciVerse ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/biocon

Short communication

Too early to call it success: An evaluation of the natural regeneration of the endangered *Metasequoia glyptostroboides*

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ARTICLE INFO

Article history:
Received 18 September 2011
Received in revised form 14 February 2012
Accepted 23 February 2012

Keywords:
Restoration
Natural regeneration
Threatened species
Gymnosperms
Metasequoia glyptostroboides

ABSTRACT

Metasequoia glyptostroboides is a famous living fossil. It is one of the most successfully recovered endangered species based on the number of extant individuals and the distribution range. However, previous studies have revealed low genetic variation in restored populations. This paper evaluates the natural regeneration ability of the natural and restored populations. The seed masses and germination rates of restored populations were found to be significantly lower than those in natural populations, indicating decreased regeneration ability in the restored populations. The decreased germination rate in the restored populations may be due to inbreeding depression. Very low seed germination rates show that it is very difficult for the restored populations to regenerate naturally, consistent with field surveys. This is the first report on a species that has successfully produced hundreds of millions of individuals but has difficulty in regenerating naturally. Our study highlights the role of population viability analysis in delisting or downlisting species under protection.

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1. Introduction

Human activities have extensively altered the global environment and caused the extinction of species at a rate of 100–1000 times pre-human rates (Chapin et al., 2000). As extinctions have become apparent, many governments have imposed regulatory restrictions on activities that harm declining species (Scott et al., 2005). In addition, many efforts have been made to protect and recover these species to the point of self-sustainability. Number of individuals and geographical range are the simplest measurements used to evaluate the outcome of conservation efforts and have been employed in setting goals for many recovery plans. The US Fish and Wildlife Service has removed 26 species from the Endangered Species List based on these indices (http://ecos.fws.gov/tess_public/DelistingReport.do). However, the recovery in individual numbers and a wide distribution range does not necessarily mean the recovery of the self-sustainability of a threatened species, especially a long-lived species, in which the negative consequences may not be observed for a rather long time. Completion of the life cycle through flowering, fruiting, dispersal and seedling recruitment is a key benchmark of the potential self-sustainability of a plant population (Menges, 2008), and thus natural regeneration is considered a vital indicator of recovery success (Godefroid et al., 2011).

Metasequoia glyptostroboides (Taxodiaceae), the dawn redwood, is a famous "living fossil". The fossil record suggests that this species was once distributed widely through most of the Northern Hemisphere, including North America and eastern and middle Eurasia, and was believed to have been extinct for several million years (Yang, 1998/1999). The discovery of living individuals in the 1940s was one of the greatest botanical discoveries of the 20th century (Ma and Shao, 2003). Currently, it occurs naturally only in the boundary of Hubei Province, Hunan Province and Chongqing Municipality, China. Approximately 90% of the wild trees (about 5000 individuals) are in an area of about 500 km^2 in Zhonglu Town, Lichuan City, Hubei Province. Since the late 1940s, many efforts have been made to increase the number of *M. glyptostroboides* and expand its distribution range. At least six hundred million individuals have been bred (Wen et al., 2001) and are distributed in about 50 countries across the world (Ma, 2008), over a much wider range than the fossils indicate they had ever been (Yang, 1998/1999). In China, it is now a common landscape tree, particularly along the middle and lower reaches of the Yangtze River. At present, if evaluated by the number of individuals and their range, *M. glyptostroboides* is one of the most successfully recovered endangered plant species. However, using molecular markers, lower within- and among-population variation was found in restored populations than in natural ones (Kuser et al., 1997; Li et al., 2003, 2005), as frequently observed in restored populations of other plants (e.g., Kettle et al., 2008). For example, Li et al. (2003) found that the mean average percentages of polymorphic loci were 10.53–33.33% and 8.77–10.53% in natural and restored *M. glyptostroboides* populations

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http://dx.doi.org/10.1016/j.biocon.2012.02.020

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respectively, using RAPD markers. Decreased genetic variation may lead to biparental inbreeding and thus inbreeding depression, especially in gymnosperms because of their outbreeding manner (del Castillo et al., 2009; Husband and Schemske, 1996). Furthermore, although many restored populations are more than 50 years old and bear seeds, hardly any naturally regenerated seedlings or saplings have been observed in restored populations. Given similar conditions in soil and climate, we assumed that such a natural regeneration difficulty was caused by intrinsic factors in the restored *M. glyptostroboides* populations.

This study was designed to identify whether seed mass, seed germination rate or seedling growth rate were the likely limiting factors in preventing regeneration in restored populations of *M. glyptostroboides*.

2. Methods

M. glyptostroboides has a narrow natural distribution range, but has been widely introduced around the world (Fig. S1). In early November 2004, we collected seeds of *M. glyptostroboides* from natural and restored populations. The two natural populations selected had higher numbers of individuals than other populations, both of which are located in Zhonglu Town, Lichuan City. In population Guihua (GH), seeds were collected from ten trees. In population QT, which is located ~15 km away from population GH, we also harvested seeds from ten trees. Two restored populations were chosen for seed collections. Population TK is located in the village of Tangkou of Huangshan City in Anhui Province, where seeds were harvested from 13 individuals. The other population is located on the campus of the East China Normal University in Shanghai (ECNU), where seeds were collected from 18 trees. The two restored populations were both about 55 years old. In both the natural and restored populations, trees for seed collection were separated from each other by at least 30 m. To test replicability, we again collected seeds from the natural population (GH) in early October and restored populations in early November of 2009, respectively. We harvested the seeds of 30 trees from the natural population GH in Lichuan City. Four restored populations were harvested from a larger range than the previous 2004 collection, including 23 trees in TK and 24 trees in ECNU, using the same locations as in 2004. In addition, we harvested seeds from 23 trees (*M. glyptostroboides,* each about 50 years old) of Nanjing University in Nanjing City, Jiangsu Province (NJU) and from 19 trees (about 30 years old) at the Institute of Botany of the Chinese Academy of Sciences in Beijing (IB).

Weights per 200 seeds were measured to the nearest 0.0001 g. Seed were germinated using the following procedure: after being soaked in water at 45 °C for 48 h, the seeds were sown on March 23 and 24, 2005, and March 30, 2010 in 10 cm × 20 cm pots filled with garden soil and covered with sand. The first germinations were observed on April 5, 2005 and April 7, 2010, respectively. In our replication study, the germination rate and height of each seedling were estimated every week until May 20, 2010, when no newly germinated seedlings had been observed for 3 weeks. When the 2005 seedlings were about 5 cm in height, they were transferred to pots 7 cm in diameter. The 2010 seedlings were transferred to 72-cell propagation trays with 4 cm × 4 cm cells. The seedlings were watered every day to keep them wet. In September 2005, the seedlings were transplanted to a common garden. Once a month, beginning in June of each year and ending in December 2005 in the original study and in August 2010 in the replication, the height of each plant was recorded. The basal diameter of the stem and canopy cover of each seedling was monitored monthly from September to December in 2005.

Because some data were not normally distributed (Shapiro–Wilk normality test) or had unequal variance (Levene's test), the difference between each population pair was assessed by Wilcoxon signed-rank test. The relationship between germination rate and seed mass was analyzed by regression analysis. All statistics were performed using R language (R Development Core Team, 2010).

3. Results

The seeds collected in 2004 from the restored populations ECNU and TK weighed 1.06 ± 0.07 and 1.83 ± 0.16 g per 1000 seeds (PTS), respectively, which was significantly lower than those from the natural populations of GH (2.63 ± 0.11, $P < 0.001$ for both comparisons by Wilcoxon signed-rank test) and QT (2.53 ± 0.17 g PTS, $P < 0.01$ for both comparisons) (Fig. 1a). No significant difference was found between the two natural populations. However, there was a significant difference between the two restored populations ($P < 0.001$). Seeds collected in 2009 from restored populations NJU, IB and ECNU weighed 1.12 ± 0.06, 0.84 ± 0.08 and 1.16 ± 0.07 g PTS, respectively, which was significantly lower than those from another restored population, TK (1.61 ± 0.07 g PTS, $P < 0.001$ for all comparisons) (Fig. 1a). As in 2004, the seed masses of restored populations were significantly lower than those of the natural population GH (2.31 ± 0.09 g PTS, $P < 0.001$ for all comparisons) (Fig. 1a).

For seeds collected in 2004, no significant difference in the germination rate was found between the natural populations ($P = 0.762$) or between the restored populations ($P = 0.255$) (Fig. 1b). However, the germination rates of seeds from the natural populations were significantly higher (GH: 32.85 ± 3.30%, QT: 27.78 ± 4.96%) than for those from restored populations (ECNU: 1.93 ± 0.79%, TK: 5.26 ± 2.08%) (Fig. 1b). The findings were similar for seeds collected in 2009. The seed germination rate of the GH population in 2009 (8.98 ± 0.94%) was significantly lower than that of 2004 ($P < 0.001$). However, this value was still significantly higher than those for the four restored populations in the 2009 study (Fig. 1b). There was a significant relationship between germination rate and seed mass (adjusted $R^2 = 0.87$, Fig. S2).

There were no significant differences in the height of 1-year-old seedlings between populations (27.68 ± 1.42 cm, 28.04 ± 2.43 cm, 28.54 ± 1.69 cm and 29.84 ± 1.64 cm for populations ECNU, TK, GH and QT, respectively, $P > 0.05$). No significant differences were observed in either the basal diameter or the canopy cover of the 1-year-old seedlings of the restored and natural populations (the data are not shown).

4. Discussion

Natural regeneration via seed is the basis for both self-sustainability and the evolution of a species as it deals with changing environments. Seed siring and seed germination are critical for natural regeneration. In restored populations of *M. glyptostroboides*, seed siring has been observed in 17-year-old plantations (Botany Group of Nanjing Forestry College and Botany Group of Ningxia Agricultural College, 1977). In all of the studied populations, the seeds were collected from trees that were at least 30 years old, old enough to sire seeds in 2004 and 2009.

In the natural population GH, the germination rate of seeds collected in 2009 was lower than that of the seeds collected in 2004. This variation may have been due to the difference in the time of year that the seeds were collected. Seeds of *M. glyptostroboides* mature from late October to early November (Zhang and Zhang, 1980). In 2009, the seeds were collected during early October and were not fully mature, probably causing their lower germination rate.

Although there are no detailed data, germination rates in field conditions were thought to be much lower than those germinated in laboratory conditions (Zhang, 2000). The seed masses and germination rates of the restored populations were significantly lower

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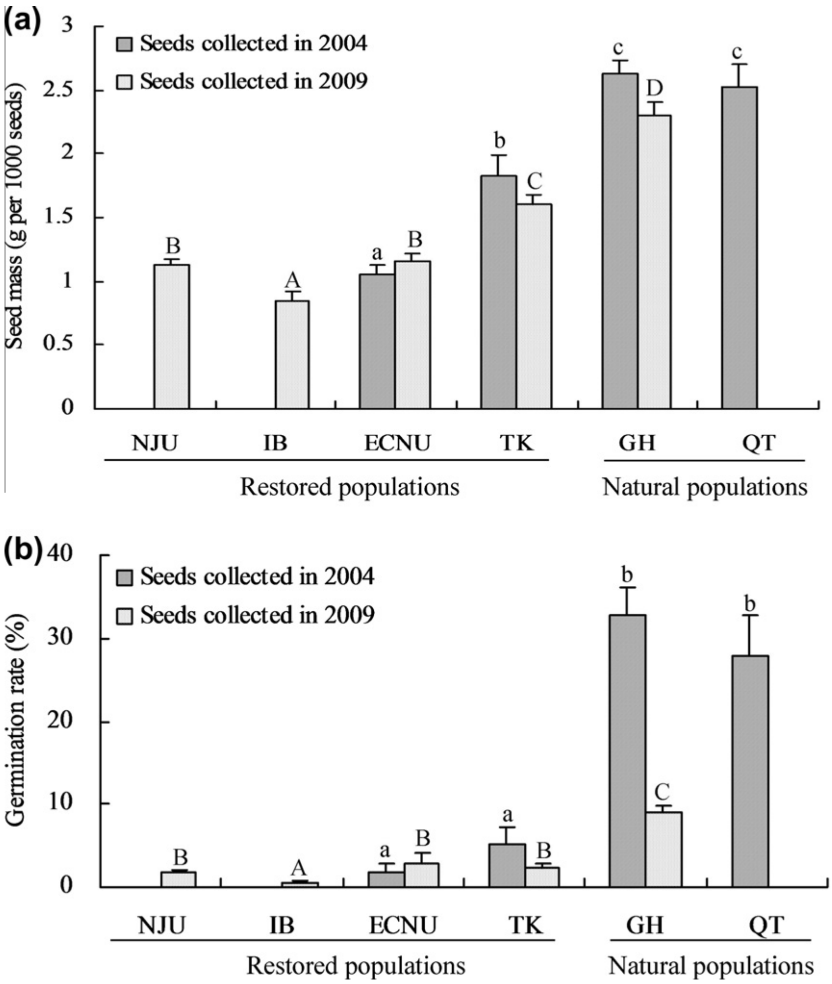

Fig. 1. Seed mass (a) and germination rates (b) of the restored and natural populations of *Metasequoia glyptostroboides* in 2004 and 2009. Bars indicate 1 SE, and different lowercase and uppercase letters show significantly different ($P < 0.05$) for values of 2004 and 2009, respectively, using Wilcoxon signed rank test.

than those of the natural ones in both 2004 and 2009. Such a pattern in the restored populations may indicate inbreeding depression, a negative consequence of high levels of genetic similarity among individual trees. The high levels of similarity in restored populations were due to inappropriate recovery methods. Traditionally, collecting seeds from advantageous trees is frequently used as a standard in tree breeding in China. This method has also been used in the recovery of *M. glyptostroboides*. Furthermore, unisexual reproduction methods, such as cutting, have also been used in seedling production, and at least 160 million seedlings have been produced in this way (Wen et al., 2001). This is evidenced by lower genetic differentiation among restored populations than among natural populations (Li et al., 2005) and by the highly similar genetic composition of individuals planted in different decades (Chen et al., 2003). High levels of genetic similarity among individuals may lead to serious inbreeding depression (Amos et al., 2001; Takagawa et al., 2006), as manifested by the smaller seeds and reduced germination rates found in the present study.

Decreased germination rates and no differences at the seedling stage indicate that inbreeding depression may have purged most inferior individuals during the early stages, such as seed production and seed germination. Many gymnosperms are wind-pollinated monoecious, and self-fertilization generally occurs at a low to moderate rate. However, most selfed seeds succumb to inbreeding depression, and the amount of inbreeding is very low at the seedling stage (Karkkainen and Savolainen, 1993; Koski, 1970; Sorensen, 1982). Vogl et al. (2002) estimated individual inbreeding coefficients for *Pinus radiata*, for example, a species that has a restricted natural distribution (there are only five natural populations), but has been widely planted across the southern hemisphere. They found that most individuals result from selfing, although some are unlikely to be selfed due to purging by inbreeding depression. For the endangered but long-lived gymnosperms, the consequences of inappropriate recovery methods will only become obvious decades later, as has been the case with *M. glyptostroboides*.

Although laboratory studies may not reflect the real status in the field, comparative studies in the same conditions can provide an assessment for conservation management. Our laboratory study may overestimate germination rates in the field because selection purging is more effective in less suitable environments (Husband and Schemske, 1996). Compared to the natural populations, significantly lowered seed germination rates suggest the decreased regeneration ability of the restored populations. Under field conditions, the percentage of successfully germinated seeds is likely to be much lower for the restored populations. In fact, we surveyed dozens of restored populations of dawn redwood and found no naturally regenerated seedlings, though one natural regenerated seedling was reported in the 1960s in a restored population (Botany Group of Nanjing Forestry College and Botany Group of Ningxia Agricultural College, 1977). However, abundant seedlings and saplings were observed in natural populations (Chu and Cooper, 1950), though a regeneration difficulty was also observed recently in natural populations (Tang et al., 2011), as a result of extensive anthropogenic disturbance.

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The present study is the first to highlight that conservation efforts have succeeded in producing several hundred millions of individuals, but have failed to recover the species' natural ability to regenerate. Reproductive failure in captive animals has made conservationists and managers consider inbreeding depression in captive animal breeding (Hedrick and Kalinowski, 2000). However, the successful reproduction of hundreds of millions of dawn redwood trees and their great longevity suggest that the consequences of inbreeding depression can be masked when negative consequences are delayed for dozens of years. Our findings also hint that we should reconsider apparent successes in recovering threatened species, particularly in cases involving long-lived plants. The criteria used to define success and the criteria for delisting species should not be based solely on the number of extant plants and their distribution ranges, but on population viability analyses that integrate demographic, genetic, environmental and catastrophic factors (Godefroid et al., 2011; Reed et al., 2002; Volis et al., 2005).

Acknowledgements

The authors thank Shenhou Fan and Lichuan Metasequoia Management Office for their help with seed collection, Guisheng Shen and Jing Li for their help with experiments. This study was supported by the National Natural Science Foundation of China (30700101, 31170492) and the Fundamental Research Funds for the Central Universities.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biocon.2012.02.020.

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