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Toward the restoration of a sustainable population of a threatened aquatic plant, *Nymphoides peltata*: Integrated genetic/demographic studies and practices

Jun Nishihiro[a,*], Ryuji Uesugi[b], Shinichi Takagawa[c], Izumi Washitani[a]

[a] Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi Bunkyo-ku, Tokyo 113-8657, Japan
[b] Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
[c] The Nature Conservation Society of Japan, Mitoyo Bldg. 2F 1-16-10 Shinkawa Chuo-ku, Tokyo 104-0033, Japan

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ABSTRACT

We provide a brief review of practices and relevant studies for restoration of *Nymphoides peltata* (yellow floating heart, Asaza), a distylous floating-leaf clonal plant, in Lake Kasumigaura, Japan to develop a model of integrated genetic and demographic conservation for threatened plants. Several theoretically expected issues, such as sudden extinction of small populations with low genetic diversity, limited seed production in small-sized local populations, and significantly high heterozygosity in adults that have survived environmental change, were ascertained through integrated studies on demography with discrimination of genets and genetics using highly polymorphic genetic markers. Investigation of genetic properties of the remnant soil seed bank suggested that the seed bank could potentially restore genetic diversity, although the fitness reduction of seed banks caused by inbreeding could affect the success of restoration. As a result of restoration efforts, increases in the number of local populations and genets in the Lake Kasumigaura metapopulation have led to population recovery.

1. Introduction

The ultimate goal for conservation of threatened plants is to establish or maintain self-sustaining natural populations that are genetically diverse and demographically stable. The processes required to achieve this goal include several steps: assessing the status of each population to set restoration goals, hypothesizing mechanisms of the decline, and implementing and managing adaptively. To assess the status of a population appropriately, integrated demographic and genetic studies are indispensable (Lande, 1988; Schemske et al., 1994; Oostermeijer et al., 2003). Because the degradation of a population is generally recognized by a decrease in its size, a primary plan for restoration usually includes measures to recover the number of individuals. However, even if population size can be recovered, negative genetic effects, such as loss of original genetic variation, may potentially cause reductions in the ability of a population to adapt to a changing environment (Barret and Kohn, 1991; Lande and Shannon, 1996). Furthermore, a reduction in the immediate fitness of progeny due to inbreeding depression (Charlesworth and Charlesworth, 1987; Keller and Waller, 2002) may persist for several or even hundreds of generations (Nei et al., 1975; Newman and Pilson, 1997; Kirkpatrick and Jarne, 2000). Hence, genetic assessment using appropriate genetic markers and indices can help to assess the risk of such impacts (Frankham et al., 2002).

In the case of demographic assessment of a clonal plant, investigations should be based on the genet rather than the ramet. The genet number fundamentally affects viability (Charlesworth and Charlesworth, 1987; Lande and Shannon, 1996) and evolvability (Frankel, 1974; Avise, 1994) of a population, while the ramet number contributes to population persistence via the longevity of a genet (Honney and Bossuyt, 2005). Hence, highly polymorphic genetic markers, which allow discrimination of genets, are useful for precise assessment of the status of clonal plant populations, especially in species without apparent morphological variations among genets.

Plans designed to recover degraded plant populations should cover all key life-history stages of the target plant, not only 'above-ground stages' from germination to reproduction, but also 'below-ground stages' or the soil seed bank, which is an important component of population dynamics and persistence (Silvertown and Charlesworth, 2001). Persistent soil seed banks seem to be an ideal source for recovering a plant population because they preserve some of the genetic diversity that the aboveground population had before its degradation (Levin, 1990; McCue and Holtsford, 1998). However, the effectiveness of seed banks for

* Corresponding author. Tel.: +81 (0)3 5841 8915; fax: +81 (0)3 5841 8916.
E-mail addresses: ajn@mail.ecc.u-tokyo.ac.jp (J. Nishihiro), consbiol@yahoo.co.jp (R. Uesugi), takagawa@wine.plala.or.jp (S. Takagawa), awashi@mail.ecc.u-tokyo.ac.jp (I. Washitani).

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genetic restoration varies, depending on species traits and population histories. If a seed bank is largely the result of inbreeding caused by population size reduction, seedlings from the seed bank and the immediately following generations may have low fitness caused by inbreeding depression (Apparicio and Guisande, 1997). Therefore, genetic properties of a seed bank population, as well as seed bank size, should be incorporated in plans for restoring a sustainable population.

Restoration programs should be conducted using an adaptive management approach, in which experimental restoration measures are conducted to test hypotheses on the mechanisms of the decline, because such programs are usually planned under conditions of uncertainty (Washitani, 2001a; Heywood and Iriondo, 2003). In the adaptive management cycle, citizen involvement in the implementation of management measures, as well as in goal setting, planning, and monitoring is recognized as an important component (Ludwig et al., 2001; Stringer et al., 2006).

Although a large number of studies on the demographic and genetic studies concerning conservation of threatened plants, case studies on population restoration fulfilling all above mentioned criteria are rare. Here, we provide a review of practices and relevant studies for the restoration of a threatened aquatic plant, *Nymphoides peltata* (yellow floating heart; Japanese common name, Asaza), in Lake Kasumigaura, Japan, to contribute to develop an integrated genetic/demographic study model. This species has advantages as a model species. First, the highly polymorphic genetic markers are available. Second, the demographic and genetic status of the population is well studied. Thus, hypotheses on the mechanisms of the population decline can be developed. Third, a comparatively large scaled restoration practice using soil seed banks has been implemented by The Japan Ministry of Land, Infrastructure, and Transport. Finally, this species has been used as a flagship species in an ecosystem restoration program coordinated by a NGO and many citizens, including school children, have participated in the program.

2. Study species

N. peltata (Gmel.) O. Kuntze, Menyanthaceae, a floating-leaf perennial plant, is widely distributed in temperate and subtropical zones of Eurasia including Japan. This species has a heterostylous (distylous) reproductive system: seed production therefore usually requires cross-pollination between long- and short-styled floral morphs (Ornduff, 1966). Although an unusual homostylous morph, occurring at a low frequency, can self-fertilize, the fitness of the offspring from self-pollination is suggested to be significantly lower due to inbreeding depression (Takagawa et al., 2006).

Individual genets of *N. peltata* often occupy a large area through extensive clonal growth by rhizomes to form patches of floating leaves. Aggregation of patches within several hundred meters and a group of such aggregations in a single water body provides sub- and meta-populations (Hanski and Gilpin, 1991), respectively, from a reproductive ecological view.

The most frequent flower visitors are solitary bees (Marui and Washitani, 1993) that generally forage within relatively small areas (Gathmann and Tscharntke, 2002), although an infrequent visitor, *Parnara guttata*, can fly long distances (Nakasuji and Nakano, 1990). On the other hand, seed and shoot dispersal by water allows episodic long-distance gene flow within a water body.

The seeds of the species have physiological dormancy (Smits et al., 1990). Germination is facilitated by chilling (stratification) and alternating temperatures, and is inhibited by submergence even in shallow water (Smits et al., 1990; Nishihiro et al., 2004a).

3. Importance of the Lake Kasumigaura population for conservation of *N. peltata* in Japan

In Japan, *N. peltata* was once common in freshwater habitats such as lakes, ponds, and brooks. However, most populations have disappeared as a result of anthropogenic habitat loss, so that the species is now listed as vulnerable in the Japanese Red Data Book (Environmental Agency of Japan, 2000). A nationwide population survey in 2001–2003 revealed that 27 metapopulations consisting of 64 local populations remained in the Japanese archipelago (Uesugi et al., 2009). In the survey, local populations and metapopulations were operationally defined as aggregations of patches occurring <500 m apart from each other and groups of local populations in a single water body, respectively. Among the recognized local populations, only one in Lake Kasumigaura, a large (220 km^2 surface area) and shallow (4 m average depth) freshwater lake near Tokyo (Fig. 1), contained both the long- and short-styled morphs and thus had the potential for sound sexual reproduction (Uesugi et al., 2009). The number of genets identified using ten microsatellite loci developed by Uesugi et al. (2005) was 61 in Japan, and 18 of them are in Lake Kasumigaura (Uesugi et al., 2007). These results suggest that Lake Kasumigaura is the most important habitat of the species in Japan.

N. peltata is a flagship species in a collaborative ecosystem restoration program for Lake Kasumigaura and its catchments (Washitani and Iijima, 1999), named 'Asaza project' <http://www.kasumigaura.net/asaza/en/>.

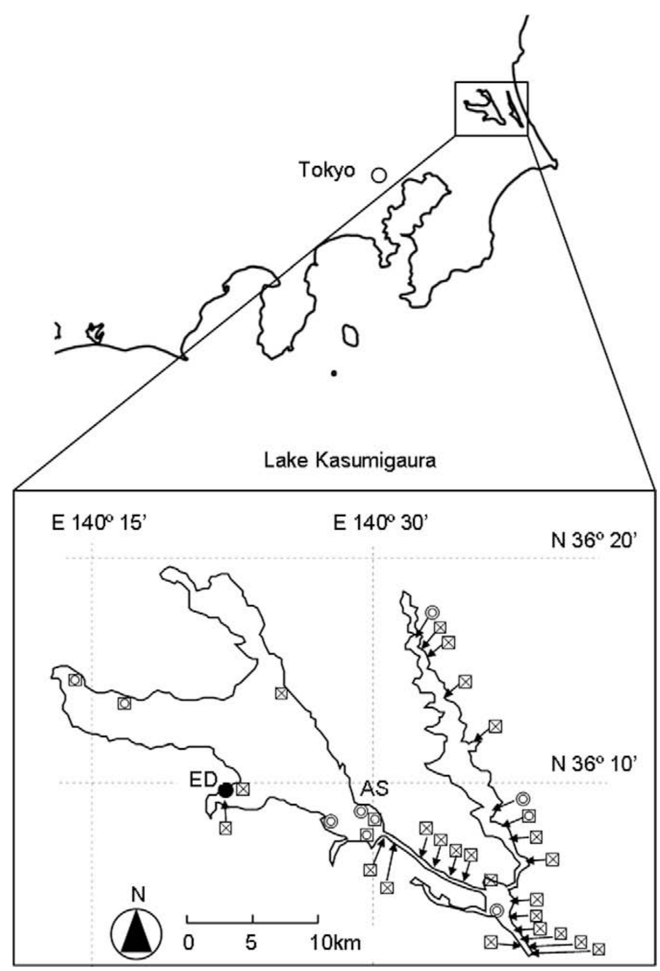

Fig. 1. Location of Lake Kasumigaura and the study sites. *N. peltata* local populations are shown as squares or circles: ⊠, extinct in 1996–2000; ▣, extinct in 1996–2000 but restored by planting collected ramets; ●, extinct until 2000 but restored from the soil seed bank; ◎, surviving in 2007.