Ecology and conservation of the endangered quillwort *Isoetes sinensis* in China

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**Abstract**

*Isoetes sinensis* Palmer is an East Asia endemic fern that faces impending extirpation from the expansive area of Mainland China. Presently, only two natural populations are known to exist in this vast region. Both populations have decreased by about 50% within the last 4 years. This reduction is most probably related to the decline in the size of the wetlands since reproductive activity, spore production, and recruitment were noticeably lower at the microsites occurring in smaller ponds. The decline in population size seems to be associated with succession from marsh to dry land at the habitats of both populations. The study revealed that individuals in both populations produced copious numbers of spores suggesting adequate fecundity, furthermore high levels of spore germination were obtained in laboratory experiments. However, inefficient spore dispersal appears to have contributed to the limited spread of the species. The sex ratio in both populations was male-biased. Several factors are identified here as posing a threat to *Isoetes sinensis* populations in China, chiefly the loss of habitat due to infrastructure construction and competition with adventive weed species for establishment on available sites. Disturbance by introduced mammals during browsing and trampling by livestock farm workers, as well as tourists, further contribute to the diminishing numbers of *Isoetes sinensis* at the study sites.

**Keywords:** China, conservation, ecology, *Isoetes sinensis*, rare fern

**Introduction**

*Isoetes* is classified in the subdivision Lycopsida, which is an ancient group extending back to the Devonian (Foster and Gifford 1974). It is a small cosmopolitan genus with heterosporous with an estimated 150 extant species (Taylor and Hickey 1992). The genus is recognized as the only remaining living representative of ancient taxa that were distinguished by a strongly reduced plant body, and it occupies a unique position in plant evolution as the closest relative of the famous tree Lycopods (Takhtajan 1956; Pigg 1992,
2001). *Isoetes* also has some of the limited number of aquatic plants that have crassulacean acid metabolism (Keeley 1998), which makes the genus especially valuable for research into this unique metabolic pathway. In addition, quillwort are the only surviving epibiotic members of family Isoetaceae and are therefore of great importance for scientific research. The genus has one of the most endangered components in the flora of East Asia. The species is likely to become extinct if it is not protected (Fu 1992).

*Isoetes sinensis* is an evergreen perennial, submersed or emergent marsh plant with glossy leaves. The plant is 15–30 cm high. The main body of the plant is a corm which is rhizomatous and tri-lobed. The leaves are imbricate, 15–30 cm long, 1–2 mm in width, bright green, and linear, highly reduced. The ligules are triangular, cuspidate, 1–2.5 × 1.5–3.0 mm. The velums are rudimentary, covering only the distal edge of the sporangium. The sporangia are basal, oval, 2–7 × 1.5–4.5 mm. The megaspores are grey when wet, and change to white when dry with a mean diameter of 406.7 μm. Microspores are dusty grey, elliptic, laevigate to granulate with a mean diameter of 29.6 μm. Few sporangia contain both megaspores and microspores.

Currently, *I. sinensis* Palmer is restricted to eastern Asia (Japan, China, and Korea). In China, *I. sinensis* grows mainly in freshwater or wetland habitats in the Qiantangjiang River basin. In recent time, habitats of *I. sinensis* in China have become degraded, and the plant has disappeared altogether from several locations (Hao et al. 2000). The species is now considered to be rare and endangered (Fu 1992; Yu 1999). Recently, calls have been made for urgent conservation measures to avert its impending extirpation from continental China (Wen et al. 2003). Decline in *I. sinensis* populations has been attributed to: competition for nutrition with adventive weeds, deterioration in water quality, and the loss of genetic diversity (Chen et al. 2004).

In addition to its inherent importance as a key component of the pteridophytes biodiversity in China, the rarity of *I. sinensis* renders it of considerable interest in studies into the phylogeny of pteridophytes. Previous studies on *I. sinensis* have largely concentrated on morphology, cytology, and physiology (He et al. 2002; Liu et al. 2002, 2003; Pang et al. 2003). In the present study, we investigated aspects of the ecology of *I. sinensis* as a basis for better understanding the biology of this rare plant as a prerequisite for formulating appropriate strategies for its conservation. In particular, we focus on the habitat characteristics and regeneration status of the species and make inferences regarding prospects for its conservation.

**Methods**

**Study sites and populations**

The geographical location of both study sites was mapped using GPS (Figure 1). The larger of the two study sites is in Jiande on the banks of the Xinanjiang River (29°28’N, 119°15’E), Zhejiang Province of southern China. The study site extends for 100 m along the western bank. The flow of the river is regulated by the Xinanjiang dam (Cui 1991). The quillwort population at this site was discovered by our research team during a reconnaissance tour in 2000 and has been monitored since that time. The majority of the individuals at the site occur within a 200 m² area. The habitat is dominated predominantly by *Eclipta prostrata* (Linn.) Linn. and *Alternanthera philoxeroides* (Mart.) Griseb., however, large patches of ground at the site are almost bare owing to low soil fertility (Wen et al. 2003). At this site most of the individuals of *I. sinensis* occur in a 14 m² plot at the centre of the quillwort population (core plot) and in another 40 m² plot close to the edge of the population (edge plot).
The second study site is in a marsh in an abandoned rice field; the marsh lies in a low-lying area surrounded by hills and borders a creek and farmland (29°30′N, 118°09′E). The soil at the site is a fertile swampy loam, and the dominant species are *Eriocaulon buergerianum* Koern., *Rotala indica* (Wild.) Koehne, and *Lemna minor* Linn. *Isoetes sinensis* plants at this site are scattered and generally appeared not to be robust, with no individuals exceeding 20 cm in height.

**Field studies**

From 2002 to 2005, the number of individuals of *I. sinensis* in each of the study sites was recorded. The location of each individual was indicated on a map. The density of *I. sinensis* in both populations was calculated. Demographic data were obtained for each season during the duration of the study. We also estimated the population size, and described the general health and distribution of individuals within the population. The height and the number of leaves of all individuals were also recorded. The location of each site and the numbers of mature, fertile, and juvenile individuals were recorded. Data on environmental parameters including water temperatures, pH, conductivity, dissolved oxygen, dissolved carbon dioxide, alkalinity, acidity, and hardness, as well as air temperature, were obtained at the study sites. Air and water temperature were measured with a thermometer, pH was measured with a portable pH meter (HI98107, Hanna Co., Italy). Conductivity was measured with a portable conductivity meter (HI983004, Hanna Co.). The remaining water parameters were analysed with a portable test kit (HI3814, Hanna Co.). Cases when the parameter in the sample was below the lowest threshold of the meter measurements were indicated as zero. Altitude, aspect, slope, topographic position, and percentage of
ground surface covered by each of the following: vascular plants, moss, litter, bare ground, and rock were recorded during each visit to the habitats. In addition, a 500 ml water sample was taken for ion analyses in the laboratory during each visit. Water analysis was performed on sub-samples using a multi-parameter ion-specific photometer (C200, Hanna Co.) at room temperature.

Spore germination experiments were done following the methods of Kott (1981). Approximately 500 megaspores were obtained from the Jiande population. Plants were collected and kept in the laboratory in glass tanks filled with distilled water. Mature microspores were obtained from the same population. The contents of ten mature sporangia from each plant were emptied into plastic vials filled with distilled water. Microspores were scraped with the edge of a razor blade into small vials containing distilled water. The vials were placed in a refrigerator at 4°C for 84 days and thereafter transferred to a cabinet and maintained at 24°C. Counts of germinated spores were taken 60 days after the end of the cold treatment. Groups of megaspores and microspores that had been kept at room temperature (ca 24°C) were used at the control.

During megaspore germination counts each vial was emptied into a clean Petri dish and the contents examined under a dissecting microscope. Megaspores were considered to have germinated then they showed a split spore coat and evidence of development of young archegonia. Readings were recorded as the percentage of germinated spores to the total number of spores in each vial. Microspore germination counts were made under a microscope from slides smeared with 10 μl of the suspension pipetted from the vials. The vials were shaken to agitate their contents before pipetting. Microspores were considered as having germinated when they appeared empty and clear with an opening on the side. Roughly 150 spores were scored per slide and the percentage of spores germinated was recorded.

For the purpose of data analysis, plants were categorized as sporelings, saplings, or adults depending on their number of leaves. Individuals with less than nine leaves were categorized as sporelings, those with between nine and 17 leaves were categorized as saplings, while those with 18 or more leaves were categorized as adults. Reproductive status was analysed against the number of leaves by nominal logistic regression. Sex ratio analysis was carried out by a standard G-test. Single classification goodness-of-fit test was carried out using William’s correction (Sokal and Rohlf 1995). Analyses were performed using the SAS statistical package (SAS Institute Inc.).

Results

Population size

Population characteristics of I. sinensis at the study sites are shown in Table I. In 2000, the estimated total population size at the Jiande site was 3000, which included 400 juvenile individuals, while at Xiuning there were only 34 individuals, including 12 juveniles (Figure 2). Other species which were found growing in close juxtaposition with I. sinensis at the study sites included Eriocaulon buergerianum Koern., Rotala indica (Wild.) Koehne, Lemna minor Linn. in the Xiuning population, and Eclipta prostrata (Linn.) Linn., Alternanthera philoxeroides (Mart.) Griseb., Oenanthe javanica (Blume) DC., and Potamogeton maackianus A. Benn. in the Jiande population.

Both the sporeling-to-adult and the juvenile-to-adult ratios are low in both populations, and show considerable homogeneity, both indicating poor sporeling recruitments at both sites (Table I).
The sex ratios of fertile individuals at both study sites were highly skewed towards males (Table II). A female: male ratio of 11:23 males was observed at the Jiande site while at the Xiuning site the ratio was 12:25. This was a significant deviation from the expected 1:1 ratio ($G_{adj} = 4.36$, df=1, $P<0.05$ for Jiande; $G_{adj} = 4.36$, df=1, $P<0.05$ for Xiuning).

**Physico-chemical characteristics of the water at the study sites**

The water pH ranged from 6.7 to 8.0, with a mean of 6.92; total dissolved solids (TDS) at the same site ranged from 0.02 to 0.2 mS cm$^{-1}$, with a mean of 0.05 mS cm$^{-1}$; while NH$_3$-N at the site ranged from 0.13 to 0.33 mg l$^{-1}$, with a mean of 0.20 mg l$^{-1}$. Fe concentration ranged from 0.00 to 0.17 mg l$^{-1}$, with a mean of 0.02 mg l$^{-1}$. Mg concentration at the site ranged from 0.11 to 2.2, with a mean of 1.84 mg l$^{-1}$. There were no significant differences ($P<0.05$) for the values between years (Table III).

**Spore germination**

At room temperature 0.84% of microspores and 4.3% of megaspores of *I. sinensis* germinated. The germination rate of both microspores and megaspores of *I. sinensis* improved dramatically when the spores were subjected to a cold treatment period.

| Site and date | Area | N | Total | Seedlings | Saplings | Adults | SE/A | SA/A |
|---------------|------|---|-------|-----------|----------|--------|------|------|
| Jiande        |      |   |       |           |          |        |      |      |
| September 2002| 14   | 424| 30.29 | 6.71      | 17       | 6.57   | 1.02 | 2.59 |
| December 2002 | 14   | 424| 30.29 | 6.71      | 17       | 6.57   | 1.02 | 2.59 |
| July 2003     | 14   | 391| 27.93 | 4.64      | 16.79    | 6.5    | 0.71 | 2.59 |
| September 2003| 14   | 654| 46.71 | 12        | 25.64    | 9.07   | 1.32 | 2.83 |
| December 2003 | 14   | 525| 37.5  | 11.14     | 18.93    | 7.43   | 1.50 | 2.55 |
| March 2004    | 14   | 571| 40.79 | 24.29     | 15.29    | 1.21   | 20.00| 12.59|
| July 2004     | 14   | 782| 55.86 | 29.71     | 11.64    | 14.5   | 2.05 | 0.80 |
| December 2004 | 14   | 291| 20.79 | 9.571     | 7.14     | 4.07   | 2.35 | 1.75 |
| Xiuning       |      |   |       |           |          |        |      |      |
| September 2002| 4    | 41 | 10.25 | 3.5       | 5        | 1.75   | 2    | 2.86 |
| December 2002 | 4    | 80 | 20    | 8.5       | 7.25     | 4.25   | 2    | 1.71 |
| July 2003     | 4    | 91 | 22.75 | 1.75      | 8        | 13     | 0.13 | 0.62 |
| September 2003| 4    | 60 | 15    | 4         | 6        | 5      | 0.8  | 1.2  |
| December 2003 | 4    | 34 | 8.5   | 4.25      | 2.75     | 1.5    | 2.83 | 1.83 |
| March 2004    | 4    | 80 | 20    | 15.5      | 4.5      | 0      | —    | —    |
| July 2004     | 4    | 68 | 17    | 6.25      | 4        | 6.75   | 0.93 | 0.59 |
| December 2004 | 4    | 17 | 4.25  | 0.5       | 3.5      | 0.25   | 2    | 14   |

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**Table II. Sex ratio in the Jiande and Xiuning populations of Isoetes sinensis.**

| Site    | Female | Male | Ratio |
|---------|--------|------|-------|
| Jiande  | 11     | 23   | 0.48  |
| Xiuning | 12     | 25   | 0.48  |
Table III. Physico-chemical parameters of water collected at the habitat of *Isoetes sinensis* and means and standard errors over all samples.

| Variable | Time          |       |       |    | All       |       |       |
|----------|---------------|-------|-------|----|-----------|-------|-------|
|          | 2002 (n=2)    | 2003 (n=3) | 2004 (n=2) |    |          | Mean | SE    |
| pH       | 7.05          | 6.7   | 7     | 0.16| 6.92      | 0.16  | 5.99  |
| TDS (mS/cm⁻¹) | 0.07          | 0.02  | 0.06  | 0.24| 0.05      | 0.02  | 72.53 |
| NH₃-N (mg·l⁻¹) | 0.22          | 0.18  | 0.19  | 0.66| 0.20      | 0.02  | 22.80 |
| NO₃-N (mg·l⁻¹) | 0.4           | 1.1   | 1.2   | 0.68| 0.9       | 0.19  | 92.63 |
| P (mg·l⁻¹) | 0.45          | 4.4   | 0.7   | 0.46| 1.85      | 0.69  | 99.90 |
| PO₄³⁻ (mg·l⁻¹) | 0.77          | 0.16  | 0.19  | 0.11| 0.37      | 0.15  | 138.79|
| NO₅⁻ (mg·l⁻¹) | 1.77          | 4.88  | 5.316 | 0.68| 3.99      | 0.84  | 92.63 |
| Cl⁻ (mg·l⁻¹) | 0.08          | 0     | 0.23  | 0.46| 0.10      | 0.01  | 183.59|
| Br⁻ (mg·l⁻¹) | 0.02          | 0     | 0     | 0.72| 0.01      | 0     | 0     |
| Ca (mg·l⁻¹) | 2             | 0.91  | 1.13  | 0.32| 1.35      | 0.34  | 75.79 |
| Mg (mg·l⁻¹) | 1.12          | 2.2   | 2.2   | 0.37| 1.84      | 0.29  | 41.75 |
| Fe (mg·l⁻¹) | 0             | 0     | 0.05  | 0.41| 0.02      | 0.02  | 133.65|
| Mn (mg·l⁻¹) | 0.25          | 1     | 0.5   | 0.68| 0.58      | 0.13  | 77.71 |
| Cu (mg·l⁻¹) | 0.05          | 0.5   | 0.14  | 0.17| 0.23      | 0.07  | 98.76 |
| Zn (mg·l⁻¹) | 0             | 0.14  | 0.17  | 0.21| 0.10      | 0.03  | 95.16 |
| Mo (mg·l⁻¹) | 2.15          | 2     | 3.3   | 0.98| 2.48      | 0.36  | 45.84 |
| Cr⁶⁺ (mg·l⁻¹) | 0             | 0     | 0     | 0   | 0         | 0     | 0     |
| Ni (mg·l⁻¹) | 0.4           | 1.1   | 1.2   | 0.68| 0.9       | 0.19  | 92.63 |

(Table IV). The germination rate compared favourably with those of several other species reported in Kott and Britton (1982).

**Spatial decline**

At the time of our discovery of the quillwort population at Jiande it was subdivided into two sub-populations. The smaller sub-population was in a pond about 300 m from the main population. One hundred *I. sinensis* individuals occurred at the pond, which was destroyed in 2003 to make way for urban infrastructure construction. In a 100 m transect, 20 m was within 10 m of the core plot, while 80 m was >10 m away. At a distance of less than 10 m from the source area the density of *I. sinensis* individuals was as high as 38 per m², while at distances >10 m the density was reduced to 10 per m². This pattern of distribution was significantly different from random dispersion (*G* _adj_ =15.188, df=1, _P_<0.001. At the Xiuning site, we recorded only 17 individuals in 2004. The average density of *I. sinensis* plants in this population was lower than that in the Jiande population. The low seedling-to-adult and sapling-to-adult ratios are indicative of a declining population (Table I). The density at Jiande was 23.6 individuals per m² at distances of up to 2 m from the edge of the population, but reduced to only three individuals per m² at
greater distances from the population border. These differences in population density were highly significant ($G_{adj} = 14.44$, df=1, $P<0.001$). At the Jiande site, the *I. sinensis* individual occurring furthest away from the main population was 120 m away, while at Xiuning the most distant individual was 30 m away from the main population.

**Status of the *Isoetes sinensis* population at the study sites**

The area coverage of the quillwort population at Jiande has decreased from 300 m$^2$ in 2001 to about 100 m$^2$ in 2004. Over the same period the Xiuning population also displayed a decrease in area coverage from 30 m$^2$ to about 3 m$^2$. The population structure at Jiande and Xiuning, although highly skewed towards fertile individuals, was consistent with a declining population (Table I). However, abundant regeneration by spores and the production of multiple trunks around stem bases was observed. Field observations also revealed a slight tendency for females to reproduce for a longer period than males (Table II).

**Discussion**

Although anomalies in the chromosome behaviour during meiosis (namely cytomixis, lagging chromosomes, chromosome bridges, chromosome fragments, and micronuclei) have been observed in *I. sinensis*, these were, however, absolved from being the major cause of decline in this species (He et al. 2004). Evidence points towards habitat degradation as being the major cause of decline in *I. sinensis*. Presently, only a few populations of the Chinese quillwort persist in China. Furthermore, the populations are fragmented and the numbers of individuals in the populations are dwindling rapidly.

*I. sinensis* is an aquatic plant with highly specific habitat requirements with regard to water quality and intensity of illumination. These conditions mostly occur in fertile swamps such as Jiande, which also commonly have thin soils (Rhazi et al. 2004). Some of the characteristics that make *I. sinensis* well adapted to these environments include an extensively well-developed root system and a relatively low, spreading growth habit. Currently, several invasive weed species threaten the *I. sinensis* habitat at the study sites, these include *Eriocaulon buergerianum* Koern., *Rotala indica* (Wild.) Koehne, *Lemma minor* Linn. in Xiuning, and *Eclipta prostrata* (Linn.) Linn., *Alternanthera philoxeroides* (Mart.) Griseb., *Oenanthe javanica* (Blume) DC., and *Potamogeton maackianus* A. Benn. in Jiande.
Due to their fast-spreading nature, these weeds, many of which have copious seed production, out-compete *I. sinensis*, gradually reducing its areal coverage in the habitat. The presence of these weeds continues to accelerate the decline of *I. sinensis* populations at the study sites.

Our results have indicated that spore production in *I. sinensis* in the wild is high, thereby dispelling the notion that the decline of *I. sinensis* may be attributed to the absence of viable spores. However, spore dispersal in the study site appears to be restricted. Similar to many species with weak spore coats, *I. sinensis* has spores that are less persistent within soil spore banks. Furthermore, the spores have been reported to display dormancy. Seeds or spores that require abrasion tend to break dormancy at different times rather than in a sudden flush (Harper 1977). This episodic germination may enable *I. sinensis* to re-establish at sites long after loss of the adult plants; however ephemeral spores and a low rate of spore germination are overall detrimental to the survival of *I. sinensis*.

The rhizome in this species does not grow to great lengths and the sporelings have been shown to establish at only short distances from the parent plant, hampering the spread of *I. sinensis* and confining it to only limited favourable sites. Leaves of *I. sinensis* can attain a horizontal spread of 30 cm and are commonly shaded by the branches of plants growing in close juxtaposition with the plant, helping to avoid direct light. These strategies assist *I. sinensis* in persisting in natural habitats until it is either eliminated by catastrophic disturbance or it is out-competed by more robust-growing weeds. This is in striking contrast to transplanted plants, which are often in a single population.

The male-biased sex ratio found in the Jiande and Xiuning population could have arisen simply by chance when the population size was small. A slight tendency for males to reproduce at an earlier stage than females might also have been involved, but this cannot be adequate to explain the strong bias observed. A male-biased sex ratio is a general phenomenon in quillwort in China.

The physico-chemical parameters of the water at the study sites supported the assertion by Keeley (1998) that *Isoetes* species are adapted to living in carbon-limited environments such as shallow seasonal pools and acidic, soft-water, oligotrophic, lacustrine habitats. These environments have low levels of dissolved inorganic carbon, where the pH is regulated by a weak buffer system of $\text{CO}_2 / \text{HCO}_3^- / \text{CO}_3^{2-}$. In addition, the environments have typically low conductance with very low phosphate, nitrogen, and carbon dioxide levels in the water and significantly higher nutrient levels in the sediment (Roelofs et al. 1984). Both our study sites have low water conductivity as well as low dissolved carbon dioxide, nitrogen, nitrate, and phosphate concentrations, and a mean pH value of 6.92 (Table I). *Isoetes* species can isolate nitrates in rhizomes from sediment-poor water, and some lacustrine species of the genus act as enticement for dissolved inorganic nitrogen (Catalan et al. 1994; Olsen and Andersen 1994). This may construe the low concentration of nitrates in the water at the study sites. The sediment supporting *I. sinensis* in both sites had high total phosphorus and low filterable phosphorus ($\text{PO}_4^{3-}$). *I. sinensis* can absorb soluble reactive phosphate (SRP) efficaciously from pool water. This has the effect of increasing the concentration of SRP in the sediment just below the surface and causing the formation of a small SRP pool with an accelerated annual turnover of phosphorus (Andersen and Olsen 1994; Christensen et al. 1998). This has important suggestions for the pattern of exchange of phosphorus between sediment and water and the phosphorus budget of entire lakes, implying that *I. sinensis* may be important for keeping lakes in an oligotrophic state. Jackson et al. (1993) demonstrated the effect of water pH on the bioavailability of aluminium to isetoid macrophytes and demonstrated that the plants
accumulated the metal at higher concentrations than those found in the sediment. This attests that *Isoetes* species may tolerate high concentrations of aluminium in these environments. Plants that grow submerged, such as *I. sinensis*, are the most affected by human-induced eutrophication (Roelofs 1996). They are particularly sensitive to changes in water quality and clarity (Gacia and Ballesteros 1993; Rorslett and Johansen 1995; Middelboe and Markager 1997). *Isoetes sinensis* has a highly specific growth strategy; like other macrophytes living in similar environments, it has developed a variety of mechanisms to adapt to the unique conditions presented by the aquatic habitat. However, evidence (Wen et al. 2003) indicates that under certain circumstances, *I. sinensis* appears to fall victim to its own insufficiently flexible growth strategy under sustained adverse environmental impacts arising from changes in water chemistry wrought by processes such as water hardening, calcification liming, eutrophication, and pollution by heavy metals. Our own preliminary experiments indicated that *I. sinensis* is highly sensitive to Cr$^{6+}$ concentrations and is killed off when concentrations reach 0.001 mol l$^{-1}$.

Humans have played a significant role in the recent decline of *I. sinensis* at several sites in China directly by destroying natural habitats, and indirectly by introducing domestic herbivores and adventive weeds. Land clearance and conversion to agricultural use in the last century has seen the loss of large areas of actual and potential habitats for *I. sinensis* in mainland China, particularly in valleys and on riverbanks. In areas where there still exists a suitable and still intact habitat, *I. sinensis* is commonly restricted to sites that introduced browsing animals cannot reach, or where animal numbers are low. The resultant small *I. sinensis* populations are vulnerable to stochastic disturbance and natural successional processes. Competition for seedling establishment sites with adventive weed species may be a further cause of decline. The rates of decline of *I. sinensis* in China are alarming. One moderate-sized population in Dongxiang Jiangxi province that was sighted by our team in 2000 has now been exterminated. Persistence of *I. sinensis* in the wild is clearly to a great extent affected by the disturbance regime in its habitat.

Spore germination experiments revealed moderate germination rates of spores of *I. sinensis*. Young plantlets were also common in the populations, these were however largely restricted to areas immediately next to the adult plants which may indicate limited spore dispersal. The rarity of *I. sinensis* in the wild appears to be controlled by its stringent habitat requirements, susceptibility to disturbance by introduced domestic herbivores, and limited dispersal ability. *Isoetes sinensis* has never been reported as common in China, and is considered to be close to extinction in the wild. Our recent intensive field searches have not revealed more plants in the wild than was previously thought, and no new populations have been located.

The conservation status of rare plant species needs to be assessed both within individual populations and at the metapopulation level (Schemske et al. 1994). The status of individual *I. sinensis* populations range from a few large stable populations with adequate regeneration, to only two individuals with no apparent regeneration which appear vulnerable to extirpation. The status of *I. sinensis* at the metapopulation level requires an assessment of the spatial distribution of populations and an understanding of population colonization rates of new habitats. Populations of *I. sinensis* in China are widely scattered and largely discrete, and migration between populations seems unlikely. The species is apparently non-specialized and poor spore dispersal ability indicates that movement to new habitats would also be infrequent.

The Department of Conservation of the Ministry of Forestry in China has prepared and initiated implementation of a recovery plan for endangered species including *I. sinensis*. The
plan hopefully will ensure the perpetuation of *I. sinensis* in the wild and the maintenance of its genetic diversity. Specifically for *I. sinensis* this programme should include the active management of existing populations, particularly to limit the growth of weeds that may choke and out-compete *I. sinensis*, as well as establishment of new populations. At one of our study sites, the pruning of weedy neighbours at the site has shown to be effective in maintaining the population, and also allowing new seedlings of *I. sinensis* to establish. Results of the present study could provide guidelines for the habitat characteristics of sites where new populations should be initiated. To maintain and increase wild genetic diversity, we recommend that material for new populations should be derived from spores rather than from vegetatively produced material. Experience gained from the conservation of *I. sinensis* will be valuable for application to the conservation of other quillworts facing similar threats in China and elsewhere.

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