

Size-class structure and variation in seed and seedling traits in relation to population size of an endangered species *Craigia yunnanensis* (Tiliaceae)

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Abstract. *Craigia yunnanensis* W. W. Sm. & W. E. Evans is an endangered canopy tree species distributed in southern China and northern Vietnam. We located and surveyed six remnant populations, all from subtropical areas of Yunnan, China. These six populations contained between 6 and 167 adult trees in 2007, plus larger numbers of seedlings and resprouts from cut trunks. Bulk seed samples were collected from these populations and examined for 10 fitness traits (e.g. seed number, seed length, width and 1000-seed weight, germination in the controlled light and darkness conditions and nursery, seedling height, root collar diameter and dry weight). All traits differed significantly among populations, but only number of seeds per capsule was significantly correlated with population size. For some populations, germination capacity in light was significantly higher than that in darkness, indicating that canopy removal might promote regeneration from seed. Examination of population size-class structures in four *C. yunnanensis* populations determined that small seedlings and resprouts are abundant, although very few of these reach even the sapling stage. Hence, the remaining populations could be expanded and conserved by allowing a proportion of seedlings and resprouts to reach maturity. However, *ex situ* conservation, informed by data from the present study, is also advisable in case the remaining wild populations are lost.

Introduction

It is well known that a practical conservation strategy for an endangered plant is based on an understanding of the threats it faces. Population size-class distribution carries a wealth of demographic information, and has been shown to be useful in evaluating the viability of populations of an endangered tree species (Souza 2007). Seed traits (seed size, germination and fitness immediately afterwards) are components of plant fitness that determine the success of the crucial seedling recruitment stage of the life cycle (Yang *et al.* 1999; Ellison 2001; Navarro and Guitián 2003), and these in turn are profoundly affected by factors such as provenance and population size (Young 2005; Krauss *et al.* 2007). Therefore, conservation and sustainable use of genetic resources for a species can be enhanced by (1) information about seed germination and seedling emergence, leading to understanding of its reproductive strategy and how populations regenerate (Yang *et al.* 2008), and (2) knowledge about provenance and genetic variability within its natural populations, and how this relates to phenotypic variation within the species (Mamo *et al.* 2006; O'Brien *et al.* 2007).

Seed production and genetic quality of seed can also be influenced by properties of the source population, such as population size and fragmentation, and this too can potentially have an impact on restoration goals (Young 2005; Krauss *et al.*

2007). Small population size should lead to lower fitness via inbreeding depression and random genetic drift (Ellstrand and Elam 1993; Reed 2005). Moreover, in small populations important interactions with mutualists such as pollinators and seed dispersers may be disrupted, reducing plant reproductive success (Young *et al.* 1996; Kearns *et al.* 1998; Cunningham 2000; Kolb 2008). Indeed, many studies have demonstrated positive relationships between population size and several aspects of fitness (Kéry *et al.* 2000; Vergeer *et al.* 2003; Kolb 2005; Leimu *et al.* 2006).

The genus *Craigia* W.W.Smith & W.E.Evans (Tiliaceae) was widespread and abundant in Europe, North America and East Asia during the Tertiary period, but is now endemic to eastern Asia, specifically southern China (Guangxi, Guizhou, Yunnan and Tibet provinces) and adjacent northernmost Vietnam (Jin *et al.* 2009). Only two species of this genus survived into modern times (Tang *et al.* 2007); of these, *C. kwangsiensis* Hsue has not been found in the wild since 1957. The second *Craigia* species, *C. yunnanensis*, is seriously threatened and has been pushed to the verge of extinction because of vegetation destruction in China, and consequent contraction of its distribution (Fu 1992). Hence, it was listed as a nationally rare and endangered plant in 1999 and has also been proposed as a second-ranked plant for national protection in China (Wang and Xie 2004; IUCN 2008).

Craigia yunnanensis is a deciduous tree that can grow up to 30 m tall, and exceed a diameter at breast height of 100 cm. It produces top-quality timber for construction and furniture because of its straight trunk and fine wood structure (Li 2005). Because it is naturally adapted to limestone mountainous habitats, it has the potential to be used for afforestation in such areas (Gong *et al.* 2006). However, because of deforestation and cash-crop cultivation in southern China, its natural population size has been reduced greatly in recent decades. The remaining wild populations of *C. yunnanensis* may therefore represent the last few populations in the world of a once widespread genus, which also has commercial value, and is a genetic resource that must be conserved. However, at present there is little information about the number and size of the remaining populations of this species, still less about their demographic structure and regeneration pattern, and none at all about their fitness related characters.

In the present study, we located six small, remnant populations of *C. yunnanensis* and assessed their status with regard to population size, size-class structure, regeneration pattern and fitness. We then looked at relationships between these traits, to analyse population viability and ascertain possible reasons for its decline. Although little information is available about natural regeneration of *C. yunnanensis* populations, pilot field surveys have shown that seedlings usually appeared in canopy gaps and open areas from clear-cutting. Thus, light is expected to be a factor potentially affecting germination in the field for this tree species. Therefore, we combined a demographic study with common laboratory and nursery experiments to address the following questions: (1) what is the size-class structure in extant *C. yunnanensis* populations, and what can this tell us about the threats to these populations and how they might best be conserved; (2) is there detectable variation in seed- and seedling-related characters among populations; (3) does seed germination require light, and if so, does this vary between populations; and (4) if considerable variation in seed- and seedling-related characters are detected, how is this related to the population size?

Materials and methods

Location and survey of extant populations

A list of localities where *C. yunnanensis* has been recorded during the past century was compiled using information from herbarium specimens, inventories from regional and local forestry

departments, records from nature reserves, and the knowledge of local botanists. Between 2005 and 2007, we conducted an extensive survey of all of these localities except for those in Tibet and northern Vietnam, which could not be searched because of travel restrictions and logistical limitations.

Of the three remaining provinces of China from which *C. yunnanensis* had been recorded, no material could be found in Guizhou or Guangxi, indicating that the species may already be extinct in these provinces. However, a total of six wild populations of *C. yunnanensis* was found in two disjunct regions of Yunnan, i.e. WenShan (south-eastern Yunnan) and DeHong (south-western Yunnan), which are more than 600 km apart (Table 1, Fig. 1).

All six extant populations occur between 1400 and 1700 m asl. The geographic locations and climatic conditions of these sites are shown in Table 1 and Fig. 1. They had all been severely disturbed, primarily as a result of increased cultivation of *Amomum tsao-ko* Crevost & Lemarie, a cash crop that is commonly grown in the understorey of primary or secondary forests where *C. yunnanensis* occurs. Cultivation of *A. tsao-ko* began during the 1980s in both WenShan and DeHong as a result of government sponsorship for cash crops, and as with the more traditional crop *A. villosum*, lead to near-total removal of herbs and shrubs plus thinning of the canopy, although some trees are left in place to provide partial shade (Liu *et al.* 2006). Felling of *C. yunnanensis* by local people for construction probably also had an impact.

The status of *C. yunnanensis* in Vietnam and Tibet is currently unknown. Furthermore, to our knowledge, this species is not in cultivation anywhere.

Biology of *C. yunnanensis*

Craigia yunnanensis is a canopy tree species. Once its diameter at breast height (DBH) reaches ~30 cm, it enters into the reproductive phase (flowers and fruits). It produces large numbers of small purplish-pink hermaphrodite flowers (>1 cm long) arranged in cymes with 2–9 flowers. The flowering period is from August to September. This species is pollinated by small insects such as bees and flies, although geitonogamy occurs and it is self-compatible; hence, it has a mixed mating system (Z. Gao and C. Q. Zhang, unpubl. data). The fruits have five wings and appear to be adapted for wind dispersal, although the fruits are

Table 1. Locations and geoclimatic variables for six remnant populations of *Craigia yunnanensis* in Yunnan

Annual mean rainfall was calculated on the basis of data from Yunnan Climatic Agency and the altitude of the population, by using formulae in Wang (2006). Annual mean temperature was calculated on the basis of data from Yunnan Climatic Agency and the altitude of the population, by using formulae in Wang (2006). The values in the population size column indicate the number of reproductively mature individuals that were present in 2005; the values in parentheses show how many of these individuals were still present (had not been cut down) in 2007. For explanation of population codes and location of populations see Fig. 1

Population code	Full name	Latitude (°N)	Longitude (°E)	Altitude (m)	Rainfall (mm)	Temperature (°C)	Population size
Wenshan region							
W-FD	FaDou	23.38	104.78	1461	1204.2	16.7	89 (89)
W-LH	LianHuaTang	23.16	104.85	1460	1275	16.1	151 (133)
W-ML	MaLiPo	23.19	104.72	1449	1406.9	15.9	125 (116)
W-MG	MaGuan	23.05	104.26	1410	1291.2	16.6	11 (6)
DeHong region							
D-JD	JiangDong	24.53	98.38	1694	2204	15.0	106 (73)
D-HG	HuGuo	24.56	98.05	1696	2190.2	15.1	173 (167)

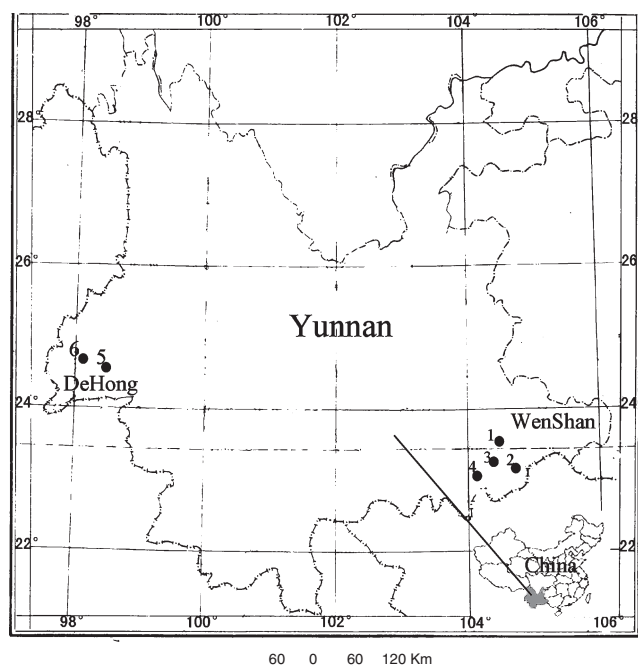


Fig. 1. Location of six populations for *Craigia yunnanensis* in Yunnan, China. Numbers represent populations as follows: WenShan region: 1 = FaDou (W-FD), 2 = LianHuaTang (W-LH), 3 = MaLiPo (W-ML), 4 = MaGuan (W-MG); Dehong region: 5 = JiangDong (D-JD), 6 = HuGuo (D-HG).

heavy and do not travel far; a single tree produces thousands of fruits in a season (Z. Gao and C. Q. Zhang, unpubl. data).

In response to felling, *C. yunnanensis* has the ability to resprout from stumps. Sprouting in trees, which results in the production of secondary trunks, is an induced response to injury or to a dramatic change in surrounding environmental conditions (Del Tredici 2001). Stems that originate from resprouting can flower when they reach 20 cm DBH (Z. Gao and C. Q. Zhang, unpubl. data). In the case of *C. yunnanensis*, both seedlings and regrowth sprouts were observed to contribute to regeneration following disturbance, e.g. in canopy gaps, forest edges, roadsides and areas opened by clear-cutting.

Population sizes, size-class structures and regeneration patterns

Sizes of all the extant populations were estimated from a detailed survey of the study areas during 2005–2007. We recorded all individuals that were present in 2005 and that flowered between 2005 and 2007, and took the total number of these as an estimate of the effective population size. Because some individuals were cut down between 2005 and 2007 (Z. Gao, pers. obs.), we further determined whether each one of those trees that had entered the reproductive phase by 2005 were still standing in 2007, so that the losses between 2005 and 2007 could be quantified.

Population structure and patterns of regeneration in *C. yunnanensis* were investigated in the following four of the six detected populations: FaDou (W-FD) and MaLiPo (W-ML) in WenShan; and JiangDong (D-JD) and HuGuo (D-HG) in DeHong. Five plots (30 m × 30 m per plot) were established in each of these populations in August 2007. Because the

distribution of the species at each locality is uneven, these plots were chosen to contain *C. yunnanensis*, and generally represent the densest concentrations of *C. yunnanensis* at each site. Of the two remaining populations, the MaGuan (W-MG) population was too small for such a survey, whereas the mountainous nature of LianHuaTang (W-LH) and the sparse distribution of the population there made such a survey impractical.

In the study plots of each population, we counted the number of *C. yunnanensis* individuals and subdivided them into categories indicating size, origin and reproductive status. The four smallest categories were based on height, i.e. 0–30 cm (small seedling), 30–60 cm (larger seedling), 60–90 cm (smaller sapling) and 90–130 cm (larger sapling). At >130 cm in height, all plants were termed juvenile or adult trees and classified according to DBH, in classes of 0–5, 5–10, 10–15 and 15–20 cm, then upwards at 10-cm intervals up to 80 cm in DBH, then 80–100 and >100 cm DBH. Within these categories, individuals were further categorised as having originated from seed, or from resprouting of cut or damaged trunks. Individuals >40 cm in DBH all appeared to be of seed origin.

Seed collection

Seeds of *C. yunnanensis* were collected and examined from all six detected sites. We would have liked to have measured the number of fruits per tree, to provide a more complete survey of reproductive fitness per individual; however, the large size of the species and the mountainous nature of its habitat made this impractical.

Seeds were taken from capsules collected between March and April 2007. The number of sampled trees per site ranged from 3 (W-MG) to 16 (D-HG), depending on the availability and accessibility of reproductively mature individuals at each site. To ensure maximum genetic variation, capsules were collected from trees spaced at least 100 m apart within a population, following FAO (1975). The capsules were collected from the northern, southern, eastern and western sides of each sampled tree to avoid the effect of capsule position on seed traits studied. At least 100 capsules were collected from each tree, which represented <10% of each individual's annual seed set. All capsules from each population were then bulked together in cotton bags. Seeds were extracted from each capsule by hand and air-dried at 6–8% moisture content. Abnormally small, shrunken or discoloured seeds were discarded. All traits were analysed by the method of Mamo *et al.* (2006), which requires data to be divided into replicates for each population.

Seed number and size

Variation in the number and quality of seeds produced, i.e. number of seeds per capsule (S-num), seed length (S-len), seed width (S-wid) and 1000-seed weight (S-Wgt) were quantified. From each site, five replicate samples of 50 randomly chosen capsules per site were taken, seeds were extracted from each capsule, and the total number of seeds in each replicate sample was determined and used to calculate S-num. Similarly, five replicates of 20 seeds each from each site were randomly selected for measuring seed length and width with a digital micro-caliper. For seed weight, eight replicates of

100 air-dried seeds each per site were weighed by electronic balance, and from this the 1000-seed weight was computed (International Seed Testing Association 2008).

Seed germination tests

Variation in germination rates of *C. yunnanensis* seeds collected from all six sites was investigated under laboratory and nursery conditions. Measuring germination in different conditions can control for atypical germination patterns in a particular artificial setting. All seeds were soaked in distilled water for 24 h and subsequently rinsed thoroughly with sterilised water before the tests (Yang *et al.* 2007).

Germination capacity in controlled light conditions (GC-light) was determined by using light applied at 1810 lux for 12 h out of 24 h (an artificial photoperiod of 12 h/12 h, light/dark). For each population, five replicates of 20 randomly sampled seeds each were placed on wet filter paper in Petri dishes (9-cm diameter \times 1.2-cm depth), which was kept continuously moist with distilled water. Petri dishes were placed in an artificial climate incubator, within which the day/night temperatures and relative humidity were maintained at $24 \pm 1^\circ\text{C}/16 \pm 1^\circ\text{C}$ and 76%/87%, respectively. The filter papers were replaced at 2-day intervals to avoid fungal growth on the seeds. To ensure no systematic effects occurred because of the position within the chamber, Petri dishes were rearranged at random every 2 days (Yang *et al.* 1999). The germination process was monitored every day for 60 days and the final score for each population was the percentage of seeds that had germinated. Seeds were counted as having germinated when the radicle reached 2 mm. Germination capacity in darkness (GC-dark) followed the protocol described earlier in all ways except that Petri dishes were covered with aluminium foil to deprive seeds of light.

Germination capacity in the nursery (GC-nursery), measured the percentage of seed that had germinated within 90 days of sowing, in the institute nursery ($25^\circ 07' 59.9''\text{N}$, $102^\circ 44' 31.8''\text{E}$, at an elevation of 1966 m). Seeds for this experiment were grown in perforated pots ($26 \times 26 \times 13$ cm), which were filled with a mixture of humus-rich forest soil, local lateritic soil and fine sand in the ratio of 3 : 1 : 1, respectively. From each population, 30 seeds were sown into each of three pots, providing three replicates of 30 seeds each. The pots were watered daily and rearranged every 2 days to avoid effects from their locations (Yang *et al.* 1999). All pots were placed in the nursery, which was 20×30 m, with day/night temperatures at $5^\circ\text{C}/21^\circ\text{C}$ and a translucent roof to protect from rain and direct sunlight.

Seedling performance tests

Seedlings that had germinated within the nursery were also used to assess the variability of seedling performance after germination. These seedlings, at 90 days old, were transplanted into polythene plastic pots of size $18 \times 18 \times 30$ cm and thinned to one seedling per pot. These pots were filled with the same soil mixture as the germination pots. All pots were placed in the same nursery as for seed germination tests (GC-nursery). These pots were rearranged at random every month to control for location effects. To maintain soil moisture at an optimal level, the pots were watered once a day, in the evening for the first 30 days; however after this, watering only every 2 days was found to be sufficient. No fertiliser or

mycorrhizal inoculation was used. After 12 months of growth, there had been no mortality of seedlings, and all appeared healthy. At this time, 30 seedlings were randomly chosen from each population for measurement of three fitness parameters, i.e. seedling height (F-hgt), root collar diameter (F-rd) and dry weight (F-dwt), which was determined via oven drying total seedling biomass for a period of 48 h at 80°C .

Statistical analysis

The general linear model (GLM) procedure of SPSS version 15.0 (SPSS Inc., Chicago, Illinois, USA) was employed for analysis of variance (ANOVA). Before ANOVA, the percentage datasets (GC-light, GC-dark and GC-nursery) were arcsine transformed to meet the normality assumption for the analysis of variance (Zar 1996).

As the interaction effect of light conditions and population was significant (two-way ANOVA: $P < 0.01$), a separate one-way ANOVA was computed for the GC-light and GC-dark characters to examine significant variation among populations for each character singly. ANOVA for each seed- and seedling-related trait was performed on the basis of the following linear model:

$$y_{ij} = \mu + P_i + e_{ij},$$

where y_{ij} is seed/seedling traits of j th replication of the i th population, μ is the overall mean, P_i the effect due to i th population ($i = 1, \dots, 6$) and e_{ij} is the error (following Mamo *et al.* 2006). Any means that exhibited significant differences were compared by using Tukey's test at the 5% level.

The magnitude of variation, as a percentage of the total variation, owing to population or environment (error) was quantified for each character by the restricted maximum-likelihood method. The phenotypic coefficient of variation (PCV) for each character was computed as the square root of total variance divided by the overall mean, multiplied by 100% (Mamo *et al.* 2006). Pearson's product-moment correlations were calculated to examine relationships between seed- and seedling-related traits. Likewise, Pearson's correlation coefficients were calculated between each of 10 fitness parameters and effective population size. For these analyses, the mean value of each character for each population was used. Bonferroni corrections were applied in multiple-corrections (Table 4).

Results

Status and population size-class structures of *C. yunnanensis*

Across the six populations of extant *C. yunnanensis* found during our study, the total number of reproductively mature individuals detected was 655, of which 584 were still present in 2007 (Table 1). The largest population, with 173 mature individuals, was D-HG from DeHong, whereas the smallest (W-MG, WenShan) had only 11 individuals. However, five of the six populations surveyed had lost reproductively mature individuals between 2005 and 2007 (Table 1); the most severe losses being in population D-JD (106 individuals reduced to 73, a loss of 31%) and the small W-MG population (11 individuals reduced to six, a loss of 45%).

On the basis of survey plot data from the four populations in which population size class was examined (see below), the

number of seedlings and saplings present was far higher. In the field, seedlings (0–30 cm tall) occurred abundantly in gaps or open areas, and were present in much larger quantities than adults in all populations surveyed; hence, the height frequency distribution of plants followed a negative exponential distribution in all investigated populations (Fig. 2). The size-class frequency distributions were often discontinuous, and the same general pattern occurred in all the investigated populations for juveniles and adults (Fig. 2). Most individuals with DBH >30 cm were of seedling origin in the four populations examined. Conversely, most individuals with height >60 cm and DBH <30 cm were of resprout origin in these populations.

Of the four populations examined in detail, D-HG was the only one that had a continuous population structure, with the number of individuals decreasing steadily as size and age increased (Fig. 2). In all populations except D-HG, there was a rapid drop-off in frequency from ~10-cm DBH upwards (Fig. 2). All three of the other populations had no individuals between 30- and 40-cm DBH, and very few between 10- and 30-cm DBH. However, D-HG was the only site with no trees of >80-cm DBH (the other sites had one or two each).

Seed- and seedling-related characters

All 10 seed- and seedling-related characters measured exhibited significant differences among *C. yunnanensis* populations (Table 2). Indeed, certain characters such as 1000-seed weight (S-Wgt), seed width (S-wid), number of seeds per capsule (S-num) and GC-dark exhibited particularly strong (>60% component of variance; Table 3) between-population variation. The phenotypic coefficients of variance (PCV) for all the studied characters were low (from 4.5% to 26.9%; Table 3).

In general, D-HG was the fittest population, scoring highest for five variables and second-highest for four more, the exception being S-wid. However, population W-LH had the highest scores for all three germination parameters and also seedling height, although it had relatively low scores for all the seed-number and -size parameters (Table 2). In general, population W-FD had the lowest fitness scores overall, although seedling-fitness scores for population W-MG were even lower (Table 2). For some populations, e.g. W-FD and W-JD, GC-light was significantly higher than GC-dark (W-FD: $P < 0.01$; W-JD: $P < 0.05$).

Some of the fitness-related characters showed fairly high correlation values with one another, although none was significant following a Bonferroni correction (Table 4). However, five fitness traits (GC-dark, GC-nursery, F-hgt, F-rd and F-dwt) showed positive correlations with effective population size of 0.66 or more, and the correlation between effective population size and number of seeds per capsule (S-num; $r = 0.93$) was significant at the 5% level following a Bonferroni correction (Table 4).

Discussion

*Population size-class structures and regeneration patterns of *C. yunnanensis**

Our field survey located just six populations of *C. yunnanensis*, all within Yunnan, containing between 6 and 167 reproductively mature individuals in 2007 (Table 1). Within these populations, *C. yunnanensis* usually existed only as scattered individuals in

isolated patches of original forest, which was consistent with Li (2005). Hence, the distribution of *C. yunnanensis* has become highly fragmented owing to agricultural practices, particularly the cultivation of *A. tsao-ko*. Furthermore, in all but one of these populations, we detected that mature trees were felled between 2005 and 2007, so destruction of most of these populations is ongoing. If no conservation measures are taken and destruction continues at the observed rates, there may be no adult plants of *C. yunnanensis* at W-MG by the end of 2010, or at D-JD by about 2014.

More detailed investigations of four populations showed that, despite widespread evidence of felling of *C. yunnanensis*, a few large trees (DBH >80 cm) still persist in each population, and these along with smaller mature trees produce enough seeds to recruit large numbers of seedlings of 30 cm or less in height (Fig. 2). The abundance of *C. yunnanensis* seedlings may be partly attributable to human disturbance, because it is a light-demanding species whose seedlings benefit, at least initially, from the opening of the canopy. Our experiments indicated that germination rates in this species are significantly enhanced by light, in at least two of the populations examined (i.e. W-FD and D-JD).

The numbers of seed-origin individuals did, however, decline sharply with increasing size (Fig. 2), indicating a high mortality rate. Although some seedling mortality is natural and to be expected, the rarity (only two individuals each in D-HG and W-ML; one individual in D-JD) of seed-origin individuals with between 5- and 30-cm DBH might further indicate that mortality rates going from seedling to sapling stage may be a problem for this species. Possible causes for this include inbreeding depression, human activity and possible longer-term effects of alterations to the habitat of this species. However, this pattern could also indicate that seedling recruitment has increased recently as a result of habitat disturbance. Hence, the ability of *C. yunnanensis* to regenerate does not seem to be impaired by small effective-population sizes at the moment, rather it might be impaired by mortality of young saplings. However, the greatest threat to the ability of this species to reproduce by seed is the continuing removal of reproductively mature trees.

The discontinuous size-class distribution within these populations, with more than one peak in the size classes (Fig. 2), suggests sporadic and intermittent recruitment events in this species. Such events could be triggered by felling of adult *C. yunnanensis*, or other episodes of heavy disturbance. A large proportion of recruitment in this species is via resprouting from cut trunks; indeed, most individuals >60 cm in height but of <30-cm DBH were of sprout origin (Fig. 2). However, larger individuals had single straight trunks, indicating that most or all were of seed origin. According to our field surveys, all of the extant populations had been severely disturbed because of habitat fragmentation caused by the increased cultivation of *A. tsao-ko*, which began during the 1980s in both WenShan and DeHong, as a result of government sponsorship for cash crops. Furthermore, destruction of most of these extant populations is ongoing. This is consistent with felling of *C. yunnanensis* trees in these populations having begun relatively recently (during the past three decades). Our work, hence, indicates that resprouting is an important means by which this species might persist in the face of logging, at least for a time.

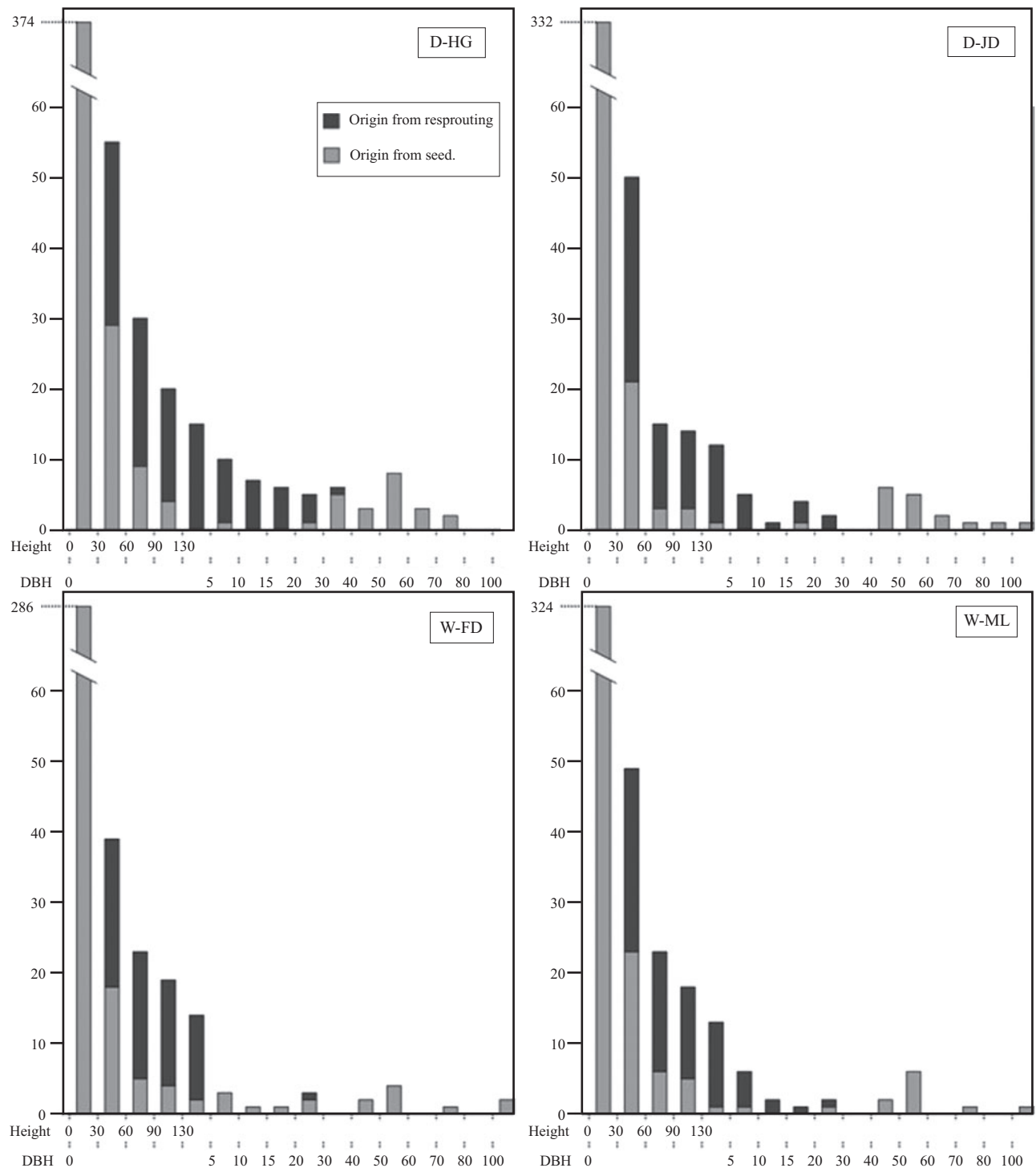


Fig. 2. Size frequency distribution for four *Craigia yunnanensis* populations. Individuals <130 cm in height (i.e. seedlings and saplings) are classed according to height; those >130 cm according to diameter at breast height (DBH), in cm. Darker bars indicate plants of sprout origin, lighter bars plants that originated directly from seed. For each population, graphs show all individuals that were present in five 30 × 30 m plots (i.e. 4500 m² in total); these plots represent in general the densest stands of *C. yunnanensis* at each locality.

Of the four populations examined in detail, D-HG might be suffering less from destruction of saplings and juvenile trees than do the other sites, even though it has been affected at least as much as the others by the destruction of mature trees.

Seed- and seedling-related characters of C. yunnanensis
Provenance has profound implications for the success of restoration efforts, because most species show adaptive genetic variation within their range (O'Brien *et al.* 2007). In the present

Table 2. Mean values (s.e. in parentheses) of seed- and seedling-fitness traits among six populations of *Craigia yunnanensis*

Seed parameters: S-num, number of seeds per capsule; S-len, seed length; S-wid, seed width; S-Wgt, 1000-seed weight. Germination parameters: GC-light, germination capacity in 12-h photoperiod; GC-dark, germination capacity in continuous darkness; GC-nursery, germination capacity in the nursery. Seedling-growth parameters: F-hgt, seedling height; F-rcd, root collar diameter; F-dwt, seedling dry weight. * $P < 0.05$, ** $P < 0.01$. The highest value for each parameter is in bold and marked ⁺; the lowest is marked ⁻.

Parameter	Population						No. of replicates ^A	<i>F</i>
	W-MG	W-FD	D-JD	W-ML	W-LH	D-HG		
Seed parameter								
S-num	1.42 (0.01) ⁻	2.04 (0.06)	1.90 (0.04)	2.55(0.08)	2.32 (0.09)	2.83 (0.20) ⁺	5 × 50	25.57**
S-len (mm)	9.44 (0.03)	9.42 (0.11)	9.59 (0.23)	8.77 (0.05) ⁻	9.02 (0.05)	9.62 (0.13) ⁺	5 × 20	7.51**
S-wid (mm)	3.94 (0.05)	3.40 (0.07)	3.04 (0.11)	3.52 (0.06)	2.99 (0.02) ⁻	3.42 (0.06)	5 × 20	29.00**
S-Wgt (g)	38.34 (0.54)	27.59 (0.15) ⁻	31.66 (0.25)	37.09 (0.48)	28.91 (0.11)	39.13 (0.48) ⁺	8 × 100	180.21**
Germination parameter (%)								
GC-light	76.00 (1.87)	72.00 (2.55) ⁻	68.00 (2.00)	72.00 (2.55) ⁻	86.00 (2.92) ⁺	83.00 (3.39)	5 × 20	6.47**
GC-dark	51.00 (3.32)	42.00 (2.55) ⁻	48.00 (6.04)	69.00 (1.87)	80.00 (2.24) ⁺	73.00 (3.39)	5 × 20	19.08**
GC-nursery	41.11 (5.56)	47.78 (5.88)	37.78 (5.88) ⁻	42.22 (4.84)	62.22 (4.01) ⁺	57.78 (2.94)	3 × 30	3.78*
Seedling parameter								
F-hgt (cm)	36.2 (0.35) ⁻	36.7 (0.42)	38.0 (0.30)	37.1 (0.38)	42.2 (0.52) ⁺	39.1 (0.33)	30	32.51**
F-rcd (mm)	8.02 (0.12) ⁻	8.11 (0.10)	8.59 (0.09)	8.23 (0.12)	8.88 (0.11)	9.57 (0.09) ⁺	30	31.62**
F-dwt (g)	7.86 (0.28) ⁻	8.23 (0.32)	8.65 (0.30)	9.42 (0.24)	10.24 (0.26)	11.95 (0.22) ⁺	30	31.17**
Effective population size	11	89	106	125	151	173		

^AFor seed and germination parameters, the first value is the number of replicate groups, the second the number of capsules/seeds within each replicate group.

Table 3. Estimates of variance components as percentage of the total variation and phenotypic coefficient of variation (PCV) for seed-size characters (S-num, S-len, S-wid, S-Wgt), germination parameters (GC-light, GC-dark, GC-nursery) and seedling-growth attributes (F-hgt, F-rcd, F-dwt)

B. For each character, the phenotypic coefficient of variation (PCV) was computed as the square root of total variance divided by the overall mean, multiplied by 100% (Mamo et al. 2006). For definition of character abbreviations, see Table 2

Character	Overall mean	Variance component (%)		PCV (%) ^B
		Population	Error	
Seed parameters				
S-num	2.00	83.1	16.9	26.9
S-len (mm)	9.31	56.6	43.4	4.5
S-wid (mm)	3.39	84.9	15.1	11.0
S-Wgt (g)	33.79	95.7	4.3	15.2
Germination parameters (%)				
GC-light	61.00	52.2	47.8	10.6
GC-dark	51.00	78.3	21.7	20.1
GC-nursery	44.00	48.1	51.9	16.1
Seedling growth parameters				
F-hgt (cm)	38.20	51.2	48.8	8.0
F-rcd (mm)	8.57	50.4	49.6	9.5
F-dwt (g)	9.39	50.1	49.9	22.5

study, seed- and seedling-related traits varied significantly among populations of *C. yunnanensis*, and this could be exploited for future breeding or selection work. Variation in seed size, germination characters and seedling traits have been found in many tree species, and the differences have been attributed to both genetic differentiation and environmental differences among seed-collection sites (e.g. Loha et al. 2006, 2008; Mamo et al. 2006). For *C. yunnanensis*, most of the phenotypic variation in seed number and seed-size characteristics (S-num, S-len, S-wid

and S-Wgt) occurs among populations. The character that varied most among individuals, as shown by phenotypic plasticity (PCV, Table 3), was seeds per capsule (S-num). This indicated that seed production is strongly influenced by other factors, such as pollinator effects, which could in turn be affected by in habitat fragmentation. Additionally, maternal effects on seed quality and germination are also possible (Guterman 2000).

In general, the highest scores were for the two largest populations, D-HG and W-LH, although the latter had relatively low scores for seed-size characters (Table 2). The smallest population, W-MG, had the lowest scores for seed number and all three seedling-size traits, indicating a possible reduction in fitness in this population. However, its scores for germination-fitness traits were mostly higher than those for the intermediately sized W-FD and D-JD populations (Table 2). Hence, although the low fitness of population W-MG might be due to its small effective-population size, variation for at least some fitness traits could be due to differences that are independent of current effective-population size and/or that existed before human activity caused a reduction in population size.

Compared with seeds incubated in 12-h photoperiod, the overall germination performance of seeds incubated in darkness was low in the laboratory. However, seeds from some populations (e.g. W-LH) germinated equally well in 12-h photoperiod and darkness. This indicated that *C. yunnanensis* populations do not all respond in the same way to the light conditions. This could be due to genotypic differences that developed in response to environmental differences among populations (Navarro and Guitián 2003).

Whereas other studies have detected significant effects of seed size on fitness during and after germination (Navarro and Guitián 2003), no such effects were detected in the current study; indeed, many correlations between seed size and germination or fitness characters were negative (Table 4). This suggested that seed traits have little importance in predicting germination capacity or

Table 4. Inter-character correlations of seed and seedling parameters of six populations of *Craigia yunnanensis*

For definition of character abbreviations, see Table 2. All values are Pearson product–moment correlation coefficients, r . For the correlation between population size and fitness traits, Bonferroni corrections were applied independently for seed characters (S-num, S-len, S-wid, S-Wgt), germination characters (GC-light, GC-dark, GC-nursery) and seedling characters (F-hgt, F-red, F-dwt). For correlations between fitness traits, a single Bonferroni correction was applied across all 45 correlation values, giving a significance threshold of $0.05/45 = 0.0011$. * $P < 0.05$, after Bonferroni correction

	Seed number/size				Germination characters			Seedling characters		
	S-num	S-len	S-wid	S-Wgt	GC-light	GC-dark	GC-nursery	F-hgt	F-red	F-dwt
Population size	0.93*	−0.17	−0.67	−0.09	0.46	0.70	0.66	0.69	0.81	0.87
S-num		−0.27	−0.36	0.16	0.42	0.71	0.58	0.46	0.71	0.88
S-len			0.05	0.07	−0.11	−0.50	−0.11	−0.21	0.29	−0.01
S-wid				0.66	−0.13	−0.27	−0.37	−0.72	−0.44	−0.32
S-Wgt					0.10	0.25	−0.18	−0.32	0.20	0.30
GC-light						0.78	0.92	0.76	0.64	0.69
GC-dark							0.72	0.76	0.64	0.80
GC-nursery								0.81	0.69	0.74
F-hgt									0.67	0.64
F-red										0.94

seedling growth of *C. yunnanensis*. Instead, variation in seed traits might reflect adaptation to dispersal, dormancy or other functions. Environmental effects on the mother plant could also be a factor (Ganatsas *et al.* 2008).

Variation in reproductive output (the total quantity of reproduction, such as flowers and fruits and/or seeds) has been shown to vary among populations, primarily owing to differences in total size and, to some extent, changes in resource-allocation patterns (Bazzaz *et al.* 2000). When resources are limited, a plant may allocate its available resources to the production of either fewer larger seeds or many smaller ones (Harper *et al.* 1970). In common with other studies (Mamo *et al.* 2006; Loha *et al.* 2008), we were unable to prove such an effect, because although seed characters were negatively correlated with population size (Table 4), the difference was non-significant. However, the smallest population (W-MG) had the highest seed width and the second-highest seed weight, so an effect of small population size on seed size cannot be discounted.

In common with certain other species examined (Hensen and Oberprieler 2005; Hirayama *et al.* 2007; Krauss *et al.* 2007), there is a detectable effect of reproductive population size on reproductive output in *C. yunnanensis*, although only in terms of the number of seeds per fruit. Small population size might have immediate effects on reproductive output, such as reducing the attention given to a species by pollinators (Kolb 2008) and shortage of heterospecific pollen, leading to increased selfing or geitonogamy, and/or reduced seed set. However, determining exactly how small population size leads to a reduced seed set, and, for example, whether pollinator behaviour is involved, would require manipulative experiments (Vergeer *et al.* 2003; Lienert and Fischer 2004).

More long-term effects of small population size would include inbreeding depression and genetic deterioration, which would be expected to have an impact on reproductive success in terms of germination success and seedling fitness. In common with similar studies on other species (Hobbs and Yates 2003; Krauss *et al.* 2007), we were not able to prove these effects in *C. yunnanensis*, although consistent positive correlations between population size and germination or seedling-fitness traits indicated that these effects might be present. The absence of a statistically significant

relationship could be due to the effects of population size on fitness being weakened in laboratory and nursery conditions (Lesbarrères *et al.* 2005), although overall it indicates that inbreeding depression, if present, is probably not yet severe (Eisto *et al.* 2000).

Sensitivity to fragmentation and population-size reduction might be greatest in predominately outcrossing species (Mathiasen *et al.* 2007), because these tend not to be adapted to cope with the effects of inbreeding (Charlesworth and Charlesworth 1987). Conversely, species that inbreed regularly may gradually purge their genetic load and, hence, be less susceptible to the negative genetic effects of small population size (Busch 2005; Leimu *et al.* 2006). Furthermore, species that are naturally rare tend to have both genetic traits and reproductive strategies that avoid the negative effects of existing as very small populations, whereas those that become rare as a result of human activity do not (Huenneke 1991). *C. yunnanensis* is not naturally rare; however, regular geitonogamy in this species might make it less susceptible to small population size than is the case for an obligate outcrossing species. However, the lack of observable effects on seedling fitness might simply be due to the contraction in population size having been too recent for inbreeding depression to take effect so far. Further work, comparing genetic diversity among populations by using molecular markers, and testing directly the effects of cross- and self-pollination on fitness, will be necessary to examine in more detail how small population size is affecting fitness in *C. yunnanensis*.

Conclusions

Craigia yunnanensis appears to face a direct extinction threat from human activity. The total number of adults estimated was 655, of which 584 were still present in 2007, plus larger numbers of seedlings and resprouts from cut trunks. Our data on population size-class structure indicated both a dearth of juvenile trees and abundant regeneration both from seed and from cut stems. The abundance of seedlings is likely to be due to increased light availability following canopy removal, which also occurs in other tree species (Stewart and Rose 1990; Haase 1999), and is

expected given that *C. yunnanensis* germinates better under light conditions *in vitro*.

Results from the present study provided evidence that seed- and seedling-related traits vary considerably among populations of *C. yunnanensis*. An effect of population size on seed production was found in *C. yunnanensis*, although no effects of population size on seed size, germination rates, or seedling fitness could be demonstrated. This indicates that some populations at least have already dropped to a size at which size effects have a negative impact on fecundity. Whether or not the fitness differences detected are directly due to population-size effects, it would appear that populations W-LH and D-HG comprise the fittest material and, hence, the most appropriate sources for seed for *ex situ* conservation.

At present, the most pressing need is to preserve the few adult trees that remain, and ensure that at least some of the seedlings and resprouts they are producing are permitted to reach maturity. *Ex situ* conservation, via seed banks and cultivated populations, may be necessary to supplement wild material and prevent extinction, should wild populations be lost.

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