

## THE INFLUENCE OF NATURAL VARIATION IN POPULATION SIZE ON ECOLOGICAL AND QUANTITATIVE GENETICS OF THE ENDANGERED ENDEMIC PLANT *HYPERICUM CUMULICOLA*

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*Premise of research.* Genetic variation for ecologically important traits is necessary for populations to adapt to environmental change. Many authors have called for a greater emphasis on directly measuring quantitative genetic variation in rare species, which are expected to have reduced amounts of genetic variation due to genetic drift in small populations. The extent of among-population differentiation for quantitative traits may also help to evaluate the likelihood that genetic rescue/translocation will be a successful conservation strategy. Despite these merits, relatively few studies measure quantitative genetic variation for ecologically important traits as a function of population size.

*Methodology.* Sixteen populations of the endangered plant *Hypericum cumulicola* were sampled, capitalizing on previous work that has estimated relative effective population sizes and demonstrated minimal migration between populations. This context allows more direct inference about the role of drift in small populations on quantitative genetic variation, the focus of this study. Using controlled pollinations and a greenhouse common garden, quantitative genetic variation within populations and differentiation among populations were estimated for six putatively ecologically important traits.

*Pivotal results.* There were few significant estimates of genetic variation for most traits irrespective of population size. There was a positive correlation between population size and genetic variation for anther-stigma distance, a floral trait expected to influence the degree of self-fertilization. There was also limited differentiation among populations for all traits.

*Conclusions.* Limited genetic variation for ecologically important traits in these populations could slow or limit adaptive responses to future environmental change, possibly increasing extinction risk. The smallest populations will be particularly sensitive to environmental/habitat changes that result in reduced pollinator visitation. Limited phenotypic differentiation, combined with previous evidence of strong heterosis in crosses between small populations, suggests little risk of outbreeding depression if genetic rescue efforts become necessary to preserve this species.

*Keywords:* adaptive potential, adaptive constraint, extinction, Allee effect, pollinator limitation, mating system evolution.

*Online enhancements:* appendix figures and tables.

### Introduction

Understanding the consequences of small population size and/or isolation for population persistence and ability to respond to changing conditions is increasingly important in the face of global change and habitat fragmentation (Kareiva et al. 1993; Lynch and Lande 1993). While habitat loss and demo-

graphic and environmental stochasticity are of immediate conservation concern (Lande 1988; Schemske et al. 1994), even species with protected status may face increased extinction risk due to genetic causes (Ellstrand and Elam 1993; Lynch and Lande 1993; Willi et al. 2006). One such risk is limited genetic variation for ecologically important traits, defined as those expected to influence fitness in the native habitat, because such genetic variation is necessary for adaptation to changing conditions. Genetic variation within and among populations of rare taxa is typically investigated using neutral genetic marker data. This is often the only feasible approach for threatened or endangered species and, in many circumstances, can provide useful information, but the relationship between neutral genetic variation and genetic variation underlying ecologically impor-

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tant traits is often weak (Reed and Frankham 2001). Neutral genetic markers can provide valuable information about long-term effective population sizes and the pattern and magnitude of migration between populations, but they cannot directly provide information about levels of genetic variation underlying ecologically important traits. More conservation genetic research that incorporates quantitative genetic variation has been repeatedly called for (Storfer 1996; Reed and Frankham 2001; Willi et al. 2006; Kramer and Havens 2009).

Genetic variation for quantitative traits is predicted to be lower in small or isolated populations relative to large populations (Willi et al. 2006) because of loss of genetic variation due to genetic drift and because of the smaller number of individuals in which beneficial mutations might arise. This paucity of potentially adaptive genetic variation is predicted to increase the risk of population extinction if the environment changes (Orr and Unckless 2008; Gomulkiewicz and Houle 2009). Direct estimation of quantitative genetic variation in populations of different sizes allows for the testing of predictions about the effects of population size on genetic variation and can provide information about relative extinction risk of managed populations. Although in a stable environment, only a complete lack of genetic variation can impose an absolute constraint on adaptive potential, in a changing environment, limited genetic variation could slow adaptation to the point where extinction is likely to occur before a new adaptive optimum is reached (e.g., Gomulkiewicz and Houle 2009). Thus, lower genetic variation in small relative to large populations would cause relatively slower adaptive responses that could increase extinction risk.

The few studies that have measured quantitative genetic variation for ecologically important traits as a function of population size in rare taxa have found no relationship (Waldmann and Andersson 1998; Podolsky 2001; Gravuer et al. 2005; Willi et al. 2006; but see Willi et al. 2007). One challenge in interpreting this lack of relationship is that measures of population size are often based on census sizes without information about long-term effective population sizes. Populations that have recently become fragmented would not be expected to have lost as much quantitative genetic variation due to genetic drift as populations that have been historically small. Another challenge is that the magnitude and pattern of gene flow is often unknown in these studies. A small population that receives gene flow and/or migrants from a large population is expected to have greater genetic variation than an isolated population of the same size. Estimating quantitative genetic variation in systems where relative effective population sizes and patterns of migration are known provides valuable context for interpreting patterns of quantitative genetic variation within populations.

Regardless of their relationship to population size, estimates of quantitative genetic variation within populations and estimates of differentiation among populations for ecologically important traits can both contribute to the conservation and management of rare species. Estimates of quantitative genetic variation within populations can identify those with relatively high levels of variation, which could be the most suitable sources for genetic rescue/translocation. Knowledge of differentiation among populations can also provide insight for management aimed at *ex situ* conservation of genetic resources be-

yond that derived from neutral genetic markers (Bekessy et al. 2003). Differentiation among populations for ecologically important traits could be used to help qualitatively evaluate the relative risk of outbreeding depression of a proposed translocation (Petit et al. 2001; Gravuer et al. 2005). For example, translocations between populations from similar habitats with similar mean values of putatively adaptive traits would be expected to have a relatively low risk of reduced fitness due to loss of local adaptation. Predicting the risk of outbreeding depression has been identified as the most important scientific challenge in the conservation genetics of wild populations (Frankham 2010).

The endangered perennial plant *Hypericum cumulicola* (Small) P.B. Adams (Hypericaceae) is uniquely suitable for studying the influence of population size on patterns of genetic diversity and differentiation for ecologically important traits. Census population sizes (ranging from 11 to >1000), relative effective population sizes, and migration between populations have been quantified for 16 populations (Oakley and Winn 2012). Previous work strongly implicates a role of genetic drift in shaping genetic variation related to fitness in small populations. Heterosis in crosses between small populations (Oakley and Winn 2012) suggests the fixation of partly recessive deleterious mutations within populations, which cannot be attributed to natural selection. The habitat requirements and demography of this species have also been well studied (Quintana-Ascencio and Morales-Hernandez 1997; Quintana-Ascencio et al. 1998, 2003, 2007), permitting identification of phenological and morphological traits likely to affect fitness in this species.

Traits expected to influence the degree of selfing in *H. cumulicola* are also of interest because pollinator visitation is reduced at low density in this species (Boyle and Menges 2001). In self-compatible species, pollinator limitation can impose selection for floral morphologies such as reduced anther-stigma distance (hereafter, ASD) and smaller flower size that promote greater autogamous selfing (Chang and Rauscher 1999; Goodwillie et al. 2010; Sicard and Lenhard 2011) as a means of reproductive assurance (e.g., Kalisz et al. 2004; Moeller and Geber 2005; reviewed in Eckert et al. 2010). Ideally, direct estimates of selfing rates could be obtained using neutral genetic markers, but limited genetic variation and high homozygosity at microsatellite markers for nearly all populations of this species (Oakley and Winn 2012) preclude such estimates. Patterns of differentiation for floral morphologies associated with increased selfing rates thus provide the only way to investigate if small populations are likely to be more highly selfing than large populations. Unless small populations of *H. cumulicola* have lesser mean ASD or smaller flower size relative to larger populations and/or possess genetic variation for these traits, they may be less likely to be able to persist in the face of reduced pollinator service.

I measured quantitative genetic variation for six ecologically relevant traits in 16 populations of *H. cumulicola* to address three questions: How is quantitative genetic variation for ecologically important traits partitioned within and among populations? Does the amount of genetic variation for traits within populations increase with population size? Do smaller populations exhibit floral morphologies expected to facilitate greater autogamy?

## Material and Methods

### Study System

*Hypericum cumulicola* is a federally endangered, short-lived perennial plant. This species is endemic to the patchily distributed rosemary scrub of the southern Lake Wales Ridge in Florida, which occurs on excessively well-drained white sands (Christman and Judd 1990). This species is a specialist of open sandy gaps (Christman and Judd 1990; Quintana-Ascencio and Menges 1996), likely because it is a poor competitor against dominant shrubs (Quintana-Ascencio and Menges 2000), some of which produce negative biochemical effects (Hunter and Menges 2002; Hewitt and Menges 2008).

This species is self-compatible, but pollinator exclusion experiments in a single population suggest that selfing is mostly pollinator mediated (Evans et al. 2003). Autogamous selfing is possible and was estimated to occur at a rate of approximately 7% (Evans et al. 2003). Reduced pollinator visitation has been observed at low density in this species (Boyle and Menges 2001), which could select for increased autogamous selfing as a means of reproductive assurance. Although the short-term selective advantage of autogamy could be diminished by inbreeding depression (reviewed in Goodwillie et al. 2005), inbreeding depression in this species has been shown to be not significantly different from zero and averages less than 28% (Oakley and Winn 2012).

The 16 focal populations of *H. cumulicola* investigated here range in census size from 11 to greater than 1000 individuals; these population sizes are representative of the range for this endangered endemic, which has a naturally patchy distribution. Previous analysis of microsatellite marker data (Oakley and Winn 2012) has shown that most populations are effectively isolated even at a scale of several hundred meters. Estimates of mutation-scaled inbreeding effective population sizes ( $4N_e\mu$ , where  $\mu$  is the mutation rate) are strongly positively correlated with census population sizes (Oakley 2013); relative  $N_e$  can be inferred if one assumes that mutation rates are similar among populations. Previous work in these populations has also shown that genetic drift promotes the fixation of recessive or nearly recessive deleterious mutations within small populations, which have 68% lower fitness than large populations and exhibit an average heterosis (increased fitness of between-population compared to within-population crosses) of 70% (Oakley and Winn 2012).

### Generation of Full-Sib Families

For each of the 16 populations, I conducted hand pollinations to generate full-sib families to estimate genetic variation within populations and differentiation among populations. I initially collected seeds from naturally pollinated fruits in the field from 5–12 maternal lines in each population for a total of 160 maternal plants. I germinated these seeds and grew the seedlings in the greenhouse for one generation to reduce environmental maternal effects. For each of the 151 maternal lines that produced flowering plants, I randomly selected one individual for pollination. Each day over the course of 2 wk, a subset of these plants was selected based on flower availability and isolated in a pollinator-free room prior to anthesis. Within

1 h of anthesis, plants were self-pollinated by brushing the stigmas of open flowers with the anthers of a different flower from the same plant. Flowers seldom last longer than 5 h (Oakley 2011), and I kept plants isolated overnight to prevent unwanted pollen movement. A total of 1139 self-pollinations yielded an average of 8 families per population (range = 4–12) that produced sufficient seeds. Production of self-sibships allows calculation of broad-sense measures of genetic variation. This method was chosen because many of the populations contained fewer than 25 individuals, precluding a paternal half-sib design. Inbreeding depression in these populations is unlikely to be a severe problem for this crossing design (Oakley and Winn 2012). Inbreeding depression is defined here as a within-population quantity, due to deleterious mutations segregating within a single population. It is different from heterosis in between-population crosses, which is caused by the masking of deleterious mutations that have been fixed within populations.

### Greenhouse Common Garden and Trait Measurement

I sowed an average of 72 seeds per family (range = 25–114) in petri dishes with moist field soil, for a total of 9225 seeds. For each family, I scored the number of days until germination for all seedlings (defined as emergence of cotyledons from the seed coat). I transplanted seedlings two per cell into 38-cell seedling trays (5.7-cm diameter, 12.7-cm-deep cells) filled with field-collected soil. Large portions of these seedlings were used for a separate field experiment (Oakley 2011) and were not able to be included here. The remaining seedlings were raised in the greenhouse under uniform conditions. After 2 wk, I thinned 194 cells to one haphazardly chosen seedling and transplanted these into 20-cm-deep, 7.5-cm square pots filled with pure field soil. These 194 plants (97 families with two seedlings per family) were watered daily with distilled water and fertilized once a month with 10% strength 20-20-20 NPK liquid fertilizer until the end of the experiment. Limited greenhouse space and logistical constraints set upper limits on the overall size of the experiment. The level of replicates per family was sacrificed to maximize the number of populations and the number of families per population needed for the power to address questions about differentiation, genetic variation, and their relationship with population size.

For 14 of the populations with sufficient progeny, an additional 334 plants (an average of 6.6 families per population, with an average of 3.6 individuals per family) were left in flats for an additional 6 wk. After 6 wk, I harvested the above- and belowground material, dried it at 60°C for 2 wk, weighed dried root and shoot biomass separately for each individual plant, and used these to calculate juvenile root-shoot ratios. Relative allocation to roots versus shoots is likely to be an important trait for adaptation to xeric conditions in this species.

I measured several growth, phenological, and floral traits on the 194 transplanted individuals. I measured stem height at thinning and again 4 wk later and calculated juvenile stem growth as the difference in the height measurements. I recorded time to first flowering every 1–2 d. All individuals survived, and 95% (184/194) flowered. I collected approximately 3 flowers from each plant, taking 1 flower per day on 3 different days. All flowers were collected within half an hour of an-

thesis, placed upright in individual vials, and stored in a cooler until they were digitally photographed the same morning. Flowers of *H. cumulicola* have 5 petals and 3 stylar branches, which depart from the base of the style perpendicularly to a central cluster of stamens (fig. A1; figs. A1–A3 available online). Top view images were made of each flower under a dissecting scope at  $\times 10$  magnification using a millimeter ruler for scale. For each flower, I measured the total diameter as the average of the distance from the tip of the longest petal to the tip of each of the two petals most closely opposite. I also measured ASD as the average of the three measured distances between each stigma and the nearest anther. Plant means were calculated from the means of the three individual flowers. All measurements were made using ImageJ software (Rasband 1997–2002).

### Statistical Analyses

I tested for differentiation among populations and among families within populations for each trait with nested ANOVA. Population and family nested within population were both treated as random effects (PROC MIXED; SAS 2008). Significance of all model terms was assessed by the difference in the  $-2$  log likelihood score between a model with the term of interest and a model without it. This test statistic is approximately  $\chi^2$  distributed with 1 degree of freedom. For each trait, proportions of total variance explained by both population and family nested within population were calculated from variance components from the full model. Separate models were then run for each trait and population to generate estimates of the variance components for family ( $V_F$ ). Significance of individual estimates of  $V_F$  was assessed with likelihood ratio tests as described above.

Population means for each trait were calculated, and broad-sense coefficients of genetic variation ( $CV_G$ ) for each population for each trait were calculated as (square root of  $V_G$ )/(trait mean)  $\times 100$ , where in a full-sib design,  $V_G$  is calculated as  $2 \times V_F$  (Podolsky 2001; Gravuer et al. 2005). Because estimates of heritability are influenced by the amount of phenotypic variance in a trait, and thus not easily comparable among traits (Houle 1992; Hansen et al. 2011), I focus on the

mean standardized estimates of broad-sense genetic variance ( $CV_G$ ). To examine the relationship between population size and broad-sense genetic variation, I examined correlations between both previously published estimates of  $4N_e\mu$  and census population size (Oakley and Winn 2012) as well as  $CV_G$  for each trait. I include correlations with census size because effective population-size estimates were not available for the second-largest population, and the correlations with  $4N_e\mu$  are therefore less powerful. To examine the relationship between population size and potential for autogamous selfing, I performed a similar set of correlations using mean values for these traits.

## Results

### Differentiation and Overall Variation

Mean time to germination differed significantly among populations (table 1); the latest-germinating population was 66% (23.1 d) slower than the earliest-germinating population (14.4 d; table A1; tables A1, A2 available online). There was also significant differentiation among families within populations (table 1). In total, 32.6% of the variation in germination time was attributed to genetic factors, with about half of the genetic variation for this trait partitioned among populations and the other half partitioned among families within populations (table 1).

There was significant differentiation among populations and among families within populations for juvenile growth traits. Aboveground growth of juvenile stems differed significantly among populations (table 1), with a range of 7.6–15.4 cm (table A1), but did not differ significantly among families within populations (table 1). Juvenile root-shoot ratio was not significantly different among populations (table 1) but was significantly different among families within populations (table 1). Family within population explained 10% of the phenotypic variation.

Flowering phenology and floral traits likewise showed significant differentiation. Time to first flower differed significantly among populations but did not differ significantly among families within populations (table 1). The effect of population

**Table 1**

**Analysis of Variance Results of the Effects of Population and Family Nested within Population for the Six Traits Measured**

Trait	Significance		% variance explained	
	Population	Family (population)	Population	Family (population)
Time to germination	48.6***	520.7***	17.0	15.6
Juvenile stem growth	4.5*	1.1	9.6	NA
Juvenile root-shoot ratio	2.0	4.2*	NA	10.3
Days to first flowering	10.5**	.3	14.8	NA
Flower diameter	5.5*	3.1+	14.0	16.7
Anther-stigma distance	7.0**	7.2**	13.8	24.5

Note. Table entries for significance are  $\chi^2$  values, while percent variance explained is given only for significant terms.

NA = not applicable.

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

\*\*\*  $P < 0.001$ .

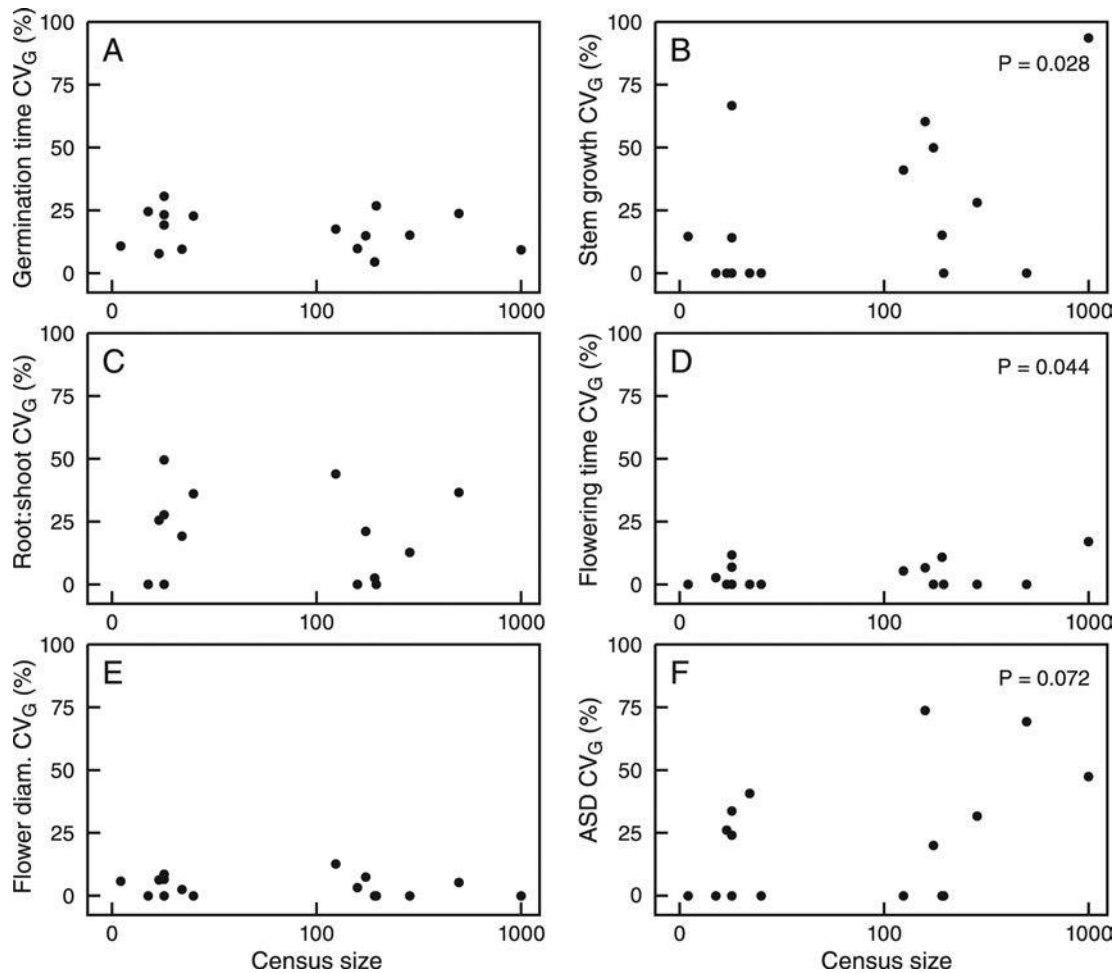
+ Nonsignificant;  $P = 0.078$ .

explained almost 15% of the phenotypic variation (table 1); mean days to first flower ranged from 143 to 168 d (table A1). Flower diameter was significantly different among populations (table 1), with a range of 10.02–11.93 mm (table A1), and there was nearly statistically significant differentiation among families within populations (table 1). Together, 30.7% of the variation in flower diameter was attributed to genetic factors, with roughly half of this partitioned among populations and the other half among families within populations (table 1). Differences among populations and among families within populations for ASD were significant (table 1) and explained a combined 38.3% of the variation in this trait. About one-third of the genetic variation for ASD was attributable to genetic differentiation among populations, and the remaining two-thirds was attributable to differences among families within populations. Anther-stigma distance had the greatest proportional differentiation among populations of any of the traits measured; ASD in the population with the largest mean was 110%

greater than mean ASD in the population with the smallest ASD, although the range of absolute values was small (0.35–0.73 mm; table A1).

#### Genetic Variation within Populations

Despite significant overall differences among families for four of the six traits (table 1), genetic variation within populations was rarely significantly greater than zero. Over 16 populations and six traits, only about 20% of the family-level variance components were found to be significantly different from zero. Overall mean  $CV_G$  was about 15% (figs. 1, A2). One exception was germination time, for which  $V_F$  was significant for 13 of the 16 populations (table A2). For the remaining traits, only 7 of the 78 estimates of  $V_F$  were significant or nearly significant; ASD had the most evidence of significant genetic variation, with three populations having significant estimates of  $V_F$  (table A2). Overall, larger populations (>124 individuals)



**Fig. 1** Population broad-sense coefficients of genetic variation ( $CV_G$ ) for the six traits measured as a function of census size. Significant or nearly significant correlations between census size and  $CV_G$  were detected for B, D, and F. Significant or nearly significant overall genetic variation within populations was detected for A, C, E, and F. Population census size estimates are from Oakley and Winn (2012). Juvenile root:shoot ratio (C) was not measured for populations 7 and 15 due to insufficient numbers of progeny. ASD = anther-stigma distance.

had about twice as many significant estimates of  $V_F$  compared to smaller populations (<25 individuals; table A2).

### Correlations with Population Size

I found no significant correlation between population size and  $CV_G$  for most traits for which there was a significant overall effect of family nested within population (tables 1, 2; figs. 1, A2), but there was a nearly significant positive correlation between census size and  $CV_G$  for anther-stigma distance (table 2; fig. 1F). The lack of a significant positive relationship between population size and  $CV_G$  for timing of germination is notable because most populations had significant  $V_F$  for this trait (tables 1, A2; fig. 1A). There was a significant positive relationship (table 2) between population size and both juvenile stem growth (figs. 1B, A2B) and days to flowering (fig. 1D).

There were no significant correlations between population size and population mean values of traits expected to influence the degree of self-fertilization (flower diameter:  $r = 0.13$ ,  $P = 0.63$ ; ASD:  $r = -0.05$ ,  $P = 0.84$ ; figs. 2, A3). Correlations between effective population size and population mean trait values were qualitatively similar (flower diameter:  $r = -0.05$ ,  $P = 0.86$ ; ASD:  $r = -0.21$ ,  $P = 0.46$ ).

### Discussion

Despite calls for a greater focus on genetic variation underlying fitness-related traits in conservation genetics (Storfer 1996; Reed and Frankham 2001; Willi et al. 2006; Kramer and Havens 2009), few studies have undertaken this task (reviewed in Willi et al. 2006). Studies that have examined the relationship between population size and either narrow or broad-sense genetic variation typically find no pattern (Waldmann and Andersson 1998; Podolsky 2001; Gravuer et al. 2005; Willi et al. 2006; but see Willi et al. 2007), but such a relationship could be obscured if estimates of census population size do not accurately reflect effective population sizes and/or if gene flow is obscuring the relationship between census and effective population sizes (e.g., source-sink migration). To my knowledge, this study is the first to incorporate estimates of effective population sizes ( $N_e$ ) and knowledge of the magnitude and pattern of migration in examining levels of quantitative genetic variation as a function of population size in a rare species. I measured quantitative genetic variation for six ecologically

important traits in 16 populations of the endangered plant *Hypericum cumulicola*. Overall, I found significant differentiation among populations for five of the traits and significant (or nearly significant) differentiation among families within populations for four of the traits. Within populations, significant estimates of broad-sense genetic variation for most traits were generally limited to one to few populations, but there was some evidence to suggest greater genetic variation in larger populations. A nearly significant positive correlation between population size and genetic variation for anther-stigma distance could indicate that smaller populations would be less able to respond to selection for increased autogamous selfing as a means of reproductive assurance.

### Population Differentiation

Overall, there was significant differentiation among populations for most of the measured traits, with an average of about 14% (range = 9.6–17.0) of the phenotypic variation explained by variation among populations. The magnitude of this differentiation was comparable to the average of 17% (range = 10.3–24.5) explained by variation among families within populations. For traits related to survival, limited differentiation is perhaps not surprising because *H. cumulicola* is narrowly endemic to one habitat type, which could suggest similar selection pressures in all populations (Petit et al. 2001; Gravuer et al. 2005). For time to first flowering, significant differentiation among populations (15% of the phenotypic variation explained) combined with a lack of significant variation among families within populations is consistent with a history of divergent natural selection for this trait, but other mechanisms such as genetic drift cannot be ruled out with the data presented here. A similar pattern, but of lesser magnitude, was observed for juvenile stem growth.

Other studies investigating population differentiation for quantitative traits in rare taxa have compared differentiation of quantitative traits ( $Q_{ST}$ ) to differentiation ( $F_{ST}$ ) of neutral genetic markers (Waldmann and Andersson 1998; Gravuer et al. 2005; Willi et al. 2007). A history of divergent selection among populations for a particular trait could be inferred if  $Q_{ST} > F_{ST}$  (Spitze 1993; Merilä and Crnokrak 2001; Leinonen et al. 2008), though this inference requires many assumptions (Hendry 2002; Whitlock 2008; Edelaar and Björklund 2011). In rare taxa,  $Q_{ST}$  has been found to exceed  $F_{ST}$  (Willi et al. 2007), but many authors attribute some role of neutral/stochastic processes in phenotypic differentiation (Waldmann and Andersson 1998; Petit et al. 2001; Willi et al. 2007). I did not compare  $Q_{ST}$  to  $F_{ST}$  for *H. cumulicola* because previous work (Oakley and Winn 2012) indicated no detectable migration between the majority of these populations. This suggests that  $F_{ST}$  (for neutral markers) is essentially 1 in most cases, making it impossible to distinguish between divergent selection and drift when comparing to  $Q_{ST}$  because the maximal value for both quantities is 1.

### Genetic Variation within Populations

Despite significant (or nearly significant) broad-sense genetic variation for four of the six traits, with the exception of time to germination and ASD, significant genetic variation was limited to a single population for each trait. For time to germination, 13

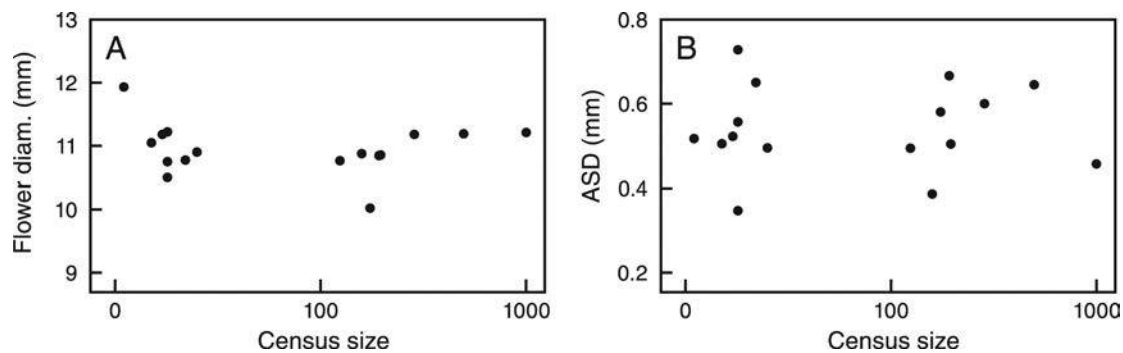
Table 2

Correlations of Population Broad-Sense Coefficients of Genetic Variation ( $CV_G$ ) with Both Census Population Size and Mutation-Scaled Effective Population Size ( $4N_e\mu$ )

Trait	Correlation	
	$CV_G$ and census size	$CV_G$ and $4N_e\mu$
Time to germination	-.21	-.24
Juvenile stem growth	.55*	.58*
Juvenile root-shoot ratio	-.00	-.30
Days to flowering	.51*	.43
Flower diameter	-.23	-.28
Anther-stigma distance	.46+	.42

\*  $P < 0.05$ .

+ Nonsignificant;  $P = 0.072$ .



**Fig. 2** Population mean flower diameter (A) and anther-stigma distance (ASD; B) as a function of census population size. Significant overall differentiation was found for both flower diameter (A) and ASD (B). Correlations between census size and coefficients of genetic variation ( $CV_G$ ) were not significant for either trait. Census size estimates are from Oakley and Winn (2012).

of the 16 populations had significant genetic variation. Temporal or spatial variation in selection on germination time within populations (Picó et al. 2003) could maintain genetic variation in this trait in spite of strong effects of genetic drift. Alternatively, the larger sample sizes for this trait (in terms of number of replicate seeds per family) may have increased the power to detect small but significant amounts of genetic variation. Estimates of  $CV_G$  for time to first germination averaged 17% (range = 5–31). Approximately 25% of the total phenotypic variation for ASD was explained by variation among families within populations. This was the greatest amount of phenotypic variance explained by either population or family nested within population for any trait.

At the population level, only three populations had significant genetic variation for ASD, but estimates of  $CV_G$  were the largest of any of the traits (mean = 23%, range = 0–74) with significant overall genetic variation.

Some caveats to the interpretation of these estimates of genetic variation within populations are necessary. First, I report broad-sense genetic variation from a full-sib design; this was a practical constraint because of small population sizes. Ultimately, future adaptive potential depends on additive genetic variation (Willi et al. 2006), but broad-sense variation constitutes an upper limit to the amount of additive genetic variation. Second, the limited number of families per population used reflects the reality of working on an endangered species and the requisite large number of populations needed to test for a relationship between genetic variation and population size. Small family sizes do reduce the power to detect significant genetic variation and may contribute to the limited number of significant estimates detected. However, the total number of populations and/or families used here is comparable to, if not larger than, the total numbers used in similar studies (Waldmann and Andersson 1998; Podolsky 2001; Gravuer et al. 2005; Willi et al. 2007) investigating quantitative genetic variation in rare taxa.

### Correlations with Population Size

There were few significant correlations between population size and  $CV_G$ . Significant positive correlations were found between population size and  $CV_G$  for both juvenile stem growth and time to first flowering, but these correlations should be

interpreted with caution because there were no significant overall effects of family nested within population for either of these traits (table 1). There was a nearly significant ( $P = 0.072$ ) positive correlation between census population size and coefficients of genetic variation for ASD, consistent with smaller populations having less genetic variation for a trait that could influence population-level selfing rate.

Significant differentiation for flower diameter and/or ASD could indicate variation in selfing rates among populations because smaller flowers and smaller ASD have been shown to be associated with increased autogamous self-fertilization (Chang and Rausher 1999; Goodwillie et al. 2010; Sicard and Lenhard 2011). Despite significant differentiation among populations in both ASD and flower size, there was at least as much genetic variation within populations as there was differentiation among populations for both traits. The relationship between ASD (and flower size) and selfing rate have not been directly studied in this species, but mean ASD in small and large populations was the same (mean = 0.54, range = 0.33–0.73 and 0.39–0.65 for small and large populations, respectively). The range in mean flower sizes among populations was also modest (about a 20% difference between the smallest and largest population mean flower sizes). There was no significant relationship between population size and the mean for either trait, which is inconsistent with smaller populations having greater autogamous self-fertilization.

Lack of genetic variation for ASD in small populations with average or greater-than-average mean values of ASD could decrease population fitness and increase extinction risk due to an inability to respond to selection for increased selfing driven by pollen limitation (cf. Moeller and Geber 2005; Eckert et al. 2010). Increased self-fertilization could also be constrained to a degree by inbreeding depression, but inbreeding depression in this species is weak (Oakley and Winn 2012), particularly in small populations. The selective advantage of increased selfing due to reproductive assurance would thus be likely to outweigh any short-term negative consequences of increased selfing (Kalisz et al. 2004).

### Conclusions and Conservation Implications

These historically small and patchily distributed populations of *Hypericum cumulicola* appear to have limited quantitative

genetic variation. Consequently, this species may be very sensitive to any further fragmentation or anthropogenic changes to the native habitat. Limited pollinator service in small populations may put them at particular risk of extinction because they possess limited genetic variation for traits expected to facilitate increased self-fertilization. With the possible exceptions of time to first flowering and juvenile stem growth, there is limited phenotypic differentiation for ecologically important traits among populations relative to variation within populations. Combined with prior evidence of heterosis in crosses between small populations, this suggests that genetic rescue via interpopulation crosses and/or translocation could prove beneficial for small populations of this species and is unlikely to result in outbreeding depression by either intrinsic or extrinsic causes. Genetic rescue as a conservation strategy is controversial (Tallmon et al. 2004; but see Frankham 2010; Frankham et al. 2011), but controlled crossing and common garden experiments as described here are a relatively low-cost way to as-

sess likelihood of success of such an approach and provide much more information than would neutral marker data alone.

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