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Fine-scale genetic structure in populations of the spring ephemeral herb *Megaleranthis saniculifolia* (Ranunculaceae)

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ABSTRACT

Fine-scale genetic structure (FSGS) in plants occurs primarily through restricted seed dispersal. Analyses of FSGS have been used retrospectively to infer seed dispersal and other ecological processes. The spring ephemeral *Megaleranthis saniculifolia*, endemic to Korea, is insect-pollinated and has no special seed dispersal mechanism, and its seedling recruitment is quite low. Given these ecological and life-history traits, we expect that there would be significant FSGS in juveniles, which would persist into adult stage. Since *M. saniculifolia* is self-compatible and many adults produce 2–3 inflorescences, we expect considerable inbreeding. To test these predictions, we used allozyme-based *Sp* statistics to compare two undisturbed populations on Mt. Deogyu (DEO) and Mt. Taebaek (TAE), as well as between juveniles (J) and adults (A) stages. We also measured genetic diversity and inbreeding in each population. The two populations exhibited significant FSGS in both life stages. Although the strength of FSGS was reduced with increasing stage (J, $Sp = 0.0313$; A, $Sp = 0.0178$ in DEO and J, $Sp = 0.0502$; A, $Sp = 0.0286$ in TAE), differences in FSGS between stages and between populations were not significant. Within-population genetic diversity (mean $\%P = 27.0$, $A = 1.27$, $H_e = 0.116$) was relatively low and comparable to reference values for both endemic and narrowly-distributed plant species. Genetic differentiation between sites, however, was high ($F_{ST} = 0.465$), and the two populations exhibited a significant deficit of heterozygotes (mean $F_{IS} = 0.289$), primarily due to selfing and biparental inbreeding (effective selfing rate was ~0.40). Our results revealed that the magnitude and spatial scale of FSGS in *M. saniculifolia* is strong and does not differ significantly with life-history stage. The strong FSGS, low within-population genetic variation, high between-population genetic differentiation, and high inbreeding are consistent with the species' limited seed dispersal and a mixed mating system.

1. Introduction

Seed plants disperse their genes by two independent life-history stages: pollen (haploid) dispersal and seed (diploid) dispersal. Because of the difference in chromosome content, each pollen dispersal event contributes only half as much as each seed dispersal event to total gene flow between populations (Hamrick and Nason, 1996). Further, pollen and seed dispersal have very different effects on the pattern and magnitude of fine-scale genetic structure (FSGS; the nonrandom spatial distribution of individual genotypes) found within populations (Kalisz et al., 2001). At one extreme, for example, a plant species may have very limited pollen dispersal (e.g., nearest-neighbor mating or even selfing) while seeds are dispersed at random within the population. In this case, there will be no development of FSGS (Chung et al., 2003a; Fuchs and Hamrick, 2010). At the other extreme, if there is extensive pollen dispersal (e.g., random mating) but very limited seed dispersal, seed shadows of half-siblings will be formed around maternal plants resulting in substantial FSGS (Hamrick and Nason, 1996; Epperson, 2000). Although long-distance pollen and seed dispersal between populations will tend to homogenize allele frequencies and reduce genetic

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differentiation between populations (Dyer, 2007), inter-population gene flow will generally have little effect on the patterns of FSGS within populations. Substantial overlap of seed shadows—which is a function of population density—is the primary factor causing low FSGS even in a species with poor seed dispersal ability (Hamrick et al., 1993; Hamrick and Trapnell, 2011).

Previous studies have demonstrated that the pattern and magnitude of FSGS found in juveniles may differ significantly from that observed in adults. Consequently, the ecological processes underlying observed patterns of FSGS can be misinterpreted when either a single life-history stage (e.g., seedlings, juveniles or adults) is studied or when data across life-history stages are pooled (Tonsor et al., 1993; Jones and Hubbell, 2006; Hampe et al., 2010). In juveniles, FSGS is shaped by the pattern and extent of seed dispersal overlain upon parental spatial demographic and genetic structure (Kalisz et al., 2001). The juvenile FSGS can be changed in subsequent life-history stages by random or competitive thinning and mortality of individuals, historical events, habitat disturbance, post-dispersal selection, or other ecological and evolutionary processes (Kalisz et al., 2001; Chung et al., 2003b; Jacquenmyn et al., 2006; Yamagishi et al., 2007). Therefore, comparisons of FSGS among different life stages provide insight into ecological and evolutionary processes underlying observed FSGS in natural populations (Tonsor et al., 1993; Kalisz et al., 2001; Chung et al., 2003b; Jacquemyn et al., 2009; Fuchs and Hamrick, 2010). To date, however, there is no consistent trend in changes in the pattern and magnitude of FSGS across life-history stages. Studies have shown that FSGS may decrease (Hamrick et al., 1993; Epperson and Alvarez-Buylla, 1997; Parker et al., 2001; Chung et al., 2003b; Hardesty et al., 2005; Yamagishi et al., 2007) or increase with age (Tonsor et al., 1993; Kalisz et al., 2001; Jacquenmyn et al., 2006; Jones and Hubbell, 2006), or not differ significantly between life-history stages (Chung et al., 2000, 2003b; Jacquemyn et al., 2009; Fuchs and Hamrick, 2010). Together, these studies show that populations of both woody and herbaceous species may exhibit these three inter-stage specific patterns of FSGS.

Spring ephemerals are characterized by short life cycles adapted to the transient availability of resources in the understory of temperate deciduous forests (i.e., flowering in early and fruiting in late spring, with leaf senescence before canopy closure; Miller et al., 2002; Sunmonu and Kudo, 2014). Spring ephemerals generally have low rates of sexual reproduction and produce few seeds (Meier et al., 1995), which are typically dispersed short distances (on the order of few a tens of centimeters to a few meters) by gravity, ants, ballistics, or heavy rains (if the populations are located on slopes) (Ohara and Higashi, 1987; Matlack, 1994; Kalisz et al., 2001; Miller et al., 2002). The localized dispersal of seeds around maternal plants may be advantageous by avoiding predation and unfavorable or unsuitable habitats (the local adaptation hypothesis; Schemske, 1984; Schmitt and Gamble, 1990). Similarly, because spring ephemerals flower and fruit before canopy closure, longer distance seed dispersal may not be favored since seeds may not need to disperse to locate more favorable (light, open) environments (D. Mladenoff, pers. comm.). Following dispersal, spring ephemerals generally have low rates of germination (less than 1%; C. Baskin, pers. comm.) and seedling establishment. Meier et al. (1995) suggests that the combination of these factors (limited seed production, dispersal, and recruitment) has contributed to their decline in eastern North America. These factors also are expected to influence the spatial scale and magnitude of FSGS within spring ephemeral populations.

Megaleranthis saniculifolia Ohwi (Ranunculaceae) is a spring ephemeral belonging to a monotypic genus endemic to Korea. The species is a small, non-clonal perennial herb (ca. 10–15 cm at flowering time; ~40 cm at fruiting period) and self-compatible under greenhouse conditions (M.Y. Chung and M.G. Chung, unpubl. data). Each adult usually produces 2–3 terminal inflorescences with a solitary white flower (ca. 2 cm at diameter) that is open about 1 week. A variety of insects have been found visiting *M. saniculifolia* flowers, including *Bombylius major*, *Eristalis tenax*, and several anthomyiid and tachinid flies (Choi, 2002; M.Y. Chung, J.M. Chung, and M.G. Chung, pers. obs.). About 6–10 pistils per flower mature into follicles, and each fruit contains 2–5 seeds, which are small (1.8 mm long, 1 mm wide), ovoid, and smooth, suggesting no special seed dispersal mechanisms. Like many spring ephemerals (Baskin and Baskin, 1988; Lee et al., 2012), *M. saniculifolia* disperses seeds (that have underdeveloped embryos) in spring under morphophysiological dormancy (Lee et al., 2003). Thus, seeds of *M. saniculifolia* go through warm (summer) and cold (winter) conditions before germinating the following spring (Lee et al., 2003). This one-year period of dispersal prior to germination increases the chance of seed mortality. The number of juveniles is considerably fewer than adults in several populations of *M. saniculifolia*, suggesting that seedling recruitment is quite low (J.M. Chung, pers. obs.).

As *M. saniculifolia* has no specialized seed dispersal mechanisms, our first expectation is for there to be significant FSGS in juveniles. Second, if seedling recruitment is low and juveniles are not subject to density-dependent mortality, we expect the significant FSGS found in juveniles to persist into adults. Third, since *M. saniculifolia* is self-compatible, many adults produce 2–3 inflorescences, and flowers are produced under cold and windy early season conditions unfavorable to pollinators, we expect considerable inbreeding (i.e., selfing and/or biparental inbreeding). To test these predictions, we conducted a comparative study of juvenile and adult *M. saniculifolia*, using genetic markers (allozymes) to estimate genetic diversity, inbreeding, and spatial patterns of FSGS for these two life stages in two undisturbed populations.

2. Materials and methods

2.1. *Study species*

The Korean endemic species *Megaleranthis saniculifolia* occurs on gently sloping (< 20°) valley floors in the mesic deciduous forests from 800 to 1500 m asl, usually accompanied by several other herbaceous perennials (e.g., *Aruncus dioicus*, *Meehania urticifolia*, and *Veratrum oxysepalum*). To date, 12 populations are known, with some being large (six populations are 5000–18000 m^2) but isolated (separated by distances ranging from ca. 8–540 km, with an average between-population distance of 162 km) along the Baekdudaegan (the main Korean mountain range) in the central and southern Korean Peninsula, including Jeju Island (Jeong et al., 2010). Following the criteria of the IUCN (International Union for Conservation of Nature) Red List Categories (IUCN, 2001), the species has been categorized as “Vulnerable” VU A2cd (MOE, 2014) and more recently as “Endangered” EN B2ab(iii) (Chang et al., 2016). *Megaleranthis saniculifolia* is also protected by the regulations of the Korea Forest Service as a “rare” plant (KNA, 2008)

2.2. *Population sampling*

To determine levels of allozyme diversity and to analyze FSGS in *M. saniculifolia*, in April 2014 we mapped and marked all individuals with aluminum tags, and collected one leaf from 217 individuals (population size) within a 30 × 50 m area (total spatial extent; Fig. 1) on a gentle east-facing slope at 1530 m asl on Mt. Deogyu, in southern Korea (hereafter referred as DEO). For replication, in May 2014 we mapped, identified, and sampled all 190 individuals (population size) within a 15 × 25 m area (total spatial extent; Fig. 1) on a gentle northern-west facing slope at 1380 m asl on Mt. Taebaek, in central Korea (hereafter referred as TAE). In DEO *M. saniculifolia* is accompanied by a variety of trees (nine species; e.g., *Taxus cuspidata*, *Picea jezoensis*, *Betula ermanii*, *Quercus mongolica*, *Acer tschonoskii* subsp. *koreanum*, *A. caudatum* subsp. *ukurundense*), shrubs (five species; e.g., *Deutzia parviflora*, *Weigela subsessilis*, *Rhododendron schlippenbachii*), and herbaceous perennials (eight species; e.g., *Dryopteris crassirhizoma*, *Veratrum oxysepalum*, *Pedicularis resupinata*, *Meehania urticifolia*, *Huperzia serrata*, *Aconitum pseudolaeve*). Nearly the same shrubs and herbaceous perennials grow in TAE, although there are some tree species not occurring in TAE, including