Population Genetic Effects of Urban Habitat Fragmentation in the Perennial Herb *Viola pubescens* (Violaceae) using ISSR Markers

**THERESA M. CULLEY**, **SARAH J. SBITA** and **ANNE WICK**

Department of Biological Sciences, University of Cincinnati, 614 Rieveschl Hall, Cincinnati, OH 45221-0006, USA

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- **Background and Aims** Fragmentation of natural habitats can negatively impact plant populations by leading to reduced genetic variation and increased genetic distance as populations become geographically and genetically isolated from one another. To test whether such detrimental effects occur within an urban landscape, the genetic structure of six populations of the perennial herb *Viola pubescens* was characterized in the metropolitan area of Greater Cincinnati in southwestern Ohio, USA.

- **Methods** Using three inter-simple sequence repeat (ISSR) markers, 51 loci amplified across all urban populations. For reference, four previously examined agricultural populations in central/northern Ohio and a geographically distant population in Michigan were also included in the analysis.

- **Key Results** Urban populations retained high levels of genetic variation (percentage of polymorphic loci, \( P_p = 80.7\% \)) with similar genetic distances among populations and an absence of unique alleles. Geographic and genetic distances were correlated with one another, and all populations grouped according to region. Individuals from urban populations clustered together and away from individuals from agricultural populations and from the Michigan population in a principle coordinates analysis. Hierarchical analysis of molecular variance (AMOVA) revealed that most of the genetic variability was partitioned within populations (69.1\%) and among groups (22.2\%) of southwestern Ohio, central/northern Ohio and Michigan groups. Mean \( F_p \) was 0.308, indicating substantial population differentiation.

- **Conclusions** It is concluded that urban fragmentation does not appear to impede gene flow in *V. pubescens* in southwestern Ohio. These results are consistent with life history traits of this species and the possibility of high insect abundance in urban habitats due to diverse floral resources and nesting sites. Combined with the cleistogamous breeding system of this species, pollinator availability in the urban matrix may buffer populations against detrimental effects of habitat fragmentation, at least in larger forest fragments. Consequently, it may be inappropriate to generalize about genetic effects of fragmentation across landscapes or even across plant species with different pollination systems.

**Key words:** Habitat fragmentation, ISSR, population genetic structure, urban effects, *Viola pubescens*.

**INTRODUCTION**

Habitat fragmentation is increasing throughout the world and has many important ecological and genetic consequences for endemic plant populations. In general, plant species in fragmented areas may experience reduced habitat and population size as well as potential disturbances in pollinator service and seed dispersal (Jennersten, 1988; Rathcke and Jules, 1993; Kwak *et al.*, 1998; Spira, 2001; Bhattacharya *et al.*, 2003). Over time, this can lead to increased inbreeding (Ellstrand and Elam, 1993), lower reproductive success (Steffan-Dewenter and Tscharntke, 1999) and disrupted gene flow. Consequently, isolated plant populations can experience loss of genetic variation, increased population differentiation and genetic drift (Young *et al.*, 1996; Mills and Tallmon, 1999). Over the long term, fragmentation may also reduce the ability of populations to adapt to changing environments (Fisher, 1930; Mills and Tallmon, 1999), thereby increasing local extinction events. In some cases, however, fragmentation may not be detrimental (Young *et al.*, 1996) and may even promote gene flow (Young and Merriam, 1994; Aldrich and Hamrick, 1998; White *et al.*, 2002).

Although genetic effects of fragmentation in plants have been examined in a variety of landscapes, including grasslands (Young *et al.*, 1999), woodlands (Prober *et al.*, 1998), deciduous forests (Foré *et al.*, 1992; Young *et al.*, 1993; Young and Merriam, 1994; Cruzan, 2001) and agricultural areas (Berg *et al.*, 1998; Culley and Grubb, 2003), urban areas have remained relatively unexplored.

Urban habitat fragmentation results in a mixture of natural areas interspersed with residential and commercial developments and often dissected by major transportation corridors. Consequently, the matrix of an urban landscape can be much more complex than the matrices of other landscapes, such as agricultural areas where crop monocultures typically separate fragments. This complexity of the urban matrix may potentially impose a more substantial barrier to gene flow than other matrices, especially if movement of pollinators and seed dispersers is negatively impacted by urbanization. On the other hand, the urban matrix may potentially promote gene flow if pollinators and seed dispersers persist and flourish within the urban matrix.

An ideal location in which to examine the genetic effects of urban fragmentation is southwestern Ohio. As with many other areas of the USA, this locality has experienced increased urbanization as the city of Cincinnati has expanded into outlying areas. The city was one of the first
settlements in the Ohio Valley circa 1778 when mixed mesophytic and beech-maple forests were common (Braun, 1950; Gordon, 1969). It is estimated that >95% of Ohio was originally covered with forests (Griffith et al., 1993), which were gradually cleared for agriculture and settlement, until by 1910 only 10% of the state was forested. Following passage of laws encouraging forest development as well as abandonment of farms as people migrated to urban locations, forest cover in Ohio gradually increased to >30% by 1991 (Griffith et al., 1993). Today, >13% of southwestern Ohio is forested (Griffith et al., 1993), a portion of which is contained within urban parks in the Greater Cincinnati metropolitan area. This area is now home to >2 million people, occupying 13 counties in Ohio, Kentucky and Indiana, and was considered the 24th largest metropolitan area in the country in 2000 (US Bureau of the Census, 2003).

Within urban parks in Greater Cincinnati can be found Viola pubescens var. scabra, the Smooth Yellow Violet. This native perennial is ideal for study because it is partially dependent upon insect pollinators for seed set (Culley, 2002), it contains substantial amounts of genetic variation relative to other species (Culley and Wolfe, 2001) and its population genetic structure was previously characterized within an agricultural landscape (Culley and Grubb, 2003). In this case, populations in small forest fragments exhibited reduced genetic variation and increased population differentiation relative to populations in larger fragments (Culley and Grubb, 2003). The goal of the current study was to quantify the genetic structure of urban populations of V. pubescens to answer the following questions. (a) Do urban populations exhibit lower levels of genetic variation relative to non-urban populations? (b) Are urban populations more genetically distant and differentiated from one another compared with non-urban populations? (c) Is gene flow impeded by the urban matrix? To our knowledge, this is the first study of genetic effects of habitat fragmentation in a plant species within an urban environment.

MATERIALS AND METHODS

The study organism

Viola pubescens var. scabra is a stemmed, non-clonal violet that is a common resident of deciduous forests in eastern North America (Ballard, 1994). Foliage is largely glabrous, which distinguishes this variety from the densely pubescent V. pubescens var. pubescens (Ballard, 1994); the varieties also differ in number of stems, basal leaves and teeth on leaf margins (Lévesque and Dansereau, 1966; Cain, 1967; Ballard, 1994). The species itself exhibits dimorphic cleistogamy (Culley and Klooster, 2007), producing two types of flowers at different times: yellow chasmogamous flowers are produced first in the early spring and inconspicuous cleistogamous flowers subsequently appear after the forest canopy forms in late spring (Culley, 2002). Individuals continue to produce automatically self-pollinated cleistogamous flowers until plant senescence in early autumn. Chasmogamous flowers are visited by a variety of generalist pollinators, including bumblebees, carpenter bees, butterflies, halictid bees and beetles (Culley, 2002). Outcrossing rates vary substantially between years, ranging from 0.40 to 0.73 (Culley, 2002). Seeds are dispersed both ballistically up to 4 m away from the maternal plant and also by ants (Culley, 2002), so long-distance gene flow probably only occurs via pollen. Individuals can live at least 5 years (Culley, 2002) and possibly longer, as in Viola sororia which has an individual life expectancy >10 years (Solbrig et al., 1980).

Sampling

Fresh leaf samples were obtained during Spring, 2003 from 39–49 individuals in each of six populations of V. pubescens var. scabra scattered throughout the Greater Cincinnati area in southwestern Ohio (Fig. 1; Table 1). These populations are found in secondary growth forest that formed after agriculture was abandoned in the region and most probably originated from relict populations inhabiting smaller farm woodlots in the area. Four of the populations (Miami Whitewater, Sharon Woods, Trillium Trails and Winton Woods) are located in urban parks owned by the Hamilton County Park District in Hamilton County, Ohio. The remaining two populations inhabit preserves owned by the University of Cincinnati in Hamilton County, Ohio (The Harris M. Benedict Botanical Preserve) and Miami University in neighbouring Butler County, Ohio (Miami University Natural Areas). Within each site, samples were collected from individual plants located at least 2 m apart to prevent sampling the same plant twice. Tissue samples were placed on ice for transport back to the University of Cincinnati, where they were stored at −75 °C.

These six southwestern Ohio populations were also compared with four different non-urban Ohio (hereafter referred to as ‘central/northern Ohio populations’) and Michigan populations of V. pubescens var. scabra and one Michigan population of V. pubescens var. pubescens that had been previously examined (Culley and Wolfe, 2001). Bohannan, a central Ohio population in Delaware and Morrow Counties, is located in Bohannan Woods, a scientific preserve owned by Ohio Wesleyan University. Three sites in northern Ohio (Etter Central, Hill and Stump) are privately owned woodlots in Crawford County, Ohio; populations in these sites inhabit isolated wooded areas within an agricultural landscape that was historically part of the Sandusky Plains (Culley and Grubb, 2003). Finally, a distant site in Emmet County, Michigan contained intermingled populations of V. pubescens var. scabra and pubescens individuals. Samples from these populations were originally analysed at The Ohio State University (Culley and Wolfe, 2001) using laboratory techniques as described below.

Generation of genetic data

DNA from the southwestern Ohio samples was extracted using a modified mini-prep cetyltrimethyl ammonium bromide (CTAB) technique of Doyle and Doyle (1987)
and then stored at $-20^\circ C$ until further analysis. Dominant inter-simple sequence repeat (ISSR) markers were used to quantify the genetic structure of all populations. ISSR markers are similar to randomly amplified polymorphic DNA (RAPD) markers (Wolfe and Liston, 1998) except that ISSR primers are typically longer nucleotide sequences, resulting in a higher primer annealing temperature that gives greater band reproducibility than RAPD markers. Three simple sequence repeats (SSRs) were used as primers to generate 51 bands in single-primer reactions. Primer Mao [(CTC)$_4$RC] yielded 15 bands, primer 17898 [(CA)$_6$RY] produced 22 bands and primer 844 [(CT)$_8$RG] generated 14 bands.

Polymerase chain reaction (PCR) was conducted individually for each primer with either 15 $\mu$L (for primers Mao and 17898) or 25 $\mu$L (for primer 844) reaction volumes using optimized conditions as follows: 0.2 mM dNTPs, 3 mM (for Mao and 17898) or 2 mM (for 844) MgCl$_2$, 0.4 $\mu$M primer, 1 $\times$ Taq DNA polymerase buffer, 0.25 U (for Mao and 844) or 0.5 U (for 17898) of Taq (Gibco/BRL; Invitrogen) and 0.3 $\mu$L (for Mao and 844) or 0.5 $\mu$L (for 844) of DNA. A larger reaction volume was used for primer 844 because it exhibited inconsistent band amplifications at lower reaction volumes. The brand of Taq polymerase and its buffer was found to be important because different brands (e.g. Promega Corp., Madison, WI, USA) may amplify only a sub-set of loci. Amplifications were performed in an Eppendorf Mastercycler using the following conditions: initial denaturation at 94 $^\circ C$ for 1.5 min; 35 cycles each of 94 $^\circ C$ for 45 s, 45 $^\circ C$ (for Mao and 17898) or 46 $^\circ C$ (for 844) for 45 s, and 72 $^\circ C$ for 1.5 min; a final cycle consisted of 94 $^\circ C$ for 45 s, 45 $^\circ C$ or 46 $^\circ C$ for 45 s, and 72 $^\circ C$ for 5 min.

Following PCR, blue/orange loading dye (Promega Corp.) was added to each reaction and samples were loaded onto a 1.2% agarose gel in 1 $\times$ TAE buffer. Additionally, 1 kb ladders (Promega Corp.) and negative and positive controls were loaded onto each gel, which were then run at constant voltage (145 V). Each gel was stained with ethidium bromide and digitized under UV light using the Kodak 1D software package and a Kodak DC290 camera (Eastman Kodak, Rochester, NY, USA). The images were analysed using the same software, which assigns a fragment size to each band using an

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**Fig. 1.** Locations of *Viola pubescens* populations examined in the current study. Shown in the left panel are reference populations in central Ohio, northern Ohio and Michigan (numerals along the border represent latitude and longitude, respectively). The right panel represents the enlarged area in southwestern Ohio that shows the location of six populations in the Greater Cincinnati metropolitan area. County lines are indicated in both panels for Ohio or Greater Cincinnati, and major roadways (dark lines) and Hamilton County parks (grey areas) are indicated in the right panel.
algorithm based on the 1 kb ladder. These fragment sizes were used to assign loci for each primer, and bands for each assigned locus were scored as diallelic (1 = band present; 0 = band absent).

Data analysis

To avoid common problems associated with the analysis of dominant data (Culley and Wolfe, 2001), analyses only used band presence or absence data and did not involve Hardy–Weinberg equilibrium. This is necessary because populations of *V. pubescens* are known to deviate from Hardy–Weinberg equilibrium with co-dominant allozyme markers (Culley and Grubb, 2003). It was assumed that there was no co-migration of alleles from different loci, alleles shared by two individuals descend from a common ancestor and each locus consisted of only two alleles that segregate in Mendelian inheritance. Genetic variation was characterized for all 12 populations by calculating the number of shared and unique bands, the percentage of polymorphic loci (*P*), the percentage of loci that were fixed for a single band, Shannon’s index of diversity (*H*), and the percentage of loci that were fixed for a single band. Shannon’s index of diversity was also estimated for each population by calculating the proportion of bands observed per locus across all individuals. Differences between southwestern Ohio and central/northern Ohio populations in *P*, *H* and the percentage of loci with fixed bands were analysed with individual Student’s 𝑡-tests in SAS version 9.1 (SAS Institute, Cary, NC, USA). To test for separate relationships between *P* or *H* with fragment area on both regional and local scales, Spearman’s coefficient of rank correlation was calculated separately in SAS for all *V. pubescens* var. scabriuscula populations and for the sub-sets of southwestern Ohio and central/northern Ohio populations.

Genetic distance was quantified using the Nei and Li (1979) coefficient to compare the number of alleles shared between individuals or populations, excluding shared band absences. This coefficient was first calculated as a distance measure for each pair of individuals in MSVP version 3.11 h (Kovach Computing Services, Anglesey, UK) and used in a principle coordinates analysis to determine whether individuals would cluster according to their designated population. The Nei and Li similarity coefficient was calculated for pairs of populations using WAVSIML (V. Ford, unpublished; see Crawford et al., 1998 for formulae), and genetic distances were computed for each pair of populations as (1 – similarity). Distance values range from 0 (no difference among groups) to 1 (complete difference among groups). Distances were used to construct a population-level UPGMA tree in NTSYSpc version 2.11a (Rohlf, 1998).

To determine if a relationship existed between genetic and geographic distances of pairs of populations, a Mantel test was conducted using the TFPGA software package (Miller, 1997). Geographic distances were calculated as straight line distances between pairs of sites using latitude and longitude of each location.

Population differentiation was examined with a hierarchical analysis of molecular variance (AMOVA) for populations of *V. pubescens* var. scabriuscula using Arlequin version 3.1 (Excoffier, 2005). This method treats dominant data as haplotypes and partitions the total variance into covariance components associated with differences among individuals within populations, among individuals in different populations within groups and among groups. These regional groups consisted of: (a) urban populations in southwestern Ohio; (b) agricultural populations in central/northern Ohio; and (c) the Michigan population. Overall *F*<sub>st</sub> was calculated and significance tests were performed in Arlequin (Excoffier et al., 1992) using the approach of Weir and Cockerham (1984) with 1023 permutations. AMOVA was also conducted separately for the southwestern OH populations and for the central/northern OH populations to generate *F*<sub>ST</sub> values for these regions.

RESULTS

Genetic variation

High levels of variation were observed in urban populations of *V. pubescens* in Greater Cincinnati (Table 2). The

### Table 1. Locations of populations of Viola pubescens var. scabriuscula in Ohio and Michigan

<table>
<thead>
<tr>
<th>Site</th>
<th>County</th>
<th>Area (ha)</th>
<th><em>N</em>&lt;sub&gt;pop&lt;/sub&gt;</th>
<th><em>N</em>&lt;sub&gt;sampled&lt;/sub&gt;</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southwestern Ohio (Urban)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benedict (BEN)</td>
<td>Hamilton</td>
<td>26.30</td>
<td>&lt;800</td>
<td>47</td>
<td>39.26577</td>
<td>-84.35498</td>
</tr>
<tr>
<td>Miami Whitewater (MWW)</td>
<td>Hamilton</td>
<td>779.22</td>
<td>&gt;900</td>
<td>49</td>
<td>39.25163</td>
<td>-84.70099</td>
</tr>
<tr>
<td>Sharon Woods (SHW)</td>
<td>Hamilton</td>
<td>192.01</td>
<td>&gt;800</td>
<td>39</td>
<td>39.27797</td>
<td>-84.40200</td>
</tr>
<tr>
<td>Trillium Trails (TT)</td>
<td>Hamilton</td>
<td>9.30</td>
<td>&lt;500</td>
<td>40</td>
<td>39.25668</td>
<td>-84.47898</td>
</tr>
<tr>
<td>Winton Woods (WW)</td>
<td>Hamilton</td>
<td>548.63</td>
<td>&gt;800</td>
<td>43</td>
<td>39.26173</td>
<td>-84.51852</td>
</tr>
<tr>
<td>Miami University (MIU)</td>
<td>Butler</td>
<td>10.50</td>
<td>&gt;600</td>
<td>46</td>
<td>39.52912</td>
<td>-84.70796</td>
</tr>
<tr>
<td>Central/Northern Ohio (Agricultural)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etter Central (ETT)</td>
<td>Crawford</td>
<td>21.13</td>
<td>&lt;800</td>
<td>37</td>
<td>40.74555</td>
<td>-82.94684</td>
</tr>
<tr>
<td>Hill (HLL)</td>
<td>Crawford</td>
<td>25.09</td>
<td>&lt;800</td>
<td>35</td>
<td>40.71079</td>
<td>-83.02582</td>
</tr>
<tr>
<td>Stump (STP)</td>
<td>Crawford</td>
<td>31.30</td>
<td>&lt;1000</td>
<td>34</td>
<td>40.75498</td>
<td>-82.92736</td>
</tr>
<tr>
<td>Bohannan (BOH)</td>
<td>Delaware/Morrow</td>
<td>40.50</td>
<td>&gt;1000</td>
<td>33</td>
<td>40.35049</td>
<td>-82.92864</td>
</tr>
<tr>
<td>Outgroup</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Michigan (MI)</td>
<td>Emmet, MI</td>
<td>&gt;500</td>
<td>&gt;1000</td>
<td>18</td>
<td>45.54386</td>
<td>-84.85573</td>
</tr>
</tbody>
</table>

Listed are the approximate area of each site that is forested, an estimate of the total number of individuals in the population (*N*<sub>pop</sub>) and the subset of individuals that were sampled (*N*<sub>sampled</sub>).
The average percentage of fixed alleles in the southwestern Ohio populations (2.4% ± 0.809) was slightly higher than in central/northern Ohio populations (2.0% ± 0.907). Similarly, the mean Shannon diversity index did not differ significantly (t = 0.323, P = 0.749) or for the subset of southwestern Ohio populations (r = 0.327; Table 3). Genetic distances between the southwestern Ohio and north/central groups of populations were higher, ranging from 0.339 to 0.587 (mean = 0.453; Table 3).

Finally, southwestern populations were most distant from the Michigan populations of either the same variety (var. scabriuscula; mean distance = 0.611) or var. pubescens (0.648; Table 3).

The UPGMA phenogram indicated that populations generally clustered according to their geographic location. Urban and agricultural populations in central/northern Ohio and Michigan populations were most different from each other, and more similar to each other than to the south/central Ohio populations (Fig. 2). Genetic distances for all pairwise comparisons of the six southwestern Ohio populations were quite similar to one another, ranging from 0.300 to 0.366 (mean = 0.327; Table 3). Distance measures between the north/central Ohio populations were more variable, ranging from 0.287 to 0.482 (mean = 0.399). Genetic distances between the southwestern Ohio and north/central groups of populations were higher, ranging from 0.339 to 0.587 (mean = 0.453; Table 3).
Genetic distances were calculated using 51 ISSR loci, based on (1-similarity) using the Nei and Li (1979) coefficient. Population abbreviations are given in Table 1.


Ohio populations (Mantel test; genetic and geographic distances for the six southwestern single Michigan population was removed (because a lower coefficient was detected when the probably due to a significant isolation

\[ F_{ST} \]

overall groups and 22.2% among groups (Table 4). Values of within populations, 8.7% among populations within

\[ r \]

revealed that genetic variability was partitioned 69.1%

western Ohio, central/northern Ohio and Michigan)

Hierarchical AMOVA of three population groups (southwestern Ohio, central/northern Ohio and Michigan) revealed that genetic variability was partitioned 69.1% within populations, 8.7% among populations within groups and 22.2% among groups (Table 4). Values of overall \( F_{ST} \) for individual loci ranged from −0.004 to 0.695, with a mean of 0.308 that was significantly different from zero (\( P < 0.00001 \)). When analysed separately, urban populations within southwestern Ohio and agricultural populations in central/northern Ohio had \( F_{ST} \) values of 0.111 and 0.113, respectively; both values were significantly greater than zero (\( P < 0.00001 \)).

**Population differentiation**

Urban effects of habitat fragmentation have received little attention to date, although fragmentation in general often lowers genetic variation and increases genetic differentiation of plant populations (Young *et al.*, 1996; Mills and Tallmon, 1999). Such effects may also occur in urban areas if the matrix imposes substantial barriers to gene flow (Ledig, 1992), leading to increased isolation and genetic drift of populations. However, in the urban landscape of southwestern Ohio, populations of *V. pubescens* exhibited high genetic variation and substantial gene flow, as indicated for example by the low amount of variation that was attributed among populations within groups (8.7%). In general, genetic variation in urban populations of *V. pubescens* was substantially higher than for other long-lived perennials with mixed-mating systems (Hamrick and Godt, 1996), a result consistent with previous investigations of this species in agricultural areas (Culley and Wolfe, 2001; Culley and Grubb, 2003). Although urban populations were genetically differentiated from one another, similar levels of differentiation were also observed for non-urban populations of the same species. Combined with the absence of unique bands, these results indicate that habitat fragmentation in urban areas does not substantially impede gene flow in *V. pubescens*.

This study is distinctive in that it is one of the first reports of whether the genetic structure of a plant species is affected by habitat fragmentation in an urban environment. Within animals, high levels of genetic variation have also been found in urban populations of butterflies (Kronforst and Fleming, 2001) and woodland beetles (Desender *et al.*, 2005), but not in toads (Hitchings and Beebee, 1998). The fact that largely deleterious effects of fragmentation were not detected in *V. pubescens* is not completely unusual in plant populations, as negligible and even positive effects of fragmentation have been found in non-urban habitats. For example, pollen dispersal increased in *Swietenia humilis* in dry forest fragments (White *et al.*, 2002), and isolated individuals of *Symphonia globulifera* were responsible for most seedlings in nearby remnant tropical forest (Aldrich and Hamrick, 1998). In temperate

**Table 4. Table of the hierarchical AMOVA examining differences among and within groups of urban and agricultural populations of Viola pubescens var. scabriuscula**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Variance component</th>
<th>Percentage variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among groups</td>
<td>2</td>
<td>438-12</td>
<td>1-846</td>
<td>22.17</td>
</tr>
<tr>
<td>Among populations</td>
<td>8</td>
<td>279-86</td>
<td>0-722</td>
<td>8.67</td>
</tr>
<tr>
<td>Within population</td>
<td>410</td>
<td>2360-90</td>
<td>5-758</td>
<td>69.16</td>
</tr>
<tr>
<td>Total</td>
<td>420</td>
<td>3078-88</td>
<td>8-326</td>
<td></td>
</tr>
</tbody>
</table>

Three groups consisted of populations in southwestern Ohio, north/central Ohio and Michigan. Values are based on 51 ISSR loci.
forest, fragmented populations of *Acer saccharum* did not exhibit reduced genetic variation (Young et al., 1993), although genetic structure was affected (Young and Merriam, 1994). Interestingly, all these species are long-lived trees while *V. pubescens*, as a herbaceous perennial with a relatively shorter generation time, would be expected to be more susceptible to deleterious effects of fragmentation.

In *V. pubescens*, a scarcity of deleterious effects of urban fragmentation could in part be explained by characteristics of the species itself and of the urban environment. First, reproductive traits of *V. pubescens* promote occasional long-distance gene flow and resistance to population extinction, thus preventing loss of genetic diversity in fragments. For example, dual production of chasmogamous and cleistogamous flowers (Culley, 2002) creates opportunities for outcrossing by generalist pollinators but also guarantees seed production independent of pollinator activity. Chasmogamous flowers also have the added benefit of delayed selfing if left unvisited (Culley, 2002). *Viola pubescens* is a long-lived perennial with a seed bank, factors that enhance population persistence. Overall, these traits may protect populations against short-term effects of habitat fragmentation, but may not be as effective in the long-term if populations continue to lose pollinators and habitat (Culley and Grubb, 2003). Other plant species may be more detrimentally affected by habitat fragmentation, especially if they are annual, obligate outcrossers with specific habitat requirements or require specialized pollinators.

Genetic variation and gene flow in *V. pubescens* may also be maintained in urban areas because insect pollinators are not as detrimentally affected by fragmentation as originally thought (Rathcke and Jules, 1993; Didham et al., 1996; Kearns et al., 1998; Kwak et al., 1998; Spira, 2001). Many previous investigations focused on non-urban landscapes in which the intervening matrix is relatively inhospitable for pollinators (e.g. Jennersten, 1988; Steffan-Dewenter and Tscharntke, 1999). In the urban landscape, the matrix may be more permeable because it contains residential yards, undeveloped land and transportation corridors that sustain pollinators with food and possibly nesting sites as they move between fragments. Urban areas enhance insect abundance but not diversity (McIntyre and Hostetler, 2001; Tommasi et al., 2004), even though both are expected to decline in fragments (Rathcke and Jules, 1993). Compared with natural areas, certain bee species are more abundant in metropolitan areas (McIntyre and Hostetler, 2001; Tommasi et al., 2004), and many insects that pollinate *V. pubescens* (Culley, 2002) have been observed in residential areas of southwestern Ohio (T. Culley, pers. obs.), including bumblebees known to fly far from urban nesting sites (Chapman et al., 2003; but see Bhattacharya et al., 2003). Although busy roadways could potentially restrict pollinator movement and hence gene flow in urban locations, this does not seem to occur in populations of *V. pubescens*. For example, the Benedict and Sharon Woods populations are separated by US Interstate 71, a busy six-lane roadway, but they exhibited one of the lowest pairwise genetic distances of all populations. Such pollinator activity in urban areas would buffer populations against expected declines in genetic variation associated with fragmentation (Young et al., 1996; Mills and Tallmon, 1999), in part by counteracting genetic isolation that has been observed in small agricultural populations (Culley and Grubb, 2003).

Substantial levels of genetic variation detected in this study may also reflect the relatively large size of urban habitat fragments (25–779 ha), many of which are large public parks. In contrast, fragments in agricultural areas are typically only 0.5–40 ha (Culley and Grubb, 2003). Larger urban fragments could buffer populations against loss of genetic variation by minimizing edge effects, maximizing population size and promoting pollinator persistence within the fragments (Rathcke and Jules, 1993; Buchmann and Nabhan, 1996; Spira, 2001). Not surprisingly, levels of genetic variation of *V. pubescens* were not correlated with fragment size in the urban landscape as they were in the agricultural area (see also Culley and Grubb, 2003). Although this could be caused in part by different glacial histories of the two regions, both areas were almost completely deforested in the early 20th century and thus it is more likely that the genetic results are due to fragmentation rather than to glaciation. Fragment size, however, is not completely responsible for genetic patterns because the smallest urban populations contained more genetic variation than the closest matching sized agricultural population. For example, urban populations at Miami University and Trillium Trails were smaller (<11 ha) than the smallest agricultural population, Etter Central (21 ha), but yet contained a higher percentage of polymorphic loci (84.3 and 80.4 %, vs. 70.6 %). This suggests that other factors, such as those described previously, are also involved in the genetic response of urban populations to fragmentation.

**CONCLUSIONS**

Populations of *V. pubescens* that inhabit urban forest fragments in southwestern Ohio are less prone to deleterious genetic effects of habitat fragmentation than previously thought. It now appears that at least some of our basic understanding regarding genetic effects of habitat fragmentation is in need of revision, especially for urban areas. Because different urban environments may not all have the same effect on plant populations, the impact of various urban settings also needs to be explored. This study suggests that it may not be appropriate to generalize about fragmentation effects across landscapes because processes affecting gene flow may differ depending upon the surrounding environmental matrix as well as the plant species itself. To appreciate more fully the genetic implications of fragmentation in the urban landscape for plants, additional investigations are needed using a variety of plant species in different urban areas. In this way, it will finally be possible to understand the genetic consequences and ecological effects of urban habitat fragmentation, an ever-increasing problem in the world today.
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LITERATURE CITED


