

ORIGINAL ARTICLE

The structural and functional connectivity of the grassland plant *Lychnis flos-cuculi*

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Understanding the relationship between structural and functional connectivity is essential for successful restoration and conservation management, particularly in intensely managed agricultural landscapes. We evaluated the relationship between structural and functional connectivity of the wetland plant *Lychnis flos-cuculi* in a fragmented agricultural landscape using landscape genetic and network approaches. First, we studied the effect of structural connectivity, such as geographic distance and various landscape elements (forest, agricultural land, settlements and ditch verges), on gene flow among populations as a measurement of functional connectivity. Second, we examined the effect of structural graph-theoretic connectivity measures on gene flow among populations and on genetic diversity within populations of *L. flos-cuculi*. Among landscape elements, forests hindered gene flow in *L. flos-cuculi*, whereas gene flow was independent of geographic distance. Among the structural graph-theoretic connectivity variables, only intrapopulation connectivity, which was based on population size, had a significant positive effect on gene flow, that is, more gene flow took place among larger populations. Unexpectedly, interpopulation connectivity of populations, which takes into account the spatial location and distance among populations, did not influence gene flow in *L. flos-cuculi*. However, higher observed heterozygosity and lower inbreeding was observed in populations characterised by higher structural interpopulation connectivity. This finding shows that a spatially coherent network of populations is significant for maintaining the genetic diversity of populations. Nevertheless, lack of significant relationships between gene flow and most of the structural connectivity measures suggests that structural connectivity does not necessarily correspond to functional connectivity.

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INTRODUCTION

The loss and fragmentation of natural and semi-natural habitats are the major causes of biodiversity decline in European agricultural landscapes (Liira *et al.*, 2008). Fragmentation of habitats has decreased genetic diversity within populations and increased genetic differentiation among populations (Leimu *et al.*, 2006) that, in turn, reduced their fitness and survival. Therefore, enhancing connectivity among fragmented habitats is one of the major goals of conservation planning (Brückmann *et al.*, 2010).

A wide range of measures has been developed to quantify habitat connectivity that either measure structural connectivity accounting for the area and spatial configuration of habitats or quantify functional connectivity reflecting the actual movement of individuals or genes among populations (Tischendorf and Fahring, 2000). Structural connectivity can be relatively easily measured, for example, by calculating landscape metrics obtained from geographic information systems, assessing the topology of habitat or population networks or simply estimating geographic distances among habitats or populations. Therefore, structural connectivity is often used as a proxy for functional connectivity, although the response of species may not necessarily be correlated to the spatial configuration and composition of a landscape (Tischendorf and Fahring, 2000). However, success in restoration can only be achieved by evaluating to which degree an

increase in structural connectivity (for example, the creation of stepping stones) improves the functional connectivity of study species. Functional connectivity can be measured by either directly observing the movement of species, for example, by radio-tracking or mark-recapture studies (Keller *et al.*, 2010), or indirectly by using population genetic data (Keller *et al.*, 2010). In plants, direct measurements of seed or pollen dispersal are very labour- and time consuming and may strongly underestimate the spatial and temporal extent of dispersal (Kamm *et al.*, 2010). Therefore, genetic information has been successfully applied to assess functional connectivity in plant populations (Kamm *et al.*, 2010).

One of the possibilities for examining the relationship between structural and functional connectivity of plants is to correlate measurements of gene flow to various measurements of landscape structure. Many studies simply correlated geographic distance with gene flow estimates among spatially isolated populations (see, for example Jacquemyn *et al.*, 2007). However, in addition to geographic distance, landscape properties influence functional connectivity among populations by either fostering or impeding gene flow. Landscape genetic approaches have been widely applied to examine the relationship between landscape structure and functional connectivity in animal species, but, to date, only few studies applied landscape genetic approaches to examine gene flow among plant

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populations (Holderegger *et al.*, 2010). This is most probably because of the fact that gene flow among plant populations is mediated through other vectors than plants themselves, for example, pollinators, wind, water and so on. Thus, it is not the plants that landscape structure acts upon, but seed and pollen dispersers, which complicates evaluating the possible role of various landscape characteristics on gene flow in plants.

Graph-theoretic connectivity metrics combine the attributes of habitats and populations with the dispersal behaviour of species (Pascual-Hortal and Saura, 2006). In graph-theoretic approaches, the habitat patches or populations (nodes) are connected by links, for example, geographic distances (Saura and Pascual-Hortal, 2007; Saura and Torne, 2009). Nodes are considered connected if the internode distance is lower than, for example, the maximum dispersal distance of a focal species (that is, structural connectivity). Based on the topology of a network, it is possible to determine the most critical network elements—nodes as well as links—for maintaining the structural connectivity of habitat patches or populations in a landscape. This approach has been suggested as a useful tool in assisting decision making for conservation purposes (Pascual-Hortal and Saura, 2006). Despite the increasing application of graph-based connectivity metrics in recent years, studies relating these measures to estimates of functional connectivity, such as gene flow, or to genetic diversity, remain scarce (Neel, 2008; Dyer *et al.*, 2012).

The current study was carried out in an intensively managed Swiss agricultural landscape where semi-natural grasslands made up only 2% of the total area. We examined whether these fragmented grasslands within a matrix of agricultural land, forests and settlements maintain gene flow and genetic diversity as measured from molecular markers from *Lychnis flos-cuculi*, an insect-pollinated and predominantly outcrossing wetland plant with no specialised seed dispersal mechanism. This plant species was chosen because insect-pollinated outcrossing species are most vulnerable to habitat fragmentation (Aguilar *et al.*, 2008). First, we evaluated whether graph-based estimates of structural connectivity reflect functional connectivity, measured as gene flow, and genetic diversity of this species. Second, we examined the effects of landscape elements on gene flow in *L. flos-cuculi* using a landscape genetic approach based on transects

(Holderegger *et al.*, 2010; Angelone *et al.*, 2011; Van Strien *et al.*, 2012). We hypothesised that settlements, agricultural land, forests and topography (that is, elevation change) negatively affect gene flow (Tewksbury *et al.*, 2002; Helm *et al.*, 2009; Keller *et al.*, 2012; Pellissier *et al.*, 2012), whereas the amount of ditch verges being one of the key habitats of this species in contemporary agricultural landscapes (Aavik *et al.*, 2008) facilitate gene flow in *L. flos-cuculi*.

MATERIALS AND METHODS

Study species, data collection and genetic analyses

L. flos-cuculi L. (syn. *Silene flos-cuculi*; Caryophyllaceae) is a diploid polycarpic perennial herb that is distributed throughout Europe (Jalas and Suominen, 1986). It usually grows in moist, open habitats such as floodplain and fen meadows. In arable landscapes, it has become scarce because of loss of habitats, but is still found in secondary habitats such as ditch and stream verges. *L. flos-cuculi* is an insect-pollinated species visited by various pollinators: Lepidoptera, Diptera and Hymenoptera (Van Rossum and Triest, 2010). The species is predominantly outcrossing, but capable of self-fertilisation (Biere, 1991). Stems are 30–90 cm tall, and flowers open between April and June in the second year after establishing. Capsules contain ~150 seeds (Biere, 1991) that are dispersed mechanically. In addition to sexual reproduction, *L. flos-cuculi* forms vegetative rosettes from axillary stem buds.

The study area with a size of ~80 km² is an agricultural landscape with a mixture of pastures and arable land (Figure 1). It is located in the Cantons of Bern and Aargau in Switzerland. We mapped the centroids of all naturally occurring populations of *L. flos-cuculi* in the study area using Global Positioning System, collected leaves from 30 individuals (where possible) of *L. flos-cuculi* in each population and estimated census population size (Table 1).

We kept collected plant material in silica gel until DNA extraction. DNA was extracted from 10 mg of dry leaf material using the Dneasy 96 Plant Kit (QIAGEN, Hombrechtikon, Switzerland). We used three microsatellite markers developed for *L. flos-cuculi* (Galeuchet *et al.*, 2002) and three markers developed for *Silene latifolia* (Moccia *et al.*, 2009), a close relative of *L. flos-cuculi*. PCR was carried out as described in Aavik *et al.* (2012). PCR products were analysed on an ABI 3730 automated sequencer (Applied Biosystems, Carlsbad, CA, USA). Allele lengths were scored using GENEMAPPER 3.7 (Applied Biosystems). Tests of linkage disequilibrium in FSTAT 2.9.3.2 (Goudet, 1995) over all populations revealed a significant linkage between three pairs of loci, but a lack of significant linkage within populations indicated



Figure 1 Location of study populations of *Lychnis flos-cuculi* in Switzerland. Grey lines mark ditches and other water bodies with flowing water; grey areas indicate forested areas.

Table 1 Coordinates (according to Swiss national grid), sample and population size, allelic richness (A_R), expected heterozygosity (H_E), observed heterozygosity (H_O) and inbreeding coefficient (F_{IS}) of the study populations of *Lychnis flos-cuculi*

Population	E	N	Sample size	Population size	A_R	H_E	H_O	F_{IS}
N10	628095	230481	30	430	5.68	0.643	0.661	-0.028
N11	621919	230642	30	100	5.23	0.658	0.622	0.056
N12	622202	230752	30	100	5.34	0.699	0.717	-0.026
N13	625045	227516	30	4300	5.37	0.682	0.620	0.093
N14	627753	231501	30	650	5.33	0.696	0.633	0.092
N15	629374	232675	30	100	5.02	0.659	0.622	0.056
N16	624406	229357	20	20	6	0.683	0.642	0.062
N17	624908	229044	30	150	5.64	0.665	0.573	0.14
N18	627475	227938	30	1170	5.11	0.659	0.628	0.048
N19	629958	227380	15	15	3.26	0.502	0.351	0.309
N20	627154	232378	30	400	5.44	0.683	0.643	0.06
N21	629437	233871	30	260	4.09	0.628	0.644	-0.026
N22	629526	232632	13	15	4.75	0.63	0.628	0.003
N23	629581	233562	29	500	5.34	0.71	0.712	-0.002
N24	629581	234200	30	70	5.74	0.693	0.650	0.063
N25	628795	230672	17	100	4.04	0.572	0.549	0.041
N26	629306	233697	30	150	5.02	0.702	0.717	-0.021

that those loci were not physically linked. FSTAT 2.9.3.2 was used for detecting deviations of loci from Hardy–Weinberg expectations, but none of the loci deviated from Hardy–Weinberg expectations. As estimates of genetic diversity within populations, we used allelic richness (A_R , indicating the number of alleles per locus based on a minimum sample size of 13 individuals), expected heterozygosity (H_E), observed heterozygosity (H_O) and inbreeding coefficient (F_{IS}), which were all calculated using FSTAT 2.9.3.2 (Goudet, 1995).

Landscape data

Transect- or corridor-based analysis is often used in landscape genetics of plants (Holderegger et al., 2010). We therefore generated pairwise straight lines between all populations of *L. flos-cuculi* in ARCGIS 9.3.1 (ESRI, Redlands, CA, USA). The lines were buffered to create corridors with different widths of 50, 100, 300, 500 and 1000 m. Within these corridors, the proportion of several landscape elements potentially influencing gene flow by seed or pollen of *L. flos-cuculi* was determined based on VECTOR25 data (Swisstopo, Switzerland; <http://www.swisstopo.admin.ch/internet/swisstopo/en/home/products/landscape/vector25.html>). VECTOR25 provides nine thematic layers among which the layer of primary surfaces was used in the present study. We calculated the proportion of the following landscape elements that can potentially hamper gene flow of plants: settlements (Helm et al., 2009), agricultural land and forests (Tewksbury et al., 2002). In addition, we calculated the area along the verges of ditches as suitable habitats of *L. flos-cuculi* in fragmented agricultural landscapes (Aavik et al., 2008) by buffering ditches by 2 m on both sides. We also calculated Euclidean distance using ARCGIS 9.3 and the ‘ArcGis toolbox’ of landscape genetics (Etherington, 2011), as well as the cumulative elevation change between all populations using a digital height model with a 25 m resolution (DHM25; Swisstopo, Switzerland), because strong elevation gradients can significantly hinder the movement of insects (Keller et al., 2012) as potential pollen vectors of *L. flos-cuculi*.

Network analysis

We used CONEFOR 2.6 (Pascual-Hortal and Saura, 2006; Saura and Pascual-Hortal, 2007; Saura and Torne, 2009) to assess the relative importance of populations (nodes) and links (geographic distance) in the population network of *L. flos-cuculi*. CONEFOR 2.6 assesses a node or a link by comparing the distance between nodes and a species-specific threshold dispersal distance for the investigated organism. If the distance between two nodes is shorter than the assigned threshold dispersal distance, nodes are considered as equally connected (Pascual-Hortal and Saura, 2006; Saura and Pascual-Hortal, 2007;

Saura and Torne, 2009). Previous observations about dispersal and pollination distances of *L. flos-cuculi* did not come up with a clear threshold distance for this species (Van Rossum and Triest, 2010; Aavik et al., 2013). Therefore, we tested various threshold distances beyond which all nodes were considered equally connected (that is, 100, 200, 500, 1000, 2000, 3000 and 5000 m). Nodes were characterised by log-transformed population sizes, and links were evaluated using Euclidean distances between populations.

Two graph-based connectivity indices, the integral index of connectivity (IIC) and the probability of connectivity (PC), have been shown to perform best for the purpose of prioritisation of habitats and links (Pascual-Hortal and Saura, 2006; Saura and Torne, 2009). We therefore chose these two indices for evaluating the importance of nodes and links in the present study. IIC ranges between 0 and 1 and is defined as:

$$IIC = \sum_{i=1}^n \sum_{j=1}^n \frac{a_i a_j}{1 + n l_{ij}} \quad (1)$$

where n is the total number of nodes in the landscape, a_i and a_j are the attributes of nodes i and j and $n l_{ij}$ is the number of links (distances) between nodes i and j . IIC is based on a binary connection model that means that two patches considered are either connected or not depending on the actual distance between them in relation to the predefined distance threshold.

Similar to IIC, PC ranges between 0 and 1 (Saura and Pascual-Hortal, 2007) and is calculated as:

$$PC = \sum_{i=1}^n \sum_{j=1}^n a_i a_j p_{ij}^* \quad (2)$$

where p_{ij}^* is defined as the maximum product probability of all possible paths between nodes i and j . In contrast to IIC, PC is based on a probabilistic connection model, where a certain probability of dispersal between the two patches considered (p_{ij}) characterises the links between nodes i and j in the graph. Because of lack of adequate background information about the potential dispersal distance of *L. flos-cuculi*, a probability of 0.5 was used for connectivity calculations that corresponds to the median dispersal distance of the study species depending on the threshold distance considered.

In CONEFOR 2.6, the prioritisation of each node for maintaining landscape connectivity is calculated as the percentage of the variation of IIC or PC resulting from the removal of a given node from the landscape:

$$dI(\%) = 100 \cdot \frac{I - I_{remove}}{I} \quad (3)$$

where I is the overall index value (both for IIC and PC) for all initially existing nodes in the landscape, and I_{remove} is the overall index value after the removal of a given node from the landscape.

The index includes the connections between nodes (interpopulation connectivity in the current study) as well as the connected area existing within the nodes themselves (intrapopulation connectivity; Saura and Pascual-Hortal, 2007). Saura and Rubio (2010) proposed a way for partitioning node importance into three fractions that quantify the way each node contributes to overall habitat connectivity. Accordingly, node importance consists of three components, depending on whether IIC or PC are considered:

$$dIIC_k = dIICintra_k + dIICflux_k + dIICconnector_k \quad (4)$$

and

$$dPC_k = dPCintra_k + dPCflux_k + dPCconnector_k \quad (5)$$

where $dIICintra_k$ and $dPCintra_k$ represent the contribution of node k to intrapopulation connectivity, for example, habitat area or population size. This value is independent of the dispersal distance of the focal species and does not depend on the connectivity of the node to other nodes. In the present study, this index was calculated on the basis of the node property population size. $dIICflux_k$ and $dPCflux_k$ indicate how well a node k is connected to other nodes in the landscape (that is, the interpopulation connectivity of a node) without considering its contribution to intrapopulation connectivity. $dIICconnector_k$ and $dPCconnector_k$ show whether a node k contributes to the connectivity

Table 2 Results of regression on distance matrices analyses on the effects of landscape variables on the genetic differentiation F_{ST} among the study populations of *Lychnis flos-cuculi* in Switzerland using different corridor widths

Corridor width	R ²	Distance	Agriculture	Forest	Ditch verges	Settlements
50 m	0.125	NS	NS	+ (**)	NS	NS
100 m	0.134	NS	NS	+ (**)	NS	NS
300 m	0.147	NS	NS	+ (**)	NS	NS
500 m	0.119	NS	NS	+ (*)	NS	NS
1000 m	0.121	+ (*)	NS	NS	NS	NS

Abbreviation: NS, nonsignificant variable.

The symbol '+' refers to the positive effect of a variable on pairwise genetic differentiation.

* $P \leq 0.05$ and ** $P \leq 0.01$.

Table 3 The γ -correlations between genetic diversity (H_E : expected heterozygosity; H_O : observed heterozygosity; A_R : allelic richness; F_{IS} : inbreeding coefficients) and node importance ($dIIC$: integral index of connectivity; dPC : probability of connectivity) and between genetic diversity and different components of node importance ($dIICintra$, $dIICflux$, $dPCintra$, $dPCflux$) with distance thresholds ranging from 100 to 5000 m

Variable	Distance threshold	H_E	H_O	A_R	F_{IS}	Variable	H_E	H_O	A_R	F_{IS}
$dIIC$	100 m	0.237	0.099	0.099	-0.106	$dIICintra/dPCintra$	0.254	0.111	0.127	-0.087
	200 m	0.215	0.070	0.092	-0.092	$dIICflux$	0.512	0.415	0.238	-0.381
$dIIC$	500 m	0.398	0.424	0.038	-0.278	$dIICflux$	-0.037	0.074	-0.309	-0.418
	1000 m	0.143	0.303	-0.143	-0.444	$dIICflux$	0.273	0.468	-0.145	-0.509
$dIIC$	2000 m	0.263	0.242	-0.023	-0.143	$dIICflux$	0.104	0.387	-0.056	-0.504
	3000 m	0.128	0.091	0.308	-0.038	$dIICflux$	0.258	0.359	0.000	-0.258
$dIIC$	5000 m	0.173	0.030	0.143	-0.038	$dIICflux$	0.143	0.061	0.098	-0.068
	dPC	100 m	0.263	0.273	-0.008	-0.218	$dPCflux$	0.173	0.030	0.143
200 m		0.328	0.368	0.000	-0.254	$dPCflux$	0.209	0.383	-0.090	-0.463
dPC	500 m	0.313	0.368	-0.030	-0.224	$dPCflux$	0.209	0.414	-0.090	-0.463
	1000 m	0.239	0.353	-0.015	-0.269	$dPCflux$	0.254	0.444	-0.030	-0.403
dPC	2000 m	0.224	0.263	0.075	-0.209	$dPCflux$	0.239	0.368	-0.045	-0.299
	3000 m	0.209	0.188	0.149	-0.164	$dPCflux$	0.254	0.308	0.060	-0.224
dPC	5000 m	0.194	0.083	0.104	-0.119	$dPCflux$	0.224	0.203	0.134	-0.179
						$dPCflux$	0.179	0.098	0.119	-0.134

Significant correlations ($P < 0.05$) are marked in bold.

between other nodes as a stepping stone (Saura and Rubio, 2010). We calculated the three fractions of connectivity for each study population as described in Equations (4) and (5). However, $dIICconnector_k$ and $dPCconnector_k$ were mostly very close to or equal to zero and were therefore not considered in further analysis.

In addition to assessing the importance of each node, CONEFOR 2.6 enables evaluating the contribution of each individual link to landscape connectivity. For calculating link importance, we selected the 'link removal' option that assesses the impact of removing each of the links for landscape connectivity. The impact of losing a link from a network was calculated using both the binary connection model (IIC) and the probabilistic connection model (PC).

Statistical analysis

We used nonparametric γ -correlations to assess the relationship between estimates of genetic diversity (A_R , H_E , H_O and F_{IS}) and node importance ($dIIC$ and dPC) as well as intra- and interpopulation components of node importance ($dPCintra$, $dPCflux$, $dIICintra$ and $dIICflux$) with different threshold distances (100, 200, 500, 1000, 2000, 3000 and 5000 m). The γ -correlations were used because of tied data in the data set.

To estimate the effects of landscape variables on gene flow as a measure of functional connectivity, we used Euclidian distance, cumulative elevation change and the landscape variables agricultural land, settlements, forests and ditch verges calculated within corridors of different widths (50, 100, 300, 500 and 1000 m). First, we aimed at determining the most representative corridor width that best explained gene flow. To avoid multicollinearity, all landscape variables were checked for Spearman's rank correlation. All correlations between landscape variables were below $r_s < 0.6$ with the exception of Euclidian distance and cumulative elevation change ($r_s > 0.8$). Therefore, the latter was excluded from further analysis. Pairwise genetic differentiation F_{ST} (Wright, 1965), a measure of past gene flow among populations, was calculated in FSTAT 2.9.3.2 (Goudet, 1995) and used as a response variable. The influence of Euclidian distance, agricultural land, forest, settlements and ditch verges on F_{ST} was then evaluated with multiple regression on distance matrices using function 'MRM' (package *ecodist*; Goslee and Urban, 2007) in R 2.15.1 (R Development Core Team, 2012). All landscape variables were rank-transformed before entering regression analysis, and the significance of regression coefficients was estimated using 10 000 permutations. Separate regressions were performed on models with different corridor widths. For further analysis, we used the landscape variables calculated within corridors of 300 m width, because they provided the best goodness of fit (Table 2).

In a next step we built regression models on distance matrices that included pairwise F_{ST} as a response variable and the following structural connectivity variables as explanatory parameters: link importance, pairwise summarised values of intra- and interpopulation connectivity and landscape variables calculated within corridors of 300 m width. Separate sets of models were built for different threshold distances (100, 200, 500, 1000, 2000, 3000 and 5000 m) for both network measures, PC and IIC. Explanatory variables exhibiting a correlation of $r_s \geq 0.7$ (Dormann et al., 2013) were excluded from analysis to avoid multicollinearity. Some variables could not be included in the analysis because of missing values (that is, summarised value of interpopulation connectivity and link importance at distance thresholds of 100 and 200 m in IIC). Explanatory variables were again rank-transformed before performing the analysis. The significance of regression coefficients was estimated using 10 000 permutations.

RESULTS

The effect of structural graph-theoretic connectivity measures on genetic diversity

Depending on the distance threshold and connectivity index considered, we detected significant correlations between node importance

and estimates of genetic diversity (Table 3). Node importance based on the binary connection model (*dIIC*) was significantly and positively related to observed (H_O) and expected heterozygosity (H_E) at the distance threshold of 500 m and negatively related to the inbreeding coefficient (F_{IS}) at a threshold of 1000 m (Table 3 and Figure 2a). Node importance based on the probability of connectivity (*dPC*) had a positive relationship with H_O at distance thresholds of 200 and 500 m (Table 3 and Figure 2b). The interpopulation connectivity component of node importance based on IIC (*dIICflux*) was positively related to H_O at distance thresholds of 500–2000 m, but had a negative relationship with F_{IS} at thresholds of 500 and 1000 m (Table 3 and Figure 2c). The interpopulation component of node importance based on PC (*dPCflux*) had a positive relationship with H_O at thresholds of 100–1000 m and a negative correlation with F_{IS} at 100–500 m (Table 3 and Figure 2d). In conclusion, correlations between estimates of genetic diversity and node importance as well as between genetic diversity and the interpopulation connectivity component of node importance were highest at distances ranging between 500 and 1000 m. Correlations were lowest at larger distance

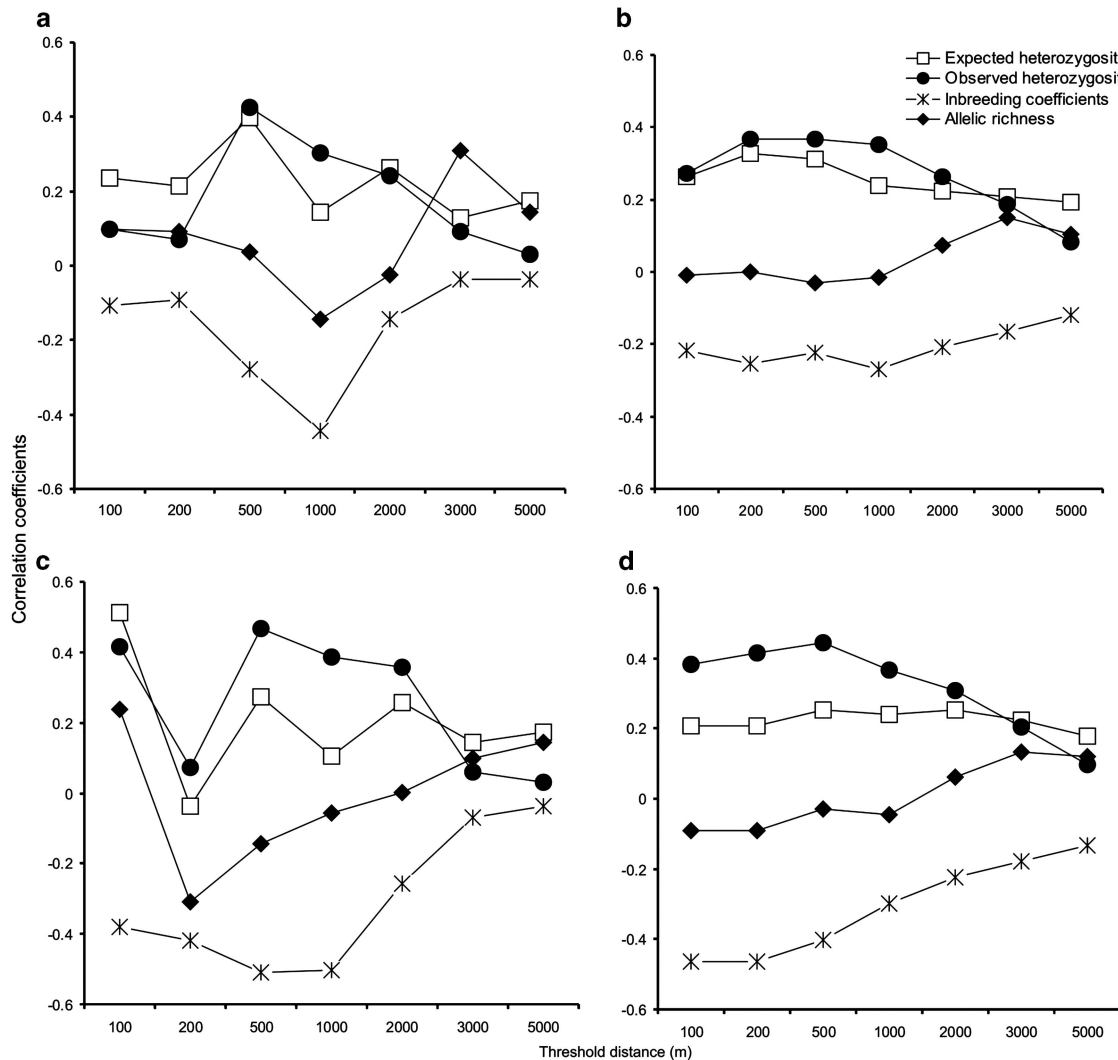


Figure 2 Correlation coefficients between estimates of genetic diversity within the populations of *Lychnis flos-cuculi* and node importance (a: integral index of connectivity (*dIIC*); b: probability connectivity index (*dPC*)) and between estimates of genetic diversity and interpopulation connectivity components of node importance (c: metric based on integral index of connectivity (*dIICflux*); d: metric based on probability connectivity index (*dPCflux*)) using different threshold distances (100–5000 m).

Table 4 Results of multiple regression on distance matrices on the effect of landscape and graph-theoretic connectivity variables on gene flow of *Lychnis flos-cuculi* using different threshold distances (100–5000 m) and connectivity indices (IIC and PC)

Model	R ²	Distance	Connectivity variables			Landscape variables						
			Link importance	Sum. flux	Sum. intra	Agriculture	Forest	Settlement	Ditch verges			
IIC(threshold = 100 m)	0.159	NS	Not incl.	Not incl.	NS	NS	+	(**)	NS	NS		
IIC(threshold = 200 m)	0.288	NS	Not incl.	Not incl.	–	(***)	NS	+	(**)	NS	+	(*)
IIC(threshold = 500 m)	0.155	NS	NS	NS	NS	NS	+	(**)	NS	NS	NS	
IIC(threshold = 1000 m)	0.298	NS	NS	NS	–	(***)	NS	+	(*)	NS	NS	
IIC(threshold = 2000 m)	0.188	NS	NS	NS	–	(*)	NS	+	(*)	NS	NS	
IIC(threshold = 3000 m)	0.289	NS	NS	Not incl.	–	(***)	NS	+	(**)	NS	+	(*)
IIC(threshold = 5000 m)	0.332	NS	Not incl.	+	(**)	–	(***)	NS	+	(*)	NS	NS
PC(threshold = 100 m)	0.184	NS	NS	NS	NS	NS	+	(**)	NS	NS	NS	
PC(threshold = 200 m)	0.187	NS	NS	NS	NS	NS	+	(*)	NS	NS	NS	
PC(threshold = 500 m)	0.241	NS	NS	NS	–	(**)	NS	+	(*)	NS	NS	
PC(threshold = 1000 m)	0.285	NS	NS	NS	–	(***)	NS	+	(*)	NS	NS	
PC(threshold = 2000 m)	0.310	NS	NS	NS	–	(***)	NS	+	(*)	NS	NS	
PC(threshold = 3000 m)	0.308	NS	NS	Not incl.	–	(***)	NS	+	(*)	NS	+	(*)
PC(threshold = 5000 m)	0.288	NS	NS	Not incl.	–	(***)	NS	+	(*)	NS	+	(*)

Abbreviations: IIC, integral index of connectivity; Link, link importance; Not incl., variables that were not included in the model because of multicollinearity or because of missing values in the data set; NS, nonsignificant variables; PC, probability of connectivity; Sum.flux, summarised value of interpopulation component of node importance (*dIICflux* or *dPCflux*); Sum.intra, summarised value of intra-population component (*dIICintra* or *dPCintra*).

The symbols '+' and '–' mark positive or negative effects of regression coefficients, respectively.

* $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$.

thresholds. The intrapopulation connectivity component (*dIICintra* and *dPCintra*) was not related to any of the variables of genetic diversity.

The effect of landscape and graph-theoretic connectivity measures on gene flow

Multiple regression on distance matrices revealed that among landscape variables, forest had a significantly positive effect on genetic differentiation F_{ST} (that is, a negative effect on gene flow) in all models (Table 4). For the remaining landscape variables, only the proportion of ditch verges had a minor negative effect on gene flow, generally in those models that included connectivity metrics calculated at larger distance thresholds (≥ 3000 m). Among variables of structural graph-based connectivity, the summarised value of intra-population component of node importance (*dIICintra* or *dPCintra*) had a significantly negative effect on genetic differentiation (that is, a positive effect on gene flow) in most of the models, particularly in models including connectivity metrics calculated at larger distance thresholds (≥ 1000 m; Table 4). The summarised pairwise value of interpopulation component (*dIICflux* and *dPCflux*) had almost no effect on genetic differentiation. Similarly, link importance had no effect on gene flow. Surprisingly, geographic distance remained nonsignificant in all models. The goodness of fit (R^2) of models (all including the same number of explanatory variables) ranged between 0.155 and 0.332, explaining a reasonable part of the variation in genetic differentiation. Goodness of fit was relatively low ($R^2 < 0.2$) in models including connectivity metrics calculated at lower distance thresholds and started to increase in models including connectivity metrics at distance thresholds beyond 1000 m.

DISCUSSION

Maintaining and enhancing spatial connectivity among fragmented natural and semi-natural habitats is one of the central targets of nature conservation. However, though the structural properties of landscapes are widely used as indicators of biodiversity in practical

landscape planning, they may not have a straightforward effect on functional connectivity, that is, the way individual species respond to landscape structure (Tischendorf and Fahring, 2000). In the present study, we used the proportion of several landscape variables and two graph-theory based indices (Pascual-Hortal and Saura, 2006)—IIC and PC—to quantify structural connectivity among the study populations of *L. flos-cuculi* occurring in fragmented grassland patches of an intensively managed agricultural landscape. In addition, gene flow among populations was used as a measure of functional connectivity. This enabled us to examine the relationship between structural and functional connectivity of this formerly common grassland species, whose abundance has recently decreased because of landscape fragmentation and habitat loss.

Relationship between structural connectivity and genetic diversity

Genetic diversity measured by expected and observed heterozygosity within populations of *L. flos-cuculi* was positively related to node importance (that is, the contribution of populations to structural connectivity) at distance thresholds between 200 and 1000 m. Similarly, the interpopulation connectivity fraction of node importance calculated at distance thresholds of 100–1000 m was more strongly related to indicators of genetic diversity than to connectivity parameters with thresholds > 1000 m. These results probably indicate that gene flow in *L. flos-cuculi* takes place at distances of < 1 km. Gene flow of this species can occur by both insect-mediated pollen flow and seed dispersal. Generally, most of the pollen flow of insect-pollinated species is restricted to the nearest vicinity of plants (Van Rossum and Triest, 2010), although occasional pollen flow events occur over much longer distances (Kamm *et al.*, 2010). Likewise, seeds of *L. flos-cuculi* seldom disperse far from source populations (Aavik *et al.*, 2013); most seeds are spread in the close neighbourhood of mother plants because of the lack of a specialised dispersal syndrome. Thus, geographic distance between isolated populations of grassland plants with seed and pollen dispersal strategies similar to *L. flos-cuculi* should not be larger than ~ 1 km in order to maintain genetic diversity within populations.

We did not detect any significant relationship between genetic diversity and intrapopulation connectivity component. In the present study, this index was based on population size, and Leimu *et al.* (2006) showed that larger populations are generally characterised by higher genetic diversity. However, small populations may still contain substantial genetic variation if they represent remnants of recently larger populations (Landerogott *et al.*, 2001; Klank *et al.*, 2012) and thus the relationship between population size or related indices and genetic diversity may not have re-established yet.

Indices based on PC have shown to perform better as compared with other graph-based connectivity measures (Saura and Pascual-Hortal, 2007), whereas the IIC may oversimplify connectivity among populations because of its binary approach. Neel (2008), in contrast, found that connectivity measures related to IIC predicted estimates of genetic diversity within plant populations better than those based on PC. In the current study, we did not detect any notable differences between these two indices.

The effects of landscape elements on gene flow

Among the studied landscape elements, only forest had a consistent and significantly negative effect on gene flow among populations of *L. flos-cuculi*. Previous studies demonstrated that not only patches of forest (Schmitt *et al.*, 2000), but also linear woody elements considerably restrict the movement of certain pollinator groups in an open landscape (Wratten *et al.*, 2003). Furthermore, the presence of open corridors within a forest matrix significantly enhanced pollen flow among otherwise spatially isolated insect-pollinated plant populations (Tewksbury *et al.*, 2002). Seeds of *L. flos-cuculi* do not have specialised dispersal mechanism and most of the seeds disperse over short distances. Thus, it is very likely that the negative effect of forest on gene flow among the study populations was because of forest inhibiting the movement of pollinators (but see Zurbuchen *et al.*, 2010) rather than the dispersal of seeds.

We hypothesised that agricultural fields and settlements would hinder gene flow among the study populations (Helm *et al.*, 2009; Pellissier *et al.*, 2012), because agricultural intensification and urbanisation have been the major processes contributing to habitat fragmentation. Surprisingly, genetic differentiation was independent of both of these variables. One possible explanation is that the changes in the spatial structure of landscapes are not yet reflected in genetic differentiation F_{ST} among populations, the estimate for functional connectivity of *L. flos-cuculi* used in the present study, because perennial plant species respond to landscape fragmentation more slowly than short-lived species (Kuussaari *et al.*, 2009). In addition, genetic differentiation does not necessarily reflect contemporary gene flow (Whitlock and McCauley, 1999). A previous study examining current levels of gene flow among the same populations of *L. flos-cuculi* as studied here revealed that present-day functional connectivity of this species is indeed low (Aavik *et al.*, 2013), and therefore it is likely that genetic differentiation F_{ST} rather represents historical than current functional connectivity in our study landscape. The most dramatic landscape changes in Switzerland took place in the second half of the twentieth century (Schultz and Dosch, 2005), and thus the effects of these changes on the genetic differentiation of perennial plants might only become traceable in the future (Honnay *et al.*, 2006; Klank *et al.*, 2012).

It is also possible that agricultural land does indeed not inhibit gene flow. Recent studies have demonstrated that open areas in European agricultural landscapes do in fact enhance pollen flow among spatially isolated insect-pollinated plants (Kamm *et al.*, 2010). Furthermore, agricultural areas with a network of structurally connected semi-

natural linear elements still contain small stepping-stone plant populations that support higher levels of gene flow (Van Geert *et al.*, 2010; Van Rossum and Triest, 2012). In our study area, field boundaries adjacent to roads or forests were the most common semi-natural habitats and could have mitigated the potential negative effect of agricultural fields on gene flow in *L. flos-cuculi*, at least to a certain extent. However, note that a higher proportion of ditch verges among our study populations had no or even a slightly negative effect on gene flow according to most models (Table 4).

The relationship between graph-theoretic connectivity measures and gene flow

We found that the intrapopulation fraction of structural connectivity had a significant positive effect on gene flow, that is, a pair of populations having a higher summarised value of intrapopulation connectivity was characterised by lower genetic differentiation. In the present study, we calculated the contribution of a population to intrapopulation connectivity based on its population size. Our findings thus indicate that more gene flow is taking place among larger populations than among smaller ones. Several mechanisms related to either seed dispersal or pollen flow may explain this observed pattern. First, larger patches of flowering plants generally enhance gene flow by pollen (Dauber *et al.*, 2010), as larger populations are visited by pollinators more frequently and thus serve as more important sources as well as sinks of pollen than smaller populations (Richards *et al.*, 1999). Second, larger populations usually produce more dispersal propagules that have therefore a higher likelihood of dispersing into the surrounding landscape than the lower number of seeds from smaller populations (Ellstrand and Elam, 1993). It has also been shown that seeds from larger populations exhibit higher germination success and thus fitness than seeds from smaller populations (Faast *et al.*, 2011), perhaps as a result of the higher genetic diversity observed in larger populations (Leimu *et al.*, 2006). None of the other connectivity metrics (link importance and summarised value of interpopulation connectivity) had any effect on gene flow in the present study, regardless of the different threshold distances or connectivity indices (IIC and PC) considered. One of the reasons for the lack of a relationship between gene flow and link importance could be the above-mentioned delayed effect of structural changes on functional connectivity (Kuussaari *et al.*, 2009).

CONCLUSIONS

We conclude that structural connectivity, as measured by graph-based approaches, influences genetic diversity within populations, but does not (or only in an indirect way) affect gene flow among populations of *L. flos-cuculi*. This suggests that structural connectivity should be used as a proxy for functional connectivity with caution (Tischendorf and Fahring, 2000). Our findings nevertheless show that a distinction between IIC and PC into fractions of intra- and inter-population connectivity could provide valuable insights into processes driving functional connectivity (Saura and Rubio, 2010).

Among the studied landscape variables, only forest had a negative effect on gene flow, most likely through hindering the movement of pollinators. Surprisingly, we did not detect any significant effect of Euclidian distance or of any other landscape variable on gene flow. Although it is possible that the negative effects of settlements and agricultural fields were partly mitigated by linear semi-natural elements connecting the spatially isolated populations of *L. flos-cuculi*, it is also possible that the effect of landscape fragmentation on genetic differentiation is subject to time lags, particularly in case of perennial species (Kuussaari *et al.*, 2009). Thus, studies focussing on gene flow

patterns of plants in recently fragmented landscapes may benefit from examining contemporary gene flow patterns.

DATA ARCHIVING

Data available from the Dryad Digital Repository: doi:10.5061/dryad.147r0.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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