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Assessing modularity in genetic networks to manage spatially structured metapopulations

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Abstract. As habitats and landscapes are becoming increasingly fragmented, it is more important than ever that the conservationists understand how organisms move across the landscape and to assess connectivity. Functional connectivity is necessary to maintain metapopulation dynamics, minimize genetic drift, maintain genetic diversity on the landscape, and ultimately for the preservation of future evolutionary potential. Graph theory and network analyses have proven to be exceptional tools for assessing functional connections among habitat patches. Ecological studies have recently begun incorporating modularity into analyses of networks. Modularity arises in networks when nodes (habitat patches) form clusters or modules wherein patches within a module interact extensively with each other, but rarely interact with patches from different modules. The goals of this study were to assess modularity in a genetic network, determine the critical scales that functional connections occur among populations, assess the contributions of populations to connectivity, and to identify habitat and landscape connectivity variables affecting network modularity. We constructed a network of genetic covariance to determine functional connections among breeding populations of *Ambystoma annulatum* (Ringed Salamander) at Fort Leonard Wood, Missouri, United States. From this network, we tested for the presence of modularity after accounting for the effects of distance between each breeding population, assessed the relative importance of each breeding population in contributing to within- and among-module movements, and tested the effects of habitat and landscape connectivity on network parameters using linear models. The genetic network consisted of four modules, and modularity was significant after accounting for distance. Individual populations generally contributed to within- or among-module movements, but not both. As within-module strength decreased, among-module connectivity increased. Habitat and connectivity parameters were generally poor predictor network parameters, suggesting that modularity may be a result of biotic or abiotic factors that affect successful recruitment from local populations. Our study highlights the importance of fully understanding the functional connections among populations on the landscape. The scale at which connections occur and the role of each population in contributing to connectivity are invaluable to making effective management and conservation decisions. Ultimately, analyses of network modularity have tremendous potential to inform these decisions.

Key words: *Ambystoma annulatum*; amphibian; critical scale; dispersal; functional connectivity; gene flow; metapopulation; network modularity; Ouachita; Ozark; population genetics; scale.

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Introduction

As natural landscapes continue to be degraded and fragmented, local populations are becoming increasingly isolated (Lindenmayer and Fischer 2006). The future persistence of populations in the face of such assaults is largely contingent upon functional connectivity to maintain metapopulation dynamics (Hanski 1998, Hanski and Ovaskainen 2000). In addition to colonization following local extinction, movement of individuals among habitat patches is necessary to minimize genetic drift, maintain genetic diversity on the landscape, and ultimately for the preservation of future evolutionary potential (Whitlock 2004). The importance of connectivity from both a demographic and genetic perspective has made it a cornerstone of landscape ecology, landscape genetics, and conservation biology (With et al. 1997, Storfer et al. 2007, Cushman et al. 2013). Assessment of connectivity in biological and ecological settings has benefitted tremendously from applications of graph theory (McRae et al. 2008, Urban et al. 2009), which has provided critical insights into the importance or resilience of habitat patches and the connections between them (e.g., Schick and Lindley 2007, Galpern et al. 2011, Peterman et al. 2013*b*, Vasudev and Fletcher 2015).

Another important aspect of effective conservation and management planning is scale. Identifying the spatial scale at which ecological processes operate is essential to making correct ecological inference and sound conservation decisions, especially when ecological and evolutionary processes may vary depending upon the scale at which they are assessed (Baguette and Van Dyck 2007, Galpern and Manseau 2013). However, identifying the scale of ecological and evolutionary processes remains remarkably challenging and is often poorly understood (Wiens 1989, Richardson et al. 2014). Depending upon the scale that a system is assessed, markedly different inferences regarding movement, species distributions, or community composition may result. To address issues of scale, researchers often assess the ecological or evolutionary processes in question at a number of arbitrarily selected scales (Wiens 1989, Keitt et al. 1997).

In an effort to more objectively assess the critical scales that control ecological processes, researchers have drawn from work in physics (Fortunato 2010), molecular biology (Hartwell et al. 1999), and social sciences (Handcock et al. 2007) to adapt the concept of modularity to objectively assess the scale at which movement and gene flow occur among populations in space (Garroway et al. 2008, Fortuna et al. 2009, Albert et al. 2013, Fletcher et al. 2013). Modularity is a network measure that describes the division of a network into modules or groups, where nodes (habitat patches) within a module are strongly connected with frequent interactions, but patches between modules have little interaction (see Table 1 for definitions). Measures that can be derived once a network is divided into modules include within-module strength and participation coefficient, which measure the importance of a patch to maintaining connectivity within a module and the importance of a patch to maintaining connectivity among modules, respectively. Studies of ecological systems have found that higher modularity increases network and metapopulation persistence and minimizes the effects of disturbances (Kininmonth et al. 2010, Stouffer and Bascompte 2011), and that modularity can have substantial effects on metapopulation viability (Fletcher et al. 2013). Superficially, assessment of modularity with genetic data appears similar to the identification of clusters using genetic algorithms (e.g., Pritchard et al. 2000, Guillot et al. 2005, Chen et al. 2007). However, identification of modules and assessment of their properties is an outcome of the data, whereas genetic clustering algorithms probabilistically assign membership of individual genotypes to a predefined number of clusters. Furthermore, the presence and spatial locations of modules and presence and strength of connections within and among modules allows for a critical evaluation of the scale at which patches are connected as well as the importance of individual populations in contributing to connectivity.

Graph theory has provided a valuable framework for assessing functional connectivity and metapopulation dynamics in many systems, but pond-breeding amphibians are particularly amenable to this framework. Breeding ponds can be considered patches on the landscape (Marsh and Trenham 2001) with dispersing juveniles providing connections among ponds (Berven and Grudzien 1990, Gamble et al. 2007). In the context of graph theory, ponds are equivalent to nodes, and the connections formed by dispersing individuals

Table 1. Definition and ecological relevance of graph and network terms.

Metric	Definition	Ecological relevance
Population graph	A graph-theoretic approach to determine the minimal set of connections between populations to describe the genetic covariance among all populations.	Connections in the population graph indicate that substantial gene flow is occurring.
Modularity	A measure of network structure describing how nodes are organized into modules or clusters. Nodes within a module tend to interact extensively with each other, but only rarely with nodes from other modules.	Determining how patches/populations are grouped and connected via movement of individuals or their alleles can be used to identify critical scales for management of ecological processes. Increased modularity increases metapopulation persistence and can minimize effects of disturbance.
Node	Elements within a network that are connected by links if they interact.	Discrete populations or habitat patches can be represented as nodes within a network.
Edge/link/ connection	A network element that connects nodes.	Indicates dispersal or gene flow between nodes.
Node/patch strength	Total movement or gene flow of a particular node, independent of network modularity.	Provides a measure of the total contribution of a patch/population to movement or gene flow across the landscape.
Within-module strength	A modularity-based measure of the importance of a node to connectivity within its module relative to all other nodes within the same module.	Measures the importance of individual patches/ populations to local connectivity, which is critical for minimizing genetic drift and for colonization and rescue processes.
Participation coefficient	A measure of how connected a node is to all other modules in the network.	Measures the importance of individual patches/ populations to landscape connectivity, which is critical for long-term metapopulation viability and maintenance of genetic diversity across the landscape.

are the links or edges in a network. Several studies have used graph theory to assess population dynamics and pond importance to amphibian metapopulations (e.g., Fortuna et al. 2006, Ribeiro et al. 2011, Peterman et al. 2013*b*). Recent analyses of genetic networks have demonstrated that amphibian populations can also exhibit significant modularity (Albert et al. 2013, Fletcher et al. 2013). Ultimately, the concepts of modularity are closely aligned with metapopulation biology (Hanski 1998), making modularity analyses a powerful and insightful tool for better understanding metapopulations.

Modularity analyses appear to have great potential for determining critical scales in ecological networks and for determining the relative role that habitat patches have in maintaining connectivity. However, only recently has the approach been generalized to explicitly accommodate distance (Fletcher et al. 2013). The likelihood of two populations interacting or exchanging genes predictably decreases as distance increases (Wright 1943, Levins 1969). As previous research has not incorporated distance into modularity analyses, it is unclear whether the existence of modularity in ecological networks is solely a consequence of well-known distance effects, or if significant modularity can remain after accounting for distance (but see Fletcher et al. 2013). In this study, we use the novel framework presented by Fletcher et al. (2013) to assess modularity in a genetic network after accounting for distance effects between populations. We apply these methods to population genetic data from *Ambystoma annulatum* (Ringed Salamander) at Fort Leonard Wood, Missouri, United States. In doing so, we (1) create a spatial network of genetic co-variance; (2) assess modularity in the network of ponds; (3) assess the scales at which ponds cluster into modules as well as the scales that functional connectivity among ponds occur; (4) assess individual pond importance to the genetic network based on pond participation coefficients, within-module strength and contribution to the genetic network; (5) assess the potential for local habitat and connectivity to affect the contribution of a pond to the genetic network.

Methods

Study species

Ambystoma annulatum is a forest-dependent species that requires fishless ponds for reproduction (Petranka 1998, Peterman et al. 2014). The species is endemic to the interior highlands

of the Ozark and Ouchita mountains of Missouri, Arkansas and Oklahoma. Breeding and oviposition occur in the fall. Larvae overwinter and develop for 7–9 months in breeding ponds and metamorphose in spring (Semlitsch et al. 2014).

Study site

Sampling occurred at Fort Leonard Wood (FLW), in the Ozark Highlands, Pulaski County, Missouri, USA (Fig. 1). FLW is an active military training facility encompassing 24,852 ha in the northern Ozark Highland. Eighty percent of FLW is forested, characterized by oak-hickory forests (*Quercus* spp., *Q. stellata*, *Carya* spp., and *C. texana* canopy; *Rhus aromatic* and *Cornus florida* understory) or short-leaf pine plantations (*Pinus echinata*). There are over 500 ponds at FLW that are either constructed or unintentional water bodies (e.g., tire ruts). Ponds vary in size from 1 to 42,549 m^2. Most are small (<0.04 ha), fishless, constructed wildlife ponds, but there are several large ponds and small lakes (>1 ha) stocked with game fish.

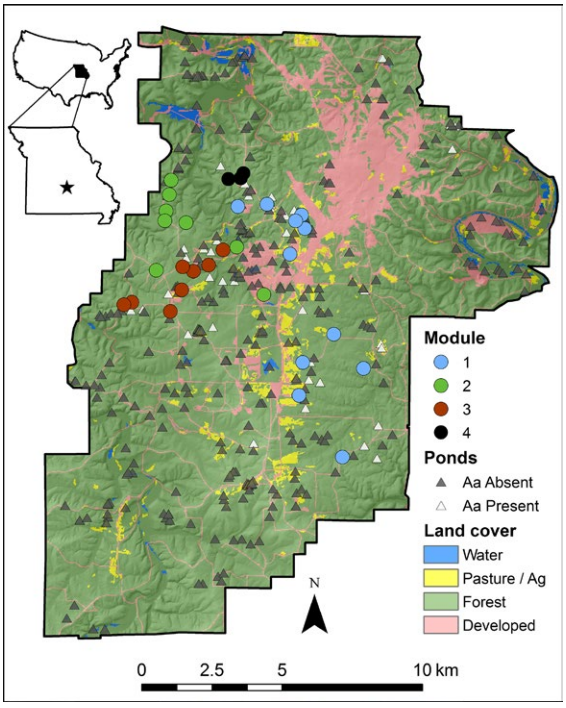

Fig. 1. Map of Fort Leonard Wood, Missouri, United States depicting major land cover classes, location of all known ponds on the landscape (triangles) as well as ponds that have been used by *Ambystoma annulatum* for reproduction (Aa present/Aa absent). Circles are ponds included in the genetic network analysis of this study with circle color indicating module membership.

Collection methods

We collected 1–65 *A. annulatum* from 89 ponds. Embryos and recent hatchlings were sampled at each pond; samples were stored in 95% EtOH and stored at −20°C until DNA extraction. To minimize sampling of siblings, samples were systematically collected from the entire perimeter of the pond. All collections were conducted October–November 2012.

Laboratory procedures

We extracted DNA using a chelex-based resin (Instagene, BioRad) as detailed by Peterman et al. (2012). Individuals were genotyped at 24 microsatellite loci (Peterman et al. 2013*a*). Locus Ac300 (Savage 2009) and Aj346 (Julian et al. 2003) were used in this study (Table 2), but were not included in (Peterman et al. 2013*a*). Primers were fluorescently labelled and arranged into two multiplex PCR reactions with conditions as described in Peterman et al. (2013*a*) and in Table 2. Positive and negative controls were included with each PCR reaction, and 10% of all tested samples were run twice to check for errors; no inconsistencies were found. Amplification products were sized on an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, California, USA) using Liz 600 size standard at the University of Missouri DNA Core Facility, and results were scored using GENEMARKER (v.1.97; Softgenetics, State College, Pennsylvania, USA).

Analyses

Genetic diversity.—Before proceeding with analyses we tested for, and removed, full siblings from our data set using COLONY (Jones and Wang 2010). In COLONY, we set both male and female mating to polygamous without inbreeding, used a long run with full likelihood and high precision, and did not include a sibship prior. We calculated rarefied llelic richness and observed and expected heterozygosity using GenoDive v2.02b24

Table 2. Rarefied allelic richness (A_r), observed and expected heterozygosity (H_O and H_E), inbreeding coefficient (F_{IS}), multiplex reaction, and fluorescent label for 24 microsatellite loci used to assess *Ambystoma annulatum* sampled from 30 breeding ponds at Fort Leonard Wood, Missiouri, USA.

Locus	Alleles	A_r	H_O	H_E	F_{IS}	Multiplex	Label
Aa_153	8	2.25	0.563	0.576	0.021	1	NED
Aa_19†	19	5.54	0.912	0.909	0.023	1	VIC
Aa_20‡	5	2.44	0.465	0.616	0.034	2	FAM
Aa_21	11	3.29	0.662	0.724	0.358	1	NED
Aa_25	8	3.52	0.736	0.743	−0.052	2	VIC
Aa_258	5	2.06	0.559	0.533	0.244	2	PET
Aa_27	4	2.29	0.558	0.585	0.010	1	NED
Aa_28	7	3.23	0.664	0.718	−0.046	1	NED
Aa_31	8	2.77	0.609	0.666	0.006	1	FAM
Aa_311	15	4.62	0.809	0.813	−0.010	1	FAM
Aa_312	11	3.54	0.754	0.744	0.037	2	VIC
Aa_314	6	2.07	0.544	0.535	−0.024	1	PET
Aa_36	13	3.62	0.743	0.751	−0.064	1	VIC
Aa_37	8	2.92	0.749	0.729	0.012	2	FAM
Aa_39	5	2.79	0.651	0.666	−0.008	2	NED
Aa_40	10	4.64	0.816	0.813	−0.013	2	PET
Aa_44	7	2.45	0.665	0.634	0.077	2	NED
Aa_46‡	10	3.29	0.469	0.731	−0.016	2	NED
Aa_50	9	3.58	0.744	0.747	0.075	2	FAM
Aa_84	4	1.97	0.528	0.510	0.007	1	PET
Aa_85	6	3.75	0.776	0.760	0.076	2	NED
Aa_86	3	1.67	0.412	0.416	−0.033	1	NED
Ac300	7	3.58	0.736	0.748	−0.003	2	VIC
Aj_346	7	2.86	0.697	0.674	−0.115	1	FAM
Overall§	7.71	3.02	0.666	0.667	0.025	–	–

† Excessive genotyping issues: >10% missing genotypes, omitted from downstream analyses.
‡ Locus significantly deviated from HWE expectation with evidence of null alleles, omitted from downstream analyses.
§ Calculated without loci that deviated from HWE expectation or excessive missing genotypes.

(Meirmans and Van Tienderen 2004). Genepop 4.2 (Raymond and Rousset 1995, Rousset 2008) was used to test for significant deviations from expected heterozygosity values under Hardy–Weinberg equilibrium (HWE) and to test for linkage disequilibrium among pairs of loci. Both tests were conducted using 250 batches with 2500 iterations following a burn-in of 2500. Significance of all tests was assessed following Bonferroni correction for the number of comparisons (Rice 1989). We tested for presence of null alleles using "PopGenReport" (Adamack and Gruber 2014).

Spatial genetic network.—To create a weighted, undirected spatial genetic network graph (population graph), we used our multilocus microsatellite data after the removal of full siblings and loci that deviated significantly from Hardy–Weinberg expectation or showed evidence of null alleles. Following the methods of Dyer and Nason (2004) as implemented in the R package "popgraph" (Dyer 2014), we constructed an incidence matrix of genetic covariance (A_{ij}), which created the network that we subsequently evaluated for modularity. Genetic covariance is an application of graph theory to codominant genetic data that identifies the most important connections between populations. This approach begins with a fully connected network in which all populations are linked to each other. The network is then reduced by removing links whose genetic covariance is shared with other populations. The final network consists of the fewest possible connections that explain the genetic covariance structure among all populations simultaneously, with retained connections identifying gene flow between populations (Dyer and Nason

2004). This approach to analyzing multilocus genetic data is unique in that it simultaneously assesses genetic variation in all individuals/populations without requiring averaging or a priori specification of hierarchical model structure.

Modularity.—Our assessment of modularity within our network closely followed the methods of Fletcher et al. (2013) and equations therein. To identify modularity, Q, within our network we used the equation described by Girvan and Newman (2002):

$$Q = \frac{1}{2m} \sum_{ij} \left(A_{ij} - P_{ij} \right) \delta \left(C_i, C_j \right)$$

where m is the total number of links possible in the undirected network, A_{ij} describes the gene flow between ponds i and j, $\delta(C_i, C_j)$ is a matrix indicating whether elements i and j are members of the same module. We calculate patch strength as:

$$w_i = \sum_j A_{ij}$$

To make our assessment of modularity spatial (Expert et al. 2011), P_{ij} is calculated as:

$$P_{ij} = w_i w_j f \left(d_{ij} \right)$$

where w is the strength of pond i and j, and $f(d_{ij})$ is a deterrence function that accounts for the variation in gene flow as a function of the distance (d) between ponds on the landscape:

$$f(d) = \frac{\sum_{i,j|dij=d}^{n} A_{ij}}{\sum_{i,j|dij=d}^{n} w_i w_j}$$

As described by Fletcher et al. (2013), this is a non-parametric function that describes the probability of gene flow between pond i and j given the inter-pond distance. This deterrence function requires that distances be binned into categories. To determine the optimal bin size, we iteratively tested bin widths ranging from 100–10 000 m in 100-m increments, and the bin width with the highest Q-value was used for subsequent analyses. By including spatial information into this analysis, we are explicitly assessing whether there is significant modularity (difference in movement within vs. among modules) after accounting for the expected decrease in movement that occurs with distance. To maximize the modularity function, a simulated annealing algorithm was used to iteratively search for the $\delta(C_i, C_j)$ (Guimerà and Amaral 2005, Fletcher et al. 2013). A zero-adjusted gamma generalized linear model was used to determine whether there was significant variation in movement within modules vs. among modules, with and without accounting for the distance between patches (hereafter, ponds). Fletcher et al. (2013) demonstrated that generalized linear models were more powerful than traditional randomization procedures at correctly identifying network modularity, especially in smaller networks. Zero-adjusted gamma models were fit with the "gamlss" package in R (Rigby and Stasinopoulis 2005).

Patch importance and modularity.—By definition, connectivity within modules of a modular network differs from connectivity between modules. Further, ponds can play critically different roles contributing to these two aspects of modular connectivity. The metrics used to describe these measures of connectivity are within-module strength and participation coefficient (Guimerà and Amaral 2005). Within-module strength is calculated as:

$$Z_i = \frac{w_{ig} - \overline{w}_g}{\sigma_{K_{gi}}}$$

and describes the relative importance of each pond i to the connectivity within its member module g, compared to all other patches within the same module g. w_{ig} is the amount of movement from pond i to all ponds within module g, and $\overline{w}_g$ is the average movement from all ponds within module g. The participation coefficient of a pond i is:

$$P_i = 1 - \sum_{i=1}^{Nm} \left(\frac{w_{ig}}{w_i} \right)^2$$

where N_m is the total number of modules within the network. The participation coefficient, P_i, will be zero when all gene flow from pond i is confined to its member module, and will approach one when gene flow is evenly distributed among all modules present in the network.

Predictors of within-module strength, participation coefficient, and patch strength.—After calculating

patch strength (W_i), within-module strength (Z_i) and participation coefficient (P_i) for each pond in the network, we used linear models to assess whether pond-level habitat variables, connectivity, or a combination of habitat and connectivity were meaningful predictors of network metrics. Previous research has shown that pond area, pond hydroperiod, the number of ponds within 300 m, and the percentage of land cover within 300 m that is forest are related to *A. annulatum* abundance (Peterman et al. 2014) or performance (Ousterhout et al. 2015). Connectivity (or conversely, isolation) has also been shown to be a significant predictor of genetic differentiation among *A. annulatum* populations at FLW (Peterman et al. 2015). For each pond, we calculated a connectivity index, S_i, using the incidence function:

$$S_i = \sum_{j \neq i} \exp\left(-\alpha d_{ij}\right)$$

where $1/\alpha$ is the mean distance between connected ponds (see results below), and d_{ij} is the distance between ponds i and j (Moilanen and Nieminen 2002). S_i is a relative measure of how connected (or isolated) a pond is. We extended this connectivity index to include local habitat features:

$$SH_i = H_i \sum_{j \neq i} \exp\left(-\alpha d_{ij}\right)$$

where H is a local habitat feature of pond i (see above). SH_i reflects the interaction between a habitat feature and the connectivity of ponds across the landscape, and was calculated separately for each habitat variable, H. All independent variables were scaled and centered to a mean of zero ±1 standard deviation and checked for collinearity prior to inclusion in linear models. The dependent variable within-module strength was log transformed prior to analysis to meet assumptions of normality.

Results

Genetic diversity

We collected 958 tissue samples from 89 breeding ponds. COLONY identified 26% of our collected samples as being from full sibling families. Following the removal of siblings and populations with fewer than 10 remaining unrelated samples, we had 578 samples from 30 ponds (mean ± SD; 19.3 ± 8.4; Table 3). There were excessive missing genotypes, evidence of null alleles or significant deviations from HWE expectation for three loci (Aa_19, Aa_20, Aa_46; Table 2); these loci were removed from downstream analyses. There was no evidence of linkage disequilibrium among any loci.

Genetic network and modularity

Overall, the ponds at FLW were highly connected with a total of 67 connections in the network and an average of 4.47 connections between ponds (range = 1–8). Our analysis of genetic network modularity identified four modules in the network of 30 *A. annulatum* breeding ponds, and these same four modules remained after accounting for distance between ponds (Fig. 2). The optimal bin width for assessing spatial modularity was determined to be 7400 m. Both the non-spatial (modularity = 0.450) and spatial (modularity = 0.475) tests for modularity were significant ($P < 0.001$, respectively). These results indicate that there is greater movement of individuals among ponds within the same module than among ponds between module, but that by accounting for physical distance between populations our estimate of modularity increased. We focus on the spatial assessment of modularity for the remainder of the paper. On average, there are 3.19 more connections between ponds within same module than between ponds in different modules. Each module contributes 0.04 to 0.18 to the total landscape modularity (Fig. 2). The average distance (mean ± SD) among ponds within the same module (4527 m ± 2193) did not differ significantly from the average distance among ponds between modules (4613 m ± 2288; $t_{311} = -0.464$, $P = 0.643$). The average distance between connected ponds in the network is 2510 m (±2140; median = 1674 m). Overall, there is a general trend for the strength of within-module connectivity (module strength) to decline as among-module connectivity (participation coefficient) increased (Pearson's $r = -0.447$, $P = 0.013$; Fig. 3a, Table 4). Patch strength is not correlated with module strength ($r = 0.245$, $P = 0.191$; Table 4) and moderately correlated with participation coefficient ($r = 0.454$, $P = 0.012$; Table 4). Furthermore, there is extensive variability in each of the network and modularity measures assessed (Fig. 2). The rank importance of ponds

Table 3. Population genetic, genetic network, and modularity summary statistics for *Ambystoma annulatum* from 30 sampled ponds at Fort Leonard Wood, Missouri, USA. All measures are calculated without loci that deviated from HWE expectation or excessive missing genotypes. Population is a unique identification number for each pond, N is the number of samples after removal of full siblings, A_r is the mean rarefied allelic richness, H_O is observed heterozygosity, H_E is expected heterozygosity, F_{IS} is the inbreeding coefficient, Z_i is the within-module strength, P_i is the between-module participation coefficient and W_i is patch (pond) strength indicating the contribution of each pond to the genetic network. UTM coordinates are provided with 100-m precision.

Pond	N	Alleles	A_r	H_O	H_E	F_{IS}	Z_i	P_i	W_i	Easting	Northing
0.002	23	5.33	3.30	0.636	0.664	0.043	0.014	0.593	18.400	572700	4178400
8	11	4.38	3.14	0.658	0.665	0.011	0.009	0.667	35.496	570200	4175200
11	18	5.91	3.40	0.719	0.697	−0.032	0.013	0.833	23.390	573300	4178700
54	19	4.29	2.73	0.599	0.599	−0.001	0.014	0.427	33.142	576500	4172900
65	15	4.48	2.75	0.590	0.590	0.000	0.010	0.500	33.356	575400	4171900
66	34	4.95	3.18	0.669	0.649	−0.031	0.016	0.000	15.528	571100	4174500
82	10	4.14	2.91	0.590	0.653	0.096	0.014	0.500	31.969	569000	4174000
110	10	2.95	2.19	0.542	0.507	−0.069	0.014	0.667	68.042	574000	4174300
120	19	5.48	3.69	0.718	0.703	−0.022	0.020	0.427	33.929	572500	4175900
126	33	5.86	3.64	0.703	0.695	−0.011	0.008	0.593	18.745	572000	4175400
127	11	4.86	3.38	0.675	0.701	0.036	0.020	0.427	31.965	571500	4175200
152	27	5.10	3.62	0.701	0.694	−0.010	0.006	0.000	3.958	570500	4177300
174	17	4.76	3.14	0.664	0.643	−0.033	0.022	0.000	32.457	577500	4171700
176	11	4.67	3.16	0.667	0.680	0.019	0.019	0.427	36.313	576800	4168500
186	20	5.67	3.62	0.717	0.704	−0.019	0.022	0.000	27.195	575400	4176700
190	16	4.14	2.82	0.666	0.619	−0.076	0.017	0.000	27.386	575200	4170700
216	14	5.57	3.38	0.668	0.686	0.025	0.014	0.593	17.421	573200	4178500
238	16	4.57	3.15	0.681	0.659	−0.033	0.010	0.593	20.322	571200	4176900
247	21	5.24	3.54	0.690	0.700	0.015	0.023	0.000	22.094	570500	4177000
264	10	3.91	2.92	0.649	0.662	0.020	0.011	0.815	43.662	569300	4174100
291	33	5.81	3.63	0.694	0.692	−0.003	0.033	0.000	31.408	571100	4175300
323	19	5.95	3.64	0.672	0.711	0.055	0.011	0.500	23.594	573100	4177500
331	19	5.38	3.56	0.677	0.690	0.019	0.015	0.427	34.352	575300	4177200
380	28	5.43	3.47	0.717	0.694	−0.034	0.020	0.370	33.732	574100	4177600
400	42	5.71	3.49	0.656	0.685	0.043	0.022	0.542	50.424	574900	4175800
407	10	4.05	2.94	0.624	0.636	0.019	0.026	0.370	42.944	570700	4173700
408	10	3.95	2.94	0.648	0.649	0.002	0.015	0.747	45.106	573000	4176000
414	21	5.48	3.44	0.678	0.693	0.022	0.008	0.640	36.120	575100	4177000
419	12	4.43	2.93	0.675	0.662	−0.020	0.015	0.747	42.946	570700	4178400
246B	29	5.29	3.43	0.646	0.702	0.080	0.011	0.000	10.703	570600	4177900
Avg	19.27	4.92	3.25	0.667	0.670	0.003	0.016	0.413	30.870	-	-

in relation to patch strength, within-module strength, and between-module participation coefficient differs substantially. While some ponds are critical regardless of the network measure assessed, on average, there is a difference of ±8.36 (±6.47 SD) in the rank importance of pond contributions to each metric (Fig. 3b, Table 4). These results indicate that different populations are critical contributors to different aspects of the network.

Predictors of within-module strength, participation coefficient, and patch strength.—Habitat and connectivity variables poorly predict any of the network metrics evaluated, with one exception (Appendix A: Table A1). The percentage of habitat that is forested within 300 m of a pond is a moderately significant parameter in the habitat only model predicting patch strength, and the combination of forested area and connectivity is a good predictor of patch strength in the habitat × connectivity model. However, the habitat × connectivity model only explains about 29% of the variation (adjusted $R^2 = 0.288$; Appendix A: Table A1). The combination of connectivity and the number ponds within 300 m of a focal pond

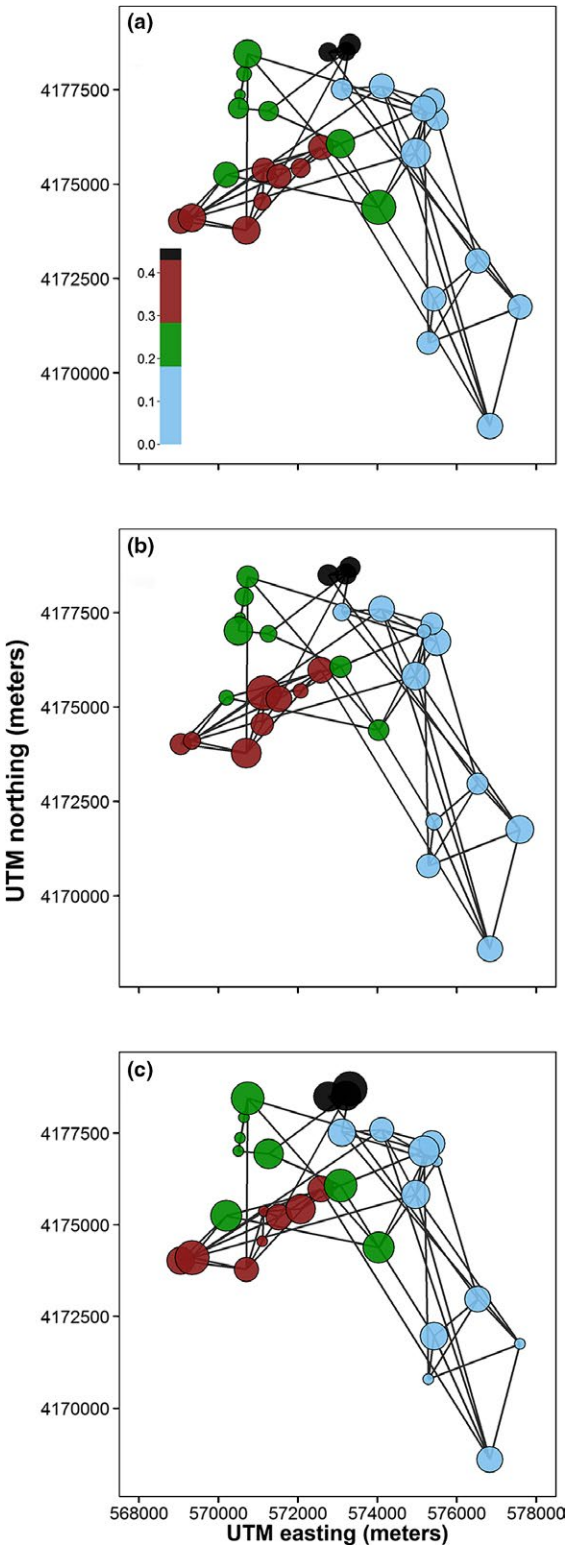

Fig. 2. Network modularity of *Ambystoma annulatum* based on gene flow among ponds. Nodes (ponds) are color-coded to represent module membership (same as Fig. 1). Node size represents contribution to total gene flow within the genetic network (patch strength) in (a), within module strength in (b), and participation coefficient in (c). Locations of nodes in plots reflect spatial locations of ponds on the FLW landscape. The scale bar indicates the cumulative contribution of each module to the total modularity (Q) of the network.

also contributed to the fit of this model. Interestingly, the forest, forest × connectivity, and pond × connectivity parameter estimates are negatively related to a population's contribution to gene flow across the landscape (i.e., patch strength), suggesting that as the amount of forest cover and number of ponds within 300 m of a pond increase, and as the degree of connectivity with other ponds on the landscape increases, the contribution of a pond to gene flow decreases.

Discussion

The presence of modularity among *A. annulatum* breeding populations on the FLW landscape is an important indication that these populations are spatially structured and provides powerful insight into the relevant spatial scale at which conservation and management should occur. The modules identified through our analysis correspond to genetic units that should be managed as a whole (Fortuna et al. 2009), and the distance between connected ponds can be used as a guide for the creation of new ponds on the landscape. Our assessment of patch strength, and within- and among-module contribution to connectivity provides a first means of ranking and prioritizing breeding ponds for conservation or management. Populations identified as having high within-module strength (Z_i) are likely critical for minimizing genetic drift and for colonization and rescue processes (Holt 1992), while ponds with high inter-module connectivity, as measured by participation coefficient (P_i), are most likely important for long-term metapopulation viability and maintenance of genetic diversity across the landscape (Fletcher et al. 2013). In addition, patch

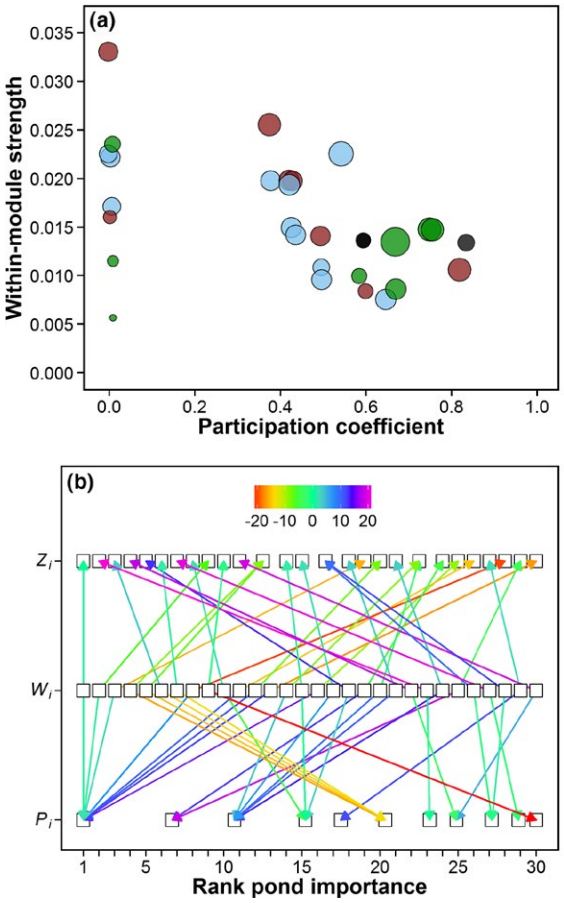

Fig. 3. (a) Importance of ponds to gene flow in *Ambystoma annulatum* in relation to within-module strength (the importance of ponds for gene flow within modules) and between-module participation coefficient (extent to which gene flow occurs to all other modules from a pond). Size of points indicate the genetic contribution of each pond to the network (patch strength) and color represents module membership (same as Fig. 1). (b) Rank importance of ponds changes depending upon whether patch strength (W_i), within-module strength (Z_i), or between-module participation coefficient (P_i) is the network metric used, indicating that populations are critical contributors to different aspects of connectivity within the network. Arrow color is scaled to the amount of rank change.

strength (W_i) provides a relative measure of the overall importance of a pond in facilitating gene flow across the landscape. An important result of our analysis, however, is that ponds are generally only meaningful contributors to either within- or among-module connectivity, but not both. It is also evident that the rank importance of patch strength, which measures the contribution of gene flow across the landscape, may have little bearing on the importance of a pond to maintaining local connections within a module or landscape connections among modules (Fig. 3b). For instance, some of the lowest ranked contributors to gene flow across the landscape are among the most important populations for maintaining within-module connections. These findings underscore the need to fully assess network modularity and the role of each population in the network to ensure that conservation and management decisions will not jeopardize connectivity and metapopulation dynamics.

In addition to understanding the contributions of specific populations to movement within a network, effective conservation strategies require a critical assessment of scale (Clark et al. 2011). Analysis of modularity in population genetic networks holds tremendous potential to identify these ecologically meaningful scales (Garroway et al. 2008, Fortuna et al. 2009, Fletcher et al. 2013). We found that *A. annulatum* populations formed spatially congruent modules at a scale of ~4500 m, but that direct connections between any two populations were, on average, ~2500 m. As additional validation of the scale at which direct population connections were identified in our network, Peterman et al. (2015) previously estimated that genetic dispersal distance in *A. annulatum* averaged 1700 m, a value that closely aligns with the median inter-population connection distance of 1674 m found in this study. There is currently little guidance for conservationists and land managers concerning where on the landscape to create new ponds for amphibian breeding habitat. While it is clear that the location of a created wetland on the landscape is critical for colonization (Brown et al. 2012), criteria for placement are often only loosely based on generic estimates of amphibian dispersal ability or core habitat use, such as described by Rittenhouse and Semlitsch (2007) or Semlitsch and Bodie (2003). The distance between connected ponds in the genetic network and the average distance between ponds within the same module provide empirically derived estimates for future landscape management and pond creation.

The location of a created pond on the landscape is important for initial colonization, but success-

Table 4. Correlation matrices of the network metrics calculated and assessed in this study. In both matrices, correlation coefficients are in the lower triangle and *P*-values are in the upper triangle. Pearson's product-moment correlations are based on the actual values of each metric at each pond, while Spearman's rank correlations are based on the rank importance of pond contributions to each metric.

	Module strength	Participation coefficient	Patch strength
Pearson's product-moment correlation			
Module strength	–	0.013	0.191
Participation coefficient	−0.447	–	0.012
Patch strength	0.245	0.454	–
Spearman's rank correlation			
Module strength	–	0.003	0.277
Participation coefficient	−0.524	–	0.022
Patch strength	0.205	0.418	–

ful amphibian reproduction in ponds is often a function of pond and local landscape habitat characteristics (Brown et al. 2012, Peterman et al. 2014). Features such as pond hydroperiod, slope of the pond basin, presence of emergent aquatic vegetation, presence of predators (fish or invertebrate), and the surrounding upland habitat can all play a role in determining the species breeding in a wetland and whether recruitment is successful (Shulse et al. 2012, Semlitsch et al. 2015). While many of these pond and landscape features have previously been found to affect *A. annulatum* (Peterman et al. 2014, Ousterhout et al. 2015), we found no evidence that pond-level habitat or connectivity parameters meaningfully related to the modularity-based measures of within-module strength and participation coefficient. However, we did find evidence that the amount of forest and number of ponds in the landscape surrounding a focal pond interact with a pond's connectivity to other ponds on the landscape to negatively affect the contribution of a population to gene flow across the landscape, as measured by patch strength. This surprising result could be indicative of a high rate of philopatry among individuals breeding and metamorphosing from ponds that are located in suitable forested habitat in close proximity to other ponds, resulting in limited long distance dispersal. Gene flow is likely high, however, among these clusters of highly connected ponds, and the genetic diversity within these populations is likely very similar. As such, more isolated populations harboring unique alleles may be greater contributors to the total genetic variability. This interpretation is highly speculative and warrants further investigation.

Our modularity analysis of a genetic network builds upon the work of Fletcher et al. (2013) and explicitly incorporates distance. As a fundamental expectation of population ecology and population genetics, the likelihood of two populations interacting or exchanging genes decreases as distance increases (Wright 1943, Levins 1969). Fletcher et al. (2013) demonstrated that significant modularity can remain after accounting for the spatial effects of inter-patch distance, and we corroborate these findings in our study. The distance between ponds within the same module did not differ from the distance between ponds in different modules. This result emphasizes that gene flow among *A. annulatum* populations is affected by more than distance alone. As such, alternative hypotheses underlying the modular structure of *A. annulatum* ponds must be considered. The first alternative hypothesis is that landscape features are imparting additional resistance above and beyond the effects of distance. Landscape resistance has been shown to play a role in spatial genetic structure of many organisms, including ambystomatid salamanders (Greenwald et al. 2009, Goldberg and Waits 2010, Richardson 2012). However, we have rigorously tested this hypothesis in *Ambystoma* spp. at FLW and have found no evidence for the landscape to affect gene flow and patterns of genetic differentiation (Peterman et al. 2015). The second alternative hypothesis would suggest that pond-level differences in *A. annulatum* reproduction and recruitment are affecting the ultimate movement of individuals and their genes across the landscape. Such differences may be biotic (e.g., Anderson et al. 2015*a*, Ousterhout et al. 2015) or abiotic

(e.g., Peterman et al. 2014). We have abundant evidence that these inter-pond differences result in variable numbers of larvae present (Peterman et al. 2014) as well as quality of metamorphs produced (Ousterhout et al. 2015). Pond and connectivity variables, however, were not meaningfully related to any of the modularity measures in this study and only moderately related to the network measure of patch strength. These alternative explanations need to be more extensively explored to better understand the underlying mechanisms generating the observed modularity in *A. annulatum*.

Beyond genetic networks, modularity has been hypothesized to be important in a variety of ecological networks (e.g., Bascompte et al. 2006, Olesen et al. 2007) and the concept of modularity is closely aligned with existing ideas of scale and connectivity in landscape ecology (Keitt et al. 1997, Bodin and Norberg 2007) and metapopulation biology (Hiebeler 2000, Kallimanis et al. 2005). While distance is expected to be an important feature of all spatial networks, genetic network and modularity analyses prior to Fletcher et al. (2013) did not explicitly account for distance, thereby making it unclear whether modularity arose from the isolating effects of distance alone. Fletcher et al. (2013) showed that significant modularity can remain even after accounting for distance, a finding that we have also confirmed in our analysis of *A. annulatum*. Further, Fletcher et al. (2013) found that the inclusion of modularity in metapopulation analyses can substantially increase estimates of metapopulation capacity and alter the estimated importance of a patch to the persistence of the metapopulation. As such, network modularity provides a powerful and flexible framework to determine the scales at which populations interact locally (within-module) and across the broader landscape (among-module) as well as the relative importance of patches in contributing to gene flow occurring at these different scales.

Conclusions

A fundamental goal of landscape genetics has been to understand how populations are connected in space and how population genetic structure is subsequently affected (Manel et al. 2003, Storfer et al. 2007). Use of genetic networks has provided insight into how genetic variation is spatially distributed as well as identified the functional connections between these populations (Dyer and Nason 2004, Dyer 2007). Modularity analyses have extended the inferences that can be gleaned from networks by identifying ecologically relevant scales for management and conservation as well as an assessment of the relative importance of individual populations in contributing to network connectivity (Garroway et al. 2008, Fortuna et al. 2009, Albert et al. 2013, Fletcher et al. 2013). Our analysis has provided novel insight into the gene flow of *A. annulatum*. However, it is important to note that genetic networks can be sensitive to both unsampled and undersampled populations (Koen et al. 2013). We are well aware that the 30 ponds included in this study are only a fraction of the >100 ponds that *A. annulatum* have been documented to breed in at FLW (Anderson et al. 2015*b*, Fig. 1). Further, the life history of *A. annulatum* have made them exceptionally challenging to obtain adequate sample sizes of unrelated individuals (Peterman et al. 2015), which in this study resulted in nearly 50% of field-collected samples being unuseable for population genetic analyses. Nonetheless, we are confident that the presence of modularity in *A. annulatum*, after accounting for distance, is not an artifact of sampling. Rather, it is most likely indicative of variable recruitment, fitness, or dispersal among populations.

We were unable to identify habitat or landscape variables that predicted within-module strength or participation coefficient, and we found only moderate support for forest cover and connectivity to negatively affect a population's contribution to gene flow. The reasons for this are unknown. However, the habitat variables are specific to the time that they were collected, while genetic data is a representation of multiple generations of dispersal and reproduction. As such, it may not be surprising that these measures are incongruent. Alternatively, our incomplete sampling of the network may have affected the estimated contributions of each population to our genetic network and its modular structure. Further research is needed to understand the habitat or population characteristics underlying

the observed patterns of modularity and is a promising direction for future research.

In most systems, like *A. annulatum* at FLW, it is unlikely that the mechanisms or processes driving modularity will be known. Despite this, important inferences related to conservation and management can be garnered by assessing modularity. First and foremost, the grouping of populations (ponds, patches, etc.) into modules identifies critical, ecologically relevant scales at which movement or gene flow occur, and consequently the scale at which conservation and management should occur. Once the modular structure of a network is known, it is then possible to further examine the relative contribution and importance of patches to within- and among-module movement. Both Fletcher et al. (2013) and this study have demonstrated that patches rarely are important contributors to both within- and among-module movement. This highlights the fact that a single "source" population that is critical to the maintenance of the entire metatpopulation is unlikely to exist. Instead, some populations are important for small-scale rescue/colonization processes among adjacent patches, and some populations are critical to forming connections to other modules across the landscape. Identification, conservation, and management of both of these patch types is critical for successful long-term management of metapopulations.

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Supporting Information

Additional supporting information may be may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.1231/supinfo