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The Spatial Structure of Antagonistic Species Affects Coevolution in Predictable Ways

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ABSTRACT: A current challenge in evolutionary ecology is to assess how the spatial structure of interacting species shapes coevolution. Previous work on the geographic mosaic of coevolution has shown that coevolution depends on the spatial structure, the strength of selection, and gene flow across populations. We used spatial subgraphs and coevolutionary models to evaluate how spatial structure and the location of coevolutionary hotspots (sites in which reciprocal selection occurs) and coldspots (sites in which unidirectional selection occurs) contribute to the dynamics of coevolution and the maintenance of polymorphisms. Specifically, we developed a new approach based on the Laplacian matrices of spatial subgraphs to explore the tendency of interacting species to evolve toward stable polymorphisms. Despite the complex interplay between gene flow and the strength of reciprocal selection, simple rules drive coevolution in small groups of spatially structured interacting populations. Hotspot location and the spatial organization of coldspots are crucial for understanding patterns in the maintenance of polymorphisms. Moreover, the degree of spatial variation in the outcomes of the coevolutionary process can be predicted from the network pattern of gene flow among sites. Our work provides us with novel tools that can be used in the field or the laboratory to predict the effects of spatial structure on coevolutionary trajectories.

Keywords: antagonistic coevolution, coevolutionary hotspot, gene flow, geographic mosaic, Laplacian matrices, subgraphs, spatial graphs.

Introduction

One of the central problems in evolutionary ecology is to understand how species interactions and the coevolutionary process shape the evolutionary dynamics of interacting species (Gandon and Nuismer 2009; Thompson 2009; Guimarães et al. 2011). The role of coevolution in shaping

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diversity depends on the interaction between ecological, evolutionary, and spatial processes (Thompson 1994, 2005; Nuismer et al. 1999, 2008). The geographic mosaic theory of coevolution formalizes this spatial dependence by partitioning these effects into three sources of spatial variation in coevolving interactions: variation in the structure of selection (selection mosaics), variation in the strength of reciprocal selection (coevolutionary hotspots and coldspots), and variation in the spatial distribution of traits through trait remixing (Thompson 1994, 2005).

In that sense, geographic mosaic theory is part of the expansion of evolutionary biology to characterize the structure of selection in more ecologically realistic ways. Species are collections of spatially distributed populations connected by dispersal of individuals (Gilpin and Hanski 1991; Liebold et al. 2004; Holyoak et al. 2005) that potentially interact with populations of other species along their geographic range (Ricklefs 2008). The spatial distribution of these interacting populations of different species influences local community assembly (e.g., Hubbell 2001; Liebold et al. 2004) and ecological dynamics (e.g., Amarasekare et al. 2004) as well as evolutionary and coevolutionary outcomes (Benkman 1999; Brodie et al. 2002; Thrall and Burdon 2002; Loeuille and Leibold 2008; King et al. 2009). Part of the goal of coevolutionary biology must therefore be to understand how the spatial distribution of populations and patterns of dispersal among interacting populations of distinct species (hereafter, spatial structure) shape the coevolutionary process.

Each of the components of spatial variation characterized by geographic mosaic theory now has some support from empirical studies. Variation among populations in the structure of selection has been shown in the interaction between the floral-parasitic moth *Greya politella* and its herbaceous host plant *Lithophragma parviflorum*. The interaction is consistently mutualistic at sites where there are few effective co-pollinators but antagonistic at sites where other pollinators are abundant (Thompson and

Cunningham 2002; Thompson et al. 2010). In recent years, selection mosaics have been shown in an increasingly wide range of other interspecific interactions (Brodie et al. 2002; Foitzik et al. 2009; Laine 2009; Hoeksema 2010).

Variation among populations in the strength of reciprocal selection has been shown, for example, in coevolving interactions between conifers (genus *Pinus*) and crossbills (genus *Loxia*) across North America and Eurasia depending on the presence of squirrels (genus *Tamiasciurus*). Where squirrels are abundant, the conifers coevolve almost exclusively with them, creating coevolutionary coldspots in the interaction between conifers and crossbills (Benkman 1999; Parchman and Benkman 2002; Mezquida and Benkman 2005).

Finally, variation in the distribution of traits among populations through gene flow, genetic drift, and metapopulation dynamics can affect the evolution and maintenance of polymorphisms (e.g., Nuismer et al. 1999; Gomulkiewicz et al. 2000; Nuismer 2006). Models describing coevolution of antagonistic interactions, such as parasites and hosts, in a single site predict that coevolutionary dynamics should lead to unstable oscillations of allele frequencies (Gavrilets and Hastings 1998; Nuismer et al. 1999; see app. A; apps. A–E available online). These oscillations could lead to fixation or elimination of alleles in real populations. In contrast, the incorporation of linear spatial structure of interacting populations (fig. 1) into experimental and theoretical models of coevolution shows that gene flow damps coevolutionary oscillations, which leads to stable polymorphic populations (e.g., Nuismer et al. 2003; Brockhurst et al. 2007; Vogwill et al. 2008). Furthermore, both dynamic and stable clines in allele frequency may appear under linear spatial structures (Nuismer et al. 2000, 2003). Recent microcosm studies on host-parasite interactions with complex spatial structures have shown that coevolutionary outcomes are strongly affected by spatial structure and patterns of gene flow (Forde et al. 2008; Vogwill et al. 2010). Therefore, a major current challenge in the study of coevolution is to assess whether different spatial structures of the populations of coevolving species affect trait evolution in predictable ways.

Here we use coevolutionary models and spatial subgraphs to explicitly consider how spatial structures and coevolutionary hotspots shape the coevolutionary process. Spatial graphs are mathematical representations describing the spatial configurations of interacting populations. The subgraphs are the building blocks of more complex, spatially structured networks of interacting species observed in nature. Each node represents a site inhabited by species and each link represents gene flow between sites (Fortuna et al. 2009; Economo and Keitt 2008, 2010). Pairwise coevolutionary dynamics within sites can then be investigated through dynamic models that take into account dif-
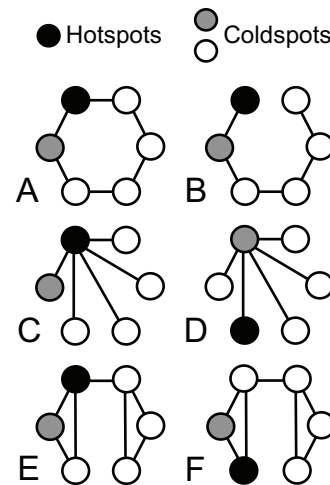

Figure 1: Spatial subgraphs analyzed. In each subgraph, lines represent gene flow between sites, black circles are coevolutionary hotspots, and gray and white circles are coevolutionary coldspots. Gray coldspots are closer to hotspots and were the ones used for coldspot stability analyses. Spatial configurations include (*A*) circular, (*B*) linear, (*C*) star with central hotspot, (*D*) star with peripheral hotspot, (*E*) modular with central hotspot, and (*F*) modular with peripheral hotspot.

ferent selective pressures among sites or the occurrence of coevolutionary coldspots and hotspots (e.g., Nuismer et al. 1999). First, we used tools derived from graph theory to investigate the effects of the spatial configuration of sites on the coevolutionary dynamics. Second, we combined numerical simulations focusing on within-site dynamics with a theoretical framework based on Laplacian matrices that generalize these results to across-site dynamics to develop testable hypotheses on how coevolutionary dynamics are affected by the spatial structure of interacting populations.

We addressed two questions: how do different spatial configurations of antagonistic interacting species affect coevolutionary dynamics, and how does the location of coevolutionary hotspots within each spatial configuration affect coevolutionary dynamics? We hypothesized that maintenance of genetic polymorphisms within and among populations is lowest when populations are distributed in ways that favor the rapid flow of alleles across sites and therefore create a cascading effect (Ferrer i Cancho and Solé 2003). We also hypothesized that coevolution at central sites has a broader impact on global coevolutionary dynamics than does coevolution at peripheral sites (Borgatti 2005). Finally, because central sites are being constantly disturbed, we also expected central sites be more prone to become monomorphic (Borgatti 2005).

Methods

The Model

Our model builds on previous versions of matching alleles models (e.g., Seger 1988; Nuismer et al. 1999; Nuismer 2006; Gavrilets and Michalakis 2008). In these models, allelic frequencies of two species change through time due to selection imposed by pairwise antagonistic interactions in a given site (see app. A for a description of the dynamics of the model). We chose to work with a matching alleles model (MAM), because it has well-known mathematical properties (Gomulkiewicz et al. 2003) and, although simple, reproduces some key aspects of the observed evolutionary dynamics (Dashiell et al. 2001; Gomulkiewicz et al. 2003). We evaluated MAM dynamics across six sites, some of which are connected by gene flow. The structure of selection imposed by the interaction varies among sites. In our model, there is a single site, the coevolutionary hotspot, in which the antagonistic interaction (e.g., host-parasite or predator-prey interaction) between species leads to reciprocal selection. In all other sites, selection is nonreciprocal. In these coevolutionary coldspots, there is a fitness gain for the consumer, whereas the interaction has no effect on the fitness of the host. We explored the role of the structure of spatial configurations in shaping the outcome of evolutionary dynamics.

The general model evaluates two interacting species at n sites ($n = 6$ in all simulations). For simplicity, we assumed that individuals of species 1 and 2 (parasites and hosts, respectively) encounter each other at random rates proportional to their relative frequencies, are semelparous and haploid, have synchronic generation times, reproduce asexually, and have populations sufficiently large to neglect genetic drift (e.g., Seger 1988). Although asynchronous generation times (Gandon and Michalakis 2002), diploidy (Nuismer 2006), sexual reproduction (Nuismer et al. 2008), and genetic drift (Gandon and Nuismer 2009) can all affect coevolutionary outcomes, these simplifying assumptions allowed us to focus on the effect of spatial structure on coevolutionary dynamics. The general life cycle for each species was as follows: species (i) interact, (ii) reproduce, then (iii) migrate.

Species Interactions. The interaction is governed in each species by a single locus with two alleles (e.g., Nuismer et al. 1999). Species 1 carries alleles X and x with frequencies $p_{1,i}$ and $1 - p_{1,i}$, whereas species 2 carries alleles Y and y with frequencies $p_{2,i}$ and $1 - p_{2,i}$ at the ith site. We assumed the effect of species interaction on fitness to be constant through time and equal across coldspots. Whenever the interaction is reciprocal (at hotspots), fitness sensitivity on the interaction is the same for both species. In all sites, an individual of species 1 experiences a fitness

gain if it successfully parasitizes an individual of species 2. A successful interaction will only occur by matching alleles. That is, an individual with genotype X can parasitize individuals with genotype Y but is unable to parasitize individuals with genotype y. An individual of species 2 that is infected by an individual of species 1 loses fitness in the hotspot. Within coldspots, however, the interaction has no effect on the fitness of individuals of species 2. As in previous studies (e.g., Nuismer et al. 1999), we assumed linear fitness functions described in equations (1), (2), (3), and (4):

$$W_{X,i} = 1 + \alpha_{1,i} p_{2,i}, \tag{1}$$

$$W_{x,i} = 1 + \alpha_{1,i}(1 - p_{2,i}), \tag{2}$$

$$W_{Y,i} = 1 - \alpha_{2,i} p_{1,i}, \tag{3}$$

$$W_{y,i} = 1 - \alpha_{2,i}(1 - p_{1,i}). \tag{4}$$

Here $\alpha_{k,i}$ is a parameter that represents the fitness sensitivity on the interaction for species k at site i. Here we defined as hotspots all sites i in which $\alpha_{1,i} > 0$ and $\alpha_{2,i} > 0$. We defined as coldspots all sites in which $\alpha_{1,i} > 0$ and $\alpha_{2,i} = 0$. Thus, coldspots are sites in which species 1 gains fitness through interacting with species 2 while species 2 remains unaffected.

Reproduction. If genetic drift is negligible compared with selective pressures, then the postselection frequency of a given allele in species k at site i ($p'_{k,i}$) can be determined by preselection frequencies weighted by the average allele fitness (eqq. [5], [6]):

$$p'_{1,i} = \frac{p_{1,i} W_{X,i}}{p_{1,i} W_{X,i} + (1 - p_{1,i})W_{x,i}}, \tag{5}$$

$$p'_{2,i} = \frac{p_{2,i} W_{Y,i}}{p_{2,i} W_{Y,i} + (1 - p_{2,i})W_{y,i}}. \tag{6}$$

Migration. We define gene flow as the flow of individuals between two contiguous sites. We assumed for simplicity that gene flow is symmetrical, constant through time, and equal for both species across all sites. Postmigration frequencies ($\overset{*}{p}_{k,i}$) are computed using the following equation:

$$\overset{*}{p}_{k,i} = \sum_{j=1}^{n} M_k(i, j) p'_{k,j}. \tag{7}$$

Here $M_k(i, j)$, or gene flow between contiguous sites, is the fraction of the population of species k in site i that came from site j. In our model, links are either present or absent; gene flow is the same across all extant links. However, to

make our results comparable to previous studies (e.g., Nuismer et al. 2000), we assumed gene flow to have a Gaussian form:

$$M_k(i, j) = \frac{\exp\left[-\frac{1}{2}\left(\frac{d_{ij}}{\sigma}\right)^2\right]}{\sum\limits_{j=1}^{n} \exp\left[-\frac{1}{2}\left(\frac{d_{ij}}{\sigma}\right)^2\right]}, \qquad (8)$$

where n is the number of sites to which site i is connected, including itself. This way, gene flow increases with the parameter σ^2 (gene flow strength hereafter) and decreases with distance between sites (d_{ij}). We assumed an inverse relationship between gene flow strength and distance between sites, in which $d_{ij} = 1$ for all connected sites and $d_{ii} = 0$ in all simulations. Therefore, gene flow between contiguous sites is fixed and constant across all simulations and all spatial configurations. The overall distribution of links among sites, however, varies across spatial configurations. We can thus explore how coevolutionary outcomes may vary due to the effects of spatial configurations that go beyond the fixed gene flow between contiguous sites (fig. 1).

Interacting Spatially Distributed Populations as Subgraphs

Spatial structure was incorporated into the model by using spatial subgraphs (Dyer and Nason 2004; Fortuna et al. 2009). Sites are depicted as nodes, and flow of individuals between contiguous sites is depicted as links between nodes (Fortuna et al. 2009; Economo and Keitt 2008, 2010; Carrara et al. 2012). Here we used four distinct subgraph configurations: circular (fig. 1A), linear (fig. 1B), star (fig. 1C), and modular (fig. 1E). We chose these subgraphs because they embody the basic building blocks (network motifs) of more complex graphs depicting larger networks (Ferrer i Cancho and Solé 2003; Solé and Valverde 2004) and the spatial distributions of interacting populations (Economo and Keitt 2008, 2010). The spatial configurations considered in this study differ between each other in three important features: hotspot location, hotspot connectivity, and coldspot spatial configuration.

First, hotspots can occur at several different locations within a given configuration. We explored the effect of hotspot location in coevolutionary dynamics within star and modular configurations by placing the hotspot in central or peripheral positions (fig. 1D–1F). We did not change the location of hotspots in the circular configuration, because all locations are dynamically equivalent. Also, the linear configuration can be considered as a special case of the circular configuration without periodic boundary conditions. Because a linear structure with central

hotspot behaves as a circular structure as the number of sites increases (results not shown), we only considered the case of a peripheral hotspot.

Second, the spatial configurations differ in the number of connections between hotspots and other sites, which we called hotspot connectivity. We used simulations in which connections were sequentially removed from a star configuration with central hotspot to evaluate the effect of hotspot connectivity on coevolution.

Third, the spatial organization of coldspots differs from subgraph to subgraph, affecting the overall distance between noncontiguous coldspots and the hotspot. For example, in a linear spatial configuration with a peripheral hotspot the average number of links between a coldspot and a hotspot, $\overline{L}$, is $\overline{L} = (c + 1)/2$, in which c is the number of coldspots, whereas in the star configuration with a peripheral hotspot $\overline{L} = 2 - 1/c$. For $c = 5$, $\overline{L}$ is 60% smaller in the star configuration, although in both configurations the hotspots have the same connectivity. We evaluated the effect of spatial organization of coldspots on coevolutionary dynamics by performing a set of simulations in which we sequentially connected coldspots and tracked the effect of the additional coldspot on coevolutionary dynamics. We started with two connected sites, a hotspot and a coldspot. Then we added new coldspots, one at a time, to the most distant coldspot with respect to the hotspot, in such a way that the organization built up from two-site to six-site linear configuration.

More complex configurations, such as the modular configuration, can show combined effects of hotspot location, hotspot connectivity, and the spatial organization of coldspots on coevolutionary dynamics. We analyzed these combined effects in modular configurations with both central and peripheral hotspots. Because the modular configuration is formed by two connected cycles, we first removed the link that connects the two modules. This first step allowed us to assess the effect of the organization of coldspots on coevolutionary dynamics, because the dynamics of the cycle with a hotspot were now uncoupled from the one composed of only coldspots. Then, we connected a coldspot to the hotspot to assess the effect of increasing hotspot connectivity within the modular configuration. Because this process leads to changes in the overall number of connections as well, we performed a control in which the coldspot was linked to another coldspot.

Baseline Parameters and Stability Analysis

In single-site MAM models, all alleles have an unstable equilibrium at frequency $1/N$, where N is the number of alleles considered within each species (Seger 1988). In our

case, $N = 2$ for all species, so a single-site MAM will show an unstable equilibrium at 0.5 for both alleles (see app. A; Seger 1988). In all of our simulations, we used fixed initial allelic frequencies, because they do not affect the stability of polymorphisms (Seger 1988). Because a single-site MAM with two alleles has an equilibrium point at 0.5, we set initial allelic frequencies to be different from 0.5 for one species, so that coevolution could occur. In particular, for species 1 we set initial allelic frequencies to 0.6 and 0.4 for alleles X and x, respectively, and we set both allele frequencies to 0.5 for species 2. Different initial conditions led to qualitatively similar results (data not shown).

We assessed the polymorphism stability of our spatially structured MAM model relative to known properties of other MAM models. Analyses of the model's transient dynamics are available in appendix E. When two sites are coupled by gene flow in a MAM, dampened oscillations toward equilibrium may also occur if gene flow is strong enough to overcome the intrinsically unstable allelic frequency oscillations of each independent site. Stable, dampened oscillations will in turn keep allelic frequencies far away from fixation or elimination frequencies, yielding polymorphic populations (whenever initial gene frequencies are different than 0 or 1; see app. A). Conversely, unstable oscillations can bring allelic frequencies close to their fixation or elimination frequencies, which increases the probability of getting monomorphic populations in finite populations.

We explored the regions of the space of parameters where dynamics lead to stable and unstable equilibria for polymorphisms. For each spatial configuration, we varied the fitness sensitivity ($\alpha_{k,i}$) and strength of gene flow between contiguous sites (σ^2), keeping track of allele frequencies within the hotspot and the nearest coldspot separately. When more than one coldspot was at the same distance from the hotspot, we randomly chose one of them to keep track of its dynamics. We varied $\alpha_{k,i}$ from 0.02 to 0.3 with a step of 0.05, allowing for the occurrence of all known dynamic behaviors of the MAM model. We varied σ^2 from 0.15 to 0.8 with a step of 0.05. Below 0.15, gene flow is not strong enough to couple the coevolutionary dynamics across sites; hence, no effect of the spatial configuration can be detected below that value. Above 0.4, sites show synchronous fluctuations for several configurations studied. Above 0.8, synchronic behavior is widely present, and all sites behave, in fact, as a single one (see apps. A and E).

We considered the dynamics of our spatially structured MAM model to be stable for a particular combination of parameters if the absolute value of the difference between the allelic frequency at a given time and the stable allelic frequency (0.5) was less than 0.001 for at least 200 consecutive generations for both species (see appendix). If the latter condition was not met, the simulation ended after 200,000 generations, and the system was considered to be unstable for that particular combination of parameters. We repeated this procedure for all combinations of values of $\alpha_{k,i}$ and σ^2.

Laplacian Matrices and Spatial Variation in the Coevolutionary Dynamics

We investigated how coevolution affects geographic variation in allele frequencies by using Laplacian matrices of subgraphs (Barrat et al. 2008). The Laplacian matrix is defined as $L = [l_{ij}]_{n \times n}$, in which $l_{ij} = -1$ if sites i and j are connected, $l_{ii} = k_i$ where k_i is the number of links connecting the site i with other sites, and $l_{ij} = 0$ if sites i and j are not connected. The eigenratio of the Laplacian matrix, λ_2/λ_{max}, is the ratio of the smallest nonzero eigenvalue (the smallest eigenvalue is always zero in a Laplacian matrix), λ_2, to the leading eigenvalue, λ_{max}, and characterizes the spatial synchronization in the coevolutionary process. The closer the eigenratio is to one, the greater the likelihood of synchronization within a graph (Barrat et al. 2008) and the lower the likelihood that coevolution leads to spatial variation in allele frequencies. We used Laplacian matrices to extend our results to spatial subgraphs that have any number of sites, and we also generalized the approach for different magnitudes of gene flow between contiguous sites (all derivations are available in apps. B–D). Our approach makes it possible to use classical indexes, such as Wright's measure of population differentiation mediated by genetic structure, to build up Laplacian matrices (app. C). This approach also makes it possible to make qualitative predictions of coevolutionary dynamics even without detailed quantitative estimates of gene flow among populations.

Results

Spatial Structure

The spatial structure of hotspots (fig. 2) and coldspots (fig. 3) had strong effects on the stability of polymorphisms. The effects of spatial structure on polymorphisms were shaped both by fitness sensitivity on species interactions within hotspots and by the level of gene flow among contiguous populations. Within hotspots, the circular configuration (fig. 2A) showed stable polymorphisms within a narrower range of values of gene flow and fitness sensitivity (i.e. the stable region is smaller) than the linear configuration (fig. 2B). In fact, the linear configuration showed a larger stable region for polymorphisms than any other configuration (fig. 2B). In contrast, the star configuration with a central hotspot (fig. 2C) showed stable polymor-
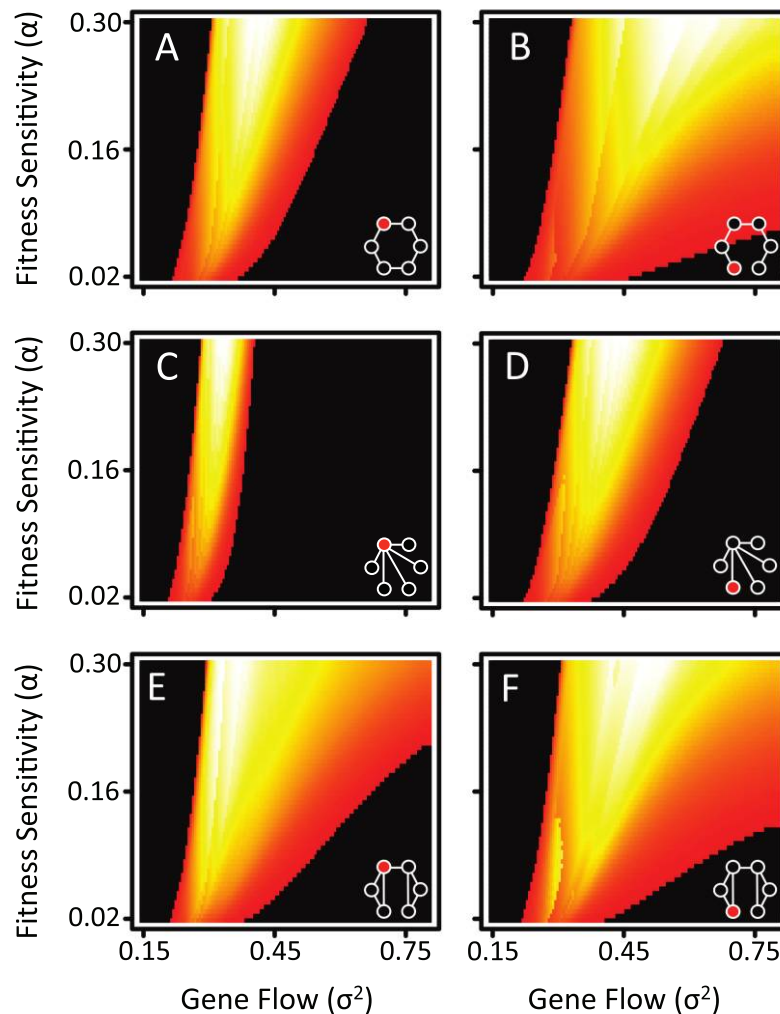
Figure 2: Stability analyses of polymorphisms within hotspots (marked as a red circle) for combinations of the strength of reciprocal selection (α) and gene flow (σ^2). We marked the regions of the space of parameters that yielded unstable oscillatory dynamics of polymorphisms (eventually monomorphic populations) in black and marked those that yielded stable polymorphic populations in color. For stable polymorphic dynamics, color gradation indicates relative time to equilibrium, with short times being colored in white and long times being colored in red. Shown are (*A*) circular, (*B*) linear, (*C*) star with central hotspot, (*D*) star with peripheral hotspot, (*E*) modular with central hotspot, and (*F*) modular with peripheral hotspot. The regions of stability of all configurations show similar qualitative general shapes, which suggests the existence of an underlying constraint on coevolutionary dynamics in terms of the strength of reciprocal selection and gene flow. However, the quantitative shape of stable regions for polymorphisms is configuration-specific. We plotted only the coevolutionary dynamics of the parasitic species to simplify the visualization, because host and parasite dynamics are qualitatively the same.

phisms within lower values and narrower ranges of gene flow and fitness sensitivity when compared to all other configurations (fig. 2). The modular configuration with a central hotspot (fig. 2*E*) or peripheral hotspot (fig. 2*F*) showed stable polymorphisms across wider ranges of gene flow and strength of reciprocal selection than the circular (fig. 2*A*) or the star configurations (fig. 2*C*, 2*D*).

The coldspots behaved in a qualitatively similar way to hotspots for intermediate to high levels of gene flow (fig. 3). At low levels of gene flow, however, all configurations showed a different behavior than the one observed in the hotspots (figs. 2, 3). All configurations presented the same behavior of stable polymorphisms at lower levels of gene flow, which indicates that the levels of gene flow needed to stabilize polymorphisms in coldspots are lower than those needed to attain polymorphism stability within hotspots. As a consequence, the spatial configuration did not affect stability at low levels of gene flow. Both star configurations showed an unstable wedge intruding into the region where stable polymorphisms occur (fig. 3) that is
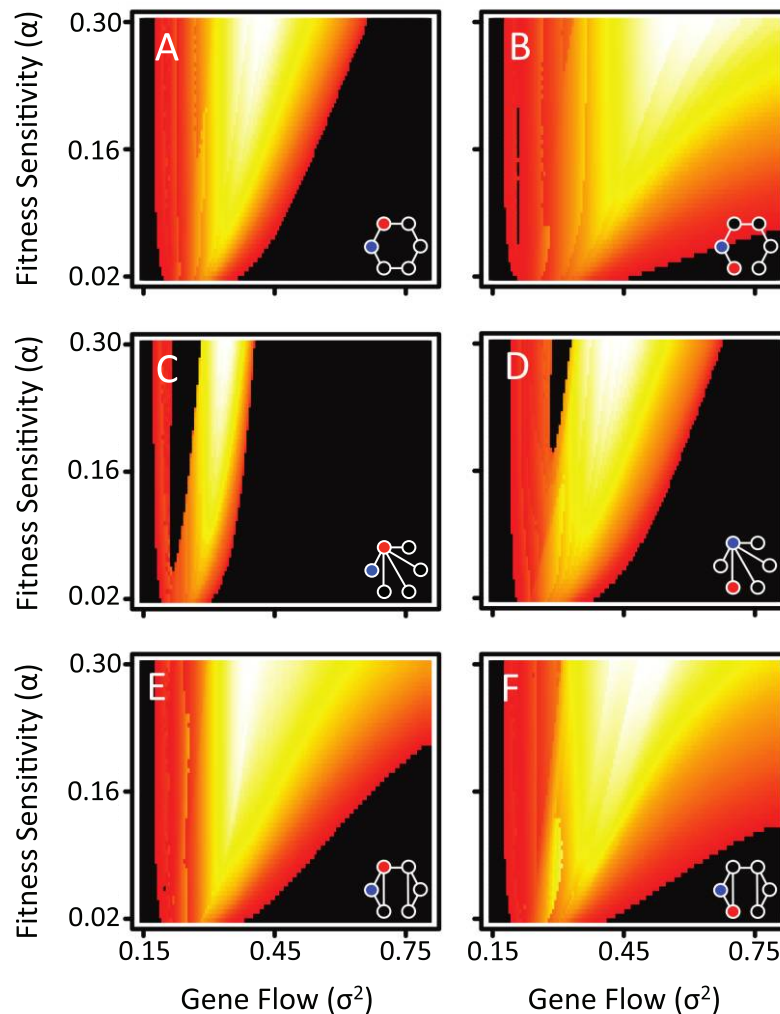
Figure 3: Stability analyses of polymorphisms within the coldspot that is closer to the hotspot (marked as a blue circle next to the hotspot, in red) for combinations of the strength of reciprocal selection (α) and gene flow (σ^2). We marked the regions of the space of parameters that yielded unstable oscillatory dynamics of polymorphisms (eventually monomorphic populations) in black and marked those that yielded stable polymorphic populations in color. For stable dynamics of polymorphisms, color gradation indicates relative time to equilibrium, with short times being colored in white and long times being colored in red. Shown are (*A*) circular, (*B*) linear, (*C*) star with central hotspot, (*D*) star with peripheral hotspot, (*E*) modular with central hotspot, and (*F*) modular with peripheral hotspot. We plotted only the coevolutionary dynamics of the parasitic species to simplify the visualization, because host and parasite dynamics are qualitatively the same. Blue nodes indicate the coldspots that we analyzed.

not present in other configurations (fig. 3). This wedge narrows the stability region within star configurations compared with all other configurations.

Hotspot Location

Hotspot location within a configuration strongly affected polymorphism stability for hotspots (fig. 2*C*–2*F*) and coldspots (fig. 3*C*–3*F*). Central hotspots tended to destabilize polymorphisms within hotspots. Both star and modular

configurations with a central hotspot were stable for polymorphisms within narrower ranges of gene flow and fitness sensitivity on interactions than their peripheral hotspot counterparts (fig. 2*C*–2*F*). Nevertheless, hotspot centrality did not necessarily result in polymorphism instability. Although the star configuration with a central hotspot showed polymorphism stability for a narrower range of parameter values than any other configurations (fig. 2*C*), the modular configuration with a central hotspot showed polymorphism stability for a wider range of pa-

rameter values than did the circular and the star configurations with a peripheral hotspot (fig. 2*A*, 2*C*, 2*E*). Similar relationships between centrality and spatial configuration held for coevolutionary dynamics within coldspots (fig. 3*C*–3*F*).

Hotspot Connectivity

Hotspot connectivity narrowed the range of parameters for which polymorphism stability was achieved. As hotspot connectivity increased, the stability region for polymorphisms within the hotspot slightly decreased (fig. 4*A*–4*E*). Hotspot connectivity affected polymorphism stability in more complex structures, such as the modular configuration, in similar ways (i.e., increasing connectivity also decreased the region of polymorphism stability; fig. 4*L*, 4*M*). Moreover, connectivity linearly increased the time needed to attain stability within the star configuration (fig. 5*A*). Thus, as connectivity increased, so did the time needed to reach stable polymorphisms.

Coldspot Spatial Organization

The spatial organization of coldspots was a major determinant of polymorphism stability within hotspots. Within the linear configuration, the stable region for polymorphisms increased with the number of coldspots connected (fig. 4*F*–4*J*). However, the effect of coldspots did not depend solely on the number of connected sites. In the simulation in which the link connecting two modules within the modular configuration was trimmed, the region for polymorphism stability within the hotspot module decayed abruptly (fig. 4*K*, 4*L*). The effect of adding an extra coldspot with the same number of links varied with the spatial organization. If the coldspot added to the cycle was directly linked to the hotspot, there was no significant change in polymorphism stability (fig. 4*M*). In contrast, the region of polymorphism stability was enlarged if the new coldspot was connected to another coldspot, transforming the hotspot in a peripheral site within the spatial configuration (fig. 4*N*). The effect of the spatial organization of coldspots also held within coldspot dynamics for intermediate to high levels of gene flow (fig. 3). Nevertheless, the effects of coldspots on polymorphism stability were distance-dependent: as distance to hotspot increased (in number of links), so did time to equilibrium within coldspots (fig. 5*B*).

Laplacian Matrices and Spatial Variation in the Coevolutionary Dynamics

Analyses of the eigenratio λ_2/λ_{max} of Laplacian matrices showed that linear and modular subgraphs are more prone to exhibit spatially variable coevolutionary dynamics than cycle and star subgraphs (fig. 6*A*). These results also hold for larger subgraphs (fig. 6*B*) and are qualitatively similar for different magnitudes of gene flow between contiguous sites (fig. 6*A*; appendix). Therefore, linear and modular subgraphs are not only the spatial configurations favoring stable polymorphisms at the local level, but also the configurations more likely to promote spatial variation in allele frequencies.

Discussion

Our results expand previous theoretical, experimental, and field studies on coevolution that have shown that geographic differences in the strength and direction of reciprocal selection can have major impacts on the coevolutionary dynamics of interacting species. Our findings support the view that the spatial configuration of interacting populations is an additional mechanism through which coevolution is continually reshaped by species interactions in nature. The spatial configuration of sites modifies the effects of the gene flow between contiguous sites and the fitness sensitivity on phenotypic matching, leading to changes in allele frequencies within and across sites.

High levels of gene flow among sites can homogenize diversity (Gomulkiewicz et al. 2000) and impede differentiation caused by evolutionary dynamics (Vogwill et al. 2008). Low levels of gene flow can impede any effect of spatial structure on coevolutionary outcomes, because within site dynamics would be independent from each other (Nuismer et al. 1999). Our results show how these outcomes change when considering different spatial structures. The spatial configuration of interacting populations affects the likelihood that gene flow between sites can overcome the intrinsic dynamic instability of hotspots. The level of gene flow between sites that allows allelic oscillations to be dampened, increasing the stability and maintenance of polymorphisms (e.g., Gandon 2002; Vogwill et al. 2010), depends on hotspot location, hotspot connectivity, and coldspot organization, all of which affect the implications of genetic connectivity at the landscape level. At very high levels of gene flow, however, the cascading effects of unstable allelic oscillations are enhanced, increasing the chance that populations will become monomorphic independently of the spatial configuration.

These findings are likely to hold in more complex systems, because our configurations capture several key aspects of natural complex spatially structured interacting populations. In fact, linear (Bergerot et al. 2010), circular (Chisholm et al. 2010), modular (Fortuna et al. 2008), and star (Economo and Keitt 2008) configurations are common components of the distribution of species assem-
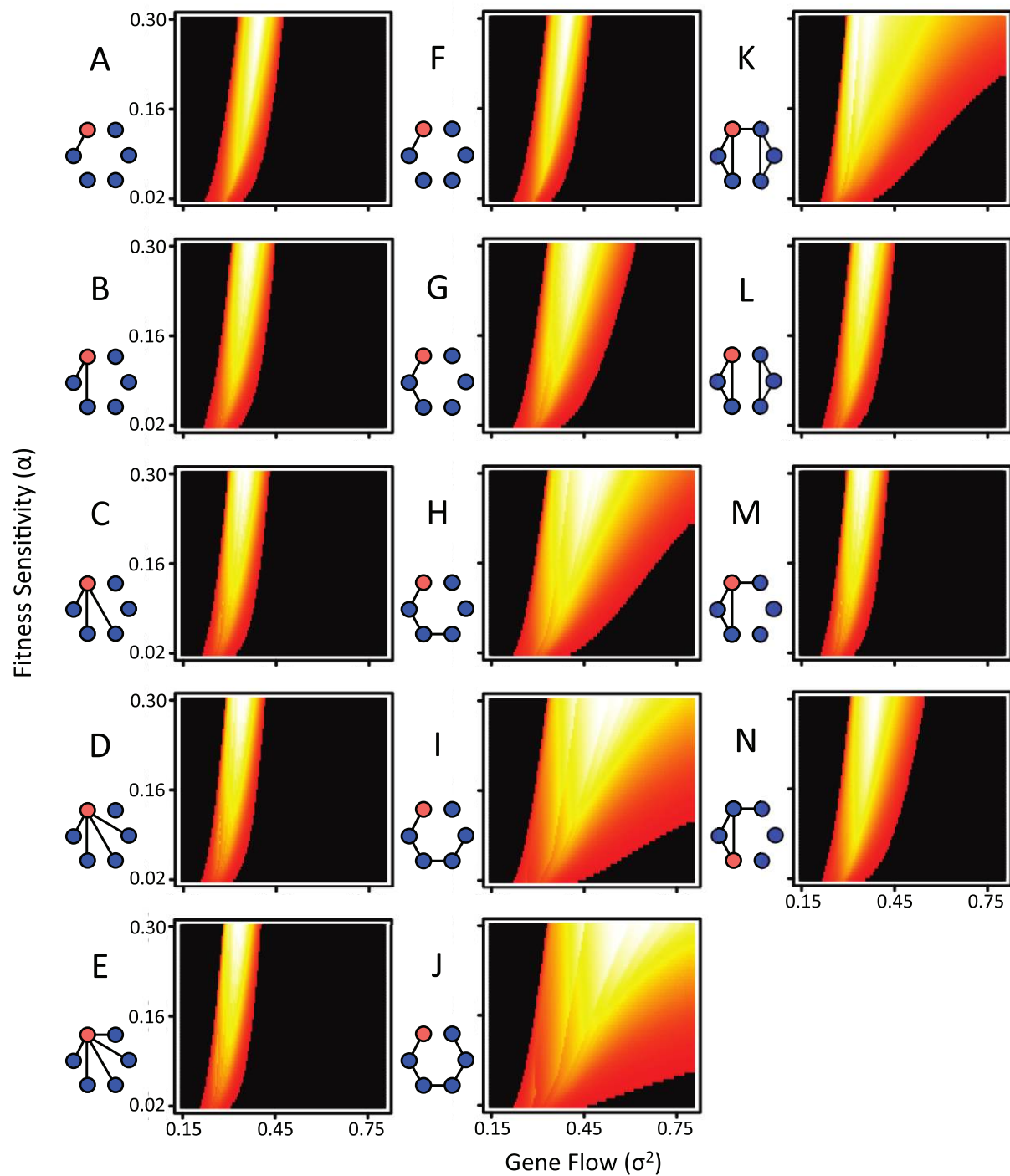
Figure 4: Each column shows the stability analyses within hotspots for three specific sets of simulations: *A–E*, hotspot connectivity simulation within the star configuration; *F–J*, coldspot organization within the linear configuration; and *K–N*, connectivity and coldspot organization experiment within the modular configuration. The axes and color code used for the stability plots are the same as those described in figures 2 and 3. The spatial configurations considered in each set of simulations are depicted near each plot, with hotspots in red and coldspots in blue. By comparing the sequence of *A* with that of *E*, it can be seen that increasing connectivity slightly decreases the stability region for polymorphisms. The sequence *F–J* shows that, as the complexity of the spatial organization of coldspots increases, so does polymorphism stability. By comparing *K* with *N* it can be seen that the effects of connectivity and coldspot organization also hold for more complex configurations, such as the modular configuration. We plotted only the coevolutionary dynamics of the parasitic species to simplify the visualization, because host and parasite dynamics are qualitatively the same.
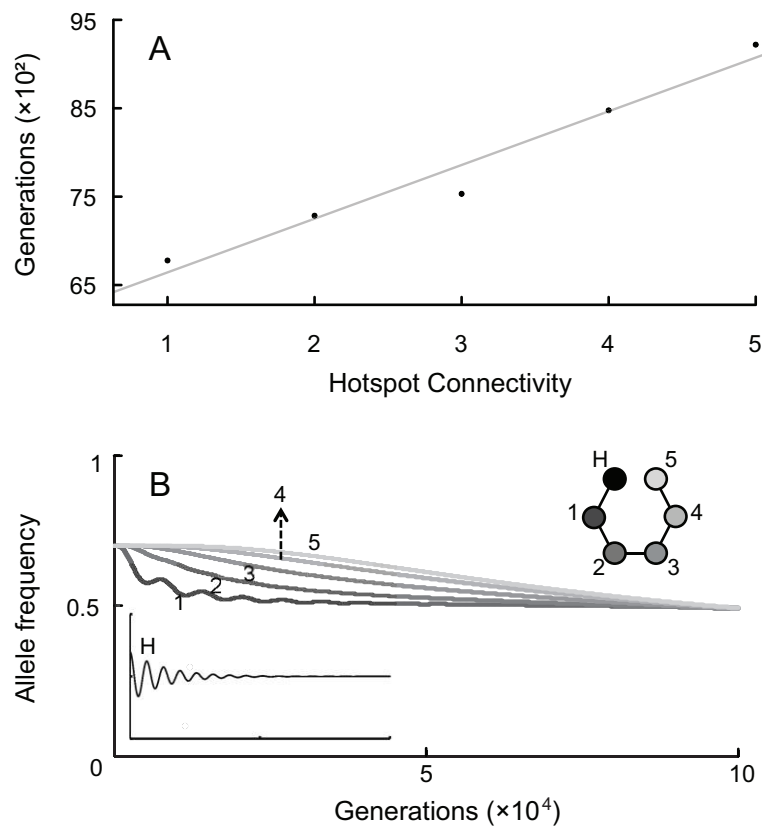
Figure 5: *A*, Mean time to polymorphism equilibrium as a function of hotspot connectivity. *B*, Parasite allele frequency change over time (in generations) for every coldspot in a linear spatial configuration is plotted in different shades of gray ($\sigma^2 = 0.23$ and $\alpha_{k,1} = 0.002$). The figure shows that coldspot dynamics depend on distance from the hotspot. The dynamics of the hotspot (black) are plotted in the lower left corner. Axes properties and model parameters are the same for both plots.

blages and are ultimately the result of ecological and phylogeographic processes shaping species distributions. The approach introduced here, however, will be less informative if the actual spatial distribution of the system cannot be approximated by discrete patches connected by gene flow.

Previous studies on geographic mosaics of coevolution with simpler spatial structure have shown that spatial structure coupled with selection mosaics affects the maintenance of polymorphisms (e.g., Nuismer et al. 1999; Gomulkiewicz et al. 2000). Those models maintain polymorphisms under conditions in which similar single-site models do not (Seger 1988; Nuismer et al. 1999; see appendix). Moreover, polymorphic populations occur under a broad range of parameter combinations, thus suggesting that it may be a common phenomenon in nature (e.g., Nuismer et al. 1999; Gomulkiewicz et al. 2000; Nuismer 2006). Our study contributes to coevolutionary theory by allowing incorporation of information from real spatial configuration of species interactions. In recent years, there

have been considerable advances in characterizing the landscape connectivity of populations in the field by integrating graph-based approaches and genetic data (Luque et al. 2012). The structure of these spatial networks combined with results of our model now make it possible to infer the potential for coevolution to maintain polymorphisms in real populations.

Our results suggest that the spatial configuration of coevolving populations affects the probability of maintaining polymorphic populations within sites through three main spatial mechanisms. First, hotspots impair the chance of maintaining polymorphic populations whenever they are central within species distributions. Higher centralization facilitates the cascading effects of reciprocal selection across all sites in a way similar to that found for the flow of information in complex networks (Ferrer i Cancho and Solé 2003; Borgatti 2005), coevolutionary cascades in mutualistic networks (Guimarães et al. 2011), or coextinction cascades in ecological networks (Solé and Montoya 2001; Allesina and Pascual 2009). The prediction that polymor-
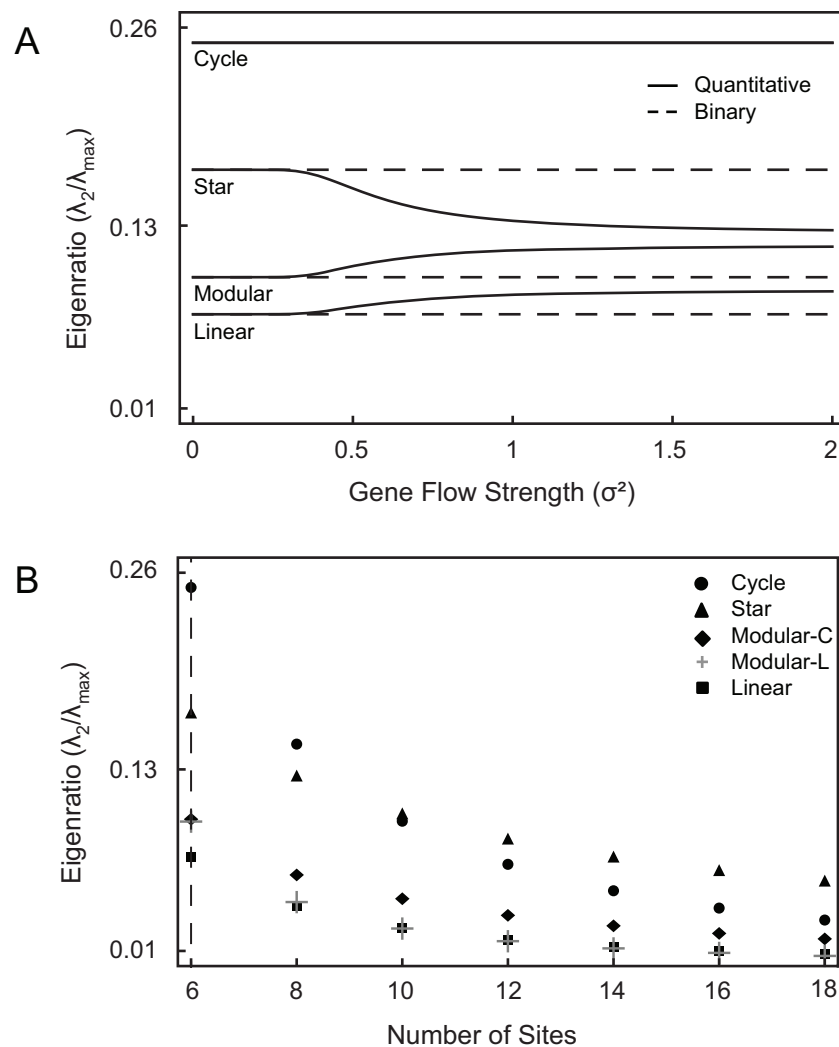
Figure 6: Eigenratio as a function of (*A*) gene flow strength for six-site subgraphs and (*B*) different numbers of sites for distinct spatial configurations. The higher the eigenratio, the lower the potential of spatial variation in the allele frequencies. Modular-C are modular configurations with an overrepresentation of sites within cycles, whereas modular-L are modular configurations with an overrepresentation of sites within the linear segment connecting the cycles. The dashed line indicates the number of sites used in most analyses of this study.

phic populations should be underrepresented in spatial configurations with central hotspots could be investigated by combining analysis of reciprocal selection in the field (Anderson and Johnson 2008) with analysis of the structure of spatial graphs describing populations in nature (Dale and Fortin 2010).

Second, increasing hotspot connectivity decreases the probability of maintaining polymorphisms either through increasing allelic fluctuations or by increasing the time at which fixed allelic frequencies are reached. In experimental microcosms, it has been shown that connectivity controls dispersal and biodiversity patterns (Carrara et al. 2012).

Connectivity can also counteract the effects of local disturbance in an experimental metacommunity by increasing global dispersal rates (Aldermatt et al. 2011). A meta-analysis has shown that local connectivity does not seem to affect species coevolution to a great extent (Urban 2011), but our results suggest that the overall effects of spatial configurations depend on the patterns of connectivity of hotspots. Together, these results underline the need for comprehensive studies aiming to reconcile the results of current meta-analyses and existing theory on spatially structured coevolution.

Third, we have shown that spatial configuration of cold-

spots increases the chance of maintaining polymorphic populations. As the number of coldspots increases, allelic frequency fluctuations are dampened within hotspots. In this sense, previous studies have suggested that coldspot organization might affect coevolutionary dynamics with respect to hotspots (e.g., Nuismer 2006; Gandon and Nuismer 2009). For example, partially overlapping ranges between coevolving populations can affect the way in which coldspots are connected, in turn affecting coevolutionary dynamics (Nuismer et al. 2003). Our results suggest that longer linear or modular arrangements of coldspots maintain polymorphisms in ways that star configurations do not. Moreover, our results show that time to equilibrium within coldspots increases as linear coldspot arrangements become larger, eventually forming temporal allele frequency clines that could yield maladaptation (Nuismer et al. 2000). This time delay in dynamical responses seems to buffer the cascading effects of reciprocal selection, sustaining genetic diversity through polymorphism maintenance. A testable prediction derived from our analysis is that sites arranged as long chains or in modules should have higher levels of polymorphisms than expected for configurations where there are highly connected central sites, such as star configurations.

The incorporation of the explicit spatial configuration of species interactions into coevolutionary models also makes it possible to predict the potential for spatial variation in allele frequencies. Spatial variation in selection and traits is now known to be a common feature of coevolving interactions (Thompson 2005). The analysis of Laplacian matrices makes it possible to explore, both in models and in empirical studies, the extent to which spatial variation in selection depends on the spatial configuration of populations. The models analyzed here suggest that spatial configurations that favor maintenance of polymorphisms at the local level also favor the maintenance of spatial variation in allele frequencies at the landscape level. In empirical studies, Laplacian matrices can be computed from any kind of spatial graph describing wild populations (Dale and Fortin 2010; app. C). Moreover, they can be easily extended to incorporate the quantitative information available on the metapopulation organization of real populations (Hanski and Ovaskainen 2000). In a way analogous to the predictive power of matrix eigenvalues for metapopulation dynamics in fragmented landscapes (Hanski and Ovaskainen 2000), the Laplacian eigenratio can be viewed as a metric that helps to predict how spatial organization affects spatial variation in the coevolutionary outcomes. The results suggest that the spatial variation in polymorphisms should be more evident in antagonistic interactions that show modular or linear spatial configurations, whereas species interactions characterized by central, highly connected sites would be expected to show less spatial variation in the outcomes of coevolution.

To test these predictions in real interactions, it is necessary to have an idea of how natural coevolving metapopulations are distributed as spatial networks (app. C). The Laplacian matrix takes into account whether gene flow exists between each pair of populations. Gene flow can be quantified using techniques such as classical F_{ST} methods (Slatkin 1985; Slatkin and Barton 1989). Quantifying the strength of gene flow is necessary only if quantitative predictions of possible coevolutionary dynamics are intended. Indeed, we have shown that the binary structure of the spatial networks is enough for a good qualitative description of these dynamics using Laplacian matrices. Because empirical depiction of spatial networks of interacting populations is hard to achieve, the approach used here could also be used with null model analysis (e.g., Dale and Fortin 2010). Although this combined approach would not solve the problem of a lack of statistical replication within networks, it could provide supporting arguments in favor of or against a given hypothesis.

A complementary approach is to use micro and mesocosm experiments to parameterize models. For example, microcosms with bacteria and phages have been successfully used in recent years to test some key components of the geographic mosaic theory of coevolution, such as the occurrence of maladaptation (Vogwill et al. 2008, 2010) or geographic mosaics of selection (Forde et al. 2008). Laplacian matrices of spatial networks can be used to characterize spatial structure in such microcosm systems and test the predictions on the role of hotspot location, connectivity, and coldspot configuration. It would also be possible to evaluate whether linear and modular networks should maintain polymorphisms in ways that more central networks, such as stars, do not. Furthermore, microcosm experiments would allow tests of predictions on the maintenance of spatial variation in coevolutionary outcomes, using the results from eigenratio analysis. Finally, microcosm experiments could test our predictions on more complex scenarios, such as conditions in which levels of gene flow vary across sites (see app. D).

The spatial configuration of connected populations has become a crucial problem in coevolutionary biology, metapopulation biology, landscape ecology, and conservation biology. Making such links between ecological patterns and coevolutionary processes is becoming increasingly important at a time when habitat fragments are increasingly losing connectivity as a result of human activities. Our results provide one way by which it is possible to use these natural experiments to test our predictions in the field and in the laboratory and assess the implications of the pattern of habitat fragmentation on the future of the coevolutionary process.

Acknowledgments

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