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Effects of Corridors on Genetics of a Butterfly in a Landscape Experiment

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Abstract - To investigate the possible role of landscape connectivity on the genetic structure of isolated populations, we examined the effects of habitat corridors on the population genetics of a vagile butterfly species, *Junonia coenia*, within a large-scale, experimental system. Using allozyme electrophoresis, a total of nine loci were identified and scored, six of which exhibited polymorphism. Our data demonstrated consistently higher levels of expected (H_e) and observed (H_o) heterozygosity in butterflies sampled from patches connected by corridors compared to unconnected patches. A *t*-test comparing H_e and H_o in connected versus unconnected patches found a marginally significant difference in one locus, the glycolytic enzyme phosphoglucose isomerase (PGI). Connected patches exhibited overall lower F_{ST} values compared to unconnected patches, indicating potentially increased levels of gene flow due to corridors. Our results support previous investigations on dispersal and population size for *J. coenia*, and show that higher dispersal through corridors promotes genetic variability at a locus (PGI) implicated in dispersal and fitness in butterflies.

Introduction

The fragmentation and outright loss of natural habitats are currently thought to be the most serious threats to global biodiversity (Fischer and Lindenmayer 2007, Saunders et al. 1991, Solé et al. 2004, Wilcove et al. 1998). Fragmentation of large contiguous areas into smaller habitat islands often results in the geographic isolation of populations, in turn limiting movement of individuals between populations (Bierregaard et al. 1992, Harris 1984). A popular management strategy to counter this type of fragmentation often involves attempts to connect habitat remnants with a corridor of similar habitat (Mann and Plummer 1995, Merriam and Saunders 1993, Noss 1992). Habitat corridors have been shown to facilitate dispersal of diverse taxa (Berggren et al. 2002, Haas 1995, Haddad 1999a, Haddad et al. 2003, Machtans et al. 1996, Sutcliffe and Thomas 1996, Tewksbury et al. 2002, Zhang and Usher 1991), reduce local extinction rates (Fahrig and Merriam 1985, Noss 1991), and increase levels of gene flow (Aars and Ims 1999, Hale et al. 2001, Mech and Hallett 2001). While the use of habitat corridors presents a potential benefit for species in isolated habitats, many uncertainties about their importance for use as a conservation strategy

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remain. Of particular concern is, even if corridors increase dispersal rates, does more movement have impacts on populations, including population genetic structure?

Population genetics provides a powerful approach to investigate population dynamics in a landscape context (Manel et al. 2003). Smaller, isolated habitats could be expected to contain fewer individuals than larger, more contiguous areas (see Vandewoestijne and Baguette 2004), affecting genetic diversity within species (Schmitt and Seitz 2002). Therefore, studies that examine the effects of connectivity on maintaining genetic variation are timely. Since new alleles appear in a gene pool through both the process of mutation and immigration of individuals from separated populations (Mettler et al. 1988, Wallace 1981), both mechanisms contributing to genetic variation within populations need to be considered. In the short-term, gene flow via immigration tends to increase genetic diversity within a given local population by offsetting the loss of alleles due to inbreeding and drift (Hoole et al. 1999, Peterson and Denno 1998, Slatkin 1985). Habitat degradation limits gene flow by reducing the movement of individuals and their alleles between fragmented, isolated populations (Hänfling et al. 2004; Nei 1973, 1987; Slatkin 1985). In addition to fewer alleles being introduced, the isolation of unconnected populations would likely reduce both heterozygosity and the total number of alleles in the population through the process of random genetic drift and inbreeding (Slatkin 1985, Van Rossum et al. 2004, Wright 1978). Recent work suggest distinct correlations between fitness and genetic diversity (Reed and Frankham 2003), with particular attention paid to molecular polymorphism in the glygolytic enzyme phosphoglucose isomerase (PGI), as this locus has been shown to affect fitness in butterflies. Polymorphism at the PGI locus has specifically been shown to enhance the flight performance and fitness of *Colias* butterflies (Watt 2003), as well as to act to increase metabolic rate, fecundity, and population growth in the *Melitaea cinxia* L. (Glanville Fritillary) (Haag et al. 2005, Hanski and Saccheri 2006). With the potential for habitat isolation to alter genetic structure at some loci and perhaps not others, a consideration of the role habitat connectivity plays in affecting the population genetics of previously isolated populations is important.

Allozyme electrophoresis has been a useful tool for investigating the genetic structure of numerous insect populations, including nymphalid butterflies (Britten and Brussard 1992, Britten et al. 1994, Brittnacher et al. 1978, Porter and Mattoon 1989). Allozymes are considered to be a valuable but conservative measure of genetic variation and are interpretable in terms of Mendelian inheritance for specific loci (Avise 1975, 1994). Numerous investigators have successfully used both DNA and allozyme analyses to examine the effects of habitat fragmentation on the genetic structure of various insects, including beetles (Britten and Rust 1996, Knutsen et al. 2000, Six et al. 1999), crickets (Berggren et al. 2002), and butterflies (Baguette et al. 2003, Johannesen et al. 1997, Keyghobadi et al. 1999, Kronforst and Fleming 2001, Meglécz et al. 2004, Vandewoestijne et al. 1999, Williams et al. 2003).

The main objective of this study was to examine if habitat corridors facilitated gene flow in an open-habitat specialist butterfly, *Junonia coenia* Hübner (Common Buckeye), within a large-scale experimental system at Savannah River Site (SRS), SC. This system was established within native *Pinus taeda* L. (Loblolly Pine) forest and contained replicate connected and unconnected habitat patches. Several previous studies with *J. coenia* at SRS (Haddad 1999a, 1999b, 2000; Haddad and Tewskbury 2005) showed that the establishment of corridors increased movement of butterflies, resulting in higher population densities in habitat patches that were experimentally connected by corridors (Haddad and Baum 1999, Tewksbury et al. 2002). Because the facilitation of movement between patches via corridors was previously established, we asked if the genetic structure of butterflies within isolated and connected patches would be differentially affected via connectivity. We expected that increased levels of gene flow in habitat patches connected to each other by a corridor would cause *J. coenia* populations in connected habitat patches to have higher genetic diversity than those in unconnected patches. We also collected butterflies from outside the experiment to establish a background or "source" level of variation. These butterflies also provided insight into the development of genetic structure within the experiment since the original colonization by the butterfly after experimental creation. The main questions addressed were: (1) do Common Buckeye butterflies sampled from patches connected by a corridor have a different genetic structure than butterflies sampled from isolated unconnected patches and, (2) does the model-corridor system at SRS reflect the natural genetic structure of *J. coenia*?

Materials and Methods

Study site

We examined the genetic structure of *J. coenia* in an experimental-model system established in the winter of 1999–2000 within the 1240-km^2 Savannah River Site. Eight 50-ha experimental blocks were created to examine the effects of corridors on plant and animal dispersal. Each block contained five open habitat patches within a dense matrix of pine forest. Patches are being restored to *Pinus palustris* P. Mill. (Longleaf Pine) savannah through restoration of Longleaf Pine and other herbaceous species, and through regular controlled fire. In each block, a central 1-ha patch was surrounded by four other patches, each 150 m away (Fig. 1). This central patch was connected to only one of the peripheral patches (also 1 ha) by a 25- X 150-m corridor. The three remaining patches were unconnected and equal to the size of the connected patch plus the corridor (i.e., 1.375 ha; Fig. 1). For a complete description of the site and establishment of plots, see Tewksbury et al. (2002). This model system was ideal for our questions because a previous study found movement of butterflies from the central patch to peripheral patches was greater with a corridor present (Tewksbury et al. 2002).

Butterfly Collection

The Common Buckeye is a multi-voltine, open-habitat butterfly that occurs throughout the southeastern United States (Opler 1998). Because the Common Buckeye in the southern US produces more than one generation per year, we estimate that at the time of our collection (summer 2002), from 6–7 generations could have developed within experimental patches since plot colonization in 2000. A total of 111 butterflies were collected from experimental patches during May and June of 2002. We choose this period to collect because populations likely peak in May and June in the deep south (N.M. Haddad, pers. observ.; Pyle 1981). In total, 53 butterflies were collected from patches connected by a corridor, while 58 butterflies were collected from unconnected peripheral patches (for the number of individuals collected in each patch refer to Table 1). No butterflies were sampled within the boundaries of an actual corridor. After collection, we determined that two blocks yielded too few butterflies (n = 7) and thus were excluded from analysis. Therefore, our

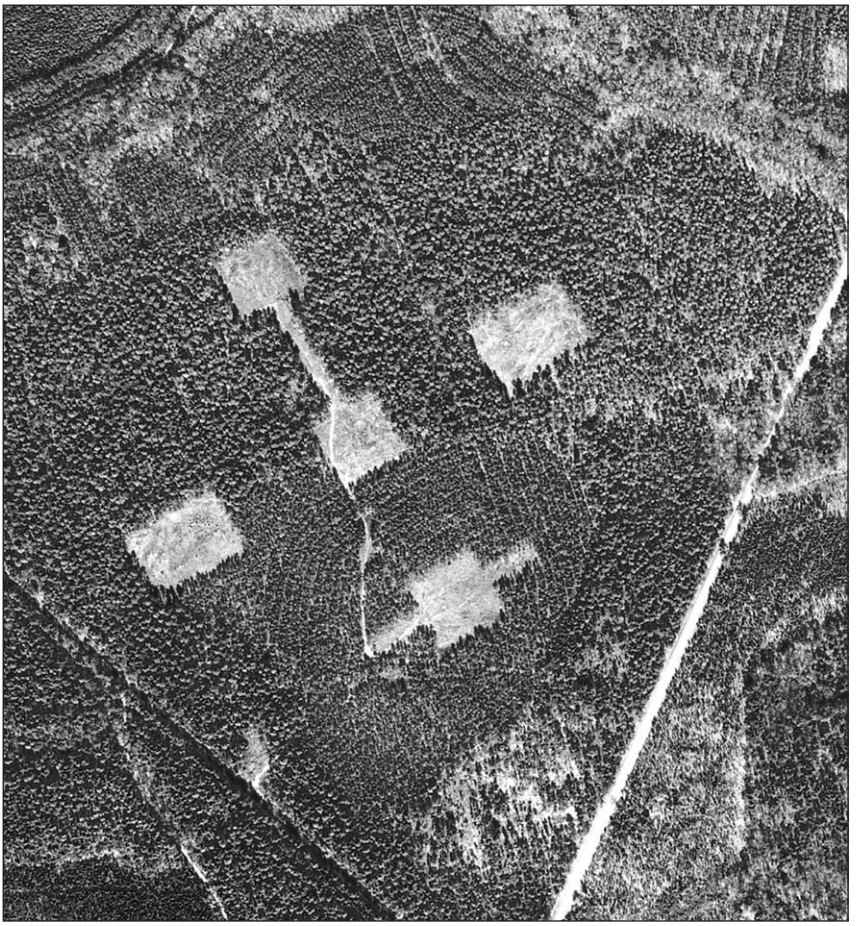

Figure 1. Patch arrangement in a single experimental block at the Savannah River Site.

genetic analysis was based on comparisons between and among six blocks, using a total of 104 butterflies. While sample size was a potentially limiting factor in our experiment, based on the numbers of alleles identified and the amount of genetic variation found across sites (see below), we feel there was a sufficient number of specimens collected to adequately address our primary questions. In order to assess genetic variation in long established populations of *J. coenia* at SRS, we sampled two populations ($n = 18$, $n = 20$) from outside of the experiment. These large, panmictic butterfly populations were sampled from wide, open power-line right-of-ways. Since butterflies from surrounding habitats founded patches within the experimental blocks, the genetic structure of "source" butterflies provides insight into mechanisms responsible for observed genetic variation within the experiment. In all cases, butterflies were captured with a handheld net and stored on ice in the field for no longer than 2 h. Individuals were then frozen and transported to Appalachian State University (ASU), Boone, NC for analysis.

Genetic analysis

Individuals were dissected to determine gender. The thorax was then partitioned into two equal-sized parts, with one half stored at -80 °C, and the remaining half ground in 2.5 ml of simple grinding buffer solution described by Werth (1985). Homogenized tissue was partitioned into two equal aliquots and stored at -80 °C until used in electrophoresis.

Nine allozyme loci were resolved on 13% starch gels (Sigma Chemical Company) using three gel/electrode buffer systems following Werth (1985) and Selander et al. (1971). Four loci, Aspartate aminotransferase (AAT) (EC 2.6.1.1), Malate dehydrogenase (MDH) (EC 1.1.1.37), Malic enzyme (ME) (EC 1.1.1.40), and Aldolase (ALD) (EC 4.1.2.13), were found to be consistently scorable on a Tris-borate-EDTA pH 9.1 gel/electrode buffer system with gel run for 6 hours at 50 mA. Phosphoglucomutase (PGM) (EC 2.7.5.1), Phosphoglucoisomerase (PGI) (EC 5.3.1.9), and Isocitrate dehydrogenase (IDH) (EC 1.1.1.42) were consistently scorable on a discontinuous Tris-citrate pH 6.3/6.7 gel/electrode buffer system with gel run for 5 hours at 100 mA. Triosephosphate isomerase (TPI) (EC 5.3.1.1) and Glycerol-3-Phosphate dehydrogenase (G3PDH or α-GPDH) (EC 1.1.1.8.) were scorable on a discontinuous Tris-citrate pH 8.2/8.7 gel/electrode buffer system with gel run for 5 hours at 100 mA. Stains were prepared according to Cardy et al. (1983), Soltis et al. (1983), and Werth (1985). The fastest-tracking allele was always scored as number one, with all subsequent alleles ordered sequentially. Zymograms were drawn immediately following staining to record all observed banding patterns. Stained gel slices were digitally photographed using a Nikon Coolpix 3500 digital camera.

Standard measures of genetic diversity, including observed heterozygosities (H_o; Levene 1949), expected heterozygosities (H_e; Nei 1978) and Wright's *F*-statistics (Wright 1951) were calculated using Popgene-32 (Yeh et al. 1997) and F-stat (Goudet 2001). Observed heterozygosity and H_e across all loci were calculated using a correction for small sample size (Nei 1978). Differences

between H_o and H_e in connected versus unconnected patches were tested for significance at each locus using a paired *t*-test (Proc GLM, SAS Institute, 2001). Butterflies were pooled in either connected or unconnected patches in an experimental block and the data analyzed using the block as a replicate ($n = 6$). All values were arcsine transformed to normalize the data. Expected and observed heterozygosities were tested for deviation from Hardy-Weinberg expectations using a chi-square goodness-of-fit analysis. Wright's *F*-statistics, specifically F_{ST}, was used to examine relative amounts of reductions in heterozygosity at a given level of the population structure relative to another more inclusive level of population structure (see Wright 1965, 1978). Results where $0.10 > P > 0.05$ are reported as marginally significant.

Results

Of the 111 butterflies sampled within the model system, eighteen females (16.2%) and 93 males (83.8%) were collected (male:female ratio of 5.2:1). The source populations contained a total of 38 butterflies, with 8 females (21.1%) and 30 males (78.9%) (male:female ratio of 3.8:1). We believe that the skewed sex ratio is caused by lower detection probabilities of females (males are pseudo-territorial and highly visible; N.M. Haddad, pers. observ.).

Allele frequencies and numbers of butterflies collected in each experimental unit for the polymorphic loci are presented in Table 1. Of the nine enzyme systems used for analysis, six displayed polymorphism at a 95% confidence level. The loci ALD, α-GPDH, and ME were all fixed for a single allele and were therefore considered monomorphic. While only two blocks (in connected patches) had butterflies polymorphic for MDH, this locus was included in the analysis (Table 1). There was considerable variation in the numbers of *J. coenia* collected in the different blocks.

We found that four loci from connected patches and two loci from the unconnected patches were not in Hardy-Weinberg equilibrium (Table 2). When comparing observed and expected heterozygosity across treatments using a student *t*-test, only one locus, PGI, differed between connected and unconnected patches (marginally significant; Table 2). Without considering other factors such as wing wear and changes due to flight season, this result possibly demonstrates an effect on some loci but not others and suggests, based on other studies with PGI, that a locus implicated in dispersal ability of butterflies is affected by connectivity between habitats.

There was considerable variation in H_o and H_e within blocks in both connected and unconnected patches (Table 3). Overall, H_o, H_e, and percent polymorphic loci were higher in connected than unconnected patches for five of the six blocks sampled. One word of caution regarding sample size: in some plots, the disproportionate number of butterflies collected in connected versus unconnected patches could have biased our results with respect to calculated H_o and H_e (see Tables 1 and 3). However, the consistent observation of higher heterozygosites in connected versus unconnected patches

Table 1. Allele frequencies for connected (C) and unconnected (U) patches in six polymorphic loci in sequentially numbered experimental blocks. *n* = number of Common Buckeye butterflies collected in patches. See Materials and Methods for a description of the loci.

		Blocks											
Locus	Allele	1-C	1-U	2-C	2-U	3-C	3-U	4-C	4-U	5-C	5-U	6-C	6-U
n		10	3	6	3	5	6	21	13	4	21	5	7
AAT	1			0.083				0.047	0.154		0.024	0.100	
	2	1.000	1.000	0.917	1.000	1.000	1.000	0.929	0.808	1.000	0.976	0.900	1.000
	3							0.024	0.039				
TPI	1								0.024	0.192	0.125	0.024	0.143
	2	1.000	1.000	1.000		1.000	1.000	0.976	0.769	0.875	0.976	0.900	0.857
	3								0.039		0.100		
PGI	1					0.100	0.083	0.120	0.077				
	2	0.450	0.500	0.417	0.500	0.500	0.250	0.452	0.462	0.625	0.452	0.100	0.357
	3	0.500	0.500	0.500	1.000	0.300	0.583	0.405	0.423	0.375	0.548	0.900	0.929
	4	0.050		0.083		0.100	0.083	0.024	0.039				0.071
PGM	1	0.050	0.167					0.024					
	2			0.250	0.167		0.250	0.024	0.115	0.125	0.143	0.100	
	3	0.700	0.677	0.583	0.833	0.800	0.667	0.050	0.577	0.625	0.738	0.900	0.928
	4	0.250	0.167	0.083			0.800	0.667	0.214	0.269	0.250	0.119	0.071
	5			0.083					0.039				
IDH	1	0.050		0.083			0.083		0.039		0.024		
	2	0.850	0.833	0.833	0.500	0.800	0.917	0.833	0.962	0.875	0.905	0.900	0.857
	3	0.100	0.167	0.083	0.500	0.200		0.167		0.125	0.048	0.100	0.143
MDH	1					0.200				0.125			
	2	1.000	1.000	1.000	1.000	0.800	1.000	1.000	1.000	0.875	1.000	1.000	1.000

with near equal numbers of butterflies collected supports our stated result. Averaging blocks, observed heterozygosity was 20% higher in the connected (H_o = 0.1928) than unconnected (H_o = 0.1547) patches. Similarly, expected heterozygosity increased by 23% in connected patches, while Wrights F_{ST} was 37% lower in connected than unconnected patches when blocks were averaged (Table 3). Finally, source-population butterflies were substantially and consistently higher in H_o, H_e, and percent polymorphic loci compared to butterflies collected within the experiment. Average values of Wrights F_{ST} for the source populations were more comparable to butterflies collected in connected than unconnected patches (Table 3).

Table 2. Statistical results from Hardy-Weinberg Equilibrium analysis (χ^2 and *P* value) for connected and unconnected patches and H_o and H_e analysis using a *t*-test (*t* and *P* value) for each locus. See Materials and Methods for a description of the analyses and loci. * = $P < 0.05$, ** = $P < 0.1$.

	Connected		Unconnected		H_o		H_e	
Locus	χ^2	*P*	χ^2	*P*	*t*	*P*	*t*	*P*
AAT	9.310	0.030*	10.31	0.020*	0.58	0.595	0.22	0.595
TPI	0.030	0.990	1.800	0.610	0.83	0.443	0.94	0.390
PGI	13.33	0.040*	21.42	0.002*	2.11	0.059**	1.15	0.275
PGM	7.050	0.720	8.080	0.620	0.51	0.621	0.37	0.718
IDH	14.27	0.003*	0.850	0.990	0.95	0.364	0.56	0.583
MDH	34.33	0.001*						

Table 3. Mean ± standard deviation (SE) of observed (H_o) and expected (H_e) heterozygosity within each block for connected (C) and unconnected (U) patches and source (S) populations. Also presented are mean ± SE for all blocks (1–6) and source populations (S1–S2) combined for H_o, H_e, percent polymorphic loic (% Ploci) and Wrights *F* statistic (F_{ST}). See Materials and Methods for a description of the analyses.

Block		H_o (mean ± SE)	H_e (mean ± SE)	% Ploci	F_{ST}
1	C	0.2393 ± 0.2827	0.2163 ± 0.2660	55.6	0.0299
	U	0.1746 ± 0.3228	0.1914 ± 0.2555	55.6	0.0913
2	C	0.1333 ± 0.1414	0.1330 ± 0.1518	55.6	0.0816
	U	0.1270 ± 0.1667	0.1202 ± 0.1652	44.4	0.0973
3	C	0.2500 ± 0.3062	0.1840 ± 0.2064	55.6	0.0462
	U	0.1270 ± 0.1813	0.1319 ± 0.1942	55.6	0.0943
4	C	0.1778 ± 0.2906	0.1778 ± 0.2325	44.4	0.1354
	U	0.1481 ± 0.2561	0.1358 ± 0.2329	33.3	0.1681
5	C	0.1778 ± 0.3073	0.1394 ± 0.2209	33.3	0.0908
	U	0.2222 ± 0.3727	0.1420 ± 0.2224	33.3	0.1001
6	C	0.2037 ± 0.3203	0.1775 ± 0.2474	44.4	0.0510
	U	0.1481 ± 0.3379	0.0864 ± 0.1803	22.2	0.0783
1–6	C	0.1928 ± 0.0177	0.1690 ± 0.0126	47.4 ± 3.72	0.0643 ± 0.0156
	U	0.1547 ± 0.0147	0.1310 ± 0.0139	38.7 ± 5.51	0.1017 ± 0.0130
S1		0.3210 ± 0.3411	0.2454 ± 0.2544	55.6	0.0185
S2		0.3278 ± 0.3392	0.2517 ± 0.2452	55.6	0.0185
S1–S2		0.3244 ± 0.0034	0.2485 ± 0.0032	55.6 ± 0.0	0.0185 ± 0.0

Discussion

We found that *J. coenia* had higher levels of genetic variation in connected patches as measured by percent polymorphic loci, observed and expected heterozygosity, and lower F_{ST} values, strongly suggesting that increased dispersal between populations influences the genetic structure of populations. Furthermore, differences in genetic variation at the PGI locus occur due to connectivity, demonstrating that variation at loci implicated for importance in fitness and dispersal in butterflies is likely due to increased connectivity provided by corridors. Our study supports a previous multi-year study conducted at the SRS that found a greater number of marked butterflies released in a central patch are recaptured in patches connected by a corridor (Tewksbury et al. 2002). In combination with those results, our evidence suggests that movement between isolated populations is important for maintaining genetic diversity of *J. coenia*.

By a number of metrics, our data suggest that habitat connectivity promotes greater genetic variability, as *J. coenia* had overall higher level of heterozygosity and polymorphic loci when corridors were present. The fixation index (i.e., F_{ST}) indicates moderate levels of genetic differentiation (see Wright 1978 for description) in populations collected within habitat patches, regardless of connectivity. Butterflies collected in connected patches did, however, have an overall lower F_{ST} than those in unconnected patches (Table 3), indicating less genetic divergence, likely because of increased gene flow. We base this conclusion in part on the fact that our results correspond with data on *J. coenia* movement within the same experimental corridor system that has shown increased movement of this butterfly in patches connected by corridors (Tewksbury et al. 2002). Although other investigators have examined the effects of habitat fragmentation on the genetic structure of insect populations (Baguette et al. 2003, Johannesen et al. 1997, Kronforst and Fleming 2001, Meglécz et al. 2004, Williams et al. 2003), few have attempted to link a genetic analysis to movement results, providing a mechanistic explanation as to why genetic changes occur (Castellón and Sieving 2006, Keyghobadi et al. 1999).

Our results also support the notion that habitat connectivity promotes polymorphism at a particular locus. Populations of *J. coenia* exhibited higher overall genetic variation at the PGI locus in connected habitats, reflected in the marginally significantly higher H_o in connected versus unconnected patches (Table 2). This result is extremely interesting in light of recent studies linking the PGI locus to dispersal capability in butterflies (Hanski and Saccheri 2006). Relevant to our investigation, patch area and spatial connectivity of habitat have been demonstrated as being important in maintaining polymorphism of PGI (Hanski et al. 2004). Our results support the conclusion that spatial configuration of habitat can contribute to the maintenance of molecular polymorphisms, even in vagile taxa like butterflies. This is especially relevant in the experimental system used in this study, which accounted for the size of the corridor, and from previous work that determined the shape of the habitat patches

had no effect on the ability to capture butterflies (Tewksbury et al. 2002). Thus, in this system, there is no evidence the corridors acted as drift fences with respect to butterfly movement. Although somewhat speculative, we conclude that variation at the PGI locus provides evidence that gene flow is the primary mechanism shaping the genetic structure of *J. coenia* in connected habitat patches.

To better understand possible mechanisms contributing to the genetic differentiation observed in our experiment, we asked how well the model system reflected source-population genetic differentiation. This is relevant to consider since the population genetic structure within experimental patches was ultimately dependent on butterflies colonizing from nearby source populations. We found that source populations had much higher genetic variation than butterflies in the experiment (Table 3). The substantially lower F_{ST} in these populations indicate higher levels of gene flow, resulting in genetic panmixia. Lower values of heterozygosity and F_{ST} values observed in our experiment are likely the result of a combination of founder effects and reduced gene flow (see Baughman et al. 1990, Nice and Shapiro 2001) compared to butterflies in the open environment outside the experimental units, which have likely passed through many generations. With no prior knowledge of genetic structure in founding butterflies, it is somewhat speculative to conclude that the genetic variation we found was due mostly to gene flow and not genetic drift (possibly due to a small population size in patches compared to the surrounding area). Nevertheless, provided that colonizing butterflies in connected and unconnected patches had similar levels of genetic variation after patches were created, the lower F_{ST} and higher heterozygosity in the connected patches two years later provide evidence that since the time of establishment, populations of *J. coenia* have differentiated more when a corridor connected patches.

In conclusion, our investigation demonstrates the value of addressing genetic questions when the role of habitat connectivity on species in fragmented landscapes is being examined. We demonstrated greater levels of genetic differentiation in butterfly populations connected by corridors and, importantly, provided evidence that variation in a key locus implicated in the fitness and dispersal of butterflies is affected by connectivity. Data from source populations suggest effects of both gene flow and possibly genetic drift acting in this system, pointing to a need to examine both population and genetic data in conservation studies. Though we studied a common species, our study suggests a need for similar experimental approaches in cases where species become rare or imperiled due to habitat fragmentation.

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