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Contributed Paper

Combining Measures of Dispersal to Identify Conservation Strategies in Fragmented Landscapes

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Abstract: *Understanding the way in which habitat fragmentation disrupts animal dispersal is key to identifying effective and efficient conservation strategies. To differentiate the potential effectiveness of 2 frequently used strategies for increasing the connectivity of populations in fragmented landscapes—corridors and stepping stones—we combined 3 complimentary methods: behavioral studies at habitat edges, mark-recapture, and genetic analyses. Each of these methods addresses different steps in the dispersal process that a single intensive study could not address. We applied the 3 methods to the case study of* Atrytonopsis *new species 1, a rare butterfly endemic to a partially urbanized stretch of barrier islands in North Carolina (U.S.A.). Results of behavioral analyses showed the butterfly flew into urban and forested areas, but not over open beach; mark-recapture showed that the butterfly dispersed successfully through short stretches of urban areas (<500 m); and genetic studies showed that longer stretches of forest (>5 km) were a dispersal barrier, but shorter stretches of urban areas (≤5 km) were not. Although results from all 3 methods indicated natural features in the landscape, not urbanization, were barriers to dispersal, when we combined the results we could determine where barriers might arise: forests restricted dispersal for the butterfly only when there were long stretches with no habitat. Therefore, urban areas have the potential to become a dispersal barrier if their extent increases, a finding that may have gone unnoticed if we had used a single approach. Protection of stepping stones should be sufficient to maintain connectivity for* Atrytonopsis *new species 1 at current levels of urbanization. Our research highlights how the use of complementary approaches for studying animal dispersal in fragmented landscapes can help identify conservation strategies.*

Keywords: *Atrytonopsis*, connectivity, crystal skipper, habitat fragmentation, mark-recapture, population genetics, stepping stones

Combinación de Medidas de Dispersión para Identificar Estrategias de Conservación en Paisajes Fragmentados

Resumen: *El entendimiento de la manera en que la fragmentación del hábitat altera la dispersión animal es clave para la identificación de estrategias de conservación efectivas y eficientes. Para diferenciar la efectividad potencial de 2 estrategias utilizadas frecuentemente para incrementar la conectividad de poblaciones en paisajes fragmentados – corredores y pasaderas – combinamos 3 métodos complementarios: estudios conductuales en bordes de hábitat, marca-recaptura y análisis genéticos. Cada unos de estos métodos aborda los diferentes pasos del proceso de dispersión que un solo estudio intensivo no podría atender. Aplicamos los 3 métodos al estudio de caso de* Atrytonopsis *especie nueva 1, una mariposa rara endémica a islas parcialmente urbanizadas en Carolina del Norte (E. U. A.). Los resultados del análisis conductual mostraron que la mariposa voló hacia áreas urbanas y bosques, pero nunca sobre la playa abierta; la marca-recaptura mostró que la mariposa se dispersó exitosamente por extensiones pequeñas de áreas urbanas (<500 m); y los estudios genéticos mostraron que extensiones grandes de bosque (>5 km) eran una barrera para la dispersión, pero extensiones de áreas urbanas más pequeñas (≤5 km) no lo fueron. Aunque los resultados de los 3 métodos indicaron que las características naturales del paisaje, no la urbanización, fueron barreras para la dispersión, cuando combinamos los resultados pudimos determinar donde podrían surgir barreras: los bosques restringieron la dispersión de la mariposa solo cuando había extensiones grandes sin hábitat.*

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Por lo tanto, las áreas urbanas tienen el potencial de convertirse en una barrera para la dispersión si su extensión incrementa, un hallazgo que hubiera pasado inadvertido si hubiésemos usado solo un método. La protección de pasaderas debería ser suficiente para mantener la conectividad para Atrytonopsis *especie nueva 1 con los actuales niveles de urbanización. Nuestra investigación destaca que el uso de métodos complementarios para estudiar la dispersión animal en paisajes fragmentados puede ayudar a identificar estrategias de conservación.*

Palabras Clave: *Atrytonopsis*, conectividad, fragmentación del hábitat, genética de poblaciones, marca-recaptura, pasaderas

Introduction

Habitat fragmentation has the potential to impede dispersal of animals and plants, thereby reducing gene flow and colonization. Consequently, there is a need to understand whether and how sources of fragmentation, including agricultural or urban development, affect dispersal (Fahrig 2007). We present a rationale, through a case study, for the use of multiple approaches to quantify dispersal for use in identifying potential conservation actions.

Three strongly contrasting methods—behavioral studies at habitat edges, mark-recapture, and genetic analyses—are often used to evaluate the effects of fragmentation on animal dispersal (e.g., Haddad et al. 2003; Keyghobadi 2007; Dover & Settele 2009). Individual studies frequently focus on specific aspects of dispersal, but then their results are used to make general recommendations for connecting populations across landscapes. Rather than focusing on a single, intensive approach, we drew on all 3 approaches to assess different aspects of the dispersal process. Our goal was to use results of the 3 methods to differentiate between the potential effectiveness of 2 commonly proposed approaches for increasing the connectivity of populations in fragmented landscapes: corridors and stepping stones (Diamond 1975; Bennett 2003). We used a rare butterfly in an urbanizing landscape as a case study.

Measuring Animal Dispersal

Successful dispersal between habitat patches in fragmented landscapes involves several steps (Ims & Yoccoz 1997): (1) an individual moves through an edge that it has encountered between its current habitat patch and the matrix, which contains no habitat, (2) it survives in the matrix, (3) it finds another habitat patch, and (4) it becomes part of or contributes to formation of the reproductive population in the new patch. Behavioral, mark-recapture, and population-genetic approaches address different dispersal steps.

The first step of dispersal, emigration from habitat to the matrix, can be measured by observing the behavior of individuals at habitat edges. The probability of encountering an edge and edge permeability affect emigration rates (Stamps et al. 1987). Empirical evidence suggests that edge permeability for a species depends on the type of and structural contrast between habitat and adjacent matrix (Ries & Debinski 2001; Stevens et al. 2006). Although edge behavior may be relatively easy to measure, it rarely provides information on survival in the matrix or on an individual's ability to find other habitat patches.

Mark-recapture methods are commonly applied to assess dispersal rates (Stevens et al. 2010). Despite their popularity, mark-recapture studies can be costly and time intensive and are often challenging to use for species sensitive to physical handling (Haddad et al. 2008). It can also be difficult to distinguish whether low movement rates between populations are due to high emigration rates and high mortality in the matrix or to low emigration rates and low or high mortality in the matrix (but see Ovaskainen et al. 2008). Additionally, mark-recapture typically underestimates long-distance dispersal (Koenig et al. 1996). These dispersal events are particularly important for genetic exchange in rare species with low population densities and low dispersal rates.

Techniques used to study population genetics provide insight into the dispersal rate of a species throughout its range. Populations that are relatively more differentiated genetically have exchanged fewer individuals, which indicates lower dispersal rates (Slatkin 1985). The study of genetics at the landscape extent can be used to investigate whether natural (e.g., mountains) or human-made landscape features (e.g., urbanization) affect gene flow and population structure (Manel et al. 2003; Epps et al. 2005). Techniques used to study population genetics have the capacity to provide information about dispersal across more extensive areas than mark-recapture techniques. Unlike studies of behavior at habitat edges and the mark-recapture method, which focus on individuals, genetic studies typically address populations, although the source of dispersing individuals can potentially be determined with assignment tests (Manel et al. 2003).

Multiple Approaches to Understand Dispersal

Combining approaches to study dispersal can be difficult because of the different spatial extents that each approach addresses (Lima & Zollner 1996). Consequently, mark-recapture often is combined with either population genetics or behavioral studies at habitat edges.

We are aware of no other studies that draw on behavioral, mark-recapture, and genetic approaches in one assessment.

Some researchers have used one approach to validate the findings of another. For example, behaviors of butterflies and birds at habitat edges have been used to predict dispersal across more extensive areas as measured via mark-recapture studies (Haddad 1999; Levey et al. 2005). Watts et al. (2004) in their study of damselflies found significant genetic differentiation between populations separated by short distances (≤8 km) and then used mark-recapture to corroborate their findings.

In other cases, combining 2 approaches provided complementary insights about dispersal. Howeth et al. (2008) used genetics to measure historical levels of connectivity among box turtle (*Terrapenecoahuila*) populations and used mark-recapture to quantify the effects of recent isolation. Miller et al. (2002) used dispersal data from aquatic insects caught in traps to distinguish among hypotheses about the lack of genetic differentiation between stream catchments.

Choosing Conservation Strategies

Conservation strategies, such as corridors and stepping stones, can mitigate the effects of fragmentation on populations by improving connectivity among them. Corridors, linear strips of habitat that connect larger habitat patches, increase dispersal rates for a number of taxa (Haddad et al. 2003; Gilbert-Norton et al. 2010). However, corridors can be difficult to establish when the acquisition or restoration of specific areas is required. In urbanizing landscapes with high property values and nearly irreversible land conversion (e.g., parking lots), it may be impossible to establish a corridor between natural areas. Furthermore, the presence of a corridor alone does not necessarily translate to increased dispersal (Tischendorf & Fahrig 2000).

Theoretical and empirical evidence also demonstrates that stepping stones, small patches of habitat located between larger habitat patches, can improve connectivity across a landscape (Gilpin 1980; Schultz 1998; Uezu et al. 2008). Stepping stones may be easier to implement than corridors because they can be established more opportunistically via habitat restoration, the purchase of less expensive land, and by working with cooperative landowners. To date, more conservation plans and empirical research have focused on corridors than stepping stones (Beier et al. 2008). The most effective conservation strategy for a given species depends on how the species disperses through the landscape.

Using multiple approaches to study dispersal can help distinguish among the potential effects of corridors and stepping stones (Fig. 1). Behavioral studies at habitat edges can determine the probability that an individual will enter the matrix. If individuals are unlikely to enter the matrix, then corridors might be more effective. However, entering the matrix does not automatically suggest stepping stones as an effective strategy because individuals may die or return to their source location before finding another habitat patch. Mark-recapture studies can indicate whether individuals that enter the matrix encounter habitat. Population genetics can then determine whether individuals can disperse through the matrix over longer distances than can be tested with mark-recapture and assess whether dispersing individuals become part of a reproductive population. Yet, without the behavioral studies, it would be difficult to separate dispersal effects from local adaptation. Combining studies of behavior of individuals at edges and population genetics can also help differentiate the effects of dispersal distance and matrix composition.

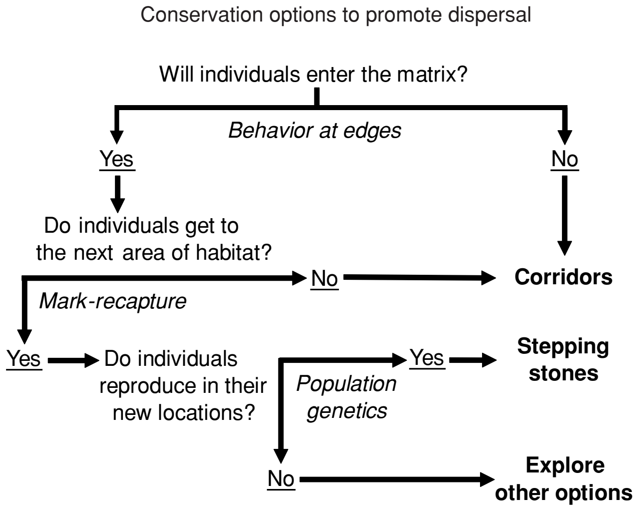

Figure 1. Relations among the 3 different approaches (behavior at edges, mark-recapture, and population genetics) we used to examine dispersal of Atrytonopsis sp1*, the research questions we asked, and 2 possible conservation strategies (corridors and stepping stones [bold]).*

Case Study

Atrytonopsis new species 1 (Hesperiidae), commonly referred to as the crystal skipper, is a rare butterfly endemic to Bogue Banks, Bear Island, and a few small dredge-spoil islands within a 50-km stretch of barrier islands in North Carolina (U.S.A.) (Fig. 2). Locally, the butterfly is abundant, and throughout much of the butterfly's 50-km-long, 50- to 500-m-wide range, urban development has fragmented its sand-dune habitat such that many populations occur only on remnant patches of dune. Our research was motivated by a desire to understand the degree to which urbanization limits dispersal in this species and to identify potential conservation options.

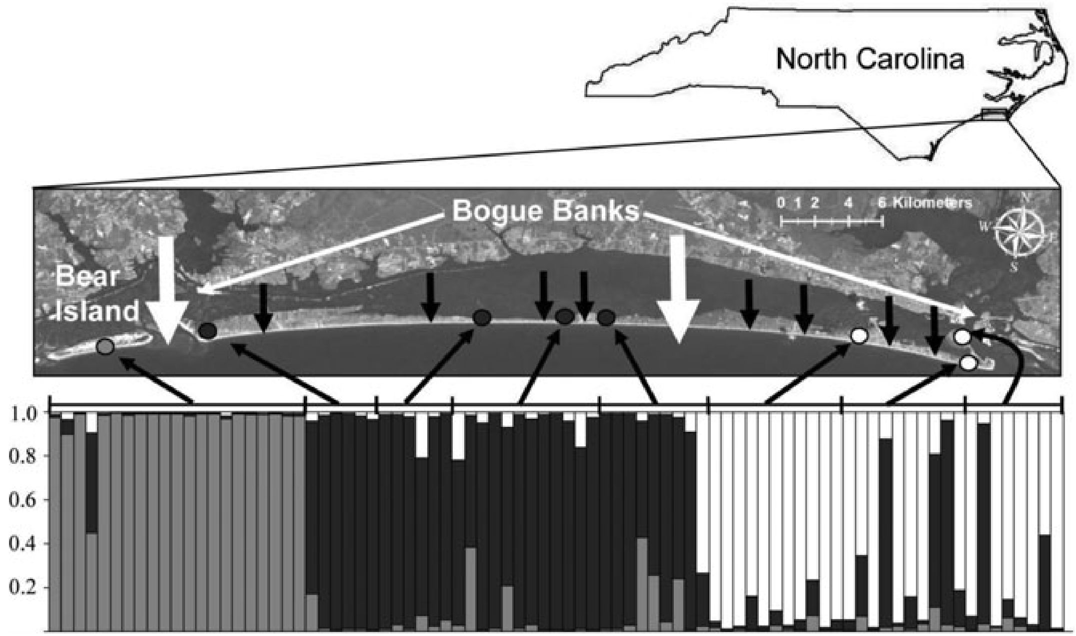

Figure 2. Range of Atrytonopsis sp1 *(Bogue Banks and Bear Island, North Carolina, U.S.A.) and sampling sites for the genetic analyses (circles). Circles are shaded according to the population genetic structure results, which identified 3 genetically distinct regions. Each bar in the lower part of the figure corresponds to an individual and the* y*-axis shows the proportion of that individuals' genome assigned to 1 of the 3 genetically distinct regions. Individuals are arranged along a west-east axis and are grouped according to their sampling location (black arrows pointing up). The black arrows pointing down indicate areas with high-intensity urban development. White arrows correspond to natural barriers (i.e., ocean, maritime forest).*

However, our synthesis does not include an intensive investigation with each of the 3 approaches we used to study the butterfly's dispersal. The narrow focus with each approach is consistent with what may typically be feasible in conservation research; applying all 3 approaches in-depth would consume considerable time and resources.

Methods

Study System and Species

Atrytonopsis new species 1 (hereafter *Atrytonopsis* sp1) was first identified in 1978 (E. Quinter, personal communication; specimens available at the National Museum of Natural History, Washington, D.C.). The butterfly was previously identified by the U.S. Fish and Wildlife Service (USFWS) as a federal species of concern, a category that is no longer in use. However, the Raleigh Field Office of USFWS continues to believe that *Atrytonopsis* sp1 is a species of concern and is working to learn more about this imperiled species. The butterfly is bivoltine and flies from mid-April to mid-May and mid-July to mid-August (Hall 2004; Leidner & Haddad 2006). Two state parks, located at either end of the butterfly's range, support thousands of adults, and many other small patches of sand dunes between the parks support hundreds of adults (Hall 2004; Leidner & Haddad 2006).

High-quality *Atrytonopsis* sp1 habitat is within the primary and secondary sand dunes, where its host plant, seaside little bluestem (*Schizachyrium littorale*), is one of the dominant grasses. Sand dunes within a barrier island are usually continuous and run parallel to the shoreline. Within an island, natural disturbances prevent sand dunes dominated by grasses from undergoing succession to maritime forest. However, human-made structures, such as jetties and seawalls, can disrupt the continuity of sand dunes by altering erosion rates (Pilkey et al. 1975). On Bogue Banks, erosion has contributed to the near total loss of sand dunes along a continuous 10-km stretch of the 35-km-long island. The maritime forest begins directly above the high tide line, serving as a potential barrier to dispersal of *Atrytonopsis* sp1. Natural ocean inlets are also likely to be dispersal barriers. Additionally, sand dunes on Bogue Banks are fragmented by residential and commercial development, leaving many populations of *Atrytonopsis* sp1 isolated by hundreds to thousands of meters of urban area.

Behavioral Studies at Habitat Edges

We released *Atrytonopsis* sp1 at the boundaries of 4 different edge types to determine whether the species would enter the matrix. These edges separated the sand dunes occupied by *Atrytonopsis* sp1 and 4 dominant matrix types: beach, maritime forest, urban area (e.g., parking lots or housing developments), and open ocean. Edges between sand dunes and beach or maritime forest are potential natural barriers to *Atrytonopsis* sp1 dispersal. The beach-ocean edge was the only edge we tested that was not adjacent to sand dunes. We released individuals at this edge because for *Atrytonopsis* sp1 to move between islands, it must cross both open beach and open ocean. We spread release sites across the butterfly's range and chose sites on the basis of accessibility and similarity of vegetation structure within edge type. All edges ran along a north-south or east-west axis.

We caught butterflies with hand nets, placed them in glassine envelopes, held the envelopes in a dark, cool location, and released butterflies <1 h after capture. For each edge type, we released individuals at 3–6 sites. We used this release procedure because butterflies were not abundant at each type of edge. We marked individuals to avoid their reuse in a release. We always released individuals into the same patch of habitat from which they were captured. We caught and released butterflies during the summer (mid-July to mid-August) 2007, spring (mid-April to mid-May) 2008, and summer (mid-July to mid-August) 2008 flight periods. To release butterflies, we placed a single individual into a small plastic container with a lid. After allowing the butterfly time to acclimate, we removed the lid from a distance with a string to limit the effect of the observer on the butterfly's behavior (protocols were similar to those of Conradt et al. [2000] and Kuefler et al. [2010]). Butterflies were scored as moving to the dune or matrix if they flew >1 m from the release site (most flew much farther). Data were recorded until the butterfly rested for >7 min or was lost from sight. Although the butterflies we released may have been exhibiting some stress response to handling, the movement we recorded is an effective measure of habitat preference (Conradt et al. 2000; Kuefler et al. 2010). Due to the lack of consistent and obvious morphological differences between male and female *Atrytonopsis* sp1, our team could not consistently identify sex in the field. Data on sex and other intraspecific differences might explain some variation in movement rates. Yet given the number of individuals in both the behavioral ($n = 194$) and mark-recapture procedures ($n = 535$), and the observations of those in our group who could differentiate sex, both males and females dispersed into and through the matrix.

We used a backward stepwise logistic regression to test whether preference for sand dunes was affected by edge type (beach, urban, ocean, forest), season (summer 2007, spring 2008, summer 2008), release site, wind speed (continuous variable), direction of the wind relative to the edge (whether the wind was blowing into the dune, into the matrix, or along the edge), and the interaction of wind speed and the direction of the wind relative to the edge. We used a p value of 0.15 to determine whether a variable was retained in the model. A significant intercept would indicate an overall preference for sand dune relative to edge types.

Mark-Recapture

We used mark-recapture to determine the proportion of *Atrytonopsis* sp1 that moved through continuous dune and urban areas. We established 15 marking locations that formed 9 adjacent pairs of sites, each separated by approximately 400 m. All marking locations were in sand dunes. Three pairs of sites were separated by continuous sand dunes and 6 pairs by urban areas. Our aims were more limited than a typical mark-recapture study because we were interested only in relative movement rates and whether butterflies would disperse across urban areas. Furthermore, we only assessed movement between adjacent pairs of sites, not among all 15 locations.

Each marking location was approximately 1 ha of dunes, although some of these locations were situated within larger dune areas. Sites were surveyed on 26 and 27 April and 3 and 4 May 2008. Over the 4 days, there were 15 observers split into groups of 2–3. A large group of observers was needed to survey all sites in a single day, so there were a limited number of days over which we could conduct the study. On each day, the same observers surveyed both sites within a pair. When possible, a given pair of sites was surveyed twice each day. In each group one person was highly experienced at marking butterflies and observers were rotated to different groups and sites among days. At each location, we searched for butterflies for 50 min, with the exception of 4 small sites in which survey time was adjusted downward in proportion to the area of the site. To account for time spent recording data and handling butterflies, we added 3 min to the base survey time for each individual marked and 2 min for each individual recaptured. Locations were surveyed for a maximum of 120 min. We captured butterflies with hand nets and used a black ultrafine-point marker to mark each with a unique alphanumeric combination (Ehrlich & Davidson 1960).

We assumed survival and detection probability were equivalent in the 2 locations that formed a pair and that movement of a butterfly was not directionally biased. We believe these are reasonable assumptions because the 2 locations comprising a pair were similar in topography and vegetation structure. On the basis of these assumptions, we calculated the dispersal rate between 2 locations within a site as the number of recaptured butterflies that moved divided by the total number of recaptures (survival- and detection-probability terms cancel out). This metric of movement, also called dispersal fraction or exchange rate, is used commonly in butterfly studies (reviewed by Stevens et al. 2010). We did not assume detection probability among the 9 pairs of sites was equivalent. However, one does not need equal detection probability to compare movement rates among pairs of sites. We used a t test (assuming unequal variance) to compare the dispersal rates in the 3 pairs of sites separated by continuous dunes to the 6 pairs of sites separated by urban development.

Population Genetics

The goal of our genetic analyses was to determine whether there was significant population structure across the range of *Atrytonopsis* sp1 and, if so, to identify any barriers that might explain population structure. We sampled *Atrytonopsis* sp1 individuals at 8 locations throughout the extent of its range. Because of the rarity of the butterfly, we never collected more than 5% of a local population during a flight period. Results of preliminary field work showed that seasonal adult abundances at each sampling location were>100 (A.K.L., unpublished data). Sampled individuals were frozen at −80°C. We extracted DNA from the thorax with a Quiagen DN easy 96 Tissue Kit (QIAGEN, Valencia, California), following a modified mouse tail protocol (Sheck et al. 2006). We used amplified fragment length polymorphisms as genetic markers. Our amplification procedure followed the protocol of Sheck et al. (2006) and Vos et al. (1995). We used *Mse*1 and *Eco*RI as restriction enzymes. For selective amplification, we used 4 primer combinations (*Eco*R1+ AAC/*Mse*1 + CAT; *Eco*R1+ AAC/*Mse*1 + CCT; *Eco*R1+ AGC/*Mse*1 + CAC; and *Eco*R1+ AGC/*Mse*1 + CCT) and visualized the markers on Li-Cor 4200 and 4300 sequencers.

We examined the population structure with a Bayesian clustering algorithm (STRUCTURE 2.2, Pritchard et al. 2000). This method applies Markov Chain Monte Carlo (MCMC) and reveals k hidden populations within the data, estimates the probability that there are k populations, and calculates the proportion of each individual's genome that is associated with one of the k populations. We used the admixture and recessive alleles models (Falush et al. 2003, 2007). Using 1×10^4 MCMC cycles for burn-in and 5×10^4 MCMC cycles for data analyses (longer burn-in and run lengths produced highly similar parameter estimates), we tested k between $k = 1$ and $k = 6$ and selected the k with the lowest log probability averaged over 10 runs. We then mapped the population structure results relative to natural and anthropogenic barriers in the landscape.

Results

Behavioral Studies at Habitat Edges

We collected data from 194 butterflies at the 4 edge types (beach $n = 26$, urban $n = 89$, forest $n = 54$, ocean $n = 25$). Only the interaction of wind speed and the direction of the wind relative to the edge was significant in the final model ($\chi^2 = 9.02$, df = 3, $r^2 = 0.04$, $p = 0.03$). The interaction indicated that at higher wind speeds, butterflies were more likely to fly in the direction of the prevailing wind; thus, we excluded 28 releases made when winds were >11 km/h (Table 1). The intercept was significant in the full model (slope of 1.29 [SE 0.20], $p < 0.001$), which indicated that overall *Atrytonopsis* sp1 chose sand dune over any of the 4 matrix types. The strength of this choice among edge types did not vary because edge type did not emerge as a significant variable in the regression.

Table 1. ***Atrytonopsis*** **sp.1 choice of area type after release at the boundaries of dunes and 4 matrix (nonhabitat) types (wind speed at time of release was ≤11 km/h).**

Choice edge type	*n*	*Dune*	*Matrix*	*Percent dune*
Dune/beach	21	21	0	100
Dune/urban	77	56	21	73
Dune/forest	49	38	11	78
Beach (dune)/ocean	19	15	4	79

Mark-Recapture

The 15 sites were surveyed for approximately 80 h, during which time 535 butterflies were marked and 58 individuals were recaptured across all sites (Table 2). Although the proportion of marked individuals that moved through urban areas or continuous dunes did not differ significantly ($t_{[3]} = 1.859$, $p = 0.165$), the proportion of marked individuals that moved through continuous dunes was nearly 3 times higher than the proportion that moved through urban areas. Only one individual was recaptured more than 400 m (the distance between pairs of sites) from its release site.

Population Genetics

We collected 83 individuals from 8 locations (average 10.4/location) (Fig. 2). The 4 primer combinations produced 103 polymorphic loci. We found 3 genetically distinct regions within the range of *Atrytonopsis* sp1 ($k = 3$, posterior probability approximately 1) (Supporting Information). When we mapped the results relative to urban and natural features in the landscape, natural features, not urbanization, correlated most closely with the detected population structure (Fig. 2). The western break between populations was located at Bogue Inlet, which separates Bear Island from Bogue Banks. The eastern break occurred in an area of maritime forest, where severe erosion had contributed to the loss of all sand dunes. The data presented here are a subset of those in Leidner and Haddad (2010).

Table 2. ***Atrytonopsis*** **sp1 dispersal measured with mark-recapture in marking locations separated by either 400 m of continuous dunes or 400 m of urban area.**

Area type between marking locations	*Site*	*Survey time (minutes)*[a]	*Marked*[a]	*Recaptured*[a]	*Moved*	*Proportion moved*	*Average time between recaptures (days)*
Continuous sand dunes	1	909	187	3	1	0.33	3.00
	2	500	41	4	2	0.50	3.75
	3	883	72	8	1	0.13	3.75
					0.32 (0.19)[b]		
Urban	1	669	56	18	5	0.28	1.50
	2	548	28	5	0	0.00	0.80
	3	621	44	10	2	0.20	1.33
	4	635	66	13	0	0.00	1.36
	5	494	46	1	0	0.00	7.00
	6	685	82	9	1	0.11	2.22
					0.10 (0.12)[b]		

[a] *Values do not sum to values in the text because some sites were counted in surveys for 2 separate pairs.*
[b] *Average (SD).*

Discussion

Results from behavioral, mark-recapture, and genetic methods all led to the same general conclusion: natural features in the landscape, not urbanization, are barriers to *Atrytonopsis* sp1 dispersal (Table 3). Beaches, open water, and maritime forest restricted dispersal, and there was no difference between *Atrytonopsis* sp1 dispersal rates in urban areas and continuous dune areas. The results from the 3 approaches can be used in concert to explore in detail whether corridors and stepping stones may be effective and efficient for maintaining connectivity among populations.

Stepping stones only improve connectivity if a species enters the matrix. Only open beach was a strong dispersal barrier for *Atrytonopsis* sp1. Although individuals were more likely to move into dunes at all of the edge types than into any other type of area, between 21% and 27% of individuals flew over urban areas, maritime forest, and the ocean (Table 1). In isolation, these results suggest that stepping stones could be an effective conservation strategy because *Atrytonopsis* sp1's limited dispersal through urban areas is roughly equivalent to its limited dispersal through many of the natural barriers with which it evolved. One reason that so many butterflies moved into urban areas may be low structural contrast between sand dunes and urban areas. In contrast to beaches, which provide little shelter, urbanized areas have ground cover and structures for camouflage. Furthermore, roads are not dispersal barriers for many grassland butterflies (Ries & Debinski 2001; Severns 2008).

Knowing that *Atrytonopsis* sp1 will enter the matrix is insufficient to determine whether stepping stones may be an effective conservation strategy because emigrating individuals may never arrive in a different habitat patch. Our results from the mark-recapture study again suggest that stepping stones might be effective because individuals occasionally move 400 m through urban areas (Table 2). Although the difference in movement rates was not statistically significant, movement rates in continuous dune were higher. Still, butterflies dispersed across urban landscapes in numbers sufficient to maintain gene flow and colonize unoccupied habitat.

The recapture rate in marking locations surrounded by urban areas was notably higher than the recapture rate in locations surrounded by continuous dune habitat (Table 2). We believe this is a result of the general differences in population size between these 2 types of sites. For example, continuous site 1 (Table 2) had a low recapture rate (1.6%). This site is in a state park that has large tracts of intact sand dunes and an adult *Atrytonopsis* sp1 population size in the thousands. Consequently, we could catch only a small percentage of individuals in the population. The high recapture rate at many of the

Table 3. Summary of results for the 3 methods used to measure ***Atrytonopsis*** **sp1 dispersal into the 4 matrix (nonhabitat) types tested.**

		*Matrix type**			
Question: Will Atrytonopsis sp1	*Method*	*ocean*	*beach*	*forest*	*urban*
Enter matrix	behavior at edges	Y	N	Y	Y
Successfully disperse through matrix	mark-recapture				Y
Regularly disperse/reproduce	genetics (distances ≤5 km)	N			Y
	genetics (distances >5 km)			N	

**Key: yes (Y), matrix type favorable for* Atrytonopsis *sp1 dispersal; no (N), matrix type unfavorable; blank, unable to use the given method or to test the matrix-type combination given logistical or landscape constraints.*

urban sites reflects the small population sizes at these sites. Our standardization of survey effort across sites was imperfect, but our metric of movement (recaptures that moved divided by the total number of recaptures) was unaffected by this limitation.

The spatial arrangement of stepping stones is key to their effectiveness because there is usually a limited distance over which individuals can disperse. Mark-recapture studies could provide insight, but are logistically difficult and expensive to implement over large extents, and they cannot confirm that immigrants reproduce in their new population. The results of our genetic analyses showed that ocean and maritime forest were strong barriers to dispersal and were associated with genetically distinct populations, but that urbanization was not a barrier to dispersal (Fig. 2). There was some evidence of genetic exchange among the 3 regions, but the level was low compared with genetic exchange within each region.

A critical issue with studies of population genetics in urban environments is determining whether a lack of genetic structure indicates no effect of urbanization or that an effect has not yet developed. Here, we believe the absence of structure was due to a lack of effect of urbanization. Both the behavioral and mark-recapture studies were consistent with the results of our genetic analyses. Additionally, some urban areas have been present for over 70 years along eastern Bogue Banks and 30 years on the along the western side (140 and 60 generations of *Atrytonopsis* sp1, respectively; Pilkey et al. 1975).

Integration of Methods and Conservation Recommendations

In isolation the 3 methods we used would result in similar conclusions, but none by itself would provide compelling evidence that stepping stones may be an effective conservation strategy (Table 3). Unlike mark-recapture studies, the study of behavior at edges could not have determined whether individuals that move into urban areas eventually immigrate to a different habitat patch. The mark-recapture results also showed that the results of the edge-release study provided a realistic measure of the willingness of *Atrytonopsis* sp1 to enter various matrix types. This evaluation is important because the configuration of *Atrytonopsis* sp1 habitat was not conducive to mark-recapture studies in areas where patches of sand dune were isolated by ocean or maritime forest.

A difference between the edge behavior and population genetic results shows additional advantages of combining research approaches. The genetic results indicated that urban areas were more permeable than forest, yet the behavior of butterflies at edges showed that forest and urban areas were equally permeable (Table 3). We believe the distance (about 8 km) of maritime forest separating *Atrytonopsis* sp1 populations, within which there were no patches of sand dune, contributed to the genetic differentiation between eastern and western Bogue Banks. Although urbanization separates some sampling sites by similar distances, habitat for *Atrytonopsis* sp1 exists in the urban matrix and provides stepping stones. If development in the region were to intensify and remove *Atrytonopsis* sp1 habitat within urban areas, those areas could become dispersal barriers. No comparable arrangement of sand-dune habitat and maritime forest exists within the range of *Atrytonopsis* sp1, so we could not directly test with mark-recapture or genetic analyses whether small areas of forest and small urban areas similarly impeded dispersal. Only by drawing on results of multiple methods were we able to conclude that intensive urbanization has the potential to become a dispersal barrier.

Because *Atrytonopsis* sp1 enters and traverses urban areas and reproduces after dispersal, we recommend stepping stones be preserved or restored throughout the range of the species. Retaining small undeveloped areas, maintaining wide dune lines, and encouraging homeowners to use native dune plants for landscaping could help promote butterfly dispersal. Corridors may still allow *Atrytonopsis* sp1 to disperse, but given the logistical and financial constraints of establishing corridors in urban landscapes (Beier et al. 2008), we believe stepping stones offer a more realistic strategy.

Using multiple methods to understand dispersal is particularly important in landscapes where it is not feasible to conduct highly controlled, replicated experiments to measure effects of habitat fragmentation. We examined behavior of individuals at edges and used mark-recapture methods and genetic analyses, but other approaches to studying dispersal (e.g., fitting random walks, geographic-positioning-system tracking) could be combined in a similar fashion. By drawing on multiple research approaches, one can corroborate the conclusions of a single study, develop a more complete picture of the effects of fragmentation, and identify potentially effective conservation strategies.

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Supporting Information

STRUCTURE results for the average log probability of the data and posterior probability for each *k* are available online (Appendix S1). The authors are responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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