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RESEARCH ARTICLE

# Natural, not urban, barriers define population structure for a coastal endemic butterfly

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**Abstract** Habitat loss and fragmentation are the major causes of biodiversity loss, and, increasingly, habitat is fragmented by urbanization. Yet, the degree to which urbanization creates barriers to animal dispersal remains poorly understood. We used population genetic techniques to determine whether urbanization and/or natural landscape features are dispersal barriers to a butterfly, *Atrytonopsis* new species 1, throughout its range on coastal sand dunes that are increasingly threatened by development. Using AFLP markers that produced 89 polymorphic loci, we found significant population structure across the range of *Atrytonopsis* sp1. We found no indication that existing levels of urbanization were barriers to *Atrytonopsis* sp1 dispersal. Rather, two natural barriers, an ocean inlet and maritime forest, explained the genetic structure. Even in areas with long histories of urbanization, we found no significant isolation-by-distance relationship, and there was very low genetic differentiation between sampling locations. Consequently, conservation strategies for *Atrytonopsis* sp1, and potentially for other mobile insects that use open-structured habitats, should not focus explicitly on habitat corridors through urban areas, but rather should seek to preserve and restore as much habitat as possible across the butterfly's range.

**Keywords** Population genetics · Habitat fragmentation · Urbanization · Conservation · *Atrytonopsis* · Population structure

## Introduction

Among its many consequences, habitat fragmentation can negatively affect species by reducing their dispersal (Caughley 1994). Reduced dispersal can isolate populations, which in turn leads to an increased chance of local extirpation due to genetic drift, inbreeding, and susceptibility to natural disasters (Saunders et al. 1991; Caughley 1994; Hanski and Gilpin 1997; DeSalle and Amato 2004). One way that habitat is fragmented is by conversion of natural areas to urban development. We tested the effects of urbanization on the population genetic structure of a rare butterfly endemic to coastal North Carolina.

Habitat fragmentation is often studied in the context of converting land to semi-natural vegetation via agricultural activities and logging, but less often in the context of urbanization. However, as the world's population and the proportion of people living in urban areas have increased (UNDP 2006), so has the need for understanding the fragmentation that results from urbanization. Coastal areas have been particularly hard hit with urbanization; in the United States, 53% of people live in coastal counties, although coastal counties (excluding Alaska) only account for 17% of the land area (Crossett et al. 2004).

Urban fragmentation studies often investigate the effects of roads on dispersal. For many species, such as desert bighorn sheep, violet ground beetles, red-backed

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salamanders, bobcats, and coyotes, roads have been shown to depress movement rates (Keller and Largiadèr 2003; Epps et al. 2005; Riley et al. 2006; Marsh et al. 2008). More generally, some populations of species in urban areas have lower genetic diversity and are more isolated compared to populations in rural or natural areas (Caughley 1994; Hitchings and Beebee 1997; Wood and Pullin 2002; Takami et al. 2004; Desender et al. 2005; Noel et al. 2007; Vandergast et al. 2007).

Despite the seemingly impenetrable nature of urban areas, small natural areas in urbanized landscapes can harbor many species, in particular insects, depending on the size of the natural area and its proximity to other natural areas (Faeth and Kane 1978; Bolger et al. 2000; Connor et al. 2002; Koh and Sodhi 2004; Dunn 2005). Consequently, the dispersal rates of some insects may be only minimally affected by urbanization. For beetles and butterflies, comparative studies indicate that the dispersal of habitat specialists and sedentary species are more affected by urbanization than vagile species that are habitat generalists (Takami et al. 2004; Desender et al. 2005). Even insect pollinated plants may be relatively unaffected by urbanization (Culley et al. 2007). Understanding if urbanization limits dispersal, and the degree to which it does limit dispersal, can be used to help guide the type and location of urban development so as to limit urbanization's effects on species.

Detecting whether habitat fragmentation yields long-term consequences for populations is commonly addressed with genetic data. Population genetic techniques are increasingly used to assess the effects of both natural and human-caused fragmentation (DeSalle and Amato 2004; Holderegger and Wagner 2006; Bonin et al. 2007; Keyghobadi 2007). The benefit of genetic approaches is that they permit range-wide assessments of dispersal in cases where traditional mark-recapture or radio-telemetry techniques are difficult or costly to implement. Such techniques can also be used to detect the potential erosion of genetic diversity that may result from habitat fragmentation. Moreover, while studies of animal movement at potential urban barriers are useful in understanding short-term behavioral responses to fragmentation, genetic responses can indicate if individuals survive the dispersal process and contribute to gene flow between populations. As a first step, population genetic studies elucidate whether a species occurs as distinct genetic populations across a landscape, or whether there is a single panmictic population. If there is structure, the way in which it is partitioned can explain what landscape features—natural or anthropogenic—should be regarded as barriers to dispersal, which would ultimately result in reduced gene flow (Manel et al. 2003).

Here, we present a study of the population genetic structure of a newly identified and globally rare butterfly whose habitat is rapidly being converted to residential and commercial development. *Atrytonopsis* new species 1, the crystal skipper, is endemic to a 50-km stretch of barrier islands in North Carolina (Fig. 1). It primarily occupies sand dune habitat that is ~25–100 m wide. Historically, sand dune habitat was continuous within islands, and only punctured by areas of severe erosion or ocean inlets. However, over the last 70 years sand dunes within the range of *Atrytonopsis* new species 1 have largely been converted to residential and commercial development. The first goal of our study was to use population genetic data to determine whether urbanization and/or natural features are barriers to *Atrytonopsis* new species 1 dispersal. The second goal was to use this information to identify conservation strategies that will increase the long-term persistence of the butterfly.

## Methods

### Study system and species

*Atrytonopsis* new species 1 (hereafter *Atrytonopsis* sp1) is a butterfly in the skipper family (Hesperiidae). Primary *Atrytonopsis* sp1 habitat consists of sand dunes with the larval hostplant *Schizachyrium littorale* (seaside little bluestem). *S. littorale* is a dune grass that forms the dominant vegetation cover on open sand dunes within the known range of the butterfly. *Atrytonopsis* sp1 is a federal species of concern and may become a candidate for listing under the Endangered Species Act, as its total range is less than 3,300 ha. Although *Atrytonopsis* sp1 is globally rare, it is locally abundant, with population sizes in the hundreds to thousands. This butterfly has two generations a year (Hall 2004; Leidner and Haddad 2006). The taxonomic status of the species is not yet resolved, but current genetic, morphological, and ecological research indicates that it is a distinct species (J. Burns, personal communication).

Barrier islands are characterized by ~25–100 m wide linear vegetation bands that run parallel to the ocean (Ehrenfeld 1990). Above the high-tide line is open beach, followed by primary and secondary sand dunes, followed by shrub thickets. Behind the shrub thickets is maritime forest, followed by marsh, followed by the open sound. Unlike oceanic islands, the vegetation bands on barrier islands do not form rings around the island.

*Atrytonopsis* sp1 is restricted to two barrier islands in North Carolina—Bogue Banks and Bear Island (Hall 2004; Leidner and Haddad 2006). The butterfly is also found on several small human-made dredge spoil islands. The two sites that support the largest numbers of *Atrytonopsis* sp1 are located in state parks at either end of the species' range. The western-most site is on Bear Island, a ~5 km long undeveloped barrier island. The eastern-most site is at the

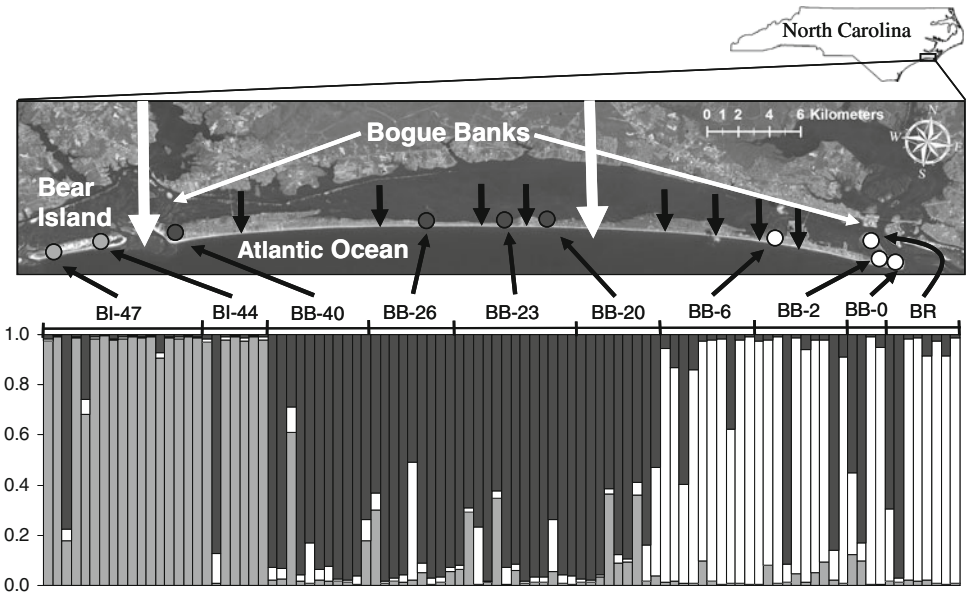


**Fig. 1** Map of the known range of *Atrytonopsis* sp1 in North Carolina. The *filled circles* indicate sampling locations for the population genetic analysis. Sampling locations are *shaded* based on the results from STRUCTURE, which indicates that three populations ($k = 3$) is the best fit for the data. The lower part of the figure represents the individual population assignments from STRUCTURE. Each individual is represented by a column and arranged along a west-east axis. The proportion of each individual's genome that is assigned to the three populations is represented by the shading (*dark gray*, *light gray*, *white*). The *white arrows* show the location of natural barriers and the *black arrows* indicate areas with heavy urban development

eastern end of Bogue Banks, within Ft. Macon State Park. Other areas with *Atrytonopsis* sp1 are smaller and often surrounded by urban development (e.g., housing complexes, hotels, strip malls, parking lots), which can separate patches of sand dune habitat by many kilometers. There is a long history of urbanization on Bogue Banks. Development on the eastern end began more than 70 years ago and development on the western end is at least 30 years old (Pilkey et al. 1975). Within *Atrytonopsis* sp1's range, there is one section of maritime forest where severe erosion (possibly caused by a combination of storms and urbanization) has eliminated the primary and secondary dunes to the point where there is an abrupt transition from the maritime forest to the beach. There is virtually no *S. littorale* present in this area.

## Collection procedure

We sampled individuals from ten locations throughout the range of *Atrytonopsis* sp1 (Fig. 1). Sampling locations are labeled by a two-letter code according to the island and, with the exception of Brandt Island, are also numbered according to their distance in kilometers along an east–west axis (east = 0). Nine locations occurred in natural habitat on Bear Island (BI) and Bogue Banks (BB) and one location was on Brandt Island (BR), a dredge spoil island. The two areas with the largest number of *Atrytonopsis* sp1 are also the only areas where we could establish sampling locations separated by only continuous sand dune habitat. One set of locations, BB-0/BB-2, occurs within a state park that has a ~2 km stretch of sand dunes. The other set of locations, BI-44/BI-47, occurs within a state park that has a ~5 km stretch of sand dunes (Fig. 1). The remainder of the sampling locations had fewer *Atrytonopsis* sp1, thus limiting the number of individuals we could collect. Sampling was spread out over 2 or 3 flight periods to reduce the impact at a given location and, when possible, we sampled older individuals that would have less reproductive impact on small populations. Butterflies were captured using hand nets and brought live to a −80°C freezer, where they were stored until DNA extraction.

## DNA extraction and AFLP protocol

There is no published genetic research on any species in the *Atrytonopsis* genus, and microsatellites are notoriously difficulty to develop in Lepidoptera (Zhang 2004; but see Keyghobadi et al. 1999). Therefore, we used amplified fragment length polymorphisms (AFLP) as genetic markers (Vos et al. 1995), which require little development time (Mueller and Wolfenbarger 1999; Lowe et al. 2004).

DNA was extracted from half of each thorax using a Qiagen DNeasy 96 Tissue Kit, following a modified mouse tail protocol as described in Sheck et al. (2006), with the exception that we incubated tissue in 170 µl of lysis buffer and 30 µl of proteinase K. We also followed the methods

outlined in Sheck et al. (2006) for the AFLP procedure, which was in turn modified from Vos et al. (1995) and Remington et al. (1999). However, for pre-amplification we used 3 µl of DNA and for selective amplification we used 24 µl of dNTP. We used four primer combinations for selective amplification (*Eco*RI + AAC/*Mse*1 + CAT, *Eco*RI + AAC/*Mse*1 + CCT, *Eco*RI + AGC/*Mse*1 + CAC, and *Eco*RI + AGC/*Mse*1 + CCT) and visualized the markers on Li-Cor 4200 and 4300 sequencers. Each gel had a standard (Li-Cor STR marker, 50–700 bp) loaded at either end. Gels were scored using Quantar 1.08 (KeyGene Products), a semiautomatic image analysis program.

To ensure scoring accuracy of markers for individuals run on different gels, we ran duplicates of 2–11 individuals for each primer combination (AGC/CAC = 2 individuals/26 markers, AGC/CTT = 11 individuals/35 markers, AAC/CCT = 5 individuals/31 markers, AAC/CAT = 5 individuals/17 markers). We calculated the error rate of each marker by dividing the number of individuals that did not match by the total number of individuals that could be compared. We then eliminated markers that had an error rate greater than 10%. The final error rate averaged over all markers was 1.7% (standard deviation = 3.2%). We repeated the analyses using a dataset that included markers with higher error rates and found nearly identical results. Only markers with a frequency between 5 and 95% were retained in the final analyses.

## Genetic diversity

We estimated allele frequencies for each sampling location in AFLP-Surv v1.0 using a Bayesian approach with non-uniform priors that is suitable for dominant markers (Zhivotovsky 1999; Vekemans et al. 2002). Using these values, we calculated expected heterozygosity ($H_j$) for each location, following the method of Lynch and Milligan (1994), and pairwise $F_{ST}$ for all locations.

## Population structure

We used a Bayesian clustering method implemented in Structure v2.3.1 (Pritchard et al. 2000) to test for population structure across the range of *Atrytonopsis* sp1. Structure groups individuals based on their genotype and determines the most likely number of populations ($k$) given the data. The $k$ with the highest ln probability (ln Pr(X|$k$)) is considered to be the best fit. For a given dataset, we averaged the values of ln Pr(X|$k$) over 10 runs. We did not assign individuals to populations prior to analyzing the data (i.e., we used naïve clustering). Each run of the dataset for each $k$ used $1 \times 10^4$ Markov Chain Monte Carlo (MCMC) cycles for burnin and $5 \times 10^4$ MCMC cycles for data analysis. These burnin and run lengths provided consistent parameter estimates and were almost identical to estimates derived from longer burnin and run lengths. We analyzed data using correlated allele frequency and admixture models, which helps to account for migration and shared ancestry between putative populations (Falush et al. 2003), and the recessive alleles option designed specifically for AFLP data (Falush et al. 2007). Recently, Hubisz et al. (2009) developed a new extension of the Pritchard et al. (2000) and Falush et al. (2007) algorithms that can better detect weak population structure, especially in cases with limited data. Their method takes into account the location from which individuals were sampled and was not shown to detect artificial population structure. Although we rely on the traditional method for detecting population structure, we present the results of the new algorithm in the supplemental text.

We also looked at the partitioning of genetic diversity using Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992) implemented in Arlequin v3.11 (Excoffier et al. 2005). AMOVA is similar in concept to ANOVA, and can be used to compare the proportion of genetic diversity accounted for by each level of a hierarchical analysis (within sampling locations, among locations/within regions, and among regions). Because we had missing data at some loci, we used a locus-by-locus AMOVA (Excoffier et al. 2005). We tested for statistical significance using 10,000 permutations of the data. Input files for Arlequin were prepared using AFLPDat (Ehrich 2006).

## Isolation-by-distance

We used Mantel tests to examine the correlation between pairwise $F_{ST}$, the existence of potential barriers (coded as 1 if the barrier was present and 0 if it was not), and the distance between sampling locations (isolation-by-distance; Mantel 1967; Smouse et al. 1986). Although more complex approaches have been developed to look at gradients of barriers (e.g., developing least cost surfaces), we found no difference in responses to gradients in urbanization (Leidner and Haddad, in review). Furthermore, the variation within each landscape type—including urban areas—is far smaller than the variation between landscape types. We measured geographic distance following the shoreline, rather than straight-line distance, although the difference between these two measurements was negligible. We then used partial Mantel tests to examine whether adding potential barriers to dispersal (i.e., urban areas or natural barriers) explained additional variation beyond the effects of distance. All Mantel tests were conducted in Fstat v.2.9.3.2 with 20,000 randomizations to test for statistical significance (Goudet 1995). Most sampling

locations were separated by some urbanization, so we used a Mantel test to look at the isolation-by-distance relationship for sites only separated by urbanization, but not natural barriers. Finally, we used ANOVA with a post-hoc Tukey test to examine the average pairwise $F_{ST}$ values for sampling locations separated by various combinations of urban and natural barriers, and continuous habitat. We excluded Brandt Island (BR) from the isolation-by-distance analyses because it is situated north of the main east–west axis of the barrier islands, making it inconsistent to measure distances between sampling locations. Additionally, it was the only sampling location on human-made habitat (dredge spoil) and may therefore be affected by unique genetic circumstances (e.g., founder effects).

## Results

In total, we sampled 98 individual butterflies from ten locations (Table 1). Across four primer combinations, we scored markers for 89 polymorphic loci. The results from all of the population genetic analyses point to the same conclusion—that ocean inlets isolate *Atrytonopsis* sp1, and urbanization does not limit butterfly dispersal.

### Genetic diversity

Overall, there was little difference in genetic diversity among sampling locations (range 0.274–0.416, Table 1). However, genetic diversity of the western-most, isolated sampling location (BI-44 and BI-47 combined) appears to be lower than the other locations.

### Population structure

The population structure analysis shows that the data are best fit by a model that assumes three populations ($k = 3$) across the range of *Atrytonopsis* sp1 (Table 2, Fig. 1). The finding that there were three populations was robust to whether we used the traditional algorithm or the Hubisz et al. (2009) algorithm that takes into account sampling location (Table 2, Fig S1). The three populations are geographically distinct, and their boundaries are associated with two natural barriers in the landscape. The western barrier, an inlet, has separated the two main barrier islands in *Atrytonopsis* sp1's range since at least 1585 (Fisher 1962). The eastern barrier corresponds to a stretch of beach with no sand dunes (see "Methods"). Despite the population structure, there was evidence of migrants, or recent descendants of migrants, in all three regions (Fig. 1).

To ensure that there was no additional structure within each of the three genetically distinct regions, we analyzed the population structure of each region individually. The eastern and central region had no significant population structure ($k = 1$, Table S1, Figs. S2–S3). Although the western region did have significant population structure ($k = 2$), when we visually examined the results of $k = 2$ for that region, there was no indication of population structure correlated with geographic distance or urbanization (Table S1, Fig. S4).

We used AMOVA to examine the partitioning of genetic variance based on natural and urban barriers (Table 3). For the natural barriers, we used the three regions identified in the population structure analysis (Fig. 1, Table 2). For the urban barriers, we assigned sampling locations to six

**Table 1** Sample sizes (*n*), average number of individuals scored per loci (avg *n*), percent polymorphic loci at the 5% level (%P), and expected heterozygosity (Hj) with standard error (SE) for each sampling location (and subsites within)

| ID | Sampling location | *n* | avg *n* | %P | Hj (SE) | Region (natural barrier) | Region (urban barrier) |
|---|---|---|---|---|---|---|---|
| BR | Brandt Island | 8 | 7 | 89.9 | 0.375 (0.016) | Eastern | 1 |
| BB-0 | Ft. Macon (east) | 4 | 3 | 100.0 | 0.416 (0.015) | Eastern | 1 |
| BB-2 | Ft. Macon (west) | 10 | 9 | 97.8 | 0.409 (0.014) | Eastern | 1 |
| | *Ft. Macon (combined)* | 14 | 13 | 97.8 | 0.404 (0.014) | | |
| BB-6 | Atlantic Beach | 10 | 9 | 87.6 | 0.351 (0.016) | Eastern | 2 |
| BB-20 | Salter Path | 9 | 8 | 92.1 | 0.385 (0.016) | Central | 3 |
| BB-23 | Indian Beach | 13 | 12 | 91.0 | 0.360 (0.017) | Central | 4 |
| BB-26 | Emerald Isle east | 9 | 8 | 88.8 | 0.364 (0.017) | Central | 5 |
| BB-40 | Emerald Isle west | 11 | 10 | 92.1 | 0.366 (0.016) | Central | 6 |
| BI-44 | Bear Island (east) | 7 | 6 | 83.5 | 0.329 (0.018) | Western | 6 |
| BI-47 | Bear Island (west) | 17 | 16 | 68.5 | 0.274 (0.021) | Western | 6 |
| | *Bear Island (combined)* | 24 | 23 | 74.2 | 0.279 (0.020) | | |

Sample sizes (*n*) do not match the average number of individuals scored (avg *n*) because of missing data. Populations grouped by natural and urban barriers for the AMOVA (see Table 3) are indicated by the last two columns. Combined data for Ft. Macon and Bear Island are presented at the bottom of the table

**Table 2** Results from STRUCTURE showing that three populations ($k = 3$) is the best fit across the range of *Atrytonopsis* sp1

| $k$ | Average (SE) of ln Pr(X\|k) | |
|---|---|---|
| | Without group information | With group information |
| 1 | −6182.24 (0.90) | −6183.18 (0.97) |
| 2 | −5841.20 (1.74) | −5827.00 (13.27) |
| 3 | −5728.99 (1.90) | −5741.86 (4.36) |
| 4 | −5867.69 (12.10) | −5874.80 (18.39) |
| 5 | −6060.55 (17.36) | −6071.12 (43.50) |

The values for each $k$, with standard error (SE), were averaged across 10 runs

regions (Table 1). Better models explain more variance among regions, while minimizing variance within regions. Although a significant amount of variation was explained by region in both the natural and urban barriers models, more variance was accounted for using natural barriers relative to urban barriers (13.9 vs. 4.4%, Table 3). Using natural barriers, sampling location within a region explained very little variance (1.4%) and was not significant ($P = 0.085$), whereas, when using urban barriers, sampling location within a region explained more variance (8.1%) and was significant ($P < 0.001$).

### Isolation-by-distance

Full Mantel tests revealed a significant correlation between pairwise $F_{ST}$ and distance across the range of *Atrytonopsis* sp 1 (Table 4, dashed line in Fig. 2). There was also a significant correlation between pairwise $F_{ST}$ and the presence of an ocean barrier, but no correlation between pairwise $F_{ST}$ and the presence of urban or forest barriers (Table 4). For sites only separated by urban areas, but not natural barriers ($n = 8$, a subset of the data in Table 4), we found no significant isolation-by-distance relationship (slope = 0.001, $r = 0.411$, $P = 0.316$, $r^2 = 0.169$).

Given the role of distance in explaining pairwise $F_{ST}$, we used partial Mantel tests to look at the combined effect of distance and potential barriers (Table 4). After controlling for the effect of distance, ocean was still significant in explaining pairwise $F_{ST}$, but neither forest nor urban areas were significant. Similarly, after controlling for the effect of ocean, distance was still significant in explaining pairwise $F_{ST}$, but urban areas and forest were not significant.

**Table 4** Full and partial Mantel tests for pairwise $F_{ST}$ between sites on Bogue Banks and Bear Island (excluding Brandt Island)

| | $r$ | $P$ | $r^2$ |
|---|---|---|---|
| Mantel tests | | | |
| Distance | 0.639 | <0.001 | 0.409 |
| Ocean | 0.833 | <0.001 | 0.693 |
| Forest | 0.255 | 0.133 | 0.065 |
| Urban | 0.061 | 0.734 | 0.004 |
| Partial mantel tests | | | |
| After controlling for distance | | | |
| Ocean | 0.833 | <0.001 | 0.787 |
| Forest | −0.255 | 0.135 | 0.470 |
| Urban | −0.061 | 0.727 | 0.477 |
| After controlling for ocean | | | |
| Distance | 0.639 | <0.001 | 0.787 |
| Forest | 0.255 | 0.135 | 0.817 |
| Urban | 0.061 | 0.729 | 0.710 |

For the partial Mantel tests, the model $r^2$ is provided. In total, there were 36 pairwise comparisons

There were significant differences in average pairwise $F_{ST}$ among sites separated by different combinations of natural (ocean or forest) and urban barriers (ANOVA, $F_{[5,30]} = 27.710$, $P < 0.001$, $r^2 = 0.822$, Fig. 3). Most notably, sites separated by only urban areas had a much lower average pairwise $F_{ST}$ than sites separated by ocean (Fig. 3). When we tested the same model using distance as a covariate, distance was not significant ($P = 0.746$).

## Discussion

Our results demonstrate that natural features in the landscape, including ocean inlets and maritime forest, are

**Table 3** Locus-by-locus AMOVA results for *Atrytonopsis* sp1

| Source of variation | df[a] | Sum of squares | Variance | Percent of variance | $P$ |
|---|---|---|---|---|---|
| Natural barriers | | | | | |
| Among regions | 2 | 168.268 | 2.252 | 13.894 | < 0.001 |
| Among locations within regions | 7 | 109.786 | 0.225 | 1.387 | 0.085 |
| Within sampling locations | 82 | 1127.332 | 13.732 | 84.719 | < 0.001 |
| Urban barriers | | | | | |
| Among regions | 5 | 180.267 | 0.691 | 4.404 | 0.006 |
| Among locations within regions | 4 | 97.787 | 1.273 | 8.109 | < 0.001 |
| Within sampling locations | 82 | 1127.332 | 13.732 | 87.487 | < 0.001 |

[a] Degrees of freedom averaged across all loci

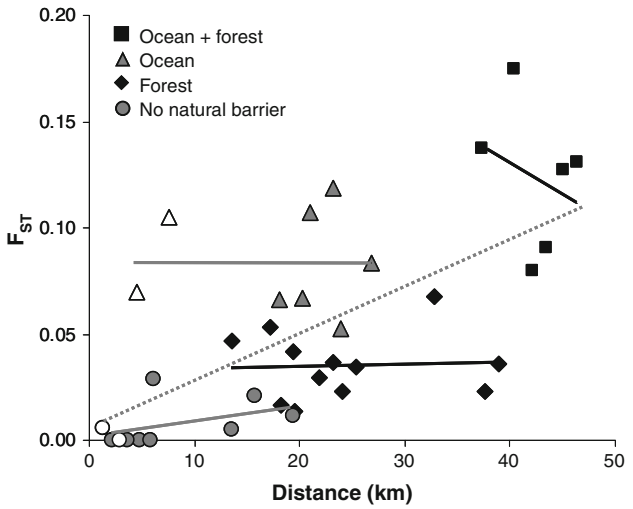


**Fig. 2** Isolation-by-distance relationship for all sites on natural habitat (i.e., excluding Brandt Island). *Gray circles* are $F_{ST}$ values for sampling locations separated by no natural barriers. *Black diamonds* and gray triangles are sampling locations separated by one natural barrier (forest and ocean, respectively). *Black squares* are sampling locations separated by both forest and ocean. All sites are separated by urban development, except for the two *open circles* (BB-0/BB-2 and BI-44/BI-47) and the two *open triangles* (BB-40/BI-44 and BB-40/BI-47). Trend *lines* are shown for all four groupings (*solid lines*) and for the overall dataset (*dashed line*)

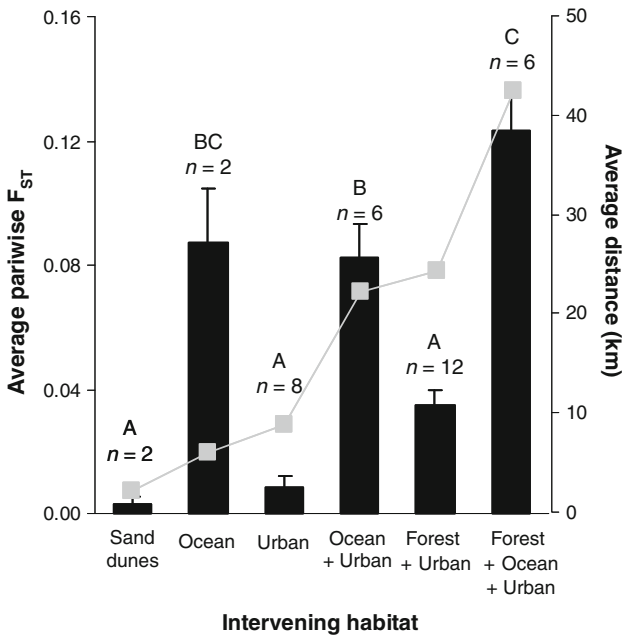


**Fig. 3** Average pairwise $F_{ST}$ (*left y-axis*) for sites separated only by sand dunes (BB-0/BB-2 and BI-44/BI-47), one type of potential barrier, or combinations of potential barriers. *Error bars* represent standard error. Means were compared using a Tukey post-hoc test. *Gray squares* (*right y-axis*) show the average pairwise distance between sites. Pairwise $F_{ST}$ values are arranged by increasing average distance between sites

barriers to *Atrytonopsis* sp1 dispersal, whereas urbanization (at least in its current intensity and extent) is not. Both STRUCTURE and AMOVA show that we can reject a null hypothesis of panmixia among *Atrytonopsis* sp1 populations and STRUCTURE indicates that there are three genetically distinct groupings of *Atrytonopsis* sp1. The partial and full Mantel tests also reveal that ocean accounts for genetic differentiation, whereas urbanization does not. Furthermore, there is no isolation-by-distance relationship in sites separated by only urban areas.

*Atrytonopsis* sp1 appears to be a mobile butterfly, and several lines of additional evidence support the finding that current urbanization is not a barrier to its dispersal. This is important because a main concern about use of genetic studies to test the effects of human-caused fragmentation is whether enough time has elapsed to detect effects on genetic structure (Keyghobadi 2007). As part of our effort to determine the number of individuals we could sample from a given location, we conducted several small mark-recapture studies. Although we did not intend to look at dispersal, we found that individually marked butterflies regularly moved hundreds of meters to kilometers through urban and natural areas over short time spans (1–3 days). As part of another study, we examined *Atrytonopsis* sp1 behavior at the edge of sand dune habitat and urban development, and found that individuals regularly flew into urban areas (Leidner and Haddad, in review).

The lack of an isolation-by-distance relationship among sites separated by urban areas might be generated in two ways: (1) there is so much dispersal of *Atrytonopsis* sp1 between populations that there is no genetic differentiation, or (2) there is so little dispersal between populations that drift dominates over migration (*sensu* Keyghobadi et al. 2005). A comparison of the genetic differentiation between sites (Figs. 2, 3) and genetic diversity within sites (Table 1) can help differentiate between these possibilities. High levels of isolation between *Atrytonopsis* sp1 populations would result in relatively high pairwise $F_{ST}$ between sites and possibly low genetic diversity values within sites. However, the average pairwise $F_{ST}$ of sites separated by only urban areas is low, and similar to the average pairwise $F_{ST}$ of sites separated by only continuous habitat. Sites separated by any kind of natural barrier (forest and/or ocean) had significantly higher pairwise $F_{ST}$. Our isolation-by-distance analysis was admittedly limited by both the paucity and proximity of sampling locations separated by only sand dune habitat (open circles, Fig. 2). However, if there had been a strong, natural isolation-by-distance relationship prior to human disturbance, we would have likely detected this signal in the sites currently fragmented by urbanization (closed circles, Fig. 2). Combined, these results support the hypothesis that high movement rates of

*Atrytonopsis* sp1 result in no isolation-by-distance relationship among locations separated by urbanization.

If anything, the negative correlation between urban barriers and $F_{ST}$ we observed suggest that urban fragmentation may increase movement rates of *Atrytonopsis* sp1 over short distances, leading to a total breakdown of isolation-by-distance in the absence of natural barriers (Table 4). Butterflies, and other species, have been theorized (Zollner and Lima 1999; Fahrig 2003) and observed (Schtickzelle et al. 2007; Kuefler et al. 2010) to exhibit persistent, directional movement in non-habitat areas, relative to more "wandering" movement within habitat. Consequently, once *Atrytonopsis* sp1 enters an urban area, it may move further and faster than it would within continuous habitat.

*Why is current urbanization not a barrier to* Atrytonopsis *sp1 dispersal*? Urbanization on barrier islands often levels sand dunes and replaces them with open, flat areas, such as managed lawns and parking lots, which are virtual oceans for butterflies. These environments provide little protection from predators or shelter from harsh weather conditions. Yet urbanization is not always this drastic, as residential development can leave remnant habitat for *Atrytonopsis* sp1. For example, ornamental flowers and shrubs provide nectar resources and cover from predators and bad weather. Unlandscaped yards and undeveloped lots retain native vegetation cover, including host plants. In this respect, urban development is far more hospitable to butterflies than open beaches and ocean.

*Why are natural features barriers to* Atrytonopsis *sp1 dispersal?* Ocean is likely a barrier to *Atrytonopsis* sp1 because of strong selection pressure for the butterfly to avoid flying over it. A butterfly flying over the ocean or sound would almost never find suitable habitat and would face higher wind speeds compared to flying over land, which could limit successful dispersal events. Forest might be a barrier to dispersal because of its contrasting structure with *Atrytonopsis* sp1's open habitat. Studies of other butterflies that use open habitat (e.g., meadows) have found that forest was a barrier to dispersal (Haddad 1999; Keyghobadi et al. 1999, 2005; Ries and Debinski 2001).

Forest appears to be a weaker barrier to *Atrytonopsis* sp1 dispersal relative to ocean. The average pairwise $F_{ST}$ between *Atrytonopsis* sp1 populations separated by forest and urbanization is lower than the average pairwise $F_{ST}$ between populations separated by ocean (Fig. 3). This difference is particularly notable given that the distance of the ocean barrier is much shorter than the distance of the forest barrier. One reason that forest may be less of a barrier is that the mortality risk of flying over forest may be relatively low, as there are likely some nectar resources and high probability of finding habitat. In fact, over short distances forest may not be a strong barrier to dispersal; rather, the barrier could result from the long distance over which there is no suitable *Atrytonopsis* sp1 habitat.

Although ocean and forest are barriers to dispersal, in that they generate genetically distinct regions within the range of *Atrytonopsis* sp1, they are not complete barriers to dispersal. As indicated in the results from the population structure analysis (Fig. 1), there is evidence that individuals move among the three regions.

## Conservation implications for insects in urbanizing landscapes

The fate of *Atrytonopsis* sp1 in an increasingly urbanized landscape relies on both the maintenance of populations in large natural areas and on the ability of butterflies to disperse between habitat patches. If large stretches of habitat are destroyed, *Atrytonopsis* sp1 populations are likely to be isolated, increasing extinction threats. In combination with natural demographic crashes experienced by insects, this threat is increased by the susceptibility of barrier islands to hurricanes. However, the current intensity and pattern of development within the range of *Atrytonopsis* sp1 does not isolate populations. The small patches of sand dunes, interspersed between large natural areas, help maintain connectivity, suggesting that *Atrytonopsis* sp1 does not need explicit structural corridors to maintain movement. Instead, smaller natural areas, undeveloped lots, and houses with unlandscaped yards, can all support small butterfly populations and maintain connections by serving as stepping stones. Consequently, instead of concentrating explicitly on corridors, we recommend the preservation and restoration of as much habitat as possible, and encouraging homeowners to retain native vegetation as landscaping.

Small natural areas within an urban landscape may serve a similar function as remnant forest fragments in agricultural landscapes. Indeed, one study showed that the effects of fragmentation resulting from urbanization on insects were no different than fragmentation resulting from agriculture (Rickman and Connor 2003). In agricultural landscapes, research on "countryside biogeography" provides compelling evidence that forest fragments can retain species diversity for several taxa, and even benefit surrounding agricultural areas via ecosystem services such as pollination (Daily et al. 2001; Ricketts et al. 2001; Daily et al. 2003; Horner-Devine et al. 2003). Similarly, "urban biogeography" could be applied to small natural or semi-natural areas embedded in an urban landscape, particularly for species that use open-structured habitat. A common theme among many studies of insects in urban areas, like ours, is that resource availability (i.e. nectar and host plants) is an important determinate of species richness and abundance (Wood and Pullin 2002; Koh and Sodhi 2004; McFrederick and LeBuhn 2006).

The good news for *Atrytonopsis* sp1, and perhaps other mobile insects in urban landscapes, is that small natural areas (<1 ha) could play an important role in promoting dispersal, and thus gene flow, in fragmented landscapes. If urbanization becomes extremely intense, with more dense urbanization or more non-native landscaping, the connectivity of *Atrytonopsis* sp1 across its range may severely diminish, placing the species at greater risk of extinction. However, these small but important natural areas for insects are often overlooked in conservation because they may provide little benefit to the larger, more charismatic vertebrates for which conservation efforts are often focused (Dunn 2005). Especially for mobile insects in urban landscapes, small-scale conservation efforts may go a long way to conserving insect diversity and to promoting local awareness of conservation issues. Ultimately, conservation situations similar to *Atrytonopsis* sp1 provide excellent examples of how conservation and development can be reconciled.

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