Assessing genetic variation among *Bombus Impatiens* (Hymenoptera: Apidae) in two boroughs of New York City using mitochondrial DNA

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Abstract

Due to a decline in pollinators worldwide, urban habitats have been suggested as potential conservation areas for generalist pollinators, such as bumblebees. Bumblebees have been shown to have an increased abundance in urban areas compared to surrounding natural habitats, but little is known about the genetic variation of bumblebees in urban habitats. To investigate the genetic variation of *Bombus impatiens* in New York City, sequences of the mitochondrial gene Cytochrome b (Cytb) were analyzed from bees sampled from four locations in the Bronx and Harlem. The aligned Cytb gene sequences indicate that there is some genetic variation in these populations. However, further analysis is required to clearly determine the genetic structure of *B. impatiens* populations in New York City.

Introduction

Currently, there is great concern over pollinator declines worldwide (Kearns *et al.* 1998), mainly due to declines in populations of *Apis mellifera* (honeybee), the major pollinator in agricultural systems (Kremen *et al.* 2004). A loss of honeybees coupled with reported declines of cultivated (Kremen *et al.* 2004) and native bees (Allen-Wardell *et al.* 1998) have led some to refer to the issue as a 'pollination crisis' (Kearns *et al.* 1998). Due to the predicted loss of pollination services, conservation of pollinators has become a high priority.

Bombus species (bumblebees) are important targets for conservation because many wild flowers and commercial crops depend on them for pollination (Matheson *et al.* 1996). Urban habitats have been suggested as potentially important for conservation of bumblebees because of the increased diversity of flowering species in gardens and parks due to the addition of horticultural varieties and exotic species (Thompson 2003). Recent assessments of bee diversity

in urban systems (McIntyre and Hostetler 2001, Frankie *et al.* 2005, Cane *et al.* 2006) suggest that certain generalist species, such as bumblebees, have a greater abundance in urban areas, compared to surrounding natural habitats. Despite a possible abundance of bumblebees in urban habitats, little is known about the genetic variation of urban bees.

This study investigates the genetic diversity of *Bombus impatiens* worker bees sampled from four locations in New York City by sequencing a fragment of the mitochondrially encoded Cytochrome b gene. This approach was chosen because most species exhibit high levels of diversity in mtDNA (Avise 1986), and intraspecific variation is expected in populations that are geographically separated. The objective of this study was to determine whether bees sampled in two boroughs of New York City were of the same population, or if there is a sufficient distance between the populations such that gene flow is prevented.

Materials and methods

Collection of specimens

Bees for this study were generously provided by Kevin C. Matteson. The bees were netted by hand in August and September of 2005, and pinned shortly thereafter. Eight dried specimens of *Bombus impatiens* workers were selected from the insect collection maintained at the Louis Calder Biological Field Station of Fordham University in Armonk, NY. These specimens were chosen to represent four community gardens located in Bronx, NY and Harlem, NY. Four specimens were sampled in two locations in Bronx, NY: Garden of Happiness (GH) and Drew Garden (DR), which are separated by a distance of approximately 1km. In addition, four samples were taken from two locations in Harlem, NY: Peaceful Valley (PV) and Papo's

Garden (PA), which are separated by approximately 0.5km. The distance between the Bronx and Harlem gardens is approximately 8km.

DNA isolation, PCR, PCR purification, and sequencing

To successfully extract DNA, only the head of each specimen was used. After removing the head with forceps, the specimens were briefly frozen in liquid nitrogen and ground in individual 1.5ml Eppendorf tubes. The DNA extraction was performed using the DNeasy Animal Blood and Tissue Kit (Qiagen), following the manufacturer's protocol for tissue extraction. To avoid clogging the spin column to be used, the lysate was allowed to settle for one minute before loading the sample.

Fragments of the mitochondrial gene Cytochrome b (Cytb) were amplified using polymerase chain reactions (PCR). PCR reactions were performed in a final volume of 25µL. The reaction mixtures contained 2ng of DNA, 12.5µL of Promega GoTaq Green Master Mix, 0.5µL of forward primer (10 pmol) and 0.5µL of reverse primer (10 pmol) and dH_20. The set of primers (forward and reverse) that corresponded to Cytb were expected to produce a 434bp product. These primers were designed using mtDNA sequences of *Apis mellifera* (Crozier and Crozier 1993). PCR amplifications were performed with an initial period of denaturation at 94°C for 5 minutes, and then the following conditions for 50 cycles: 94°C, 45s denaturation; 50°C, 45s annealing; 72°C, 90s extension. This was followed by an additional extension period of 7 minutes at 72°C. The PCR products were then maintained at 4°C until they were run on a 1.5% agarose gel and visualized. PCR products of Cytb, which showed clear bands of the expected size, were purified using the Rapid PCR Purification Protocol (Marligen).

PCR-purified products were sequenced by Genewiz, Inc. (South Plainfield, NJ). To confirm that the obtained sequences were indeed of the genes of interest, BlastN was used to screen the NCBI database GenBank for highly similar sequences. Alignments of confirmed sequences from each specimen were conducted using ClustalW (EMBL-EBI, 2007).

Results

DNA was successfully extracted for six of the eight worker bees used in this study. Primers designed using a sequence from the Cytb gene of *Apis mellifera*, produced a band of the expected size (approximately 450bp) for five of the six specimens analyzed (Fig.1).

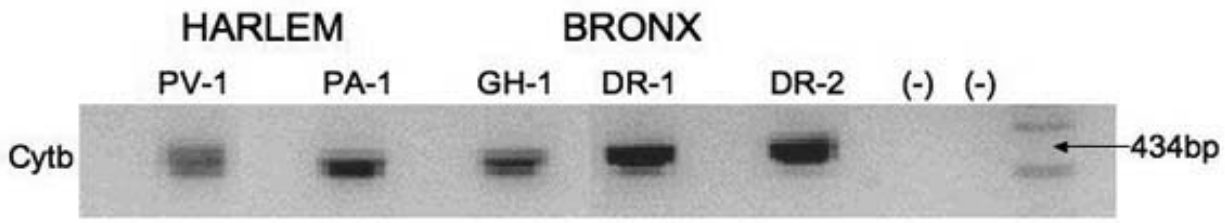

Figure 1. Successfully extracted bee mtDNA was amplified using PCR. Here PCR products of the mitochondrial gene Cytochrome b are visualized.

The primers, designed to amplify a 434bp fragment based on *Apis mellifera* sequence data, had the following sequences:
Forward: 5'-TACTACCATGAGGACAAATATC-3'
Reverse: 5'-ATTACACCTCCTAATTTATTAG-3'

Five bees sampled from four locations in New York City were analyzed. Locations of bees are shown above gel. (PV: Peaceful Valley, PA:Papo's Garden, GH: Garden of Happiness, DR: Drew Garden.)

Sequence data from the bands of the size corresponding to the Cytb gene were confirmed to be Cytb using a BlastN search. These five sequences yielded highly similar sequences to a partial coding sequence (CDS) of 488bp of *Bombus impatiens* (Accession no. AF281169). The sequences of the five specimens sampled in gardens in New York City were aligned using

ClustalW. The alignment showed nearly exact nucleotide match, with the exception of two base changes in the sequence of one specimen from Garden of Happiness in the Bronx (Fig. 2). The alignment also showed a single base change in one specimen collected from Papo's Garden in Harlem (Fig. 2).

Figure 2. Sequences (360 bp) from the Cytochrome b gene for five *B. impatiens* specimens were obtained from purified PCR products and aligned using ClustalW.

Bees were sampled from two gardens in the Bronx, Drew Garden (DR) and Garden of Happiness (GH), and two gardens in Harlem, Peaceful Valley (PV) and Papo's Garden (PA).

Discussion

Partial sequences of a mitochondrial gene of *Bombus impatiens* specimens were analyzed to determine the genetic heterogeneity of bees sampled in the Bronx and Manhattan, two boroughs of New York City. The minimal heterogeneity shown in the alignment of the Cytochrome b sequences indicates that there is some genetic variation in these populations. However, further analysis is required to clearly determine the genetic structure of *B. impatiens* populations in New York City.

The findings of this study indicate that there is or has been extensive gene flow among bees across New York City, implying that workers fly far and may mix across sites (Chapman *et al*. 2003). Two studies of *Bombus terrestris* have shown that workers fly an average distance of 663m and 275m from their nests to forage for pollen (Osborne et al. 1999; Walther-Hellwig and Frankl 2000). Based on the distances reported in those studies, *B. impatiens* may be expected to cover the distance between gardens within the Bronx and Harlem (1.5km and 0.5km, respectively), but not between the two boroughs (approximately 8km). In fact, in a mark-recapture study, *B. impatiens* individuals were consistently recaptured in the same gardens, while no bees were recaptured in a garden different from the original site (Matteson, personal communication). This suggests that most B. impatiens are not flying long distances, and thus would not likely contribute to gene flow across the sampling locations in New York City. Further genetic analysis of this species must be conducted to more clearly understand the genetic structure of *B. impatiens* in New York City.

The mitochondrial Cytb gene fragments sequenced in this study may not be the most appropriate method for assessing genetic heterogeneity across such a small geographic area. Pirounakis *et al.* demonstrated that sequences of the Cytb gene could successfully reveal

geographic subdivision in *Bombus pascuorum* (1998). However, the geographic range of the bee populations assessed in their study is much larger than the range of this study. Another study of the population structure of *Bombus Terrestris* on two islands off of the coast of Africa utilized sequences of the Cytb gene to demonstrate genetic heterogeneity (Widmer et al. 1998). However, in a study assessing the genetic structure of Asian populations of *Bombus ignitus*, Shao et al. found that microsatellite markers revealed high sequence heterogeneity, while the Cytb gene of the mitochondrial DNA showed no genetic heterogeneity (2004). In future studies, microsatellite markers may provide more information about the genetic structure of *Bombus impatiens* in New York City.

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