Assessing the genetic relationships between mayfly (*Baetis*) populations

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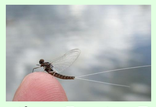
Introduction

Populations experience different accumulations of random mutations if they are physically separated and inter-population breeding is prevented (by physical, geographical, weather, etc. barriers). The accumulation of random mutations/change in gene frequency over time in a population is called genetic drift. Two populations that are prevented from interbreeding experience independent genetic drift and, given enough time, can evolve into separate species or at least genetically distinct sub-species (Honnay, et. al, 2005). This process happens frequently to island species because of their obvious physical isolation in breeding. A contributing factor is that, usually, the founding population of an island is quite small, a phenomenon called founder effect.

In the prairie of the central United States, islands are hard to find. However, ecological habitats such as small streams or watersheds can function as ecological islands – islands of habitat in a sea of land (Allan, 1995; Barbour and Brown, 1974; Brown, 1971; Brönmark, et. al, 1970; Sepkoski and Rex, 1974; Vuilleumier, 1973).

Our ongoing studies are examining the behavioral and genetic differences between mayflies on Santa Cruz Island and mayflies of the nearby California mainland. Although mayflies "can" fly, they are weak fliers and only fly for a few days at the end of their 3-6 month life cycle. Their scientific name "Ephemeroptera" refers to the ephemeral nature of their winged stage. In addition, their immature stages are confined to freshwater.

Immature Mayfly 

Adult Mayfly 

Therefore the 20 miles of water forming the Santa Barbara Channel separating the mayflies inhabiting mainland streams and mayflies inhabiting island streams is a significant barrier to population interbreeding (Figure 1).

Because mayflies are extremely sensitive to poor water quality they are classified as a bioindicator species (Menetrey, et. al, 2008). As clear water streams become increasingly rare in Iowa, mayfly populations may be experiencing genetic isolation similar to mayflies on the California coast, and thus, are undergoing genetic drift as they are confined to "islands" of good habitat. If the Iowa mayfly populations are genetically isolated we would expect to see greater genetic differences between mayflies inhabiting waters of different watersheds compared to mayflies inhabiting streams within a single watershed.

In order to measure genetic drift, we are analyzing the DNA sequences of mtCOI (mitochondrial cytochrome C oxidase I) from mayflies collected from various sites. mtCOI has been effectively used to distinguish closely related species (Ball et. al, 2005). We are attempting to select Iowa sites that mimic the 20-mile barrier between Santa Cruz Island and the nearby mainland by selecting sites within a watershed separated by ~20 miles of agricultural land and sites between watersheds also separated by ~20 miles of agricultural land.

Materials and Methods

Mayfly nymphs were collected, then preserved in 70% ethanol at -20ºC. DNA was isolated from mayfly nymphs using ZR Insect/Tissue DNA Kit-5 (Zymo Research Corp., Orange, CA).

Amplification of a 650 bp fragment of cytochrome C oxidase I (COI) from the mitochondrial genome was performed using PCR with primer pairs LC01490 and HC02198 (Hughes, et. al, 2003) 0.75µl of 20µM, 22µl nuclease free water, 5 µl 25µM $MgCl_2$, 10µl 5x Go Taq buffer, 0.5µl 10mM dNTPs,, 1 µl Go Taq (Flexi) Polymerase (Promega, Madison, WI), and 10 µl template DNA. PCR products were visualized on ethidium bromide-stained 1% agarose gels, photographed and purified from gels using Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI).

Gel purified DNA fragments were cloned into pGEM-T Easy (Promega, Madison , WI) and transformed into *E. coli*. Transformants were selected based on growth in the presence of ampicillin and white (vs. blue) colony color. Selected white, ampicillin resistant colonies were picked, grown and plasmid was isolated using Zippy Plasmid Miniprep Kit (Zymo Research, Orange , CA). Cloned fragments were liberated by cutting with BstZ1 and analyzed on 1% agarose gels.

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Abstract

As Midwestern prairie habitats were largely converted into an agricultural monoculture, their lotic systems were degraded through sedimentation and eutrophication. These changes created ecological islands—remnants of suitable habitat in large areas of degraded habitat. Populations restricted to these isolated patches of habitat can accumulate random genetic changes leading to genetic drift.

Baetis mayflies may be particularly sensitive to such habitat degradation and isolation. They spend the majority of their life cycle as aquatic nymphs with narrow habitat requirements. Their adult dispersal capabilities are low, as they are weak fliers and this life stage is relatively short (few days). Therefore, we hypothesize that habitat loss and fragmentation in Iowa has created ecological islands of mayfly habitat. We believe these isolated groups of mayflies are experiencing genetic drift that can be detected by molecular genetics analysis. We are amplifying fragments of the mitochondrial cytochrome oxidase I (mtCOI) gene from mayflies collected within and between four different Northwest Iowa watersheds. We are cloning the fragments into pGEM-T vectors so that the mayfly mtCOI gene fragments can be sequenced. We predict that mayflies within a watershed will exhibit fewer genetic differences than mayflies in different watersheds.

*contributed equally to the work presented

Figure 1. Locations of collections in California. Mainland collection sites indicated by **BROWN** spots. Island collection sites indicated by **PURPLE** spots.

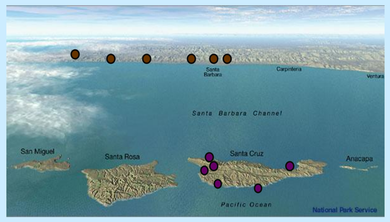

Figure 2. Locations of mayfly collection sites in Northwest Iowa,. Sites represent the Big Sioux, Little Sioux, Rock and Floyd Rivers.

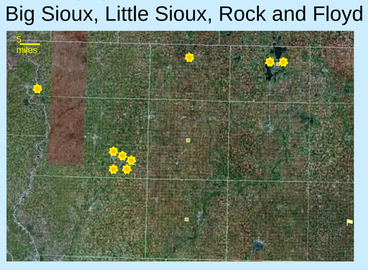

Figure 3. Mayfly collection.

Future work

We have plans to collect mayflies from additional tributaries of the Northwest Iowa Rivers in this study throughout the summer of 2011. We will isolate DNA and amplify COI fragments from these mayflies and from additional, stored, mayflies collected in 2008 - 2010. We are working on cloning the amplified fragments after gel purification and hope to have fragments in sequencing vectors ready to send for sequence analysis during the summer of 2011.

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Results

Mayflies were collected from six Santa Cruz Island streams and six California mainland streams in July 2008 (Figure 1). Mayflies were collected from five Northwest Iowa river sites representing the Big Sioux, Little Sioux, Rock and Floyd Rivers 2009-2010 (Figures 2 and 3). Mayflies were also collected from the James River, French Creek and Battle Creek (South Dakota).

High molecular weight mayfly DNA was successfully isolated from approximately 150 whole organisms, legs, or mayflies in which the gut was removed (Figure 4).

A 650-bp fragment of the mtCOI gene was successfully amplified from the isolated DNA by PCR using the primer pairs LCOI490 (5'-ggtcaacaaatcataaagatattgg-3') and HCO2198 (5'-taaacttcagggtgaccaaaaaatca-3') (Figure 5).

PCR products were purified from gels and cloned into pGEM-T easy vector. (Figure 6A). Ligation products were transformed into *E.coli*. (Figure 6A and B). Representative ampicillin-resistant, white colonies were selected, grown and the presence of an insert of the expected size was confirmed by liberating the cloned fragment by digestion with BstZ1. (Figure 7).

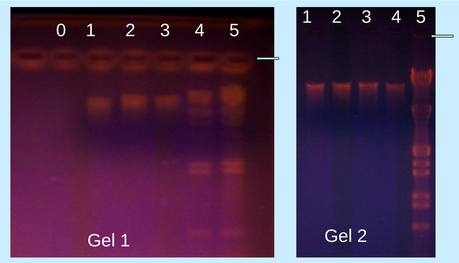

Figure 4. High molecular weight DNA from mayflies. Gel 1: DNA isolated from mayflies collected from Little Sioux watershed Gull Point (lanes 1-2); Spillway (lane 3). Lanes 4 and 5 represent DNA molecular weight markers. Lane 0 is no DNA control. Gel 2: DNA isolated from mayflies collected from the Floyd River (lanes 1-4). Lane 5 represents DNA molecular weight markers. Arrows indicate position of wells.

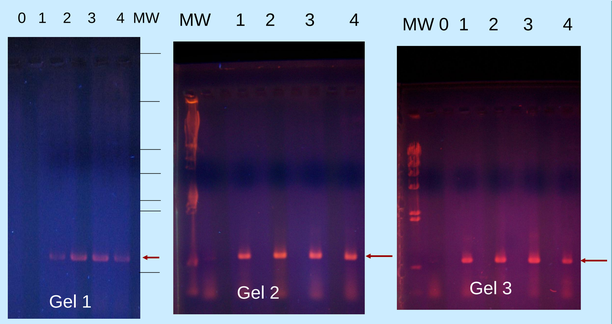

Figure 5. A 650 bp fragment of the mtCOI gene is amplified from mayfly DNA by PCR using the primer pair LCOI490 and HCO2198 (red arrows). Gel 1: lanes 1-4 are PCR products amplified from DNA isolated from 4 mayflies collected from Santa Cruz Island. Gel 2: Lanes 1-4 are mayflies collected from the Floyd River. Gel 3: PCR products from 4 mayflies collected from the Big Sioux River. No DNA controls are indicated by 0. Molecular weight markers are indicated by MW.

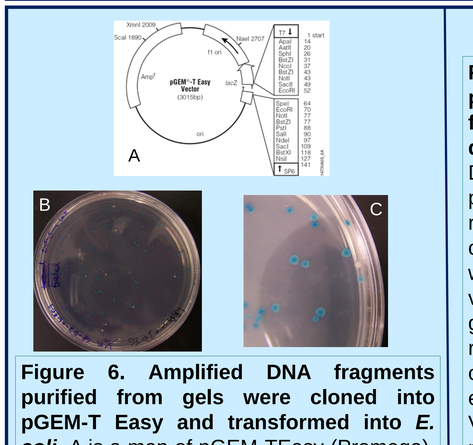

Figure 6. Amplified DNA fragments purified from gels were cloned into pGEM-T Easy and transformed into *E. coli*. A is a map of pGEM-TEasy (Promega). B and C are representative plates of transformed E. coli after incubation.

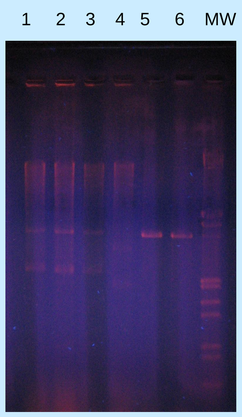

Figure 7. Cloned PCR products were liberated from vector DNA by digestion with BstZ1. DNA mini preps were performed on ampicillin-resistant, white bacterial colonies and then digested with BstZ1. DNA was visualized on 1% agarose gels. Lanes 1-3: recombinant plasmids containing an insert of the expected size. Lanes 5-6: Vector only. MW indicates molecular weight markers.

Discussion

We have initiated a project in which we propose to study genetic drift in mayflies separated by a ~20 mile water barrier and mayflies separated by ~20 mile agricultural barrier.

We have successfully collected over 250 mayfly nymphs from 12 California sites and 9 Iowa sites. We are able to consistently isolate amplifiable DNA from our samples and have done this with nearly 150 mayflies. We have designed a protocol in which we are able to consistently amplify the desired fragment from the COI gene and have done so for nearly 70 of these samples. We have begun to work on cloning the amplified DNA into pGEM-TEasy and have successfully accomplished this for a handful of samples. We are anxious to expand the number of cloned mayfly DNA fragments so that we can begin sequence analysis