A DNA Barcode Approach to Ascertain the Foraging Patterns of Native Bees

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Abstract

With increased use of insecticides, climate change, and the difficulty in maintaining domestic honeybee hives due to Colony Collapse Disorder, supporting the native bee population has become of critical importance. This study sought to determine whether DNA analysis of single pollen grains can be effectively used to determine the floral preferences of various pollinators. If targeted sequences of DNA from pollen grains selected randomly from native bees can be amplified, sequenced, and identified, it can provide information that could be used to support the native bee population through companion planting. Pollen grains were harvested from three Lasioglossum and stained with dye in petri dishes. 15 pollen grains, individually sampled from each petri dish, were photographed and categorized into broad morphological groups, then placed into PCR tubes. Gel electrophoresis showed that of the 45 pollen grains sampled, an average of 48.89% were successfully amplified per bee following first stage PCR, 60.49% of these were successfully amplified after second stage PCR, and 38.89% of samples sent for sequencing were successfully identified. Sequenced pollen grains were identified as Betula Kenaica, Pisum Sativum, and Veronica Arvensis. These three successful identifications support that single pollen grain DNA amplification is a possible method for identifying flora native bees forage on. However, the high failure rate increases costs and time required to use the methodology, making it essential to increase the success rate before the method can be effectively applied on a larger scale.

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Introduction and Hypothesis

As a keystone species, native bees are an essential part of our ecosystem, and help pollinate many food crops. However, with increased use of insecticides, climate change, and the destruction of natural habitat, the bee population has been declining, presenting a danger to the ecosystem. Promoting the native bee population also has an increased importance now that domestic honeybees, which have traditionally been used for pollinating food crops, are subject to Colony Collapse Disorder (Jacobson, Tucker, Mathiasson, & Rehan, 2018). Lasioglossum are a very common genus of native bee in Massachusetts and are an important local pollinator due to their high levels of pollinator effectiveness (Ballantyne, Baldock, Rendell, & Willmer, 2017). They are also known to pollinate essential local crops, such as commercial cranberry bogs (Hicks & Sircom, 2016). However, as with most native bees, research regarding this genus remains very limited. Identifying what native bees such as Lasioglossum prefer to pollinate could have an important effect on reducing the decline in the bee population, as well as improving crop yields through companion planting.

Due to the high importance of promoting the native bee population, the relatively low cost of single pollen grain DNA amplification, and the method's potential to quantify foraging preference, the present project aims to determine whether single pollen grain DNA amplification can be effectively utilized to identity what local flora Lasgioglossum forage on, and to begin composing a regionally tuned list of plants that will support the native bee population. It is hypothesized that if pollen grains can be isolated from Lasioglossum bees collected at a site with an abundant Lasioglossum population, then DNA barcoding can be used to identify the plants attracting the Lasgioglossum. Because native bees coevolve with native plants, identified flora will have a higher likelihood of supporting the native bee population than flowers in standard "Save the Bees" seed mixes, which are often sold nationally and lack regional specificity.

Background Research

Pollen identification techniques using light microscopy require a high level of specialized expertise and are severely limited by the lack of distinct morphological features across plant species in many plant groups (Rahl, 2008). As a result, there has been a turn in favor of amplifying pollen DNA for sequencing, typically through traditional Sanger sequencing or using more recent developments in high throughput sequencing (Bell, De Vere, Keller, Richardson, Gous, Burgess, & Brose, 2016). While high throughput sequencing allows for stronger amplification of mixed DNA species, it has drawbacks in assessing foraging preference. Due to the small size of pollen grains and high amplification rate of high throughput sequencing, both minor traces of contamination and pollen grains that only made up a small portion of the bee's total pollen load will be amplified, which can produce misleading results. (Bell et al., 2016). While risk of contamination is high for sanger sequencing as well, the method allows for better analysis of proportionality between pollen types, allowing probability to be used to determine a bee's preference, assuming pollen grains are randomly sampled. While next generation sequencing can produce pollinator networks by matching bees to plant species identified through amplification, it cannot quantify preference among these plant species, assess if different pollen species amplify at different rates, or determine which reads resulted from pollen that made up a very minor portion of a bee's total load. (Bell, Fowler, Burgess, Dobbs, Gruenwald, Lawley, Brose, 2017).

For sanger sequencing to be applied for pollen amplification, individual pollen grains must first be isolated from the mixture of pollen species typically found within a bee's pollen load (Matsuki et al. 2007; Aziz & Sauve 2007). While single pollen samples from known sources have been successfully amplified (Petersen, Johansen, & Seberg, 1996), this approach has not been utilized to assess foraging patterns of native bees using randomly samples individual pollen grains. This method has additional benefits in assessing floral preferences of bees such as Lasioglossum, which do not have pollen baskets and therefore typically don't carry as large pollen loads (Gibbs, 2011).

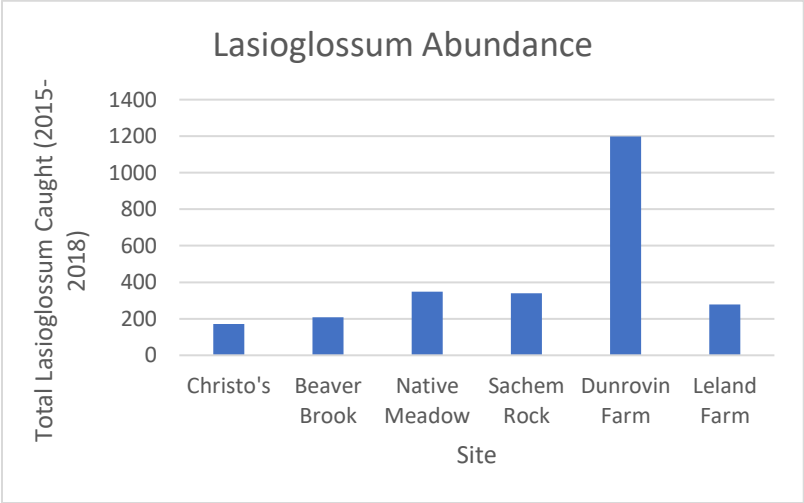
If single pollen grains' DNA are successfully amplified, the DNA can be sequenced, and a reference library can be used to match the sequencing data to a plant species, such as the BLAST database (Mcginnis & Madden, 2004).

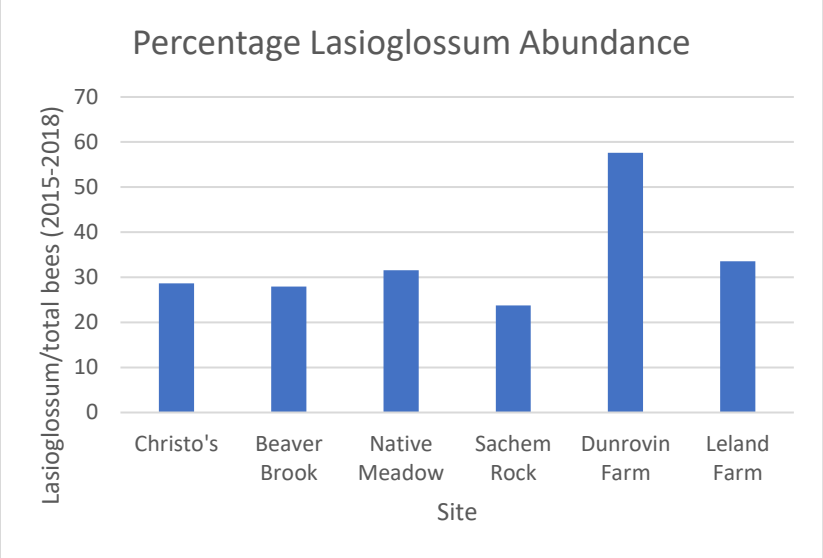
Risks

This experiment will involve collecting bees in the field, which carries inherent safety concerns, such as encountering poisonous flora, ticks, or being stung by a bee. As a safety precaution, field work should never be completed alone. Wearing long pants and bug spray during field work is also essential to repel harmful species. Skin should be checked for rashes and/or bug bites following field work.

Site Selection

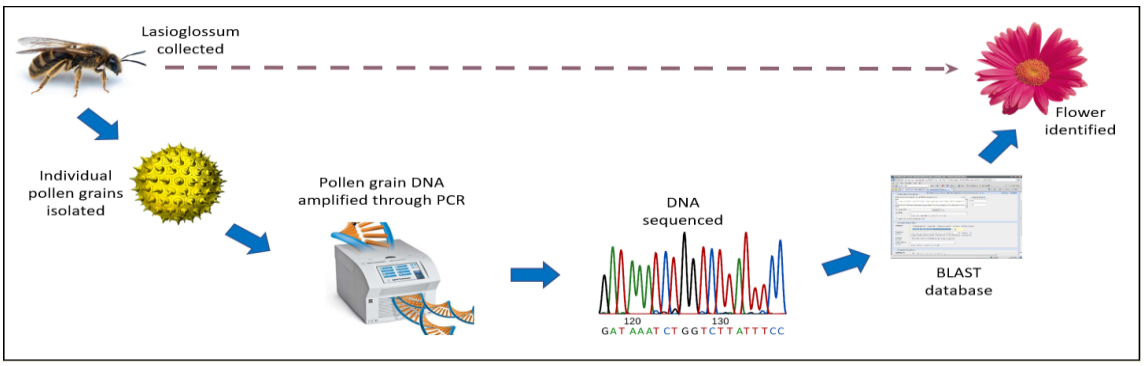
Dunrovin Farm had both the highest Lasioglossum abundance and the highest ratio of Lasioglossum to total bees out of 6 sites in Southeastern MA, making it an ideal site for this project. Because it is shown that Dunrovin Farm is already known to have a high Lasioglossum population, the current project can be used to identify plants that may be attracting Lasioglossum to the site, increasing the likelihood that identified flora will positively impact the native bee population. Information regarding Lasioglossum abundance at these 6 sites between 2015 and 2018 was taken from Massasoit Community College's database.





Experimental Methods and Procedures

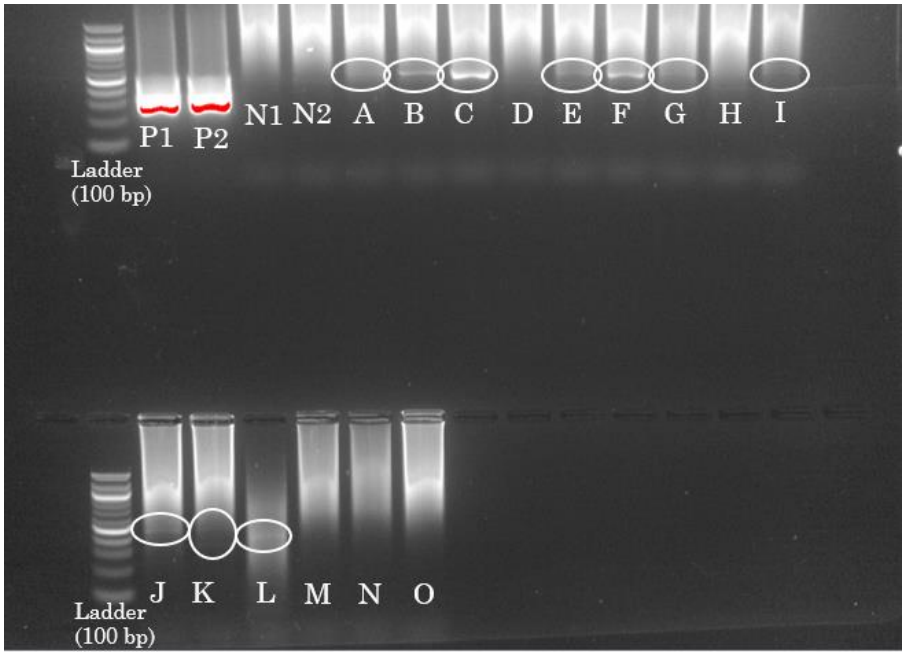
Bees were collected at Dunrovin Farm, Halifax, MA on August 28th, 2018. Lasioglossum were caught via aerial sweep netting and placed into ethyl acetate jars. Genus was confirmed using a dissecting microscope. To remove the Lasioglossums' pollen, each bee was placed into a 1.5 mL tube, submerged in 200 µL of nuclease free water, and vortexed at maximum speed for one minute. The bees were then removed from the tube, and the remaining water was centrifuged at 15000 rpm for two minutes. For each bee, 10 µL of the centrifuged water were pipetted from the bottom of the tube onto a small petri dish along with 10 µL of safranin dye. A pipette tip was then used to spread out the solution, and the petri dish was left to dry. The samples created were examined using an inverted compound microscope, allowing for free manual manipulation of the petri dish. 15 pollen grains were individually picked up from each petri dish using a size 1 pin and suspended in PCR tubes with 5 µL of water. To avoid bias in pollen grain selection, the petri dish was moved about randomly until a pollen grain came into view before it was selected. If more than one pollen grain came into the field of vision at the same time, both were sampled. Each pollen grain was photographed (using an iPhone X camera), labeled (A-O with respect to each bee), and categorized as tetrad or round based on morphological structure before being placed into a PCR tube. If 15 pollen grains could not be found on the petri dish, a second sample was prepared using another 10 µL of the remaining centrifuged water and 10 µL of safranin dye. PCR was completed for 15 pollen grains at a time, with separate reactions for each bee. Each amplification set used ITS2 and psbA-trnH primer at concentrations of .5 µM, contained two negative controls, and contained two positive controls. Negative controls contained the same ingredients as the experimental tubes but did not have a DNA source. Positive controls also had the same composition except they contained purified genomic pea DNA as the template. Cocktails were vortexed, then pipetted into their respective PCR tubes to create a 25 µL final volume. After each PCR tube was prepared, samples were loaded into a thermocycler to undergo PCR. Amplifications were performed for 40 cycles, at an annealing temperature of 62°C, and an extension time of 20 seconds. PCR results were visualized using 1% agarose gel and the BioRad Gel Doc EZ Imager software. A second round of PCR amplifications were conducted for samples with bands, using 2 µL of the multiplex product as the DNA source, to confirm and separate the products by primer. Products from the second round of PCR were again visualized using 1% agarose gel and the BioRad Gel Doc EZ Imager software. Samples which displayed bands were purified and sequenced. Sequencing results were analyzed using the Basic Local Alignment Search Tool (BLASTn) database to identify the plant species each pollen grain originated from. The PCR success rate throughout the experiment was calculated and compared across pollen morphological groups to assess amplification bias.



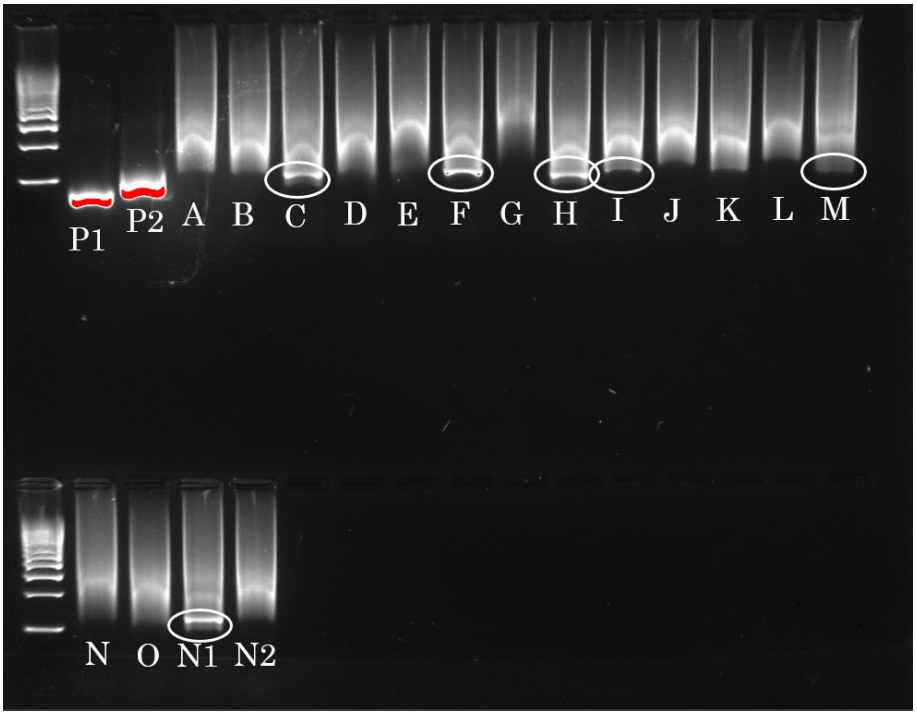
Overview of procedure

Data

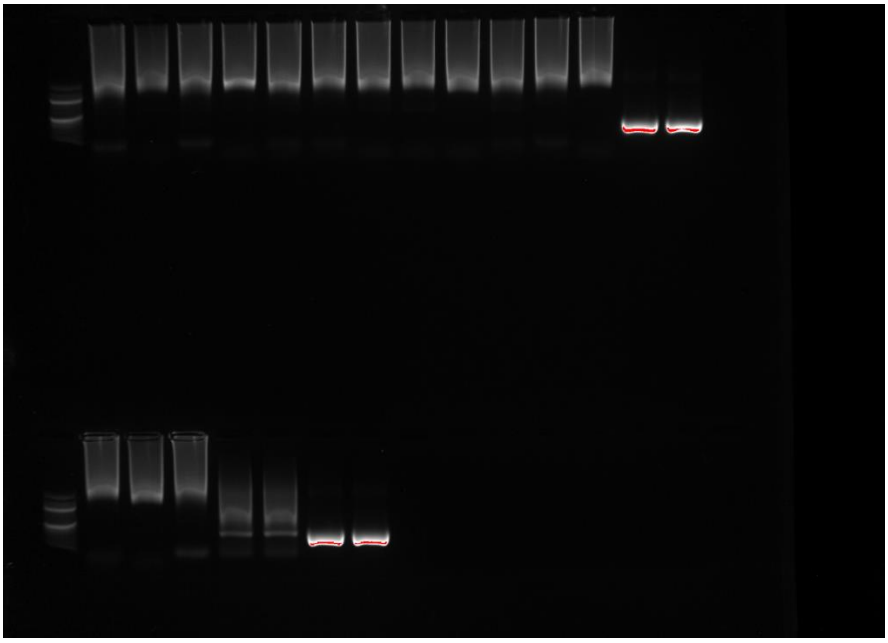
(Gel images are shown in order they were created)



First Stage PCR: Bee S2, Pollen grains A-O



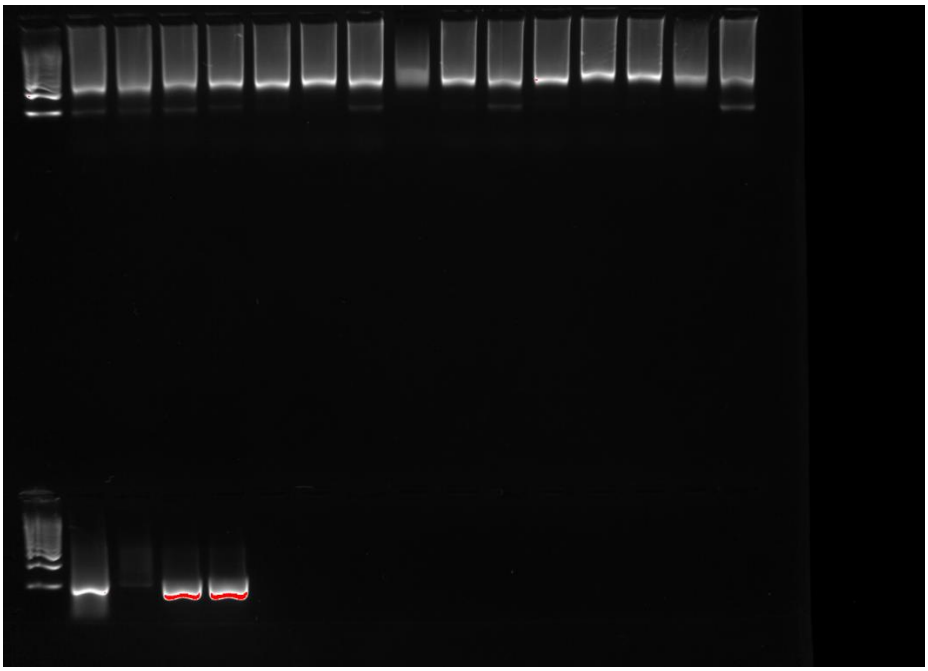
First Stage PCR: Bee S1, Pollen grains A-O



Second Stage PCR: Samples with bands from S2 and S1 first stage, psbA only

Top row, left to right: Ladder, 2A, 2B, 2C, 2E, 2F, 2G, 2I, 2J, 2, 2L, 2C, 1F, P1, P2

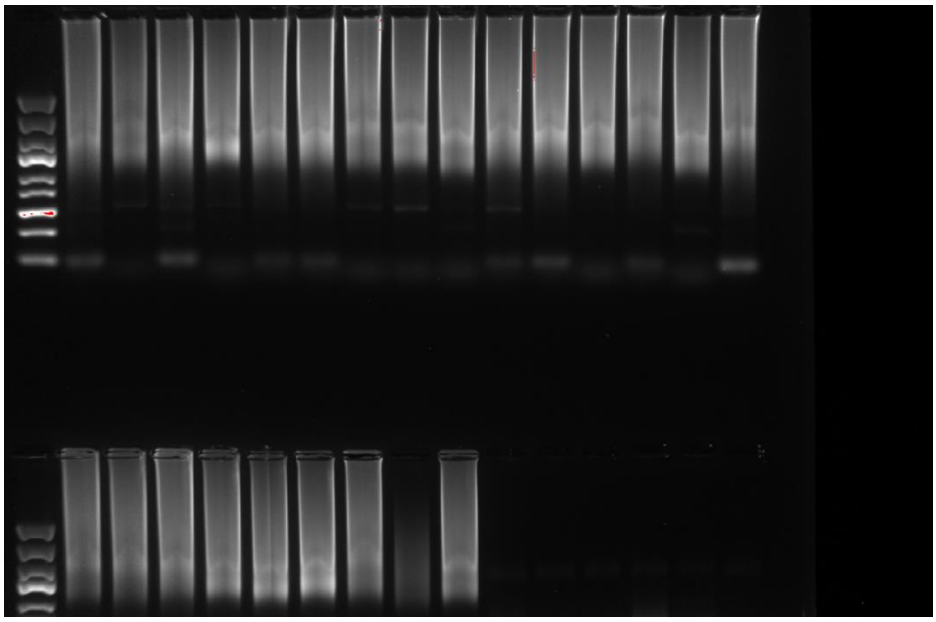
Bottom row, left to right: IH, 1I, 1M, N1, N2, P1, P2



Second Stage PCR: Samples with bands from S2 and S1 first stage, ITS2 only

Top row, left to right: Ladder, 2A, 2B, 2C, 2E, 2F, 2G, 2I, 2J, 2, 2L, 2C, 1F, 1H, 1I, 1M

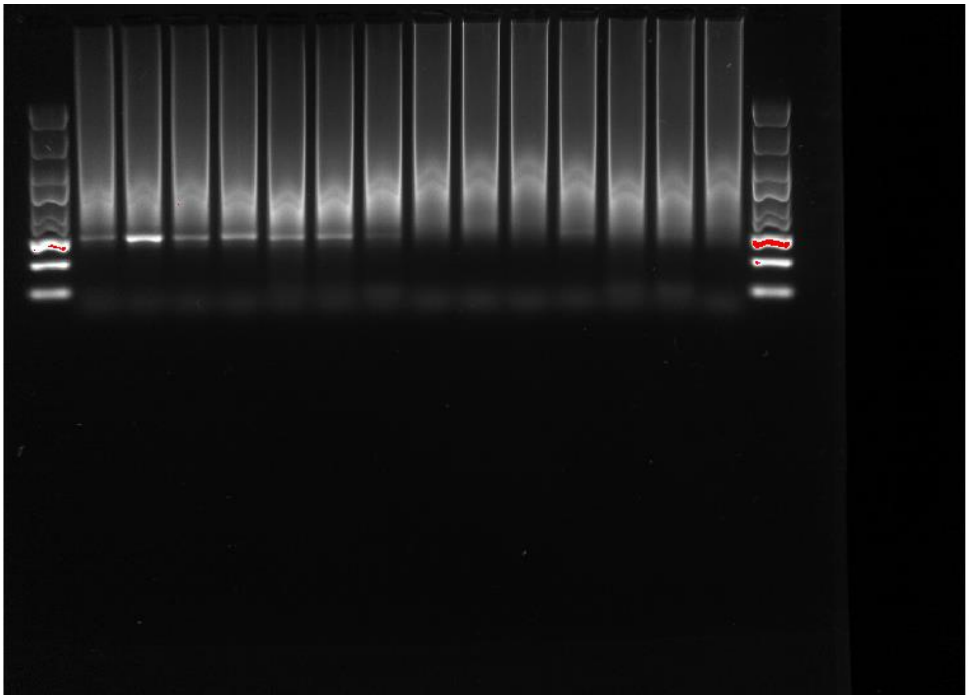
Bottom row, left to right: N1, N2, P1, P2



Second Stage PCR: Samples with bands from S2 and S1 second stage RERUN

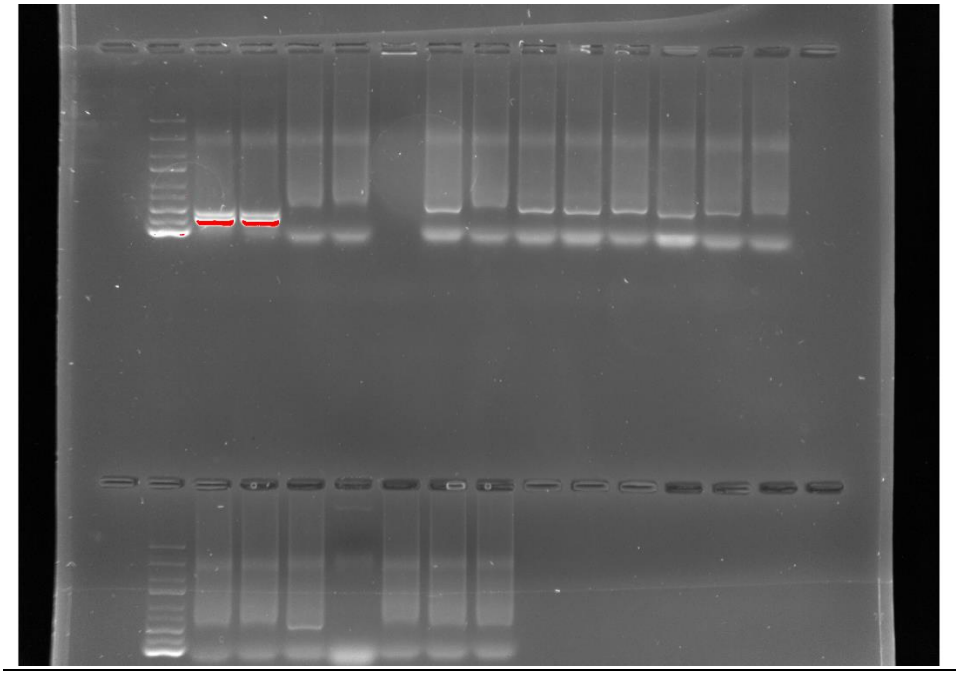
Top row, left to right: 1C, 1F, 1H, 1I, 1M, 2A, 2B, 2C, 2E, 2F, 2G, 2I, 2J, 2K, 1L

Bottom row, left to right: 1F, 1C, 1H, 1I, 1M, 2A, 2B, 2C, 2E



Successful samples from S1 and S2 second stage PCR purified (left) vs not purified (right)

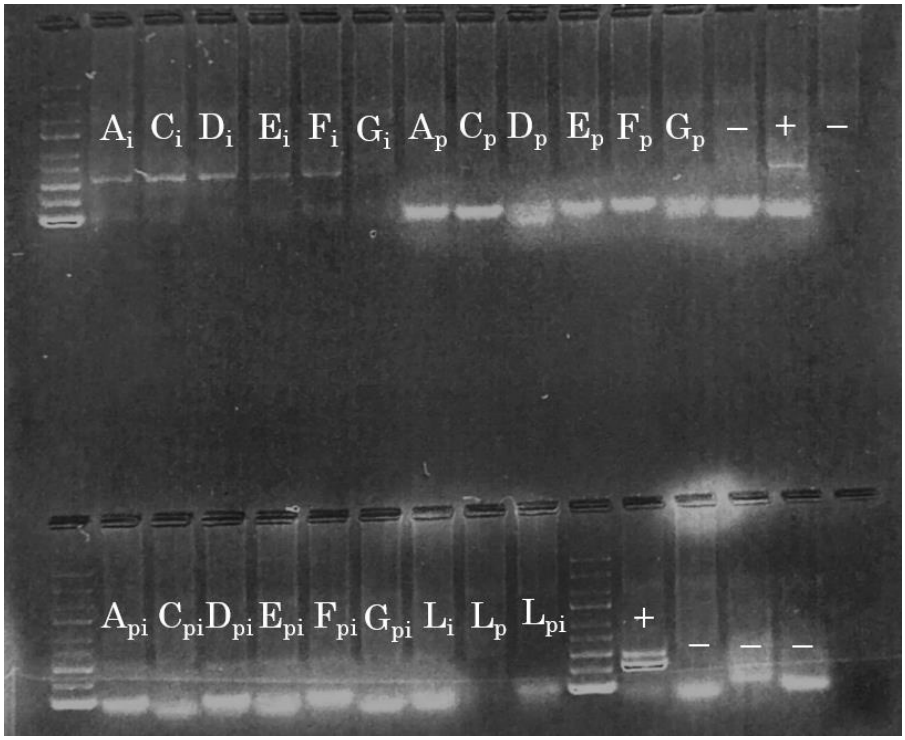
Top row, left to right: Ladder, purified 1F, purified 2B, purified 2C, purified 1F, purified 1C, purified 1H, purified 2B, 1F, 2B, 2C, 1F, 1C, 1H, 2B, ladder



First Stage PCR: Bee S4, Pollen grains A-O

Top row, left to right: Ladder, P1, P2, N1, N2, 4A, 4B, 4C 4D, 4E, 4F, 4G, 4I

Bottom row, left to right: Ladder, 4J, 4K, 4L, 4M, 4O, 3A, 3B



Second Stage PCR: Bee S4; Pollen grains A, C, D, E, F, G, and L; ITS2 only, psbA only, and pairwise

Bee S2

Pollen Grain	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	Success Rate
First Stage PCR Successful?	✓	✓	✓	X	✓	✓	✓	X	✓	✓	✓	✓	X	X	X	0.66667
Second Stage PCR Successful?	X	✓	✓	—	X	✓	X	—	X	X	X	X	—	—	—	0.3
Sequencing Successful?	—	X	✓	—	—	—	—	—	—	—	—	—	—	—	—	0.5

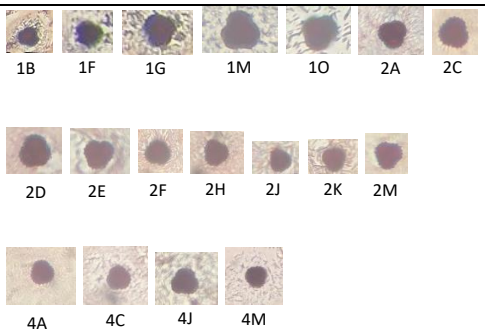
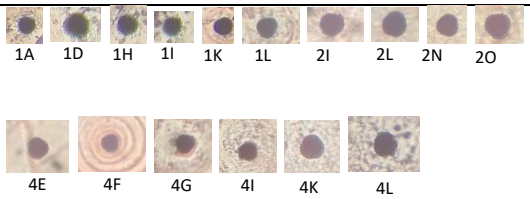
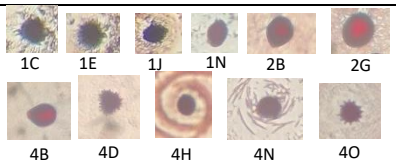
Bee S1

Pollen Grain	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	Success Rate
First Stage PCR Successful?	X	X	✓	X	X	✓	X	✓	✓	X	X	X	✓	X	X	0.33333
Second Stage PCR Successful?	—	—	✓	—	—	✓	—	✓	✓	—	—	—	X	—	—	0.8
Sequencing Successful?	—	—	✓	—	—	✓	—	X	—	—	—	—	—	—	—	0.66667

Bee S4

Pollen Grain	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	Success Rate
First Stage PCR Successful?	✓	X	✓	✓	✓	✓	✓	X	X	X	X	✓	X	X	X	0.466667
Second Stage PCR Successful?	✓	—	✓	✓	✓	✓	X	—	—	—	—	X	—	—	—	0.71429
Sequencing Successful?	X	—	X	X	X	X	—	—	—	—	—	—	—	—	—	0

	Bee S2	Bee S1	BeeS4	Average
First Stage PCR Success Rate	0.66667	0.33333	0.46667	0.48889
Second Stage PCR Success Rate	0.3	0.8	0.71429	0.60476
Sequencing Success Rate	0.5	0.66667	0	0.38889

Group	Pollen Grains in Group	Amplification Success Rate
Tetrad		9/18, .50
Round		9/16, .5625
Unrepresentative (Not large enough sample size to make own group)		4/11, .36
All	All (1A-1O, 2A-2O, 4A-4O,)	.4889

First stage PCR amplification success rate based on morphological structure

Pollen Grain	Identification	Confidence
S1F	 Betula Kenaica (Kenai Birch)	83%
S1C	 Pisum Sativum (Pea)	89%
S2C	 Vernonica Arvensis (Corn Speedwell)	94%

Sequencing Results: Identified Pollen Grains

Analysis

Overall, PCR had a high failure rate throughout this experiment, with a 49% average success rate per bee following first stage PCR, and a 60% average success rate per bee following second stage PCR. The high first stage PCR failure rate could have resulted from error when isolating pollen grains. After picking up individual pollen grains with a pin and suspending them in water in a PCR tube, the pollen grains could not be confirmed to be in the tube, because the safranin dye used to stain them was highly water soluble and appeared to wash off the pollen immediately after it hit the water. Because of this, determining whether a pollen grain was or was not in a PCR tube, or if it got stuck to the side of the PCR tube, was not feasible. Therefore, it is possible some pollen grains fell off the pin as they were transferred from the petri dish to the PCR tube, explaining one possible cause for the high failure rate of first stage PCR. However, this doesn't account for the high failure rate in second stage PCR. While the 60% success rate of second stage PCR is higher than the 49% rate of second stage PCR, error in pollen grain isolation would not have been a factor. The low success rate throughout PCR reactions may also be attributed to an insufficient starting DNA concentration. Since reactions included single pollen grains rather than a specific starting DNA concentration, lack of sufficient copies of ITS2 and psbA-trnH in pollen grains may have contributed to low amplification success rate.

Despite the high failed amplification rate, single pollen grain amplification could still be effectively used to identify plants Lasioglossum commonly forage on if the failures are random, because this would not affect the average proportionality of pollen types among results. However, if the failures resulted from a bias towards certain pollen types, the effectiveness of the method would decrease since the randomness of pollen grain selection would be lost. Pollen grains not being successfully placed into PCR tubes would be a random failure, whereas variability between certain morphological structures may introduce bias towards certain pollen types. No significant amplification bias was seen between tetrad and round pollen grain types, since tetrad pollen types had a success rate of 56.25%, and round pollen types had a success rate of 50%. However, this analysis is severely limited by the low resolution of pollen images, which introduced subjectivity into pollen grain groupings. Furthermore, tetrad and round are two extremely broad morphological groupings. It remains possible amplification bias exists between pollen grains within these two groups, that of which is not currently quantifiable.

Despite two primers being used in each reaction, all the wells (with the exception of bee 2, pollen grain K) only showed a single band following first stage PCR. Although the positive controls for bee 2 (PCR stage one) also only show a single band, first stage PCR for bee S1 confirmed both primers were working, since P1 contained only psbA-trnH and P2 contained only ITS2, both of which resulted in strong bands. In first stage PCR for Bee S2, it was likely that the difference in amplicon size was too minimal to be distinguishable, so the bands overlapped each other, explaining the thickness of bands for the two positive controls. While this could be a possibility for other wells, the thinness of the bands suggests this is not the case. Rather, it is likely the DNA from the pollen grains was simply not amplified substantially enough to result in two strong bands. Additionally, as the second stage PCR image for bee S4 shows, 5/7 ITS2 amplifications were successful, whereas 0/7 psbA-trnH amplifications were successful. This indicates ITS2 was a significantly more effective primer than psbA-trnH. Since psbA-trnH is an intergenic spacer region found in the plastids, it is likely to have more variability regarding initial copies of the spacer region per pollen grain than ITS2 (which is found in the nucleus), further accounting for the lack of amplification success.

Only 1 out of 2 amplified pollen DNA samples sent for sequencing from bee 1 were successful for bee 2. 2 of 3 samples were successfully sequenced for bee 1, and 0 of 5 were successful from bee 4, resulting in a 39% sequenced success rate per bee on average. The most likely cause for this high failure is that samples were sent with the incorrect concentrations. In particular, bee 4 had 5 samples sent for sequencing, yet none of them were successful. These samples were purified as a batch, suggesting an error during purification could be the cause of the failure.

Of the 45 pollen grains isolated, only 3 resulted in successful identifications. Pollen grain S1F was identified as Betula Kenaica – commonly known as kenai birch – at an 83% confidence level. Kenai birch is a tree and is typically wind-pollinated it doesn't grow any flowers. However, this result shows that bees can still benefit from tree pollen, even though trees don't require bee pollination to survive.

Pollen grain S1C was identified as Pisum Sativum (pea) at an 89% confidence level. However, because pea DNA was used as a positive control throughout this experiment, this result could have been the result of contamination. Pollen grain S1C was never directly next to the positive control, and S1A and S1B (which were closer to the pea DNA) did not show any

bands. Furthermore, the band S1C displayed following first stage PCR was not in line with the band from pea DNA for ITS2 or psbA, suggesting it had a different number of base pairs. While this suggests the identification is not the result of contamination, it cannot be conclusively determined, since the pea DNA used as a positive control did not specify what species of pea is used.

Pollen Grain S2C was identified as Veronica Arvensis (Corn Speedwell) at a 94% confidence level. Corn speedwell are not native, but they have been introduced and naturalized in the US and have been found in Massachusetts. However, corn speedwell is a weed, so it is not ideal for supporting the native bee population and local agriculture through companion planting.

Conclusion

Based on the results of the current project, single pollen grain DNA amplification is a possible method for identifying flora native bees forage on, but it is not yet efficient. Attaining 3 identifications through the methodology support that it is possible, however, the low average rate of success per bee during first stage PCR (49%), second stage PCR (60%), and sequencing (39%) demonstrates there are currently significant limitations. If the high failure rate is the result of random error, the methodology could still be effective, because it would not compromise the proportionality of pollen grain sampling. However, it is still a considerable drawback in terms of the method's feasibility, as this would significantly increase the time and cost needed to use the methodology. If the high failure rate is the result of amplification bias, however, this would compromise the randomness of the sampling, decreasing the method's effectiveness. Although no amplification bias was seen between tetrad and round pollen types, further analysis is needed to assess the potential for amplification bias more conclusively.

For this method to be effective, the amplification success rate does not have to be 100%. However, the failure does have to be random. Furthermore, improvement in the success rate would significantly increase the practicality of the method by lowering the time and cost required to conduct it, making it applicable on a larger scale.

Currently, despite three sequences, there is not enough data to conclusively compose a list of regionally tuned plant species that may better support the native bee population. Although confidence levels of identifications averaged 89%, the plants identified are not all likely to be appropriate for supporting native bees, as one pollen grain was identified as a weed (Veronica arvensis) and would therefore not be suitable for a seed mix designed to support local agriculture. This demonstrates that for the methodology to be successful, it is essential additional research is conducted on each identification to determine whether identified plant species are likely to benefit the native bee population. Furthermore, each identified plant is currently supported by a single pollen grain identification. To determine Lasioglossum preference, multiple pollen grains identifications referring to the same plant are imperative.

Further Investigations

The methodology used in this experiment will be repeated to identify additional flora Lasioglossum forage on, increase the confidence level of flora identification, and work to identify any weak points throughout the procedure that could be contributing to the low PCR success rate.

Increasing the quality of pollen grain pictures taken before sampling could allow further analysis regarding whether amplification bias was present between various pollen grains, or whether amplification failure was the result from random human error.

Since Lasioglossum could be attracted to Dunrovin Farm because of factors other than foraging – such as habitat – future research must be conducted to assess whether identified flora have a positive effect on native bee populations and/or local agriculture

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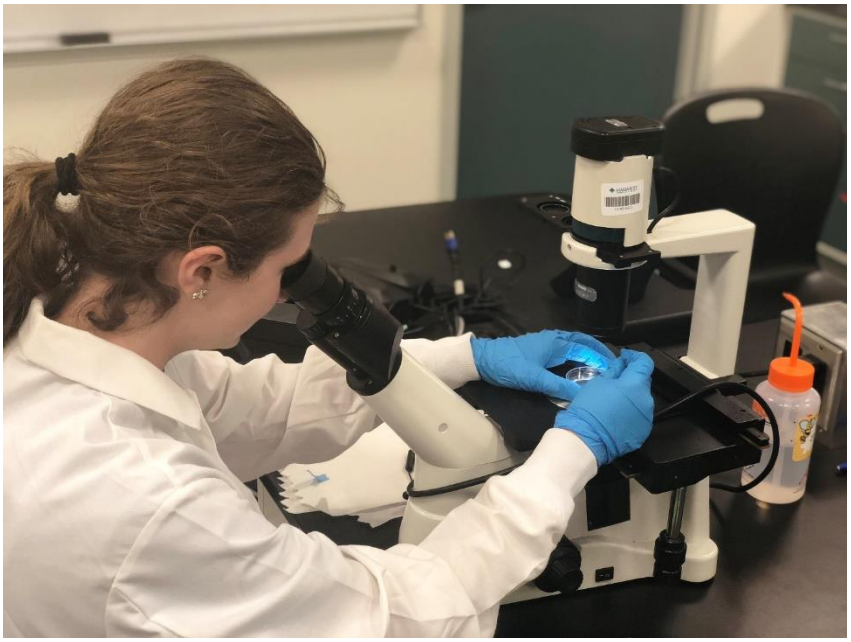
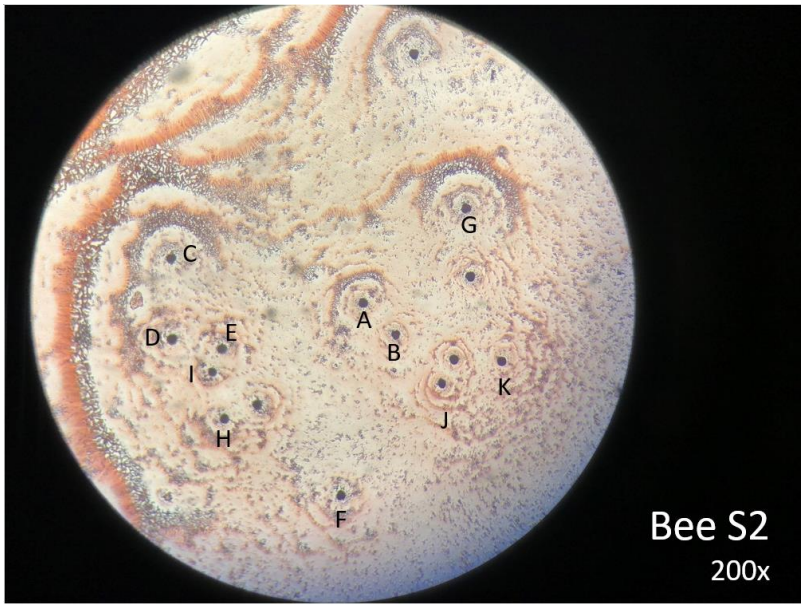
Appendix

Photos



Isolating Pollen Grains



Stained Pollen Grains: Bee S2, pollen grains A-K

Bee Collection Info

Lasioglossum were caught via aerial sweep netting and placed into ethyl acetate jars.

Dunrovin Farm

Collection Date: 8/28/18

Weather: Sunny, 85 degrees (F)

Location: Dunrovin Farm, Halifax, MA

Time: 9:37 AM – 10:23 AM

Latitude and Longitude: 41.967, -70.877

Additional Sequencing Information

S1F

Betula kenaica voucher CCDB-18325-D1 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: MG236820.1Length: 455Number of Matches: 1

Range 1: 89 to 418GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
384 bits(425)	2e-102	273/330(83%)	0/330(0%)	Plus/Plus

```
Query  1    TCGTTGCCCCCAACCCCNTCNCCTTGNAAAGGGACNAGAGGGCCANTGGGGTANAAATTG  60
            |||||||||||||||| || ||||| |||||||| || |||||  ||||| | ||||||
Sbjct  89   TCGTTGCCCCCAACCCCATCTCCTTGCAAAGGGACGAGGGGGCCTGTGGGGCAGAAATTG  148

Query  61   GCCTCCCGNGAGCTCATGNNTGCGGTTGGCCTAAAAGCGAGTCCTCGGNNACNCGCGCCA  120
            |||||||| |||||||||  ||||||||||||||||||||||||||||  || |||||||
Sbjct  149  GCCTCCCGTGAGCTCATGCATGCGGTTGGCCTAAAAGCGAGTCCTCGGCGACGCGCGCCA  208

Query  121  CNACAATCGGNGGTTGACAAACCCTCNNGTCCNNNNNNGNGTGCNNNNNNNNTCATCNNG  180
            | |||||||| ||||| |||||||||  ||||      | ||||        |||||  |
Sbjct  209  CGACAATCGGTGGTTGTCAAACCCTCGTGTCCCGTCGTGCGTGCCGCGTCGCTCATCGTG  268

Query  181  TGCTCTTTGACCCTGTTGTGTCNCGCTANCNATGCTTCCAATGCGACCCCAGGTCAGGCG  240
            ||||| |||||||||||||||| ||||| | | |||||||| ||||||||||||||||||
Sbjct  269  TGCTCCTTGACCCTGTTGTGTCGCGCTAGCGACGCTTCCAACGCGACCCCAGGTCAGGCG  328

Query  241  GGACTACCCGCTGANTTNAANCATATCAATAANCGNANGaaaanaaaCTTACAAGGATTC  300
            |||||||||||||| || || |||||||||||| || | ||||| ||||||||||||||||
Sbjct  329  GGACTACCCGCTGAATTTAAGCATATCAATAAGCGGAGGAAAAGAAACTTACAAGGATTC  388

Query  301  CCTTANNAACGGNNANCGANCCGGGANTTA  330
            |||||  |||||  | ||| |||||| |||
Sbjct  389  CCTTAGTAACGGCGAGCGAACCGGGATTTA  418
```

S1C

DownloadGenBankGraphicsNextPreviousDescriptions

Pisum sativum subsp. elatius 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Sequence ID: JN617189.1Length: 489Number of Matches: 1

Range 1: 58 to 413GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
502 bits(556)	3e-138	319/357(89%)	1/357(0%)	Plus/Minus

```
Query  4    CGNTACTAANGNNAATCCTTGTTANTTTCNNTTCCTCCGCTTATTGATATGCTTAAATTC  63
            || |||||| |  |||||||||||| ||||  ||||||||||||||||||||||||||||
Sbjct  413  CGTTACTAAGGG-AATCCTTGTTAGTTTCTTTTCCTCCGCTTATTGATATGCTTAAATTC  355

Query  64   AGCGGGTAGCCCCGCCNGACCTGAGGTCTCATCACGAGCGTTTANAAACGCAAATGGGTA  123
            ||||||||||||||| |||||||||||||||||||||||||||| ||||||||||||||
Sbjct  354  AGCGGGTAGCCCCGCCTGACCTGAGGTCTCATCACGAGCGTTTAGAAACGCAAATGGGTA  295

Query  124  AAAGAGCCCAAATTTNATAGAGCAACACATGATTGGTCTCGTGGGTCACACAACCACCAT  183
            ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  294  AAAGAGCCCAAATTTAATAGAGCAACACATGATTGGTCTCGTGGGTCACACAACCACCAT  235

Query  184  TTATCATGGNNCACCCTACCAAGGTCTCAATTTTCAACCAACCATGANACNAAANANAGC  243
            |||||||||  |||||||||||||||||||||||||||||||||| || ||| | |||
Sbjct  234  TTATCATGGCACACCCTACCAAGGTCTCAATTTTCAACCAACCATGAGACGAAAGAGAGC  175

Query  244  TCNNGGGAGGCCNACATCCACCCTGNNNANTANCTGNCAAAAGGAAATTGNNNNGNGGCT  303
            ||  |||||||| ||||||||||||   | || ||| ||||||||||||    | ||||
Sbjct  174  TCACGGGAGGCCAACATCCACCCTGCACAATACCTGTCAAAAGGAAATTGGCAGGAGGCT  115

Query  304  TCAATATGTGACACCNANGCANACGTGCCCTCNANCTAATGGCATCNGGTGCANCTT  360
            ||||||||||||||| | ||| |||||||||| | |||||||||| || ||| |||
Sbjct  114  TCAATATGTGACACCCAGGCAGACGTGCCCTCAACCTAATGGCATCGGGCGCAACTT  58
```

S2C

Veronica arvensis voucher CAL1811WP 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: MG218716.1Length: 340Number of Matches: 1

Range 1: 110 to 340GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
361 bits(400)	4e-96	216/231(94%)	0/231(0%)	Plus/Minus

```
Query  14   TTAAATTCAGCGGGTANCCCCGCCTGACCTGAGGTCTCATCACNAGCGTTTACAAACGCA  73
            |||||||||||||||| |||||||||||||||||||||||||| |||||||| |||||||
Sbjct  340  TTAAATTCAGCGGGTAGCCCCGCCTGACCTGAGGTCTCATCACGAGCGTTTAGAAACGCA  281

Query  74   AATGGGTAAAANAGCCCANATTNAATANAACAACACATGATTGGTCTCGTGGGTCACACN  133
            |||||||||||| |||||| ||| |||| | ||||||||||||||||||||||||||||
Sbjct  280  AATGGGTAAAAGAGCCCAAATTTAATAGAGCAACACATGATTGGTCTCGTGGGTCACACA  221

Query  134  ACCACCATTTATCATGGCACACCCTACCAAGGTCTCAATTTTCAACCAACCATGANACGA  193
            |||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||
Sbjct  220  ACCACCATTTATCATGGCACACCCTACCAAGGTCTCAATTTTCAACCAACCATGAGACGA  161

Query  194  ANNANAGCTCACGGGAGGCCAACATCCACCCTGCACNANACCTGTCAAAAG  244
            |  | |||||||||||||||||||||||||||| | ||||||||||||
Sbjct  160  AAGAGAGCTCACGGGAGGCCAACATCCACCCTGCACAATACCTGTCAAAAG  110
```