Developing microsatellite primers for two *Tilia* species in order to assess ascertainment bias

William Stephenson, W.G.Stephenson1@newcatle.ac.uk

Newcastle University

genetics society

What are Microsatellites?

Microsatellites are continuous repeats of the nucleotide bases: Adenine, Thymine, Cytosine and Guanine (represented by letters A, T, C and G) that make up DNA. These could be mononucleotide (single repeats) such as:
CTTCGACTCAG**AAAAAAAAAAAAAAAAA**CTGAAGT
Or more complex Dinucleotides (two repeating units) such as
CTTCGACTCAG**TATATATATATATATATATA**CTGAAGT
Due to the repeating nature of microsatellites, enzymes that carry out the duplication of DNA can end up forgetting where on the repeat section they are. This can lead to nucleotides being lost or gained as the enzyme carries on from the wrong part of the microsatellite. Consequently, mutations in microsatellites happen extremely fast compared to the rest of the DNA. These high speed mutations are important when looking at differences between populations as the level of difference indicates how populations are interacting and diverge. This can tell us how species have migrated and originated and can even identify new species.

Project Aims

This research project aimed to create microsatellite DNA primers. These nucleotides are used to amplify microsatellite containing DNA multiple times in order for a Gene Analyser to determine the microsatellite length. If lengths are taken from multiple SSR primers then individuals can be grouped into populations based upon the similarity of their microsatellites. This data can then be used statistically to assess, for example, diversity or the level of inbreeding.
In particular this project looked to see if primers developed for one species could be effective diversity indicators when used in a closely related species. This is called ascertainment bias.

Figure 1: Tilia cordata (small leaved lime), http://web03.bruns.de/bruns/en/EUR//Pflanzen/TILIA-cordata-MILL-%2C-Small-leaved-lime/p/1860

What is *Tilia*?

Tilia are the genus of trees commonly referred to as lime trees. While the fruit bearing limes may be your first thought, *Tilia* are actually a completely unrelated group of northern hemisphere trees. These trees are an important part of our heritage being thought to have once made up 40% of the ancient deciduous woodland in the UK. The trees have several important uses, which include providing resources and habitats for other species, particularly through their leaf litter, which has been found to contain more species than oak, and the production of goods. *Tilia* are the source of lime tree tea, honey and have historically been a popular wood to carve from.

Table 1: raw size scores in base pairs for microsatellites. Meaningful data can be obtained through applying statistics based upon these sizes.

Samples	Tcprimer1		Tcprimer2		Tcprimer3		Tcprimer4		Tcprimer5		Tpprimer1		Tpprimer2		Tpprimer3		Tpprimer4	
Tilia cordata																		
Tc5	114.77	114.77	192.84	192.84	227.79	241.78	259.98	267.87	260.9	260.9	129.94	129.94	120.24	120.24	202.81	206.75	238	238
Tc6	110.53	117.52	213.08	220.09	227.44	235.63	260.12	263.91	260.78	260.78	129.7	129.7	120.01	120.01	202.93	202.93	238	238
Tc7	110.32	112.55	198.76	216.17	227.74	235.84	260.19	263.99	260.85	260.85	127.48	127.48	120.01	120.01	202.71	212.53	238.25	238.25
Tc8	112.55	114.9	192.62	199.32	235.73	241.79	260.17	260.17	260.83	260.83	127.48	136.51	120.14	120.14	203.04	203.04	238	238
Tc9	112.68	117.39	198.44	198.44	235.55	235.55	260.11	260.11	260.82	260.82	127.48	136.51	120.14	120.14	203.04	203.04	238	238
Tc10	114.88	114.88	192.92	198.61	227.69	237.74	260.15	260.15	260.73	260.73	131.98	131.98	120.05	120.05	202.86	202.86	238	238
Tc11	107.99	119.39	192.51	192.51	241.7	249.83	260.14	260.14	260.78	260.78	125.25	125.25	119.92	119.92	202.97	202.97	238.1	238.1
Tc12	117.39	124.07	192.62	192.62	212.23	227.26	260.19	267.81	260.67	260.67	127.2	127.2	120.01	120.01	202.94	206.8	238	238
Tc13	110.4	115.06	192.85	198.33	235.94	233.92	260.28	260.28	261	261	132.45	132.45	121.44	121.44	203.13	219.95	237.67	237.67
Tc14	110.4	114.93	192.62	192.62	235.81	237.84	260.19	267.92			128.43	128.43	121.44	121.44	202.68	221.85		
Tc15	114.77	114.77	191.88	191.88	235.88	235.88	259.91	266.06	260.89	260.89	128.2	132.43	121.44	121.44	202.8	220.14	237.67	237.67
Tc16	108.2	112.73	192.7	211.01	235.89	235.89	260.19	260.19	260.85	260.85	130.17	130.17	121.44	121.44	203.14	212.57		
Tc17	115.32	115.32	201.24	213.13	237.77	237.77	260.21	263.66	260.76	260.76	124.01	124.01	121.68	121.68	202.82	202.82	237.93	237.93
Tc18	110.45	112.81	198.41	198.41	231.84	235.84	260.19	260.19	260.86	260.86	130.16	130.16	121.39	121.39	202.84	212.5	238.1	238.1
Tc19	110.11	110.11			231.8	231.8	260.17	260.17			128.2	128.2	121.49	121.49	202.73	221.9	237.87	237.87
Tc20	107.96	117.13	191.85	191.85	227.82	235.87	260.33	260.33			121.94	121.94	121.57	121.57	202.93	202.93	238.17	238.17
Tilia platyphylos																		
Tp5	114.75	114.75	192.88	192.88	228.14	241.8	260.33	260.33	261.06	261.06	88.32	96.18			204.83	210	238.1	255.69
Tp6	110.24	117.27	213.15	220.21	227.85	235.63	260.33	260.33	261.04	261.04	97.62	100.11	120.14	120.14	204.61	209.9	243.8	249.75
Tp7	110.24	112.49	198.65	216.31	227.84	235.88	260.28	260.28	261.04	261.04	97.49	97.49	120.36	120.36	203.16	209.83	249.78	255.75
Tp8	112.63	114.88	192.8	192.8	235.98	241.73	260.19	260.19	260.93	260.93	89.24	97.23	119.78	119.78	203.04	208.69	249.66	255.65
Tp9	112.44	117.13	198.62	198.62	235.91	235.91	260.22	260.22	260.96	260.96	89.63	97.49	120.14	120.14	202.95	205	249.71	257.59
Tp10	114.85	114.85	192.48	198.64	227.89	237.79	260.15	260.15	260.85	260.85	111.3	113.69	120.05	120.05	202.97	204.8	257.58	257.58
Tp11	108.01	119.55	192.85	192.85	241.97	250.03	260.17	260.17	260.8	260.8	100.4	100.4	119.92	119.92	202.97	202.97	249.63	257.48
Tp12	117.4	124.16	192.62	192.62	227.6	227.6	260.14	267.84	260.84	260.84	97.62	100.11	119.92	119.92	202.72	202.72	249.69	257.51
Tp13	110.66	115.19	193.18	193.18	206.94	227.07	260.23	260.23	259.11	274.89	95.13	97.36	120.24	120.24	203.56	203.56	249.75	257.67
Tp14	110.19	110.19	193.27	193.27	208.87	227.03	260.03	260.03	259.11	261.17	98.11	98.11	120.14	120.14	203.23	210.04	243.95	257.61
Tp15	124.07	124.07	193.13	193.13	208.79	227.02	260.28	260.28	271.07	274.89	97.49	97.49	120.14	120.14	205.05	208.87	249.83	257.61
Tp16	117.26	117.26	192.99	192.99	208.88	231.03	260.21	260.21	265.35	275.01	97.62	97.62	120.14	120.14	203.12	205.02	249.66	257.45
Tp17	115.17	124.2	192.91	192.91	208.83	226.91	260.01	260.01	257.01	274.81	97.88	97.88	120.27	120.27	205.05	208.87	243.93	249.69
Tp18	115.17	124.2	192.77	192.77			260.08	260.08	257.01	274.81	97.52	97.52	120.05	120.05	209.97	209.97	257.57	257.57
Tp19	115.01	124.02	192.77	192.77	224.9	224.9	260.01	260.01	257.01	274.81	97.52	97.52	120.05	120.05	210.05	210.05	257.57	257.57
Tp20	103.77	110.58	193.19	193.19	207	209.03	260.03	260.03	260.67	270.83	97.75	97.75	120	120	203.13	208.83	251.75	257.59

Results

It was found that the working primers developed from *T. cordata* showed a similar level of diversity as those that were developed from *T. platyphylos* and vice versa (Table 2). This would suggest that *Tilia* primers derived from RNA can be used as effective diversity indicators between species. Consequently this helps validate previous *Tilia* studies that have used primers developed from genomic DNA and could help inform future research projects that are constrained by the expense and time consuming nature of producing species specific primers. Additionally, as part of the project and to help future research, the first microsatellite primers for *T. cordata* and additional ones for *T. platyphylos* were developed. While several primers failed to work, one of the primers only amplified in *Tilia platyphylos*, this can be used in the future to identify unknown *Tilia* samples.

Table 2: comparison of heterozygosity, a measure of diversity, for newly developed *Tilia* primers compared to original primers. The similar expected heterozygosity suggests that there is no bias occurring.

species tested on	DNA or RNA derived primers	primers developed from	number of samples	heterozygosity observed	heterozygosity expected
T. platyphylos	RNA	T. platyphylos	15	0.484	0.485
T. cordata	RNA	T. platyphylos	15	0.179	0.427
T. platyphylos	RNA	T.cordata	15	0.448	0.538
T. cordata	RNA	T.cordata	15	0.471	0.534

Figure 2: The Angel Raphael and the young Tobias. Limewood. 97 cm, (Germanisches Nationalmuseum, Nuremberg), ca 1500

Method

This project used the two *Tilia* species *Tilia cordata* (small leaved lime) and *Tilia platyphlos* (large leaved lime) to assess the ascertainment bias. While the dinucleotide (Logan 2016) microsatellites used were identified from provided RNA sequences of these species(Logan 2016), using microsatellite searching software MISA and primer development program Primer 3. The primers developed were then searched against the RNA sequences of the other *Tilia* species to increase the chance of finding microsatellites that amplified in both species (this was done using the program CLC genomics workbench). Following this, 10 primers were developed for both species and were tested on both species DNA. For the working primers, scores were assigned according to the microsatellite length in the DNA (Table 1) and the diversity between samples assessed using this. Comparisons were then made between primers developed from one species compared to the other, for both species.

Future work

The study only looked at one population and two species of *Tilia*. To make a definitive assessment of the primers, multiple populations will need to be looked at. Further work could also compare the diversity found from these new RNA derived primers with the *T. platyphylos* primers developed in earlier experiments using genomic DNA.

Acknowledgements

I would like to thank my supervisor Dr Kirsten Wolff, and Dr Samuel Logan for providing help and encouragement throughout the project. I am also grateful to the Genetics Society whom funded this research project and made it possible.

Reference

Samuel A. Logan (2016). Ancient relicts in the limelight: an evolutionary study of diversity and demographic history in species of broad-leaved temperate forest tree genus *Tilia*. PhD thesis Newcatle University.