

2021-22

BSURP The BioScientific Undergraduate Research Publication





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What is **BSURP**?

The BioScientific Undergraduate Research Publication (BSURP) is a collection of summaries detailing research projects that were undertaken by Biological Sciences undergraduate students at the University of Calgary. As one of the top research universities in Canada, the University of Calgary provides many students with the incredible opportunity to conduct innovative research in many diverse areas. Many students in the Biological Sciences have taken up research as part of summer programs or courses for their degree.

The Biology Students' Association (BSA) began the BSURP during the 2020-21 academic year in order to highlight the amazing research being done by our fellow Biological Sciences students. Each one of the six programs in Biological Sciences (Biochemistry; Biological Sciences; Cellular, Molecular and Microbial Biology; Ecology; Plant Biology; and Zoology) is represented in our publication. Projects were conducted in research labs both within and outside the Biological Sciences department, covering a diverse range of interesting and ground-breaking topics.

All previous publications of the BSURP are also available to view on the BSA website at <u>https://www.bsaucalgary.ca/bsurp</u>. We hope this publication not only showcases the fantastic work of Biological Sciences students over the years and encourages other students to get involved in research, but also provides insight into what research is about and what students can achieve.

Eligibility

To be eligible to submit to BSURP, applicants had to be undergraduate Biological Sciences students at the University of Calgary for the duration of the project. The project had to take place between May 2021 and May 2022, and both research courses (507/528/530) and non-credit projects were acceptable. Research could occur in any department so long as the student was a Biological Sciences student.



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Contributors

The following University of Calgary undergraduate students and alumni are featured in this publication:

Emily Bartram Ginny Li Ihncheol Jung Taylor Ford-Sahibzada Zoe Penno

BSURP team

Joan Chu (2021-22 BSA Vice-President Academic) designed and assembled the publication. The 2021-2022 Academic team (Joan Chu, Mackenzie Aranas, Candace Elisha Chan, Ariz Lalani, Euro Lee) collaborated to determine submission criteria and evaluation methods.

Acknowledgements

We would like to thank all applicants for participating in BSURP and sharing their amazing work! 2021-22 was a challenging year for many students with the remote-to-in person learning shift, and we really appreciate all applicants taking the time to contribute their research to this publication.

Additionally, we would like to thank the BSA 2021-2022 executive council for helping shape this publication and assisting with logistics, feedback, and marketing.

Contact us

The BSA is a Student Union sanctioned, departmental club at the University of Calgary that provides community engagement opportunities, academic resources, professional development events, and more. Our club resources and initiatives are designed to help Biological Sciences students thrive academically, connect with peers and enhance their student experience. Learn more about the BSA at <u>bsaucalgary.ca</u>



For inquiries about BSURP or the BSA, please feel free to reach out to our team at **contact@bsaucalgary.ca**.

<u>Cover photos</u> Pollination: Photo by <u>Richard Sagredo</u> on <u>Unsplash</u> Coral: Photo by <u>QUI NGUYEN</u> on <u>Unsplash</u> Connective tissue: Photo by <u>National Cancer Institute</u> on <u>Unsplash</u> Cows: Photo by <u>Kyle Mackie</u> on <u>Unsplash</u>



The BioScientific Undergraduate Research Publication

BSURP Entries

The associations between prenatal exposure to bisphenols and atopic diseases in children

Type of project: 507 **Supervisor(s):** Dr. Deborah Dewey, Faculty of Medicine; Dr. Gillian England-Mason, Faculty of Medicine; Dr. Hamid Habibi (co-supervisors)

About the Researcher Emily Bartram



Hey! I'm a Biology major with a minor in Gender & Sexuality studies heading into my fourth year at the University of Calgary. I really love learning about the immune and endocrine systems; this project allowed me to combine these two systems and do a deep dive into how they connect and what can happen when things don't work perfectly. This project was especially interesting as it involved a lot of research into plastics – we are beginning to see the outcomes of our exposure to plastics now and it really makes you think about how pervasive plastic use is in our daily lives. The most challenging part of this project for me was learning how to write for academic research— it took me a while to get the knack of it and it's something I am still working on. Outside of research, I coach a swim club and love to swim! I also enjoy reading murder mysteries and watching true crime shows.

Background

It has been suggested that prenatal exposure to endocrine disrupting chemicals (EDCs), such as bisphenols, may be associated with the development of atopic diseases in children. Atopy is a predisposition to produce an exaggerated immunoglobulin E (IgE) response to harmless allergens, which typically manifests in clinical conditions (i.e., atopic disease) such as eczema, asthma, and allergies (Owen &



Kuby, 2013). Additionally, the development of atopic diseases in children could be influenced by the interactions of maternal history of atopic diseases, such as asthma, with bisphenols. Human research suggests that prenatal exposure to BPA may be associated with the development of atopic diseases in children, but the findings are mixed - for example, a 2014 study by Gascon et al. examined the relationship between prenatal BPA exposure in the first and third trimesters of pregnancy and development of eczema, asthma, and wheeze in children at multiple timepoints. Positive associations were also reported in a 2017 study by Zhou et al., where they found that maternal exposure to BPA measured within 3 days of delivery was associated with increased risk of atopic disease (eczema, wheeze, or both) in female infants during the first 6 months of life. However, there is also evidence of negative associations between prenatal exposure to BPA and the occurrence of atopic disease during childhood. A 2013 study by Donahue et al. found that maternal BPA exposure measured during the third trimester was associated with decreased risk of wheeze in children at 5 years. Our study's objective was to investigate the associations between prenatal exposure to bisphenol A (BPA) and bisphenol S (BPS) and the development of atopic diseases in children at 12, 24, and 36 months of age; maternal history of asthma was examined as a potential interacting variable with BPA and BPS.

Methods

This study included a subsample of maternal-child pairs (N = 488) from the Alberta Pregnancy Outcomes and Nutrition (APrON) study (Kaplan et al., 2014). At the time of recruitment, information on maternal age, ethnicity, education level, income, other sociodemographic characteristics, and history of maternal asthma was collected. Urine samples were collected from the mothers during their second trimester of pregnancy (average gestational weeks = 17, standard deviation = 2.1 weeks). Bisphenols were quantified using established methods (England-Mason et al., 2021; Liu et al., 2018; Liu et al., 2021). In brief, liquid chromatography-high resolution mass spectrophotometry (Orbitrap Elite, Thermo Fisher Scientific) was performed to quantify the levels of BPA and BPS. Aliquots of the urine samples (1mL) were analyzed for creatinine.

Mothers completed a child health questionnaire when children were 12 months, 24 months, and 36 months of age. The questionnaire asked about common health issues and conditions, such as whether the child had ever experienced rashes or skin problems, respiratory/breathing problems including asthma, and food or other allergies. Based on these responses to these questions, conditions were coded as not atopic (0) or atopic (1). The categories of atopic diseases considered were: i) dermatological



conditions, ii) respiratory conditions, iii) food allergies, and iv) other allergies. A coding scheme was developed based on the scientific literature and used to classify the parent-reported childhood health conditions in the APrON cohort as either an atopic disease (e.g., eczema, asthma, wheeze, peanut allergy) or not an atopic disease (e.g., cradle cap, diaper rash, croup, gluten sensitivity) (see Table 1 for prevalence).

Descriptives and frequencies were computed using Statistical Packaging for the Social Sciences (SPSS) Version 28 (IBM Corp, 2019)). Binary logistic regressions were conducted to examine the associations between prenatal exposure to bisphenols and the development of atopic diseases in children at 12, 24, and 36 months of age, as well as the development of atopic diseases at any age (i.e., 12, 24, and/or 36 months).

Results

Logistic regression models revealed that there were no significant associations between BPA or BPS and atopic outcomes at any time point; however, in these models, maternal history of asthma was significantly associated with increased odds of children developing atopic dermatological conditions at 12 months (aOR = 1.9, 95% CI = 0.9, 3.9) and atopic respiratory conditions at 24 months of age (aOR = 10.7, 95% CI = 2.1, 54.5). At 36 months of age, maternal history of asthma was significantly associated with higher odds of children developing atopic dermatological conditions (aOR = 3.5, 95% CI = 1.8, 6.8), atopic respiratory conditions (aOR = 7.8, 95% CI = 2.1, 28.8), as well as food allergies (aOR = 3.8, 95% CI = 1.5, 9.5). Across all time points, maternal history of asthma was found to be associated with increased odds of children developing atopic dermatological conditions (aOR = 2.7, 95% = 1.5, 4.9), atopic respiratory conditions (aOR = 2.9, 95% CI = 1.4, 6.0).

Significance

The primary objective of this study was to examine the associations between prenatal exposure to bisphenols and the development of atopic diseases in children at 12, 24 and 36 months of age. We did not find any significant associations between prenatal exposure to BPA or BPS and the odds of developing atopic disease (dermatological, respiratory, food allergy, and other allergy) in early childhood. However, maternal history of asthma was significantly associated with increased odds of developing atopic diseases (dermatological, respiratory, food allergy, and other allergy) at 12, 24, and 36 months. Our secondary objective examined whether maternal history of asthma interacted with prenatal exposure to BPA and BPS to predict the development of atopic disease at ages 12, 24, and 36 months. Our analyses did not find any



significant interactions; however, dermatological conditions at 12 months and respiratory conditions at 36 months both approached significance (p = 0.08, p = 0.07 respectively).

This project is significant because it adds to the existing literature on prenatal exposure to bisphenols and atopic diseases in children. Thus far, the published literature has been mixed, so our study, along with past studies, provides a basis for future research to continue looking into this increasingly important area of research. Our study also suggests that parental history of asthma should be examined when studying the associations between bisphenols and the development of atopic disease. As the majority of research so far has focused on BPA, future research may consider examining multiple bisphenols - as we replace BPA in some of our plastic products with other bisphenols such as BPS or BPF, it is important to consider the associations these chemicals may have with child health outcomes. Additionally, other research suggests that other EDCs, such as phthalates, may also be associated with child health outcomes and current literature continues to examine this.

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Figures

Table 1. Prevalence of atopic dermatological conditions, respiratory conditions, food allergies and other allergies in children at 12, 24 and 36 months of age.

	Conditions classified as atopic disease	Conditions that were not classified as atopic disease	
Data collected at 12 months			
Dermatological	22.0%	78.0%	
	(85)	(302)	
Respiratory	1.9	98.1	
	(8)	(422)	
Food Allergy	7.5%	92.5%	
	(32)	(395)	
Other Allergy	2.6%	97.4%	
	(11)	(414)	
Data collected at 24 months			
Dermatological ^a			
Respiratory	2.2%	97.8%	
	(8)	(359)	
Food Allergy	6.4%	93.6%	
	(23)	(339)	
Other Allergy	2.4%	97.6%	
	(9)	(364)	
Data collected at 36 months			
Dermatological	26.1%	73.9%	
	(92)	(260)	
Respiratory	3.2%	96.8%	
	(13)	(392)	
Food Allergy	7.1%	92.9%	
	(29)	(382)	
Other Allergy	4.1%	95.9%	
	(17)	(395)	

^a No dermatological data collected at 24 months.

Where to find out more about the project?

More information about the general cohort and similar published projects can be found at <u>https://apronstudy.ca</u>.



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Full thesis writeup: https://docs.google.com/document/d/1t70Ob9yATwdpZ5DjFVaK28MhrEh4zmS2czsF3j2 3soY/edit?usp=sharing



Inferring the evolutionary relationship between Aquilegias and hummingbirds with regards to lineage age and range size

Type of project: 530 Supervisor(s): Dr. Jana Vamosi

About the Researcher Ginny Li



I graduated from my 4th year of my Bachelors in Biological Sciences. Out of the upper year courses, plant sciences stood out to me along with the theory of coevolution from Principles of Ecology which taught the Red Queen Hypothesis: "it takes all the running you can do, to keep in the same place". It means that a species must evolve for survival because other organisms are also evolving. Connecting literature to sciences resonated with me since I like reading in my spare time (my two hour commute). Studying plants and evolution is interesting because we get to reveal the hidden evolutionary depths that it takes to be a sessile plant and how they must "run" evolutionarily in order to stay rooted. I enjoyed learning more about R and trying to troubleshoot issues with my code since many of the packages were new to me! I hope that other graduates also continue running forward and evolving no matter how little change you see at first.

Background

Coevolution, or the evolution between two or more different species where a change in one species affects another species, occurs often between plants and their pollinators which often leads to species divergence and specialized relationships (Fründ et al., 2010). One of the model organismal groups in evolutionary biology are the *Aquilegia spp.* or commonly known as columbine flowers. They are commonly found in

Alberta in the alpine regions. Columbines are a model species as they have a wide range of colour combinations as they have a set of five sepals and five petals that could be modified to have spurs with nectar tips.

Major pollinator guilds, such as birds compared to insects, exhibit different behaviours and may affect species stability of angiosperms, leading to rapid range changes. Red floral parts likely indicate that they have hummingbird pollinators as birds possess red cones, whereas many insects do not sense red so they are not believed to pollinate these flowers as often (Gegear et al., 2017). Hummingbird pollination relationships are thought to be younger than insect pollination. Recently diverged species often have small ranges given the fact that they have not received the necessary time to diffuse further away from their parent species (Sheth et al., 2020). Similarly, older lineages may become more robust to localized disturbance as they generate more genetic variation as they expand their range (Sheth et al., 2020). Pollinators also affect range as hummingbirds would be able to cross-pollinate flowers that are a farther distance and produce more genetic diversity for the plant compared to insects and self-fertilization so there is a higher chance of evolution to fit a new environment (Fründ, 2010).

With the previous knowledge in mind, it is hypothesized that younger *Aquilegia* lineages, flowers with red traits, have smaller ranges than non-red flowers. Yet this conflicts with the second hypothesis where hummingbird pollinated plants will have larger ranges than insect pollinated ones. The final hypothesis is that different pollinator guilds will affect the lineage age of *Aquilegia* species.

Methods

To collect a list of *Aquilegias* with the estimated age of lineages, Timetree was used as they aggregate estimates from published research papers (Bastida et al., 2010, Clark et al., 2015). The parameters of range will be defined as the extent of occurrence (EOO) and area of occupancy (AOO) ranges. EOO is the shortest continuous boundary that surrounds all of the current locations where the plant is located (Bachman & Moat, 2012). The AOO is located within the EOO and it is the smallest area that the plant is occupying. The Global Biodiversity Information Facility (GBIF) databases were used for locating the data in Geocat, a mapping tool that plots points of occurrences for a taxon using open source data to allow geospatial estimates for EOO and AOO (Bachman & Moat, 2012). *Aquilegias* were separated based on petal and sepal colours using images found on the GBIF herbarium site, iNaturalist and botanical garden images. R version 4.0.2 was used to perform statistical analyses, including t-tests and ANCOVAs. The data



was simplified to comparisons between red and "not_red" (with "not_red" including the purple, yellow and white sepals).

Data on hummingbirds that lived in the same range as *Aquilegias* were collected the same way. It is assumed that this sample of hummingbirds is representative of the age of lineage of hummingbirds that pollinate *Aquilegias*.

Results

The phylogenetic tree revealed that the oldest lineages were purple flowers, nonred flowers, while the red flowers showed up more recently (Figure 1). The age of the red flowers compared to non-red flowers were considered to be significantly younger as the red flowers had a mean lineage age of 0.647 million years ago, while the mean of the non-red flowers was 1.03 million years ago (Two-sample t-test: t = 2.5239, df = 45.127, p-value = 0.01519). The differences in log EOO and log AOO mean ranges of red sepals were significantly larger EOO than non-red sepal species (Two-sample t-test: t = 31.119, df = 1, p-value = 0.02045), (Two-sample t-test: t = 15.356, df = 1, p-value = 0.0414). When we compare the red *Aquilegias* to hummingbird EOO, the results were not significant (Two-sample t-test: t = -1.329, df = 8.3016, p-value = 0.2192). However, hummingbird AOO was significantly larger than red *Aquilegias* (Two-sample t-test: t = -15.715, df = 10.834, p-value = 8.434 x 10-9).

A one-way ANCOVA was performed to analyze if there was any significant interaction between the age of the *Aquilegia* lineages and the EOO of red and other colours of *Aquilegias*. The two main effects of sepal colour and age of lineage was not significant, but the interaction between the two was significant. The relationship between EOO size and age depended on whether the species had red sepals (ANCOVA one-way: F = 2.99, df = 3, 46 p = 0.041). The EOO was higher for the red *Aquilegias* compared to the other colours. There were no significant interactions with the AOO ANCOVA test between age of lineage and the sepal colour. The relationship between AOO size and age did not depend on the sepal colour (ANCOVA one-way: F = 1.65, df = 3, 46, p = 0.19). The scatterplots show a summary where the red lineages are younger than purple lineages, but younger red lineages did better than non-red lineages as a whole (Figure 4, 5).

Significance

The results of the geospatial analysis demonstrated with the ANCOVA that the sepal colour had a significant interaction with age of lineage when focused on EOO. For



EOO, the interaction between age and sepal colours was significant, with the red sepal flower species having a larger range than non-red ones. This may suggest that hummingbird pollinators have an effect on speciation and preferentially pick red flowers. Having hummingbird pollinators may also explain the larger range size as they would be able to maintain high gene flow between flower patches. As the t-test showed, *Aquilegias* with red sepals were younger than non-red sepal *Aquilegias* supporting that there was a factor that led to their diversification, which is likely the acquisition of hummingbird pollinators.

The scatterplots summarize the trend where red columbines are younger than non-red columbines (Figure 4, 5). This shows visual evidence that the different pollinator guilds cause a difference in lineage ages. The negative slope of the red columbines in both figures represents how older red flowers have smaller ranges than younger flowers. The hypothesis where younger lineages have smaller ranges for red flowers is rejected but is still viable for non-red flowers. Pollinators may be the predominant determiner for range size in this case.

The data suggest that pollinator types play a major role in species stability and range expansion. Using *Aquilegias* as a model organism can inform studies of plants with similar patterns of coevolution such as *Digitalis* which has become an invasive species in the United States. Researchers attributed this population growth to novel hummingbird pollinators (Mackin et al., 2020). For endangered flower populations, studying biological controls and pollinator interactions may be a potential avenue for population conservation. Introducing novel pollinators may encourage species stability.

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Figures



Figure 1. Phylogenetic tree from Timetree that shows the petal and sepal colour of the *Aquilegia* spp. The grey circles represent unknown colours as the pictures of the flowers could not be located. Each node in the tree has a time point that was used to estimate lineage ages.



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Figure 2. A box plot comparing non-red (purple, white, yellow), and red-sepal Aquilegias with their EOO area in ln km².



Figure 3. A box plot comparing non-red (purple, white, yellow), and red sepal Aquilegias with their AOO area in ln km².



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Figure 4. The scatterplot comparing the log EOO of the non-red and red Aquilegias while taking age of lineages in millions of years into account.



Figure 5. The scatterplot comparing the log AOO of the non-red and red Aquilegias while taking age of lineages in millions of years into account.



Obtaining body condition from photos: yay or neigh? Repeatability of horse body condition scoring from photos

Type of project: 530 Supervisor(s): Dr. Jocelyn Poissant and Dr. Kathreen Ruckstuhl Department: Ecosystem and Public Health, Faculty of Veterinary Medicine



About the Researcher

Ihncheol Jung

I am a 5th year zoology major, and my research interests are focused on wildlife and domestic animal health, and how the two interact and influence each other. As we increase our interactions with wildlife populations, we are faced with new public health and wildlife management challenges. This project on the Sable Island feral horses was a wonderful experience generating new knowledge that can benefit both wildlife, and domestic equine health. I aim to become a veterinary pathologist, working with both livestock and wildlife to manage public health, and protect our precious wildlife. This project has given me insight into how research on wildlife can benefit livestock, and the importance of understanding both animal physiology, and population level ecology in managing the challenges associated with wildlifelivestock interactions. This insight will help me guide my career towards becoming a veterinary pathologist. One of the main takeaways from my project was that stepping outside of our comfort zones and being willing to learn new skills can lead us to achieve much more than we may think we are capable of. I believe that although undergraduate research may feel daunting, students should trust in their abilities and give it a shot. It is a chance to uncover knowledge that no one has before as a real scientist. More importantly, it is a chance to challenge yourself and gain confidence in your scientific skills. Outside of science, I love to swim, travel, eat good food, watch Marvel movies, bake bread, and spend time with my family.

Background

Body condition is a measure of body fat on a numerical scale, with higher scores associated with greater amounts of body fat. Body condition scoring in horses and other livestock have many potential uses including weight management and maximizing



reproductive performance (Wildman et al., 1982; Roche et al., 2007; Hussein et al., 2013; Kenyon et al., 2014). Horse body condition has also been applied on feral populations to study evolutionary ecology and to manage population sizes (Gold et al., 2019; Debeffe et al., 2017; Ransom et al.,2010). Feral horses with higher body condition are associated with greater reproductive success (Debeffe et al., 2017) as well as lower parasite load. Body condition is therefore used as an indication of horse fitness in studying the evolution of reproductive strategies (Debeffe et al., 2017) and parasite resistance (Gold et al., 2019).

Horse body condition scoring is on a scale of 0, thin, to 5, obese, assessing the hips, ribs, and spine (Figure 32) through palpation and visual assessment (Carroll and Huntington, 1988). This system has adapted for photo assessment of body condition in multiple studies (Debeffe et al., 2016; Gold et al., 2019).

Assessing repeatability is crucial in determining whether photo body condition estimates can be used systematically and for communicating amounts of body fat amongst horse owners, veterinarians, and researchers. Repeatability of photo body condition estimates has been assessed in cows and goats, reporting good repeatability, but there are no comparable studies on horses (Ferguson et al., 2006; Vieira et al., 2015). Reliable body condition scores from photos would allow for remote health assessments by veterinarians and collect fitness data of feral horses through camera traps (Debeffe et al., 2017; Gold et al., 2019).

There are eight research questions with rationales that determine whether horse body condition can be obtained from photos, and what strategies should be used to obtain scores.

1. How do body regions vary in inter scorer repeatability? Some body regions may be harder to score than others from photos which can in part be due to inadequate reference photos in the scoring guide. This difference in scoring difficulty between body regions can cause differences in inter scorer repeatability.

3. What is the effect of view angle on body condition scores? Body regions may appear differently across view angles, which may bias the body condition scores.

4. What is the effect of view angle on inter scorer repeatability? Body condition may be harder to score from certain view angles, resulting in differences in inter scorer repeatability.

5. What is the effect of coat colour on body condition scores? Levels of body fat across body regions may appear differently depending on coat color which can bias the scores. There may also be actual differences in body condition between horses of different coat



colors.

6. What is the effect of coat colour on inter scorer repeatability? Some body regions may be harder to score depending on the coat colour, which can cause differences in inter scorer repeatability.

7. What is the effect of sex on inter scorer repeatability? The musculature of male horses may cause some scorers to overestimate body condition, which would reduce inter scorer repeatability compared to females.

8. How consistent are scorers when scoring the same photos multiple times? Vagueness in the scoring guide, or lack of training may result in differences between scores on the same photos, by the same scorer.

Methods

800 photos of 200 adult Sable Island feral horses consisting of 100 males, and 100 females, each with 50 dark and 50 light coloured horses were selected from an archive of photos collected from 2016 to 2019. The Sable Island horses are an unmanaged feral horse population that have not received human care since the 1960s allowing for a wide range of body conditions to be possible. For each horse, lateralanterior, lateral, lateral-posterior and posterior view photos were selected. Lateral photos were those taken near perpendicular to the anterior-posterior axis of the animal. Posterior photos were those taken from directly behind the animal such that a near equal proportion of the left and right sides of the hips were visible. Lateral-anterior photos were at an angle between directly in front of and side of the animal. Lateral posterior photos were those at an angle between directly behind and side of the animal.

Six individuals in Dr. Poissant's lab used the online platform "Zooniverse" to score the 800 photos twice across two rounds of scoring, separated by a minimum of 7 days to ensure scores from the two rounds are independent of each other. The scorers practiced on photos of Sable Island horses until they felt comfortable using the body condition scoring system (Figure 1). The ribs, spine, and hips were scored separately such that the scorer must finish scoring the ribs for all 800 photos, before moving onto the spine, then the hips to reduce bias in body region scores from one another. Photos were presented in random order one at a time.

Six mixed linear models were created to explain the variation in male and female rib, spine, and hip body condition scores. The fixed effects included in the model were round, color, scorer, angle, as well as each of the interactions between them. The random effects of horse ID and photo ID were included to account for the nonindependence of scores from the same horse and photo. To measure repeatability, the



intraclass correlation coefficient (ICC), which is on a scale from 0 to 1, with 1 representing perfect repeatability, was used. A mixed linear model that describes the variation in ICC values calculated for each photo group based on sex, color, angle, and body region was created. The model included sex, color, angle, body region, round, and the interactions as fixed effects. It included photo group ID as a random effect to account for the non-independence of ICC values from the same photo group calculated in rounds 1 and 2. ANOVA analyses were conducted to determine significant relationships. The models were simplified using Akaike Information Criterion model selection, and only the top model explaining variation in body condition scores or ICC values were used to generate plots of significant fixed effects and interactions.

Results

The ribs had higher ICC values compared to the spine and hips across all view angles (Figure 2). The lateral anterior view generally had lower body condition score across all body regions for both sexes when variation due to all other variables are accounted for (Figure 3, Figure 4, Figure 5). The lateral, lateral posterior, and posterior views were generally closer in score, with model predictions within 0.5 of each other (Figure 3, Figure 4, Figure 5). The lateral view yielded the lowest ICC values for the hips and spine at around 0.4 when variation due to all other variables are accounted for but yielded a higher ribs ICC value of around 0.75 (Figure 2). The lateral anterior view resulted in the highest ICC values among the view angle for all body regions at around 0.6 for the hips and spine, and 0.75 for the ribs (Figure 2). The lateral posterior view vielded moderate ICC values around 0.5 and 0.6 for the ribs, spine, and hips (Figure 2). The posterior view yielded an ICC value of around 0.55 for the hips (Figure 2). Dark males had higher scores compared to light males across all body regions by around 0.5 when variation due to other variables are accounted for, while the reverse was observed in females with light females having higher scores compared to dark females (Figure 6, Figure 7). Light coat colour was associated with lower ICC values compared to the dark coat colour across all body regions when other variables are controlled for (Figure 8). The difference in ICC values between coat colours was larger in the ribs and spine, at around 0.1 for the ribs, and 0.15 for the spine (Figure 8). Dark males had lower ICC compared to females and light males by around 0.3 when other variables are controlled for (Figure 9). Generally, at least one scorer and usually 2 scorers had differences in scores between rounds 1 and 2 at around 0.4 across the body regions for both sexes when other variables are controlled for (Figure 10, Figure 11, Figure 12).



Significance

This study has revealed which variables are causing variation in body condition scores and inter scorer repeatability, and that intra scorer repeatability can vary across scorers. The higher inter scorer repeatability of the rib scores from all view angles suggests that they can be used as reliable body condition data. The lower inter scorer repeatability of the hip and spine scores suggests that the benefit of improving accuracy when using all body region scores, may not outweigh the efforts needed to account for scorer biases such as training new scorers to match previous scorers. The general trend of lateral anterior view underestimation emphasizes the importance of accounting for view angle biases. Also, scorer biases evident in the interactions between score and view angle need to be accounted for when generating body condition data. If these biases are accounted for, a single scorer is sufficient in generating accurate scores, although score averaging from multiple scorers would improve precision as there is considerable score variation around model predictions. It is important to note that biases are specific to a group of scorers. Therefore, scores from one group cannot be directly compared to scores from another group. The reversed effect of color on body condition scores between males and females suggests that the difference in scores may be reflecting actual body fat differences instead of coat colour biasing scores. Dark coat colours being associated with higher repeatability than light colours may be due to more variation in subtle colour patterning in light horses compared to dark horses, which have more uniform colorations resulting in more similar scores across scorers. Repeatability of dark horses being lower than females and light males may be due to reduced visibility of musculature being confused for body fat in dark horses, resulting in more score variation across scorers. Varying intra scorer repeatability across scorers suggests that it is important to select for consistent scorers. The study findings can be used to implement training protocols and scoring guide revisions to improve both inter and intra scorer repeatability for accurate and precise body condition scores. Future studies should focus on further developing body condition scoring guidelines, which would make photo derived body condition a valid assessment and correlate of body fat.

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The BioScientific Undergraduate Research Publication

Figures

Ribs



0. Skin hangs tightly over ribs



1. Prominent ribs with little fat



2. Ribs barely visible



3. Smooth ribs

4



4. Ribs ripple with fat





0. Prominent and angular spine



1. Prominent but smooth spine



2. Spine apparent with small ridge



3. Flat back, no ridge/gutter



4. Slight gutter on back

Hips



0. Skin tight over angular hips. Very concave sides



1. Flat hips visible points. Slightly concave sides



2. Points barely visible. Flat sides.



3. Round hips and sides. Points not visible.



4. Round hips with 'gutter'

Figure 1. Horse (*Equus caballus*) body condition scoring guide adapted from the scale described in Carroll and Huntington (1988). The scale is from 0 to 4, for assessment of three body regions: ribs, spine, and hips. Reference photos are of Sable Island feral horses.





Figure 2. Mixed linear model predictions of Sable Island feral horses (*Equus caballus*) body condition score inter-rater reliability ICC values across hips, ribs, and spine for lateral, lateral anterior, lateral posterior, and posterior views. Red represents lateral, green represents lateral anterior, blue represents lateral posterior, and purple represents posterior view angles. Black dots represent individual ICC values after controlling for variation due to other fixed effects, and photo group random effect.







Figure 3. Rib body condition scores of female adult Sable Island feral horses (*Equus caballus*) from 2016 to 2019 across view angles. Black dots represent jittered individual rib scores of photos that fit its respective category. The blue lines represent the mixed linear model predictions, with no changes applied to the other variables. Changes in predicted scores are significant across view angles.



Figure 4. Spine body condition scores of male adult Sable Island feral horses (*Equus caballus*) from 2016 to 2019 across view angles. Black dots represent jittered individual rib scores of photos that fit its respective category. The blue lines represents the mixed linear model prediction, with no changes applied to the other variables. Changes in predicted scores are significant across view angles.





angle

Figure 5. Hip body condition scores of female adult Sable Island feral horses (*Equus caballus*) from 2016 to 2019 across view angles. Black dots represent jittered individual rib scores of photos that fit its respective category. The blue lines represents the mixed linear model prediction, with no changes applied to the other variables. Changes in predicted scores are significant across view angles.





Figure 6. Rib, spine, and hip body condition scores of male adult Sable Island feral horses (*Equus caballus*) from 2016 to 2019 across coat colours. Black dots represent jittered individual rib scores of photos that fit its respective category. The blue lines represent the mixed linear model prediction, with no changes applied to the other variables. Changes in predicted scores are significant across colour.





Figure 7. Rib, spine, and hip body condition scores of female adult Sable Island feral horses (*Equus caballus*) from 2016 to 2019 across coat colours. Black dots represent jittered individual rib scores of photos that fit its respective category. The blue lines represent the mixed linear model prediction, with no changes applied to the other variables. Changes in predicted scores are significant across colour.





Figure 8. Mixed linear model predictions of Sable Island feral horses (*Equus caballus*) body condition score inter-rater reliability ICC values across hips, ribs, and spine for dark and light horses. The model controlled for variation due to other fixed effects and the photo group random effect. Red represents dark horse body parts, while blue represents light horse body parts. Black dots represent individual ICC values.





Figure 9. Mixed linear model predictions of Sable Island feral horses (*Equus caballus*) body condition score inter-rater reliability ICC values of dark and light horses across females and males. The model had the fixed effect of photo group. All other variables other than sex, and colour were kept constant, and their variation was accounted for. Red represents dark horses, and blue represents light horses. Black dots represent individual ICC values





Figure 10. Male rib scores of Sable Island feral horses (*Equus caballus*) for rounds 1 and 2 across six scorers. Black dots represent individual rib body condition scores. Red represents mixed linear model prediction scores for round 1 across scorers, and blue represents model prediction scores for round 2 across scorers. The mixed linear model had individual horses and horse photos as random effects. No other variables other than scorer and round were manipulated.





Figure 11. Female spine scores of adult Sable Island feral horses (*Equus caballus*) for rounds 1 and 2 across six scorers. Black dots represent individual rib body condition scores. Red represents mixed linear model prediction scores for round 1 across scorers, and blue represents model prediction scores for round 2 across scorers. The mixed linear model had individual horses and horse photos as random effects. No other variables other than scorer and round were manipulated.





Figure 12. Male hip scores of adult Sable Island feral horses (*Equus caballus*) for rounds 1 and 2 across six scorers. Black dots represent individual rib body condition scores. Red represents mixed linear model prediction scores for round 1 across scorers, and blue represents model prediction scores for round 2 across scorers. The mixed linear model had individual horses and horse photos as random effects. No other variables other than scorer and round were manipulated.



The buzz about Bombus rufocinctus: Examining colour patterns across Alberta, Canada

Type of project: 528/530

Supervisor(s): Dr. Mindi Summers & Dr. Paul Galpern

About the Researcher Taylor Ford-Sahibzada

My name is Taylor Ford-Sahibzada and I am going into my 5th year of Zoology at the University of Calgary. I have always loved animals and throughout my degree I have taken various courses that have elevated my interest in animals and ecosystems. Outside of science I enjoy playing competitive women's soccer, working at the Calgary Farmyard where I take care of farm animals, and volunteering at a dog and cat rescue called Megan's Rescue Efforts Society.

Throughout my studies I have had various opportunities to perform zoological research. This includes working with Dr. Mindi Summers on a plant-pollinator research project during the summer of 2021, my 528/530 project on the distribution of Bombus rufocinctus colour morphs across Alberta, gathering and curating an insect collection for an entomology course, conducting various literature reviews, and learning about various animal behaviour and mammalogy field methods. These experiences have been very important to my professional and academic development and have helped me gain insight as to whether or not I would like to pursue a Master's program and/or a career in zoological research, which is the main reason I got involved in undergraduate research. If you are a student who wants to get involved with research, the most important thing to do is to make yourself known to professors who you may want to work with by attending their office hours, class representative meetings, asking/answering questions during class, and ensure that you express to them your interest in getting into research.

Background

Bumble bees are in the genus *Bombus* and are important pollinators that are native to Alberta, Canada (Williams et al., 2014; Neame et al., 2021). *Bombus* species are often differentiated based on the colour of the hair that covers their bodies (Williams et al., 2014; Neame et al., 2021). This hair is called pile, and pile colour patterns are often conserved within *Bombus* species (Williams et al., 2014; Neame et al., 2021). Colour patterns that repeat throughout a species are called colour morphs. The presence of colour morphs is due to selection pressures which favour the survival of bees with certain colour patterns over others (Briolat et al., 2019). *Bombus* species are



often monomorphic or dimorphic, meaning that they have one or two colour morphs throughout their species respectively (Badejo et al., 2020; Briolat et al., 2020). Despite its common name, the red-belted bumble bee, *Bombus rufocinctus* is inconsistent with other *Bombus* as they have a large range of colour options that occur in varying patterns, thus *B. rufocincus* is referred to as a polymorphic species (Plowright & Owen, 1980; Owen & Plowright, 1988). Previous research has found connections between *Bombus* colour patterns and geography and various reasons have been suggested to explain the correlation, however studies on the colour patterns of Alberta *Bombus rufocinctus* have not yet been conducted (Ezray et al. 2019; Williams, 2007; Plowright & Owen, 1980).

In this study, the colour patterns of *Bombus rufocinctus* are catalogued using a simple colour system to determine if there are common colour morphs that are connected to regionality in Alberta, Canada. Due to the fact that previous research has catalogued distinct colour morphs of *B. rufocinctus* and the colour patterns of *Bombus* have been found to be regionally grouped, it is predicted that there will be distinct morphotypes of *Bombus rufocinctus* in Alberta that are clustered across the province. I therefore hypothesize that there are common *Bombus rufocinctus* colour morphs in Alberta that are regionally grouped because of environmental differences.

Methods

Methods were designed in collaboration with a partner study that examined colour patterns across 10 Alberta *Bombus* species (Archibald, 2022). Bumble bee sampling occurred as part of a separate study conducted in June, July, and August between 2015 and 2018 (Vickruck et al., 2019; Purvis et al., 2020; Galpern et al., 2021). *Bombus* individuals were continuously collected using blue vane traps located in 330 sites across Alberta, pinned, and identified (Vickruck et al., 2019; Purvis et al., 2020; Galpern et al., 2020; Galpern et al., 2021). The sites were grouped into seven broad regions, South of Calgary, Kinsella, Duchess, Grande Prairie, Vauxhall-Taber, Red Deer, and Claresholm (Figure 1) (Vickruck et al., 2019; Purvis et al., 2020; Galpern et al., 2021). *Bombus rufocinctus* individuals were haphazardly collected from this collection for my study. The colour patterns of 502 *B. rufocinctus* individuals from 155 sites across Alberta were catalogued (Figure 1).

Colour patterns of *Bombus rufocinctus* individuals were scored using a simple letter system. Each part of the bee was visually sectioned into 27 parts and assigned a letter code after being examined under an Infinity 1, Olympus SZ61 Microscope and a Cole Parmer light (Figure 2). Five colour code options were used in this study, orange



(O), yellow (Y), black (B), mixed (M, interspersed black and yellow hairs), and brown (R). Each section of the bee was assigned a letter colour code as follows. One letter was recorded for a section containing only one colour (ex. a yellow section = Y). Two letters separated with a slash were recorded for a section with a >50% majority colour and a <50% minority colour. (ex. major black, minor orange = B/O). Lastly, if a section had an even split of two colours, two uppercase letters with no slash were recorded (ex. YB).

To test for distinct colour morphs within the data an R coding software (R version 4.0.3) called Allelematch was used (Galpern et al., 2012). To test for correlations between colour patterns and geography I used an R coding software (R version 4.0.3) called MEMGENE (Galpern et al., 2014). I conducted a general additive model (GAM) with a Gaussian error distribution using R coding software (R version 4.0.3) to assess the relationship between colour indices and latitude (Wood, 2017). The analysis tested the percentage of a single colour (either black, yellow, or orange) on each bee to determine if there were significant differences (p < 0.05) when tested with latitude.

Results

Running allelematch on the data (discluding bees with missing colour data) rendered no distinct colour morphs within the Alberta *B. rufocinctus* population. Instead, allelematch revealed that there were 155 unique colour patterns, none of which were repeated consistently (n = 269), thus there were no distinct colour morphs within the data.

The MEMGENE analysis examined the entire colour pattern on each bumble bee and concluded that 1% of the variation in *B. rufocinctus* colour patterns was significantly explained by latitude (Figure 3) (Galpern et al., 2014).

The colour indices analyses revealed that the variation in the mean percentages of black, yellow, and orange colouration on each bee was found to shift significantly with latitude. The GAM model with a Gaussian error distribution uncovered that 5.07% of the deviance in mean black colouration was significantly explained by latitude, thus when going from the southern to northern parts of Alberta, *Bombus rufocinctus* individuals have a larger amount of black colouration (Wood, 2017) (f=6.23, P < 0.001: Figure 4). 2.30% of the deviance in mean yellow colouration was significantly explained by latitude, thus when going from the southern to northern parts of Alberta, *Bombus rufocinctus* individuals have a lesser amount of yellow colouration (Wood, 2017) (f= 2.57, P < 0.001, Figure 4). Lastly, 1.68% of the deviance in mean orange colouration was significantly explained by latitude, thus when going from the southern to norther parts of the deviance in mean orange colouration was significantly explained by latitude, thus when going from the souther to norther parts of the deviance in mean orange colouration was significantly explained by latitude, thus when going from the souther to norther parts of the deviance in mean orange colouration was significantly explained by latitude, thus when going from the souther parts of the deviance in mean orange colouration was significantly explained by latitude, thus when going from the souther to norther parts of the deviance in mean orange colouration was significantly explained by latitude, thus when going from the souther to norther parts of the deviance in mean orange colouration was significantly explained by latitude, thus when going from the souther to norther parts



of Alberta, *Bombus rufocinctus* individuals have a lesser amount of orange colouration (Wood, 2017) (f= 1.81, p = 0.04: Figure 4).

Significance

This research project revealed previously undiscovered information about Alberta Bombus rufocinctus bumble bee colour patterns. It was found that these bees did not have distinct colour morphs but their colour patterns did correlate with latitude. Two main reasons were proposed for the lack of distinct colour morphs, relaxed selection and imperfect mimicry (Chittka & Osorio, 2007; Kikuchi, D. W., & Pfennig, 2013; Clusella-Trullas & Nielsen, 2020). MEMGENE found that one percent of the variation of B. rufocinctus colour patterns was explained by latitude. This is a very small trend which means that human perception is unlikely to identify it, however this is an intriguing result as it suggests that there is some sort of biologically significant mechanism driving the shift in colour patterns that we have not yet discovered. A limitation of MEMGENE is that it does not allow the visualization of the correlation between colour patterns and latitude - it just tells us that the correlation exists. To try to visualize how *B. rufocinctus* colour patterns shift with latitude, I analyzed the colour indices of each bee and found significant shifts with latitude. Potential reasons for the correlations between colour patterns and colour indices with latitude include varying predator populations, imperfect mimicry with different models, thermoregulation, and habitat differences (Williams, 2007; Duennes et al., 2012; Rapti, 2014; Ding, 2019; Ezray et al., 2019; Briolat et al., 2019; Badejo et al., 2020). There is plenty of future work to be conducted on this data set as well as on each of the factors that were proposed to be impacting *B. rufocinctus* colour patterns. Bumble bee numbers are declining, with many species being listed as vulnerable or critically endangered, thus any research conducted on these animals is important to help bring awareness and encourage their conservation (Van Hook, 1997; Cameron et al., 2011; Clusella-Trullas & Nielsen, 2020). In addition, bumble bees are generally well-liked insects among the public, and the novelty of the colourful Bombus rufocinctus means that it can potentially act as a charismatic species to promote insect and pollinator conservation as a whole, thus it is important to continue researching these fascinating insects (Van Hook, 1997).

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Figure 1. (A) Pie chart of the number of *B. rufocinctus* individuals collected from seven regions in Alberta including Vauxhall-Taber, Grande Prairie, Claresholm, Red Deer, Duchess, Kinsella, and South of Calgary. (B) Map of *B. rufocinctus* individuals collected in Alberta overlaid with rough outlines of the seven regions used in this study.



Figure 2. Representation of the sectioning of *Bombus rufocinctus* individuals. Each bumble bee was visually sectioned into 27 parts, the face, the head, the thorax split into three sections (scutum, inter-alar space, and scutellum), and the abdomen split into six tergites. The thorax and abdomen were further split into a left, middle, and right section excluding the sixth tergite because it was too small to split.





Figure 3. MEMGENE1 plot showing the variation in Alberta *Bombus rufocinctus* colour patterns explained by latitude. Points are coloured by their MEMGENE1 scores. MEMGENE1 scores closer in value represent colour patterns that are more similar. (Galpern et al., 2014).





Figure 4. Scatterplots showing the colour indices for black (A), yellow (B), and orange (C) plotted against latitude. Each point represents the percentage of colour on a single bee collected from one of Grande Prairie, Vauxhall-Taber, Claresholm, South of Calgary, Duchess, Red Deer, or Kinsella. The red line represents the mean colour index per latitude. Analysis was conducted using a general additive model (GAM) with a Gaussian error distribution (Wood, 2017).

Where to find out more about the project?



If you'd like to learn more about this project, please email me at <u>taylor.fordsahibzada@ucalgary.ca</u> and I can send you my full paper and answer any questions that you may have.



Hemiparasitism by Castilleja (Paintbrush) species influences plant community diversity

Type of project: 530 Supervisor(s): Dr. Jana Vamosi

About the Researcher Zoe Penno



I am in my fifth year and will graduate from the U of C in Spring of 2022 with a degree in Biological Sciences (Honours). This research along with other research projects completed during my undergrad have inspired me to pursue research further through graduate studies and possibly a research career. My main topics of interest are wildlife biology, conservation biology, and evolutionary biology. I plan to keep my topics of study broad as I am interested in a whole-picture approach. Growing up in Alberta with the Canadian Rockies in my backyard fueled my interest in the natural world and has given me the initiative to protect it. With experience in Indigenous studies and ethnobotany throughout my undergraduate, I hope to integrate Indigenous voices and an Indigenous pedagogy into my scientific research. As I am a lifelong explorer, this research reinforced my desire to ask questions, seek answers, and explore biology topics through science.

Background

Castilleja, commonly known as 'Red paintbrush' is a hemiparasitic plant native to Alberta and other Western parts of North America. Previous research suggests hemiparasites such as *Castilleja* have the potential to influence community composition; more specifically, *Castilleja* has the ability to increase local biodiversity in terms of



species richness and species evenness (Reed, 2012; McKibben & Henning, 2018). Through hijacking the nutrient and water supply of the host while also competing for light and environmental nutrients -- hemiparasites reduce host growth and survivability. Based on this knowledge of fitness reduction as caused by parasitism and the results of previous studies I predicted that the parasitic interactions of *Castilleja* species will increase local biodiversity where present.

Methods

A dataset was sourced from Alberta Biodiversity Monitoring Institute (ABMI) which included observations of terrestrial, vascular plants located within 1656 sites as part of a systematic grid. Data was collected during the second visit to the ABMI site (rotation 2) between 2015 and 2018. The data showed *Castilleja* present in 22 sites across 3 different *Castilleja* species: *Castilleja Lutescens, Castilleja miniata,* and *Castilleja raupii* (Figure 1). Sites with at least one observation of the genus *Castilleja*-absent. A site deemed *Castilleja*-present was paired with the next closest site that was Castilleja-absent.

Species richness was determined by comparing species counts between *Castilleja*-present and *Castilleja*-absent sites. A paired-t-test was conducted using statistical software R and R studio (R Core Team 2020).

Species evenness was determined by first creating a proxy for species abundance as the ABMI data only shows species as being present, common, or dominant. Species evenness for each site was determined using the Probability of Interspecific Encounter (PIE) which quantifies the probability, if you pick two species from a sample randomly, they will be two different species (Hurlbert 1971). The PIE measured for each site ranges from 0, perfectly uneven, to 1, perfectly even. PIE was compared between *Castilleja*-present sites and *Castilleja*-absent sites using a paired t-test in R and R studio (R Core Team 2020).

Results

Species Richness

Between sites there was no significant difference within species richness of *Castilleja* present sites versus *Castilleja* absent sites (t = 1.5836, df = 21, p-value =



0.1282). There was a trend of greater species richness in *Castilleja*-present sites when compared with *Castilleja*-absent sites with a mean difference of 12.86 and a 95 percent confidence interval -4.029174 29.756446 (Figure 2).

Species Evenness

Between sites there was no significant difference within the PIE value of *Castilleja* present sites versus *Castilleja* absent sites (t = 1.2372, df = 14, p-value = 0.2364). There was a trend of a greater PIE value in *Castilleja*-present sites when compared with *Castilleja*-absent sites with a mean difference of 0.08 and a 95 percent confidence interval -0.05915349 0.22041422 (Figure 3).

Significance

The purpose of this study was to investigate if the presence of *Castilleja* species had an effect on species diversity in specific Albertan ecosystems. Species richness and species evenness show trends towards increases in both richness and evenness in *Castilleja* containing plots, however neither was significantly significantly different in plots that contained *Castilleja* versus plots absent of the genus. Given that the data set used to conduct this research was not collected for the intent of this specific research, therefore not optimal with regards to standardizing *Castilleja* individuals present, the trends found are promising to conduct further studies on the topic. In order to have more certainty with regards to *Castilleja* presence contributing to changes in community composition, repeating the experiment with a standard density of individuals per site may allow for more informative results. Alternately, conducting the same experiment on a smaller scale may give us more confidence that the hemiparasitic individuals are in fact interacting with other plant species in the site.

More research on *Castilleja* species, specifically determining its behaviour as a generalist or specialist, host preference, in what environments, and at what abundance may give us more insight into how and when the hemiparasite can influence community diversity. Understanding the ways in which hemiparasites such as *Castilleja* have an effect on plant community composition can inform conservation and ecological restoration efforts and remind us that there are important studies to be done about plant interactions – especially on the topic of these underground hemiparasitic relationships within plant ecosystems.



References

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Figures



Esri, USGS | Esri, HERE, Garmin, FAO, NOAA, USGS, EPA, NRCan, Parks Canada

Figure 1. General locations of *Castilleja*-present (blue) and *Castilleja*-absent (red) sites across Alberta with a random distance and directional offset of up to 4 km.





Figure 2. Box-plot for visualization of species richness (number of species) for sites containing at least one *Castilleja* individual present and sites absent of the genus. (N=22)





Figure 3. Box-plot for visualization of species evenness as quantified by Probability of Interspecific Encounter for sites containing at least one *Castilleja* individual present and sites absent of the genus. (N=15)

Where to find out more about the project?

Raw Vascular Plants dataset from Rotation 2 (2015-2018 inclusive) from the Alberta Biodiversity Monitoring Institute was used, in whole or part, to create this project. More information on the Institute can be found at: http://www.abmi.ca.



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