Estimating heritability of pest resistance in forest trees: exploring potential biases from methodological and ecological factors

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Abstract

Heritability describes the proportion of phenotypic trait variance attributed to genetic effects, and is used to identify traits likely to respond to selection. Selectively breeding for increased pest resistance is often appealing to plant breeders. Because resistance is a multifactorial trait (influenced by many host attributes) and cannot be measured directly, to estimate its heritability researchers must select a proxy variable that captures variance in resistance among individuals. Frequently, this proxy is quantified on a non-Gaussian (i.e., non-normal) scale, which can be problematic because non-normal data do not strictly meet the assumptions of the linear models traditionally used to estimate heritability. As a result, non-normal data are either transformed to fit the model or a generalized linear mixed effect model (GLMM) – which can accommodate non-normal data – is used instead. In this thesis, I identify and compare the common methods used to estimate heritability of pest resistance in forest trees. From the literature, no clear evidence suggests that the scale of the proxy or choice of statistical method has a strong influence on estimates. However, my analysis of field data suggests that choice of statistical model for a percent trait can influence estimates of heritability. With field data, the true value of heritability is unknown, making it impossible to determine which model produces a more accurate estimate. As such, to determine how choice of methods (scale and modeling technique) bias estimates of heritability I used data simulations and found that GLMMs can dramatically underestimate heritability.

Heritability of pest resistance has an additional level of complexity, as its
variance is affected by factors influencing expression in the host and factors influencing the pest population (e.g., fluctuations in pest density or distribution). Using data simulations, I examine how changes in pest densities or predictable environmental patterns in pest distribution, such as edge effects, influence estimates of heritability. I find that pest density and heritability have a parabolic relationship and heritability estimates are strongly reduced when pest damage is influenced by edge effects.

Taken together, this thesis contributes toward a better understanding of the factors that contribute to variation in estimates of heritability of non-normal traits. Overall, the data presented within, will help breeders and ecologists better estimate and interpret estimates of heritability, allowing them to make more accurate predictions about how traits will respond to selection.
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We use quantitative genetics to determine the extent to which phenotypic variation in the expression of a continuous trait is influenced by genetics. Quantitative traits are polygenic, meaning they are under the influence of multiple genes at different loci, and are expressed on a continuous scale or have an underlying continuous distribution (Falconer and Mackay 1996). Quantitative traits are often important to animal and plant breeders: many desirable traits (e.g., growth, milk production, fruit production etc.) are polygenic and knowing the degree to which phenotype is determined by genetics helps breeders estimate possible gains that could be achieved through selective breeding. Much of the research in quantitative genetics is related to breeding programs, but the general principles also apply to natural populations and are used to study selection (and evolution) of traits in the wild.

Classical genetic theory – reviewed in Falconer and MacKay (1996) – states that phenotypic variance \( V_P \) of quantitative traits can be broken down into two general components, variance from genetic effects \( V_G \) and variance from environmental effects \( V_E \).

\[
V_P = V_G + V_E
\]  

Total genetic variance is generally thought of as a sum of three key components. First is additive genetic variance \( V_A \) that captures variance attributed to the
inheritance of alleles, each having a direct (and additive) effect on the phenotypic expression of the trait. Second is genetic variance from dominant alleles \((V_D)\), a dominant allele has a strong phenotypic effect, which conceals the presence of other alleles. Lastly is genetic variance caused by interactions between genes, or epistatic variance \((V_I)\).

\[
V_G = V_A + V_D + V_I
\]  

(1.2)

Heritability is a dimensionless measure used to describe the proportion of phenotypic variance that can be attributed to genetic effects. Heritability in the broad-sense \((H^2)\) estimates the extent to which an individual’s genotype influences phenotypic expression.

\[
H^2 = \frac{V_G}{V_P}
\]

(1.3)

Estimates of broad-sense heritability are used when individuals are clonally replicated from a parent (e.g., clonal rhizome reproduction in plants) because cloned individuals have identical alleles and share all sources of genetic variance \((V_A, V_D, \text{and } V_I)\). Since most populations are composed of related individuals, which share only a portion of common alleles, it is more common to find heritability estimated in the narrow sense \((h^2)\). Narrow-sense heritability estimates the proportion of phenotypic variance attributed to additive genetic effects.

\[
h^2 = \frac{V_A}{V_P}
\]

(1.4)

Only the additive genetic effects are used because narrow-sense heritability is used to model how a sexually reproductive population would respond to selection at the individual level. Dominant genetic effects and epistatic genetic interactions are not selected for at this level and are therefore not included in estimates of narrow-sense heritability (Hartl and Clark 1997).
Though heritability estimates can be very useful, they can be difficult to interpret and it’s important to understand their meaning and limitations. An estimate of heritability describes a particular population at a given point in time in a particular environment and the parameter should not be generalized to other populations. Heritability depends on both genetic and environmental sources of variation and both of these parameters can vary among and within populations. Genetic variation is dependent on allele frequency, which is not necessarily similar among populations and can change within a population over time. Environmental variance is influenced by many different factors including climate, quality and quantity of resources, competition, predation and herbivory. As such we would expect to find different levels of environmental variation among populations experiencing different conditions or within a population over time. There is also the added complication that arises when genetic effects change in response to environmental conditions and organisms show phenotypic plasticity in response to environmental conditions ("genotype by environment interactions"). Interactions between genetic and environmental effects may change the magnitude of genetic effects or change the ranking of genotypes (see Hoffmann and Merilä 1999). Despite this, estimates of heritability are useful as they help breeders identify traits that are likely to respond to selection and can be a useful indicator of response to selection when derived from experiments that are adequately replicated in time and space (Dudley and Moll 1969; Talbert 1992). Additionally, genetic improvement programs can be a cost-effective method that benefits producers (e.g., Ledig and Porterfield 1981; Talbert et al. 1985; Conington et al. 2004; Gjedrem et al. 2012).
Heritability of pest resistance:

Selectively breeding for increased pest resistance is appealing to plant breeders because it helps reduce pest damage and can decrease the need for pesticides. Applying pesticides is generally considered a cost-effective method for pest management, as the costs of application are outweighed by the product gains (see Pimentel et al. 2005). However, there are many indirect costs to pesticide use. There is extensive literature on the indirect cost of pesticide use and how pesticides can negatively impact human health, wildlife, and ecosystems (see Pimentel et al. 1992; Freemark and Boutin 1995; Wilson and Tisdell 2001; Pimentel et al. 2005). Government regulations have limited the availability of many pesticides out of concerns for human health and the environment but consumers are still pressuring farmers to further reduce their use (Loureiro et al. 2002). Incorporating genetically based resistance into breeding strategies can naturally reduce pest damage and decrease the need for pesticides, making it beneficial from both an economic and environmental perspective (Eigenbrode and Trumble 1994; Reinert et al. 2003; Molinari 2011).

Despite the advantages, host plant resistance is often underutilized as a method of pest management (Eigenbrode and Trumble 1994). In forest trees, breeding programs often focus on improving traits related to growth and wood quality (Carson and Carson 1989; White et al. 2007; Li et al. 2017), and few programs actively select for traits related to resistance (Larsson 2002). In general, life history theory predicts that selection for resistance in trees is likely to limit investment in other traits, such as reproduction, maintenance and growth (Stearns 1976). Potential tradeoffs between resistance and growth rate are of particular concern to tree breeders as the economic costs of slow growth could easily exceed the potential benefits of reduced herbivory. Several recent studies, however, suggest
that selection for resistance does not always entail a sacrifice of vigorous growth. For instance, resistance against white pine weevil (*Pissodes strobi* (Peck)) in interior spruce (a hybrid of *Picea glauca* (Moench) Voss and *Picea engelmannii* Parry) is positively related to tree growth rate (King et al. 1997). Additionally, DeHayes (1981) found that resistance in balsam fir (*Abies balsamea* (L.) Mill) to balsam twig aphid (*Mindarus abietinus* Koch.) is unrelated to tree height, suggesting that resistance in this instance is unrelated to tree growth rate. Tree breeding for resistance against herbivory will likely appeal most in systems where the relationship between host resistance and growth rate is either neutral or positive, or in systems where trees are largely valued for their aesthetics (such as urban landscaping or Christmas tree production).

I start this thesis by testing the hypothesis that resistance to the balsam twig aphid is a heritable trait in balsam fir trees. Balsam fir trees are commonly used as Christmas trees and for holiday greenery, which are products highly valued for their aesthetics (Chastagner and Benson 2000). Selectively breeding for increased resistance could help reduce unsightly pest damage in balsam fir Christmas tree plantations. In Chapter 2, I estimate heritability of aphid resistance in two populations of balsam fir trees over four consecutive years. Few studies have been published on heritability of insect resistance in coniferous trees and our study is unique because we examined the same individual trees over time, whereas most studies examining heritability of resistance are replicated in space but not in time. Estimating resistance over time is important because defenses (whether constitutive or induced) can draw heavily on available carbon stores (see Carson and Carson 1989) and if increased resistance in one year comes with a cost of increased susceptibility for subsequent attacks genetic gains would not be appreciable to growers.
In Chapter 3, I ask what methods are commonly used to estimate heritability of resistance, testing the hypothesis that methodological effects could influence estimates of heritability. Host resistance is likely due to a combination of different morphological and biochemical mechanisms (see Hanover 1975; War et al. 2012). For example, in conifers resistance to white pine weevil damage has been linked to different mechanisms including density of resin canals (Alfaro et al. 1997; Tomlin and Borden 1997), leader morphology (Tomlin and Borden 1994), chemical defenses (Alfaro et al. 1980; Tomlin et al. 1996, 2000) and host phenology (Hulme 1995; Alfaro et al. 2000). Because host plant resistance is a multifactorial trait it cannot be measured directly, and often signs or symptoms of pest damage are used as a proxy variable to quantify differences in resistance among individuals. Following the central limit theorem, which states that the sum of a large number of independent random quantities will converge to a normal distribution, it is assumed that resistance will follow a normal distribution as it is influenced by many genes and environmental effects (see Hartl and Clark 1997). Interestingly, we often find that proxy variables used to represent resistance are expressed or quantified on non-normal (i.e., non-Gaussian) scales (e.g., binary, binomial, or Poisson). Traditionally, variance components used to estimate heritability are found using linear models – analysis of variance (ANOVA) or linear mixed effect models (LMMs) – although recently generalized linear mixed effect models (GLMMs) have been gaining in popularity. Chapter 3 of this thesis is a review of the literature on pest resistance in forest trees where I determine how pest resistance is typically measured and test for effects of methodological choices (i.e., choice of measurement scale, modeling technique, and treatment of data) on estimates of heritability among studies. Methodological effects are seldom discussed in quantitative genetics (Houle et al. 2011) and our review is the first to outline and critique current practices used to estimate heritability of resistance in forest trees and offer
suggestions for methods of best practice.

In Chapter 4, I explore potential discrepancies in estimates among models when estimating heritability of percent traits. During preliminary data analysis for Chapter 2, I found that choice of statistical modeling technique for a percent trait (i.e., percent shoots damaged) influenced estimates of heritability (Appendix B). GLMMs appeared to be more appropriate for percent data because they can accommodate non-normal distributions, whereas linear models often require transformation of the data to meet model assumptions. However, since with field data the true value of heritability is unknown, it is impossible to determine which model produced a more accurate estimate. During my literature review (Chapter 3) I found that percent traits are often quantified as a proportion (i.e., x out of n shoots damaged) or on a categorical scale. As such, in Chapter 4 I test the hypothesis that choice of methods (scale and modeling technique) can bias estimates of heritability for percent traits. I tested my hypothesis by simulating pest damage (percent shoot damage) as a trait of known heritability within a population of trees. This allows for comparison between expected and estimated values of heritability to determine which methods produce more accurate estimates. Chapter 4 is an important part of my research as it identifies methods that potentially produce inaccurate estimates of heritability and it will help researchers avoid potential biases when estimating heritability of percent traits.

In Chapter 5, I examine whether changes in pest densities or predictable environmental patterns in pest distribution, such as edge effects, influence estimates of heritability. The occurrence of symptoms and signs of pest damage used to quantify host resistance depend not only on host susceptibility but also on pest populations and their prevalence in the environment (Kennedy and Barbour 1992), which is often overlooked (White et al. 2007). Environmental variance in
pest resistance may be caused by factors relating to the pest population, such as large fluctuations in pest density or distribution. For example, if there is heterogeneity of pests within a stand or overall low pest density, plants that appear resistant may possess some level of resistance or may have simply escaped damage by chance, making truly resistant genotypes difficult to detect. In this chapter, I demonstrate that our ability to accurately estimate heritability can largely depend on the density and distribution of the pest population.

In summary, this thesis demonstrates how heritability of resistance can be influenced by ecological factors relating to the pest population (e.g., density and distribution) and how choice of methods used to estimate heritability of non-normal traits can bias estimates. Estimates of heritability are used to predict how a trait will respond to selection. When heritability is underestimated, breeders may overlook desired traits that could be improved. If heritability is overestimated, resources may be devoted to selectively breeding for traits that will not produce appreciable gains. A better understanding of the factors contributing to variation in estimates of the heritability will help breeders and ecologists better estimate and interpret its value, allowing them to make more accurate predictions about how traits will respond to selection.

This thesis consists of four manuscript-style chapters, Chapter 2 was published in The Canadian Entomologist in 2016 (Edwards et al. 2016). I intend to submit Chapter 3 to Heredity, Chapter 4 to Methods in Ecology and Evolution, and Chapter 5 to Tree Genetics & Genomes. Chapters 2, 4 and 5 are multi-authored papers. My personal contribution to each manuscript is as follows:

- Chapter 2 – collected and analyzed data, produced figures, wrote draft and revised manuscript with co-author’s comments
• Chapter 4 & 5 – ran simulations, analyzed data, produced figures, wrote
drafts and revised manuscripts with co-author’s comments
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Chapter 2

Genetically-based resistance of balsam fir (Pinaceae) to damage from the balsam twig aphid (Hemiptera: Aphididae)

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2.1 Abstract

We evaluated the effect of tree genotype on the resistance of balsam fir, *Abies balsamea* (Linnaeus) Miller (Pinaceae), to damage from the balsam twig aphid, *Mindarus abietinus* Koch (Hemiptera: Aphididae), by visually assessing aphid damage in clonal seed orchards located in New Brunswick and Nova Scotia, Canada, during four consecutive years. Estimates of clone mean heritability were moderate, suggesting that heritability of resistance is influenced by genetic factors. In New Brunswick, positive phenotypic and genetic correlations of clone-mean damage among years indicate that clones rank similarly each year. Our results suggest that selectively breeding for increased resistance could result in genetic gains.
2.2 Introduction

Genetically-based resistance to insect pests is well documented for many agricultural crop species (Maxwell et al. 1972). However, despite the large number of forest tree breeding programmes, little is known about the heritability of resistance against insects in coniferous trees. Forest geneticists have evaluated the heritability of many traits of conifers but most of this research was focussed on traits associated with growth and wood properties. Heritability of insect resistance in conifers has only been reported for a few species of herbivorous insects, including moths (Lepidoptera) (Quiring et al. 1991), sawflies (Hymenoptera) (Leinekugel le Cocq et al. 2005), aphids (Hemiptera: Aphididae) (Jensen et al. 1997), adelgids (Hemiptera: Adelgidae) (Nielsen et al. 2002; Bains et al. 2009), and weevils (Coleoptera: Curculionidae) (King et al. 1997; Zas et al. 2005; Verrez et al. 2010).

Balsam fir, *Abies balsamea* (Linnaeus) Miller (Pinaceae), is a common tree species in the forests through northeastern North America. It is an ecologically and economically important species making up a large proportion of the boreal and Acadian forest in this area (Bakuzis and Hansen 1965). This species is harvested mainly for lumber and pulpwood but is also used for Christmas trees and holiday greenery. Reforestation of harvested balsam fir stands is achieved primarily through silvicultural techniques used to promote natural regeneration, which in eastern North America yields between 6150 and 48 025 stems/ha (Pothier 2002). Because it regenerates prolifically, balsam fir has not been included in tree improvement programmes and few seedlings are cultivated for use in reforestation (Bakuzis and Hansen 1965). However, Christmas trees are grown in intensively managed balsam fir stands with over 95% coming from plantations (Chastagner and Benson 2000) and thus breeding strategies aimed at reducing pest damage would be beneficial to
Christmas tree growers.

The balsam twig aphid, *Mindarus abietinus* Koch (Hemiptera: Aphididae), feeds on *Abies* sp. in North America and is a major pest in Christmas tree plantations of balsam fir and Fraser fir, *Abies fraseri* (Pursh) Poir, (Fondren and McCullough 2003). Twig aphids feed on current-year shoots as they develop, causing needles to twist and distort, leading to severe aesthetic damage and reduced growth (Saunders 1969). Christmas tree growers frequently use pesticides to reduce aphid populations and mitigate damage (Fondren and McCullough 2003). A significant proportion of variation in twig aphid damage has been attributed to provenance for both balsam fir and white fir, *Abies concolor* (Gordon and Glendinning) Lindley ex Hildebrand (DeHayes 1981; Ferrell 1989), suggesting that there may be genetically-based resistance in firs to this pest.

Here we describe results of a four-year field study, carried out to determine: (i) the heritability of resistance of balsam fir to damage caused by the balsam fir twig aphid; and (ii) whether genetically-based resistance is consistently expressed by clones among years.

### 2.3 Methods

#### 2.3.1 Clonal seed orchards

We evaluated the susceptibility of balsam fir genotypes to the balsam fir twig aphid in two clonal seed orchards located in Atlantic Canada. Trees in each orchard were 5-10m tall and reproductively mature. The orchard in Kingsclear, New Brunswick, Canada (45.9661°N, 66.7999°W) was established in 1992 by the New Brunswick
Tree Improvement Council. It contained 24 clones with 6-10 ramets ($\bar{x} = 8$) per clone planted randomly throughout the orchard. Tree spacing was 3m within rows and 6m between rows. Clones were grafted from selected parent trees located throughout New Brunswick. Parent trees were plus-trees; a plus-tree is one that has been selected based on a subjective judgment of its phenotype but has not been tested to determine if desirable traits are heritable. Orchards were established for Christmas tree breeding purposes and initial parent tree selection was based on a number of traits, including growth rate, foliage arrangement, colour, needle retention, and branch form. Damage from insect pests was not directly considered during selection, although trees showing signs of heavy damage would not have been selected. Parent trees were selected from different stands and are not likely to be closely related.

The second orchard located in Debert, Nova Scotia (45.4172°N, 63.4117°W) was established between 1994 and 1997 by the Nova Scotia Department of Natural Resources. This orchard contained 187 clones, planted randomly with rows separated by 5m and trees within rows by 2.5m. Clones were from selected parent trees (plus-trees) within Nova Scotia; selection criteria were similar to that mentioned above for New Brunswick. Twenty-five clones were used in this site; clone selection was based on the number of ramets available and only clones that had $\geq 9$ separate ramets were used.

### 2.3.2 Damage assessment

Aphid damage was assessed in the fall or early winter each year from 2009 to 2012. All trees were sampled at Kingsclear whereas 9-10 ramets of selected clones were sampled at Debert. Each year, one mid-crown branch from each ramet was cut from the southwest side of the tree. Branches were cut just below the fifth node.
along the main axis of the branch in 2009, and just below the third node in the subsequent three years. The current-year foliage of each branch was inspected and the percentage of shoots with distinctive needle curling, caused by aphid feeding, was recorded. Aphid damage at Debert was negligible in 2011 and 2012 and thus data for these two years were not included in analyses.

2.3.3 Statistical analysis

Traditionally most analyses of heritability use random (or mixed) effects linear models to estimate variance components within or between clones. While this is appropriate for normally distributed continuous traits, our data are proportions and are bound by zero and one. While many studies transform these data to perform standard tests, we chose statistical tests that would account for a binomial distribution. Our statistical analyses were carried out using generalised linear mixed models with a Bayesian estimation framework. We used the MCMCglmm package (Hadfield 2010) in R version 3.0.3 (R Development Core Team 2014), which uses Markov chain Monte Carlo (MCMC) methods to estimate distributions of variance components. This method allows both a binomial response (i.e., the number of non-damaged shoots was considered a “success” and the number of damaged shoots was considered a “fail”) and the inclusion of both fixed (year) and random (clone and tree) effects. They also account for overdispersion (using an additive model) and allowed for specification of correlation matrices for the variance components (Hadfield 2010). Overdispersion can occur if variance exceeds that predicted by the model. In additive models an additional random term is included into the model to capture any overdispersion (Browne et al. 2005).

To determine the most appropriate model for our data we first modelled all the variables (fixed effect of year and random effects of clone, tree, and the clone x
year interaction) in a single full model. We then compared sequentially reduced models (each with one term removed) to our full model using deviance information criterion values and examined variance distribution plots to assess overall model performance. From this we determined that a final model without the effect of tree or the clone x year interaction was the most appropriate. All of our models were fit with uninformative priors for both fixed and random effects and ran with a 10000 burn-in period, followed by 100 000 iterations and a thinning rate of 100.

In each year we had different levels of aphid damage within our orchards. Previously, both Strong et al. (1993) and Leinekugel le Cocq et al. (2005) reported a positive relationship between heritability of host plant resistance and insect density. This suggests that at both very high and low densities of insects, genetically-based resistance may not be detectable, as variance among trees would be reduced. We wanted to estimate heritability for each year individually so we needed separate estimates of variance for each year. By default in MCMCglmm random variables are assumed to have a structured identity matrix (with ones on the diagonal and zeros on the offsets), this assumes independence among the levels but only one variance component is estimated (Hadfield 2010). To estimate variance components for each year individually we changed the variance-covariance matrices for the random (clone) and residual variance in our model. Clone variance was expected to vary by year but also covary among years so an unstructured variance-covariance matrix was used, which results in one estimate of clone variance for each year and provides estimates of covariance between years. Residual variance was also estimated for each year individually using a covariance structure fixed to zero, as we had no expectation that residual variance would be correlated among years.

MCMCglmm models produce posterior distributions of estimated variance components (as opposed to point estimates). These variance distributions were
used to estimate broad-sense heritability, the proportion of total genetic variation to total phenotypic variation ($V_g/V_p$), at both the individual ($H^2_i$) and clone-mean ($H^2_c$) level for each year for both the New Brunswick and Nova Scotia orchards following White et al. (2007):

$$H^2_i = \frac{\sigma^2_c}{\sigma^2_c + \sigma^2_e}$$  \hspace{1cm} (2.1)

$$H^2_c = \frac{\sigma^2_c}{\sigma^2_c + \frac{\pi^2}{3}}$$  \hspace{1cm} (2.2)

where $\sigma^2_c$, $\sigma^2_e$, and $r$ are, respectively, the estimated clone variance, estimated random error, and harmonic mean number of ramets per clone. Random error of a GLMM is not directly given but can be taken as:

$$\sigma^2_e = \sigma^2_e + \pi^2/3$$  \hspace{1cm} (2.3)

where $\sigma^2_e$ is the additive dispersion component and $\pi^2/3$ is the distribution specific variation for a binomial model (Nakagawa and Schielzeth 2010). Because we used variance distributions and not point estimates we created a posterior distribution of heritability estimates. The mode of the distribution was used to estimate heritability and 95% credible intervals were calculated.

To determine if clone performance (i.e., low or high aphid damage) is consistent over time we examined the correlation of damage levels over time. Pearson’s phenotypic correlations based on clone means, and genetic correlations of damage between all possible pair-wise combinations of years were estimated. Since aphid damage was measured on the same individual trees we used a type A (or “age-age”) genetic correlation.
We treated aphid damage in each year as a separate trait, as in Falconer and Mackay (1996):

\[
\begin{align*}
    r_a &= \frac{\text{cov}_{xy}}{\sqrt{\sigma_{cx}^2 \sigma_{cy}^2}} \\

\end{align*}
\]

where \(x\) and \(y\) are two traits (twig aphid damage in two separate years), \(\text{cov}_{xy}\) the clonal covariance between the traits, and \(\sigma_{cx}^2\) and \(\sigma_{cy}^2\) are the clonal variance components for each trait. Estimates of variance and covariance were taken as the mode of the corresponding posterior distributions given by our statistical mode. Standard errors for the genetic correlations were not calculated since sampling errors are large and difficult to specify (Falconer and Mackay 1996).

\section{2.4 Results}

In New Brunswick the mean percentage of shoots damaged by twig aphid decreased during our four-year study from 27.1\% in 2009 to 6.3\% in 2012 (Fig. 2.1). Overall, estimates of individual heritability were very low and varied between 0.03 (credible interval (CI): 0.02–0.07) and 0.06 (CI: 0.03–0.16); clone explained little of the observed phenotypic variation within each year. Estimates of clone-mean heritability were moderate, and varied between 0.20 (CI: 0.08–0.48) and 0.50 (CI: 0.23–0.64) (Fig. 2.1). The 95\% credible intervals increased as damage within the orchard decreased, suggesting estimates of heritability are more robust as damage increases. Although, this trend only applies to the range of damage observed in this study as heritabilities will start to decline as damage increases past some point (as discussed in Chapter 5).

In Nova Scotia the mean percentage of shoots damaged was low in 2009 and 2010 (8.0\% and 1.3\% respectively) and estimates of heritability were comparable to what was found in the New Brunswick orchard (Fig. 2.1). In 2010, posterior
distribution plots showed that variance estimates for the effect of clone were stuck on zero and the chain did not converge. As we could not get reliable estimates of variance components for this year, we considered them to be effectively zero, and consequently heritability estimates were found to be near zero for Nova Scotia in 2010.

In New Brunswick, both pairwise phenotypic and genetic correlations between the mean damage per clone by the twig aphid in one versus another year were positive, although not always significant (Fig. 2.2). In Nova Scotia, there was no significant relationship between mean clone damage in 2009 and 2010 ($r_p = -0.089$, $p = 0.672$).

### 2.5 Discussion

There was a moderate genetic basis in balsam fir for resistance to damage from balsam twig aphid. Individual estimates of heritability were low, suggesting that susceptibility to damage is not consistently expressed among ramets within a clone. Low individual heritability suggests that resistance to aphid damage is a phenotypically plastic trait. However, it is also possible that patchy distribution of aphids obscured reliable estimates of heritability especially when damage levels were low. Estimates of clone mean heritability were moderate. These estimates are naturally higher than those of individual heritability since within clone variance (error variance) is reduced while the variance between clones is unchanged. Moderate estimates of clone mean heritability suggest that despite the variation among individual ramets, the mean phenotypic value of resistance among clones is moderately influenced by genetics and that selection for resistance to aphid damage could result in genetic gains.
In the New Brunswick orchard, where damage by the twig aphid was highest, correlations of mean clone damage among years were positive and generally significant, which indicates that the resistance ranking of the different clones remained similar among years. Twenty-five per cent of clones consistently ranked in the top 50% of clones during each of the four years, further suggesting that selection for increased resistance is possible.

Low to moderate estimates of heritability may be a reflection of the complexity of herbivore-host interactions. Resistance to herbivores is a multivariate trait that is often influenced by many constitutive and induced defence mechanisms (see Hanover 1975; Larsson 2002). If multiple traits function together to influence tree resistance to aphid damage, each trait would presumably show some degree of phenotypic plasticity and each will be influenced by environmental (non-genetic) factors. Price and Schluter (1991) theoretically demonstrated that life-history traits, presumably influenced by many individual metric traits (e.g., morphological, behavioural, and physiological), are expected to show lower levels of heritability when compared to metric traits. They suggest that environmental variance accumulates from each metric trait involved, increasing environmental variance of life-history traits and thus reducing heritability estimates. Classical theory also suggests that fitness related traits may be under strong selective pressure with stabilising selection reducing the genetic variation among individuals and thus decreasing genetic variance and heritability estimates (see Mousseau and Roff 1987).

We do not know which traits confer resistance of balsam fir to twig aphid damage. Variation in tree susceptibility could be due to variations in the preference of the twig aphid for susceptible clones, similar to what has been reported for aphids on melon (*Cucumis melo* Linnaeus; Cucurbitaceae) (Kennedy and Kishaba
1977), alfalfa (*Medicago sativa* Linnaeus; Fabaceae), and sweetclover (*Melilotus Linnaeus*; Fabaceae) (Kishaba and Manglitz 1965), or to variation in aphid performance on different clones, as seen in cottonwood hybrids (Larson and Whitham 1997) and tomato plants (Kaloshian et al. 1997). Alternatively, variations in the susceptibility of balsam fir to the twig aphid may be due to genetically-based variations in tolerance of the host tree. Nettleton and Hain (1982) found that Fraser fir could outgrow up to 55% of twig-aphid damage. Similarly, large variations in the amount of damage found between groups of related (half-sibling) Nordmann fir (*Abies nordmanniana* (Steven) Spach (Pinaceae)) exposed to similar densities of silver fir woolly adelgid *Dreyfusia nordmannianae* (Eckstein) (Hemiptera: Adelgidae), which causes needle curl damage similar to that of twig aphid, suggests that there is genetically-based variation in tolerance of the host tree to this pest (Nielsen et al. 2002).

If resistance of balsam fir to twig-aphid damage were in part due to tree tolerance, this would be beneficial to breeding programmes, as tolerance does not put selective pressure on pests to adapt and overcome host resistance (Rausher 2001). If balsam fir is able to outgrow twig aphid damage our heritability values may be underestimated, because trees used in this study were previously selected as “plus-trees”. Parent trees were largely chosen for desirable growth characteristics (i.e., internodal and shoot length) and selecting trees for growth characteristics could have reduced the variation in resistance to aphid damage if resistance is correlated with growth related traits. Further research exploring the mechanisms of balsam fir resistance to aphid damage and the relative influences of insect preference, performance, and tree tolerance should be explored.

This study was conducted on older mature trees and heritability estimates can be influenced by organism age. Age dependent variation in genetic expression and
heritability has been observed in animals (e.g., Charmantier et al. 2006; Serbezov et al. 2010) and plants (e.g., Boege and Marquis 2005). Serbezov et al. (2010) suggests that increases in variation with age may be attributed to differences in environmental factors that accumulate over time, resulting in greater phenotypic variation of traits. In trees these environmental effects could be due to a number of differing conditions including (but not limited to) microclimate, herbivore damage, pathogen/fungus attack, mechanical damage (e.g., ice or wind), and resource competition. Accumulated differences in environmental effects over time may reduce estimates of trait heritability in older trees. Also, resource allocation to plant defences can change over time during plant development (Boege and Marquis 2005). As trees used in this study were much older than marketable Christmas trees, further work will be needed to verify that the estimates of heritability of balsam fir to twig aphid damage observed in this study are similar to that of younger trees.

The present study was replicated temporally but not spatially, as clones planted in New Brunswick were not the same as the ones planted in Nova Scotia. Environmental conditions can influence host plant phenotype and pest populations, and thus trees found to be resistant in one location may not be resistant in another if resistance is only expressed under certain conditions (Fritz 1990). Significant genotype environment interactions in the resistance of conifers to other pests has been previously reported (Burdon 1977; Quiring et al. 1991; Alfaro et al. 2002) and should be investigated in any tree-breeding programme.

In summary, our study provides evidence that incorporating genetically-based resistance into pest management strategies may be possible for reducing twig aphid damage within balsam fir Christmas tree stands. Further work is needed to determine the mechanisms of resistance to aphid damage and how these traits are
expressed in younger (market-sized) trees. Also, future work should determine if selection for resistance to the twig aphid is compatible with selection for other commercially desirable traits, such as branch form, growth, needle retention, and resistance to other pests.

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2.7 References


Figure 2.1: Relationship between (a) clone mean and (b) individual broad-sense heritability estimates (95% credible intervals) and mean percentage of shoots damaged per tree each year by the balsam twig aphid in one clonal balsam fir orchard, located in New Brunswick (●) and one in Nova Scotia (△).
Figure 2.2: Pair-wise correlations of mean clone damage from the balsam twig aphid between years in a New Brunswick balsam fir orchard. Both phenotypic ($r_p$) and genetic ($r_g$) correlations are reported where appropriate. Significant correlations ($p < 0.05$) are indicated by an asterisk for $r_p$; significance was not calculated for $r_g$ (see text for details).
Chapter 3

Investigating methodological effects on estimates of heritability: a review of pest resistance in forest trees.

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3.1 Abstract

Pest resistance is a multifactorial trait depending on many different host plant attributes and cannot usually be measured directly. When estimating heritability of resistance, a proxy variable must therefore be selected to represent the underlying resistance. Often, the proxy variable is quantified on a non-Gaussian (i.e., non-normal) scale, which can be problematic because non-normal data do not strictly meet the assumptions of the linear models often used to estimate heritability. Consequently, data used to estimate heritability of resistance are treated in numerous ways that range from changing its scale to using modern statistical techniques like generalized linear mixed effect models. I ask whether differences in methods for handling resistance data affect estimates of heritability. Here, I review the literature on heritability of pest resistance in forest trees to determine: 1) how resistance is typically measured and quantified, 2) how data are analyzed, and 3) whether the choice of methods affects estimates of heritability. There is a diverse set of methods used to quantify and estimate heritability of pest resistance in the literature. The scale used to measure the proxy did not have a strong influence on estimates of heritability. However, when data were transformed prior to analysis estimates of heritability were significantly lower than estimates from untransformed data. Although this does suggest that transforming data affects estimates of heritability, because there are so many dimensions along which methodologies vary (measurement scale, type of transformation, statistical model, etc.), it is difficult to make clean comparisons among methods without robust sample sizes.
3.2 Introduction

How we choose to quantify the phenotypic expression of a trait may affect estimates of its variance and heritability. For most traits, quantifying phenotypic variance is rather straightforward; for example, traits such as height or biomass can be clearly defined and measured on a continuous scale. However, this is not the case with multifactorial traits, which are problematic because they cannot be measured directly. How we define and quantify these types of traits could affect estimates of heritability. For example, many different attributes collectively make up pest resistance in plants, including morphological, biochemical, and molecular mechanisms of resistance (War et al. 2012; Wiseman 1985; Beck 1965). To estimate heritability of resistance we first must select a method to quantify its expression. One issue, seldom discussed, is how methodological choices could affect estimates of heritability.

Pest resistance can be reported in many ways (see Tingey 1986; Kranz 1988), as such, the choice of proxy variable used to represent resistance and its measurement scale may not be obvious (Fig. 3.1). Arguably, it may not be the choice of the variable itself but rather the scale on which variance is reported that has the greatest effect on estimates of heritability. For instance, we could reasonably expect signs and symptoms of pest activity to be highly correlated (e.g., positive correlation between number of insects and amount of damage per host plant) and using either as a proxy should reflect the underlying host resistance. However, choice of measurement scale used to quantify the proxy variable could affect estimates of heritability. One reason is because different scales may vary in their accuracy of capturing trait variance. For example, ordinal (i.e., ordered categorical) scales simply rank individuals and have a lower accuracy in capturing
variance than if the same trait were to be measured on a quantitative scale (e.g., count, percent, and continuous; see Shaw et al. 1987). Besides differences in accuracy, data collected on different scales can follow different types of distributions (normal, binomial, Poisson, negative binomial, etc.– see Sokal and Rohlf 2012). When resistance is a polygenic trait – influenced by many additive genetic effects and not inherited solely from dominant alleles – it is assumed to follow a normal distribution (Falconer and Mackay 1996). This is true for traits phenotypically expressed on a continuum (with individuals showing varying degrees of a trait) or for threshold traits (which are discontinuous with distinct phenotypic classes). Additive genetic effects of continuous and threshold traits are inherited in the same manner and when estimating heritability of resistance, we ultimately want to capture these effects which make up the underlying distribution. This raises the question, if the proxy variable used to quantify a trait is not on a scale that accurately reflects the underlying distribution of resistance will that bias estimates heritability?

The scale of the proxy variable largely dictates how data are treated during statistical analyses. Traditionally, variance components used to estimate heritability (from groups of siblings or clones) are found using linear models (LMs). Originally this was done using analysis of variance (ANOVA), but over time there has been a transition towards mixed effect models (Boisgontier and Cheval 2016). The linear mixed effect model (LMM) has two key advantages over classical ANOVA design. First, LMM provides a more appropriate framework for handling random effects; and, second, LMMs are able to handle incomplete or unbalanced data (see Lynch and Walsh 1998). Both ANOVA and LMM are predicated on assumptions of normality. When data lack normality often they can be transformed to meet this statistical assumption (e.g., arcsine, log or square root transformations – see Sokal and Rohlf 2012). However, qualitative data – such as categorical and
binary\(^1\) – cannot be normalized through transformations and needs to be recoded\(^2\) to a quantitative scale prior to analysis. Recoding categorical data from qualitative to quantitative can be achieved in two ways, using either integer scoring or midpoint scoring (Powers and Xie 2008). In integer scoring, categories are simply recoded to an integer value based on rank order. For example, the categories low, medium and high would become 1-3 respectively. Midpoint scoring is used when categories represent a range of quantitative values, with the midpoint value of each range used to approximate the quantitative scale. For example, the categories 0-25%, 26-50%, 51-75% and 76-100% would become 12.5%, 38%, 63% and 88% respectively. To recode binary data to a quantitative scale, data need to be grouped by an additional factor (e.g., blocks or years) and are then expressed as counts (e.g., King et al. 2004; Vivas et al. 2012) or proportions (e.g., Kiss and Yanchuk 1991; Leinekugel Le Cocq et al. 2005; Verrez et al. 2010).

An alternative to transforming or recoding data to meet the assumptions of normality is to use a generalized linear mixed model (GLMM). The GLMM is an extension of the LMM that can accommodate non-normally distributed data. GLMMs use a link function, which allows the model to explicitly fit the distribution of the data (see Zuur et al. 2009). The downside to GLMMs is that they are complex and can be difficult to specify and, until recently, availability of capable software and computing power were limiting factors in their use (Bolker et al. 2009). Although, with recent technological improvements, GLMMs are growing in popularity.

\(^1\)Binary data (dichotomous variable) is a special case of categorical data. Since binomial and categorical data (with >2 categories) are treated differently during statistical analyses (see Sokal and Rohlf 2012) I will refer to each separately. For the purposes of this chapter, I define categorical data as having >2 categories.

\(^2\)In this chapter, I will define data transformations as the application of mathematical functions to the data and recoding as simply relabeling or reorganizing (e.g., grouping) variables to present values on an alternate scale.
The choice of statistical methods used will determine the scale that variance components (used to estimate heritability) are reported on. Estimates of heritability from transformed data will be produced on the transformed scale, while estimates coming from GLMMs will be on the scale of the link function. However, because heritability is a dimensionless ratio (Chapter 1), the scale on which variance is reported should not affect it. However, it has been suggested that discrepancies may occur when trait heritability is estimated from data expressed on different scales (Nakagawa and Schielzeth 2010). One advantage of the GLMM is that estimates of variance (and heritability) from binary, proportion and count data can be back-transformed to their original scale (see Nakagawa and Schielzeth 2010). Although, usually only one estimate is presented and it is rare to find estimates of heritability reported from both the link and original scale, making it difficult to determine if the scale of the variance components has an effect on its estimation.

Given that there is no exact method for measuring pest resistance and that there is a wide range of methods used to quantify pest resistance, I conducted a review of the literature to document the variety of methods used and to test the hypothesis that choice of methods used to measure pest resistance can affect estimates of heritability. Here, I present a literature review of heritability of pest (insect and disease) resistance in forest trees where I address the following questions: 1) how is pest resistance typically quantified, 2) what are the most common methods used to treat resistance data when estimating its variance, and 3) is there any effect of choice of method or data treatment on estimates of heritability?
3.3 Methods

3.3.1 Data sources

To identify as many studies on heritability of pest resistance in forest trees as possible, I used keyword searches in Scopus® and Google Scholar®. For the purpose of this paper, pest resistance was considered a reduction in signs or symptoms associated with a pest (insect or disease) in host trees. Each search included at least one of the following terms: heritability and/or genetic variation in various combinations with one or more of the following additional search terms: resistance, pest, disease, insect, and trees. I also included any relevant papers that I found referenced within papers returned from the primary search. In this review, I include estimates of both broad-sense (the ratio of total genetic effects to total phenotypic variance) and narrow-sense (the ratio of additive genetic effects to total phenotypic variance) heritability reported on an individual level. Estimates of family mean heritability were not included because they average the environmental variance across individuals within a family. Heritability reported for family means are typically higher than estimates of individual level heritability and these two estimates are not directly comparable (see Wright 1976). Because individual tree measurements are more commonly used (and reported) in forestry (van Buijtenen 1992) I chose to use estimates of individual level heritability over those produced from family means.

Since my primary interest was in the methods used to estimate heritability of resistance, if the data collection methods, treatment of data (e.g., scale changes such as transformations or recoding) or statistical methods were not clear, I omitted the paper from the review. Additionally, since my focus was on heritability
of pest resistance in forest trees, papers reporting on agroforestry species (i.e., trees used for fruit production) were not included in this review.

### 3.3.2 Data collection

**Heritability**

I extracted all the estimates of heritability recorded in each paper. When multiple estimates were presented in a paper instead of recording each estimate individually, to avoid bias, I pooled estimates if they were from the same host species and for similar pests and used the median value. Insect pests were considered similar if they were from the same feeding guild and estimates of disease resistance were pooled if they exhibited similar symptoms and damaged the same plant modules. When I pooled multiple estimates of heritability I also reported the number of estimates used to calculate the median value.

**Data collection and analysis methods**

For each study, I recorded the type of data collected (binary, categorical, continuous, count, percent or scores), type of data analyzed, any transformations that were applied and the type of analysis used (ANOVA, LMM, GLMM or multiple). The factor level “multiple” was used when papers reported multiple estimates of heritability but they used different tests for different proxy variables. For example, Balmelli et al. (2014), reported estimates of heritability for resistance using both LMMs (for traits measuring disease severity and defoliation) and GLMMs (used to estimate heritability from binary survival data). For the type of data collected, if percent values were assigned by visually assessing damage and assigned a percent value that represented a range (e.g., 10% if damage was between
I considered it to be categorical data. Additionally, the term “scores” was used when resistance values were derived by combining multiple measures into one numeric value. For example, Carnegie et al. (2004) quantified damage as a combination of disease incidence and severity (both estimated by eye) to produce one representative value of damage per tree. To use an LM (ANOVA or LMM) data must be on a numeric scale. As such, ordinal categorical data are either treated as ranked integers or, if categories represent a range of values (e.g., 0-25%, 26-50% etc.), the midpoint value of each category may be used. When reporting the type of data analyzed, studies that collected data categorically (and used an LM) were coded as either “integer” or “midpoint” to represent the method used to recode the data to a quantitative measure.

Host and pest

Because heritability estimates from closely related species are likely to be correlated, I reported both host plant family and genus. Additionally, because resistance to insects and disease could be caused by different mechanisms of resistance (see Acquaah 2012) I reported the type of pest (insect or disease) as similar mechanisms may have correlated responses. I also reported if pest populations were naturally occurring or if they were placed onto trees in a controlled manner. Controlled introduction of pests onto trees reduces environmental variance by creating a homogeneous density and encounter rate of host plants with a pest, which could have an effect on heritability.

Families: type and number

The relationship between individuals within a family (clones or siblings) was included in the data set. Broad-sense estimates of heritability (from clones) can be
considered an upper limit to trait heritability, as they include all sources of genetic variance (additive, dominant and epistatic genetic effects) and not just additive effects (see Falconer and Mackay 1996). As such, I expected broad-sense estimates of heritability to be higher than narrow-sense estimates, and therefore needed to account for the distinction.

The number of families was reported categorically as: small (<50), medium (50-100), and large (>100). Studies that included more families would have more power to detect differences among families increasing the accuracy in estimates of genetic variance. However, larger plantations cover more area and heterogeneous environmental conditions (e.g., soil conditions, microclimate, etc.) could increase environmental effects. If increased environmental heterogeneity is not accounted for (e.g., by incorporating blocking factors or spatial autocorrelation into statistical models) it could cause a downward bias in estimates. I did not include number of individuals per family in the data set, as there was often variation within individual studies in the number of individuals measured.

3.3.3 Data analysis

It has been suggested that estimates of heritability may not be suitable for a formal meta-analysis because heritability typically has large standard errors and underlying patterns can be obscured when estimates are standardized by incorporating their variance (see Markow and Clarke 1997; Whitlock and Fowler 1997). Additionally, because median values were used when multiple estimates of heritability were reported, there is no meaningful standard error associated with them and they cannot be standardized. Instead of conducting a meta-analysis, I focused on exploring trends in estimates of heritability.
To determine what methods are most frequently used for quantifying pest resistance and how data are treated prior to analysis I used simple descriptive statistics to examine trends in the type of data collected and changes made to the data prior to analysis. To test for effects of methods on estimated heritability, individual linear models were used to test for significance of: number of estimates, family relation, pest type, pest source, type of data collected, if data were transformed (yes/no), if data were recoded (yes/no), and type of statistical model used. Each variable was treated as a fixed effect and data were not transformed prior to analysis.

3.4 Results

The data set contained 31 estimates of heritability from 28 papers (Appendix A). Three papers were represented by two separate estimates of heritability, either because two different host species were examined or because heritabilities of resistance to both disease and insect pests were reported. In these cases, the estimates of heritability were considered independent data points. The number of individual estimates of heritability reported per paper varied from 1-14 (median 3), with the majority of studies (71%) reporting two or more estimates (Fig. 3.2).

3.4.1 Host

There isn’t a wide range of host species represented in the data; only eight genera of trees were present in the data (Fig. 3.3), the majority of estimates (74%) come from the family Pinaceae (Appendix A). Pine (Pinus sp.) and spruce (Picea sp.) are the most well represented groups, with 35% of estimates coming from studies
done on pine and 23% of estimates from spruce. There are no apparent trends in estimates among related groups but, because some groups (family and genera) are only represented by one (or very few) estimates I could not formally test for differences in heritability among groups.

The majority of estimates of heritability were narrow-sense (84%), with few estimates coming from clonal studies (i.e., broad-sense). No significant effect of relatedness between hosts was found on estimates of heritability ($p = 0.54$). One estimate was omitted from this analysis as it was the median value reported from both narrow-sense and broad-sense estimates of heritability.

### 3.4.2 Pests

Resistance to diseases showed higher levels of heritability than resistance to insect pests (Fig. 3.4). Most studies relied on natural pest occurrence (74%) rather than controlled introduction (26%). There was no significant effect of source of pest (natural or added) on estimates of heritability ($p = 0.42$).

### 3.4.3 Methods used

The majority of estimates of heritability of pest resistance come from data originally collected on non-Gaussian scales (Fig. 3.5). The type of data collected and type analyzed had no apparent effect on estimates of heritability ($p = 0.63$ and $0.93$, respectively). Pest resistance was reported qualitatively (i.e., categorical and binary scales) at least once in the majority of studies (73%) (Appendix A). When traits were assessed categorically the median number of categories used was 5 (min 4 and max 21; Appendix A). Categorical data was always recoded to a quantitative
scale and analyzed using LMs. The type of analysis used for binary data was split evenly between LMMs and GLMMs (50:50; Appendix A). When LMs are used, recoding to a quantitative scale is done by applying a grouping factor (blocks) and reporting percent affected family members per block. Recoding the data had no apparent effects on estimates of heritability \( (p = 0.75) \). Transformations were a common occurrence for non-Gaussian data analyzed in an LM (Appendix A). Among papers that used an LM for non-Gaussian data I did find that estimates coming from transformed data were significantly lower (Fig. 3.6).

Linear models (LMM and ANOVA) are more commonly used to estimate heritability (Fig. 3.5). GLMMs were not as common, and when they were used it was often to estimate heritability from binary data (Fig. 3.5). I did not find an effect of statistical method on estimates of heritability \( (p = 0.90) \). Often methods for estimating heritability were not consistent within a paper, 26% of estimates came from papers that used different methods to estimate heritability within a study (Fig. 3.5).

Most estimates of heritability were from studies conducted on either a small or large number of families (41% and 38% respectively) with few studies of intermediate size. The number of families examined did not affect estimates of heritability \( (p = 0.33) \).

### 3.5 Discussion

There is a diverse range of methods used to collect and analyse resistance data in forest trees for the purpose of estimating heritability. Resistance data are often collected on non-Gaussian scales and analyzed using LMs. As a result, recoding and transforming data to meet the assumptions of normality is a common
occurrence. I did not find any apparent biases in estimates of heritability for data collected on different scales. However, given the overall variability of estimates in the data set and the low number of estimates among the different scale types I lack the power to detect any small effects that may exist. My analysis suggests that estimates of heritability found using transformed data tend to be lower, although I have reason to believe this effect may not be real (discussed below). In the sections to follow I will start by discussing general trends in estimates across studies, then discuss the methods commonly used, and then discuss the effects found to influence estimates of heritability for these data. I will conclude with general recommendations on how to improve data collection.

### 3.5.1 Heritability of pest resistance

My analyses suggest that pest resistance is a moderately heritable trait (median 0.31). As more estimates are included in a paper, I find that values of heritability appear to converge toward the median (Fig. 3.2). High estimates of heritability are more likely to occur in papers reporting a single estimate, suggesting that higher estimates may simply be due to sampling artifacts rather than high levels of underlying trait heritability. It has been suggested that life history traits (such as resistance) should possess lower levels of heritability. Because life history traits collectively depend on a number of individual metric traits (with each one being influenced by both genetic and environmental effects), environmental variance is compounded across traits leading to a decrease in estimates of heritability (Price and Schluter 1991). Additional environmental variance is also introduced into estimates of pest resistance because the occurrence of symptoms and signs of pest damage rely not only on host susceptibility but also on the pest population itself and its prevalence in the environment (see Carson and Carson 1989; Kennedy and
Barbour 1992). For example, pest populations can change among years and can be considered an additional source of environmental variation (Strong et al. 1993; Leinekugel Le Cocq et al. 2005). Despite the increase in environmental variance introduced into estimates of heritability of pest resistance, overall it appears to be a moderately heritable trait. This is promising for tree breeders, as it suggests that improvement through selective breeding could produce genetic gains.

These data could suggest publication bias in the heritability literature. Although I did find papers reporting low estimates, they were in papers that reported multiple estimates, suggesting they were more robust estimates (Fig. 3.2). I found few papers reporting single estimates of heritability that were low. Low estimates may be seen as uninteresting if the goal of the research is to find heritable traits and predict gains that could be achieved through selective breeding. I want to acknowledge that under-reporting of lower estimates of heritability could mask possible biases in methodological effects.

### 3.5.2 How is pest resistance reported & analyzed?

Categorical and binary scales are arguably the easiest methods to employ in the field, as large numbers of individuals can be assessed in a relatively short period of time. It was not surprising to find that the majority of papers used categorical or binary methods to assess pest resistance (Fig. 3.5; Appendix A). Although qualitative data are easy to collect they can present challenges when it comes to accurately estimating trait variance. When a quantitative variable (e.g., percent foliage damage) is broken up into categories the width of intervals and the number of categories used can affect how much information is retained (Shaw et al. 1987). I often found that few (median 5) categories were used when reporting pest resistance (Appendix A). Using fewer groupings helps increase repeatability of
individual measurements, but it also reduces precision and accuracy of variance estimates as information is ultimately lost when traits are reported on a broad scale. I did not find any papers that used GLMM methods to estimate variance from categorical data, as such all categorical data was recoded to a quantitative scale prior to analysis. Recoding can be a problem if it changes relationships between the original values and does not reflect realistic differences among categories. For example, if foliage damage from an insect pest was reported using the categories: 0%, 1-10%, 11-50%, >50%, the integer scale would be inappropriate to apply as the magnitude of difference between categories is not reflected on an integer scale. Midpoint scaling would more accurately reflect differences among groups, but if values are skewed within a category, estimates of variance may be biased. For example, using the categories above midpoint scores become 0%, 5.5%, 30.5% and 75% respectively. If the majority of individuals had 40-60% damage the midpoint values of 30.5% and 75% would not accurately capture variance among individuals and bias estimates of heritability. Careful consideration of the spacing and number of categories can help reduce possible bias when estimating heritability. When grouping continuous data into discrete intervals Shaw et al. (1987) found that the amount of information retained depends largely on the number of intervals selected. They suggest a reasonable method for categorization of a continuous trait is to use seven categories, balancing the need for few categories to improve repeatability of estimates and the need to retain information. Additionally, because recoding could bias estimates of variance, careful scrutiny of methods used to recode categorical data are necessary to ensure recoded data are representative of the original variable.

Reporting resistance as a binary trait is the simplest method to carry out in the field, but it comes at a cost. Binary data are not able to capture variance among individuals and it is also more difficult to analyze. Linear modeling requires
binary data to be recoded to a quantitative scale and this may not be appropriate if sample size is small or if no apparent grouping structure exists. Not surprisingly, I found that GLMMs were more likely to be used on binary data (Fig. 3.5; Appendix A), as these models can handle binary responses. When resistance is treated as binary, variance in the degree of pest damage is lost. Though some traits can only be reported on a binary scale (e.g., mortality), often pest resistance is captured on a binary scale out of convenience. If the goal of a breeding program is to reduce the level of damage, we recommend methods that would more accurately capture variance in the level of damage among individuals, such as using a categorical scale that adequately breaks up the distribution of damage or using a measure of resistance that can be measured on a quantitative scale.

3.5.3 Scale used to estimate heritability

Overall, the estimates of genetic parameters and heritability are rarely presented on the original data scale. Recoding and transforming of data is common when data are analyzed using LMs, but since these data cannot be back-transformed to their original scale it is difficult to determine what effect this has on estimates of heritability. There are methods available to back-transform variance from the link scale of a GLMM (see Nakagawa and Schielzeth 2010), however, no reports that used back-transformations for estimates of heritability were found during this review. This makes it difficult to determine what effect, if any, changing the scale of the variance has on estimates. Even outside of the pest resistance literature few papers report both link and original scale estimates of heritability. As a result of this in the next chapter I start looking at the effect of the scale used on estimates of heritability through data simulations (Chapter 4).
3.5.4 Factors influencing estimates

Two variables had a significant effect on estimates of heritability: type of pest (disease or insect) and if data were transformed prior to analysis in an LM. One finding that could be biological rather than methodological is that resistance to disease appeared to be a more heritable trait than resistance to insect pests (Fig. 3.4). This difference may be due to the mechanisms of resistance under selection for different types of pests. Resistance can occur via two general mechanisms: antixenosis and antibiosis. Antixenosis (often referred to as non-preference) is a type of resistance achieved through pest avoidance, as host plants reduce the probability of contact with a pest by making themselves unattractive for feeding, oviposition and/or shelter (see Acquaah 2012). Antibiosis refers to mechanisms of resistance directly affecting the biology of the pest – reducing pest prevalence, growth and/or development. Avoidance mechanisms of resistance primarily deter insect (or other animal) attacks, rather than pathogens. It could be that antibiosis is a more heritable trait or it could be that variation in avoidance is difficult to detect when pest populations are high or there are no alternate (preferred) hosts available for insects, making heritability of avoidance more difficult to detect.

Because pest resistance is often assessed as an individual’s susceptibility to pest damage, host tolerance cannot be overlooked as an additional mechanism contributing toward resistance. Where studies have identified heritable variation in pest tolerance, the mechanisms of tolerance are often not identified (Strauss and Agrawal 1999). This makes it difficult to speculate if differences in the heritability of pest tolerance contribute toward the difference in estimates of resistance found for different types of pests. As I had no measures of the numbers of pests and subsequent damage levels for most studies, it is hard to tease apart potential causes (antixenosis, antibiosis and tolerance) of differences between estimates of
heritability from insect and disease pests. Reporting on pest density and damage per individual could help elucidate differences between mechanisms of resistance, which is an area that is yet to be thoroughly explored in the literature.

Estimates of heritability from data transformed prior to analysis in an LM were significantly lower than estimates that did not undergo transformation (Fig. 3.6). This result is difficult to interpret, although it appears to indicate a methodological effect. It could be that data that needed to be transformed were problematic and low estimates are caused by “messy” data and are not a result of transformation per se. For example, data collected from a population with low pest density and damage skewed toward zero would need to be transformed to meet assumptions of normality prior to analysis. If a low estimate of heritability was produced from these data it would not be fair to say that the transformation had an effect because heritability has been shown to have a positive relationship with pest density (Strong et al. 1993 and Leinekugel Le Cocq et al. 2005).

3.6 Conclusions

There are many dimensions along which methods can vary (e.g., measurement scale, transformation type, statistical model, etc.) and few papers use similar methods overall. This makes it difficult to make clear comparisons among estimates of heritability to determine if methodological effects exist. I did find evidence suggesting that estimates of heritability from transformed data were lower, but this effect could be confounded with additional factors. Overall, changes to the original data are often made without justification, or apparent consideration into how they could affect estimates of heritability. When estimating heritability of pest resistance there is a need to balance ease of data collection with the need for statistical rigour.
The proxy variable used to represent resistance is often measured on a non-Gaussian scale but the underlying distribution of additive genetic effects that make up host resistance are assumed to follow a normal distribution. I am unable to draw strong conclusions about the choice of measurement scale of the proxy variable and its ability to capture the underlying resistance. I believe this shows that there is a clear need for simulation-based studies to determine how methodological effects could influence estimates of genetic variance and heritability of resistance.

### 3.7 Acknowledgements

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3.8 References


Figure 3.1: Methodological choices for estimating heritability of pest resistance. Estimating heritability of resistance requires that a proxy variable (host damage or the success of the pest itself) and a measurement scale be chosen. Genetic parameters used to estimate heritability can be found using either a linear model (LM) – including both analysis of variance and linear mixed effect models – or a generalized linear mixed effect model (GLMM), if data are non-normal. The choice of the proxy variable, scale of measurement and statistical analysis are subjective and it is unclear how choices could affect estimates of heritability.
Figure 3.2: Effect of number of estimates reported per paper on estimate of heritability of pest resistance. When >1 value was presented in a paper the median value was used. Dashed line represents median value for heritability across studies.
Figure 3.3: Box and whisker plots showing heritability of pest resistance estimated from different host trees. Boxes represent 25 and 75% quartiles and the middle line represent the median. Whiskers show 2.5 and 97.5% quantiles. Where few estimates were recorded for a host species boxes were not possible and points (single estimate) or lines (< 4 estimates) are used.
Figure 3.4: Heritability estimates recorded from papers reporting resistance to disease and insect pests. Heritability to disease is significantly greater than heritability to insect pests ($p = 0.02$). Boxes represent 25 and 75% quartiles and the middle line represent the median. Whiskers show 2.5 and 97.5% quantiles.
Figure 3.5: Summary of methods used to estimate heritability of pest resistance. No apparent effects of original data format or model type were found on estimates of heritability, although meaningful comparisons of estimates among groups is difficult due to low sample size.
Figure 3.6: Heritability estimates recorded from papers using LMs with transformed and untransformed data. Heritability estimates from transformed data were significantly lower than those from untransformed data ($p = 0.01$). However, this effect is difficult to interpret because it could be confounded with other effects we are unable to account for (see text for details). Boxes represent 25 and 75% quartiles and the middle line represent the median. Whiskers show 2.5 and 97.5% quantiles.
Chapter 4

Comparing LMM and GLMM methods for estimating heritability of pest resistance: a simulation study of traits quantified on percent and categorical scales.

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4.1 Abstract

In plants pest resistance is often quantified using a percent (e.g., percent shoot damage) or categorical scale. Estimating heritability for traits that are not quantified on a Gaussian (i.e., normal) scale can be problematic as non-Gaussian data do not strictly meet the assumptions of linear mixed effect models (LMMs), which are often used to estimate heritability. As such, we often see these types of data transformed or recoded (e.g., changed from categorical to numeric) prior to analysis. Alternatively, generalized linear mixed effect models (GLMMs) – which can accommodate non-normal data – can be used. Currently, it is unclear which modeling technique produces more accurate estimates of heritability. Here we demonstrate how choice of measurement scale (percent or categorical) and modeling techniques (LMM and GLMM) affect estimates of heritability using data simulations. Our findings suggest that for percent and categorical traits GLMMs underestimate heritability. Given that LMMs accurately estimate heritability, we would suggest the use of LMMs over more complex GLMMs for these types of traits. Despite the growing literature supporting the use of GLMM models for estimating genetic parameters we would advise caution in their application and use.
4.2 Introduction

Quantitative genetic theory has largely been developed to estimate genetic parameters – such as additive genetic variance and heritability – of Gaussian (i.e., normally distributed) traits. Often statistical models used to estimate genetic parameters are linear models (LMs) – analysis of variance (ANOVA) or linear mixed models (LMMs) – which are predicated on assumptions of normality (Sokal and Rohlf 2012). Estimating genetic parameters of traits that are phenotypically expressed or quantified as percentages can pose a problem, as these traits do not strictly meet the assumptions of normality. Recently GLMMs have been gaining in popularity as a method for estimating heritability of non-normally distributed traits (see Nakagawa and Schielzeth 2010). However, there is a philosophical issue: if the genetic effects of quantitative traits are assumed to follow a normal distribution, is estimating genetic effects using a binomial distribution appropriate? For example, resistance is often a polygenic trait (i.e., under the control of many genes with additive effects and not solely inherited from dominant genes) and following central limit theorem, which states that the sum of a large number of independent random quantities will converge to a normal distribution, polygenic traits should follow a normal distribution (see Hartl and Clark 1997). However, in plants resistance is due to a combination of different morphological and biochemical mechanisms (see Hanover 1975; War et al. 2012), and as such it is a multifactorial trait that cannot be measured directly. Often in plants we measure traits, such as percent foliage damage, to serve as a proxy variable for the underlying resistance. We know that traits derived from an underlying normal distribution but expressed on a non-normal scale (e.g., displaying skew or kurtosis) can produce reasonable estimates of trait heritability (Roff 2001). However, it is still unclear which type of statistical modeling technique (LMs or GLMMs)
produce more accurate estimates of heritability for non-normal traits. Here, we use simulation studies to explore the accuracy of different methods of estimating heritability for a percent trait quantified on either a proportion or categorical scale.

In the literature, we find a variety of methods used to estimate genetic parameters from traits that follow a non-normal distribution (see Chapter 3). Because proportion data are bound between 0 and 1 and the variance of proportion data is dependent on the mean (i.e., variance is a function of the mean), they do not strictly meet the assumptions of normality and homogeneity of variance that LMs are based on. Often with proportions, a variance stabilizing transformation – usually the arcsine square root transformation (“arcsine transformation”) – is applied to normalize data prior to analysis (see Sokal and Rohlf 2012). The use of transformations has been widely accepted, but the interpretation of model parameters is often difficult as they are reported on the scale of the transformation and can’t be back-transformed to a proportion scale (see Steel and Torrie 1960). Recently there has been some debate about the general use of arcsine transformations in ecology. For example, Warton and Hui (2011) suggest that instead of transforming data to meet the assumptions of normality for an LMM, a GLMM (which can accommodate for non-normal distributions) should be used instead. GLMMs are an extension of the LMM that allow for non-Gaussian data by allowing the model to fit the distribution of the data using a link function (for more details see Zuur et al. 2009). Although variance components are reported on the link scale, one advantage of the GLMM is that they can often be back-transformed to their original scale using an inverse of the link function (see Nakagawa and Schielzeth 2010). GLMMs are computationally complex, but they are gaining in popularity and are generally considered a more appropriate method than transformed-data LMMs for analyzing non-Gaussian data (Bolker et al. 2009).
Often percent traits are grouped and reported categorically and the resulting data have been treated in various ways by analysts. For example, when looking at resistance to tree diseases we often find that traits, such as percent defoliation (e.g., Balmelli et al. 2014; Kjær et al. 2012; McKinney et al. 2011) or percent infected foliage (e.g., Balmelli et al. 2014; Milgate et al. 2005; Wu and Ying 1997) are reported categorically (see Chapter 2). Scoring percent traits categorically is appealing because of the relative ease at which data collection methods can be implemented. However, ease of implementation comes with a cost as information is ultimately lost when the distribution is partitioned and values are grouped into categories. Furthermore, estimating genetic parameters such as heritability from categorical data can be problematic. To use an LM, data must first be converted to a numeric scale. This can be done in one of two ways, using either midpoint or integer scoring. Midpoint scoring uses the midpoint value of each categories’ range, creating a variable that represents an approximation of the percent scale. With integer scoring, categories are simply assigned an integer value based on their rank order. Converting the data to a numeric scale does not necessarily normalize the data and sometimes transformations (e.g., arcsine or square root) are applied to the numeric trait values. GLMMs can be used to estimate genetic parameters from categorical data, but there is no method to back-transform estimates of variance from an ordinal GLMM to their original (categorical) scale, as such, heritability can only be estimated from variance reported on the link scale.

Traits reported on a non-Gaussian scale can be problematic when estimating heritability. Although GLMMs are often recommended for data that does not follow a Gaussian distribution, it is unclear if these models have greater accuracy or precision than LMs when estimating heritability. Our motivation for assessing methodological effects on estimates of heritability largely stems from previous work estimating heritability of aphid resistance in balsam fir trees. We found large
discrepancies between estimates of heritability from the two modelling approaches (Appendix B) but ultimately published our results using GLMMs as these models appeared to be more widely accepted for data like ours (Edwards et al. 2016). The discrepancies in estimates that we found suggested that the choice of modeling technique could bias heritability estimates. Here, we test the hypothesis that methods used to quantify percent traits (proportion or categorical scale) and the choice of statistical modeling technique and/or transformation can influence heritability estimates.

4.3 Methods

4.3.1 Data simulations

To assess accuracy of heritability estimated from different methods of trait quantification and statistical analysis we simulated traits of known heritability for a population of 80 half-sibling families with 10 individuals per family. To simulate resistance values, we started by simulating a normally distributed trait of known heritability, following Roff (2006):

\[ X_{i,j} = a_{x,i} \sqrt{t} + b_{x,ij} \sqrt{1 - t} \]  

(4.1)

Where:

- \( X_{i,j} \) is the trait value for the \( j^{th} \) individual in family \( i \)
- \( a_{x,i} \) is the random family effect of the \( i^{th} \) family \( a_{x,i} \sim N(0, 1) \)
- \( b_{x,ij} \) is the random individual effect of the \( j^{th} \) individual from the \( i^{th} \) family \( b_{x,i} \sim N(0, 1) \)
$t$ is the correlation of siblings expressed as a proportion of trait heritability. For a half-sibling pedigree structure, where related individuals (on average) share $\frac{1}{4}$ of their DNA: $t = \frac{1}{4} h^2_x$, where $h^2_x$ is the heritability of trait $x$, which we set to 0.40.

This normally distributed trait was considered to represent the underlying (and never directly observed) resistance of each plant to insect attack. To simulate phenotypic values of the sort normally observed (in this case, percent shoot damage) we recoded the underlying normal distribution of resistance to a logistic distribution. We chose a logistic distribution because, in nature, supposedly binomial traits often display more variance than expected from the mean-variance relationship of the binomial distribution (i.e., data are overdispersed) (Zuur et al. 2007). Because the logistic distribution is defined by two parameters: location and scale, determining its mean and variance respectively – it allows us to simulate percent traits with overdispersion. To recode the underlying resistance to a percent trait, first we created two ideal distributions: one normal (mean = 0, sd = 1 and n = 10000), and one logistic (location = 60, scale = 4 and n = 10000). The ideal normal distribution was broken up into percentiles (i.e., 100 equal portions) and values from each percentile were matched to location along the ideal logistic distribution. Resistance trait values were recoded by reporting the corresponding logistic scale value for the grouping they fell into on the ideal normal scale. Trait values were rounded to the nearest whole number so we could treat percent values as though they came from a sample of 100 shoots. This was important for GLMM methods, which require the number of successes (damaged shoots) and number of failures (undamaged shoots). Because the logistic distribution can exceed the bounds of 0 and 100, trait values $<0$ or $>100$ were considered equal to 0 or 100 respectively. The recoding process was repeated using logistic distributions with scale = 8, 10 and 12 to create four different phenotypic distributions of pest damage from the same underlying resistance (Fig. 4.1). We will refer to our simulated traits
as S4, S8, S10 and S12; names reflect the scale parameter used to define each trait.

We also ran simulations with data recoded to a binomial scale and with our trait simulated directly from a binomial distribution (instead of recoding from a normal underlying distribution). However, the method used for trait simulation did not change our results (see Appendix C for alternative methods of trait simulation).

To test the effect of categorizing a percent trait on estimates of heritability, phenotypic trait values were recoded to categorical scales. In total, each trait was recoded to eight different categorical scales by varying number of categories (3, 5, 7, or 10) and the spacing between categories (using even or uneven spacing) (Table 4.1).

### 4.3.2 Heritability estimates

Heritability estimates the proportion of phenotypic variance that can be attributed to additive genetic effects. For half-sibling families, heritability is estimated as:

\[
h^2 = \frac{4 \cdot \sigma_f^2}{\sigma_f^2 + \sigma_e^2}\]  \hspace{1cm} (4.2)

Phenotypic variance is a sum of family variance ($\sigma_f^2$) and random error ($\sigma_e^2$), which captures any unexplained variance. Additive genetic effects among half-sibling families are taken as four times $\sigma_f^2$ (see Hartl and Clark 1997 for details). Additive genetic effects are used to estimate $h^2$ because selection targets additive effects from breeding individuals. Dominant genetic effects and epistatic interactions are not selected for at this level and therefore are not included in estimates of heritability.
4.3.3 Statistical modeling

For each trait, an LMM was used to estimate $\sigma_f^2$ and $\sigma_e^2$ using the following formula:

$$y_{ij} = \beta_0 + f_i + \epsilon_{ij}$$

where $y_{ij}$ is shoot damage from the $j^{th}$ tree, from the $i^{th}$ family, $\beta_0$ is the intercept, $f_i$ is the random effect of family (assumed to be normally distributed with mean of 0 and variance of $\sigma_f^2$) and $\epsilon_{ij}$ is the random error (also assumed to be normally distributed with mean of 0 and variance of $\sigma_e^2$). Variance components for damage quantified on the percent scale were estimated using both untransformed and arcsine transformed ($\sin^{-1}\sqrt{y_{ij}/100}$) values of shoot damage. Both integer scoring (assigning integer values based on rank order of categories) and midpoint scoring (using midpoint values of category ranges as an approximation of damage on a percent scale) were used to convert traits quantified on a categorical scale to a numeric scale prior to analysis.

GLMMs are an extension of the LMM, which use a link function to link an expected scale (e.g., binomial) to an underlying (normally distributed) latent scale (see de Villemereuil et al. 2016). Random error (i.e., residual variance) is not directly estimated in a GLMM but overdispersion can be modeled and random error can be taken as the sum of the overdispersion parameter and the distribution specific variance (see Nakagawa and Schielzeth 2010). We treated percent traits as though they came from a sample of 100 shoots. Percent damage was converted to number of successes (damaged shoots) and number of failures (undamaged shoots) and analyzed in a binomial GLMMs with a logit link function. Categorical traits were analyzed using an ordinal GLMM with logit link.
Estimates of heritability from GLMMs for our percent traits were back-transformed from the link scale to the original (proportion) scale using the formula:

\[
h_{\text{prop}}^2 = \frac{\sigma_a^2 \cdot P^2/(1 + \exp(\beta_0))^2}{(\sigma_a^2 + \sigma_e^2) \cdot P^2/(1 + \exp(\beta_0))^2 + P(1 - P)}
\]  

(4.4)

\[
P = \frac{\exp(\beta_0)}{1 + \exp(\beta_0)}
\]  

(4.5)

Where \(h_{\text{prop}}^2\) is the proportion scale heritability, and \(P\) is the inverse-logit transformation of the link scale intercept \((\beta_0)\) (see Nakagawa and Schielzeth 2010). Estimates obtained from ordinal GLMMs were not back-transformed, as far as we know, there is no method for back-transforming heritability obtained from ordinal GLMMs.

We ran all statistical models using R version 3.4 (R Core Team 2017). LMMs were run using the lmer function from the lme4 package version 1.1-13 (Bates et al. 2015). GLMMs were conducted using the MCMCglmm function from the MCMCglmm package version 2.24 (Hadfield 2010), which uses Bayesian modeling to estimate model parameters. MCMCglmm was used because it can estimate overdispersion and it is capable of handling both binomial and ordinal data. GLMMs for proportion traits were fit with uninformative priors and ordinal models were fit with residual variance fixed to 1, following recommendations from the package author (Hadfield 2015). Each model was run with 100 000 iterations, with a burn-in period of 5 000 and a thinning rate of 100.

We ran our simulations 500 times (population/trait generation and associated heritability estimates). Heritability estimates were averaged across simulations to generate point estimates of heritability. Paired t-tests were used to compare estimates of heritability from LMMs using raw and arcsine transformed data and
for LMMs using integer and midpoint scoring for categorical traits.

Because our models were run using different statistical frameworks (frequentist and Bayesian for LMMs and GLMMs respectively) we needed to ensure that choice framework was not biasing estimates. Using one sample population, we estimated heritability of our percent traits using different packages in R, using both frequentist and Bayesian approaches (Table 4.2). LMMs using different frameworks or packages produced consistent results, as did GLMMs, suggesting no biases could be attributed to the choice of framework (or R package). Because we used Bayesian methods for our GLMMs we also tested different priors (both informative and uninformative) as prior specification can bias estimates (van Dongen 2006). We found no effect of prior specification on estimates of heritability.

4.4 Results and Discussion

LMMs produced accurate estimates of heritability for all proportion traits (Fig. 4.2). However, it is interesting to know that estimates of uncertainty around estimates of heritability did not change with increasing trait variance. As the variance in our trait increased residual plots from our models suggested increased non-normality of the untransformed trait (this was most apparent for traits S10 and S12). The arcsine transformation did normalize residuals, but we found no significant difference ($p = 0.53$) in estimates of heritability from transformed and untransformed data. This was not surprising as LMMs are known to be fairly robust to violations in assumptions of normality (Sokal and Rohlf 2012). GLMMs, on the other hand, consistently and often dramatically underestimated heritability of percent traits (Fig. 4.2). Proportion data met the assumptions of a binomial distribution and as such a binomial GLMM should (in theory) be an appropriate
method for estimating heritability. We found that estimates from GLMMs improved as overdispersion increased; however, overall LMMs consistently outperformed GLMMs when estimating heritability for traits expressed on a percent scale. It is not clear why overdispersed data perform better in GLMMs as overdispersion is normally thought to compromise statistical estimation. However, we note that while the improvement moves estimates from GLMMs closer to the true value of heritability, it never led GLMMs to recover its true value (95% confidence intervals never overlap 0.40).

For categorical traits LMMs also produced more accurate estimates of heritability than GLMMs. As with proportion traits, GLMMs consistently underestimated heritability (Fig. 4.3). When using LMMs we found no significant difference in estimates of heritability when data was scaled using midpoint estimates or integer scaling ($p = 0.99$ and 0.65 for even and unevenly spaced categories, respectively). In general, the accuracy of heritability estimates increased as the number of categories increased, and this was especially true for traits with lower phenotypic variance (Fig. 4.3). The number of categories appears to be a more important factor for accurately estimating heritability than the choice of spacing (even vs. unevenly spaced groupings). Using more categories increases the probability of breaking up the center of trait’s distribution (where the majority of individuals are found); if the center of the distribution is not broken most individuals will fall into one category and variance among the majority of the population is not captured (Shaw et al. 1987). While heritability could sometimes be accurately estimated using few categories, this was only true for traits with higher levels of phenotypic variance. Because trait variance is usually unknown prior to sampling we would recommend using no fewer than five categories when categorizing traits. This is consistent with recommendations from Shaw et al. (1987), who suggested using seven categories to quantify a continuous trait on a
categorical scale, balancing the need for few categories to improve repeatability and the need to accurately capture variance among individuals.

While we have shown that LMMs and GLMMs can differ dramatically in the analysis of percent and categorical traits, Weng et al. (2017) found that for a categorical trait ordinal GLMM and LMM techniques produced very similar estimates of heritability. Additionally, in a review paper by Nakagawa and Schielzeth (2010) similar estimates of heritability were produced from GLMMs and LMMs for a proportion trait. Although both papers suggest GLMMs are appropriate methods for estimating heritability, they did not find any evidence to suggest that LMMs were a less accurate method of parameter estimation. Synthesizing the results of the three studies (Weng et al. 2017, Nakagawa and Schielzeth 2010, and ours), we suggest that GLMMs are indeed useful for some datasets but fail in other cases. Unfortunately, it is not clear in which cases GLMMs will fail. LMMs, in contrast, appear to be more robust, providing good estimates in all cases examined. Therefore, despite the apparent advantages of GLMMs in fitting percent and categorical data, LMMs should be the preferred method of analysis.

4.5 Acknowledgements

Computational resources were provided by ACENET, the regional advanced research computing consortium for post-secondary institutions in Atlantic Canada. ACENET is funded by the Canada Foundation for Innovation (CFI), the Atlantic Canada Opportunities Agency (ACOA), and the provinces of Newfoundland & Labrador, Nova Scotia, and New Brunswick. This project was supported by funding from the University of New Brunswick.
4.6 References


Table 4.1: Details of the groupings used to report percent shoot damage on a categorical scale.

<table>
<thead>
<tr>
<th>No. categories</th>
<th>Even</th>
<th>Odd</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>&lt;33, 33-66, &gt;66</td>
<td>&lt;20, 20-80, &gt;80</td>
</tr>
<tr>
<td>5</td>
<td>&lt;20, 20-39, 40-59, 60-80, &gt;80</td>
<td>&lt;5, 5-20, 21-50, 51-75, &gt;75</td>
</tr>
<tr>
<td>7</td>
<td>&lt;14, 14-28, 29-42, 43-57, 58-71, 72-85, &gt;85</td>
<td>&lt;5, 5-15, 16-50, 51-60, 61-80, 81-90, &gt;90</td>
</tr>
<tr>
<td>10</td>
<td>&lt;10, 10-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90, &gt;90</td>
<td>&lt;5, 5-15, 16-35, 36-50, 51-60, 61-75, 76-80, 81-90, 91-95, &gt;90</td>
</tr>
</tbody>
</table>
Table 4.2: Estimates of heritability from one sample population using different methods to estimate heritability for each trait (S4, S8, S10 and S12) derived from the same underlying resistance.

<table>
<thead>
<tr>
<th>Data</th>
<th>Model</th>
<th>R function</th>
<th>R package</th>
<th>Trait heritability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S4</td>
</tr>
<tr>
<td>Proportion</td>
<td>LMM using REML estimation</td>
<td>lmer</td>
<td>lme4</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>Baysean LMM(^1)</td>
<td>MCMCglmm (family='gaussian')</td>
<td>MCMCglmm</td>
<td>0.40</td>
</tr>
<tr>
<td>GLMM with logit link</td>
<td>rpt.rptProportion</td>
<td>rptR</td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>GLMM with logit link(^2)</td>
<td>glmer</td>
<td>lme4</td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Baysean GLMM with logit link</td>
<td>MCMCglmm</td>
<td>MCMCglmm</td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Arcsine-transformed proportion</td>
<td>LMM using REML estimation</td>
<td>lmer</td>
<td>lme4</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Baysean LMM</td>
<td>MCMCglmm (family='gaussian')</td>
<td>MCMCglmm</td>
<td>0.47</td>
</tr>
</tbody>
</table>

\(^1\) ran using default prior

\(^2\) effect of tree added to account for overdispersion
Figure 4.1: An example of simulated traits (percent pest damage) for a sample population of trees. Solid lines represent the ideal phenotypic distributions traits were assumed to follow and histogram bars show the actual trait values (see methods for details). Phenotypic trait values were all derived from the same underlying resistance recoded to a logistic scale ($\mu = 60$) and with varying scale parameters ($s = 4, 8, 10$ and $12$, for traits S4, S8, S10 and S12 respectively).
Figure 4.2: Heritability estimates (mean ± SD) from simulated populations (n = 500) with percent traits derived from an underlying resistance of known heritability ($h^2 = 0.40$; dashed line). For each population, the underlying resistance was recoded to a percent scale using logistic distributions with scale parameters of 4, 8, 10 and 12 (traits S4, S8, S10 & S12 respectively). Heritability was estimated using LMM and GLMM methods. No significant difference was found between estimates of $h^2$ from LMMs using untransformed and arcsine transformed values ($p = 0.53$), as such only untransformed values are presented.
Figure 4.3: Heritability estimates (mean ± SD) from simulated populations (n = 500) with percent traits derived from an underlying resistance of known heritability ($h^2 = 0.40$; dashed line). Each percent trait (S4, S8, S10 & S12 – see methods for details) was expressed as a categorical variable with 3, 5, 7 or 10 categories and with either even (●) or uneven (○) spacing between groups. Heritability of categorical traits was estimated using both LMM and GLMM methods. With LMMs no significant difference was found between midpoint and integer scaling methods; as such only one set of estimates (midpoint scaling) are presented.
Chapter 5

The effect of pest density and distribution on estimates of heritability of resistance

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5.1 Abstract

Heritability estimates help us describe how genetics and environment influence offspring’s resemblance to their parents. Estimating heritability of traits such as tree resistance to insect pests is not straightforward. In general, heritability estimates can be influenced by many environmental effects and can be misleading if these effects are ignored or underestimated. For example, variance in pest resistance may be caused by factors relating to the pest population, such as large fluctuations in pest density or distribution. Here we examine whether estimates of heritability are influenced by changes in pest density or predictable environmental patterns in pest distribution, such as edge effects. Our findings suggest a parabolic relationship between estimated heritability and pest density. We also found that heritability estimates were strongly reduced when pest damage was influenced by edge effects. Estimates of heritability can be more informative if these effects are taken into consideration.
5.2 Introduction

Spatial and temporal variation in pest populations is a source of environmental variance that is often overlooked when estimating heritability of pest resistance. The occurrence of signs and symptoms of pest damage depend not only on host susceptibility (i.e., genetic factors) but also on the numbers and distribution of the pest population. For example, at low pest density, undamaged plants may have escaped damage simply by chance or they may possess some level of genetically-based resistance and distinguishing these alternatives, and thus detecting resistant genotypes, becomes an extremely difficult task. Previous research has shown that positive linear relationships exist between estimates of heritability of insect resistance and insect pest density (e.g., see Strong et al. 1993; Leinekugel le Cocq et al. 2005). However, in these studies estimates of heritability were only reported for moderate to low pest densities, and so it is likely that at high pest densities, estimates of heritability would not continue to increase. While resistant individuals are less susceptible to pest damage, they are not completely immune and so at high pest densities, the differences between resistant and susceptible individuals become difficult to detect as pest damage on more resistant individuals increases. Moreover, at very high pest density it is likely that susceptible individuals are no longer available to pests (e.g., if they be become completely defoliated) and as a result, pests would be forced to attack more resistant individuals. Detecting genetic contributions to resistance should, therefore be difficult at very high pest densities. Thus, we would predict that the relationship between pest density and heritability of resistance is parabolic instead of linear, or at least that estimates of heritability are more variable at very high or very low densities of the pest population.
In addition to pest density, there are other ecological factors that may affect estimation of the heritability of plant resistance. For example, spatial heterogeneity across a landscape (e.g., environmental gradients or patchy pest distributions) is a ubiquitous feature of natural populations. Edge effects in arthropod distributions may be an important contributor to environmental variance in forest plant-insect systems (Kremsater and Bunnell 1999). Environmental changes in pest density between the outer edge and the interior of a stand would increase the relative importance of environmental variance compared to inherent genetic variance and thus decrease estimates of heritability. However, whether this is a consistent feature of edge effect, and how much heritability is likely to be affected is still to be determined.

While there are likely to be many environmental conditions that reduce estimates of heritability of pest resistance, in this study we focus on whether pest densities or predictable environmental patterns such as edge effect are likely to consistently influence estimates of heritability. We use simulations to ask: 1) is there a parabolic relationship between estimates of pest density and heritability, and 2) to what degree can predictable environmental patterns such as edge effects underestimate heritability?

5.3 Methods

5.3.1 Population simulations

To demonstrate how ecological factors can influence estimates of heritability we simulated a population of trees with 80 half-sibling families and 10 individuals per family. Underlying pest resistance was considered to be a normally distributed trait
(following Chapter 4, also see Falconer and MacKay 1998) using the following formula (Roff 2006):

\[
X_{i,j} = a_{x,i} \sqrt{t} + b_{x,ij} \sqrt{1-t}
\]  

(5.1)

Where:

\(X_{i,j}\) is the trait value for the \(j^{th}\) individual in family \(i\)

\(a_{x,i}\) is the random family effect of the \(i^{th}\) family, \(a_{x,i} \sim N(0, 1)\)

\(b_{x,ij}\) is the random individual effect of the \(j^{th}\) individual from the \(i^{th}\) family, \(b_{x,i} \sim N(0, 1)\)

\(t\) is the correlation of siblings expressed as a proportion of trait heritability.

For a half-sibling pedigree structure, where related individuals (on average) share \(\frac{1}{4}\) of their DNA: \(t = \frac{1}{4} h^2_x\), where \(h^2_x\) is the heritability of trait \(X\), which we set to 0.40. Simulated trait values followed a standard normal distribution \((X \sim N(0, 1))\). Using a rearrangement of the Z-transformation we converted our simulated trait values to a proportion scale, assuming probability values \((Y_{ij})\) followed a normal distribution with mean = 0.5 (\(\mu_y\)) and standard deviation = 0.166 (\(\sigma_y\)).

\[Y_{ij} = X_{ij} \cdot \sigma_y + \mu_y\]  

(5.2)

Following the empirical rule, approximately 99.7% of values fall within 3 * \(\sigma_y\) from the mean (i.e., 0.002 and 0.998) so the distribution of \(Y\) is not strictly bounded between 0 and 1 and only approximates a distribution of probability. As such, if \(Y_{ij}\) was < 0 or > 1 it was changed to 0 or 1 respectively, to ensure all values were on a probability scale.

We created 500 populations and for each population we simulated insect
attack and estimated heritability of pest resistance following the methods described below. Simulations were built using R version 3.4.0 (R Core Team 2017).

5.3.2 Simulations of the Effect of Density

To test the effect of pest density on estimates of heritability we assumed each tree had 100 shoots and simulated ten separate pest attacks per population that would produce 5, 10, 20, 30, 40, 50, 60, 70, 80, and 90% mean damage across the stand. For each pest density, we simulated shoot damage by first sampling a tree from within the stand. We established a regime more likely to sample trees that were more susceptible (i.e., higher \( Y_{ij} \)), as we would expect pests to cue in on more susceptible individuals (see Bernays and Chapman 1994). An individual’s probability of being sampled was taken as:

\[
Y_{ij} / \sum Y
\]  

(5.3)

For each sampled individual, we randomly generated a binary outcome with the probability of success equal to its probability of damage (\( Y_{ij} \)) to determine if our pest was successful at inflicting damage on one shoot. If an insect failed to damage the sampled tree we then sampled another tree from the population – for simplicity, we assumed that there was no cost of movement of insects between host trees. Damaged shoots became unavailable to subsequent pest attack and sampling continued until the simulated stand reached the desired level of pest damage. We then calculated the total number of damaged shoots per tree and expressed this as percent shoots damaged. Damage was reset to zero before each simulated level of pest attack.
The heritability of resistance was calculated for each pest density following Wright (1976):

\[ h^2 = \frac{V_G}{V_P} \]  

(5.4)

\[ h^2 = \frac{4 \cdot \sigma^2_f}{\sigma^2_f + \sigma^2_e} \]  

(5.5)

\( h^2 \) is an estimate of the proportion of total phenotypic variance \( (V_P) \) that can be attributed to genetic effects \( (V_G) \). Phenotypic variance is taken as the sum of the estimated family and random error variance \( (\sigma^2_f \) and \( \sigma^2_e \) respectively), and for half sibling families, total genetic variance is estimated as four times the family variance (see Hartl and Clark 1997 for details). Variance components were estimated using linear mixed effect models.

### 5.3.3 Edge Effects

To simulate edge effects in insect attack, we randomly arranged trees, with their underlying resistance set as in the last section, into a 40 by 20 grid. We simulated two levels of edge effect by increasing the probability of damage in the outer two rows of the stand perimeter. For a strong edge effect, the probability of damage \( (Y_{ij}) \) was increased by 0.4 in the outer row and by 0.2 in the second row; a lower effect was simulated by increasing probability of damage by 0.25 and 0.10 in the first and second rows respectively. Using the same methods outlined above we simulated pest attack at a level of 40% stand damage under the following conditions: strong edge effect, weaker edge effect and no edge effect. When an edge effect was present, heritability of pest resistance was calculated using five different linear mixed effect models: 1) using all trees in the stand; 2) removing trees from
the outer perimeter prior to analysis; 3) removing all trees affected by edge prior to analysis; 4) adding a fixed effect indicating whether a tree is affected by edge (yes/no); and 5) adding a spatial autocorrelation structure. Spatial autocorrelation was implemented using a Gaussian correlation structure that used row and column values as location coordinates for individuals within the stand. When no edge effect was present heritability of resistance was estimated using all trees in the stand.

5.4 Results & Discussion

As predicted, we find a parabolic relationship between pest density and heritability of resistance (Fig. 5.1a). When pest density is low, the frequency of pest arrival on all trees (resistant and susceptible) is low and most trees escape damage by chance alone. Phenotypic variance among individuals is therefore low and most of the variance is attributed to random effects (Fig. 5.1b). When pest density is high, all trees (regardless of their probability of being sampled by pests) experience an increase in pest arrivals. Individual trees are less likely to escape damage simply because a pest failed to arrive. Resistant trees still have a reduced probability of being sampled by a pest (compared to susceptible trees) and a reduced probability of suffering damage when a pest arrives but they are not immune to damage. When pest density is moderate the difference between resistant and susceptible trees becomes more apparent as trees do not simply escape damage by chance alone. However, at very high pest density, when susceptible individuals become unavailable (i.e., 100% damaged) the probability of pest arrival on resistant individuals increases. Because our simulations allow pests to sample trees until they are successful, resistant individuals inevitably suffer more damage at high densities. Estimates of heritability are consequently lower because the difference
between resistant and susceptible families becomes less apparent. Although the expected value of heritability is 0.40 (equation 5.1), by simulating pest arrival and damage at either low or high pest density we increased the random error (environmental variance) in realized damage. This reduced estimated heritability, leaving 0.40 as an upper limit of heritability in our simulations. The interquartile range of heritability estimates only overlaps the expected heritability (0.40) when pest damage is between 30-70% (Fig. 5.1), suggesting that moderate pest densities are more likely to produce more accurate estimates of the underlying genetic control of resistance. However, we were surprised that at relatively low (5% damage) and high densities (90% damage) we were still able to recover estimates of heritability that were well above 0 (Fig. 5.1).

Interestingly, although the relationship between heritability and pest density is symmetrical, we find a skewed relationship between variance and density (Fig. 5.1b). At low pest density, no individuals are likely to suffer high amounts of damage, and so all individuals have low (or zero) damage and there is little variance within the data. However, as density increases some individuals will suffer greatly, with more resistant trees still escaping damage since they initially have a lower probability of being sampled (the probability of being sampled changes as susceptible trees become 100% damaged) and a lower probability of a pest causing damage. Therefore, we find more variance at higher levels of pest density (Fig. 5.2). Heritability and phenotypic variance of a trait are used to predict possible gains that could be achieved through selective breeding. The response to selection is calculated as the product of trait heritability and selection intensity, which is a measure of the difference between the mean of the study population and mean of the proposed breeding individuals. If gains were estimated using the top 20% of resistant individuals as the proposed breeding population, predicted gains would be much higher for a population experiencing moderate pest attack compared to
estimates for a population under low attack, even if the estimates of heritability are similar. Thus, as phenotypic variance increases, so too would the maximum selection intensity. If predicted gains are low, breeders may overlook resistance when designing selection programs, however, if gains were estimated when a population was experiencing low pest damage, gains may be higher than predicted if the pest population increases.

As we expected, edge effects downwardly biased estimates of heritability, with stronger edge effects having a greater impact (Fig. 5.3). Where an edge effect exists, removing trees from the most affected area (i.e., outer perimeter) prior to analysis improved estimates, but when all affected trees were removed from the analysis we were much more likely to recover the expected estimate of heritability (Fig. 5.3). Adding a spatial autocorrelation term to the models did little to improve estimates (Fig. 5.2). Although edge effects represent a spatial pattern within a landscape, spatial autocorrelation based on row and column coordinates may not accurately capture the covariance structure that exists. This is because with edge effects it is not the distance between two trees that reflects the correlation structure, but rather the shared edge/interior nature of their locations. For example, two trees on opposite sides of the field (but both along the edge) are far apart but correlated, while an edge tree and a nearby interior tree experience different attack rates. Using a much simpler model that simply includes a fixed effect of tree location (edge/interior), improves estimates of heritability (Fig. 5.3).

Edge effects are not uncommon in forestry. White et al. (2007) recommends that for experimental design of genetic tests, two or more rows of border trees should be planted around the outer perimeter of the test sites to mitigate possible effects. However, this requires prior knowledge of the spatial distribution of patterns between edge and interior. In practice, edge effects are often overlooked in
the experimental design and analysis of trait heritability. This may be partly due to the complex nature of spatial analysis (see Dutkowski et al. 2002; Gilmour et al. 1997). However, here we have demonstrated a simple approach of adding an additional factor for location can produce accurate estimates of heritability even in the presence of strong edge effects.

Although we can increase estimates of heritability by removing spatial effects it is important to consider if doing so is appropriate. Low estimates of heritability in the presence of spatial effects do not represent an error in its estimation, but rather reflect how the trait is naturally influenced by an environmental effect. Many spatial effects cannot be mitigated and removing environmental variance attributed to these factors would not be advisable. For example, it would probably be impossible to change the distribution of a pest with a naturally clumped distribution to one that is homogeneous. However, edge effects are one particular instance where the spatial distribution of the pest could be managed (at least partially) and it may be of interest for breeders to estimate heritability after accounting for its effect. Reducing the ratio of edge to interior trees (e.g., having larger test sites or sites that are more square in shape) or planting trees in a location where the adjacent ecosystem is forested, could reduce (or remove) edge effects. Estimating heritability in the absence of spatial effects in these instances may help breeders better predict how trees will respond to selection and could serve as an upper limit to potential gains that could be achieved if effects are mitigated.

Trees are long lived and suffer repeated pest attacks from year to year. As a result, defense (whether constitutive or induced) is expected to draw heavily on the available carbon stores, which can be costly (Carson and Carson 1989). Thus, changes to the rank order of susceptible and resistant individuals over time can occur if resistance (i.e., increased defense) in one year comes with a cost of
increased susceptibility in subsequent years as stored resources are depleted. In our simulations, the ranking of resistant and susceptible individuals within a stand did not change, but in nature these relationships should be examined. While Edwards et al. (2016; see Chapter 2) showed positive correlations of family rankings among years, with higher costs of defense we might expect family rankings to decline or even reverse sign. Whether defense costs are high enough to erode or reverse inter-year correlations is an empirical question, and unfortunately resistance estimates for trees are still uncommon (Chapter 3). We recommend caution when practitioners assess heritability of resistance, because for long-lived species only one year of observations may not accurately represent trait heritability.

5.4.1 Conclusions

Ecological factors can make it difficult to detect genetic differences among individuals. Single point estimates of heritability can be misleading because of the need to match the ecological conditions under which selection programs will take place with the conditions under which heritability is estimated. Failing such matching, we should expect realized gains to frequently fall short of expectations – or, perhaps equally unfortunate, we should expect to pass up chances for significant gains because of discouraging estimates of heritability. Taking multiple estimates of heritability across time and space will allow for more accurate predictions for selection programs, and of the future gains to be had from manipulating ecological conditions under which artificial selection is applied.
5.5 Acknowledgements

This project was supported by funding from the University of New Brunswick. Computational resources were provided by ACENET, the regional advanced research computing consortium for post-secondary institutions in Atlantic Canada. ACENET is funded by the Canada Foundation for Innovation (CFI), the Atlantic Canada Opportunities Agency (ACOA), and the provinces of Newfoundland & Labrador, Nova Scotia, and New Brunswick.
5.6 References


Figure 5.1: Effect of mean stand damage on estimates of (a) heritability of pest resistance and (b) estimated variance components - phenotypic ($V_P$), genetic ($V_G$) and environmental ($V_E$). The dashed line represents the expected heritability (0.40). Points represent median values generated from 500 simulations, error bars represent 95% confidence intervals. Genetic variance was taken as takes $4*\sigma_j^2$ following equation 5.5.
Figure 5.2: Simulated damage per tree for a population experiencing both low (10% mean stand damage) and high levels of pest density (90% mean stand damage).
Figure 5.3: Heritability of pest resistance estimated using five methods for simulated populations (n = 500) experiencing both a strong and relatively lower edge effect (see methods for details). Boxes represent 25 and 75% quartiles and the middle line represent the median. Whiskers show 2.5 and 97.5% quantiles.
Breeders rely on estimates of heritability to identify traits that will respond to selection and predict genetic gains that could be achieved through selective breeding. However, this can be complicated for multifactorial traits, such as pest resistance, which are difficult to assess because they cannot be measured directly. As a result, there is a diverse range of methods used to capture trait variance and estimate genetic parameters. In this thesis, I have used a combination of field data, estimates of heritability from the literature, and data simulations, to demonstrate that the choice of scale and statistical methods used, as well as the ecological characteristics of pest populations (i.e., density and distribution) can bias estimates of heritability of pest resistance. Although the focus of this work is on forest trees, the scope of this thesis is much broader as it applies to quantitative traits in general and contributes to a better understanding of the factors contributing to variation in estimates of heritability.

In recent years there has been a shift away from traditional statistical modeling techniques in favour of more technical and complex analyses as a result of the availability of intensive computational methods for statistical analysis (see Murtaugh 2007; McGill 2012). For example, generalized linear mixed effect models (GLMMs) have recently gained in popularity, and researchers often advocate for their use over traditional methods (Ives 2015). There are caveats to this: GLMMs
are computationally complex (Bolker 2009) and prone to misspecification errors, such as improper specification of random effects and covariance structures (see Baral 2006). Their popularity may be due to a general attitude that a more complex analysis will produce more convincing results (Murtaugh 2007). Simple models are often sufficient to convey key features of the data and are less prone to model misspecifications because they are easy to apply and understand (Murtaugh 2007; Ives 2015). My findings support the notion that simpler LMMs can provide very robust estimates of heritability. In contrast, more complex GLMMs often underestimate the true value of the heritability of a quantitative trait when expressed on a percent scale. I limited the scope of this thesis to traits expressed as a percent of some maximum and, as an extension, simulations studies should be conducted to determine how estimates of heritability compare between models for traits quantified on other types of non-Gaussian scales, such as Poisson and binary.

When estimating heritability of pest resistance there is a need to balance ease of data collection with the need for statistical rigour. Fortunately (for breeders), I found that when categorical scales are used to capture a quantitative trait (e.g., percent defoliation) they can produce accurate estimates of heritability (Chapter 4). However, these estimates may be negatively biased when fewer categories are used, especially when the phenotypic variance of the trait is low. This is encouraging for researchers, as categorical data are less labour intensive and relatively easy to collect compared to quantitative measures. My findings support the recommendation from Shaw et al. (1987) that seven categories should be used to quantify a continuous trait on a categorical scale. This recommendation balances the need for few categories to improve repeatability and the need to accurately capture variance among individuals. In contrast, when resistance is treated as binary, much of the variance in the degree of pest damage is lost. Some traits can only be reported on a binary scale (e.g., mortality), but out of convenience, pest
resistance is often captured on a binary scale. If the goal of a breeding program is
to reduce the level of damage, I recommend the usage of methods that more
accurately capture variance in the level of damage among individuals, such as using
a categorical scale that adequately breaks up the distribution of damage or using a
measure of resistance that can be measured on a quantitative scale.

The heritability of resistance can be biased by ecological factors such as pest
density. Consistent with previous predictions, I found that there is a parabolic
relationship of heritability with pest density: there is a downward bias in estimates
of heritability at both low and high levels of pest attack. Phenotypic variance is
also low in populations experiencing low levels of pest attack. Because estimates of
genetic gains are the product of heritability and selection intensity – with selection
intensity being the difference between the mean trait value in the original
population and that of the selected breeding population – at low levels of pest
density little (or no) gains would be predicted through genetic improvement. At
low levels of trait variance selection intensity will inevitably be low, as the
population mean and that of the top individuals (i.e., top 20%) would not be very
different, resulting in low estimates of gains. Therefore, gains achieved through
selective breeding may actually be higher than predicted if gains are estimated
under low levels of pest attack.

The spatial distribution of pests will also influence estimates of heritability.
Heterogeneous spatial distribution of the pest (i.e., increased pest density along the
edge of a stand) can negatively effects estimates of heritability by increasing the
environmental variance, and can mask genetic effects that might actually be
available for selection. Accounting for spatial variance, such as edge effects, may
help in identifying more resistant genotypes by reducing the environmental
component of trait variance. Whether identifying resistant genotypes can result in
substantial gains depends on whether the spatial arrangement of pests (1) contributes to a substantial proportion of the phenotypic variance and (2) can be mitigated in breeding programs. For example, accounting for the spatial distribution of a pest that has a clumped distribution could improve estimates of heritability by lowering the variance attributed to random environmental effects, but if the location of a tree within a stand contributes more to its level of damage, then the gains predicted through genetic improvement would be much lower than predicted. When strong edge effects exist but can be mitigated, it may be more useful to estimate heritability and genetic gains after removing the edge effects (Chapter 5).

Forest trees have not been subjected to extensive breeding and for many species, substantial amounts of natural variance exists and can be exploited by breeders for gains (Neale and Kremer 2011). Pest resistance appears to be a moderately heritable trait, suggesting that selection for pest resistance would respond positively to selection in most instances. However, pests have a short generation time – especially in comparison to long lived tree species. As a result, we need to consider the potential for pests to adapt to increased levels of resistance over time. In general, variation in host susceptibility to pest damage can be attributed to three general mechanisms: pest preference, pest performance and host tolerance (see Acquaah 2012). Resistance attributed to reduced pest preference (e.g., being unattractive for feeding, oviposition and/or shelter) or reduced pest performance (e.g., directly affecting the biology of the pest through reductions in pest growth and/or development) puts selective pressure on pest populations to adapt if no alternative hosts (i.e., more susceptible trees) are available. In tree breeding, resistance to pest damage that is based largely on increased levels of pest tolerance would be beneficial, as tolerance does not put selective pressure on pests to adapt and overcome host resistance (Rausher 2001). Further research into the
mechanisms of resistance and the relative influence of preference, performance and
tolerance would be valuable.

Breeders need to exercise caution when interpreting estimates of heritability,
largely because the performance of genotypes is not necessarily consistent across
different environments. Not only can the magnitude of difference between resistant
and susceptible genotypes change (Chapter 5), but their ranking can also change in
response to different environmental conditions (see Hoffmann and Merilä 1999).
Changes in rank are more problematic than changes in the magnitude of differences
between genotypes. Changes in the magnitude will make gains difficult to predict
but changes in genetic ranking could reduce or eliminate genetic gain in some
environments. These genetic-by-environment interactions can complicate breeding
programs. However, when they are well understood they provide an opportunity for
breeders to optimize breeding strategies to suit particular environments (Li et al.
2017). Where breeding programs are used for selecting genotypes that are to be
broadly utilized across different environments, estimates of spatial and temporal
variation in genetic parameters across these environments, i.e., genetic correlations
between environments and heritability in different environments, need to be
understood in order to predict how traits will respond to selection.

Conclusions

Choice of methods used to quantify and estimate genetic parameters can influence
estimates of heritability. Estimating heritability of pest resistance is further
complicated by ecological factors relating to the pest population. Careful
consideration into the choices of trait measurement scale and statistical analysis
can mitigate these effects, although when dealing with complex traits, such as
resistance, caution should be exercised in how estimates are generated and interpreted.

Because forest products are a major contributor to the Canadian and world economies (see Klemper 1996), and because losses to pests can be substantial, selectively breeding for increased resistance could help reduce economic losses caused by pest damage. One major conclusion from my thesis work is that much remains to be learned and as such, I have outlined areas where attention should be drawn and show how data simulations are a promising method to approach additional questions.
References


https://dynamicecology.wordpress.com/2012/09/11/statistical-machismo/


Appendix A

Literature review data

Table A1: Summary of papers used in literature review. When the original data was reported on a categorical scale the number of categories used is listed in brackets. Data analyzed are on the same scale as the original data unless stated otherwise.
Table A.1: Summary of papers used in literature review. When the original data was reported on a categorical scale the number of categories used is listed in brackets. Data analyzed are on the same scale as the original data unless stated otherwise. Table continued on next page.

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<sup>1</sup> Mycosphaerella spp., Teratosphaeria spp., and Puccinia psidii

<sup>2</sup> Endocronartium harknessii (Western gall rust), Teratosphaeria spp., and Puccinia psidii

<sup>3</sup> Complex composed of seven species of adelgid (Adelges cooleyi, Adelges lariciatus, Adelges abietis, Adelges strobiolobus, Piceus floccus, Piceus similis, and Piceus pinifolius)

<sup>4</sup> Euson lasiolepis, Euson sp., Pontania sp., & Phyillodula sp.

<sup>5</sup> Interior spruce is a complex of Picea glauca, Picea engelmanii and their hybrids

<sup>6</sup> Three of four test sites had a medium number of families but one had only a small number of families
References


25. Mottet, M.-J., J. DeBlois, and M. Perron. 2015. High genetic variation and moderate to high values for genetic parameters of Picea abies resistance to


Appendix B

Estimating heritability of aphid resistance using LMMs

Overview

In Edwards et al. (2016) we estimated heritability of resistance in balsam fir trees (Abies balsamea) to damage caused by the balsam twig aphid (Mindarus abietinus). Trees from two clonal orchards (located in Brunswick and Nova Scotia) were evaluated for percent shoot damage over four consecutive years. Aphid damage in Nova Scotia was negligible in two years of our study and data for these two years were not included in our analyses. In the published manuscript, heritability was estimated using generalized linear mixed effect models (GLMMs). Here we present an alternative analysis of these data using linear mixed effect models (LMMs). For detailed methods and GLMM specifications see Edwards et al. (2016; Chapter 2).
Statistical Analysis

For each orchard (New Brunswick and Nova Scotia), heritability of aphid resistance in balsam fir trees was estimated using the following linear model:

\[ Y_{ij} = \mu + C_i + e_{ij} \]  

(B.1)

where \( Y_{ij} \) is the proportion of shoots damaged on the \( j^{th} \) ramet of the \( i^{th} \) clone; \( \mu \) is the overall mean; \( C_i \) is the random clone effect of the \( i^{th} \) clone; and \( e_{ij} \) the random error. Separate analyses were carried out for each year. Proportions were transformed prior to analysis using the arcsine square-root transformation \((\sin^{-1}\sqrt{Y})\), as preliminary analyses suggested that transformed data better met the assumptions of normality.

Broad-sense heritability, the proportion of total genetic variation to total phenotypic variation \((V_g/V_p)\), was estimated at both the individual \((H^2_i)\) and clone-mean \((H^2_c)\) level for each year for both orchards following White et al. (2007):

\[ H^2_i = \frac{\sigma^2_c}{\sigma^2_c + \sigma^2_e} \]  

(B.2)

\[ H^2_c = \frac{\sigma^2_c}{\sigma^2_c + \sigma^2_r} \]  

(B.3)

where \( \sigma^2_c \), \( \sigma^2_e \), and \( r \) are, respectively, the estimated clone variance, estimated random error and harmonic mean number of ramets per clone. Parametric bootstrapping was used to estimate 95% confidence intervals following Nakagawa and Schielzeth (2010). Statistical models were run in R version 3.4.0 (R Core Team 2017) using the lmer function from the lme4 package version 1.1-13 (Bates et al. 2015).
Results

Broad-sense individual and clone-mean heritability estimates varied between years in each orchard, due in large part to yearly variations in total twig aphid damage. Heritability estimates were positively correlated to the mean percentage of shoots damaged by twig aphid (Fig. B1a & B1b). When > 15% of shoots exhibited symptoms of feeding by twig aphid, heritability estimates were relatively high (>0.20 and >0.70 for $H^2_i$ and $H^2_c$, respectively). No significant effect of clone was found when shoot damage was <7%, estimates of ($H^2_i$) are near zero in both cases with confidence intervals (for both $H^2_i$ and $H^2_c$) overlapping 0 (Fig. B1).

Discussion

Interpretation of these data largely depend on which model is selected. LMMs suggest a relatively strong genetic basis in balsam fir for resistance to damage from the twig aphid. Individual and clone mean estimates of heritability are moderate to high; high estimates of clone mean heritability, in particular, suggest that gains from selective breeding could be achieved. In contrast, estimates from GLMMs suggest that individual heritability is low and family mean heritability is moderate at best. The discrepancies in estimates among models demonstrate how choice of modeling technique bias estimates of heritability for percent traits, which I address in Chapter 4 of this thesis.
References


Figure B.1: Relationship between individual ($H_i^2$) and clone-mean ($H_c^2$) heritability and mean percentage of shoots damaged per tree by the balsam twig aphid in two clonal balsam fir orchards, located in New Brunswick (⊗) and Nova Scotia (△). Heritability was estimated using both LMMs (a & b) and GLMMs (c & d), error bars represent 95% confidence and 95% credible intervals for LMMs and GLMMs respectively.
Appendix C

Alternative methods for simulating a percent trait of known heritability

In addition to the method of trait simulations presented in Chapter 4, three additional methods for simulating a percent trait of known heritability were tested. This was done to ensure that estimates of heritability were not influenced by our choice of method for trait simulation.

Recall that Roff (2006) demonstrated that a normally distributed trait of known heritability could be simulated using the formula:

\[ X_{i,j} = a_{x,i}\sqrt{t} + b_{x,ij}\sqrt{1-t} \]  

(C.1)

Where:

\( X_{i,j} \) is the trait value for the \( j^{th} \) individual in family \( i \)

\( a_{x,i} \) is the random family effect of the \( i^{th} \) family \( a_{x,i} \sim N(0,1) \)

\( b_{x,ij} \) is the random individual effect of the \( j^{th} \) individual from the \( i^{th} \) family \( b_{x,i} \sim N(0,1) \)

\( t \) is the correlation of siblings expressed as a proportion of trait heritability.

For a half-sibling pedigree structure, where related individuals (on average) share \( \frac{1}{4} \)
of their DNA: \( t = \frac{1}{4} h_x^2 \), where \( h_x^2 \) is the heritability of trait x, which we set to 0.40.

In Chapter 4 we expanded on these methods to create a percent trait with known heritability. To do this, we first simulated an underlying resistance trait (normally distributed; \( h^2 = 0.40 \)) and recoded the values to a logistic distribution to simulate percent shoot damage per tree (see Chapter 4 for details). Three alternative methods for trait simulation were tested to determine if method of trait generation could be influencing estimates of heritability. The first alternative method we used for trait generation was to simply recode the underlying resistance to values bounded between 0 and 100, the second method was to recode the underlying resistance to a binomial distribution (\( n = 100, p = 0.5 \)) and our third method was to generate traits directly from a binomial distribution. Each method is described below.

**Method 1: Recode underlying resistance to percent values bound between 0 and 100**

First, we created a normally distributed trait of known heritability following Roff (2006), which we used as the underlying resistance for our trait. Phenotypic values of pest damage were simulated by cutting the range of the underlying resistance values into 100 equally spaced groupings. Values contained within in each grouping were assigned their corresponding values from 0-100%.
Alternative method 2: Recode underlying resistance directly to a binomial scale

This method of trait simulation directly follows the methods described in Chapter 4, but instead of recoding the underlying resistance to a logistic distribution we used a binomial distribution. First, we created an ideal normal distribution (mean $= 0$ & sd $= 1$) and an ideal binomial distribution ($n = 100$ & $p = 0.5$). The ideal normal distribution was cut by 100 equally spaced cut sites, creating equally spaced groupings, 101 equally spaced values spanning the range of the ideal binomial distribution were matched to these groupings. Using these values as a look up table, the underlying resistance trait values were matched to the ideal normal distribution then changed to the corresponding percent value.

Alternative method 3: Simulate trait values directly from a binomial distribution

Instead of simulating an underlying distribution of resistance, we simply generated $a_{x,i}$ and $b_{x,ij}$ values directly from a binomial distribution ($n = 100$ & $p = 0.50$) and created percent trait values directly.

Heritability of percent traits generated using all three methods was estimated using both LMM and GLMM methods (following Chapter 4). Trait values were rounded to the nearest whole number prior to analysis, and percent traits were assumed to come from a sample of 100 shoots as GLMMs require percent traits to be expressed as number of successes (damaged shoots) and number of failures (undamaged shoots).
Results

Simulating by recoding the underlying distribution to values between 0-100% (Method 1) creates an overdispersed binomial distribution (Fig. C1b). Recoding to a binomial scale (Method 2) and drawing family and individual effects directly from a binomial distribution (Method 3) produced traits which follow binomial distributions (Fig. C1c & d). Phenotypic trait values are not centered around 50% when data were simulated using Method 3. Method 3 uses family effects ($a_x$) from a binomial distribution centered at 0.5 (probability of damage), individual effects are also drawn from a binomial distribution and are therefore all positive values. Summing family and individual effects (following formula B1) to get probability of damage per tree creates a distribution centered above 0.5, resulting in a phenotypic trait distribution of shoot damage centered above 50%.

The method used to simulate our trait did not have an effect on estimates of heritability. For each percent trait LMM methods produced estimates of heritability close to the expected to value of 0.40 and GLMM methods greatly underestimated its value (Table C1).

Conclusion

Using an alternative method for trait simulation would not change the outcome of our simulation study (Chapter 4). Recoding the underlying resistance to a logistic distribution allowed us to test different levels of variance because it is defined by two parameters - location and scale, determining its mean and variance respectively. This allowed us to model different levels of trait overdispersion within our population.
References

Table C.1: Heritability estimates from percent traits generated using three different methods of trait simulation. Heritability for each trait was estimated using LMM and GLMM methods. Methods used to simulate traits were as follows: 1) Recode underlying resistance to percent values bound between 0 and 100, 2) recode underlying resistance to a binomial scale \( n = 100, p = 0.5 \), and 3) simulate the trait directly from a binomial distribution.

<table>
<thead>
<tr>
<th>Statistical test</th>
<th>Method</th>
<th>( h^2 ) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMM</td>
<td>1</td>
<td>0.44 (0.21 - 0.63)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.44 (0.24 - 0.65)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.44 (0.28 - 0.72)</td>
</tr>
<tr>
<td>GLMM</td>
<td>1</td>
<td>0.07 (0.03 - 0.11)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.01 (0.00 - 0.01)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.01 (0.00 - 0.01)</td>
</tr>
</tbody>
</table>
Figure C.1: Distributions of the underlying population resistance (a) and percent traits derived using three different methods: 1) recoding the underlying resistance to the values between 0-100% (b), 2) recoding the underlying resistance to a binomial distribution (n = 100, p = 0.5) (c), and 3) simulating a binomial trait directly by generating family and individual level effects directly from a binomial distribution (n = 100, p = 0.5) (d).
Vita

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Fraser, S. 2012. Genetically-based resistance of balsam fir (Abies balsamea) to three insect pests. Northeast Forest Pest Council Annual Meeting. April 3-6. Amherst, Massachusetts, USA.


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