Field frequency and pattern of inheritance of the herbivory-defence trait "resistance-by-ducking" in the giant goldenrod (*Solidago gigantea,* Asteraceae)

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Background and aims – For a more complete understanding of the eco-evolutionary dynamics of plant-herbivore interactions, it is important to know the genetic mechanisms that control defence traits, as well as the levels of genetic variation for these traits in plant populations. Here, I present results of a study of the occurrence and pattern of inheritance of the recently discovered trait of 'resistance-by-ducking' in the goldenrod *Solidago gigantea* (Asteraceae).

Methods – I grew maternal families of seedlings from fruits collected in a large field population of *S. gigantea* in southwestern Virginia, USA. I determined stem phenotype (ducking or erect) for 704 plants across 36 maternal families.

Key results – Of the 704 plants, 72% had ducking stems and 28% had erect stems. Employing bootstrapping with Hardy-Weinberg principles, I found that the pattern of inheritance was consistent with stem phenotype being controlled by a major gene, with the ducking morph being recessive to the erect morph. The allele frequencies for stem phenotype in the source population were estimated to be 0.85 ducking and 0.15 erect alleles.

Conclusions – These findings not only help inform ecological studies of ducking in *S. gigantea*, but they lay the groundwork for comparative studies of similar goldenrod species whose populations have differing proportions of ducking stems. For example, in all previous studies on populations of *S. altissima*, ducking stems have been the minority morph, occurring at a frequency of less than 20%. These results suggest that ducking may be costlier in *S. altissima*, while *S. gigantea* may face different ecological pressures, or has somehow overcome some of the costs of ducking.

Key words – Candy-cane stems, ducking stems, goldenrod, inheritance pattern, resistance to herbivory, *Solidago*.

INTRODUCTION

Plants may resist herbivory through a wide variety of strategies, including chemicals that poison or reduce digestibility of tissues, and physical traits like thorns, trichomes, and resins that either slow feeding or deter herbivores entirely. There has been a long history of studying the genetic control and variation for resistance traits in crop plants for the purpose of increasing yield, as well as in natural populations for the more basic goal of understanding one of the most ubiquitous ecological interactions on the planet (Painter 1958, Denno & McClure 1983, Gould 1983, Maddox & Root 1987, Kennedy & Barbour 1992, Kliebenstein 2014). Continuously varying resistance traits, such as the concentration of alkaloids in potato leaves, are generally controlled by the combined action of multiple genes, while discrete traits, such as the presence or absence of glandular trichomes, are more likely than continuous traits to be controlled by a single major gene (Kennedy & Barbour 1992, van Dam et al. 1999, Kliebenstein 2014). The mechanism by which resistance traits are inherited affects the manner in which they can evolve in response to natural or artificial selection, as well as the methods scientists use to study these responses.

"Resistance-by-ducking" is a discrete type of defence trait that has recently been recognized to occur in several species of goldenrods (*Solidago* – a genus of Asteraceae native to North America) (Wise & Abrahamson 2008, Wise et al. 2010a). Ducking involves a temporary nodding of the stem (i.e. "candy-cane" stem) in late spring and early summer when the apical leaf bud is vulnerable to herbivores.

Ducking plants have been found to be significantly less susceptible to attack by apex-galling dipterans than are those that remain erect (Wise & Abrahamson 2008, Wise 2009). Despite the apparent benefit of ducking, ducking individuals have consistently been in the minority (usually < 20%) in the natural populations of *Solidago altissima* L. that have been studied in the eastern United States (Wise & Abrahamson 2008, Wise 2009). The ecological or genetic factors that constrain ducking from becoming more common have thus far not been determined (Wise et al. 2009, 2010b).

Across more than a decade of studies involving vegetative propagation of *S. altissima* in greenhouse, garden, and field settings, I have found that all clonal progeny of a ducking genet have exclusively ducking stems, while no clonal progeny of erect genets have been observed to duck. While this pattern suggests that stem morph has a genetic basis, it does not hint at what type of genes control the trait. Because stem phenotype involves two discrete morphs (ducking or erect), it is reasonable to start with the hypothesis that the phenotype is governed by a single major gene with two alleles. Such a gene could be found in cytoplasmic DNA (e.g. in mitochondria or chloroplasts) or nuclear DNA. If stem phenotype is controlled by nuclear DNA, then ducking may be either recessive or dominant.

Basic genetic information such as this is important for understanding the interactions between goldenrod and its

Figure 1 – Cluster of ducking (candy-cane) stems in a roadside population of *Solidago gigantea* in Roanoke County, Virginia, USA. These purple-stemmed ramets are most likely clones of a single genet.

herbivores, which constitute one of the best-studied model systems in plant-herbivore evolutionary ecology (Maddox & Root 1990, Abrahamson & Weis 1997). To that end, I investigated the pattern of inheritance of stem morph in a population of the goldenrod *Solidago gigantea* Aiton. Specifically, this study had two main goals: (1) document the prevalence of ducking stems in a natural population; and (2) provide insight into the genetic control of ducking, particularly whether the pattern of inheritance is more consistent with a cytoplasmic or nuclear gene, and whether ducking is more likely be dominant or recessive.

MATERIALS AND METHODS

Natural history

Solidago gigantea (giant goldenrod) is a perennial herb abundant in fields and disturbed habitats in its native range in eastern North America, as well in its expanding ranges in Europe and eastern Asia (Weber 2001, Abrahamson et al. 2005, Schlaepfer et al. 2008, Hull-Sanders et al. 2009, Szymura & Szymura 2016, Uesugi & Kessler 2016). Like its close relative *S. altissima*, stems in populations of *S. gigantea* occur as a mixture of ducking (i.e. candy-cane) and erect morphs. Because these goldenrod species are attacked by a suite of galling dipterans (Abrahamson & Weis 1997, Dorchin et al. 2007, 2009, 2015), it is likely that ducking also provides resistance in *S. gigantea*.

Goldenrod populations spread vegetatively via rhizomes, resulting in clumps of ramets that contain clones of the same genet (fig. 1). Goldenrods reproduce sexually through insect-pollinated, obligately outcrossing flowers, and a single ramet can remain in bloom longer than a month. Inflorescences of *S. gigantea* are branching panicles that can possess several hundred small capitula, each containing from ~15–30 florets (Strausbaugh & Core 1978). Each fertilized floret matures into a single-seeded, wind-dispersed fruit called an achene. Seed dispersal begins in autumn, but many seeds remain on senescent infructescences into the following spring.

Experimental design and data collection

In mid-March of 2015, I collected achenes of *S. gigantea* from senesced ramets across Green Hill Park (a 79.6-ha public park in Roanoke County, Virginia, USA). I included achenes from only one ramet per discrete cluster (genet) of goldenrod stems to ensure that within each bag, seeds shared the same ovule-parent (dam), but likely originated from numerous different pollen-parents (sires). Thus, the seeds in each bag represented a maternal family of half-sibs and full-sibs. The current study includes offspring of 36 dams (i.e. 36 maternal families).

On 21 Apr. 2015, I sowed seeds into 5-cm (2-inch) square plastic pots (one pot per family) in Miracle-Gro® potting mix (The Scotts Company LLC, Marysville, OH, USA) and placed the pots in plastic flats on top of wooden pallets in an outdoor plot exposed to full sunlight. In mid-May, I transplanted up to 21 seedlings from each pot singly into 5-cm square pots. After ~4 weeks, I transplanted each seedling individually into 15-cm (6-inch) plastic azalea pots and ar-

ranged them in randomized positions with respect to maternal families. I checked each plant regularly until flowering to record which ones ducked and which ones remained erect. (All ducking plants straighten back up prior to producing flower buds, such that they are then indistinguishable from erect plants.) The apices of several plants were damaged by herbivores or had developmental abnormalities that precluded a definitive identification of stem morph. In total, stem morph was determined for 704 plants (a mean of ~19.6 per maternal family), with a minimum of 12 plants per family. To assess whether the frequency of stem morphs differed among families, a nominal-logistic analysis was run with family as the sole explanatory variable and stem morph as the response variable, using JMP-IN 4.04 (SAS Institute, Cary, NC, USA).

Hypothesis testing

The hypothesis that ducking is maternally inherited can be tested quite easily, even though the stem phenotypes of the dams were not known because the seeds were collected from the source population after the plants had senesced—long after the ducking period. This hypothesis predicts that all offspring within a maternal family will have the same stem
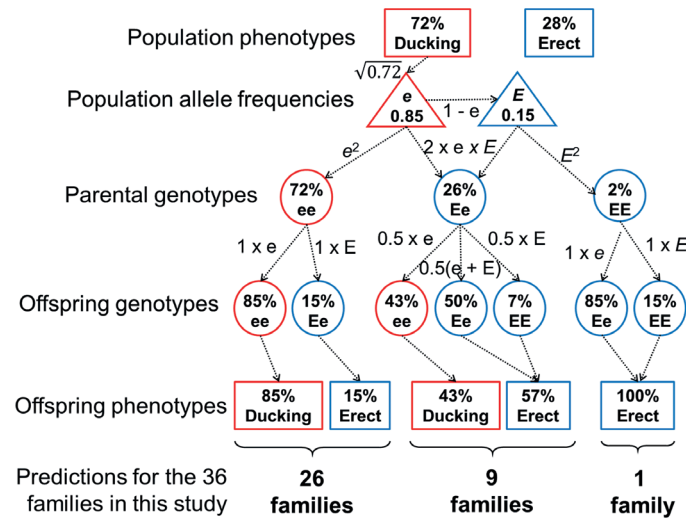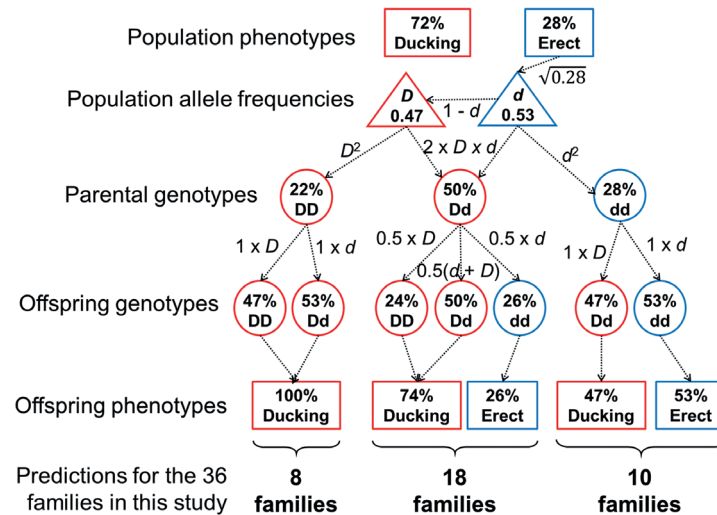
Figure 2 – Calculations of expected distributions of stem morphs for maternal families under two hypotheses: A, ducking is recessive; and B, ducking is dominant. Rectangular, triangular, and oval boxes represent phenotypic, allelic, and genotypic frequencies, respectively. Red and blue boxes represent values associated with ducking and erect stems, respectively. An arrow from one box to a second box shows the calculation used to determine value in the second box. For each hypothesis, alleles are coded as single, italicized letters, with the allele for the dominant morph capitalized and the allele for the recessive morph in lower case.

morph. If the siblings within a family consist of both ducking and erect individuals, then the hypothesis of maternal inheritance of ducking can be rejected.

Testing the hypotheses that ducking is a recessive or a dominant trait is more complex, but it can be done using Hardy-Weinberg principles to calculate allele frequencies and expected distributions of offspring morphs for each hypothesis. I used the observed proportion of 72% ducking and 28% erect stems among the 704 offspring plants across the 36 maternal families (See Results and Discussion) to represent the phenotypic frequencies of stem morphs in the parental population. I calculated the expected allele frequencies and the genotypic frequencies of the parents and offspring (fig. 2). The bottom lines display the expected number of dams that would exhibit particular ratios of ducking and erect offspring under the hypothesis that ducking is recessive (fig. 2A) versus the hypothesis that ducking is dominant (fig. 2B). Note that the ducking-is-dominant hypothesis predicts a greater number of dams to have exclusively ducking progeny, while none are expected to have more than 53% erect-stemmed progeny. In contrast, the ducking-is-recessive hypothesis predicts that one out of the 36 dams would have 100% erect progeny.

While these predictions represent expected values for percentages, they do not indicate the range of variation expected around these values. Without an estimate of this variation, it is difficult to infer whether the data are consistent with, or significantly deviate from, the predictions of these two hypotheses. In order to make these inferences, I used bootstrapping to construct confidence intervals around the expected values. Specifically, I used the same allele frequencies and calculations described in fig. 2 to simulate data sets of ducking and erect stems for 20 offspring of each of 36 dams. I ran 1000 iterations for the ducking-is-recessive hypothesis, then repeated the process for the ducking-is-dominant hypothesis.

I then superimposed the 95% confidence intervals from the bootstrapping analyses onto plots of the empirical data from the study.

RESULTS AND DISCUSSION

Frequency of stem morphs

Of the 704 plants whose stem phenotype was determined, 72% had ducking stems and 28% had erect stems. The proportion of ducking stems varied significantly among the 36 maternal families (likelihood ratio $X^2 = 191.374$, $p < 0.0001$; fig. 3). While four families had exclusively ducking stems, five families had fewer than 50% ducking stems, and one family had exclusively erect stems. Because stem phenotypes were not identical within all maternal families, stem phenotype was not inherited solely through the dam's genome. That is, the gene for morph must be located on a nuclear chromosome rather than in mitochondria or chloroplasts.

Genetic control of stem morph

If stem morph is indeed controlled by the nuclear genome, the next logical questions are whether the inheritance pattern suggests that stem morph is controlled by a single major gene, and whether ducking is a recessive or a dominant trait. Superficially, the inheritance patterns in this experiment fit reasonably well within the bootstrapped 95% confidence intervals for both the recessive and dominant hypotheses (fig. 4). It is at this right-hand tail of the distributions where the data are at odds with the predictions of one of the hypotheses. Specifically, the 95% CI for the ducking-is-dominant hypothesis does not encompass any families with more than 80% erect plants (fig. 4B). In contrast, the expectation for the
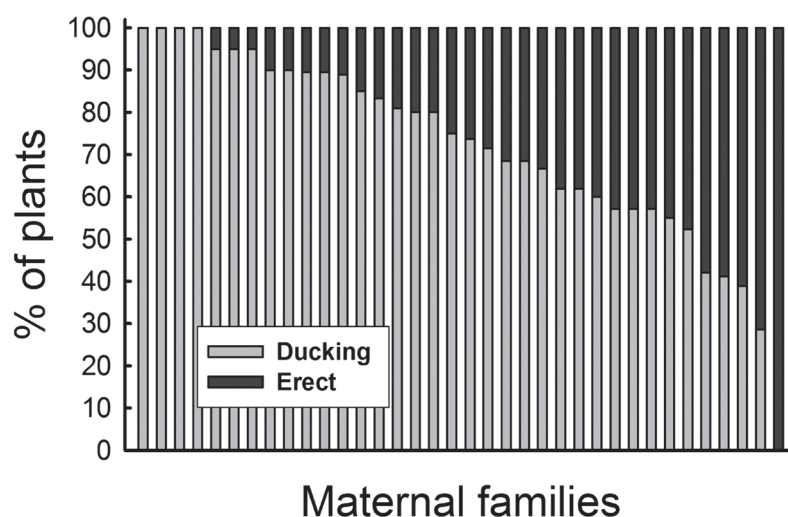
Figure 3 – Stacked-bar graph of relative percentages of ducking and erect-stemmed ramets for the 36 maternal families in this experiment, in order from highest to lowest percentage of ducking ramets. The light-gray bars represent ducking, and the dark-gray bars represent erect-stemmed ramets. Maternal families consisted of 12 to 21 ramets whose stem type could be confirmed (total of 704 ramets, mean of 19.6 ramets per family).

ducking-is-recessive hypothesis is that one out of 36 maternal families would contain 100% erect plants (figs 2 & 4A).

While a family with only erect offspring falls outside of the 95% CI for the predictions of the ducking-is-dominant hypothesis, further calculations show just how unlikely this finding of a family with only erect offspring would be if ducking were indeed dominant. The maternal family that consisted of 100% erect plants included 15 individuals. For simplicity, assume that the dam was homozygous recessive for an erect stem; thus, that dam would contribute an erect allele to all of her offspring. For her offspring to be erect (i.e., homozygous recessive), the sire would have to contribute an erect allele as well. The probability of that happening for any given offspring of this dam is simply the allele frequency for erect stems in the population (namely 0.53 under the ducking-is-dominant hypothesis). The probability that all 15 offspring would be erect would then be 0.53^{15}, or 0.000073. Because this probability is so low, it is safe to reject the hypothesis that ducking is the dominant trait.

In contrast, the inheritance patterns are quite consistent with the hypothesis that ducking is the recessive trait. The only result that falls outside of the 95% CI for this hypothesis is that four families (rather than three) consisted of 100% ducking plants (fig. 4A). Nevertheless, having four exclusively ducking plants does fall within the 98% CI for the ducking-is-recessive hypothesis.

Further considerations on the genetics of ducking

Both *S. gigantea* and *S. altissima* comprise several cytotypes, including populations of diploid, tetraploid, and hexaploid individuals (Semple et al. 1984, Halverson et al. 2008, Hull-Sanders et al. 2009, Semple 2016). In the United States, each of the three cytotypes occupies its own large geographic area, but three relatively small areas have been identified in which two cytotypes intergrade (Schlaepfer et al. 2008). The cytotypes of the plants in the current study were not determined, but the location of the source population (Roanoke County, Virginia) is close to the eastern region of diploids identified by Schlaepfer et al. (2008). Therefore, the analyses described in this paper were performed under the assumption that the plants were diploid—or at least that individuals are functionally diploid for a stem-morph gene. However, because the source population was not far from a contact zone where diploids and tetraploids intergrade (Schlaepfer et al. 2008), it is worth also looking at the predictions of the ducking-is-recessive and ducking-is-dominant hypotheses if the plants were tetraploid (and assuming that the chromosomes paired in a multivalent fashion).

If ducking-is-recessive, then the genotype for a tetraploid ducking plant must be homozygous with four ducking alleles (*eeee*). Following the analogous steps displayed in fig. 2A, the allele frequencies in the source population would be calculated as 0.92 for *e* and 0.08 for *E* (the dominant, erect allele). The predicted spread of phenotypes among the 36 families for the tetraploid cytotype is remarkably similar to the predicted spread for the diploid cytotype (bottom line of fig. 2A). For both ploidy levels, the expectation is 26 families with 85% ducking individuals and nine families with 43% ducking individuals. The only difference is that the diploid

prediction is that 1 of the 36 families is expected to have 100% erect stems, while the tetraploid prediction is that 1 of the 36 families is expected to have 86% erect stems. Thus, the ducking-is-recessive hypothesis was well supported by the empirical data whether the plants are diploid or tetraploid.

If ducking-is-dominant, then the genotype for a tetraploid erect-stemmed plant must be homozygous with four erect alleles (*dddd*; fig 2B). For the source population in this study, the allele frequencies would be 0.73 for *d* and 0.27 for *D* (the dominant, ducking allele). The predicted spread of phenotypes among the 36 families for the tetraploid cytotype with ducking being dominant is slightly more complex, but still quite similar to the predicted spread for the diploid cytotype (bottom line of fig. 2B). For tetraploids, the prediction is for 2 families with 100% ducking; 9 families with 91% ducking; 15 families with 73% ducking; and 10 families with 47% ducking individuals. For both the diploid and tetraploid cytotypes, if ducking were dominant, then no families are expected to have more than 53% erect-stemmed individuals. Thus, the finding of the exclusively erect family in this study
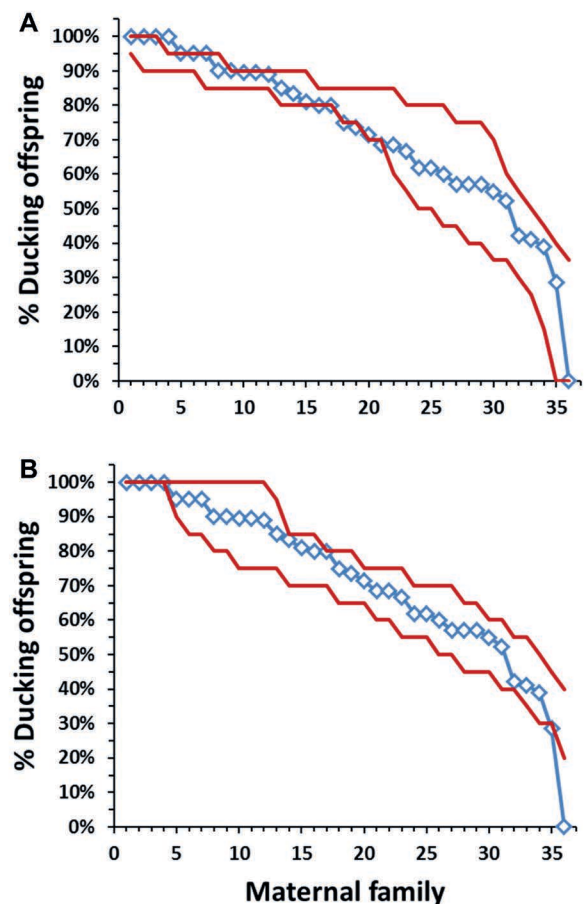
Figure 4 – Ninety-five percent confidence intervals (red lines) generated by bootstrap analyses for distributions of stem morphs for 36 maternal families under two hypotheses: A, ducking is recessive; and B, ducking is dominant. Blue diamonds show empirical data from the study.

makes the ducking-is-dominant hypothesis highly unlikely to be true whether the plants were diploid or tetraploid.

Although this study provides compelling evidence that stem morph is controlled by the nuclear genome, and strongly suggests that ducking is a recessive trait caused by a major gene, it is certainly not the end of the story in the genetics of ducking in *Solidago*. For instance, I have informally observed that goldenrod genets differ in several aspects of ducking, such as how old a stem is when it begins to duck, how long it remains ducking, and the angle of bending in the stem. Thus, while the ability to duck may be controlled by a major gene, there are likely to be other modifier and regulatory genes that affect aspects of the expression of ducking.

Eco-evolutionary relevance of stem morph

Previous studies in *Solidago altissima* have found that ducking stems are much less abundant than erect stems (Wise & Abrahamson 2008, Wise 2009, Wise et al. 2010b). Thus, one focus has been identifying costs that may constrain the evolutionary spread of this resistance trait (Wise 2009, Wise et al. 2009, 2010b). The current study on *S. gigantea*, in contrast, showed that ducking stems enjoyed a substantial majority in a large field population, and this high proportion of ducking stems is consistent with numerous personal observations of natural *S. gigantea* populations. One could still ask what factors constrain ducking from spreading to fixation in *S. gigantea*. Of even more interest would be investigations of the ecological or genetic factors that cause such a big difference in frequency of ducking and erect stems between these two goldenrod species.

I have observed the same stem dimorphism expressed by *S. gigantea* and *S. altissima* in populations of other old-field goldenrod species, including *S. rugosa* and *S. juncea*. These species are subjected to some of the same types of galling insects that ducking has proven effective against in *S. altissima*. Comparative studies of the genetic controls, geographic distributions, ploidy levels, and relative frequencies of ducking and erect stems in these species would help elucidate the eco-evolutionary dynamics of a fascinating resistance trait in a widespread and important genus of plants.

ACKNOWLEDGEMENTS

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