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Adaptive Variation in Desiccation Resistance in Rhagoletis

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ADAPTIVE VARIATION IN DESICCATION RESISTANCE IN RHAGOLETIS

By

Jennifer Lynn Hill

Accepted in Partial Completion

of the Requirements for the Degree

Master of Science

Kathleen L. Kitto, Dean of the Graduate School

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MASTER'S THESIS

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Jennifer Hill

January 14, 2016

ADAPTIVE VARIATION IN DESICCATION RESISTANCE IN RHAGOLETIS

A Thesis

Presented to

The Faculty of

Western Washington University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

By

Jennifer Lynn Hill

January 2016

ABSTRACT

Despite the ever-present challenges associated with invasive species, many environmental barriers exist that limit the spread of exotics. However, there is a growing number of examples of species overcoming these constraints via adaptive evolution years or decades after their initial introduction. The necessary genetic variation stems either from hybridization with a closely related species, or from shifting allele frequencies from standing variation in the population. Since its introduction to the Pacific Northwest, the apple maggot fly, *Rhagoletis pomonella* (Walsh 1867), has invaded all of coastal Washington, but has only small, isolated populations in the central and eastern parts of the state. The Cascade Mountains form a rain shadow that restricts the amount of precipitation in these regions, making it much drier than the western parts of Washington. I investigated aridity as an environmental constraint for the spread of *R. pomonella*, as well as potential sources of genetic variation for desiccation resistance in sympatric populations. First, I tested the potential for dry conditions, like those in the interior of Washington, to influence fitness in *Rhagoletis* flies, and act as a factor limiting their distribution. I found that individuals from a wetter part of Washington did not survive as well in dry conditions, but that individuals from a drier location were unaffected by desiccation treatment. The percent of weight that each pupa had remaining after treatment was the best predictor of survival. Second, I examined the variation in desiccation resistance in *R. pomonella's* native sister-species, *R. zephyria* across a finer environmental gradient, to look at possible adaptive variation within the species. I found that *R. zephyria* pupae from west of the Cascade Range show less desiccation resistance than those east of the Range, and that this pattern is indicative of local adaptation. Average annual precipitation and elevation of each transect site were the best predictors of how much weight each pupa would retain after desiccation treatment.

Finally, I measured desiccation resistance between apple-infesting *R. pomonella*, and hawthorn-infesting *R. pomonella* to begin to gauge the possible standing variation present in the species' genome. I found that the hawthorn host-race shows significantly more desiccation resistance than the apple host-race. This could be because the hawthorn host-race must endure a longer pre-winter diapause period, when conditions would be less favorable for water-balance strategies. The factors limiting the spread of *R. pomonella* are complex, but variation exists in both a native sister species and a sympatric host race. Determining whether there is a genetic factor associated with resistance would allow us to begin to gauge the relative importance of introgression and standing variation in the invasion of *R. pomonella* into the Pacific Northwest.

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INTRODUCTION

Invasions by exotic species have been of great interest to ecologists and evolutionary biologists for decades. As our world has become ever more globalized, humans have carried new species into virtually every environment on earth. Invasives provide unintended experimental systems for the study of interspecies interactions, but often have drastic negative effects as well (Ellstrand & Schierenbeck 2000). These species can deplete biodiversity by outcompeting or preying upon native species (Thompson 1991; Elliott *et al.* 2001; Inoue *et al.* 2007; Karatayev *et al.* 2014). They can also cause problems for humans, increasing health risks through the introduction of new disease vectors, as well as endangering cultural heritage through the extinction of native species (Manachini *et al.* 2013; Montarsi *et al.* 2013). Invasive species also pose a serious threat as agricultural pests, and can cost billions of dollars in control measures and damaged crops (Pimentel *et al.* 2001; Oliveira *et al.* 2013).

Despite the nearly ubiquitous presence of exotic species, there are nevertheless many limitations to invasions, and the vast majority of introduced species never spread aggressively (Williamson 1993). Even those that do spread often experience a lag of years or decades after initial introduction and before their subsequent invasion of surrounding habitats (Ewel *et al.* 1999). Competition with an already well-established community can pose a serious obstacle to a species trying to gain a foothold in a new environment. For example, Eskelinen and Harrison (2014) found that benefits to invasive grasses from experimentally increased rainfall and nutrients were reduced or completely offset by competition from native species. Similarly, Argentine ants decreased in number in the presence of a native species of ant, unless their colony was at least 5-10 times larger than that of the natives (Walters & Mackay 2005). Another problem, for invasive parasites or parasitoids, is host compatibility. Some exotics are host generalists, like *Drosophila simulans*, a human commensal that has been spread all over the world and that will mate on a wide variety of fruits and vegetables (Matute & Ayroles 2014). Others are more specialized, and must find a suitable host for feeding or mating upon arrival to the new location. This can be accomplished through the introduction of the original host prior to the exotic insect's arrival, or through the expansion of the insect's niche breadth to include other hosts (Mattson *et al.* 2007).

Equally important limitations to invasion can arise simply from the abiotic factors present at the site of introduction. Regional differences in temperature, soil nutrients, or rainfall amount and frequency can have important effects on a species' range, particularly one recently introduced into the environment. For example, though Argentine ants are successful invaders in many habitats, they have a high level of cuticular permeability compared to native Californian species, and are therefore susceptible to desiccation stress (Schilman *et al.* 2005). This is likely what has limited their invasions into hotter, drier locations, even on a relatively fine spatial scale (Schilman *et al.* 2007). Likewise, *Lantana camara*, a highly successful invasive shrub from tropical America, has a distribution in the Galapagos that is limited by its drought stress strategy. The plant relies on its deep root system to avoid the effects of dry conditions, however, in areas that receive less than 500mm of rainfall each year, the water table is too low even for *L. camara*, and it has failed to colonize the driest parts of the islands where its more drought-tolerant relative, *L. peduncularis* thrives (Castillo *et al.* 2006). It may actually be very common for certain environments to have a decreased susceptibility to invasions. Sites that have harsher abiotic conditions often have fewer invasions and while there are likely a number of limiting factors at work in these environments, abiotic stressors play a central role (reviewed in Zefferman *et al.* 2015).

It is, however, possible for an invasive species to overcome such constraints. There is increasing evidence that certain species have evolved to be more invasive after their initial introduction into an environment (Ellstrand & Schierenbeck 2000). Two major sources of genetic variation are available to these exotic species that could help them relieve the abiotic stress of a potential habitat: hybridization with a locally adapted species, and standing genetic variation within their own population.

Hybridization between an invasive species and its native relative can be a difficult phenomenon to study, particularly in animals, as it is often a rapid evolutionary event and is viewed as relatively rare in animals (Mallet 2005). Baseline data from before an invasion can be difficult to obtain, and until recently, backcrossed individuals could be difficult to identify with certainty (Rhymer & Simberloff 1996). Scientists also disagree on what constitutes a separate species, as hybridization events producing fertile offspring violate the Biological Species Concept. In extreme cases, hybridization can fuse populations to create a new, separate population, or hybrids may replace one or both of the parental groups (Rhymer & Simberloff 1996; Grant & Grant 2014). At other times, hybridization does not have as dramatic an outcome, and introgression via fertile hybrids backcrossing with parental species can spread novel alleles into one or both parental populations (Lee 2002; Currat *et al.* 2008; Excoffier *et al.* 2009; Pardo-Diaz *et al.* 2012). When this introgression passes traits that increase the fitness of an organism in an unfamiliar habitat, it can help exotic species become more aggressive in their spread into new environments (Abbott 1992; Perry *et al.* 2001). There are many examples of exotic species hybridizing with one another to create enhanced invasiveness, but situations in which a potential invasive interbreeds with a native species are comparatively rare (Ellstrand & Schierenbeck 2000). One likely example of this phenomenon in animals is hybridization between Neanderthals (*Homo neanderthalensis*) and modern humans (*H. sapiens*). As *Homo sapiens* first began their migration into Europe and Asia from Africa, they encountered and interbred with *H. neanderthalensis* to the point that people originating from places other than sub-Saharan Africa today can attribute 1-4% of their genomes to Neanderthal ancestors (Lowery *et al.* 2013). Neanderthal genes introgressed into the *H. sapiens* populations, and were incorporated into our early ancestors' genome, affecting traits from metabolism to cognitive development (Green *et al.* 2014). Some of these were detrimental to the fitness of the resulting generations, but many were selected for, including alleles that would have helped make early *H. sapiens* more fit to live in a non-African environment (Sankararaman *et al.* 2014). This introgression may have been part of what allowed our predecessors to spread so successfully across the globe.

Another major source of genetic variation is the standing variation that may already be present in a population's gene pool. If a species finds itself in a new environment where one previously uncommon allele increases fitness in those individuals, that allele can quickly become much more common in the population. Indeed, adaptation arising from standing variation in the gene pool could be the most rapid way a species can respond to new environmental challenges (Barrett & Schluter 2008). The potential speed of such adaptation is evident in the case of the invasive copepod, *Eurytemora affinis*. This coastal species has invaded freshwater ecosystems in multiple independent instances, showing extreme adaptability between saline and freshwater environments (Lee 1999). One possible explanation for this is standing variation in the genes controlling relevant physiological traits, such as ion transport, in the original populations (Lee *et al.* 2012). If some individuals within the introduced population possess a genotype that positively influences their fitness in the new environment, those genes will be disproportionately passed to the next generation. In the case of *Eurytemora affinis*, this shift to survival in a freshwater environment and an intolerance for a saline one can take place in as little as 12 generations in a lab setting, and over just a few decades in the wild (Lee *et al.* 2011).

Another notable example of adaptation through standing variation is in the *Rhagoletis* fruit fly complex. An ancestral host race of *Rhagoletis pomonella* (Walsh) infested hawthorn (*Crataegus spp.*) in the eastern United States, but a subset shifted hosts ~170 years ago to the introduced apple (*Malus pumila*), a tree that fruits earlier than hawthorn (Feder *et al.* 1997). Genomic inversions arose in a population of these flies in Mexico about 1.5 million years ago that are strongly associated with the duration of pupal diapause, and were then introduced into eastern populations through gene flow (Michel *et al.* 2007). That variation in diapause length allowed *R. pomonella* to colonize this novel fruit environment through adaptation to the earlier fruiting schedule (Feder *et al.* 2003). Populations also show variation in diapause length in latitudinal clines, with individuals in more southern locations having longer diapause durations (Dambroski & Feder 2007). This means that, though there will be warmer temperatures for a longer period of time before the southern flies' hosts become available, their emergence will still align with fruiting time. Further, variation in diapause length is present within single populations, allowing *R. pomonella* to colonize host plants that fruit at different times (Feder *et al.* 2003). Here, I investigate the potential for that same species to overcome climatic constraints and invade the major apple-growing regions in the arid part of Washington State.

Rhagoletis fruit flies in the *pomonella* species group depend completely on their specific fruit hosts. They live their entire larval stage feeding inside the fruit until they are developed enough to pupariate and overwinter. In addition, the adult flies forage on the surface of the plants' leaves, oviposit eggs into the fruit, and mate on the host plant (Bush 1966). Each species infests a different host plant species, or small group of species, and while the flies show strong host loyalty, they do occasionally explore other hosts (Huddleston 2013). Historical host shifts have led to sympatric speciation events within the species group, and resulted in very closely related, but genetically distinct populations living on separate hosts, sometimes in close proximity to one another (Bush, 1969; Prokopy & Bush 1972; Schwarz *et al.* 2007).

In the Pacific Northwest, the invasive *Rhagoletis pomonella* infests the fruits of apple trees (*Malus sp.*) and native and ornamental hawthorn bushes (*Crataegus douglasii* and *Crataegus suchsdorfii* [both native], *Crataegus monogyna* [introduced ornamental]) almost exclusively in the coastal parts of Washington and Oregon. Its native relative, *R. zephyria* Snow*,* infests snowberry fruits (*Symphoricarpos albus laevigatus*) throughout the region (Hood *et al.* 2013). *Rhagoletis pomonella* was likely introduced to the western part of North America via infested apples brought into Portland, Oregon in the late 1970s, and has expanded its range north to British Columbia and south to California (AliNiazee & Wescott 1987). So far, *R. pomonella* has been found only in small populations living on hawthorn in the central and eastern parts of Washington, and not in the large commercial orchards in the same region (Hood *et al.* 2013).

Climatically, central and eastern Washington are very different from the western third of the state due to the rain shadow formed by Cascade Mountains (Siler *et al.* 2013). For example, on the western side, Bellingham receives 90cm of rain annually on average, while Yakima, on the eastern side, only receives 22cm (Arguez *et al.* 2010). This results in a vastly different environment with which flies undergoing diapause must contend. It is possible that the harsher drought-like conditions in the interior of Washington act as a limiting factor for *R. pomonella* that keeps them largely bounded west of the Cascade Range.

Variation in climatic and geographic factors can be a major challenge for univoltine insects like *Rhagoletis* flies that spend the majority of their lives as pupae in diapause (Hahn & Denlinger 2007; Ragland *et al.* 2012; Kleynhans *et al.* 2014). During the first days of pupariation, *Rhagoletis* pupae lose much of their water weight in preparation for diapause (Bush 1966). Because the flies are in an immobile stage for many months, they are largely at the mercy of the environmental conditions surrounding them, particularly initially when sclerotization of the puparium is not complete (Neilson 1964; Ashley *et al.* 1976; Hulthen & Clarke 2006; Yee 2013b). Pupae are unable to augment their surroundings behaviorally or relocate to more favorable locations, so the water they do retain is due in large part to the moisture level in their environment at this time (Fitt 1981). They tend to have small body sizes and therefore large surface area/volume ratios that result in increased exchanges with the surroundings (Gibbs 2011). Pupae that undergo a long overwintering period also must keep enough body moisture to last throughout this time and too much desiccation can cause mortality during this stage, or influence adult fitness upon eclosion (Hahn & Denlinger 2007). Other species of insect have shown preference for a certain moisture level in which to enter diapause as a strategy to resist desiccation. In its larval stage, *Bactrocera tryoni*, the Queensland fruit fly, chooses soil of 75% moisture over both 0% and 100% moisture in which to pupariate, and shows a much higher mortality rate when pupariating at either extreme (Hulthen & Clarke 2006).

The impact of dry conditions on survival can be a limiting factor for the distributions of insects. *Glossina pallidipes*, a tsetse fly, has a distribution in eastern Africa that is likely limited by the intensity and duration of the weather patterns it experiences during pupariation.

The species exhibits enough phenotypic plasticity to withstand a fairly wide range of temperatures and relative humidity, but still shows significant changes in survival in hotter, dryer conditions (Kleynhans *et al.* 2014). If *Rhagoletis* fruit flies are affected in a similar way, a difference in desiccation resistance could be integral to a species' ability to live in a much drier environment, like that on the lee side of the Cascade Range. If *R. zephyria* has a more robust desiccation resistance than *R. pomonella*, it could help to explain the wide distribution of the former, and the limited distribution of the latter (Gibbs 2011).

While these two sister species remain ecologically distinct, they are capable of hybridizing and producing fertile offspring in the wild (Feder *et al.* 1999; Green *et al.* 2013; Arcella *et al.* 2015). Through these hybrids and subsequent backcrossing events, genetic introgression is possible, and has been demonstrated to happen disproportionately in certain genomic locations (Green *et al.* 2013). If the genomic regions correlate to regions that are associated with resistance to environmental stressors, such as drought conditions, *R. pomonella* may be able to gain favorable traits from its sister species, *R. zephyria,* that would allow it to expand its range further into Washington. This possibility presents a serious hazard for the apple industry of the state, which ranks as the most valuable agricultural commodity produced in Washington, a value of $2.25 billion in 2012 (Mertz *et al.* 2013). Currently, there is a zero-tolerance policy for apple flies in commercial orchards, and any detection of *R. pomonella* could lead to intense quarantine measures (WSDA 2014).

Despite the economic threat, few have sought to determine experimentally which environmental factors limit the range of the apple fly in Washington, or to what extent those environmental barriers might hold in light of hybridization and standing variation. To begin addressing this complex of questions, I examined desiccation resistance in the early pupal stage of *Rhagoletis* development. Immediately after pupariation, flies are highly vulnerable to desiccation, making it more likely to see differences in desiccation resistance during this time (Boller & Prokopy 1976). My study was composed of three separate experiments. First, I sought to test whether dry conditions and resistance to water loss under those conditions influence survival in *Rhagoletis* flies by testing for desiccation resistance and examining the relationship between water loss and survival probability (Survival Experiment). Second, I examined the hypothesis that *R. zephyria* has adaptive variation in desiccation resistance across an environmental gradient. If such variation exists, *R. pomonella* may be able to access these adaptive traits via hybridization and introgression (Cascade Transect). Third, I compared the relative desiccation resistance of the black hawthorn- and apple-infesting populations of *R. pomonella*, to explore whether there is already standing variation within the species. Such variation could increase their invasion potential through gene flow between host races (Host Comparison). And finally, I have laid the groundwork for a genome-wide association study that would allow examination of whether specific genomic regions are associated with desiccation resistance, and if those regions show disproportionate introgression (see Appendix).

METHODS

I conducted three experiments in order to assess whether dry conditions like those in central Washington could be a limiting factor to *R. pomonella* and examine the sources of variation in desiccation resistance that may be available to these invasive populations. I used the Survival Experiment to gauge aridity as a limiting factor, the Cascade Transect to explore geographic variation in *R. zephyria* desiccation resistance, and the Host Comparison to look at standing variation already present within *R. pomonella*'s host races.

Collection and desiccation treatment for all experiments

I collected all *Rhagoletis* fly individuals from snowberry bushes and apple and hawthorn trees in Washington State during August and September 2014-2015 (Table 1). Although parasitization rates of *R. pomonella* flies are extremely low in the Pacific Northwest (<1% in a single sample, [D. Schwarz, personal communication]), *R. zephyria* are commonly infested with parasitoid wasps (mainly *Opius lectoides* and *Opius downesi* [Braconidae]) which, to my knowledge, are undetectable during diapause without dissection of the puparium (AliNiazee 1985). I collected more snowberry flies than apple and hawthorn flies when relevant to account for this phenomenon. I haphazardly picked ripe or overripe fruits and spread the berries or apples into screens so that larvae would egress and fall into plastic dishes below. I collected all egressed individuals daily between 10am and 2pm for use in the desiccation experiments. During this time, most individuals pupariated and the remaining individuals pupariated shortly after they were collected. Thus, these collection procedures ensured that I used only pupae that had been in that stage for less than 24 hours. I collected the samples used in the Survival Experiment from Bellingham and Yakima, while those for the Host Comparison were all from Bellingham. Pupae for the Cascade Transect I collected from Bellingham and Yakima, as well as five sites between them (from west to east): Issaquah, Snoqualmie, Easton, Cle Elum, and Ellensburg (Table 1 and Figure 1).

Pupae in all three experiments were haphazardly assigned in equal numbers to either a high or low relative humidity (rh). The conditions were chosen to be representations of the respective humidities in both the western and central parts of Washington. The humidity chambers were kept constant through the use of saturated salt solutions, NaCl (~75% rh) and K_2CO_3 (~43% rh) for the Survival Experiment, and KCl (~85% rh) and K_2CO_3

Table 1 – Number of pupae used in each experiment, their respective sampling location, and treatment humidity. Number of samples analyzed reflects sample size after pupae with parasitoids and any treatment errors were excluded. Note that no *R. pomonella* samples were used in the analysis of the Survival Experiment (see Methods), and that parasitoids have not been excluded from the samples collected in

Survival Experiment		Samples			
Species	Location	Collected	Analyzed	RH%	Year Collected
R. zephyria	Bellingham	100	82	75	2014
R. zephyria	Bellingham	100	44	43	2014
R. pomonella	Bellingham	50	0	75	2014
R. pomonella	Bellingham	50	0	43	2014
R. zephyria	Yakima	100	74	75	2014
R. zephyria	Yakima	100	74	43	2014
Cascade Transect		Samples			
Species	Location	Collected	Analyzed	RH%	Year Collected
R. zephyria	Bellingham	200	193	85	2015
R. zephyria	Bellingham	100	44	43	2014
R. zephyria	Issaquah	100	100	85	2015
R. zephyria	Issaquah	100	97	43	2015
R. zephyria	Snoqualmie	100	98	85	2015
R. zephyria	Snoqualmie	100	96	43	2015
R. zephyria	Easton	100	99	85	2015
R. zephyria	Easton	100	97	43	2015
R. zephyria	Cle Elum	100	98	85	2015
R. zephyria	Cle Elum	100	99	43	2015
R. zephyria	Ellensburg	60	56	85	2015
R. zephyria	Ellensburg	60	60	43	2015
R. zephyria	Yakima	100	99	85	2015
R. zephyria	Yakima	100	98	43	2014-2015
Host Comparison		Samples			
Species	Location	Collected	Analyzed	RH%	Year Collected
R. pomonella (apple)	Bellingham	250	249	85	2015
R. pomonella (apple)	Bellingham	250	247	43	2015
R. pomonella (hawthorn)	Bellingham	110	110	85	2015
R. pomonella (hawthorn)	Bellingham	110	107	43	2015

Latitude and longitude of each collection site. Bellingham samples were collected and pooled from multiple sites within two miles of each other. Bellingham: 48.730451, -122.51986; Issaquah: 47.53505, -122.03008; Snoqualmie: 47.39678, -121.47952; Easton: 47.23402, -121.17654; Cle Elum: 47.20163, -120.98397; Ellensburg: 47.00676, -120.54217; Yakima: 46.82055, -120.92803.

Figure 1 – Map showing collection sites for the Cascade Transect in Washington State.

Map created by Neal Shaffer in Google Earth.

(~43% rh) for the Cascade Transect and Host Comparison (Carotenuto & DellIsola 1996; Ragland *et al.* 2012). The chambers were monitored for humidity fluctuations using iButtons (Maxim Integrated, San Jose, CA) set to record humidity every half hour throughout treatment, and were opened everyday to weigh pupae, but were kept closed otherwise. After opening, the high humidity tended to be lower (~7%) and the low humidity tended to be higher (~5%) than prescribed levels, however they generally returned to treatment humidity within three hours. The chambers were kept at 22° C in a 16:8 light:dark cycle throughout the treatment. I placed each pupa into an open 0.67mL microfuge tube, in which they remained for the duration of the experiment, and weighed them to 0.01 mg on the initial day they were collected, and again once they had been in treatment for eight full days. Pupae were not kept in soil (as they would live under natural conditions) so that I could better control the humidity of the treatment. It was necessary to run the experiments using cohorts of flies, as the necessary numbers for the entire study did not emerge from the fruit in a single day. As soon as the pupae had been weighed the second time, I placed them all, regardless of treatment, into a chamber held at their respective high humidity treatments and 4° C for five months to overwinter.

The Survival Experiment was conducted first and used pupae from Bellingham snowberries and apples, as well as snowberries from Yakima (Table 1). In this experiment I used 75% rh as the high humidity treatment. This humidity was designed to be a fairly benign humidity for flies of both species and was similar to standard *Rhagoletis* rearing conditions (Schwarz *et al.* 2007). However, the *R. pomonella* pupae faired poorly even in the high humidity treatment (mean percentage of weight remaining after treatment was 55.2±2%, compared to 65.1±2% and 69.1±2% for Bellingham and Yakima *R. zephyria*, respectively [values=mean±SE]). In addition, only two flies from the high humidity treatment survived through diapause and emerged in the spring even though the same rearing conditions normally result in substantially higher overwinter survival (D. Schwarz, personal communication). It is possible that these pupae were injured during collection, as they can occasionally get trapped in the residue from the apples. Given the uncertainty regarding the treatment of *R. pomonella,* I excluded these flies from the rest of the analysis. In the host comparison that I conducted in 2015, I took extra precautions to avoid any fruit residue in the collection dishes, and to collect individuals as gently as possible. I also adjusted the high humidity treatment to a 85% rh for the Cascade Transect and Host Comparison (Table 1) to make sure the higher humidity treatment was more benign.

Survival Experiment – Does desiccation resistance enhance fitness under drought conditions?

This first experiment aimed to test whether dry conditions and resistance to those conditions influence fitness in *Rhagoletis* flies, using overwintering survival as a proxy for fitness. I split the Bellingham and Yakima *R. zephyria* populations into two groups and placed one group into a chamber held at high relative humidity conditions (~75% rh, 22° C), and one into a chamber with low humidity conditions (~43% rh, 22° C). After eight days, I placed these samples into 75% rh (4° C) and allowed them to overwinter for five months. I then removed the pupae from their overwintering conditions and placed them back into incubation at 22° C with an 16:8 light:dark cycle. They began to eclose as adult flies starting four weeks after this removal, and had finished emerging by seven weeks afterward. I then measured the length and width of all pupal cases, and dissected those pupae that had not emerged in order to verify that they had not been parasitized by wasps. Samples that were parasitized were excluded. I also calculated the percentage of weight each pupa had remaining (PWR) at the end of the desiccation or higher humidity treatment.

I tested for differences in emergence for each population and treatment from the 2014 sampling season using a Chi square test, and then ran three post-hoc Chi square tests with a Bonferroni correction to further explore the pattern after the initial null hypothesis had been rejected. I also ran Analyses of Variance (ANOVAs) on the initial weights, surface area/volume ratios, and PWR of the various populations. I used logistic regression to identify factors that determine survival of all flies in the study, as well as those with a high PWR after treatment. I included length, initial weight of each pupa, treatment (high or low humidity), population (Bellingham or Yakima), and PWR as potential factors. I completed all statistical analyses, for this and the following experiments, in R, with “car”, “MASS” and “ape” packages (Venables & Ripley 2002; Paradis *et al.* 2004; Fox & Weisberg 2011; Team 2015).

Cascade Transect – Is there adaptive variation in desiccation resistance in *R. zephyria*?

The goal of the Cascade Transect was to explore the possibility of adaptive variation in desiccation resistance in *R. zephyria* that might be beneficial to *R. pomonella* through introgression. I treated half of the samples from each of the seven transect sites (Table 1, Figure 1) at high humidity (85% rh) and half at low humidity (43% rh). After treatment, these samples were placed into 85% rh and were overwintering at the time this thesis was written. I calculated their PWR and compared them using an Analysis of Covariance (ANCOVA) with initial weight as a covariate. I also compared them using Tukey’s HSD contrasts to look at more specific differences in the *R. zephyria* populations. These data violate the assumption of equal variances, so I adopted a stricter alpha value (0.025) to make up for artificial inflation (Gamst *et al.* 2008). For each sampling site along the transect, I calculated the mean initial weight of the pupae, and gathered data on mean annual precipitation, elevation, and mean daily average August/September temperature (MAST) from other sources (Arguez *et al.* 2010). I ran exploratory pairwise correlations between all of these potential factors to determine whether they could be used in a regression. I then performed linear regressions on both the high and low humidity data to identify predictors of PWR. Finally, I tested the regression residuals for spatial autocorrelation using Moran's I (Grant & Little 1992).

Host Comparison – Is there variation between different host races of R. pomonella?

In the Host Comparison I was interested in standing variation in desiccation resistance within *R. pomonella* that might provide adaptive potential for this species. I used populations of *R. pomonella* from Bellingham that I collected from apples, and those I collected from black hawthorns. I divided samples from both populations into two groups and subjected one to high humidity (85% rh, 22° C) and one to low (43% rh, 22° C). These samples were also placed into 85% rh (4° C) after treatment to overwinter. I calculated PWR and I compared the initial weights of the pupae using an ANOVA. I ran an ANCOVA on the PWR of the pupae using their initial weight as a covariate. These PWR data also violate the assumption of equal variances, so I again adopted a stricter alpha value (0.025) to account for artificial inflation (Gamst *et al.* 2008).

RESULTS

Survival Experiment – Does desiccation resistance enhance fitness under drought conditions?

The initial Chi square test showed significant differences in survival among the treatment groups ($p<<0.0001$, $\chi^2=40.5$, df=3). The pupae from the Bellingham population that were kept in 43% rh had a percentage of emergence less than a third of those in all other treatment groups, which was significantly less than the 75% rh treatment in the same population ($p<0.001$, $\chi^2=11.9$, df=1). Though the high humidity Bellingham population had slightly lower percent emergence compared with both treatment groups in Yakima, none of these three were significantly different after correction ($p=0.03$, $\chi^2=4.9$, df=1 between Bellingham 75% rh and Yakima; $p=0.82$, $\chi^2=0.05$, df=1 between Yakima 75% and 43% rh) (Figure 2). The PWR was also significantly different between treatment groups ($F_{3, 271}=14.24$, $p<<0.001$). Tukey's HSD contrasts showed that the PWR of the Bellingham 43% rh group was significantly lower compared to all other treatment groups, while the Yakima 43% rh group showed no difference from the 75% rh treatments from both locations (Figure 3).

Initial puparial weights did not differ between Bellingham and Yakima populations (although they neared significance, $F_{1, 273}=3.15$, $p=0.077$), but their surface area/volume ratios did ($F_{1, 273}=11.60$, $p<0.001$). However, surface area/volume ratios were based on length and width measurements taken after desiccation treatment (see above). To test for distortions of pupal shape during treatment, I calculated surface area/volume ratios for 40 untreated samples from each population, and found that the distributions were statistically indistinguishable from one another ($F_{1, 78}=1.25$, $p=0.27$). Thus, the differences in the surface area/volume of the pupae following treatment was likely due to the puparia shrinking to

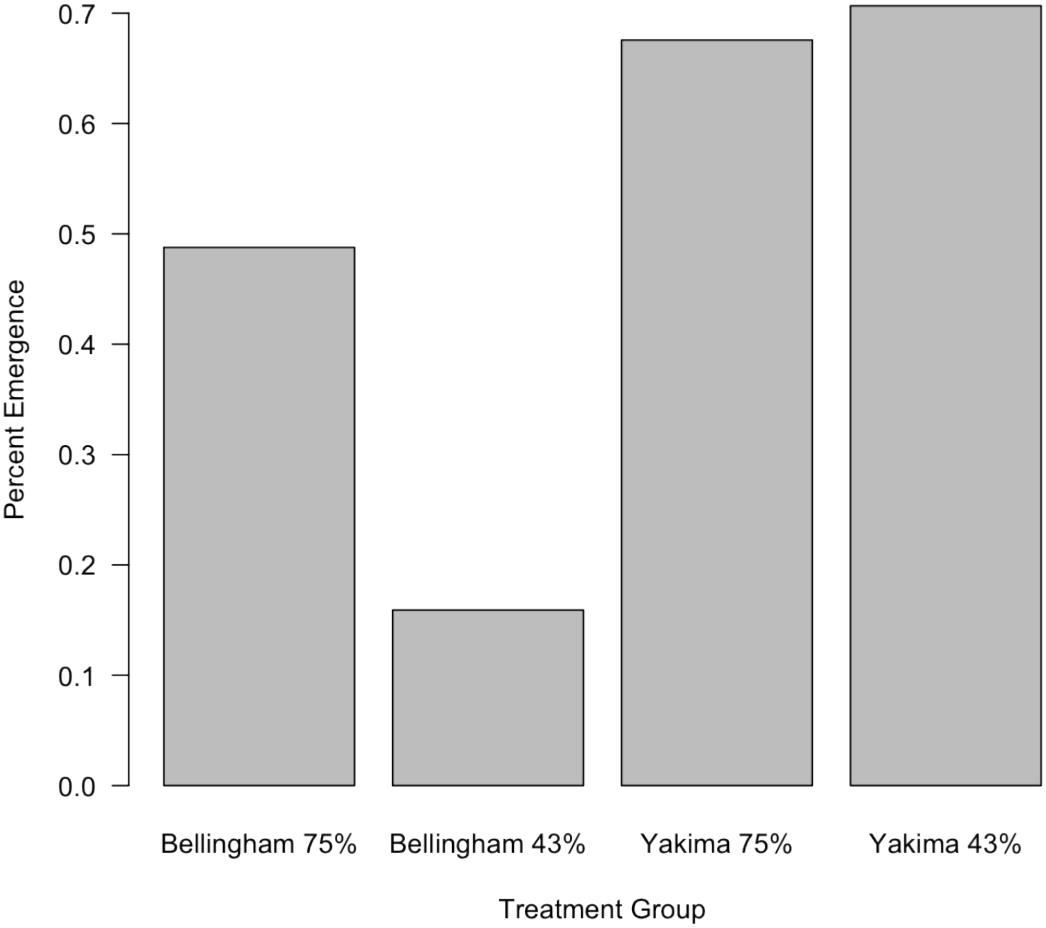

Figure 2 – Percentage of successfully eclosing flies in each treatment group, shown by location and treatment relative humidity. Emergence in different treatment groups differs significantly ($p<<0.0001$; $\chi^2=40.5$; df=3; see Results; Bellingham 75% n=82, Bellingham 43% n=44, Yakima 75% n=74, Yakima 43% n=74).

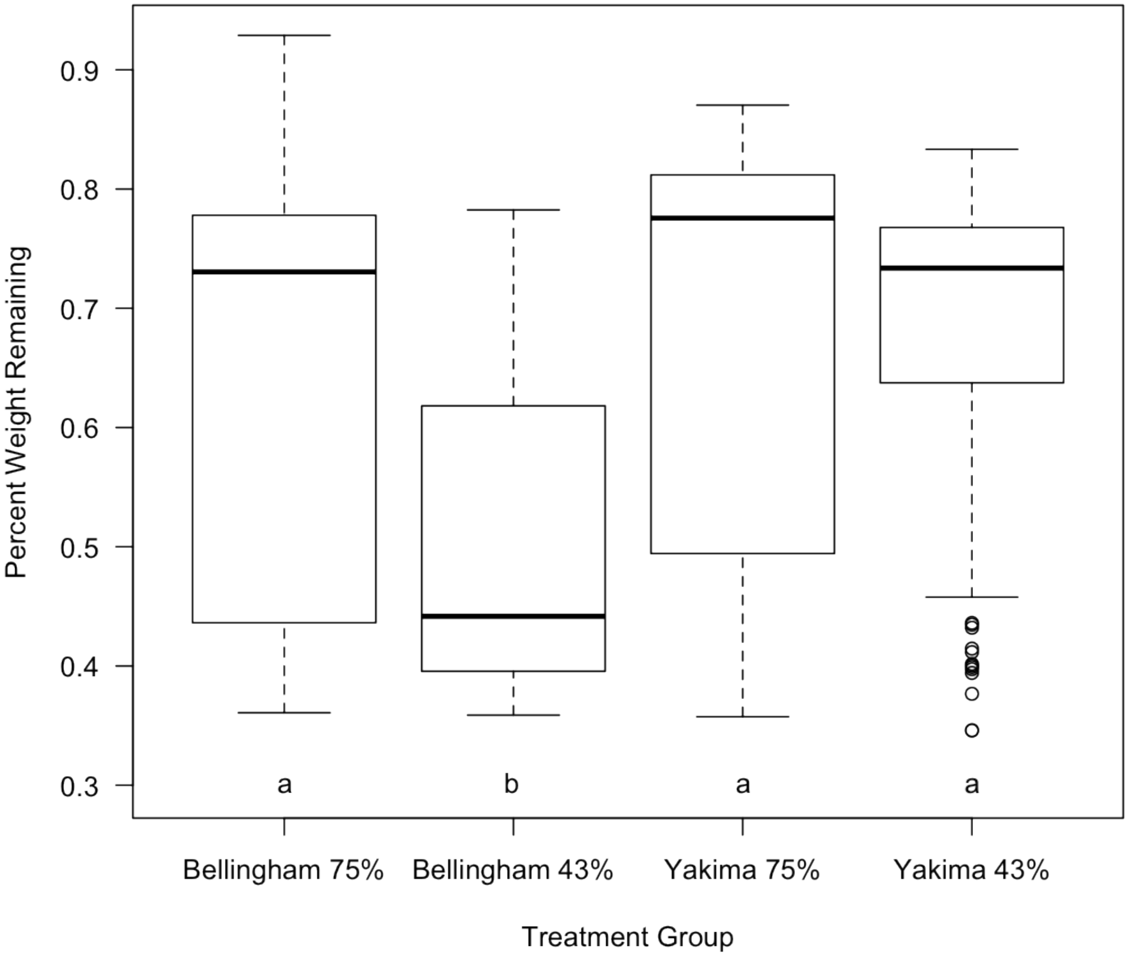

Figure 3 – Percent weight remaining for two populations of *R. zephyria* (Bellingham or Yakima) after treatment at high (75%) or low (43%) relative humidity for eight days. Treatment groups with the same letter (below boxes) have no significant difference, while those with different letters vary significantly from one another (according to Tukey's HSD contrasts, see Methods). Bellingham 75% n=82, Bellingham 43% n=44, Yakima 75% n=74, Yakima 43% n=74

varying degrees during treatment and suggest that post-treatment sizes may not be accurate representations of pre-treatment sizes; in subsequent analyses, I used each sample's initial weight as the most accurate measure of pre-treatment pupal size.

Because I know that initial weight is the more reliable measure (due to deformation of the puparium as it dries), I dropped length from the analysis, leaving initial weight, PWR, treatment, and population as potential factors. The regression showed that PWR ($p<0.0001$), population ($p<0.01$), and initial weight ($p<0.01$) were the factors included in the best fitting model according to Akaike information criterion (AIC) values (Table 2 and Figure 4). When I ran the logistic regression using only the pupae with a high PWR (≥ 65%), the same three factors were included, but with population as the most important, followed by initial weight and PWR (all $p<0.01$, Table 2).

Table 2 – AIC values for logistic regressions of factors predicting survival to eclosion of each pupa. The first table shows model selection for all samples, while the second shows model selection for only the high-PWR (>65%, see Methods). Asterisks show significant drops in AIC values when factors were added to the model (** denotes p<0.01 and *** denotes p<0.001).

Model for all Survival Experiment samples	AIC value
Survival ~ 1	380.96
Survival ~ PWR	159.86***
Survival ~ PWR + Population	152.48**
Survival ~ PWR + Population + Initial Weight	145.52**
Model for high-PWR Survival Experiment samples	
Survival ~ 1	132.71
Survival ~ Population	123.46**
Survival ~ Population + Initial Weight	120.37**
Survival ~ Population + Initial Weight + PWR	113.40**

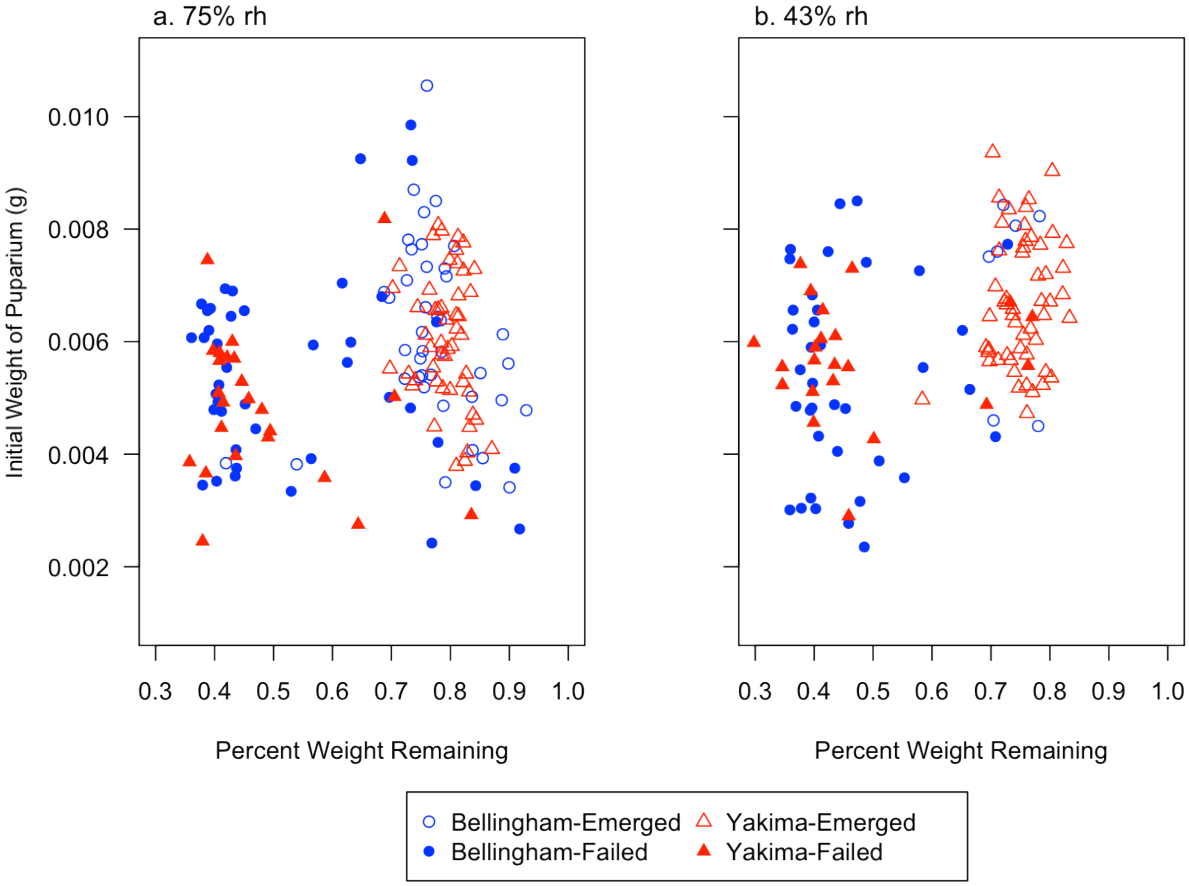

Figure 4 – Scatterplots representing the factors (Percent Weight Remaining and Initial Weight) included in the best fitting logistic regression model that predicts survival in *R. zephyria* populations in both high (panel a) and low (panel b) relative humidities.

Cascade Transect – Is there adaptive variation in desiccation resistance in R. zephyria?

Initial puparial weights of the populations were significantly different (Figure 5), so I used initial weights of each individual as a covariate in the ANCOVA to examine difference in PWR among all transect locations. For both high and low humidity treatments, the PWR was significantly different (high humidity: $F_{1,732}$=53.65, p<<0.001; low humidity: $F_{1,601}$=25.88, p<<0.001) among locations. Tukey's HSD contrasts show the PWR distributions falling into two distinct groups: Bellingham, Issaquah, Snoqualmie, and Cle Elum, opposite Easton, Ellensburg, and Yakima for high humidity (Figure 6a); Bellingham, Issaquah, and Snoqualmie opposite Easton, Cle Elum, Ellensburg, and Yakima for low humidity (Figure 6b).

In contrast to the ANCOVA where I included initial puparial weight as a covariate, I chose to include mean initial weight as an independent variable in the linear regression to gauge its importance as a predictor of mean PWR. Since I cannot include highly correlated factors in the same regression, I ran correlations on all of the potential factors. I found that precipitation correlated significantly with both initial weight (r=-0.8, p<0.05) and MAST (r=-0.79, p<0.05). Thus, I removed initial weight and MAST from the regression, leaving only precipitation and elevation. I chose to keep precipitation because it is one of the most useful factors for looking at desiccation potential in these transect sites. In the high humidity treatment, none of the factors tested decreased the AIC values significantly. However in the low humidity treatment, both elevation and precipitation (p<0.05 and p<0.01, respectively) were included in the best fitting model (Table 3).

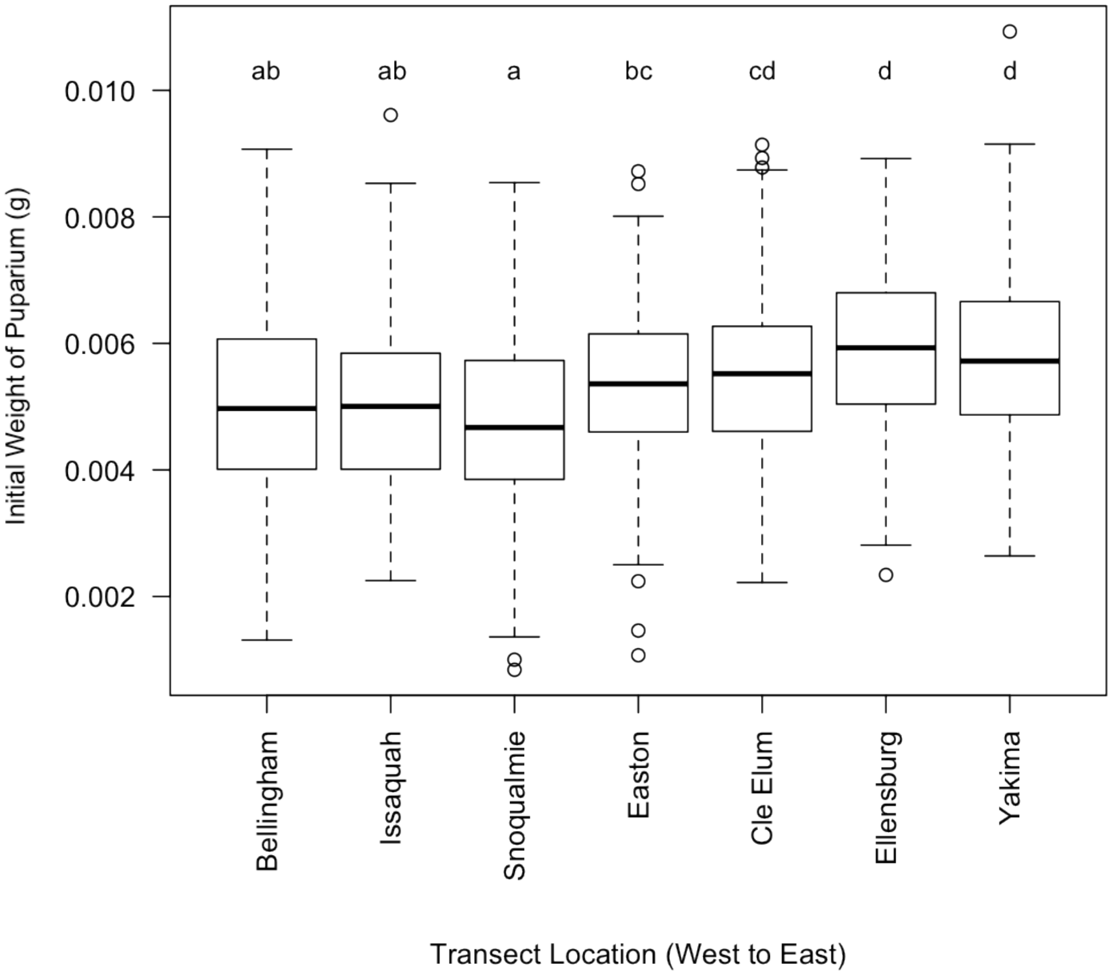

Figure 5 – Initial weight for different populations of *Rhagoletis zephyria* flies collected across a Cascade transect before relative humidity treatment. Populations with the same letter (above boxes) have no significant difference in initial weight, while those with different letters vary significantly from one another (according to Tukey's HSD contrasts, see Methods). Bellingham n=237, Issaquah n=197, Snoqualmie n=194, Easton n=196, Cle Elum n=197, Ellensburg n=116 Yakima n=197.

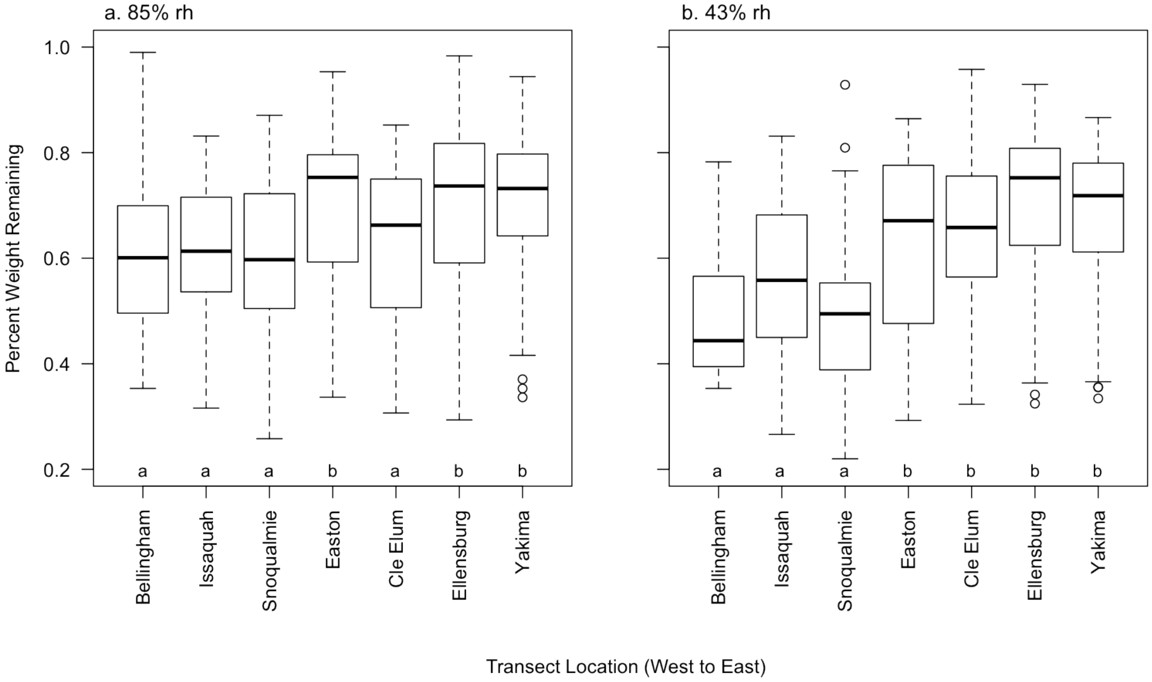

Figure 6 – Percent weight remaining (PWR) for different populations of *Rhagoletis* zephyria flies collected along a Cascade transect and kept at either high humidity (85%, panel a) or low humidity (43%, panel b) for eight days. Populations with the same letter (below boxes) have no significant difference, while those with different letters vary significantly from one another (according to Tukey's HSD contrasts, see Methods). For 85% rh: Bellingham n=193, Issaquah n=100, Snoqualmie n=98, Easton n=99, Cle Elum n=98, Ellensburg n=56 Yakima n=99. For 43% rh: Bellingham n=44, Issaquah n=97, Snoqualmie n=96, Easton n=97, Cle Elum n=99, Ellensburg n=60, Yakima n=98.

Table 3 – Table of AIC values for linear regressions of mean PWR against environmental factors for populations forming the Cascade Transect. Asterisks show significant drops in AIC values when factors were added to the model (* denotes p<0.05 and ** denotes p<0.01).

Model for 85% rh treatment Transect samples	AIC value
PWR ~ 1	-41.92
PWR ~ Precipitation	-44.03
PWR ~ Precipitation + Elevation	-45.66
Model for 43% rh treatment Transect samples	
PWR ~ 1	-33.74
PWR ~ Precipitation	-37.73**
PWR ~ Precipitation + Elevation	-46.28*

Because there is a spatial component to this experiment, I calculated Moran's I using the regression residuals to determine if the data showed spatial autocorrelation. If they did, the pattern in PWR would suggest that the differences in desiccation resistance were consistent with genetic isolation by distance of alleles conferring desiccation resistance. For both high and low humidity treatments, this was not significant ($p>0.4$ for both).

Host Comparison – Is there variation between different host races of R. pomonella?

The initial weights of the pupae were consistent within each host race, but different across host races, with apples yielding the largest pupae (9.9±0.15mg), and hawthorns producing pupae ~75% as large (7.4±0.23mg [values=mean±SE). The ANCOVA of their PWR (with initial puparial weight as a covariate) shows that host and treatment (but not the interaction between the two) both affect how much weight each pupa will have at the end of the experiment (host: $F_{1, 711}=132.40$, $p<<0.001$; treatment: $F_{1, 711}=308.39$, $p<<0.001$). Both host races had similar differences between their PWR in high and low humidity treatments. Pupae that came from hawthorn however, had ~15.6% higher PWR on average in the high humidity treatment, and ~14.2% higher PWR on average in the low humidity treatment compared to the apple host race (Figure 7).

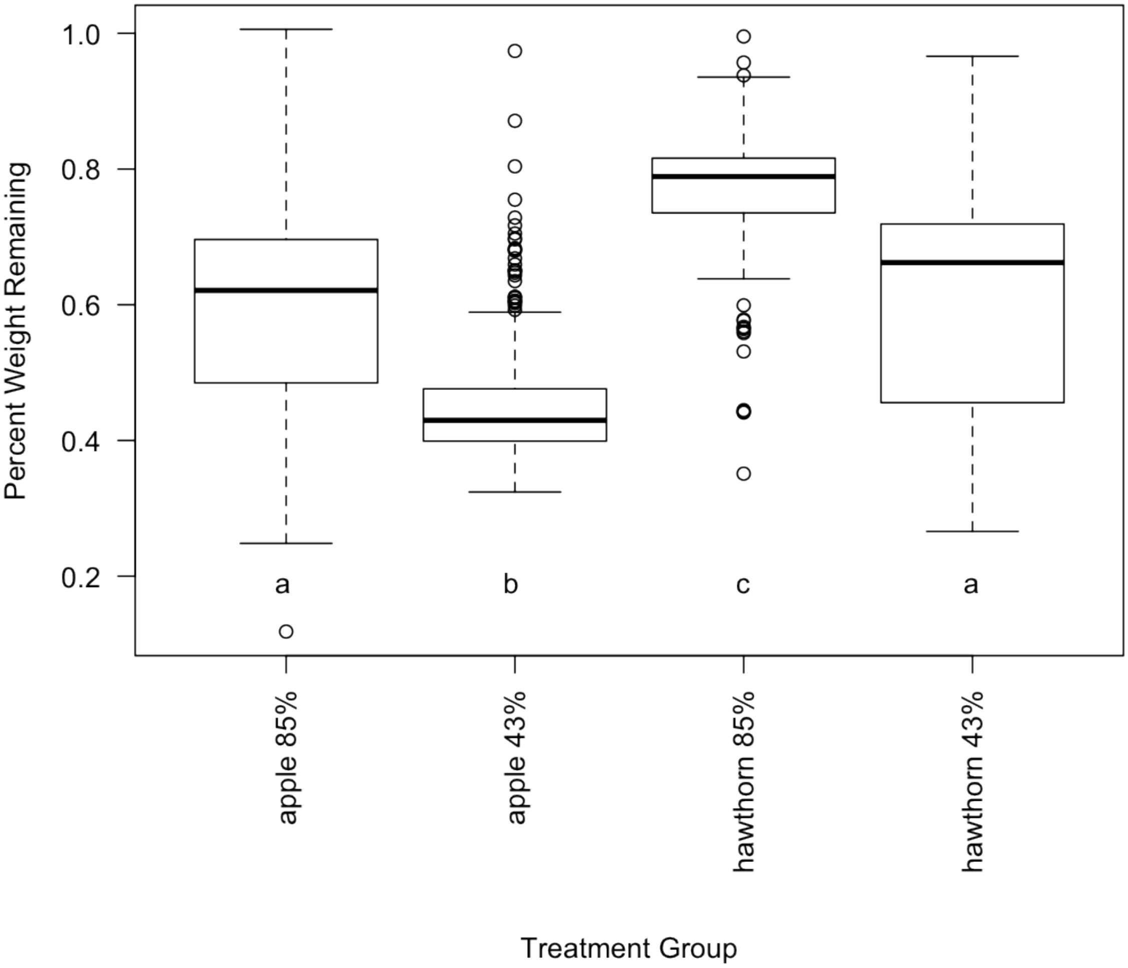

Figure 7 – Percent weight remaining (PWR) for different host races of *Rhagoletis* flies collected in Bellingham and kept at either high humidity (85%) or low humidity (43%) for eight days. Treatment Groups with the same letter (below boxes) have no significant difference in PWR, while those with different letters vary significantly from one another (according to Tukey's HSD contrasts, see Methods). Apple 85% n=249, apple 43% n=247, hawthorn 85% n=110, hawthorn 43% n=107.

DISCUSSION

My experiments show key differences in early pupal desiccation resistance among different populations and host races of *Rhagoletis* flies. Dry conditions negatively impact *R. zephyria* and the apple-infesting population of *R. pomonella* from the wet coastal regions of Washington, but do not affect *R. zephyria* from the arid interior of the state. Similarly, *R. pomonella* from early-fruiting black hawthorn shows greater desiccation resistance than *R. pomonella* from later-fruiting apple. As described below, these patterns collectively suggest an interaction between resistance to dry conditions and diapause that may cause flies with a longer pre-winter period to exhibit the same desiccation resistance as those that live in an arid climate.

Survival Experiment – Does desiccation resistance enhance fitness under drought conditions?

Rhagoletis zephyria flies, which are native to the Pacific Northwest, are able to survive a wide variety of environmental conditions, as demonstrated by their extensive range in Washington State. They have robust populations in the cool, wet climate of the coast, as well as the hotter, arid interior of the state. As evidenced by among-population differences in puparial water loss, populations from the two different climates differ drastically in their ability to withstand desiccation; individuals collected from Yakima are better able to resist desiccation in a low humidity treatment than their Bellingham counterparts. This variable desiccation resistance affects an individual's ability to survive successfully after treatment and eclose as an adult the following season. Survival is, in fact, most accurately predicted by the percentage of weight that each fly had remaining after being treated in one of the humidity conditions, with population and initial weight also playing a role. This is unsurprising, as pupae in preliminary studies that showed PWR below ~50% after eight days were visibly dried out and dead upon dissection of the puparium (data not shown). I later eliminated low-weight flies from the analysis, to gauge whether an increasing PWR would still have an effect amongst individuals who were most likely alive at the end of desiccation treatment. Percent weight remaining was still a significant factor, suggesting that although flies need to be above a certain threshold to have a chance at survival, the likelihood of eclosion goes up with higher PWR. A better predictor of survival in this group of flies is the initial weight of each pupa. Larger, heavier pupae tended to survive more often than those that were smaller. Both of these factors can predict survival in high-weight individuals due to the extended amount of time pupae still have before eclosion, when their metabolism is slowed but not ceased.

Treatment (high or low humidity) did not appear in the best fitting model for these emergence data. This is because flies from Yakima were very successful regardless of the relative humidity. By contrast, survival of flies from Bellingham was very dependent on high or low treatments and only ~23% of individuals in the low humidity treatment had PWR of over 65% (see results). This leaves them at a drastic disadvantage in dry conditions compared with the Yakima flies, which must tolerate low humidities more often. My data show that each of these populations of *Rhagoletis zephyria* is well adapted to the typical climatic conditions in its home range, and while those from Bellingham are detrimentally affected by placement in a more Yakima-like condition, those from Yakima are unaffected when placed in a more Bellingham-like condition. These patterns are consistent with those found in desiccation experiments in other systems (Schilman *et al.* 2005; Terblanche & Kleynhans 2009; Nyamukondiwa *et al.* 2010; Chown *et al.* 2011; Hidalgo *et al.* 2014;

Kleynhans *et al.* 2014). Because the humidity treatments in this study represent an initial test under artificial conditions, a study in which pupae from each location are transplanted into the opposite environment would be a more realistic test of these patterns.

Cascade Transect – Is there adaptive variation in desiccation resistance in R. zephyria?

Through the Cascade Transect I was able to examine similar patterns with finer geographic resolution. Precipitation at the source location was the strongest predictor of PWR at the end of treatment in low humidity, and elevation was also included as a significant factor. Pupae from locations with lower precipitation and higher elevation lost less weight in desiccation treatment than those from wetter, lower sites (Figure 7). Together these factors can account for approximately 86% of the variation in PWR. It is important to remember, however, that precipitation, MAST, and initial weight all correlate, and that precipitation as a factor stands for all three variables in this analysis. It may be that initial weight varies according to environmental conditions, and that this forms part of the mechanism of desiccation resistance in the more central populations of *R. zephyria.* In addition to having a higher ending weight, pupae from Yakima are also better able to survive and eclose in the spring than those in Bellingham after being exposed to desiccating conditions (see Survival Experiment, Figure 2). The results from the Cascade Transect imply that there is local adaptation in populations that must routinely deal with harsher drought conditions. This explanation best accounts for the patterns in the varying levels of desiccation resistance, both in the extreme eastern and western sites and in the intermediate ones along the transect.

One alternative explanation for the pattern in PWR after low humidity treatment could be a simple geographic gradient that is not an adaptation to different abiotic conditions, but the result of isolation by distance due to limited dispersal of flies. This is unlikely however, because the PWR from each of the seven sites shows a gradual longitudinal cline except at and just east of Snoqualmie Pass where there is instead an abrupt shift in phenotype. If the response was due to isolation by distance, the cline should extend uninterrupted. The second alternative explanation is the complete absence of gene flow, resulting in two separate populations of *R. zephyria.* If the Cascade Mountains act as a barrier to gene flow, the two populations on either side of the mountains may vary from one another phenotypically because of genetic drift, and not as the result of ecological adaptation. This is also unlikely due to the ubiquitous distribution of snowberries across the Cascade Transect, including at the top of the mountain pass and along the Columbia River Gorge, providing a corridor to facilitate at least some gene flow between the sides (Arcella *et al.* 2015). In the absence of selection for local adaptation, the expected gene flow would likely be sufficient to counteract the effect of genetic drift. The data currently available are not sufficient to test for the degree to which the Cascade Range acts as a barrier to gene flow (Green *et al.* 2013). With genomic data for the transect sites we would be able to better understand potential interactions between natural selection and gene flow.

Finally, the observed pattern along the transect may be due purely to phenotypic plasticity. *Rhagoletis zephyria* may simply be able to regulate its desiccation resistance in response to a dry pupariation environment. In this case, the differing PWR on either side of the transect would be explained by the beneficial acclimation hypothesis, which asserts that organisms that have had a chance to acclimate to harsh conditions will perform better than those who have not (Terblanche & Kleynhans 2009). If those flies from dry regions have acclimated to that level of moisture prior to collection, then they might be at an advantage over flies collected from wetter areas. After collection, flies from all populations were treated in a standardized lab environment in a common garden experiment, so phenotypic plasticity would not have arisen in separate populations based on experimental conditions unless the response occurred prior to collection (Pelini *et al.* 2012). Important to remember is that *Rhagoletis* flies spend their early lives entirely inside their fruit hosts, so the fruit growing in a dry region would need to be somehow different internally from one growing elsewhere in order for this acclimation response to take effect. The phenotypic plasticity could also take the form of a maternal effect, as appears to be the case with *Stator limbatus*, the seed beetle. Fox et al. (1997) found that by rearing the parental population on a certain plant, they could increase larval survival on a different plant, and further, that these differences were plastic responses and not genetic effects. However, others have shown that maternal gene transcripts decreased significantly by a few hours after egg deposition (Arbeitman *et al.* 2002). Because I examined a pupal phenotype, maternal effects may be less likely to explain the observed differences.

Given the available information, local adaptation is the most probable explanation of the observed pattern in PWR across the Cascade Transect. *Rhagoletis* flies have shown local adaptation in previous studies. Dambroski and Feder (2007) showed that *Rhagoletis* pupae from different host races emerged in the order that their host fruits would ripen, even when reared in standardized conditions. They hypothesized that an unknown genetic element must be responsible for this local adaptation to fruiting time (Dambroski & Feder 2007). Similarly, a genetic element for desiccation resistance may be common in the eastern populations, but may only exist at low levels in the west where it is not under as much selective pressure. To determine which explanation reflects the pattern in this transect most definitively, future studies should include a full transplant experiment between sites spanning multiple generations (Dambroski *et al.* 2005).

Unfortunately, because I lack emergence data for the Cascade Transect and Host Comparison, there are two caveats when interpreting these results. The first is the unknown level of parasitization by parasitoid wasps. In the Survival Experiment I found approximately equal rates of parasitoids between the Bellingham and Yakima populations upon dissection, but I do not yet know if the rate will remain consistent between sampling seasons or across the entire transect. I tested the distributions of the survival experiment samples against those same samples before I excluded parasitoids, and they showed no significant difference ($p>0.4$, data not shown). The second is that I cannot say with certainty that a high percent weight remaining in a given population leads to high survival and eclosion in the following season. If other populations respond similarly to those in the Survival Experiment, then this is likely to be the case, but it is also possible that there are other factors that are more important indicators of success than PWR. Nevertheless, it seems likely that the patterns I have found through these experiments will hold when I am able to gauge survival and parasitization percentages in the Cascade Transect and Host Comparison.

Host Comparison – Is there variation between different host races of R. pomonella?

Pupae that came from hawthorn, though they are 25% smaller than apple fly pupae, retain more of their weight in both humidity treatments. Unlike the *R. zephyria* from Yakima, these hawthorn-infesting *R. pomonella* show a significant difference in their response to high and low humidity. Their response to humidity is similar to that of the apple host race population, though the hawthorn pupae maintain higher weight in both treatments. A possible explanation for the increase in desiccation resistance is that hawthorn-infesting *R. pomonella* flies have adapted to their host plant's earlier fruiting time, and therefore spend longer times in diapause in pre-winter conditions. Ragland et al. (2012) explored the relationship between host fruit and seasonal shifts in Midwestern *R. pomonella,* where hawthorns fruit approximately three weeks later than apples, and found that flies feeding in apples were able to accumulate greater lipid content. This lipid accumulation helped to mitigate some of the harsher conditions the pupae experienced as a result of a longer pre-winter diapause period (Ragland *et al.* 2012). Interestingly, hawthorns in the Pacific Northwest fruit approximately three weeks earlier than apples, and the local hawthorn species' fruit is much smaller than those in the Eastern United States (Sim *et al.* 2012), so the potential trade-off may not be as favorable in this region. This suggests that the western hawthorn host race is using some other mechanism to alleviate the harsher pre-winter conditions.

Potential Mechanisms of Desiccation Resistance

Insect desiccation and desiccation resistance have been extensively studied, although very little is know about the actual mechanism of desiccation resistance in *Rhagoletis* (Hulthen & Clarke 2006; Terblanche & Kleynhans 2009; Kawano *et al.* 2010; Chown *et al.* 2011; Parkash *et al.* 2011; Yee 2013a; Hidalgo *et al.* 2014; Kleynhans *et al.* 2014). All else being equal, flies with smaller SA/V ratios should lose less water to the surrounding environment than those with larger SA/V ratios. In the Host Comparison, this is clearly not the case. Hawthorn-infesting *R. pomonella* are ~75% the size of the apple host-race, yet retain a much higher percent weight remaining at the end of desiccation treatment

(Figure 5). However a difference in size may explain the differences in PWR in *R. zephyria* across the Cascade Transect. In this species, populations that had higher initial weight also retained a higher percentage of weight after treatment (Figure 6, 7). The more favorable SA/V ratio of bigger pupae will by itself increase desiccation resistance, but higher initial weight may also suggest increased water weight as a mechanism used to resist desiccation. *Drosophila melanogaster* adults that have been selected for desiccation resistance contain approximately 30% more body water than non-selected flies (Gibbs *et al.* 1997). Variation of this kind may account for the difference in initial weight among the *R. zephyria* populations as well. This study did not test, however, the nature of the weight the flies lost. If the percent loss was due mainly to water weight, then the higher survival of the flies from Yakima and in higher humidity conditions in the Survival Experiment may be due to those individuals containing a greater amount of water at the beginning of treatment. If the percent loss was due instead to dry weight, then resisting desiccation may be a very costly response to adverse conditions and the flies with greater energy reserves may be the more successful group. When I looked at factors that best predict survival in only the high PWR individuals from the Survival Experiment, I found that those flies with higher initial weight and higher PWR were more likely to survive to eclosion. Treatment still did not appear as a significant factor, showing that at least in the Yakima sample, resisting desiccation does not appear to be a particularly costly response. A study examining differences in dry weight of populations with different desiccation resistances would help to clarify this issue.

Regardless of the type of weight loss, all pupae in these experiments lost weight to some degree because of their current developmental stage. Just after *Rhagoletis* flies pupariate, they begin to drastically reduce their metabolic rate (measured in daily CO_2 production) to conserve resources as they overwinter. It takes approximately five days of steady decline before the decrease in metabolic rate begins to level out, and at around seven days after pupariation, the flies are at diapause levels (Ragland *et al.* 2009). This means that the treatments in this study extended throughout the descent into diapause for typical *Rhagoletis* fruit flies, and that they were in a very different state at the beginning and end of the experiments. These differences can have vast implications for how the pupae might be resisting desiccation.

In quiescent insects, cuticular transpiration accounts for around 80% of the total water loss (Gibbs 2011). In this case, all mechanisms associated with desiccation resistance would need to be passive, to slow or stop cuticular transpiration or spiracular evaporation, though these strategies are also useful and common in active insects (Quinlan & Gibbs 2006; Gibbs 2011). Parkash et al. (2011) explored the various mechanisms of water balance in adults of *Drosophila* species in the Himalayan Mountains and found that while *D. melanogaster* uses an increased melanization to minimize water loss, the sympatric *D. busckii* uses instead an increased amount of cuticular lipids to achieve the same end. Both of these mechanisms work by limiting diffusion through the epicuticle, either with more melanin granules, or with more lipid content in the epicuticle, respectively (Parkash *et al.* 2011). These substances are hydrophobic, and so water passes through them less readily than through other tissues (Gibbs 2011). Though a difference in melanization is unlikely due to the lack of visible color variation of the *Rhagoletis* puparia used in this study, the different populations or host races may employ other hydrophobic compounds to decrease cuticular permeability. An analysis of the cuticular composition would be useful in determining if lipids in the epicuticle contribute to desiccation resistance.

Because *Rhagoletis* take approximately seven days to enter diapause, active mechanisms of water balance, associated with active respiration and metabolism, will also be important (Gibbs 2011). In addition, since *Rhagoletis'* diapause is facultative, it is likely that a subset of pupae did not go into diapause, or entered only a shallow diapause (Boller & Prokopy 1976; Dambroski & Feder 2007; Ragland *et al.* 2009). In these cases, those pupae would have to rely even more heavily on additional methods of desiccation resistance. Females of an *Anopheles* mosquito measured at the onset of the dry season tended to increase the expression of enzymes associated with the breakdown of glycogen, suggesting increased energetic demands associated with desiccation resistance. However they also adjusted their cuticular permeability with changes in expression of cuticular proteins, suggesting that a combination of responses to desiccation may be important for survival (Hidalgo *et al.* 2014). It is likely that if *Rhagoletis* flies that do not go into diapause exhibit desiccation resistance, they do it through multiple physiological changes.

Active mechanisms of water balance would be less helpful to the pupae in this study, as they have no way of taking in more nutrients or water after pupariation (Boller & Prokopy 1976). More probable is that any flies that did not go into diapause died before the end of the desiccation treatment, and that the patterns seen in these experiments, particularly the Host Comparison, were actually due to an interaction between desiccation resistance and diapause regulation. Ragland et al. (2009) found that about 12% of an eastern population of hawthorn-infesting *Rhagoletis pomonella* did not go into diapause at all, but continued their development in a way that mimicked normal diapause termination and adult eclosion. Dambroski and Feder (2007) found latitudinal differences in percentages of the population of flies that would forego diapause when placed into warmer, summer-like conditions. Flies from lower latitudes nearly always diapaused, even in warm conditions, while those from farther north had larger subsets that would continue development and emerge ~30 days after pupariation. They also found that flies infesting fruit that ripened earlier (because of latitudinal changes or simply from different fruit phenology) had a lessened tendency to forego diapause than populations infesting later-season fruits. (Dambroski & Feder 2007). Though the proportions of flies foregoing diapause are unknown in the Pacific Northwest, the fact that hawthorns fruit before apples do suggests that the hawthorn host-race of *R. pomonella* may have a smaller percentage of non-diapausing individuals than the apple host-race.

The physiological choice to enter diapause or not could actually be a mechanism of desiccation resistance. Pupae will be able to survive dry and warm pre-winter conditions much more easily in a quiescent state (Guppy & Withers 1999; Irwin & Lee Jr 2003; Hahn & Denlinger 2007), so those that enter diapause sooner after pupariation, despite a long pre-winter period, will be at an advantage compared to those that retain active levels of respiration and metabolism. The latitudinal and host-related differences in the percentage of individuals that do not go into diapause could reflect a population-wide strategy to resist harsh, desiccating pre-winter conditions. The hawthorn host-race of *R. pomonella* may have higher mean desiccation resistance than the apple host-race because a smaller subset of hawthorn-infesting flies will forego diapause when placed in warm conditions. This would mean that they would reach inactive levels of respiration and metabolism more quickly, conserving resources that could be vital during the overwintering process.

Diapause cannot, however, be the only mechanism at work in these populations, as variation still exists within populations and between treatments. The interaction between diapause and desiccation resistance is likely a complex and nuanced one, and will require careful testing to discover the relative effects of each one.

Introgression or standing variation?

To assess whether the invasive apple fly could gain beneficial traits that would allow it to invade the drier regions of Washington, all sources of genetic variation must be taken into account. It is possible that the desiccation resistance in the hawthorn host race of *R. pomonella* is due to a higher rate of hybridization with *R. zephyria*, and that hawthorn flies have gained this trait as a result of genetic backcrosses. The fruit hosts of these two populations occur sympatrically both geographically and temporally, and hybridization occurs between them at an estimated rate of 1.44% per generation (Arcella *et al.* 2015). Because snowberry flies have an increased desiccation resistance in certain regions of Washington, hybridization with these neighbors may be conferring an adaptive advantage. While *R.* zephyria flies in central Washington show greater desiccation resistance than those in western Washington, they are not isolated populations. Though the central populations may have higher frequencies of alleles associated with desiccation resistance than those in the west, the western populations would likely still possess those beneficial alleles in lower frequencies. This means that although hybridization with *R. zephyria* in central Washington may move the alleles more efficiently into the *R. pomonella* population, the increased resistance would still be accessible were introgression limited to the western populations.

Despite the proximity and hybridization in the Pacific Northwest however, Arcella et al. (2015) have also shown that most of the alleles that are more "*R. zephyria*-like" in the hawthorn populations are present in *R. pomonella* populations in the Midwestern United States, making it more likely that the alleles have existed in low frequencies within the species since its introduction into the Pacific Northwest. This would mean that the variation in desiccation resistance in *R. pomonella* is actually the result of standing variation providing a low-frequency allele that became more widespread in the hawthorn population. Important to remember is that the dichotomy between these two phenomena could be considered a false one. The entire *R. pomonella* species group is closely related, and while the divergence of *R. pomonella* and *R. zephyria* was certainly before the divergence of the two *R. pomonella* host races in this study, they still fall along a continuum of genetic differences (Bush & Smith 1998; Feder *et al.* 1999). Thus, hybridization with *R. zephyria* could be considered by some to constitute adaptation from standing variation in a slightly more removed population (Barrett & Schluter 2008). Also, at this time we do not know which genetic loci are associated with desiccation resistance traits, so it is impossible to say in which populations these alleles exist, or in which species they originated.

Conclusions

Both *R. zephyria* and *R. pomonella* have varying resistances to desiccation in different environments within Washington State. The native *R. zephyria* shows variation between sites that have different pupariation conditions, with flies originating from harsher habitats performing better under the drier experimentally induced conditions. *Rhagoletis pomonella* reacts differently to desiccation stress depending on fruit host and diapause length, with the early-pupating hawthorn host race retaining more weight than the apple race in both high and low humidity treatments. Previous studies of genetic variation in these two species have confirmed that they are hybridizing at low but significant levels and have speculated as to the contribution such hybridization has on the spread of the invasive *R. pomonella* in Washington (Green *et al.* 2013; Hood *et al.* 2013; Arcella *et al.* 2015; Ragland *et al.* 2015). Here, I examined the desiccation resistances of each species in conditions like those where snowberry flies are widespread and apple flies are absent. My results suggest that variation in desiccation resistance is already present within the *R. pomonella* genome, either from hybridization with *R. zephyria* or from standing variation that has increased in response to a host shift to hawthorn fruit. I also found that *R. zephyria* flies likely have adapted to local conditions and that desiccation resistance is not universal across the populations. More work is needed to determine if there is indeed a genetic factor determining resistance, and the regions of the genome with which this factor is associated. This research, in turn, would allow us to begin to gauge the relative importance of introgression in the invasion of *R. pomonella* into the Pacific Northwest.

LITERATURE CITED

Abbott RJ (1992) Plant invasions, interspecific hybridization and the evolution of new plant taxa. *Trends in Ecology & Evolution*, **7**, 401–405.

AliNiazee MT (1985) Opiine parasitoids (Hymenoptera, Braconidae) of *Rhagoletis pomonella* and *Rhagoletis zephyria* (Diptera, Tephritidae) in the Willamette Valley, Oregon. *The Canadian Entomologist*, **117**, 163–166.

AliNiazee MT, Wescott RL (1987) Flight period and seasonal development of the apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae), in Oregon. *Annals of the Entomological Society of America*, **80**, 823–828.

Arbeitman MN, Furlong EEM, Imam F, Johnson E, Null BH, Baker BS, Krasnow MA Scott MP, Davis RW, White KP (2002) Gene expression during the life cycle of *Drosophila melanogaster*. *Science*, **297**, 2270–2275.

Arcella T, Hood GR, Powell THQ, Sim SB, Yee WL, Schwarz D, Egan SP, Goughnour RB, Smith JJ, Feder JL (2015) Hybridization and the spread of the apple maggot fly, *Rhagoletis pomonella* (Diptera: Tephritidae), in the northwestern United States. *Evolutionary Applications*, **8**, 834–846.

Arguez A, Durre I, Applequist S, Squires M, Vose R, Yin X, Bilota R (2010) NOAA’s U.S. Climate Normals (1981-2010). [monthly normals].

Ashley TR, Greany PD, Chambers DL (1976) Adult emergence in *Biosteres (Opius) longicaudatus* and *Anastrepha suspensa* in relation to the temperature and moisture concentration of the pupation medium. *The Florida Entomologist*, **59**, 391–396.

Barrett RDH, Schluter D (2008) Adaptation from standing genetic variation. *Trends in Ecology & Evolution*, **23**, 38–44.

Boller EF, Prokopy RJ (1976) Bionomics and management of *Rhagoletis*. *Annual Review of Entomology*, **21**, 223–246.

Bush GL (1966) The taxonomy, cytology, and evolution of the genus *Rhagoletis* in North America (Diptera, Tephritidae). *Museum of Comparative Zoology*, **134**, 431–562.

Bush G (1969) Mating behavior, host specificity, and the ecological significance of sibling species in fruigivorous flies of the genus *Rhagoletis* (Diptera-tephritidae). *The American Naturalist*, **103**, 669–672.

Bush GL, Smith JJ (1998) The genetics and ecology of sympatric speciation: A case study. *Researches on Population Ecology*, **40**, 175–187.

Carotenuto A, DellIsola M (1996) An experimental verification of saturated salt solution-based humidity fixed points. *International Journal of Thermophysics*, **17**, 1423–1439.

Castillo JM, Leira-Doce P, Carrión-Tacuri J, Munoz-Guacho E, Arroyo-Solis A, Curado G, Doblas D, Rubio-Casal AE, Alvares-Lopes A, Redondo-Gomez S, Berjano R, Guerrero G, De Cires A, Figueroa E, Tye A (2006) Contrasting strategies to cope with drought by invasive and endemic species of *Lantana* in Galapagos. *Biodiversity and Conservation*, **16**, 2123–2136.

Chown SL, Sørensen JG, Terblanche JS (2011) Water loss in insects: An environmental change perspective. *Journal of Insect Physiology*, **57**, 1070–1084.

Currat M, Ruedi M, Petit RJ, Excoffier L (2008) The hidden side of invasions: massive introgression by local genes. *Evolution*, **62**, 1908–1920.

Dambroski HR, Feder JL (2007) Host plant and latitude-related diapause variation in *Rhagoletis pomonella*: A test for multifaceted life history adaptation on different stages of diapause development. *Journal of Evolutionary Biology*, **20**, 2101–2112.

Dambroski HR, Linn C, Berlocher SH, Forbes AA, Roelofs W, Feder JL (2005) The genetic basis for fruit odor discrimination in *Rhagoletis* flies and its significance for sympatric host shifts. *Evolution*, **59**, 1953–64.

Elliott GP, Merton D V., Jansen PW (2001) Intensive management of a critically endangered species: The kakapo. *Biological Conservation*, **99**, 121–133.

Ellstrand NC, Schierenbeck KA (2000) Hybridization as a stimulus for the evolution of invasiveness in plants ? *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 7043–7050.

Ewel JJ, O'Dowd DJ, Bergelson J, Daehler CC, D'Antonio CM, Gomez Ld, Gordon DR, Hobbs RJ, Holt A, Hopper KR, Hughes CE, LaHart M, Leakey RRB, Lee WG, Loope LL, Lorence DH, Louda SM, Lugo AE, McEvoy PB, Richardson DM, Vitousek PM (1999) Introductions of Species : Research needs - Benefits can be reaped, but risks are high. *BioScience*, **49**, 619–630.

Excoffier L, Foll M, Petit RJ (2009) Genetic consequences of range expansions. *The Annual Review of Ecology, Evolution, and Systematics*, **40**, 481–501.

Feder JL, Berlocher SH, Roethele JB, Dambroski H, Smith JJ, Perry WL, Gavrilovic V, Filchak KE, Rull J, Aluja M (2003) Allopatric genetic origins for sympatric host-plant shifts and race formation in *Rhagoletis*. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 10314–10319.

Feder JL, Roethele JB, Wlazlo B, Berlocher SH (1997) Selective maintenance of allozyme differences among sympatric host races of the apple maggot fly. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 11417–11421.

Feder JL, Williams SM, Berlocher SH, Mcpheron BA, Bush GL (1999) The population genetics of the apple maggot fly , *Rhagoletis pomonella* and the snowberry maggot , *R . zephyria*: implications for models of sympatric speciation. *Entomologia Experimentalis et Applicata*, **90**, 9–24.

Fitt GP (1981) Pupal survival of two northern australian tephritid species and its relationship to soil-conditions. *Journal of the Australian Entomological Society*, **20**, 139–144.

Fox J, Weisberg S (2011) *An {R} Companion to Applied Regression*. Sage, Thousand Oaks, CA.

Gamst G, Meyers LS, Guarino AJ (2008) *Analysis of Variance Designs*. Cambridge University Press.

Gibbs AG (2011) Thermodynamics of cuticular transpiration. *Journal of Insect Physiology*, **57**, 1066–9.

Gibbs AG, Chippendale AK, Rose MR (1997) Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *The Journal of Experimental Biology*, **200**, 1821–1832.

GoogleImageLandsat Washington. *GOOGLE EARTH*.

Grant PR, Grant BR (2014) Synergism of natural selection and introgression in the origin of a new species. *The American Naturalist*, **183**, 671–681.

Grant WS, Little RM (1992) How sedentary are greywing francolins (*Francolinus africanus*)? *Evolution*, **46**, 1477–1491.

Green E, Almskaar K, Sim SB, Arcella T, Yee WL, Feder JL, Schwarz D (2013) Molecular species identification of cryptic apple and snowberry maggots (Diptera: Tephritidae) in Western and Central Washington. *Environmental Entomology*, **42**, 1100–1109.

Green RE, Krause J, Briggs AW, Maricic T, Stenzel U, Kircher M, Petterson N, Li H, Zhai W, Fritz MH, Hansen NF, Durand EY, Malaspinas A, Jensen JD, Marques-Bonet T, Alkan C, Prufer K, Meyer M, Burbano HA, Good JM, Schultz R, Aximu-Petri A, Butthof A, Hober B, Hoffner B, Siegemund M, Weihmann A, Nusbaum C, Lander ES, Russ C, Novod N, Affourtit J, Egholm M, Verna C, Rudan P, Brajkovic D, Kucan Z, Gusic I, Doronichev VB, Golovanova LV, Lalueza-Fox C, de la Rasilla M, Fortea J, Rosas A, Schmitz RW, Johnson PLF Eichler EE, Falush D, Birney E, Mullikin JC, Slatkin M, Nielsen R, Kelso J, Lachmann M, Reich D, Paabo S (2014) A Draft Sequence of the Neandertal Genome. *Science*, **328**, 710–722.

Guppy M, Withers P (1999) Metabolic depression in animals: physiological perspectives and biochemical generalizations. *Biological reviews of the Cambridge Philosophical Society*, **74**, 1–40.

Hahn DA, Denlinger DL (2007) Meeting the energetic demands of insect diapause: Nutrient storage and utilization. *Journal of Insect Physiology*, **53**, 760–773.

Hidalgo K, Mouline K, Mamai W, Foucreau N, Dabire KR, Bouchereau A, Simard F, Renault D (2014) Novel insights into the metabolic and biochemical underpinnings assisting dry-season survival in female malaria mosquitoes of the *Anopheles gambiae* complex. *Journal of Insect Physiology*, **70**, 102–16.

Hood GR, Yee W, Goughnour RB, Sim SB, Egan SP, Arcella T, Saint-Jean G, Powell THQ, XU CCY, Feder JL (2013) The geographic distribution of *Rhagoletis pomonella* (Diptera: Tephritidae) in the Western United States: Introduced species or native population? *Annals of the Entomological Society of America*, **106**, 59–65.

Huddleston JL (2013) Short-range host preference in snowberry maggot flies depends on life history stage [master's thesis]. Western Washington University, 46 p.

Hulthen AD, Clarke AR (2006) The influence of soil type and moisture on pupal survival of *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). *Australian Journal of Entomology*, **45**, 16–19.

Inoue MN, Yokoyama J, Washitani I (2007) Displacement of Japanese native bumblebees by the recently introduced *Bombus terrestris* (L.) (Hymenoptera: Apidae). *Journal of Insect Conservation*, **12**, 135–146.

Irwin J, Lee Jr R (2003) Cold winter microenvironments conserve energy and improve overwintering survival and potential fecundity of the goldenrod gall fly, *Eurosta* *solidaginis*. *Oikos*, **100**, 71–78.

Karatayev AY, Burlakova LE, Padilla DK (2014) Zebra versus quagga mussels: a review of their spread, population dynamics, and ecosystem impacts. *Hydrobiologia*, **746**, 97–112.

Kawano T, Shimoda M, Matsumoto H, Ryuda M, Tsuzuki S, Hayakawa Y (2010) Identification of a gene, Desiccate, contributing to desiccation resistance in *Drosophila melanogaster*. *The Journal of Biological Chemistry*, **285**, 38889–97.

Kleynhans E, Clusella-Trullas S, Terblanche JS (2014) Impacts of environmental variability on desiccation rate, plastic responses and population dynamics of *Glossina pallidipes*. *Journal of Evolutionary Biology*, **27**, 337–48.

Lee CE (1999) Rapid and repeated invasions of fresh water by the copepod *Eurytemora affinis*. *Evolution*, **53**, 1423–1434.

Lee CE (2002) Evolutionary genetics of invasive species. *Trends in Ecology & Evolution*, **17**, 386–391.

Lee CE, Kiergaard M, Gelembiuk GW, Eads BD, Posavi M (2011) Pumping Ions: Rapid parallel evolution of ionic regulation following habitat invasions. *Evolution*, **65**, 2229–2244.

Lee CE, Posavi M, Charmantier G (2012) Rapid evolution of body fluid regulation following independent invasions into freshwater habitats. *Journal of Evolutionary Biology*, **25**, 625–633.

Lowery RK, Uribe G, Jimenez EB, Weiss MA, Herrera KJ, Regueiro M, Herrera RJ (2013) Neanderthal and Denisova genetic affinities with contemporary humans: Introgression versus common ancestral polymorphisms. *Gene*, **530**, 83–94.

Mallet J (2005) Hybridization as an invasion of the genome. *Trends in Ecology and Evolution*, **20**, 229–237.

Manachini B, Billeci N, Palla F (2013) Exotic insect pests: The impact of the Red Palm Weevil on natural and cultural heritage in Palermo (Italy). *Journal of Cultural Heritage*, **14**, 177–182.

Mattson W, Vanhanen H, Veteli T, Sivonen S, Niemelä P (2007) Few immigrant phytophagous insects on woody plants in Europe: Legacy of the European crucible? *Biological Invasions*, **9**, 957–974.

Matute DR, Ayroles JF (2014) Hybridization occurs between *Drosophila simulans* and *D. sechellia* in the Seychelles archipelago. *Journal of Evolutionary Biology*, **27**, 1057–1068.

Mertz C, Koong D, Anderson S (2013) *2013 Washington Annual Agriculture Bulletin*. Olympia, WA.

Michel AP, Rull J, Aluja M, Feder JL (2007) The genetic structure of hawthorn-infesting *Rhagoletis pomonella* populations in Mexico: Implications for sympatric host race formation. *Molecular Ecology*, **16**, 2867–2878.

Montarsi F, Martini S, Dal Pont M, Delai N, Milone NF, Mazzucato M, Soppelsa F, Cazzola L, Cazzin S, Ravagnan S, Ciocchetta S, Russo F, Capelli G (2013) Distribution and habitat characterization of the recently introduced invasive mosquito *Aedes koreicus* [Hulecoeteomyia koreica], a new potential vector and pest in north-eastern Italy. *Parasites & Vectors*, **6**, 292.

Neilson WTA (1964) Some effects of relative humidity on development of pupae of the apple maggot, *Rhagoletis pomonella* (Walsh). *The Canadian Entomologist*, **96**, 810–811.

Nyamukondiwa C, Kleynhans E, Terblanche JS (2010) Phenotypic plasticity of thermal tolerance contributes to the invasion potential of Mediterranean fruit flies (*Ceratitis capitata*). *Ecological Entomology*, **35**, 565–575.

Oliveira CM, Auad a. M, Mendes SM, Frizzas MR (2013) Economic impact of exotic insect pests in Brazilian agriculture. *Journal of Applied Entomology*, **137**, 1–15.

Paradis E, Claude J, Strimmer K (2004) APE: Analysis of phylogenetics and evolution in R language. *Bioinformatics*, **20**, 289–290.

Pardo-Diaz C, Salazar C, Baxter SW, Merot C, Figueiredo-Ready W, Joron M, McMillian WO, Jiggins CD (2012) Adaptive introgression across species boundaries in Heliconius butterflies. *PLoS GENETICS*, **8**, e1002752.

Parkash R, Aggarwal DD, Kalra B, Ranga P (2011) Divergence of water balance mechanisms in two melanic Drosophila species from the western Himalayas. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, **158**, 531–541.

Pelini SL, Diamond SE, Maclean H, Ellison AM, Gotelli NJ, Sanders NJ, Dunn RR (2012) Common garden experiments reveal uncommon responses across temperatures, locations, and species of ants. *Ecology and Evolution*, **2**, 3009–15.

Perry WL, Feder JL, Dwyer G, Lodge DM (2001) Hybrid zone dynamics and species replacement between orconectes crayfishes in a northern Wisconsin lake. *Evolution*, **55**, 1153–1166.

Pimentel D, McNair S, Janecka J, Wightman J, Simmonds C, O'Connell C, Wong E, Russel L, Zern J, Aquino T, Tsomondo T (2001) Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems and Environment*, **84**, 1–20.

Prokopy RJ, Bush GL (1972) Apple maggot infestation of pear. *Journal of Economic Entomology*, **65**, 597.

Quinlan MC, Gibbs AG (2006) Discontinuous gas exchange in insects. *Respiratory Physiology & Neurobiology*, **154**, 18–29.

Ragland GJ, Almskaar K, Vertacnik KL, Gough HM, Feder JL, Hahn DA, Schwarz D (2015) Differences in performance and transcriptome-wide gene expression associated with *Rhagoletis* (Diptera: Tephritidae) larvae feeding in alternate host fruit environments. *Molecular Ecology*, **24**, 2759–76.

Ragland GJ, Fuller J, Feder JL, Hahn DA (2009) Biphasic metabolic rate trajectory of pupal diapause termination and post-diapause development in a tephritid fly. *Journal of Insect Physiology*, **55**, 344–350.

Ragland GJ, Sim SB, Goudarzi S, Feder JL, Hahn DA (2012) Environmental interactions during host race formation: host fruit environment moderates a seasonal shift in phenology in host races of *Rhagoletis pomonella*. *Functional Ecology*, **26**, 921–931.

Rhymer JM, Simberloff D (1996) Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics*, **27**, 83–109.

Sankararaman S, Mallick S, Dannemann M, Prufer K, Kelso J, Paabo S, Patterson N, Reich D (2014) The genomic landscape of Neanderthal ancestry in present-day humans. *Nature-Research Letter,* **507**, 354-357.

Schilman PE, Lighton JRB, Holway DA (2005) Respiratory and cuticular water loss in insects with continuous gas exchange: Comparison across five ant species. *Journal of Insect Physiology*, **51**, 1295–1305.

Schilman PE, Lighton JRB, Holway DA (2007) Water balance in the Argentine ant (*Linepithema humile*) compared with five common native ant species from southern California. *Physiological Entomology*, **32**, 1–7.

Schwarz D, Shoemaker KD, Botteri NL, McPheron BA (2007) A novel preference for an invasive plant as a mechanism for animal hybrid speciation. *Evolution*, **61**, 245–56.

Siler N, Roe G, Durran D (2013) On the dynamical causes of variability in the rain-shadow effect: A case study of the Washington Cascades. *Journal of Hydrometeorology*, **14**, 122–139.

Sim SB, Mattsson M, Feder JL, CHA DH, Yee WL, Goughnour RB, Linn Jr. CE, Feder JL (2012) A field test for host fruit odour discrimination and avoidance behaviour for *Rhagoletis pomonella* flies in the western United States. *Journal of Evolutionary Biology*, **25**, 961–971.

Team RC (2015) R: A language and environment for statistical computing.

Terblanche JS, Kleynhans E (2009) Phenotypic plasticity of desiccation resistance in *Glossina* puparia: are there ecotype constraints on acclimation responses? *Journal of Evolutionary Biology*, **22**, 1636–1648.

Thompson JD (1991) the Biology of an invasive plant - What makes spartina-anglica so successful. *Bioscience*, **41**, 393–401.

Venables WN, Ripley BD (2002) *Modern Applied Statistics with S*. Springer, New York.

Walsh B (1867) The apple-worm and the apple maggot. *Journal of Horticulture*, 338–343.

Walters AC, Mackay DA (2005) Importance of large colony size for successful invasion by Argentine ants (Hymenoptera: Formicidae): Evidence for biotic resistance by native ants. *Austral Ecology*, **30**, 395–406.

Williamson M (1993) Invaders, weeds and the risk from genetically manipulated organisms. *Experientia*, **49**, 219–224.

WSDA (2014) *Proposed Rule Making*. WSDA.

Yee WL (2013a) Influence of media type and moisture on adult development in *Rhagoletis indifferens* (Diptera: Tephritidae). *Environmental Entomology*, **42**, 595–604.

Yee WL (2013b) Soil moisture and relative humidity effects during postdiapause on the emergence of western cherry fruit fly (Diptera: Tephritidae). *The Canadian*

Entomologist, **145**, 317–326.

Zefferman E, Stevens JT, Charles GK, Dunbar-Irwin M, Emam T, Fick S, Morales LV, Wolf KM, Young DJN, Young TP (2015) Plant communities in harsh sites are less invaded: a summary of observations and proposed explanations. *AoB PLANTS*, **7**, plv056.

APPENDIX

Genome Association Study

In order to provide evidence for a genetic element associated with desiccation resistance in *Rhagoletis* flies, I laid the groundwork for a genome-wide association study. The aim of such a study is to discover which genomic regions are associated with desiccation resistance and also whether those regions correlate with those of disproportionate introgression between the species (Green *et al.* 2013).

For this larger-scale study, I collected *Rhagoletis zephyria* pupae in the same way as before, and collected *R. pomonella* pupae from apples fallen from infested trees. The larvae were allowed to mature and egress from the fruit just as before (see methods). I collected 1600 *R. zephyria* pupae and 800 *R. pomonella* pupae during September 2014, all from Bellingham, Washington. All pupae were placed into 0.67mL microcentrifuge tubes, weighed initially, and then subjected to 43.16% rh at 22° C in a chamber with an 16:8 light:dark cycle for eight days. I weighed all of the pupae again on the last day of treatment, and immediately froze them for further analysis. For this study I used 15 cohorts total.

After freezing, I measured the length and width of each pupal case, and dissected all of the *R. zephyria* pupae, discarding any parasitoid wasps found. I calculated the percentage of weight each pupa had remaining at the end of the treatment, and then randomly selected 200 *R. zephyria* individuals with 65%-85% weight remaining, and 200 with 25%-45% weight remaining. Too few pupae fell into the 65%-85% category in the *R. pomonella* samples, so I randomly selected 200 individuals with 55%-75% weight remaining and 200 with 25%-45% weight remaining.

I extracted DNA from all of these selected samples using a standard Qiagen DNeasy Blood and Tissue kit (Qiagen, Valencia, California).

Appendix Table 1 – Table of Genome Association samples, showing the individual identifier, initial pupal weight, and percent weight remaining after treatment for each sample.

Rhagoletis zephyria – low weight			*Rhagoletis pomonella* – low weight		
Sample ID	Initial Weight	PWR	Sample ID	Initial Weight	PWR
F414	0.42967	26.36%	C162	0.44023	32.85%
F411	0.42948	29.86%	C151	0.43211	33.80%
F688	0.42587	30.00%	C39	0.43143	34.43%
F225	0.42335	31.19%	C538	0.43666	34.45%
F196	0.42927	32.08%	C337	0.43557	35.06%
F707	0.42128	32.08%	C161	0.43472	35.12%
F716	0.42714	32.90%	C30	0.43288	35.12%
F353	0.43193	33.67%	C182	0.43704	35.17%
F421	0.43013	34.03%	C138	0.43763	35.28%
F1436	0.42809	34.36%	C287	0.43028	35.30%
F202	0.43407	34.55%	C140	0.43402	35.58%
F213	0.42944	34.80%	C511	0.42626	35.91%
F215	0.43223	34.82%	C753	0.42937	36.16%
F212	0.42677	34.85%	C322	0.43518	36.19%
F705	0.42366	34.97%	C775	0.42999	36.21%
F1177	0.42907	35.02%	C701	0.42531	36.59%
F1007	0.43061	35.23%	C29	0.43665	36.65%
F564	0.42735	35.24%	C99	0.43290	36.78%
F329	0.42372	35.25%	C57	0.43830	36.79%
F578	0.42897	35.41%	C4	0.43111	36.83%
F306	0.42729	35.44%	C641	0.43114	36.86%
F217	0.42857	35.48%	C241	0.43364	36.92%
F1219	0.42490	35.51%	C190	0.43588	36.93%
F338	0.42922	35.56%	C521	0.42730	36.94%
F1453	0.43087	35.61%	C66	0.43524	37.04%
F239	0.43139	35.67%	C46	0.43477	37.10%
F257	0.42678	35.73%	C547	0.42842	37.24%

F1508	0.42555	35.79%	C512	0.43047	37.39%
F437	0.43156	35.82%	C87	0.43397	37.52%
F315	0.43392	35.86%	C751	0.42937	37.56%
F1113	0.42563	35.86%	C157	0.42881	37.63%
F712	0.42514	35.92%	C673	0.43001	37.66%
F503	0.42157	35.94%	C129	0.43766	37.74%
F203	0.43131	36.01%	C770	0.44019	37.82%
F630	0.42925	36.02%	C394	0.43220	37.86%
F1492	0.42435	36.02%	C54	0.43727	37.86%
F389	0.43045	36.13%	C164	0.43194	37.89%
F760	0.43349	36.18%	C764	0.43447	37.98%
F221	0.43014	36.19%	C108	0.43755	37.99%
F1137	0.42481	36.19%	C68	0.42925	38.10%
F835	0.42667	36.19%	C536	0.43058	38.12%
F344	0.42459	36.33%	C629	0.43172	38.16%
F1252	0.43377	36.34%	C11	0.43712	38.21%
F301	0.42527	36.35%	C59	0.43305	38.27%
F457	0.42360	36.36%	C3	0.42963	38.29%
F726	0.42577	36.44%	C74	0.43488	38.30%
F349	0.43189	36.53%	C85	0.43623	38.37%
F393	0.42788	36.58%	C651	0.43335	38.60%
F333	0.43007	36.67%	C53	0.43564	38.65%
F206	0.42839	36.75%	C383	0.43412	38.76%
F1490	0.42235	36.75%	C694	0.43092	38.77%
F214	0.42144	36.75%	C44	0.43631	38.81%
F385	0.42227	36.79%	C369	0.43013	38.83%
F384	0.42862	36.80%	C95	0.42838	38.91%
F235	0.42773	36.89%	C532	0.43366	38.97%
F228	0.42421	36.93%	C329	0.44285	39.00%
F300	0.42486	36.94%	C685	0.42752	39.09%
F381	0.43058	36.97%	C77	0.44074	39.14%
F1524	0.43293	37.00%	C67	0.43689	39.19%
F455	0.42341	37.03%	C346	0.43521	39.25%

F1019	0.42197	37.12%	C545	0.43701	39.29%
F331	0.42471	37.19%	C305	0.43914	39.33%
F334	0.42636	37.25%	C194	0.43389	39.54%
F87	0.42565	37.26%	C635	0.42488	39.55%
F693	0.42428	37.33%	C69	0.43241	39.61%
F219	0.42709	37.41%	C568	0.44242	39.72%
F1363	0.42379	37.46%	C463	0.43910	39.83%
F369	0.42918	37.48%	C163	0.43938	39.86%
F445	0.42572	37.54%	C231	0.43954	39.93%
F1243	0.43236	37.56%	C627	0.43277	39.93%
F930	0.43251	37.63%	C144	0.43614	39.95%
F1462	0.42977	37.66%	C776	0.43311	39.96%
F1370	0.42478	37.82%	C779	0.43343	39.96%
F1067	0.42423	37.86%	C767	0.43282	40.02%
F752	0.42467	37.88%	C331	0.43161	40.03%
F439	0.42407	37.90%	C131	0.44341	40.06%
F1329	0.42618	37.94%	C90	0.43203	40.12%
F1115	0.42501	37.95%	C613	0.42840	40.15%
F490	0.42940	38.00%	C430	0.42817	40.24%
F1189	0.42832	38.01%	C768	0.43925	40.29%
F157	0.42439	38.03%	C193	0.42896	40.39%
F1400	0.42836	38.04%	C32	0.42946	40.42%
F1481	0.42569	38.08%	C732	0.43562	40.44%
F511	0.43516	38.12%	C577	0.43072	40.45%
F604	0.42783	38.24%	C741	0.43649	40.52%
F224	0.42605	38.29%	C415	0.43545	40.59%
F526	0.43627	38.32%	C65	0.43540	40.65%
F447	0.42798	38.32%	C52	0.43328	40.66%
F1449	0.43030	38.35%	C201	0.43189	40.82%
F1473	0.43013	38.36%	C666	0.43544	40.94%
F750	0.42605	38.36%	C469	0.43761	40.98%
F226	0.42704	38.41%	C509	0.42882	41.05%
F1451	0.42916	38.43%	C128	0.43434	41.08%

F701	0.43728	38.44%	C619	0.43029	41.09%
F867	0.42732	38.51%	C5	0.43409	41.12%
F263	0.42635	38.52%	C648	0.43386	41.12%
F825	0.42644	38.54%	C143	0.43892	41.14%
F1487	0.42695	38.54%	C609	0.43072	41.18%
F368	0.42295	38.58%	C2	0.43277	41.18%
F999	0.42722	38.64%	C323	0.43238	41.24%
F264	0.42478	38.67%	C119	0.43497	41.36%
F287	0.42682	38.71%	C206	0.43605	41.43%
F1466	0.42598	38.71%	C342	0.44178	41.47%
F1173	0.42343	38.72%	C79	0.43370	41.57%
F1494	0.42458	38.73%	C252	0.43872	41.59%
F768	0.42985	38.75%	C167	0.44039	41.69%
F773	0.42423	38.79%	C467	0.43211	41.69%
F277	0.42580	38.80%	C630	0.43061	41.86%
F122	0.43086	38.87%	C738	0.42618	41.96%
F836	0.43543	38.88%	C220	0.43021	41.99%
F1477	0.42436	38.95%	C616	0.42977	42.00%
F390	0.42963	38.99%	C624	0.42778	42.05%
F1270	0.42603	39.03%	C437	0.42887	42.05%
F1392	0.42424	39.12%	C12	0.42837	42.06%
F1457	0.42517	39.22%	C458	0.44183	42.16%
F1165	0.43115	39.24%	C606	0.42864	42.20%
F153	0.42619	39.31%	C93	0.43481	42.24%
F1397	0.42854	39.32%	C543	0.43200	42.26%
F508	0.43092	39.37%	C433	0.44116	42.31%
F1513	0.42800	39.38%	C598	0.43940	42.31%
F878	0.43041	39.41%	C513	0.42741	42.32%
F1378	0.42377	39.43%	C145	0.44010	42.33%
F233	0.43402	39.52%	C522	0.43452	42.34%
F1206	0.42856	39.53%	C372	0.42827	42.34%
F435	0.43107	39.55%	C381	0.42787	42.39%
F1139	0.43252	39.66%	C755	0.43600	42.44%

F440	0.42940	39.73%	C626	0.42812	42.45%
F967	0.43014	39.87%	C183	0.43176	42.46%
F872	0.42526	39.96%	C793	0.42700	42.48%
F298	0.43146	39.96%	C97	0.43764	42.51%
F1204	0.42486	40.26%	C554	0.42670	42.59%
F271	0.42940	40.27%	C604	0.43447	42.60%
F559	0.42925	40.32%	C291	0.43631	42.61%
F567	0.42267	40.33%	C796	0.43504	42.65%
F1256	0.42284	40.36%	C794	0.43693	42.73%
F391	0.42425	40.45%	C279	0.43638	42.78%
F730	0.42380	40.45%	C175	0.42909	42.84%
F1008	0.42336	40.50%	C155	0.43334	42.86%
F471	0.42455	40.50%	C621	0.44706	42.93%
F1216	0.43464	40.53%	C740	0.43261	42.97%
F399	0.42537	40.58%	C243	0.43346	43.00%
F409	0.42935	40.63%	C385	0.43145	43.04%
F1398	0.42765	40.69%	C476	0.43858	43.07%
F561	0.42274	40.69%	C450	0.43006	43.08%
F232	0.42844	40.70%	C98	0.42928	43.08%
F1182	0.43372	40.79%	C103	0.42747	43.08%
F755	0.42101	40.86%	C750	0.43412	43.10%
F297	0.42695	40.87%	C640	0.43868	43.18%
F1450	0.42725	40.92%	C245	0.43301	43.20%
F261	0.42432	40.95%	C16	0.43900	43.21%
F1377	0.42992	40.96%	C8	0.42353	43.26%
F1414	0.42259	41.02%	C533	0.42947	43.27%
F781	0.42188	41.07%	C551	0.42967	43.33%
F103	0.42967	41.13%	C445	0.43479	43.40%
F589	0.43281	41.22%	C112	0.43203	43.43%
F1495	0.42246	41.28%	C165	0.43241	43.53%
F283	0.42321	41.36%	C781	0.43141	43.57%
F229	0.42953	41.38%	C211	0.43080	43.59%
F736	0.42291	41.52%	C303	0.42976	43.62%

F998	0.42844	41.53%	C31	0.42935	43.63%
F208	0.42891	41.58%	C217	0.42693	43.64%
F234	0.42989	41.71%	C307	0.42882	43.76%
F351	0.42571	41.76%	C153	0.43147	43.76%
F127	0.42855	42.00%	C360	0.42915	43.79%
F1376	0.42323	42.06%	C362	0.42937	43.79%
F318	0.42313	42.15%	C209	0.44064	43.80%
F325	0.43075	42.22%	C498	0.43094	43.86%
F373	0.42416	42.29%	C50	0.42734	43.90%
F1516	0.42132	42.34%	C202	0.44416	43.91%
F565	0.42642	42.38%	C136	0.43413	43.92%
F303	0.42812	42.42%	C443	0.43042	43.94%
F407	0.42922	42.57%	C330	0.42935	44.00%
F1558	0.43165	42.68%	C601	0.44368	44.01%
F345	0.42925	42.68%	C608	0.42823	44.01%
F434	0.42492	42.69%	C72	0.43629	44.10%
F802	0.42781	42.74%	C58	0.43579	44.17%
F348	0.42628	42.86%	C527	0.43071	44.19%
F290	0.42356	42.99%	C124	0.43636	44.25%
F310	0.43056	43.02%	C91	0.43223	44.25%
F327	0.42630	43.05%	C324	0.43122	44.30%
F171	0.42567	43.11%	C127	0.42822	44.30%
F1426	0.42477	43.11%	C152	0.43392	44.32%
F400	0.42424	43.23%	C524	0.43713	44.38%
F324	0.42868	43.26%	C37	0.42617	44.40%
F342	0.43158	43.47%	C747	0.43165	44.44%
F987	0.42181	43.60%	C370	0.44064	44.45%
F458	0.43171	43.62%	C709	0.43551	44.50%
F227	0.42260	43.68%	C457	0.42911	44.51%
F816	0.42156	43.69%	C646	0.42951	44.55%
F1283	0.42418	43.74%	C599	0.44090	44.57%
F995	0.43720	44.12%	C528	0.43097	44.62%
F319	0.42384	44.16%	C356	0.43006	44.66%

F763	0.42224	44.38%	C149	0.43191	44.68%
F352	0.43030	44.39%	C421	0.42929	44.69%
F363	0.42415	44.41%	C758	0.43067	44.71%
F475	0.42268	44.53%	C605	0.42924	44.73%
F1087	0.42602	44.54%	C82	0.43661	44.78%
F574	0.42260	44.83%	C683	0.43515	44.88%
F1464	0.43016	44.88%	C196	0.44179	44.89%
F764	0.42960	44.91%	C546	0.43048	44.91%

Rhagoletis zephyria – high weight			*Rhagoletis pomonella* – high weight		
Sample ID	Initial Weight	PWR	Sample ID	Initial Weight	PWR
F1187	0.42563	65.21%	C413	0.42414	55.11%
F218	0.43213	65.27%	C462	0.43066	55.20%
F744	0.42489	65.29%	C23	0.43320	55.31%
F442	0.42689	65.32%	C508	0.43795	55.35%
F92	0.42645	65.55%	C625	0.43202	55.50%
F1446	0.43619	65.59%	C432	0.43200	55.57%
F1432	0.43383	66.06%	C663	0.44038	55.70%
F1437	0.43124	66.22%	C708	0.44089	55.91%
F1503	0.42754	66.23%	C264	0.43620	55.93%
F1214	0.42607	66.23%	C607	0.43378	56.05%
F992	0.42519	66.28%	C237	0.43526	56.12%
F572	0.42466	66.38%	C657	0.42894	56.20%
F1248	0.42228	66.38%	C268	0.42678	56.34%
F111	0.43405	66.50%	C774	0.43325	56.35%
F1468	0.42760	66.57%	C670	0.43279	56.35%
F204	0.42825	66.60%	C18	0.43605	56.39%
F1476	0.42664	66.90%	C471	0.43789	56.40%
F147	0.43518	66.94%	C419	0.43123	56.42%
F216	0.43240	66.96%	C137	0.43039	56.44%
F1027	0.42650	67.09%	C130	0.42483	56.44%
F1285	0.43066	67.14%	C60	0.43392	56.47%
F374	0.42255	67.24%	C168	0.43410	56.48%

F1510	0.42314	67.39%	C288	0.43331	56.56%
F1234	0.42707	67.57%	C240	0.43201	56.57%
F1266	0.43386	67.73%	C744	0.43233	56.57%
F1458	0.42443	68.02%	C280	0.42822	56.61%
F1131	0.42660	68.17%	C251	0.43194	56.65%
F1514	0.43588	68.18%	C478	0.43058	56.69%
F1343	0.42318	68.26%	C361	0.44182	56.77%
F1318	0.42444	68.27%	C120	0.43711	56.79%
F723	0.42524	68.32%	C414	0.42712	57.06%
F571	0.42722	68.35%	C38	0.43331	57.10%
F1103	0.42636	68.42%	C392	0.42718	57.18%
F1515	0.42970	68.42%	C126	0.43504	57.23%
F460	0.42845	68.50%	C671	0.42978	57.55%
F1156	0.43138	68.53%	C766	0.44157	57.59%
F230	0.42977	68.56%	C9	0.44048	57.60%
F916	0.42769	68.57%	C797	0.42467	57.61%
F799	0.43582	68.65%	C587	0.42899	57.69%
F388	0.42616	68.75%	C431	0.43258	57.81%
F1518	0.42937	68.80%	C302	0.43315	57.86%
F1250	0.42562	68.91%	C632	0.42622	57.99%
F943	0.42290	68.92%	C636	0.43270	58.10%
F174	0.43090	68.97%	C173	0.43526	58.14%
F118	0.43041	69.04%	C212	0.43551	58.55%
F154	0.43373	69.06%	C583	0.42840	58.59%
F1459	0.43190	69.08%	C200	0.43266	58.60%
F983	0.43716	69.11%	C345	0.42339	58.62%
F713	0.42737	69.16%	C222	0.43049	58.62%
F1482	0.42833	69.17%	C51	0.43244	58.65%
F155	0.42863	69.35%	C572	0.43651	58.72%
F117	0.43147	69.40%	C523	0.42282	58.80%
F900	0.42446	69.57%	C725	0.43408	58.97%
F1465	0.42671	69.90%	C763	0.43486	59.06%
F977	0.42482	69.96%	C489	0.44608	59.13%

F129	0.42658	69.98%	C301	0.44117	59.15%
F27	0.42106	70.00%	C376	0.43226	59.15%
F237	0.42905	70.00%	C261	0.43339	59.30%
F191	0.42994	70.01%	C477	0.43757	59.32%
F1351	0.42636	70.14%	C771	0.43893	59.67%
F1529	0.42336	70.22%	C448	0.43275	59.69%
F942	0.43332	70.27%	C660	0.43471	59.69%
F950	0.41972	70.49%	C492	0.43823	59.77%
F1332	0.42653	70.69%	C475	0.43889	59.78%
F125	0.42424	70.77%	C581	0.43713	59.94%
F537	0.42284	70.80%	C295	0.42949	60.04%
F1474	0.42980	70.85%	C435	0.42961	60.04%
F1415	0.42300	70.88%	C790	0.44247	60.05%
F722	0.42511	71.01%	C238	0.42690	60.23%
F238	0.43504	71.10%	C782	0.43573	60.26%
F1467	0.42719	71.16%	C633	0.44537	60.31%
F798	0.42100	71.16%	C304	0.42822	60.32%
F1444	0.42538	71.21%	C562	0.42961	60.33%
F194	0.42848	71.29%	C416	0.43176	60.38%
F850	0.43130	71.31%	C690	0.43538	60.54%
F52	0.42818	71.36%	C226	0.43170	60.54%
F1174	0.42604	71.49%	C319	0.42871	60.54%
F667	0.42508	71.56%	C269	0.42752	60.58%
F443	0.42536	71.65%	C6	0.43585	60.70%
F160	0.42628	71.71%	C668	0.43852	60.76%
F1441	0.42834	71.75%	C45	0.44504	60.77%
F1434	0.42440	71.80%	C736	0.42859	60.78%
F1073	0.43089	71.82%	C674	0.43099	60.83%
F1366	0.42915	71.98%	C354	0.43565	60.85%
F1143	0.43468	72.14%	C464	0.44239	60.86%
F797	0.42273	72.15%	C702	0.43706	60.97%
F1284	0.42833	72.19%	C267	0.42820	60.98%
F968	0.42254	72.32%	C205	0.42695	60.98%

F1291	0.42693	72.33%	C47	0.43397	60.99%
F378	0.42367	72.35%	C756	0.44136	61.03%
F728	0.43556	72.44%	C61	0.43175	61.13%
F1136	0.43055	72.45%	C70	0.43643	61.16%
F1424	0.42132	72.46%	C550	0.43138	61.19%
F3	0.43160	72.50%	C210	0.43600	61.24%
F924	0.42783	72.51%	C25	0.44161	61.33%
F211	0.43445	72.51%	C773	0.44107	61.36%
F1371	0.43702	72.51%	C761	0.43768	61.38%
F1407	0.42417	72.62%	C620	0.44230	61.38%
F1417	0.42298	72.73%	C681	0.43939	61.42%
F1324	0.43155	72.73%	C617	0.43433	61.50%
F734	0.42463	72.79%	C754	0.43766	61.54%
F1440	0.43152	72.83%	C647	0.43325	61.55%
F1098	0.42171	72.93%	C27	0.43290	61.59%
F1395	0.43417	72.94%	C495	0.43540	61.63%
F1036	0.43782	72.98%	C592	0.42776	61.65%
F1251	0.42495	73.16%	C658	0.43193	62.01%
F372	0.43838	73.26%	C618	0.44205	62.05%
F1389	0.43395	73.32%	C780	0.42921	62.07%
F1438	0.43049	73.38%	C564	0.42571	62.26%
F1221	0.42768	73.51%	C20	0.43301	62.34%
F1229	0.43327	73.51%	C353	0.43061	62.35%
F985	0.42248	73.55%	C349	0.42680	62.40%
F1521	0.42535	73.63%	C314	0.42611	62.43%
F1361	0.43001	73.63%	C391	0.43288	62.67%
F1068	0.42487	73.64%	C215	0.43317	62.67%
F170	0.43113	73.66%	C714	0.42782	62.69%
F1421	0.42409	73.68%	C255	0.42499	62.71%
F79	0.43189	73.76%	C488	0.44563	62.75%
F1083	0.42781	73.95%	C661	0.43089	62.80%
F468	0.43271	73.97%	C122	0.43214	62.90%
F1299	0.42949	74.00%	C33	0.43391	62.92%

F138	0.43279	74.01%	C586	0.43419	63.06%
F732	0.42602	74.01%	C272	0.43127	63.23%
F1443	0.42841	74.04%	C672	0.42962	63.29%
F491	0.42441	74.11%	C100	0.43799	63.55%
F90	0.42461	74.12%	C700	0.44105	63.57%
F162	0.42839	74.13%	C344	0.42783	63.65%
F1472	0.43394	74.20%	C313	0.42763	63.74%
F383	0.42188	74.22%	C216	0.43113	63.76%
F1517	0.43209	74.27%	C440	0.43429	63.93%
F463	0.43160	74.29%	C246	0.42691	63.96%
F1489	0.42174	74.32%	C503	0.42591	63.98%
F1280	0.43119	74.35%	C299	0.43291	64.03%
F824	0.42962	74.37%	C306	0.42668	64.29%
F1099	0.43322	74.39%	C585	0.42454	64.39%
F149	0.43316	74.41%	C612	0.44869	64.49%
F912	0.43259	74.52%	C176	0.43293	64.60%
F179	0.43143	74.54%	C717	0.44213	64.73%
F1138	0.42441	74.55%	C772	0.43622	64.74%
F777	0.42415	74.68%	C340	0.43686	64.77%
F1427	0.42403	74.73%	C712	0.44531	64.80%
F1	0.42438	74.77%	C271	0.43063	65.06%
F151	0.42458	74.83%	C470	0.42986	65.18%
F546	0.42465	74.87%	C223	0.43728	65.19%
F1298	0.42334	74.89%	C593	0.43040	65.26%
F1306	0.42440	75.00%	C227	0.43177	65.60%
F974	0.43128	75.19%	C310	0.42774	65.69%
F438	0.42410	75.20%	C762	0.43519	65.89%
F624	0.42307	75.21%	C684	0.42970	65.90%
F623	0.42858	75.24%	C36	0.42975	65.92%
F1319	0.43671	75.25%	C214	0.42690	66.04%
F986	0.42756	75.35%	C682	0.44095	66.05%
F163	0.42968	75.36%	C567	0.44108	66.08%
F962	0.42810	75.45%	C233	0.43354	66.11%

F223	0.42463	75.52%	C327	0.43245	66.42%
F917	0.42896	75.71%	C248	0.42738	66.46%
F486	0.43366	75.73%	C159	0.42929	66.50%
F432	0.42078	75.92%	C177	0.42904	66.78%
F161	0.43346	76.04%	C746	0.43588	66.90%
F991	0.42198	76.06%	C715	0.43525	66.99%
F1520	0.42512	76.06%	C596	0.43211	67.47%
F167	0.42668	76.23%	C544	0.43316	67.51%
F499	0.43211	76.30%	C221	0.43547	67.55%
F788	0.42893	76.54%	C622	0.42888	67.64%
F1247	0.42404	76.57%	C679	0.43888	67.65%
F988	0.42924	76.63%	C610	0.43039	67.72%
F13	0.42973	76.67%	C719	0.43962	67.74%
F926	0.43127	76.72%	C265	0.43379	67.80%
F1107	0.42681	76.77%	C518	0.42559	67.84%
F1228	0.43392	77.00%	C654	0.43549	68.30%
F180	0.42669	77.01%	C174	0.43110	68.69%
F1001	0.42577	77.03%	C678	0.43965	68.70%
F981	0.42737	77.06%	C500	0.44145	68.76%
F181	0.43624	77.24%	C436	0.43397	68.77%
F1171	0.42371	77.37%	C484	0.44340	68.86%
F7	0.42660	77.48%	C460	0.42479	69.37%
F1257	0.43341	77.62%	C290	0.43804	69.47%
F1157	0.43597	77.89%	C374	0.42542	69.54%
F1382	0.42427	77.91%	C711	0.43738	69.81%
F969	0.43253	77.94%	C358	0.43667	70.08%
F1531	0.42395	78.02%	C689	0.42647	70.08%
F260	0.42392	78.03%	C473	0.44088	70.25%
F1396	0.42311	78.18%	C465	0.43874	70.61%
F201	0.43396	78.40%	C703	0.43454	70.74%
F868	0.42368	78.79%	C667	0.43628	70.83%
F951	0.43354	79.16%	C341	0.43086	70.86%
F849	0.42387	79.17%	C615	0.43630	71.19%

F970	0.42508	79.31%	C28	0.43351	71.26%
F148	0.42741	79.55%	C692	0.43897	71.58%
F1002	0.42294	79.85%	C499	0.44057	71.74%
F1112	0.42514	80.00%	C800	0.44684	72.85%
F1144	0.43110	80.26%	C257	0.42544	73.23%
F1419	0.42786	80.31%	C491	0.43355	73.42%
F1152	0.42976	80.56%	C286	0.42233	73.47%
F804	0.42957	80.62%	C502	0.42563	73.81%
F534	0.42458	80.73%	C298	0.43423	73.96%
F724	0.42553	81.37%	C281	0.43455	74.02%
F1272	0.42187	82.63%	C644	0.44867	74.05%
F854	0.42889	83.25%	C707	0.43104	74.35%
F1295	0.42562	83.49%	C114	0.43521	74.79%