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Physiological Mechanisms of Desiccation Resistance in Fruit-Parasitic Rhagoletis Flies

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PHYSIOLOGICAL MECHANISMS AND EVOLUTION OF DESICCATION RESISTANCE IN FRUIT-PARASITIC RHAGOLETIS FLIES

By

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Christa Kohnert

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A Thesis

Presented to

The Faculty of

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ABSTRACT

Understanding drought adaptation in fruit-parasitic *Rhagoletis* flies is essential for evaluating the potential for eastward spread of the invasive apple maggot fly, *R. pomonella,* from coastal Washington into arid central Washington, which poses a threat to the largest crop of U.S. apples. A closely related native species, *R. zephyria*, provides an opportunity to study existing drought adaptation in the region as it is locally adapted to drought conditions in central Washington. Here, I aim to elucidate physiological mechanisms underlying desiccation resistance in *R. pomonella* and *R.* zephyria, as well as determine if the trait is plastic or canalized in *R. zephyria.* Pupal diapause could be an advantageous state under drought stress because metabolisms are suppressed, limiting active water loss. To test diapause regulation as a mechanism contributing to desiccation resistance, I observed the proportions of diapausers (vs. direct developers) under high (drought) and low (non-drought) vapor pressure deficits in three host races of invasive *R. pomonella* and two populations of native *R. zephyria* to determine if 1) there was past selection on diapause regulation that led to higher proportions of diapausers in drought resistant populations and 2) drought stress affected diapause regulation. *R. zephyria* lacked direct development completely so diapause regulation cannot account for greater desiccation resistance in populations from arid vs. humid regions in Washington. The proportions of diapausers in *R. pomonella* were greatest among black hawthorn infesting flies (high desiccation resistance) and similar between apple infesting flies (low desiccation resistance) and ornamental hawthorn infesting flies (intermediate desiccation

resistance), and not affected by drought treatment, suggesting diapause regulation is not the primary mechanism contributing to desiccation resistance in the invasive species. Next, I conducted a differential gene expression experiment to explore additional mechanisms and to categorize canalized versus plastic transcriptional responses to drought stress. Gene expression in newly egressed *R. zephyria* larvae was largely canalized in drought resistant and susceptible populations, though drought resistant larvae responded more to low humidity conditions (relative to humid conditions) than drought susceptible larvae, suggesting that local drought adaptation in *R. zephyria* is impacted by a genotype x environment interaction. Annotation of differentially expressed genes suggest differences in cuticular hydrocarbon profiles could underlie variable desiccation resistance and highlighted potential differences in development speeds between populations. In conclusion, desiccation resistance in *R. zephyria* and *R. pomonella* is likely multi-faceted and the primary mechanism that accounts for variation in desiccation resistance among populations is yet to be identified. Furthermore, desiccation resistance appears to be adaptive to local climates in *R. zephyria* and potentially constrained by host related fitness tradeoffs in *R. pomonella*.

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INTRODUCTION

The introduction and establishment of a non-native species in a novel environment can have far reaching, often negative, impacts within that environment These impacts can range from displacing native species (e.g. Mack 1981; Holway *et al.* 2002) to larger disruptions of ecosystems (e.g. Holway *et al.* 2002; Aukema *et al.* 2010) or even impacts on the environment itself (e.g. Strong & Ayres 2013). When negatively affecting existing biota of agricultural or commercial importance there are often extensive economic ramifications (Osteen *et al.* 2012; USFS 2013). Mitigating the negative effects of invasive pests can have large costs that increase with time since introduction (Pimentel *et al.* 2000; Osteen *et al.* 2012; Simberloff *et al.* 2013; USFS 2013). Therefore, it is crucial to understand the mechanisms for establishment and spread of invasive species.

Invasive species face many challenges in a novel environment that can make proliferating in a new range difficult, but several mechanisms help make establishment possible. Abiotic conditions, competition, or trophic interactions with existing biota can limit initial survival and bottleneck or founder effects can limit the long-term persistence of the invading population (reviewed in Sax & Brown 2000). These challenges are so extensive that the majority of introductions fail (Sax & Brown 2000). Successful invaders are thought to overcome these challenges by several mechanisms, including relief from native pressures or enemy release (Keane & Crawley 2002; Diwakar *et al.* 2015; Mason 2016), underlying genetic architecture favorable for rapid adaptation (reviewed in Lee, 2002), and phenotypic plasticity.

Genetic architecture, which is defined at a population level by the number of loci, variation in alleles, allelic and genic interactions, and pleiotropy (Bradshaw & Holzapfel 2000), can facilitate invasion if it results in traits already advantageous or amenable to selection in the novel environment.

Phenotypic plasticity and genetic architecture favorable for rapid adaptation in populations can play key roles in invasion success. Faced with a novel environment and a new set of abiotic and biotic challenges, an invader must be able to express phenotypes associated with high fitness in the introduced range. If the novel and home environment are similar, phenotypes are likely already well suited in the introduced range. If the environments do not match, phenotypic plasticity can allow the invader to survive (Bock *et al.* 2015) and natural selection can act to move the invader closer to the adaptive optimum of the new environment. For example, the copepod *Eurytemora affinis*, which normally inhabits saline and hypersaline environments, is able to withstand low salinity conditions and has successfully invaded freshwater habitats over the last 100 years (Lee 1999). While expressing a wide-range of salinity tolerance, phenotypic plasticity in a single generation alone cannot explain a freshwater phenotype of *E. affinis*. Rather, a gene x environment interaction contributes to creating freshwater phenotypes, suggesting plasticity in salinity tolerance has underlying genetic variation on which natural selection acts to increase freshwater invasibility (Lee & Petersen 2002).

Invasive success is enhanced by a species' ability to rapidly adapt to a new environment (Reznick & Ghalambor 2001; Novy *et al.* 2013; Krehenwinkel *et al.* 2015).

Various mechanisms can act on genetic architecture to produce these rapid changes including, but not limited to, genomic rearrangements, additive genetic variance, and hybridization (reviewed in Lee, 2002). Greater additive genetic variance provides more phenotypic variation for selection to act, and can aid in rapid adaption in this regard; such is the case for cold tolerance in the invasive hemlock woolly adelgid (Lombardo & Elkinton 2017), and flowering time in Pyrenean Rocket (Vandepitte *et al.* 2014). For invasive species, this variation is inherited from the founding population(s) or arises via mutation or hybridization with another species (reviewed in Bock *et al.* 2015).

***Rhagoletis pomonella,* an invader in the Pacific Northwest**

Rhagoletis pomonella is an invasive fruit-parasitic fly of particular concern in the Pacific Northwest of the United States as it threatens agriculture in the region. As a phytophagus insect, *R. pomonella* infests apples (among other hosts), causing substantial damage to the host fruit as larvae. In 2013, Washington produced 57% of the total U.S. apple crop at a value of $2.19 billion (USDA 2015). Washington State is consistently the top producer of U.S. apples, so the spread of apple flies into central where the vast majority of the state's apples are grown is of great economic concern. Currently, *R. pomonella* has a limited distribution in these crucial apple growing regions, but is spread throughout coastal regions of the state (Yee *et al.* 2012).

The distribution of *R. pomonella* throughout the state differs among the three prominent host races of the species. A host race is designated as a population in partial reproductive isolation from conspecifics because the populations utilize

different hosts that fruit at different times (Diehl & Bush 1984), thus, there is temporal separation in peak emergence of adults for each population. Since introduction and establishment in the Pacific Northwest, most likely via infested apples around the 1960s, *R. pomonella* shifted from apples (*Malus spp.*) to also infest earlier fruiting black hawthorns (*Crataegus douglasii* and *C. suksdorfii*) and later fruiting English ornamental hawthorns (*C. monogyna*) hosts (Hood *et al.* 2013). Fruiting times of these three hosts, and, therefore, peak emergence time of these host races, are offset such that black hawthorns generally fruit ~3-4 weeks before apples and ~6-8 weeks before ornamental hawthorns. These three host races of *R. pomonella* (hereafter referred to as black hawthorn flies, apple flies, and ornamental hawthorn flies) are genetically distinct despite gene flow (Feder *et al.* 1997, 1999; Egan *et al.* 2015). All three host races can be found throughout coastal regions of the Pacific Northwest, but only black hawthorn flies have established, albeit small, populations east of the Cascade mountain range in central Washington (Yee *et al.* 2012).

Higher desiccation stress in central Washington appears to be a major abiotic challenge posed to the apple fly and, therefore, understanding drought adaptation in *Rhagoletis* may help evaluate the potential for eastward spread of this quarantine pest into apple growing regions. Central Washington lies in the rain shadow of the Cascade mountain range and, consequently, is drier and warmer than coastal regions during the summer months when *R. pomonella* flies are active. The arid climate of central Washington is a likely abiotic challenge that is limiting the eastward spread of the apple fly as pupae placed under desiccation stress show poor survival and adult emergence (Hill 2016). In order to spread into eastward into critical apple growing

regions, it seems the apple fly must adapt to these drier conditions. In this context, desiccation resistance is a crucial phenotype to understand in *R. pomonella.*

Variation in desiccation resistance in *Rhagoletis*

The range of invasive *R. pomonella* overlaps with its native sister species, *R. zephyria,* and this native relative offers further insight into drought adaptation in *Rhagoletis*. The native fly, *R. zephyria,* is found widely dispersed throughout Washington State on its snowberry host (*Symphoricarpos albus*), including drier regions east of the Cascades (Gavrilovic *et al.* 2007; Yee *et al.* 2012). Because it already occupies drier climates in the Pacific Northwest, this native relative provides a study system for understanding existing drought adaptation in *Rhagoletis* in central Washington. Furthermore, the two species hybridize at low rates (~1% per generation) such that snowberry fly (native, *R. zephyria*) alleles introgress asymmetrically into populations of the invasive fly (*R. pomonella*) (Green *et al.* 2013; Arcella *et al.* 2015), which could potentially confer enhanced desiccation resistance to the invasive fly, as proposed by Arcella *et al.* (2015).

Native *R. zephyria* in Washington State appear to be locally adapted to drought conditions. Hill (2016) found that snowberry fly pupae from arid climates east of the Cascades lost less weight under low relative humidity conditions, thus exhibiting greater desiccation resistance, compared to their counterparts from more humid climates west of the Cascades. While maternal effects are a possible explanation for that variation in desiccation resistance, they seem unlikely given that the life stage

used went through several molts before treatment. All pupae were reared out of fruit under similar lab conditions and had no detectable population structure expected by geographic isolation (Green *et al.* 2013; Arcella *et al.* 2015), suggesting that this variation in desiccation resistance is adaptive to local drought conditions, which could be canalized or derived from differences in plasticity among populations.

It is currently unknown if desiccation resistance in invasive *R. pomonella* shows the same patterns of geographic variation observed in its native relative, *R. zephyria*, but there is variation in desiccation resistance between host races of the invasive fly. While the black hawthorn fly (invasive, *R. pomonella*) has established east of the Cascades (Hood *et al.* 2013), desiccation resistance in those populations is unreported due to limited distributions and low infestation rates. However, invasive *R. pomonella* displays variation in desiccation resistance between apple and black hawthorn flies from west of the Cascades, with black hawthorn fly pupae exhibiting greater desiccation resistance than apple fly pupae (desiccation resistance in ornamental hawthorn flies is unreported) (Hill 2016). The mechanisms underlying desiccation resistance are still unknown, however, differences in host phenology and corresponding pre-winter length implicate diapause regulation as a possible factor involved in desiccation resistance. In addition to not knowing the mechanisms involved in desiccation resistance in both invasive and native species of *Rhagoletis,* it is unclear if variation in desiccation resistance among populations is canalized or due to differences in phenotypic plasticity. The goals of this study were two-fold; first, to elucidate mechanisms involved in desiccation resistance (specifically testing diapause regulation as a possible mechanism and also examining gene expression patterns to

identify other potential mechanisms) and, second, to evaluate canalized versus plastic responses to desiccation stress.

Diapause as a potential water conservation strategy

Diapause regulation is a key feature of the *Rhagoletis* life cycle. As with many phytophagus insects, the host-specialist, fruit-parasitic flies in the genus *Rhagoletis* have a univoltine life-cycle that is dependent upon timing of adult emergence to match host phenology (Smith 1988). After adults emerge (eclose), they live for 4-6 weeks to mate on and oviposit in host fruit. Once eggs hatch, larvae eat inside the fruit until they leave the fruit (egress), bury several centimeters into the soil, and pupate. Pupae generally remain in the soil overwinter in diapause, a state of depressed metabolic activity and suspended development until they emerge as adults the next season when host fruits are available again. Due to the importance of host fruit for mating and as a food source for larvae, it is crucial for flies to regulate diapause in a way that ensures adults eclose when hosts are fruiting and environmental conditions are hospitable (Feder & Filchak 1999; Dambroski & Feder 2007; Ragland *et al.* 2012).

This study explores diapause regulation as a strategy to minimize water loss under desiccation stress, specifically through increased selection for diapause development over direct development. In the eastern U.S., diapause length varies within populations and a portion of pupae forego diapause (do not overwinter and develop directly into adults), as *R. pomonella* is a facultative diapauser (Feder *et al.* 1997; Dambroski & Feder 2007). Natural selection favors diapause regulation that

ensures adult emergence coincides with host and mate availability and hospitable environmental conditions (Feder *et al.* 1997; Feder & Filchak 1999; Dambroski & Feder 2007). While this selection heavily favors diapause development over direct development—to the extent that some *Rhagoletis* species are obligate diapausers (Teixeira & Polavarapu 2005a; Moraiti *et al.* 2014)—the strength of selection against direct development varies depending on the host (Feder *et al.* 1997; Dambroski & Feder 2007) and diapause development does impose energetic costs that can adversely affect fecundity and metabolic stores (Hahn & Denlinger 2007). Here, diapause regulation is proposed as a mechanism involved in desiccation resistance for the following three reasons: (1) insect diapause is associated with greater stress tolerance and resistance (Denlinger & Yocum 1998; Danks 2000; Hahn & Denlinger 2007; Rinehart *et al.* 2007), (2) diapause regulation in *R. pomonella* varies with pre-winter length (Feder *et al.* 1997; Dambroski & Feder 2007), and (3) between host races, *R. pomonella* shows variation in desiccation resistance with different mean pre-winter lengths (Hill 2016).

Although the relationship between diapause regulation and desiccation resistance has not been studied in *Rhagoletis*, diapause has been linked to increased tolerance to environmental stresses in many insects. Generally, stress-resistance and tolerance-related pathways are up-regulated in insects during diapause to withstand thermal stresses (reviewed in Denlinger & Yocum 1998; Hahn & Denlinger 2007). For example, insects from multiple orders up-regulate heat shock proteins just before initiating diapause to enhance thermotolerance in preparation for winter temperatures (Rinehart *et al.* 2007). Increases in heat shock proteins have been documented in *R.*

pomonella (Ragland *et al.* 2011), as well as another relative, *R. mendax* (Teixeira & Polavarapu 2005b). While insects have such adaptations to deal with extreme temperatures during diapause, they also have adaptations to maintain water and energy stores during diapause, including minimized metabolisms (Danks 2000). Water is actively lost with gas exchange (Woods & Smith 2010), a process that is limited during diapause because intermediary and respiratory metabolisms are depressed in this state (by ~90% in *R. pomonella* (Ragland *et al.* 2009)). With a depressed metabolism and therefore limited gas exchange, diapausers might effectively achieve greater desiccation resistance compared to direct developers that maintain an active metabolism. Thus, tighter diapause regulation that further favors diapause development could be favorable under conditions that evoke greater desiccation stress such as increased temperature, decreased humidity, or prolonged exposure to stressful conditions.

Diapause regulation in *R. pomonella* varies with host phenology, which ultimately dictates the length of pre-winter periods (the time between pupation and the onset of winter) during which pupae are exposed to drier, hotter environmental conditions. *R. pomonella* pupae that consistently face longer pre-winter periods due to host phenology or geographic location have less variable diapause regulation (more diapausers). Dambroski and Feder (2007) observed in the eastern U.S. that *R. pomonella* flies from earlier fruiting hosts have more diapausers than flies from later fruiting hosts. This pattern holds within host races; the proportion of diapausers increases towards lower latitudes as temperatures increase and host phenology shifts

earlier in the season. In other words, strong selection for diapausers becomes even stronger as pre-winter lengths increase.

Why would pre-winter length affect the strength of selection for diapause? For one, to keep adult eclosion synchronized with host phenology. Because temperature influences insect development, flies from earlier fruiting hosts are selected for more recalcitrant diapause regulation (diapause regulation that is less influenced by temperature, often more diapausers and deeper diapause) to avoid pre-mature eclosion that would otherwise be induced by long exposure to warm temperatures (Feder *et al.* 1997). Another possible reason is drought stress, which has the potential to increase with increasing pre-winter length. Assuming that prolonged exposure to warm, dry pre-winter conditions increases drought stress, pupae would need to be equipped with adequate desiccation resistance. If metabolism suppression during diapause significantly contributes to maintaining water balance and overall desiccation resistance, then diapause would be even more advantageous for flies facing increased drought stress. Thus, stronger selection for diapause in earlier emerging host races could come, in part, from pressure to achieve higher desiccation resistance needed to withstand long exposure to summer conditions.

Indeed, selection for diapause development could potentially explain variation in desiccation resistance between host races of invasive *R. pomonella* and between drought susceptible and drought resistant populations of native *R. zephyria*. Early fruiting black hawthorn fly pupae (invasive, *R. pomonella*) exhibit greater desiccation resistance than later fruiting apple fly pupae (invasive, *R. pomonella*) (there is

currently no data on desiccation resistance in the latest fruiting ornamental hawthorn fly) (Hill 2016). The difference in pre-winter length between these host races suggests an interaction between desiccation resistance and diapause regulation; black hawthorn flies could compensate for a longer pre-winter period and exposure to drought stress by utilizing diapause development to limit active water loss via metabolism suppression. Stronger selection for diapause development could contribute to desiccation resistance in drought resistant populations of native *R. zephyria* as well, though diapause regulation in this species is poorly characterized and could be an obligate diapauser. However, if it is a mechanism contributing considerably to desiccation resistance in both invasive and native fly, diapause regulation should vary between drought resistant and susceptible populations. Diapausers should occur in the highest proportions within populations exhibiting greater desiccation resistance (invasive black hawthorn *R. pomonella* flies and native drought resistant *R. zephyria* flies from central Washington) (Figure 1a,b) and imposing drought stress should further increase the proportion of diapausers (Figure 1c); observing theses patterns would support the hypothesis that diapause development is selected for as a water conservation strategy and diapause regulation contributes to desiccation resistance.

Is desiccation resistance a plastic or canalized trait?

In addition to not knowing the physiological mechanisms underlying variation in desiccation resistance in both invasive *R. pomonella* and native *R. zephyria*, the

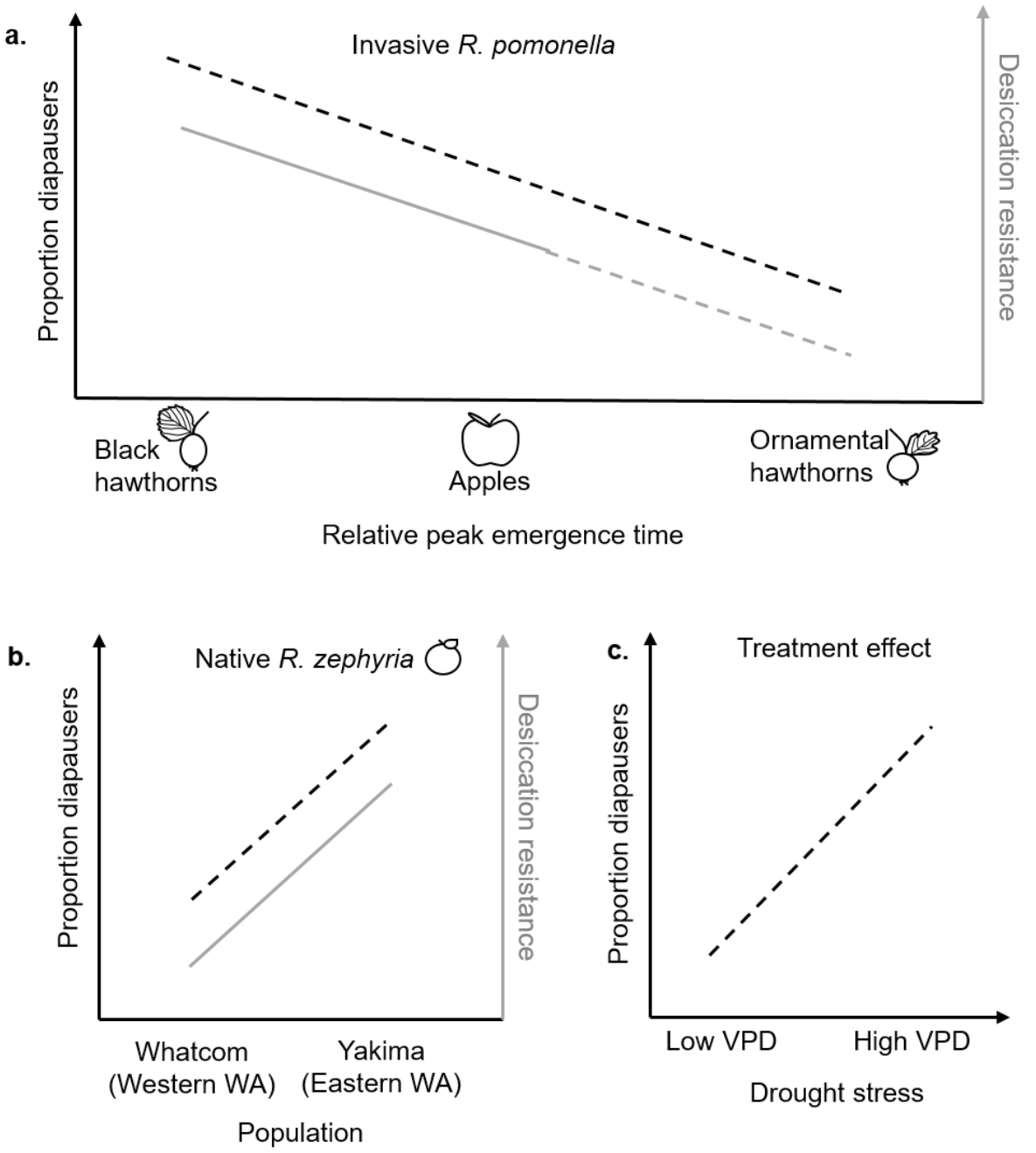

Figure 1. Hypothesized relative proportions of diapausers and desiccation resistance (plotted together as general patterns) in (a) three host races of invasive *R. pomonella* with differing pre-winter lengths, (b) two populations of native *R. zephyria*, one drought susceptible (Whatcom) and one drought resistant (Yakima), and (c) in response to increasing drought stress (VPD = vapor pressure deficit). Dashed lines indicate hypothesized patterns and solid lines indicate patterns previously observed by Hill (2016).

genetic basis of desiccation resistance is unclear. The enhanced desiccation resistance observed in drought adapted populations of native *R. zephyria* could be canalized (insensitive to environmental variability), meaning drought adapted flies have greater desiccation resistance regardless of the environment into which they are put. This pattern, would be expected if selection has acted on the underlying genetic architecture of desiccation resistance; drought susceptible and drought resistant populations would have intrinsically different abilities to cope with an arid environment. Alternatively, greater desiccation resistance in drought adapted populations of native *R. zephyria* could stem from greater phenotypic plasticity within those populations.

While selection or common garden experiments are traditionally used to tease apart genetic and environmental impacts on a trait (e.g. Lee & Petersen 2002; de Villemereuil *et al.* 2016), transcriptome analysis, such as RNAseq, offers another method for studying environmental and genetic impacts. Plastic and canalized transcriptional responses to drought stress between drought susceptible and resistant populations can be characterized by the genes which change expression in response to desiccation stress within a population (plastic transcriptional response) or by the genes that are differentially expressed between populations, regardless of desiccation stress (canalized transcriptional responses). In the context of this study, RNAseq has an added benefit of allowing for identification of physiological or molecular mechanisms that could be involved in desiccation resistance and warrant further investigation by annotating transcriptional responses to desiccation stress.

Experimental overview

This study aims to identify mechanisms contributing to variable desiccation resistance in Washington State populations of invasive *R. pomonella* and native *R. zephyria* to better understand the potential expansion of the invasive apple fly into critical apple growing regions in the arid valley of central Washington. In a phenotypic study, I tested diapause regulation as a possible mechanism for desiccation resistance in three host races of invasive *R. pomonella* and two populations of native *R. zephyria*, one drought resistant and one drought susceptible. I exposed pupae from these populations to high or low drought stress and scored diapause status (to observe diapause regulation) and weight loss (to measure desiccation resistance). This phenotypic study aims to elucidate (1) the relationship between the proportion of diapausers and desiccation resistance and (2) the effect of drought stress on the proportion of diapausers in drought resistant and drought susceptible populations of invasive *R. pomonella* and native *R. zephyria*.

To identify additional possible mechanisms involved in *Rhagoletis* desiccation resistance and observe canalized and plastic responses to desiccation, I evaluated gene expression differences between drought resistant and susceptible populations of native *R. zephyria* under high or low relative humidity (RH) treatment. Newly egressed *R. zephyria* larvae from central and western Washington populations were selected for this experiment in order to capture the greatest transcriptional response to desiccation; this targeted the life stage likely most susceptible to desiccation and the populations from Hill (2016) that exhibited the greatest difference in desiccation

resistance for either native or invasive fly. Sequencing of mRNA allowed for identification of canalized transcriptional expressions (genes differentially expressed between populations, independent of treatment) and plastic transcriptional expressions (genes differentially expressed between treatments, within a population).

Questions and hypotheses

Phenotypic study

Question 1. How do patterns of diapause regulation compare to patterns of desiccation resistance among *Rhagoletis* populations?

I hypothesized that proportions of diapausers would be greater in populations with greater desiccation resistance for both invasive *R. pomonella* and native *R. zephyria* (Figure 1a; Figure 1b). I hypothesized that ornamental hawthorn flies (shortest pre-winter period) would have lower desiccation resistance than both black hawthorn and apple flies (invasive, *R. pomonella*); otherwise, I expected to corroborate patterns of desiccation resistance thoroughly addressed in Hill (2016) (Figure 1a; Figure 1b). For diapause regulation in invasive *R. pomonella*, I hypothesized that the proportion of diapausers would increase with average pre-winter length. Specifically, I hypothesized that black hawthorn flies, which have the longest pre-winter length, would have the highest proportion of diapausers of the three host races, followed by apple flies (intermediate pre-winter length), and then ornamental hawthorn flies (shortest pre-winter length) (Figure 1a). For native *R. zephyria*, I hypothesized that drought resistant Yakima populations would have higher proportions of diapausers than drought susceptible Whatcom populations (Figure 1b).

Question 2. Does drought stress affect the proportion of diapausers?

I hypothesized that, for both species, pupae would have greater proportions of diapausers when exposed to high drought stress than when exposed to low drought stress (Figure 1c).

Expression study

Exploratory question 1. How does gene expression differ between drought resistant and drought susceptible populations of native *R. zephyria* after high or low-humidity treatments? Though this experiment was exploratory, there were specific patterns of gene expression of interest in order to determine the extent of plasticity or canalization of desiccation resistance in native *R. zephyria*:

- Canalized transcriptional expressions (genes differentially expressed between populations independent of humidity treatment)
- Plastic transcriptional expressions (genes differentially expressed within a population in response to treatment)
- Gene x environment interaction on expression (genes with plastic expressions to treatment that differ between populations)

Exploratory question 2. What molecular or physiological mechanisms might be involved in achieving desiccation resistance? What are the functions of differentially expressed genes? Here, I explored the broad function of genes that were up or down regulated.

METHODS

Overview

Rhagoletis larvae and pupae from snowberry (native, *R. zephyria)*, black hawthorn, apple, and ornamental hawthorn hosts (invasive, *R. pomonella*) were exposed to experimental pre-winter treatments designed to simulate environmental conditions east and west of the Cascade mountain range, resulting in a high and low drought stress treatment. I chose Whatcom County to represent sites west of the Cascades where drought susceptible snowberry flies (native, *R. zephyria*), black hawthorn, apple, and ornamental hawthorn flies (invasive *R. pomonella*) are widely distributed, and Yakima County to represent arid sites east of the Cascades where drought resistant snowberry flies (native, *R. zephyria*) and small populations of black hawthorn flies (invasive *R. pomonella)* can be found. In the phenotypic study, pupae were exposed to high or low vapor pressure deficit (VPD) treatments for 4 days and monitored for 61 days thereafter, scoring weight loss and diapause status throughout this time in order to measure desiccation resistance and diapause regulation.

In the differential gene expression study, snowberry larvae (native *R. zephyria*) from Whatcom and Yakima counties were exposed to high and low relative humidity (RH) treatments for three hours immediately following egression from the fruit. High-throughput sequencing of mRNA followed to compare gene expression differences between populations and humidity treatments.

Sample collection

To obtain wild samples of native *R. zephyria* and invasive *R. pomonella*, I haphazardly collected ripe or overripe host fruits from Whatcom and Yakima counties from July to September 2016 while hosts were fruiting (Table 1). Snowberries were collected in Whatcom and Yakima counties to sample drought susceptible and drought resistant populations of the native fly, *R. zephyria* (1 host x 2 locations). Black hawthorns, apples, and ornamental hawthorns were collected in Whatcom County to sample the three host races of the invasive fly, *R. pomonella* (3 hosts x 1 location). An additional sample of black hawthorns were collected in Yakima County in attempts to sample the only known established host race of *R. pomonella* east of the Cascades (Yee *et al.* 2012; Hood *et al.* 2013). However, this population was excluded from analysis due to low sample sizes (see “Design and setup” below).

All larvae egressed from the fruit under ambient lab conditions prior to treatment. Each type of fruit was spread over wire screens and placed over a plastic collection tray for the larvae to drop into once they egressed. Collection trays with apples and ornamental hawthorns had a very thin layer of dry vermiculite or sand at the bottom to prevent larvae from sticking to the tray (not deep enough to allow larvae to burrow). Larvae from snowberries and black haws did not stick excessively to the tray without a substrate. Larvae egressed in a diurnal pattern in which the majority of larvae egressed each day prior to 6 a.m. and most pupariated by 11 a.m. PDT. That egression pattern resulted in individuals being in this egression environment for less 24 hours, with most individuals held for fewer than 12 hours.

Table 1. Collection locations and dates of host fruits during the summer of 2016. Fruits were collected within two miles of site coordinates and pooled. Snowberries were collected to obtain native, *R. zephyria* flies. Black haws (*Crategus douglasii* and *C. suksdorfii*), apples (*Malus domestica*), and ornamental haws (*C. monogyna*) were collected to obtain invasive, *R. pomonella* flies from each host race within the species.

County	Host fruit	Site name	Coordinates	Collection dates
Whatcom	Snowberry	WWU	48.7335° N, 122.4873° W	Jul 21 – Aug 29
	Black hawthorn	Hovander	48.8308°N, 122.5931° W	Aug 10 – Aug 23
	Apple	York	48.7508° N, 122.4680° W	Aug 12
	Ornamental hawthorn	WWU	48.7335° N, 122.4873° W	Sept 10 – Sept 24
Yakima	Snowberry	Wenas	46.8206° N, 120.9280° W	Aug 04 – Sept 02
	Black hawthorn	Wenas	46.8206° N, 120.9280° W	Aug 04 – Sept 21

Treatment conditions

The pre-winter treatments for the phenotypic study initially imposed vapor pressure deficits (VPD) representative of conditions east and west of the Cascades. VPD is the difference between the theoretical maximum and actual amount of moisture in the air and takes into account both relative humidity (RH) and temperature, consequently better describing potential water loss than either variable alone. VPD levels were calculated based on temperatures and RHs using the following equation adapted from Allen *et al.* (1998) where RH is relative humidity, T is temperature in °C, and VPD is expressed in Pa.

$$VPD = \left(1 - \frac{RH}{100}\right) \times 610.7 \times 10^{7.5T/(273.3+T)}$$

The temperatures used were based on 2009-2015 mean daily air temperatures during August through September in Whatcom and Yakima counties (WSU AgWeatherNet, http://weather.wsu.edu). Pupae incubated at 16°C and ~85% RH (low VPD) to represent the west side and at 20°C and ~56% RH (high VPD) to represent the east side to achieve VPDs of ~0.27 and ~1.1 kPa, respectively. Each treatment had two incubators with 6 airtight, plastic boxes (18 L each) that maintained ~85% or ~56% RH with saturated salt solutions of either KCl or $MgCl_2$, respectively. After these initial treatments, all pupae were held at ~0.35 kPa VPD (20°C and ~85% RH) for observation. Temperature and humidity were monitored with iButtons to ensure that these VPDs were maintained throughout treatment and observation. To maintain

summer diurnal cues, all flies were incubated under a 14 L:10 D cycle during initial treatment and observation.

Similarly, for the expression study, conditions simulated dry conditions east of the Cascades (~43% RH) or more humid conditions west of the Cascades (~85% RH). In Hill (2016) these RH levels elicited differential weight loss between treatments of native *R. zephyria* pupae from populations used in this present study, especially among drought susceptible Whatcom pupae. Each treatment had an airtight, plastic container (946 mL) with saturated salt solutions of KCl or K_2CO_3 to maintain relative humidities of ~85% or ~43%, respectively, which incubated at 20 °C under light. RH levels were verified with iButtons that logged temperature and RH.

Phenotypic study

Design and setup

Individuals were removed from collection trays for as cohorts on a daily basis between 10 a.m. PDT and noon until 200-250 individuals from each host x location were in treatment, or in cases where infestation rates were too low to achieve those sample sizes,until egression ceased. These target sample sizes were reached for drought susceptible Whatcom snowberry flies (native, *R. zephyria*), drought resistant Yakima snowberry flies (native, *R. zephyria*), Whatcom black hawthorn flies (invasive, *R*. pomonella), and apple flies (Table 2). Half the sample size was used for ornamental hawthorn flies (invasive, *R. pomonella*) because the host is not as heavily infested as

Table 2. Pupae counts by dissection class for three host races of invasive *R. pomonella* and two populations of native *R. zephyria* following pre-winter treatment (four days in low (~0.27 kPa) or high (~1.1 kPa) vapor pressure deficit (VPD) followed by 61 days in ~0.35 kPa VPD). All *R. pomonella* samples were from Whatcom County unless otherwise noted. *R. pomonella* samples were dissected 65 days post-pupariation while *R. zephyria* samples were dried four 48 hours at 50 °C at 65 days post-pupariation prior to dissection. The last two rows indicate samples included in analyses for either diapause regulation or desiccation resistance.

	Invasive *R. pomonella*								Native *R. zephyria*			
	Black hawthorn		Black hawthorn (Yakima)		Apple		Ornamental hawthorn		Whatcom		Yakima	
	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
Pupa	156	166	-	-	42	15	27	12	-	-	-	-
Pharate adult	4	5	-	-	5	3	5	1	0	0	0	0
Pupa dried	9	14	-	2	20	14	10	4	58	100	138	160
Empty	21	14	-	-	125	165	19	48	67	26	51	31
Not dissected	-	-	-	18	-	-	24	24	50	50	50	47
Failed to pupariate	1	2	-	-	3	7	2	6	8	11	6	7
Parasitoid	0	3	-	-	0	0	8	7	64	63	1	1
Total treated	191	204	-	20	195	204	95	102	247	250	246	246
Analyzed (diapause)	159*	171*	-	0	47*	18*	32*	13*	55†	95†	128†	153†
Analyzed (desiccation)	190‡	199‡	-	0	192‡	197‡	85‡	89‡	175‡	176‡	239‡	238‡

∗ = pupae and pharate adults
† = dried pupae with 65 day weight > mean *R. pomonella* 65 day weight
‡ = total treated minus parasitoids and larvae that failed to pupariate

apples or black hawthorns in Whatcom County; not enough larvae egressed to reach 200 samples before fly activity ceased for the season. Only 20 Yakima black hawthorn fly pupae (invasive, *R. pomonella*) were obtained likely due to low infestation rates, and despite a large sampling effort (collected >80 lbs of fruit); these samples were only treated in the high VPD treatment and were excluded from any analyses.

Once removed from collection trays, individuals were haphazardly assigned to the high or low VPD pre-winter treatment (except Yakima black hawthorn fly pupae) (Figure 2). Through visual assessment, individuals were roughly paired by size and developmental progress (e.g. larvae, softer/lighter puparium, harder/darker puparium). Pairs were divided between the high and low VPD treatments haphazardly to standardize average size (Table 3) and developmental progress between treatments. Individuals that began treatment as larvae pupariated in the first 12 hours of treatment and very few were actively wandering upon collection. Once assigned to a treatment, pupae were placed in an open 1.7 mL plastic tube with 4 holes ~1mm in diameter.

After treatment assignment, pupae were assigned to a plastic box within that treatment. The two incubators within each treatment were blocked by day so that boxes were only opened every other day in order to minimize temperature and humidity fluctuations (Figure 2). Drought susceptible Whatcom snowberry pupae (native, *R. zephyria*), drought resistant Yakima snowberry pupae (native, *R. zephyria*), and apple pupae (invasive, *R. pomonella*) were randomly assigned to one of the six boxes in each incubator using a random number generator (Figure 2). Egression of larvae from black and ornamental hawthorn fruits was initially low and inconsistent (<5

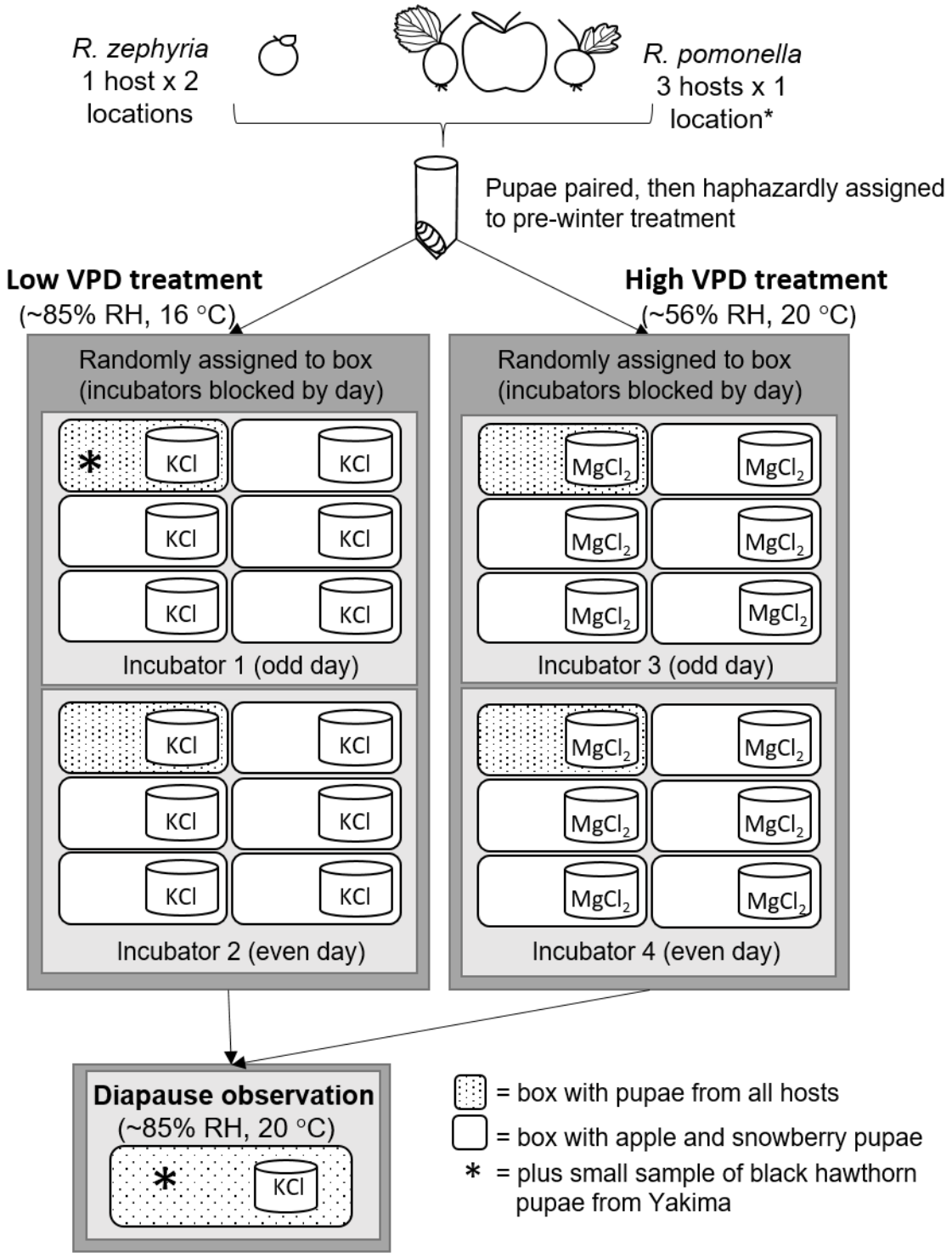

Figure 2. Schematic of phenotypic study setup. Two locations = Whatcom and Yakima counties. *R. zephyria* host = snowberries. *R. pomonella* hosts = black hawthorns, apples, and ornamental hawthorns.

Table 3. Initial pupal weights for all invasive *R. pomonella* host races and native *R. zephyria* from Whatcom and Yakima counties in the high and low VPD treatments. All values are mean weight ± standard error in milligrams. Initial pupal weight did not significantly differ between treatments for either *R. pomonella* (2-way ANOVA, $F_{(1, 964)} = 0.73$, $p = 0.39$) or *R. zephyria* (2-way ANOVA, $F_{(1,824)} = 1.47$, $p = 0.23$). Black hawthorn pupae were lighter than apple pupae and heavier than ornamental hawthorn pupae (2-way ANOVA, $F_{(1, 964)} = 127.95$, $p << 0.01$). Yakima snowberry pupae were heavier than Whatcom snowberry pupae (2-way ANOVA, $F_{(1, 824)} = 13.03$, $p << 0.01$).

			Initial pupal weight (mg)	
Species	Host	Location	Low VPD	High VPD
R. pomonella	Black hawthorn	Whatcom	7.93 ± 0.15	8.06 ± 0.14
	Black hawthorn	Yakima	-	7.38 ± 0.53
	Apple	Whatcom	9.74 ± 0.17	9.78 ± 0.16
	Ornamental hawthorn	Whatcom	6.61 ± 0.24	7.04 ± 0.21
R. zephyria	Snowberry	Whatcom	5.72 ± 0.13	5.75 ± 0.13
	Snowberry	Yakima	6.08 ± 0.12	6.32 ± 0.12

individuals a day). Due to uncertainties with sample size for these host races, black hawthorn pupae (invasive, *R. pomonella*) and ornamental hawthorn pupae (invasive, *R. pomonella*) were only put into one box per incubator (Figure 2). All 20 Yakima black hawthorn pupae (invasive, *R. pomonella*) were placed in the same box in the high VPD treatment (Figure 2). After four days of initial treatment under these conditions, tubes were closed and all pupae were moved to ~0.35 kPa VPD for 61 more days for long-term observation of diapause status (Figure 2).

Monitoring diapause regulation

Morphological markers of pupae 65 days post-pupariation indicated if the fly was a diapauser or direct developer. At this time point, each puparium was dissected to determine if it contained a pupa or a pharate adult, which were defined as diapausers and direct developers, respectively (Figure 3). I also noted if the puparium was empty or contained a dried pupa, meaning the fly was definitely dead at the time of dissection, or if it contained any parasitoid wasp (Figure 3). Multiple species of parasitoid wasps attack *Rhagoletis* flies (Forbes *et al.* 2010); here, presence of any Hymenoptera at any life stage except eggs in a *Rhagoletis* puparium was counted as a parasitoid and not identified beyond order. Parasitoids were equally common in both treatments and most common in Whatcom snowberry flies (native, *R. zephyria*) (Table 2). Parasitoids (fewer than 10% of all samples) and larvae that failed to pupariate (fewer than 3% of all samples) were excluded from all analyses (Table 2). A random

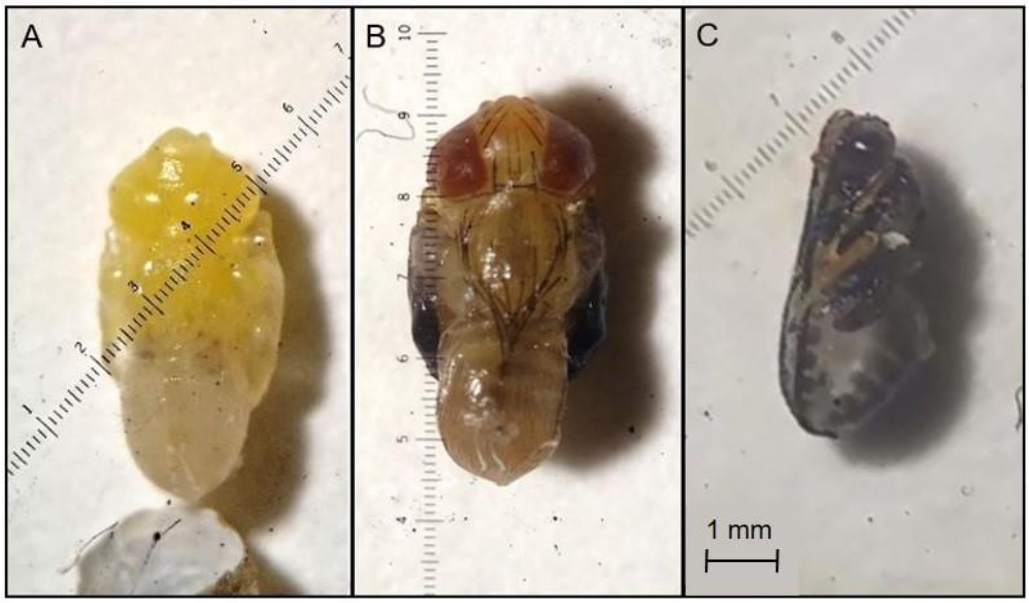

Figure 3. Contents of dissected *Rhagoletis* puparia 65 days post-pupariation were classified into one of the following categories: pupa (diapauser; A), pharate adult (direct developer; B), Hymenoptera parasitoid (excluded from analyses; any life stage—pharate adult pictured in C), dried pupa (dead; not pictured), empty (dead; not pictured), or failed to pupariate (excluded from analyses; not pictured).

subset of native *R. zephyria* and ornamental hawthorn flies (invasive, *R. pomonella*) were saved for a future experiment and, therefore, not dissected (Table 2).

The use of morphological markers to determine diapause status as described above likely underestimated direct development in these populations. Non-diapausing individuals that perished during treatment and before reaching the pharate adult stage were either misclassified as a diapausing (if dissection occurred before the pupa dried) or were excluded ("dried pupae" and "empty" dissection classes; Table 2). Misclassification of direct developers as diapausers would lead to an underestimation of direct development. Assuming that direct developers would have occurred in the same proportions in presumed living and known dead pupae, the exclusion of dead pupae does not introduce additional bias to my estimates, but respirometric data is required to test the validity of this assumption. If direct developers occurred in higher proportions among dead pupae than living pupae, the assumption that direct development is equally likely in surviving and dead pupae would result in an underestimation of the true proportion of direct developers that inflates with pupae mortality. This underestimation would have greater impacts on my estimates for apple and ornamental hawthorn pupae than for black hawthorn pupae because those host races had greater observable mortality (Table 2). For similar reasons, my chances of failing to detect direct development in native *R. zephyria* may have been higher for Whatcom pupae than Yakima pupae.

Monitoring desiccation resistance

Pupae were weighed throughout the 65 days of treatment in order to monitor desiccation resistance in each of the sampled populations to corroborate patterns observed by Hill (2016) and provide desiccation resistance data for late fruiting ornamental hawthorn flies (invasive, *R. pomonella*). Pupae were weighed initially (prior to treatment stress), at four days post-pupariation (end of treatment stress, beginning of diapause observation), and at 65 days post-pupariation (end of diapause observation). All weight measurements were made to the nearest 0.01 mg.

Additionally, the dry weights of native *R. zephyria* pupae were taken 65 days post-pupariation. At 65 days post-pupariation, snowberry fly pupae from both populations were dried at 50 °C for at least 48 hours and then weighed to the nearest 0.01 mg. These dry weights were used to explore if drought susceptible and drought resistant populations of native *R. zephyria* utilized metabolic stores differently from each other during treatment.

Statistical analysis of the phenotypic study

Analyses focused on the effects of host and drought treatment on invasive *R. pomonella* and the effects of population and drought treatment on native *R. zephyria* but also considered additional sources of natural and experimental variation that could influence diapause regulation or desiccation resistance. Start date was included as a factor in analysis of diapause regulation and weight loss in invasive *R. pomonella* because of the potential influence of pre-winter length. Start date was relative to when the first cohort of pupae began treatment within each host race, such that the first cohort of each host race had a start date of zero. Initial pupal weight (W_i) was included as a covariate in all analyses because initial size theoretically influences passive water loss (thus, desiccation resistance) and smaller individuals are more prone to non-diapause development (D. Hahn and D. Schwarz, personal communication). Box or incubator (used for initial drought treatment) was included as a random effect in case these different treatment environments had unintended effects. Box was used as a factor when considering native *R. zephyria* while incubator was used as a factor when considering invasive *R. pomonella* due to imbalance in the number of boxes used for different host races (see “Phenotypic study—Design and setup”).

Diapause regulation

Native *R. zephyria* had zero observable pharate adults (direct developers) so I only analyzed differences in diapause regulation among host races of invasive *R. pomonella*. Flies that were definitively dead upon dissection (empty puparium and

dried pupae) were excluded because diapause status could not be feasibly determined in those flies (Table 2). I used logistic regression (R Core Team 2016) on diapause status for all qualifying *R. pomonella* pupae, using host, treatment, start date, and incubator as factors, and W_i as a covariate, evaluating results with a critical value of $\alpha = 0.05$.

Desiccation resistance

For both native and invasive fly, pupal weight loss (ΔW_t at time *t,* relative to initial weight) was used to evaluate short term (4 day) and long term (65 day) desiccation resistance. Weight loss at 4 days (ΔW_4) and 65 days (ΔW_{65}) did not meet assumptions of equal variance. Transformations did not alleviate this violation, so any subsequent tests were evaluated at a stricter critical value of $\alpha = 0.01$. For invasive *R. pomonella*, differences in ΔW_4 and ΔW_{65} days were evaluated using linear mixed-effect models (LMMs) that used host and treatment as fixed effects, W_i as a covariate, and incubator as a random effect. For native *R. zephyria*, ΔW_4, ΔW_{65}, and dry weights (W_{dry}), were evaluated using LMMs that used population and treatment as fixed effects, W_i as a covariate, and box as a random effect. LMMs were conducted with the nlme R-package (Pinheir*o et al*. 2016). Conditional R^2 for LMMs, as described in (Nakagawa & Schielzeth 2013), were obtained using the MuMIn R-package (Barton 2017).

Expression study

Within 20 minutes of egression, drought susceptible Whatcom pupae and drought resistant Yakima pupae (native, *R. zephyria*) were put into the high or low RH treatment (see “Treatment conditions” above). All larvae were collected between 5 and 8 a.m. PDT over three days in September until there was a minimum of 14 individuals per treatment x population. Upon collection, larvae were placed in a closed 0.7 mL plastic tubes with four ~1mm holes to allow for air flow, and then haphazardly assigned to a RH treatment. Treatment lasted for three hours, concluding prior to visible modification of the larval skin and pupariation. Immediately following the RH treatment, individuals were ground in 200 μL Trizol, flash frozen on dry ice, and then stored at -80°C in preparation for total RNA extraction.

The following protocol was used to extract total RNA from each treated individual. To extract RNA, the tissue/Trizol slurry was spun at 12,000 x g for 10 minutes and the supernatant was transferred to a new tube and incubated at room temperature for 5 minutes. After adding 40 μL chloroform and mixing well, the samples were incubated for 2 minutes then were spun at 12,000 x g for 15 minutes. The transparent upper phase (~100 μL) was transferred to a new tube and mixed well with 100 μL ethanol (70%). After transferring to a RNeasy spin column and spinning for 30 seconds at ≥8000 x g, I used an RNA extraction kit, following the manufacturer’s protocol beginning at step 4 (RNeasy Mini Kit, Qiagen, Valencia, CA), repeating the final elution step to maximize total RNA yields.

Individual RNA extracts were systematically pooled into a total of 12 samples (3 replicates X 2 treatments x 2 population groups) and sent for mRNA sequencing. RNA extracts for 4 or 5 individuals were systematically pooled so that individuals from all three treatment/egression days were represented in a single sample (excluding extracts with low yield or contamination) and contained a total of 20 ng of RNA extract. Systematically pooling samples in this manner was intended to decrease between sample variance attributed to treatment/egression day (at the expense of within sample variance). Pooled samples were sent to the University of Minnesota Genomics Center where mRNA was isolated and libraries were prepped with the Illumina TruSeq RNA library prep kit (Illumina, San Diego, CA), then sequenced in a full lane on a HiSeq2500 Illumina platform to produce 50bp, paired end reads.

Differential expression analysis of mRNA sequences

Quality control, trimming, and alignment of reads preceded differential expression analysis and was conducted using tools in the online Galaxy platform (Afgan *et al.* 2016). FastQC (Andrews 2010), a quality control tool, identified high amounts of duplication (taken into account with read mapping and counting, below) but did not identify issues with poor average read quality, adapter contamination, or rRNA contamination. The sequencing facility removed adapter sequences from reads. Reads shorter than 36bp in length or with an average read quality of less than 30 were filtered out with Trimmomatic (Bolger *et al.* 2014). HISAT2 (Kim *et al.* 2015) mapped

unique, trimmed, and filtered reads to the *R. zephyria* genome (GenBank assembly accession: GCA_001687245.1).

R-packages constructed for sequence data were implemented to count reads and perform differential expression analysis. Reads that mapped to only one unique location in the genome were counted by gene using the GenomicAlignments package (Lawrence *et al.* 2013), generating a count matrix of reads per gene for each sample using gene models provided with the *R. zephyria* reference assembly. The DESeq2 package (Love *et al.* 2014) applied a negative binomial generalized linear model to the counts with group (the four treatment x population combos) as a factor, to identify genes differentially expressed among the four groups. Using model contrasts, I isolated treatment effects within each population (Whatcom high humidity vs. Whatcom low humidity; Yakima high humidity vs. Yakima low humidity) and population effects, independent of treatment (Whatcom high humidity vs. Yakima high humidity; Whatcom low humidity vs. Yakima low humidity). For contrasts highlighting treatment effects, genes were over or under expressed in the low treatment relative to the high treatment (e.g. positive log2 fold changes indicate upregulation in Whatcom low humidity compared to Whatcom high humidity). For contrasts highlighting population effects, genes were over or under expressed in Yakima relative to Whatcom (e.g. positive log2 fold changes indicate upregulation in Yakima high humidity compared to Whatcom high humidity). Significantly differentially expressed genes (Benjamini-Hochberg adjusted p-value < 0.1) were divided into up regulated (positive log2 fold change) and down regulated genes (negative log2 fold change).

Significantly up and down regulated genes were functionally annotated against *Drosophila melanogaster* with DAVID (Huang *et al.* 2008, 2009), which also conducted enrichment analysis. Differentially expressed genes for each group were Blasted against *D. melanogaster* to get the corresponding *D. melanogaster* proteins (top match; only included hits with e-value < 0.00001), which were then converted to genes using FlyBases's online conversion tool (http://flybase.org/static_pages/downloads/IDConv.html) to produce gene lists with gene IDs recognizable by DAVID. These eight gene lists (up and down regulation for each of four contrasts: Whatcom low vs. Whatcom high, Yakima low vs. Yakima high, Whatcom low vs. Yakima low, Whatcom high vs. Yakima high) were uploaded to DAVID for annotation referencing GO, Interpro, UniPro, COG, and SMART databases. The genes differentially expressed between humidity treatments of Whatcom larvae (Whatcom low vs. Whatcom high) were annotated in DAVID without enrichment tests because there were too few genes to conduct enrichment analysis (Huang *et al.* 2008), but genes from all other contrasts underwent enrichment analysis and were grouped into clusters of terms comprised of similar genes and presumed function with medium stringency.

RESULTS

Phenotypic study

Overview

The proportion of diapausers and pupal weight loss were used to compare patterns of diapause regulation and desiccation resistance, respectively, in invasive *R. pomonella* and native *R. zephyria* and to determine the effect of drought stress on diapause regulation. Diapause regulation did not respond to drought treatment as hypothesized, but there were differences between the two species and among host races. Black hawthorn flies (invasive, *R. pomonella*) regulated diapause differently from apple and ornamental hawthorn flies (invasive, *R. pomonella*), partially supporting the hypothesis that the proportion of diapausers would increase with average pre-winter length. Direct development was not observed in native *R. zephyria*, therefore diapause regulation did not differ between drought resistant and drought susceptible populations of the native fly as hypothesized. Patterns of desiccation were consistent with Hill (2016), but ornamental hawthorn flies were not the most drought susceptible *R. pomonella* host race as hypothesized. The hypothesis that the proportion of diapausers would be greater as desiccation resistance increased was only partially supported in invasive *R. pomonella* and rejected in native *R. zephyria.*

Diapause regulation

The proportion of diapausers 65 days post-pupariation were compared with a logistic regression to determine how host and treatment affected diapause regulation in invasive *R. pomonella*. Proportions of diapausers were best explained by a model with host and treatment (Table 4), but only host had a significant effect (Wald test, X^2=85.9, df=2, p<<0.01). The proportion of diapausing black hawthorn flies was 11% greater than in apple flies (Wald test, X^2=37.9, df=1, p<<0.01) and 13% greater than in ornamental hawthorn flies (Wald test, X^2=36.9, df=1, p<<0.01) to go into diapause; apple and ornamental hawthorn flies were equally likely to go into diapause (Wald test, X^2=0.025, df=1, p=0.88) (Figure 4). My hypothesis that the proportion of diapausers would increase with pre-winter length was only partially supported because while the earliest fruiting host race had the greatest proportion of diapausers, the latest fruiting host race, ornamental hawthorns flies, did not have lowest proportions of diapausers. The absence of a treatment effect did not support my hypothesis that drought stress would increase the proportion of diapausers.

Neither population nor drought stress affected the proportions of diapausers in native *R. zephyria* as zero non-diapausing flies were observed in 631 dissected pupae (Table 2). Consequently, the hypothesis that drought resistant Yakima flies would have more diapausers than drought susceptible Whatcom flies was rejected, as was my hypothesis that drought stress would lead to an increase in the proportion of diapausers in *R. zephyria*. It is unlikely that I failed to detect direct developers among snowberry pupae given my sample size. Assuming that *R. zephyria* flies do, in fact,

Table 4. Akaike Information Criterion (AIC) for binomial regression on diapause status of invasive *R. pomonella* with effects of host (black hawthorn, apple, or ornamental hawthorn), initial treatment (High or Low VPD), start date (relative to the date the first fly of each host was treated), initial pupal weight (W_i), and incubator. Bolded model indicates the best model (with the lowest AIC).

Model	AIC
Diapause ~ 1	182.64
Diapause ~ Host + Treatment	**174.44**
Diapause ~ Host x Treatment	177.45
Diapause ~ Host + Treatment + W_i	176.35
Diapause ~ Host + Treatment + Start date	175.56
Diapause ~ Host + Treatment + Incubator	177.62

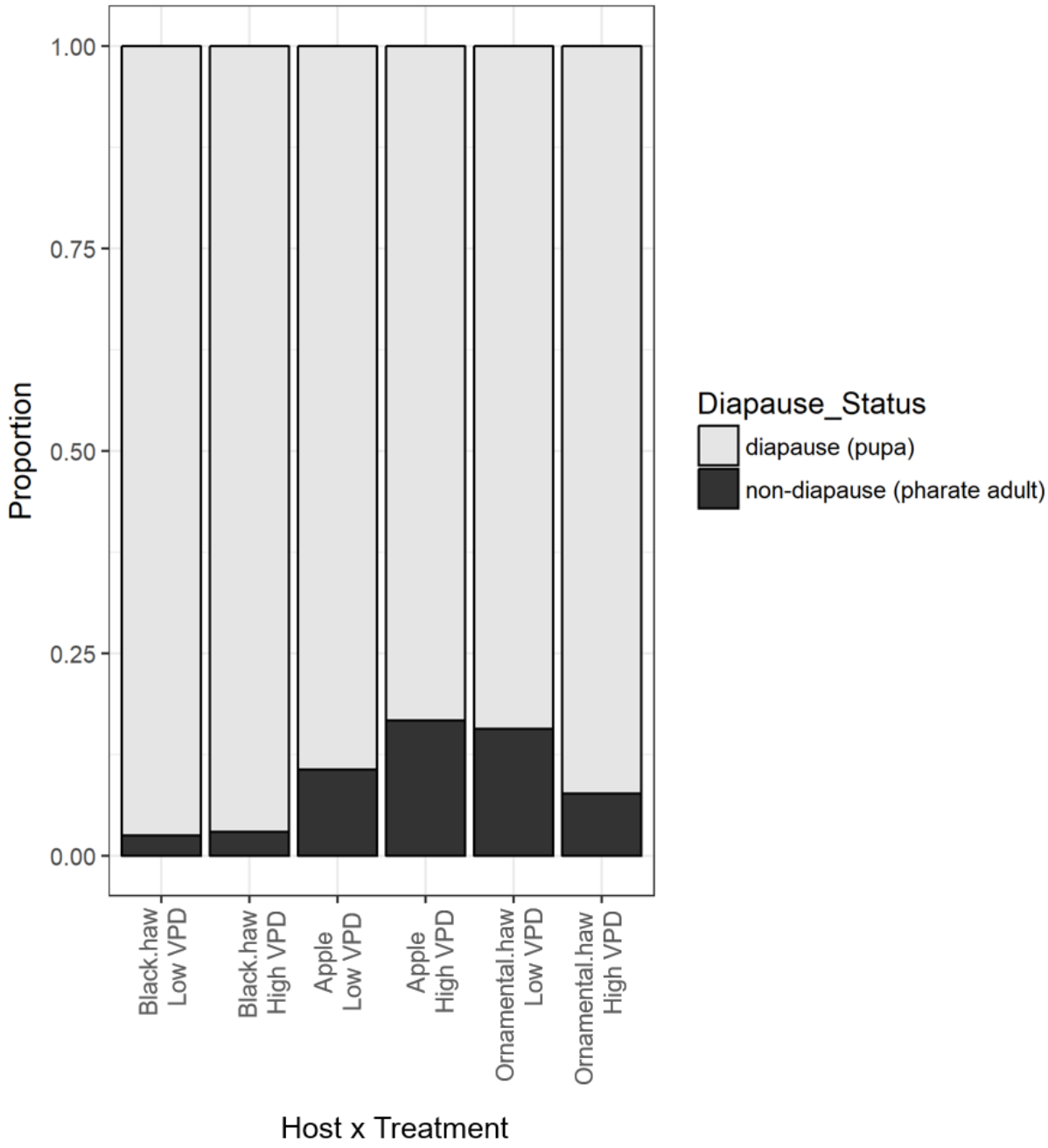

Figure 4. Proportion of invasive *R. pomonella* pupae in diapause or direct development (non-diapause) as determined by dissection 65 days post-pupariation for black hawthorn (black.haw), apple, and ornamental hawthorn (ornamental.haw) host races under an initial 4 day treatment in low VPD (~0.27 kPa) or high VPD conditions (~1.1 kPa).

have direct developers at low rates like those observed in black hawthorn flies (2.8% of the population), there was a 0.0004% chance of failing to detect directly developing *R. zephyria* flies using a binomial distribution, with a corresponding probability of 0.028 (Table 2). This does not mean that native *R. zephyria* lacks direct development, but does suggest that if direct development occurs in this species, is less common than in invasive *R. pomonella*.

Desiccation resistance in invasive *R. pomonella*

Patterns of desiccation resistance in host races of invasive *R. pomonella* were monitored with pupal weight loss to test the hypotheses that 1) desiccation resistance would be greater among host races that have longer average pre-winter periods (greatest in black hawthorn flies, intermediate in apple flies, and lowest in ornamental hawthorn flies) and 2) populations with greater desiccation resistance would have greater proportions of diapausers. Both short term (4 day) and long term (65 day) desiccation resistance were used to evaluate these hypotheses.

Pupal weight loss 4 days post-pupariation. ΔW_4 was best explained by a model that included host (LMM, $F_{2,958}=796.3$, $p<0.01$), treatment (LMM, $F_{1,2}=948.1$, $p<0.01$), W_i (LMM, $F_{1,958}=456.9$, $p<0.01$), host x treatment (LMM, $F_{2,958}=47.3$, $p<0.01$) and treatment x W_i (LMM, $F_{1,958}=52.2$, $p<0.01$) interactions, as well as a non-significant host x W_i interaction (Table 5). Among hosts in the low VPD treatment, black hawthorn and ornamental hawthorn flies lost similar amounts of weight but lost less weight than apple flies (Figure 5; Figure 6). Generally, the high VPD treatment resulted in more weight loss than in the low VPD treatment, but the magnitude of this treatment effect varied among host races (Figure 5). Black hawthorn fly weights did not vary between treatments (Figure 5). Both apple and ornamental hawthorn fly lost more weight in the high VPD treatment than the low VPD treatment by 1.7 and 2.2 times, respectively (Figure 5; Figure 6). Pupae of all host races in the high VPD

Table 5. Akaike Information Criterion (AIC) and conditional R^2 for linear-mixed effect models on invasive R. pomonella pupal weight loss 4 days (ΔW_4) and 65 days (ΔW_{65}) with host (black hawthorn, apple, or ornamental hawthorn), initial treatment (high or low VPD), and treatment start date as fixed effects, initial pupal weight (W_i) as a covariate. All models included incubator as a random effect (not shown). Bolded models indicate the best models (lowest AIC).

Model	AIC	R^2
$\Delta W_4 \sim 1$	3310.41	0.22
$\Delta W_4 \sim$ Host x Treatment	2561.67	0.64
$\Delta W_4 \sim$ Host x Treatment x W_i	2156.77	0.77
$\Delta W_4 \sim$ Host x Treatment x W_i + Start date	2156.94	0.77
$\Delta W_4 \sim$ Host x Treatment + Host: W_i + Treatment: W_i	**2155.87**	**0.77**
$\Delta W_{65} \sim 1$	4209.79	0.01
$\Delta W_{65} \sim$ Host x Treatment	3479.57	0.53
$\Delta W_{65} \sim$ Host x Treatment x W_i	2734.12	0.79
ΔW_{65} Host x Treatment x W_i + Start date	2734.81	0.79
$\Delta W_{65} \sim$ Host x Treatment + Host: W_i + Treatment: W_i	**2730.78**	**0.79**

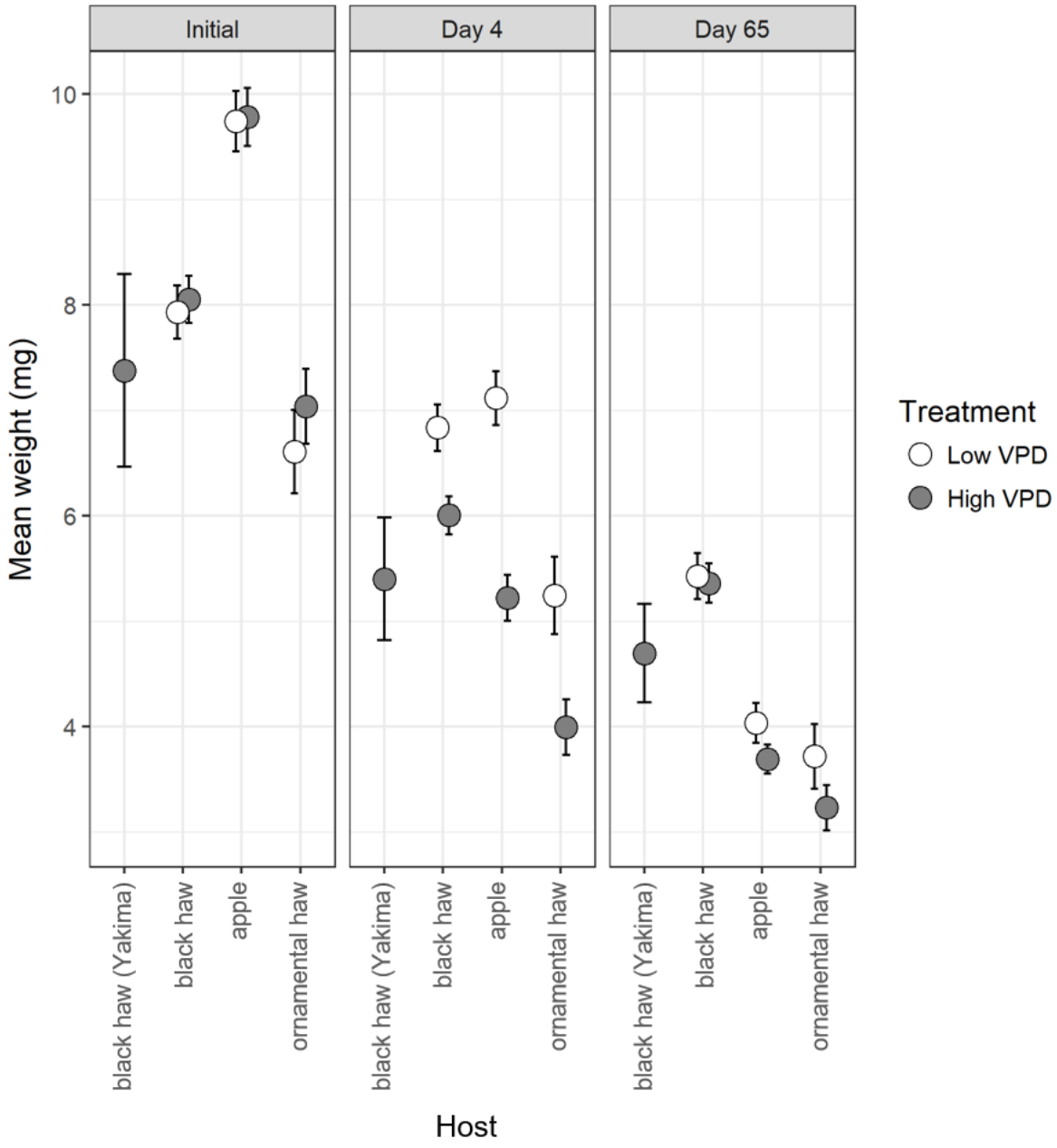

Figure 5. Mean weight in milligrams for three host races of *R. pomonella* pupae before treatment (Initial), 4 days post-pupariation following an initial low (~0.27 kPa) or high (~1.1 kPa) vapor pressure deficit (VPD) treatment, and at 65 days post-pupariation after an additional 61 days in ~0.35 kPa VPD. Error bars estimate a 95% confidence interval of the mean. Yakima black hawthorn pupae (black.haw.yak) were not included in formal analyses.

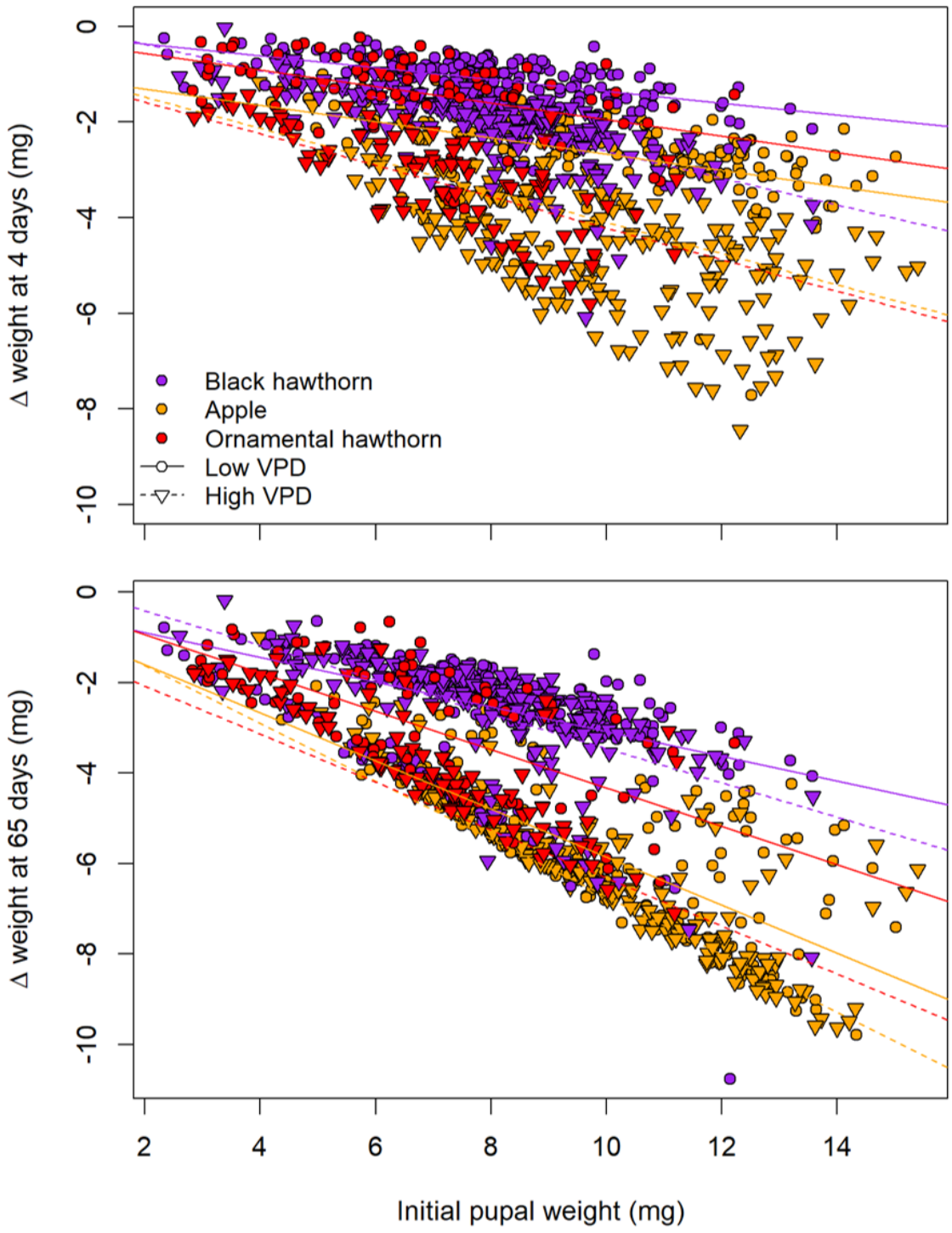

Figure 6. Weight loss of invasive *R. pomonella* pupae at 4 days (top panel) or 65 days post-pupariation (bottom panel) vs. initial pupal weights in milligrams. Fitted linear mixed-effect models account for 88% of variation in 4 day weights and 69% of variation in 65 day weights (see Table 5).

treatment lost 1.25 times more weight per mg of W_i than pupae in the low VPD treatment (Figure 6).

Pupal weight loss 65 days post-pupariation. ΔW_{65} was best explained by the model that included host (LMM, $F_{2,958}$=1182.2, p<0.01), treatment, W_i (LMM, $F_{1,958}$=1064.5, p<0.01), host x treatment (LMM, $F_{2,938}$=4.9, p<0.01), host x W_i (LMM, $F_{2,958}$=32.0, p<0.01) and treatment x W_i interactions (LMM, $F_{1,958}$=13.5, p<0.01) (Table 5). Overall, black hawthorn pupae lost 55% less weight than apple pupae and 23% less than ornamental hawthorn pupae (Figure 6). Ornamental hawthorn pupae were the only host race for which the initial treatment affected ΔW_{65} (LMM, df=938, t= -3.7, p<0.01), losing 1.3 times more weight in the high VPD treatment than in the low VPD treatment (Figure 5, Figure 6). However, all pupae in the high VPD treatment averaged 15% more weight lost per mg of W_i than pupae in the low VPD treatment (Figure 6). Black hawthorn pupae maintained 37% more weight per mg of W_i than apple pupae and 22% more weight per mg of W_i than ornamental hawthorn pupae (Figure 6). The host effects at both 4 and 65 days support patterns of desiccation resistance between black hawthorn and apple flies observed by Hill (2016), but my hypothesis that ornamental hawthorns would have the lowest desiccation resistance of the three host races was not supported.

Black hawthorn pupae from Yakima were not included in formal analyses due to low infestation and sample size, but the few samples treated in the high VPD treatment seem to be comparable to their western counterparts with similar weights initially (Table 3) and 4 and 65 days post-pupariation (Figure 5).

Desiccation resistance in native *R. zephyria*

Patterns of desiccation resistance between populations of native *R. zephyria* were compared with pupal weight loss to test the hypothesis that populations with greater desiccation resistance would have greater proportions of diapausers and to validate patterns of desiccation resistance observed in Hill (2016). Both short term (4 day) and long term (65 day) desiccation resistance were used to evaluate this hypothesis. Additionally, dry weights of pupae were used to explore differences in metabolic stores between populations and treatments.

Pupal weight loss 4 days post-pupariation. ΔW_4 in native *R. zephyria* was best explained by the model that included population (LMM, $F_{1,801}$=8.8, p<0.01), treatment, W_i (LMM, $F_{1,801}$=119.1, p<0.01), and a non-significant population x treatment interaction (Table 6). Overall, Yakima pupae lost 9% less weight than Whatcom pupae, relative to initial weights in each population(Figure 7).

Pupal weight loss 65 days post-pupariation. ΔW_{65} in native *R. zephyria* was best explained by the model that included population (LMM, $F_{1,800}$=8.8, p<0.01), treatment, W_i (LMM, $F_{1,800}$=220.3, p<0.01), population x treatment (not significant), and treatment x W_i (LMM, $F_{1,800}$=14.6, p<0.01) interactions (Table 6). Overall, Yakima pupae lost 10% less weight than Whatcom pupae (Figure 7). Counterintuitively, pupae in the high VPD treatment lost 17% less weight per mg of W_i than pupae in the low VPD treatment. The population effects at both 4 and 65 days support patterns of desiccation resistance between Whatcom and Yakima pupae observed by Hill (2016),

Table 6. Akaike Information Criterion (AIC) and conditional R^2 for linear-mixed effect models on native *R. zephyria* pupal weight loss at 4 days (ΔW_4), 65 days (ΔW_{65}), and dry weights (W_{dry}) with population (Whatcom or Yakima) and initial treatment (high or low VPD) as fixed effects, initial pupal weight (W_i) as a covariate. All models included box as a random effect (not shown). Bolded models indicate the best models (lowest AIC).

Model	AIC	R^2
$\Delta W_4 \sim 1$	2026.32	0.10
$\Delta W_4 \sim$ Population x Treatment	2019.14	0.11
$\Delta W_4 \sim$ Population x Treatment x W_i	1913.59	0.24
$\Delta W_4 \sim$ Population x Treatment + W_i	**1911.15**	**0.24**
$\Delta W_{65} \sim 1$	2340.85	0.07
$\Delta W_{65} \sim$ Population x Treatment	2334.11	0.08
$\Delta W_{65} \sim$ Population x Treatment x W_i	2132.18	0.29
$\Delta W_{65} \sim$ Population x Treatment + Location: W_i	2145.04	0.28
$\Delta W_{65} \sim$ Population x Treatment + Treatment: W_i	**2131.26**	**0.29**
$W_{dry} \sim 1$	1682.44	0.02
$W_{dry} \sim$ Population x Treatment	1662.82	0.06
$W_{dry} \sim$ Population x Treatment x W_i	**894.28**	**0.72**
$W_{dry} \sim$ Population x Treatment + Location: W_i + Treatment: W_i	895.30	0.72

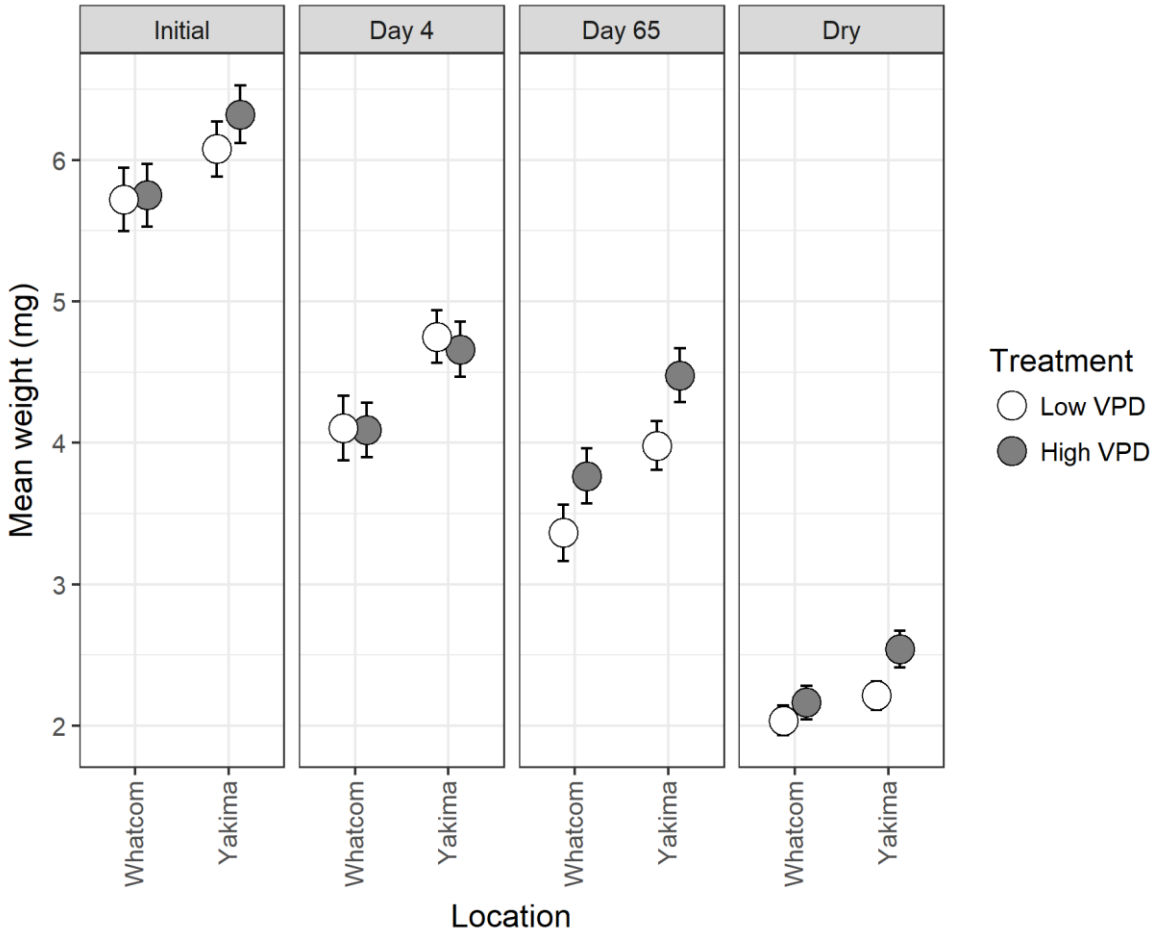

Figure 7. Mean weight in milligrams for two populations of *R. zephyria* pupae before treatment (Initial), 4 days post-pupariation following an initial low (~0.27 kPa) or high (~1.1 kPa) vapor pressure deficit (VPD) treatment, at 65 days post-pupariation after an additional 61 days in ~0.35 kPa VPD, and after drying after treatment. Error bars estimate a 95% confidence interval of the mean.

Dry weights. W_{dry} was best explained by a population x treatment x W_i model (Table 6). On average, Yakima pupae were 9% heavier than Whatcom pupae (LMM, $F_{1,602}$=51.1, p<0.01) and pupae in the high VPD treatment were 12% heavier than pupae in the low VPD treatment (LMM, $F_{1,22}$=30.9, p<0.01) (Figure 7). Dry weight covaried with W_{dry} (LMM, $F_{1,602}$=1494.4, p<0.01), but Yakima flies had 5% more dry weight per mg of W_i than Whatcom flies (LMM, $F_{1,602}$=8.2, p<0.01) and flies initially treated in the high VPD treatment had 3% more dry weight per mg of W_i than those initially in the low VPD treatment (LMM, $F_{1,602}$=7.0, p<0.01).

Expression study

Overall, gene expression in native *R. zephyria* differed more between populations than between treatments. Differences in total expression were primarily driven by population (Figure 8). In terms of the number of differentially expressed genes, population had a greater effect than humidity treatment, but there was indication of a population x environment interaction (see Appendix Table 1). On average, the difference in gene expression between populations was 5.7 times greater than it was between humidity treatments, but the effect of population was not consistent between treatments and neither was the effect of treatment between populations (Figure 9). Yakima larvae responded more to humidity treatment than Whatcom larvae, differentially expressing 8.6 times as many genes in response to low-humidity treatment as Whatcom larvae, which only had 29 genes differentially expressed between treatments (Figure 9). See Appendix for all genes differentially expressed in each contrast.

Differentially expressed genes between Yakima high and low-humidity treatment flies (Yakima high vs Yakima low) broke down into a total of 13 functional annotation clusters (six up regulated and seven down regulated in the high-humidity treatment). Five of these annotation clusters were enriched > 1.3 (Figure 10). Only one of these annotation clusters contained any terms with significant enrichment (Benjamini-Hochberg adjusted p value < 0.1), which included terms related to development and was down regulated in the low-humidity treatment.

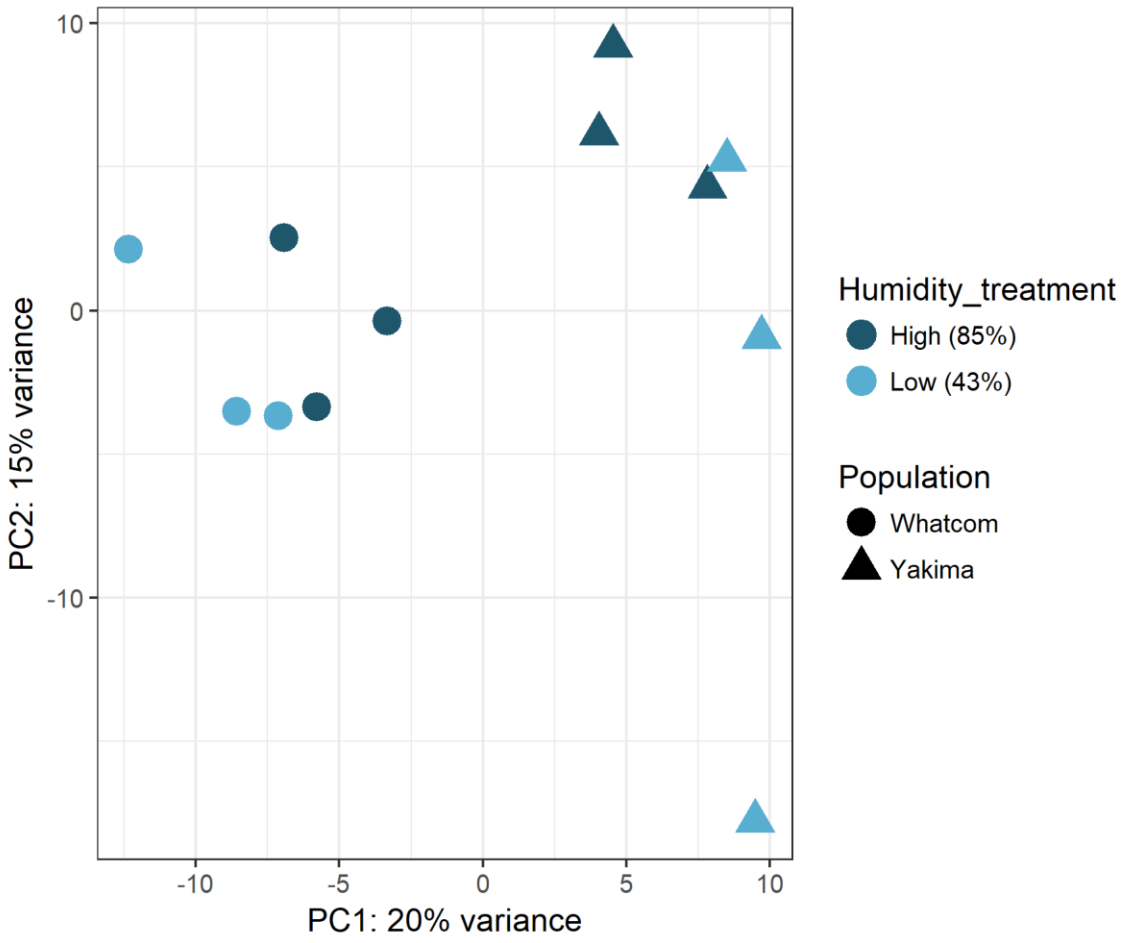

Figure 8. Principal components analysis of rlog transformed gene counts for all four relative humidity treatment x population groups of *R. zephyria* larvae. % variance on axes indicates percent variance explained by that principal component.

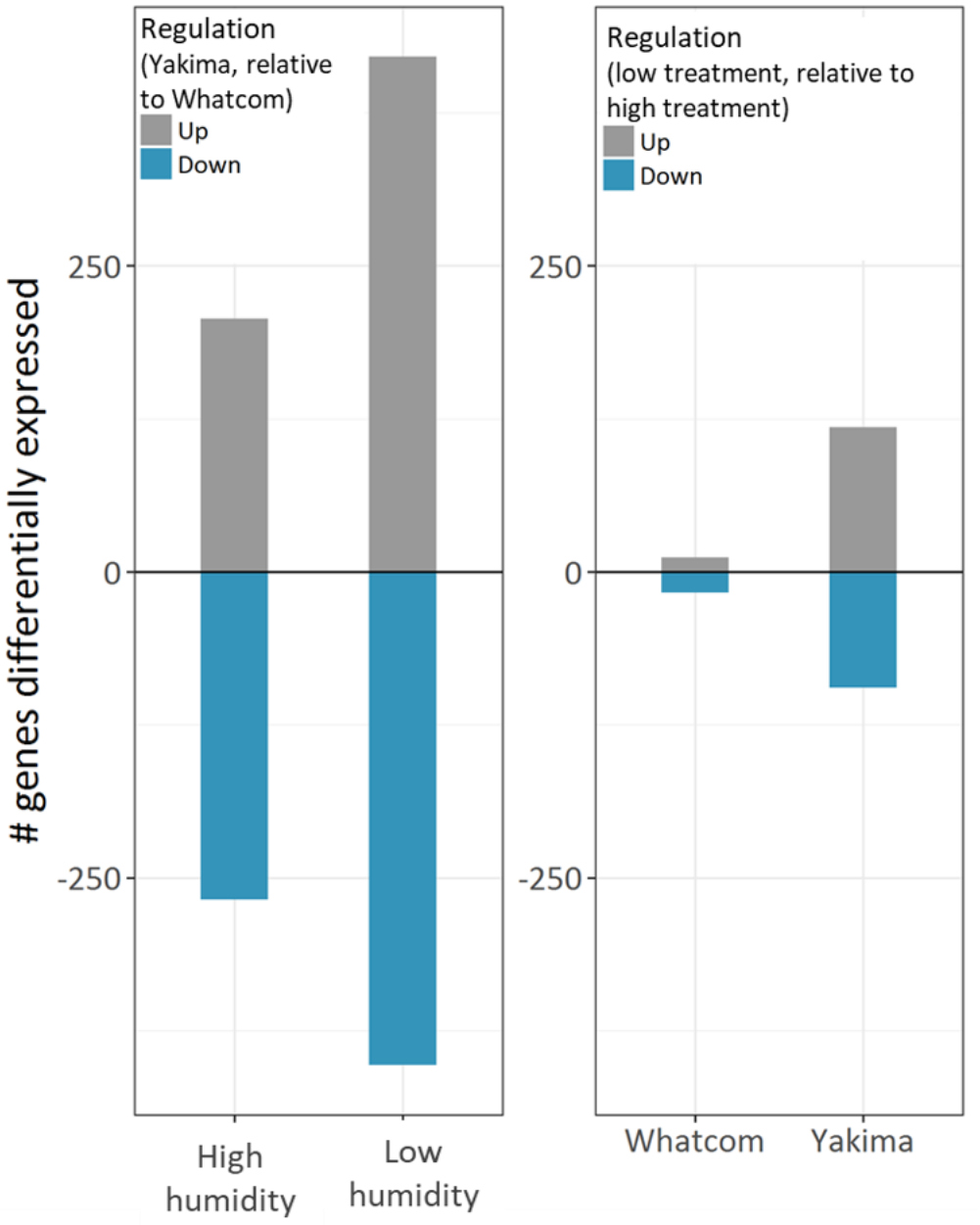

Figure 9. Number of genes significantly differentially expressed according to DESeq2 analysis between populations (left) and between treatments (right), in *R. zephyria*. Up regulation reflects genes with a positive log2 fold change and down regulation reflects a negative log2 fold change relative to Whatcom for population comparisons (left) or to the high-humidity treatment (right).

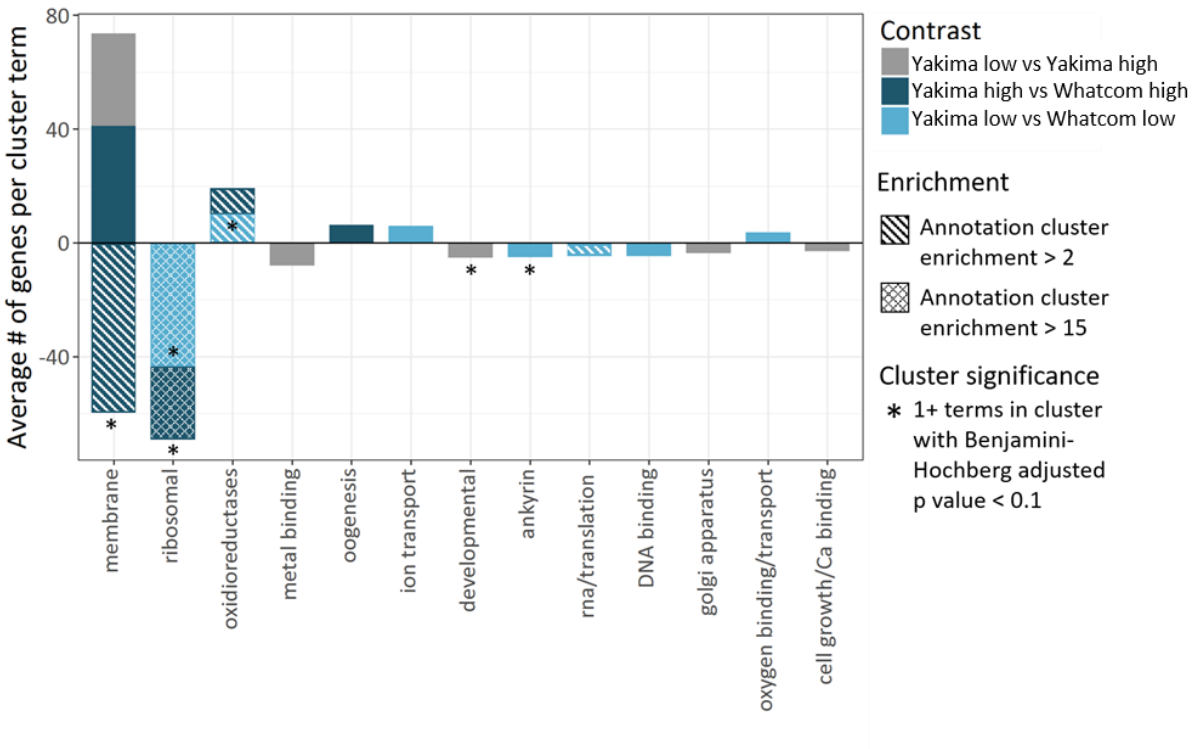

Figure 10. Average count of differentially expressed genes in a cluster of annotation terms sharing similar gene members (annotated with DAVID referencing GO, COG, UniPro, Interpro and SMART databases). Contrast indicates which two *R. zephyria* groups are being compared (population= Whatcom or Yakima, relative humidity treatment= high or low) with the second group listed as the reference group for determining up or down regulation. Positive counts represent up regulation and negative counts represent down regulation relative to the reference group. Whatcom high vs low had too few genes differentially expressed for enrichment analysis. Only annotation clusters with enrichment score ≥ 1.3 shown; strong enrichment (>2) and very strong enrichment (>15) are indicated with a hatch or cross-hatch pattern, respectively.

In the high-humidity treatment, differentially expressed genes between Whatcom and Yakima (Whatcom high vs. Yakima high) broke down into a total of 21 annotation clusters (8 up regulated and 13 down regulated in Yakima). Of these, five annotation clusters were enriched > 1.3, with clusters including membrane related terms notably enriched among both the up and down regulated genes (Figure 10). Despite annotation clusters in both up and down regulated genes, membrane-related annotation terms were only significantly enriched among the down regulated genes. The down regulated annotation cluster including ribosome associated genes was highly enriched (enrichment = 17.1) and included significantly enriched terms. Though no terms were significantly enriched, the annotation cluster including oxidioreductases was slightly more enriched than other up regulated clusters.

Some similar patterns emerged between low humidity treated Yakima and Whatcom larvae (Whatcom low vs. Yakima low). In the low-humidity treatment, differentially expressed genes between Whatcom and Yakima broke down into a total of 57 annotation clusters (26 up regulated and 31 down regulated in Yakima), but only seven of these clusters were enriched > 1.3 (Figure 10). As with the high-humidity treated larvae, the cluster including ribosome-associated genes was down regulated, substantially enriched (enrichment = 32.3), and included significantly enriched terms. RNA and translation-related genes were also down regulated with slightly greater cluster enrichment but no terms were significantly enriched. The low-humidity treated flies also up regulated oxidioreductases in Yakima larvae with greater cluster enrichment, but unlike high treated larvae, this cluster included significantly enriched terms.

DISCUSSION

Is diapause regulation a mechanism underlying desiccation resistance?

To determine if diapause regulation is a mechanism contributing to desiccation resistance in invasive *R. pomonella* and native *R. zephyria*, I tested the effect of drought stress on the proportion of diapausers within populations and compared patterns of diapause regulation to patterns of desiccation resistance. Here, I discuss how the absence of a treatment effect on diapause status suggests that greater drought stress does not necessarily lead to stronger selection for diapausers. Next, I discuss how the absence of direct development in *R. zephyria* does not support diapause regulation as a mechanism contributing to desiccation resistance in the native species. Finally, I discuss how diapause regulation in invasive *R. pomonella* can only partially account for variation in desiccation resistance among black hawthorn, apple, and ornamental hawthorn flies.

Does drought stress affect the proportion of diapausers?

To determine if drought stress affects diapause regulation, I tested for an effect of drought treatment on the proportion of diapausers within a population. Populations from both invasive *R. pomonella* and native *R. zephyria* did not respond to drought treatment; all host races of *R. pomonella* had similar proportions of diapausers after high and low drought stress as did both populations of native *R. zephyria.* The

absence of a treatment effect on the proportion of diapausers suggests that drought conditions do not directly impact diapause initiation.

How do patterns of diapause regulation compare to patterns of desiccation resistance?

For native *R. zephyria*, there was variation in desiccation resistance between pupae from western Washington and central Washington but no variation in diapause regulation between those two populations. Consistent with Hill (2016), Yakima pupae were more drought resistant than Whatcom pupae, as evidenced by short term (4 day) and long term (65 day) weight loss, yet both populations lacked direct development. The absence of direct development in *R. zephyria* is similar to other *Rhagoletis* species like *R. cerasi* and *R. mendax* that display obligate diapause (Papanastasiou *et al.* 2011) or require cold exposure to complete development (Teixeira & Polavarapu 2005a). This lack of variation in diapause regulation suggests that diapause development is not more likely to occur in drought resistant populations of *R. zephyria* than drought susceptible populations as hypothesized and cannot account for the greater desiccation resistance of pupae from arid regions of central Washington. Therefore, the results of this study do not support diapause regulation as a mechanism contributing to variation in desiccation resistance in native *R. zephyria.*

Invasive *R. pomonella*, unlike the native species, displayed variation in diapause regulation among host races. Diapause development was most common in black hawthorn flies (the host race with longest pre-winter period) as hypothesized,

but it was no more prevalent in apple flies (the host race with an intermediate pre-winter length) than it was in ornamental hawthorns (the host race with the shortest pre-winter period). Higher proportions of diapausers in black hawthorn flies than apple flies is consistent with the hypothesis that longer average pre-winter periods lead to selection for more recalcitrant diapause regulation (more and deeper diapause) (Dambroski & Feder 2007). However, diapause regulation in ornamental hawthorn flies does not fit with this hypothesis. As the host race with the shortest pre-winter period, diapause regulation should be under weaker selection than in apple flies if exposure to warmer, drier summer conditions is the primary selection pressure; the fact that direct development was not more common among ornamental hawthorns suggests that other factors besides pre-winter length contribute to selection on diapause regulation in the late-fruiting host race.

These patterns in diapause regulation were partially consistent with expected patterns of desiccation resistance among the host races of invasive *R. pomonella*. The host race with the longest pre-winter period, black hawthorns, did have the greatest desiccation resistance at both 4 and 65 days post-pupariation and the lowest proportion of direct developers, as hypothesized, suggesting diapause regulation could account for enhanced desiccation resistance in black hawthorn flies. However, desiccation resistance did not decrease among host races as average pre-winter length decreased; despite having the shortest pre-winter length, ornamental hawthorn flies exhibited greater desiccation resistance than apple flies, which have an intermediate pre-winter length. More importantly, the enhanced desiccation resistance of ornamental hawthorn flies relative to apple flies was not accompanied by an

increase in the proportion of diapausers. Given that each host race egressed onto different substrates (see Methods), it is possible that egression environment impacted patterns of desiccation resistance among the host races. However, even given this caveat and potential overestimation of the proportions of diapausers in ornamental hawthorns (see Methods), the relative difference in desiccation resistance of apple and ornamental hawthorn flies cannot be explained by differences in the proportion fo diapausing flies.

Even though diapause could still be advantageous for overall water balance, water conservation via suppressed metabolisms achieved specifically through diapause is likely not ecologically relevant in achieving desiccation resistance. There was no indication in long-term pupal weight loss that diapausers retained significantly more weight than direct developers (mean ΔW_{65} of diapausers within 95% confidence interval of mean ΔW_{65} of direct developers within each host race; data not shown). Most pupal weight loss occurs in the first eight days post-pupariation (J. Hill, unpublished data) and pupae of both species exhibit different levels of desiccation resistance within four days post-pupariation. However, respiration rates between pupae undergoing diapause development and direct development are not distinguishable until about seven days post-pupariation in *R. pomonella* (Ragland et al. 2009), therefore, diapause regulation likely does not underlie differences in desiccation resistance during this drought sensitive period in early pupal development. Water balance demands could differ between diapausing and directly developing individuals before detectable differences in respiration, but this seems unlikely.

Potential mechanisms of desiccation resistance

Here, I consider data from both the phenotypic study and expression study to discuss possible mechanisms besides diapause regulation that could contribute to desiccation resistance and warrant future consideration. First, metabolism suppression and limited gas exchange (independent of diapause) are still possible mechanisms underlying variation in desiccation resistance. Second, results from the phenotypic study suggest pupal size could account for some variation in desiccation resistance in native *R. zephyria* (but not invasive *R. pomonella*). Third, the expression study suggests that cuticular hydrocarbons might differ between drought resistant and drought susceptible populations. Finally, the expression study suggests that larvae from drought resistant populations might develop slower than larvae from drought susceptible populations and, although slower development could occur for many reasons, it has been noted in drought resistant populations of other fly species.

Metabolism suppression and limited gas exchange

This study does not eliminate metabolism suppression or limited gas exchange as mechanisms involved in minimizing active water loss but rather suggests that they are not moderated by diapause regulation. Higher dry weights in drought resistant Yakima pupae (native, *R. zephyria*) compared to drought susceptible Whatcom pupae (native, *R. zephyria*) suggests that drought resistant flies might use fewer metabolites and other storage compounds after pupariation; sustaining lower metabolisms could account for these differences in dry weights. Drought adapted populations could

sustain lower metabolisms to limit active water loss, which is not uncommon in other taxa. A multi-species analysis of *Drosophila* found that species exhibiting greater desiccation resistance have lower metabolisms and gas exchange (Hoffmann & Parsons 1989; Gibbs *et al.* 2003), although reduced respiration does not completely account for increased desiccation resistance in *Drosophila* (reviewed in Chown & Gaston 1999). Even given similar metabolic rates, many xeric insect species lose less respiratory water than mesic species (Addo-Bediako *et al.* 2001). Multiple orders of insects manipulate gas exchange cycles to reduce respiratory water loss, for example, by switching from continuous to intermittent gas exchange (Matthews & Terblanche 2015). Drought resistant pupae of either *Rhagoletis* species could sustain lower metabolisms or manipulate gas exchange cycles but respirometric data is required to determine if this is the case and, if so, to what degree it contributes to desiccation resistance.

Pupal size

Initial pupal size can account for some variation in desiccation resistance between populations of native *R. zephyria* but not invasive *R. pomonella*. Under a simple geometric model, larger pupae that have a smaller surface area to volume ratio should be more drought resistant than smaller pupae. In native *R. zephyria*, proportional weight loss was weakly, negatively correlated with initial pupal weights (Figure 11), suggesting that larger SA:V ratios are associated with more water loss relative to initial weight, thus supporting this hypothesis. Furthermore, Yakima pupae

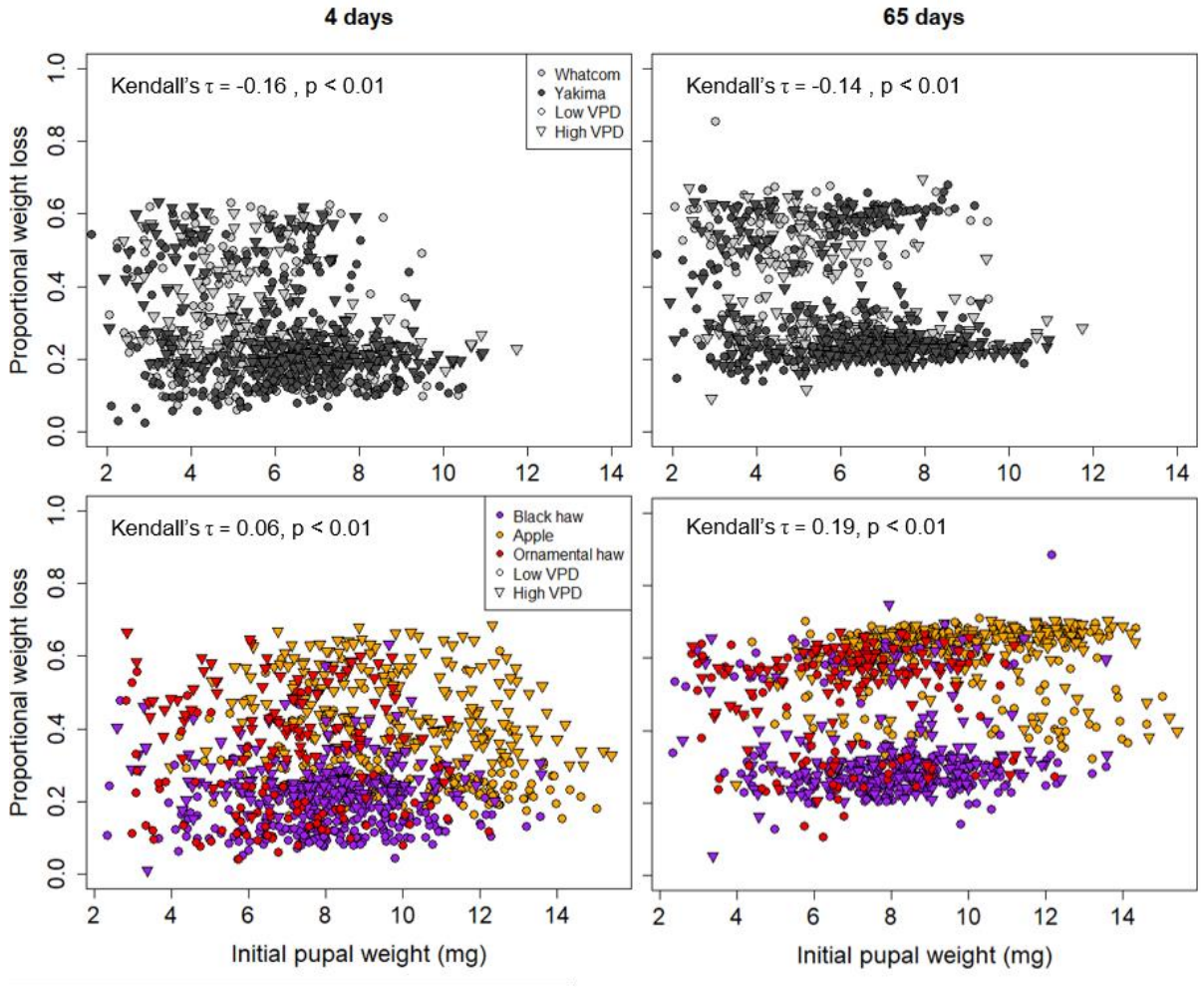

Figure 11. Proportional weight loss vs. initial pupal weight in milligrams at 4 days post-pupariation (left panels) or 65 days post-pupariation (right panels) for two populations of native *R. zephyria* (top panels) and three host races of invasive *R. pomonella*.

were, on average, heavier initially and more drought resistant than Whatcom pupae. Larger pupal size could be selected for in drought resistant populations of the native fly to limit SA:V ratios and the challenges with passive water loss that are associated with small sizes. However, this hypothesis is not supported in host races of invasive *R. pomonella*. In invasive *R. pomonella*, proportional weight loss was weakly, positively correlated with initial pupal weights (Figure 11), suggesting that larger SA:V ratios are not associated with more water loss relative to initial weight as expected. Apple pupae were initially the largest, yet both black hawthorn and ornamental hawthorn pupae exhibited greater desiccation resistance despite smaller initial sizes. Furthermore, the amount of weight lost per mg of initial weight suggests that black hawthorn pupae have intrinsically greater desiccation resistance than apple and ornamental hawthorn pupae that must be achieved through other mechanisms.

Cuticular hydrocarbons

Cuticular hydrocarbons (CHCs) have an established role in regulating passive water loss in insects by dictating cuticle permeability, thus serving as a waterproofing layer. The physical properties of the hydrocarbon molecules composing the cuticle determine cuticular permeability; longer chains, greater saturation, and less branching correspond with lower permeability and decreased water loss (reviewed in Gibbs, 1998). CHCs are derived from fatty acyl precursors and an insect-specific oxidative decarbonylase belonging to a P450 family is required in the last step of biosynthesis (Qiu *et al.* 2012). Without the gene for this oxidioreductases (CYP4G1), *D. melanogaster* is very sensitive to desiccation stress due to major shifts in CHC composition that are characterized by reduction in long-chain CHCs (Qiu *et al.* 2012).

Transcriptional differences between populations of native *R. zephyria* in the expression study suggest that differences in cuticular hydrocarbons (CHCs) might account for variation in desiccation resistance. Oxidioreductases, the category of enzyme into which CYP4G1 falls, were upregulated in Yakima larvae compared to Whatcom larvae. Furthermore, the homolog to CYP4G1 was upregulated in Yakima flies treated in low humidity. This suggests that increased desiccation resistance in Yakima flies could be achieved by increased larval investment in synthesis of CHCs, especially when exposed to drought conditions. The established role of CHCs in desiccation resistance in insects as well as the upregulation of genes involved in CHC synthesis (oxidioreductase and membrane genes) in drought resistant populations warrant further investigation of the involvement of CHCs' in desiccation resistance of

Rhagoletis. A portion of treated samples from the phenotypic study (those puparia not dissected, Table 2) were saved for future analysis with GCMS to determine CHC profiles. Future studies should also incorporate CHC profiles of *R. pomonella* to evaluate the role of CHCs in desiccation resistance of both species.

Development speed

The most striking difference in gene expression among *R. zephyria* larvae was the downregulation of ribosome associated genes in the drought resistant population regardless of humidity treatment, which suggests possible differences in development speeds. The increase in transcripts for ribosome associated genes suggests that Whatcom larvae have greater translational demands 3 hours after egression than Yakima larvae. One possible explanation for differences in translational is differences in development speed between the two populations; while this cannot be directly attributed to adaptation to environmental conditions in this study (see below), there is some precedence for drought adaption co-occurring with delays in developmental timing. In *D. melanogaster*, desiccation of larvae induces developmental heterochrony that delays larval development, extending the amount of time spent in the larval stage (Thorat *et al.* 2016). Selection for enhanced desiccation resistance in the tephritid *Anastrepha ludens* is accompanied by longer development times for pupae and delayed sexual maturation in adults (Tejeda *et al.* 2016). Yakima larvae could be developing slower or slightly delaying development compared to Whatcom larvae; this

appears to be the case as Yakima larvae take longer on average to pupariate after egression than Whatcom larvae (D. Schwarz, personal communication).

Further investigation is required to determine if upregulation of ribosome associated genes is a result of development speed and if slower development is adaptive to drought conditions. A follow up time-course RNAseq experiment that includes additional phenotypic data supporting differences in development speeds such as time spent wandering or time to pupariation could address if there is a canalized difference in development speeds between the two populations. If there is a difference in development speeds between drought resistant and susceptible populations, standardizing gene expression to the same developmental stage (rather than time post-egression) could address whether ribosome associated gene expression is attributed to development timing or a consistent difference between populations regardless of developmental stage. The advantages of delayed development under drought stress are unclear and understudied but one possibility is that slower development allows larvae to be less active and conserve water (as observed in drought resistant *Drosophila* adults (Gibbs *et al.* 2003)).

There are some caveats to interpreting results from the expression study. Differences in gene expression between populations here do not necessarily represent drought adaptation as some non-selective factors could account for transcriptional differences between drought resistant and drought susceptible populations. Primarily, differences in host quality and chemistry were not evaluated, but if present between Whatcom and Yakima snowberries, could influence larval gene

expression. In future experiments, the potential effect of host (in terms of larval diet and environment) on larval gene expression could be addressed by quantifying host quality or by rearing F1s in similar fruits. The use of F1s could also address the possibility that maternal effects account for transcriptional differences between populations. However, because all flies for a given population presumably had similar larval diets within the host fruits, differential gene expression between treatments within a population was likely caused by desiccation stress (e.g. upregulation of membrane genes and downregulation of developmental genes in Yakima flies in low humidity).

Is desiccation resistance a plastic or canalized trait?

Transcriptional differences between Yakima and Whatcom larvae (native, *R. zephyria*) were far more prevalent than either population's transcriptional response to treatment, supporting the hypothesis that transcription is largely canalized in native *R. zephyria*. Transcriptional differences between populations can likely be attributed to differences between their respective home environments, be it abiotic or biotic conditions (such as egg/larval environments in host fruits). While these transcriptional differences might not stem directly from drought adaptation (see above), these populations differ most notably in the aridity of their respective environments, which suggests that these differences might be locally adapted and that further investigation into the canalization of desiccation resistance is warranted.

In addition to possible canalization of desiccation resistance between populations of native *R. zephyria*, both phenotypic and expression data also support the hypothesis that desiccation resistance is plastic, suggesting desiccation resistance is impacted by a genotype x environment interaction. Weights after 65 days suggest that pupae of both populations respond plastically to, or at least are conditioned by, drought conditions during the first few days as pupae. Specifically, both Whatcom and Yakima pupae in the high VPD treatment maintained more weight per mg of initial pupal weight than pupae in the low VPD treatment. These results imply that initial exposure to desiccation stress potentially induced greater desiccation resistance in native *R. zephyria* pupae, allowing them to maintain more weight than unstressed counterparts when returned to neutral conditions. However, conditioning under lab imposed treatments cannot explain greater desiccation resistance in Yakima flies than Whatcom flies. Gene expression suggests that the extent of plasticity in desiccation resistance is not equal between the two populations. Yakima larvae exhibit – likely adaptive – phenotypic plasticity to desiccation stress, as evidenced by more genes differentially expressed as a result of treatment in Yakima larvae than Whatcom larvae. Differences in plasticity between populations highlight the possibility that desiccation resistance is impacted by genotype x environment interaction, such as that observed in salinity tolerance of *Eurytemora affinis* (Lee & Petersen 2002).

Desiccation resistance in invasive *R. pomonella* could be canalized or plastic and a couple caveats impede interpretation of the extent of each. Here, differences in larval environment (due to host fruit) and egression environment (due to methodology) are possible confounding factors affecting desiccation resistance among host races

that make it difficult to interpret whether variation in desiccation resistance among host races is canalized or attributed to differences in plasticity among host races. None of the host races exhibited the conditioning of pupae following high VPD treatment that was observed in native *R. zephyria*, suggesting that desiccation resistance in *R. pomonella,* if plastic, is not as plastic as it is in the native species. Furthermore, black hawthorn flies from arid climates showed no evidence of greater desiccation resistance (relative to black hawthorn flies from humid climates), suggesting that, if plastic, plasticity in desiccation resistance in this host race is not adaptive to drought conditions.

Whether due to plasticity or canalization, host fitness tradeoffs that are associated with the suite of physiological changes needed to adapt to a new host (Ragland *et al.* 2011) are important considerations concerning differences in desiccation resistance observed among host races. Host-specific traits are likely under stronger selection than desiccation resistance when climates are similar (as were the *R. pomonella* flies used in this study). It is possible that desiccation resistance is genetically correlated with host-specific traits, in which case drought adaptation in invasive *R. pomonella* could be constrained by pleiotropy or correlational selection (Walsh & Blows 2009). Even with gene flow between the host races, such genetic correlations could prevent high desiccation resistance from being passed from black hawthorn flies to apple flies.

Conclusions

Variation in desiccation resistance in both native *R. zephyria* and invasive *R. pomonella* is likely achieved through multiple mechanisms and stems from intrinsic differences among populations. This study identifies cuticular hydrocarbons as a possible mechanism involved in limiting passive water loss in *Rhagoletis* that warrants further investigation. While diapause regulation might play a role in desiccation resistance of black hawthorn flies (invasive, *R. pomonella*), it does not account for variable desiccation resistance between apple and ornamental hawthorn flies (invasive, *R. pomonella*), nor does it account for variable desiccation resistance in native *R. zephyria.* For native *R. zephyria,* differences in desiccation resistance among populations are likely driven by adaptation to local drought conditions, but for invasive *R. pomonella*, they are more likely driven by host specific adaptations. Currently, apple infesting *R. pomonella* exhibit limited desiccation resistance making them ill-equipped to spread into apple growing regions in central Washington. The nuances and sources of variation in desiccation resistance among host races of *R. pomonella* require further investigation to determine if host related fitness tradeoffs limit desiccation resistance in apple flies; if so, the eastward spread of hawthorn host races might be less of a threat to apple growers than previously thought. Similarly, if host-specific adaptations constrain drought adaptation in apple flies, introgression of *R. zephyria* fly alleles into apple fly populations alone might not be enough to enhance desiccation resistance in the invasive apple fly.

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APPENDIX

Appendix Table 1. Log2 fold change (LFC) and Benjamini-Hochberg adjusted p-value of genes significantly differentially expressed in two or more contrasts between Whatcom and Yakima flies treated in high (~85% RH) or low (~43% RH) relative humidity. Positive LFC values indicate upregulation and negative values indicate downregulation in Yakima flies for population effects and low treated flies for treatment effects. Bolded values indicate significant differences in expression (p-value < 0.1). Genes are grouped by patterns in expression across the four contrasts starting with genes differentially expressed in three contrasts.

	Population effect (LFC relative to Whatcom)		Treatment effect (LFC relative to high treatment)	
Locus	Whatcom low vs. Yakima low	Whatcom high vs. Yakima high	Yakima high vs. Yakima low	Whatcom high vs. Whatcom low
LOC108373604	**0.728 (0.06)**	**-0.976 (<0.01)**	**1.487 (<0.01)**	-0.217 (1)
LOC108374045	**0.696 (<0.01)**	**-0.665 (0.02)**	**0.776 (<0.01)**	-0.586 (0.37)
LOC108362297	**0.587 (0.08)**	**-0.761 (0.01)**	**0.995 (<0.01)**	-0.353 (1)
LOC108369415	**0.513 (0.08)**	**-0.634 (0.03)**	**0.688 (0.02)**	-0.46 (0.77)
LOC108366334	**0.478 (0.09)**	**-0.571 (0.04)**	**0.678 (0.01)**	-0.37 (1)
LOC108372546	**0.309 (<0.01)**	**-0.562 (<0.01)**	**0.723 (<0.01)**	-0.148 (0.91)
LOC108371735	**-0.605 (0.04)**	**0.781 (<0.01)**	**-1.199 (<0.01)**	0.186 (1)
LOC108367117	**-0.676 (0.07)**	**0.705 (0.07)**	**-0.997 (<0.01)**	0.385 (1)
LOC108353879	**1.026 (<0.01)**	**-0.972 (<0.01)**	0.714 (0.15)	**-1.284 (<0.01)**
LOC108380632	**-0.333 (0.02)**	**0.34 (0.03)**	-0.283 (0.16)	**0.39 (0.07)**
LOC108355455	**-0.766 (0.07)**	**1.169 (<0.01)**	-0.741 (0.19)	**1.194 (0.01)**
LOC108358463	**3.837 (<0.01)**	**1.416 (<0.01)**	0.451 (0.61)	**-1.97 (<0.01)**
LOC108367075	**-1.538 (<0.01)**	**-0.703 (0.06)**	**-0.74 (0.08)**	0.095 (1)
LOC108361351	**-0.644 (<0.01)**	**-0.734 (<0.01)**	**0.484 (0.08)**	0.394 (0.68)
LOC108363402	**-1.014 (<0.01)**	**-0.729 (0.01)**	0.552 (0.17)	**0.837 (0.02)**
LOC108370289	**-1.606 (<0.01)**	**-0.972 (<0.01)**	0.263 (0.88)	**0.897 (0.09)**
LOC108354186	**1.078 (<0.01)**	-0.222 (0.86)	**0.89 (0.06)**	-0.41 (1)
LOC108369533	**1.064 (<0.01)**	-0.234 (0.84)	**1.164 (<0.01)**	-0.134 (1)
LOC108375876	**0.941 (<0.01)**	-0.124 (0.93)	**0.928 (0.01)**	-0.137 (1)
LOC108356033	**0.922 (0.01)**	-0.338 (0.7)	**1.022 (<0.01)**	-0.239 (1)
LOC108368508	**0.875 (0.02)**	-0.307 (0.74)	**0.896 (0.04)**	-0.286 (1)
LOC108365217	**0.871 (0.03)**	-0.327 (0.73)	**1.125 (<0.01)**	-0.072 (1)
LOC108375896	**0.855 (0.03)**	-0.219 (0.86)	**0.91 (0.05)**	-0.165 (1)
LOC108379317	**0.835 (0.02)**	-0.16 (0.9)	**0.905 (0.02)**	-0.089 (1)
LOC108362514	**0.749 (0.08)**	-0.592 (0.3)	**0.875 (0.07)**	-0.465 (1)
LOC108377406	**0.732 (0.02)**	-0.117 (0.92)	**0.703 (0.07)**	-0.145 (1)

	Population effect (LFC relative to Whatcom)		Treatment effect (LFC relative to high treatment)	
LOC108373704	**0.73 (0.09)**	-0.151 (0.92)	**0.88 (0.06)**	-0.001 (1)
LOC108365795	**0.709 (<0.01)**	-0.111 (0.89)	**0.625 (0.01)**	-0.194 (1)
LOC108370886	**0.67 (0.1)**	-0.564 (0.27)	**0.925 (0.02)**	-0.308 (1)
LOC108362091	**0.653 (0.05)**	-0.398 (0.46)	**0.856 (0.01)**	-0.195 (1)
LOC108370142	**0.546 (0.08)**	-0.131 (0.89)	**0.673 (0.05)**	-0.003 (1)
LOC108373603	**0.315 (0.08)**	-0.198 (0.49)	**0.37 (0.07)**	-0.144 (1)
LOC108358845	**-0.366 (0.09)**	0.291 (0.27)	**-0.508 (0.01)**	0.149 (1)
LOC108367314	**-0.542 (<0.01)**	0.112 (0.81)	**-0.43 (0.02)**	0.225 (1)
LOC108357804	**-0.567 (0.06)**	0.125 (0.9)	**-0.631 (0.07)**	0.06 (1)
LOC108372759	**-0.568 (<0.01)**	0.051 (0.95)	**-0.505 (0.05)**	0.114 (1)
LOC108358385	**-0.591 (0.01)**	0.049 (0.96)	**-0.543 (0.07)**	0.097 (1)
LOC108371752	**-0.591 (0.02)**	0.111 (0.89)	**-0.529 (0.1)**	0.173 (1)
LOC108379045	**-0.68 (0.01)**	0.15 (0.86)	**-0.805 (<0.01)**	0.025 (1)
LOC108356423	**-0.693 (0.03)**	0.486 (0.26)	**-0.908 (<0.01)**	0.271 (1)
LOC108357417	**-0.706 (0.1)**	0.684 (0.14)	**-1.166 (<0.01)**	0.224 (1)
LOC108376467	**-0.717 (0.09)**	0.473 (0.47)	**-0.913 (0.04)**	0.277 (1)
LOC108376024	**-0.727 (<0.01)**	0.228 (0.74)	**-0.761 (0.01)**	0.194 (1)
LOC108382190	**-0.778 (0.02)**	0.355 (0.57)	**-0.875 (0.01)**	0.258 (1)
LOC108379660	**-0.807 (<0.01)**	0.453 (0.23)	**-1.041 (<0.01)**	0.219 (1)
LOC108369357	**-0.842 (<0.01)**	0.325 (0.47)	**-0.753 (<0.01)**	0.414 (0.91)
LOC108375161	**-0.854 (<0.01)**	0.134 (0.79)	**-0.755 (<0.01)**	0.234 (1)
LOC108354917	**-0.895 (0.02)**	0.508 (0.4)	**-1.167 (<0.01)**	0.237 (1)
LOC108377970	**-0.918 (<0.01)**	0.054 (0.98)	**-0.829 (0.05)**	0.143 (1)
LOC108362187	**-0.939 (<0.01)**	0.046 (0.96)	**-0.866 (<0.01)**	0.118 (1)
LOC108367725	**-1.112 (<0.01)**	0.022 (0.99)	-0.152 (0.96)	**0.983 (0.09)**
LOC108370448	0.587 (0.14)	**-0.768 (0.04)**	**0.948 (<0.01)**	-0.407 (1)
LOC108372983	0.505 (0.32)	**-0.833 (0.05)**	**1.044 (<0.01)**	-0.294 (1)
LOC108374625	0.498 (0.16)	**-0.681 (0.03)**	**0.904 (<0.01)**	-0.275 (1)
LOC108361804	0.489 (0.14)	**-0.57 (0.09)**	**0.823 (<0.01)**	-0.236 (1)
LOC108375182	0.415 (0.37)	**-0.897 (<0.01)**	**0.828 (0.02)**	-0.483 (0.98)
LOC108376702	0.367 (0.26)	**-0.68 (<0.01)**	**0.843 (<0.01)**	-0.204 (1)
LOC108354852	0.25 (0.66)	**-0.901 (<0.01)**	**0.935 (<0.01)**	-0.215 (1)
LOC108370785	0.24 (0.59)	**-1.194 (<0.01)**	**1.02 (<0.01)**	-0.414 (0.91)
LOC108374515	0.237 (0.46)	**-0.47 (0.04)**	**0.534 (0.03)**	-0.174 (1)
LOC108365920	0.236 (0.66)	**-0.693 (0.02)**	**0.657 (0.06)**	-0.272 (1)
LOC108374550	0.234 (0.46)	**-0.592 (<0.01)**	**0.476 (0.06)**	-0.35 (0.8)
LOC108370885	0.168 (0.79)	**-0.862 (<0.01)**	**0.937 (<0.01)**	-0.093 (1)
LOC108367414	0.156 (0.66)	**-0.523 (<0.01)**	**0.489 (0.02)**	-0.19 (1)
LOC108376213	0.154 (0.73)	**-0.542 (0.02)**	**0.494 (0.08)**	-0.202 (1)

	Population effect (LFC relative to Whatcom)		Treatment effect (LFC relative to high treatment)	
LOC108370765	0.153 (0.82)	**-1.088 (<0.01)**	**0.983 (<0.01)**	-0.259 (1)
LOC108365861	0.056 (0.92)	**-0.802 (<0.01)**	**0.451 (0.06)**	-0.407 (0.42)
LOC108373285	0.011 (0.98)	**-0.581 (<0.01)**	**0.514 (<0.01)**	-0.078 (1)
LOC108357256	-0.001 (1)	**0.542 (0.02)**	**-0.542 (0.04)**	0.001 (1)
LOC108364411	-0.086 (0.92)	**0.96 (<0.01)**	**-0.842 (<0.01)**	0.203 (1)
LOC108365584	-0.137 (0.89)	**0.967 (0.01)**	**-0.811 (0.1)**	0.293 (1)
LOC108376791	-0.143 (0.83)	**0.857 (<0.01)**	**-0.712 (0.01)**	0.287 (1)
LOC108356391	-0.196 (0.79)	**0.839 (0.02)**	**-0.881 (0.02)**	0.155 (1)
LOC108379954	-0.223 (0.66)	**1.241 (<0.01)**	**-1.009 (<0.01)**	0.455 (0.83)
LOC108380178	-0.225 (0.54)	**0.696 (<0.01)**	**-0.663 (<0.01)**	0.258 (1)
LOC108378947	-0.249 (0.46)	**0.475 (0.05)**	**-0.701 (<0.01)**	0.023 (1)
LOC108374340	-0.281 (0.39)	**0.616 (<0.01)**	**-0.666 (<0.01)**	0.232 (1)
LOC108357169	-0.338 (0.51)	**0.761 (0.02)**	**-1.038 (<0.01)**	0.061 (1)
LOC108380721	-0.342 (0.57)	**1.015 (<0.01)**	**-0.843 (0.05)**	0.514 (1)
LOC108378584	-0.344 (0.1)	**0.54 (<0.01)**	**-0.688 (<0.01)**	0.197 (1)
LOC108380246	-0.369 (0.5)	**0.751 (0.04)**	**-0.825 (0.04)**	0.295 (1)
LOC108371797	-0.37 (0.12)	**0.694 (<0.01)**	**-0.878 (<0.01)**	0.185 (1)
LOC108378974	-0.39 (0.44)	**0.88 (<0.01)**	**-0.864 (0.02)**	0.406 (1)
LOC108373678	-0.405 (0.29)	**0.632 (0.05)**	**-0.801 (<0.01)**	0.237 (1)
LOC108379780	-0.434 (0.12)	**0.505 (0.07)**	**-0.543 (0.07)**	0.395 (0.85)
LOC108370462	-0.533 (0.31)	**0.89 (0.03)**	**-1.077 (<0.01)**	0.346 (1)
LOC108375015	-0.567 (0.27)	**1.007 (0.01)**	**-0.971 (0.03)**	0.603 (0.95)
LOC108382153	-0.611 (0.15)	**0.903 (0.01)**	**-0.965 (0.01)**	0.548 (0.95)
LOC108373090	0.203 (0.8)	**-0.89 (0.02)**	0.057 (0.99)	**-1.036 (0.03)**
LOC108381805	-0.108 (0.86)	**0.818 (<0.01)**	-0.302 (0.6)	**0.624 (0.09)**
LOC108382796	-0.199 (0.61)	**0.548 (0.02)**	-0.16 (0.89)	**0.588 (0.09)**
LOC108361132	**0.665 (0.09)**	**-0.69 (0.09)**	0.728 (0.11)	-0.626 (0.68)
LOC108379113	**4.623 (<0.01)**	-0.061 (0.98)	**4.724 (<0.01)**	0.04 (1)
LOC108373689	**0.77 (0.07)**	-0.147 (0.92)	**0.937 (0.04)**	0.02 (1)
LOC108370242	**0.738 (0.08)**	-0.191 (0.88)	**0.98 (0.02)**	0.05 (1)
LOC108358480	**0.664 (0.05)**	-0.015 (0.99)	**0.778 (0.04)**	0.099 (1)
LOC108362082	**0.615 (0.02)**	-0.04 (0.98)	**0.668 (0.02)**	0.013 (1)
LOC108354817	**0.414 (0.03)**	-0.187 (0.63)	**0.727 (<0.01)**	0.126 (1)
LOC108373229	**-0.611 (0.07)**	0.027 (0.99)	**-0.732 (0.05)**	-0.094 (1)
LOC108371665	**-0.802 (0.05)**	0.354 (0.68)	**-1.265 (<0.01)**	-0.109 (1)
LOC108356685	**-0.827 (0.03)**	0.162 (0.9)	**-1.046 (<0.01)**	-0.058 (1)
LOC108367843	**0.795 (0.06)**	-0.163 (0.9)	-0.024 (1)	**-0.983 (0.09)**
LOC108358217	**0.719 (0.06)**	-0.138 (0.92)	-0.046 (0.99)	**-0.902 (0.09)**
LOC108359756	0.176 (0.56)	**-0.413 (0.04)**	**0.685 (<0.01)**	0.095 (1)

	Population effect (LFC relative to Whatcom)		Treatment effect (LFC relative to high treatment)	
LOC108375327	-0.104 (0.67)	**0.26 (0.09)**	**-0.367 (<0.01)**	-0.003 (1)
LOC108371664	-0.529 (0.16)	**0.958 (<0.01)**	**-1.583 (<0.01)**	-0.095 (1)
LOC108357654	0.006 (0.99)	**-0.84 (<0.01)**	-0.005 (1)	**-0.852 (<0.01)**
LOC108366491	0.002 (1)	**-0.611 (0.05)**	-0.123 (0.95)	**-0.736 (0.09)**
LOC108372540	**1.748 (<0.01)**	0.199 (0.87)	**0.772 (0.1)**	-0.776 (0.37)
LOC108368527	**1.553 (<0.01)**	0.399 (0.62)	**0.919 (0.05)**	-0.235 (1)
LOC108358479	**1.329 (<0.01)**	0.065 (0.97)	**1.156 (<0.01)**	-0.107 (1)
LOC108369615	**1.156 (<0.01)**	0.104 (0.94)	**0.943 (<0.01)**	-0.109 (1)
LOC108360568	**-0.277 (<0.01)**	-0.003 (1)	**-0.231 (0.1)**	0.043 (1)
LOC108363572	**-0.985 (<0.01)**	-0.161 (0.86)	**-0.639 (0.1)**	0.185 (1)
LOC108357979	**-1.092 (<0.01)**	-0.056 (0.97)	-0.086 (0.98)	**0.949 (0.03)**
LOC108365583	0.197 (0.83)	**1.163 (<0.01)**	**-0.914 (0.05)**	0.051 (1)
LOC108365873	-0.12 (0.71)	**-0.583 (<0.01)**	**0.439 (0.01)**	-0.024 (1)
LOC108375000	**1.455 (<0.01)**	0.352 (0.66)	**1.138 (<0.01)**	0.035 (1)
LOC108375794	**0.911 (0.01)**	0.154 (0.91)	**0.803 (0.1)**	0.045 (1)
LOC108366712	**0.857 (<0.01)**	0.204 (0.84)	**0.893 (0.01)**	0.24 (1)
LOC108368321	**0.791 (0.03)**	0.067 (0.97)	**0.742 (0.1)**	0.018 (1)
LOC108358912	**0.766 (0.01)**	0.224 (0.79)	**0.708 (0.05)**	0.166 (1)
LOC108357278	**0.547 (0.02)**	0.011 (1)	**0.567 (0.04)**	0.031 (1)
LOC108360514	**0.343 (0.05)**	0.026 (0.98)	**0.381 (0.05)**	0.064 (1)
LOC108368495	**0.273 (0.08)**	0.044 (0.94)	**0.326 (0.05)**	0.098 (1)
LOC108368690	**-0.368 (0.04)**	-0.11 (0.82)	**-0.387 (0.06)**	-0.129 (1)
LOC108359546	**-0.634 (0.06)**	-0.052 (0.97)	**-0.702 (0.07)**	-0.12 (1)
LOC108381002	**-0.842 (0.04)**	-0.237 (0.84)	**-0.865 (0.07)**	-0.26 (1)
LOC108375383	**-0.582 (0.1)**	-0.36 (0.53)	0.562 (0.24)	**0.784 (0.1)**
LOC108374913	**-0.852 (<0.01)**	-0.008 (1)	0.155 (0.95)	**0.998 (0.01)**
LOC108362356	**-1.041 (<0.01)**	-0.285 (0.76)	0.162 (0.95)	**0.917 (0.1)**
LOC108355984	**-1.064 (<0.01)**	-0.184 (0.88)	0.095 (0.98)	**0.975 (0.09)**
LOC108361223	**-1.073 (<0.01)**	-0.213 (0.87)	0.18 (0.95)	**1.04 (0.08)**
LOC108357031	**-1.122 (<0.01)**	-0.508 (0.25)	0.209 (0.9)	**0.823 (0.09)**
LOC108361126	0.169 (0.4)	**0.364 (0.01)**	0.166 (0.62)	**0.36 (0.09)**
LOC108356491	-0.006 (1)	**-1.279 (<0.01)**	-0.031 (1)	**-1.305 (<0.01)**
LOC108369411	-0.124 (0.91)	**-0.931 (0.02)**	-0.375 (0.77)	**-1.181 (0.01)**
LOC108355047	**1.532 (<0.01)**	**0.868 (0.04)**	0.358 (0.79)	-0.306 (1)
LOC108363477	**1.419 (<0.01)**	**1.818 (<0.01)**	-0.084 (0.98)	0.315 (1)
LOC108370011	**1.304 (<0.01)**	**1.652 (<0.01)**	-0.317 (0.84)	0.031 (1)
LOC108370733	**1.222 (<0.01)**	**0.805 (0.08)**	0.263 (0.9)	-0.154 (1)
LOC108379734	**1.179 (<0.01)**	**1.64 (<0.01)**	-0.285 (0.67)	0.176 (1)
LOC108366213	**0.988 (<0.01)**	**0.782 (0.09)**	0.139 (0.96)	-0.067 (1)

	Population effect (LFC relative to Whatcom)		Treatment effect (LFC relative to high treatment)	
LOC108370607	**0.934 (0.01)**	**1.725 (<0.01)**	-0.622 (0.32)	0.169 (1)
LOC108364659	**0.933 (<0.01)**	**0.774 (0.03)**	0.119 (0.96)	-0.041 (1)
LOC108382117	**0.859 (0.01)**	**1.192 (<0.01)**	-0.22 (0.9)	0.114 (1)
LOC108373827	**0.852 (0.04)**	**1.567 (<0.01)**	-0.516 (0.54)	0.199 (1)
LOC108375105	**0.803 (<0.01)**	**0.85 (<0.01)**	-0.018 (1)	0.028 (1)
LOC108358613	**0.662 (<0.01)**	**0.606 (0.03)**	0.025 (1)	-0.031 (1)
LOC108357826	**0.535 (0.09)**	**0.826 (<0.01)**	-0.1 (0.96)	0.191 (1)
LOC108366670	**0.513 (<0.01)**	**0.427 (0.03)**	0.08 (0.95)	-0.006 (1)
LOC108359345	**0.427 (0.09)**	**0.577 (0.01)**	-0.053 (0.98)	0.097 (1)
LOC108355109	**0.427 (0.01)**	**0.34 (0.09)**	0.041 (0.98)	-0.046 (1)
LOC108361735	**0.412 (<0.01)**	**0.257 (0.02)**	0.039 (0.96)	-0.116 (1)
LOC108360788	**0.372 (0.09)**	**0.506 (0.01)**	-0.009 (1)	0.125 (1)
LOC108357538	**0.199 (0.08)**	**0.239 (0.03)**	-0.031 (0.97)	0.01 (1)
LOC108377770	**-0.271 (0.06)**	**-0.363 (<0.01)**	0.019 (0.99)	-0.072 (1)
LOC108373602	**-0.312 (0.03)**	**-0.284 (0.08)**	-0.016 (0.99)	0.012 (1)
LOC108365365	**-0.323 (0.01)**	**-0.262 (0.08)**	-0.007 (1)	0.054 (1)
LOC108357665	**-0.327 (<0.01)**	**-0.247 (0.04)**	-0.043 (0.96)	0.036 (1)
LOC108377860	**-0.351 (<0.01)**	**-0.493 (<0.01)**	0.018 (0.99)	-0.124 (1)
LOC108363312	**-0.385 (<0.01)**	**-0.318 (0.03)**	-0.034 (0.98)	0.033 (1)
LOC108371533	**-0.409 (<0.01)**	**-0.288 (0.05)**	-0.12 (0.8)	0.001 (1)
LOC108373679	**-0.435 (<0.01)**	**-0.56 (<0.01)**	0.084 (0.91)	-0.041 (1)
LOC108367399	**-0.44 (0.09)**	**-0.593 (0.01)**	0.069 (0.97)	-0.085 (1)
LOC108369892	**-0.442 (0.05)**	**-0.819 (<0.01)**	0.04 (0.99)	-0.337 (0.85)
LOC108363056	**-0.443 (<0.01)**	**-0.301 (0.09)**	-0.065 (0.95)	0.077 (1)
LOC108376940	**-0.484 (<0.01)**	**-0.392 (<0.01)**	-0.013 (1)	0.079 (1)
LOC108380941	**-0.501 (0.05)**	**-0.556 (0.03)**	0.045 (0.99)	-0.01 (1)
LOC108356883	**-0.64 (<0.01)**	**-0.574 (<0.01)**	-0.041 (0.98)	0.025 (1)
LOC108368455	**-0.671 (<0.01)**	**-0.426 (<0.01)**	-0.119 (0.83)	0.125 (1)
LOC108354454	**-0.673 (<0.01)**	**-0.487 (0.1)**	-0.003 (1)	0.183 (1)
LOC108365746	**-0.68 (<0.01)**	**-0.634 (<0.01)**	-0.041 (0.97)	0.005 (1)
LOC108361815	**-0.688 (0.08)**	**-0.768 (0.05)**	0.034 (1)	-0.047 (1)
LOC108361404	**-0.689 (<0.01)**	**-0.292 (0.08)**	-0.238 (0.33)	0.159 (1)
LOC108367397	**-0.703 (<0.01)**	**-0.517 (0.06)**	-0.048 (0.99)	0.138 (1)
LOC108371691	**-0.796 (<0.01)**	**-1.187 (<0.01)**	0.026 (1)	-0.364 (1)
LOC108369888	**-0.938 (<0.01)**	**-1.295 (<0.01)**	0.053 (0.99)	-0.304 (1)
LOC108369055	**-1.023 (<0.01)**	**-1.03 (<0.01)**	0 (1)	-0.002 (1)
LOC108366142	**-1.058 (<0.01)**	**-0.872 (0.03)**	-0.126 (0.97)	0.06 (1)
LOC108378454	**-1.239 (<0.01)**	**-0.958 (0.02)**	-0.217 (0.92)	0.064 (1)
LOC108367793	**-1.351 (<0.01)**	**-1.083 (<0.01)**	-0.009 (1)	0.259 (1)

	Population effect (LFC relative to Whatcom)		Treatment effect (LFC relative to high treatment)	
LOC108355259	**-1.37 (<0.01)**	**-1.199 (<0.01)**	-0.105 (0.97)	0.066 (1)
LOC108357960	**-1.444 (<0.01)**	**-1.18 (<0.01)**	-0.064 (0.99)	0.2 (1)
LOC108373356	**-1.498 (<0.01)**	**-1.208 (<0.01)**	-0.084 (0.98)	0.206 (1)
LOC108365695	**-1.886 (<0.01)**	**-2.104 (<0.01)**	0.024 (1)	-0.195 (1)
LOC108383102	**1.268 (<0.01)**	**0.862 (0.03)**	0.43 (0.64)	0.024 (1)
LOC108360029	**1.239 (<0.01)**	**1.491 (<0.01)**	-0.58 (0.25)	-0.327 (1)
LOC108367021	**1.225 (<0.01)**	**1.479 (<0.01)**	-0.286 (0.88)	-0.032 (1)
LOC108357626	**1.147 (<0.01)**	**1 (<0.01)**	-0.142 (0.95)	-0.289 (1)
LOC108377415	**1.141 (<0.01)**	**1.027 (<0.01)**	0.202 (0.93)	0.087 (1)
LOC108381645	**1.052 (<0.01)**	**0.973 (0.01)**	-0.037 (1)	-0.116 (1)
LOC108375648	**1.051 (<0.01)**	**1.183 (<0.01)**	-0.316 (0.85)	-0.184 (1)
LOC108364656	**1.013 (<0.01)**	**0.745 (<0.01)**	0.544 (0.12)	0.275 (1)
LOC108365375	**0.973 (<0.01)**	**0.923 (<0.01)**	0.059 (0.99)	0.009 (1)
LOC108378718	**0.961 (<0.01)**	**0.754 (0.09)**	0.26 (0.89)	0.053 (1)
LOC108376342	**0.872 (0.03)**	**0.871 (0.04)**	0.096 (0.98)	0.095 (1)
LOC108376675	**0.869 (0.03)**	**0.93 (0.02)**	-0.272 (0.89)	-0.211 (1)
LOC108354621	**0.867 (0.02)**	**0.771 (0.06)**	0.158 (0.95)	0.061 (1)
LOC108361732	**0.812 (0.01)**	**0.817 (0.02)**	0.154 (0.95)	0.158 (1)
LOC108372747	**0.784 (0.07)**	**0.792 (0.08)**	0.185 (0.94)	0.193 (1)
LOC108369038	**0.782 (<0.01)**	**0.596 (0.02)**	0.228 (0.78)	0.043 (1)
LOC108360022	**0.76 (0.01)**	**0.809 (0.01)**	-0.076 (0.98)	-0.027 (1)
LOC108369040	**0.738 (<0.01)**	**0.643 (<0.01)**	0.147 (0.91)	0.053 (1)
LOC108380571	**0.733 (<0.01)**	**0.664 (0.03)**	0.332 (0.65)	0.263 (1)
LOC108376980	**0.731 (0.02)**	**0.659 (0.06)**	0.232 (0.88)	0.16 (1)
LOC108368426	**0.727 (0.03)**	**0.824 (0.01)**	-0.218 (0.89)	-0.121 (1)
LOC108379456	**0.706 (0.09)**	**0.798 (0.06)**	0.032 (1)	0.124 (1)
LOC108364742	**0.698 (0.05)**	**0.75 (0.04)**	0.225 (0.9)	0.277 (1)
LOC108357185	**0.691 (0.06)**	**0.689 (0.07)**	-0.128 (0.96)	-0.129 (1)
LOC108373227	**0.681 (0.02)**	**0.741 (0.01)**	0.201 (0.9)	0.261 (1)
LOC108368137	**0.657 (<0.01)**	**0.557 (0.01)**	-0.049 (0.98)	-0.15 (1)
LOC108361214	**0.653 (<0.01)**	**0.539 (0.02)**	0.159 (0.88)	0.045 (1)
LOC108373867	**0.641 (<0.01)**	**0.435 (0.01)**	0.266 (0.37)	0.06 (1)
LOC108379237	**0.639 (0.04)**	**0.735 (0.02)**	-0.17 (0.92)	-0.074 (1)
LOC108354949	**0.632 (0.09)**	**0.714 (0.05)**	0.004 (1)	0.086 (1)
LOC108370828	**0.59 (<0.01)**	**0.593 (<0.01)**	-0.142 (0.9)	-0.139 (1)
LOC108359294	**0.586 (0.04)**	**0.593 (0.05)**	0.062 (0.98)	0.069 (1)
LOC108369166	**0.564 (<0.01)**	**0.553 (<0.01)**	-0.139 (0.89)	-0.151 (1)
LOC108379413	**0.561 (<0.01)**	**0.488 (<0.01)**	-0.06 (0.96)	-0.134 (1)
LOC108373491	**0.512 (0.01)**	**0.432 (0.07)**	0.084 (0.96)	0.003 (1)

	Population effect (LFC relative to Whatcom)		Treatment effect (LFC relative to high treatment)	
LOC108382103	**0.476 (0.02)**	**0.601 (<0.01)**	0.11 (0.92)	0.235 (1)
LOC108363408	**0.44 (<0.01)**	**0.297 (0.04)**	0.159 (0.61)	0.017 (1)
LOC108374853	**0.439 (0.07)**	**0.47 (0.05)**	-0.212 (0.76)	-0.181 (1)
LOC108362763	**0.439 (0.06)**	**0.449 (0.07)**	0.183 (0.83)	0.193 (1)
LOC108364711	**0.425 (<0.01)**	**0.455 (<0.01)**	0.064 (0.96)	0.094 (1)
LOC108362218	**0.42 (<0.01)**	**0.37 (<0.01)**	0.186 (0.46)	0.136 (1)
LOC108366305	**0.407 (<0.01)**	**0.467 (<0.01)**	-0.124 (0.85)	-0.064 (1)
LOC108370430	**0.407 (<0.01)**	**0.332 (0.04)**	-0.152 (0.73)	-0.227 (0.98)
LOC108376247	**0.39 (0.07)**	**0.507 (<0.01)**	-0.187 (0.75)	-0.07 (1)
LOC108366823	**0.383 (0.02)**	**0.35 (0.05)**	0.298 (0.21)	0.265 (0.85)
LOC108370435	**0.379 (<0.01)**	**0.347 (<0.01)**	0.136 (0.71)	0.104 (1)
LOC108365387	**0.366 (0.07)**	**0.366 (0.09)**	-0.098 (0.93)	-0.099 (1)
LOC108366741	**0.345 (0.07)**	**0.409 (0.02)**	0.025 (0.99)	0.089 (1)
LOC108371444	**0.288 (0.09)**	**0.331 (0.05)**	0.136 (0.8)	0.179 (1)
LOC108364290	**0.26 (0.06)**	**0.252 (0.09)**	-0.107 (0.83)	-0.115 (1)
LOC108361327	**-0.185 (0.05)**	**-0.177 (0.09)**	0.007 (1)	0.015 (1)
LOC108361230	**-0.201 (0.1)**	**-0.235 (0.05)**	0.055 (0.94)	0.021 (1)
LOC108355977	**-0.246 (0.09)**	**-0.353 (<0.01)**	0.107 (0.84)	0 (1)
LOC108369292	**-0.255 (<0.01)**	**-0.31 (<0.01)**	0.059 (0.91)	0.004 (1)
LOC108380887	**-0.256 (<0.01)**	**-0.229 (0.03)**	0.009 (1)	0.036 (1)
LOC108369770	**-0.258 (0.07)**	**-0.29 (0.04)**	0.068 (0.93)	0.036 (1)
LOC108366756	**-0.266 (<0.01)**	**-0.291 (<0.01)**	0.129 (0.61)	0.104 (1)
LOC108366416	**-0.269 (0.02)**	**-0.262 (0.03)**	0.04 (0.96)	0.048 (1)
LOC108354327	**-0.28 (<0.01)**	**-0.251 (0.03)**	0.006 (1)	0.035 (1)
LOC108371431	**-0.29 (0.04)**	**-0.259 (0.1)**	-0.136 (0.74)	-0.104 (1)
LOC108366824	**-0.294 (0.05)**	**-0.277 (0.09)**	0.025 (0.99)	0.043 (1)
LOC108357969	**-0.3 (0.08)**	**-0.334 (0.05)**	0.085 (0.92)	0.051 (1)
LOC108373157	**-0.314 (0.02)**	**-0.272 (0.08)**	0.018 (0.99)	0.06 (1)
LOC108377545	**-0.317 (0.04)**	**-0.288 (0.09)**	0.007 (1)	0.036 (1)
LOC108377053	**-0.321 (<0.01)**	**-0.315 (<0.01)**	0.029 (0.97)	0.035 (1)
LOC108367005	**-0.324 (0.02)**	**-0.275 (0.07)**	-0.235 (0.26)	-0.187 (1)
LOC108360880	**-0.335 (<0.01)**	**-0.335 (0.01)**	0.129 (0.75)	0.129 (1)
LOC108368309	**-0.336 (<0.01)**	**-0.254 (0.01)**	-0.135 (0.53)	-0.053 (1)
LOC108377454	**-0.339 (<0.01)**	**-0.328 (0.02)**	0.043 (0.97)	0.054 (1)
LOC108371454	**-0.367 (0.01)**	**-0.303 (0.08)**	-0.066 (0.95)	-0.002 (1)
LOC108362462	**-0.369 (<0.01)**	**-0.323 (0.04)**	-0.093 (0.91)	-0.047 (1)
LOC108359481	**-0.372 (0.03)**	**-0.369 (0.04)**	0.048 (0.97)	0.052 (1)
LOC108361642	**-0.385 (0.04)**	**-0.428 (0.02)**	-0.07 (0.96)	-0.113 (1)
LOC108374304	**-0.39 (0.03)**	**-0.377 (0.06)**	0.064 (0.96)	0.077 (1)

	Population effect (LFC relative to Whatcom)		Treatment effect (LFC relative to high treatment)	
LOC108363635	**-0.393 (<0.01)**	**-0.318 (0.06)**	-0.09 (0.92)	-0.015 (1)
LOC108368902	**-0.408 (0.06)**	**-0.428 (0.06)**	0.4 (0.16)	0.38 (0.6)
LOC108370847	**-0.413 (<0.01)**	**-0.335 (0.05)**	0.017 (1)	0.095 (1)
LOC108374076	**-0.413 (0.04)**	**-0.424 (0.04)**	0.119 (0.91)	0.108 (1)
LOC108361850	**-0.418 (0.02)**	**-0.407 (0.03)**	-0.022 (0.99)	-0.011 (1)
LOC108366776	**-0.435 (0.08)**	**-0.726 (<0.01)**	0.364 (0.37)	0.072 (1)
LOC108372055	**-0.446 (<0.01)**	**-0.365 (<0.01)**	-0.109 (0.86)	-0.029 (1)
LOC108358955	**-0.459 (<0.01)**	**-0.315 (0.05)**	-0.172 (0.64)	-0.028 (1)
LOC108358831	**-0.471 (<0.01)**	**-0.456 (<0.01)**	0.224 (0.56)	0.239 (1)
LOC108368594	**-0.486 (<0.01)**	**-0.363 (0.06)**	-0.142 (0.84)	-0.019 (1)
LOC108370694	**-0.498 (0.01)**	**-0.531 (<0.01)**	0.062 (0.97)	0.029 (1)
LOC108368631	**-0.51 (<0.01)**	**-0.414 (<0.01)**	0.061 (0.94)	0.156 (1)
LOC108369895	**-0.51 (<0.01)**	**-0.642 (<0.01)**	-0.051 (0.98)	-0.183 (1)
LOC108367258	**-0.511 (0.1)**	**-0.578 (0.05)**	-0.133 (0.94)	-0.2 (1)
LOC108354743	**-0.519 (0.1)**	**-0.791 (<0.01)**	0.41 (0.43)	0.137 (1)
LOC108374681	**-0.52 (<0.01)**	**-0.474 (<0.01)**	0.036 (0.97)	0.082 (1)
LOC108374645	**-0.526 (0.05)**	**-0.612 (0.02)**	-0.101 (0.96)	-0.187 (1)
LOC108370094	**-0.547 (0.02)**	**-0.629 (<0.01)**	0.097 (0.96)	0.015 (1)
LOC108361677	**-0.567 (0.02)**	**-0.544 (0.05)**	0.186 (0.88)	0.209 (1)
LOC108358936	**-0.586 (0.02)**	**-0.583 (0.03)**	-0.053 (0.99)	-0.049 (1)
LOC108371102	**-0.599 (0.02)**	**-0.64 (0.01)**	0.069 (0.97)	0.028 (1)
LOC108371537	**-0.601 (0.04)**	**-0.551 (0.09)**	0.182 (0.9)	0.232 (1)
LOC108378491	**-0.63 (0.09)**	**-0.949 (<0.01)**	-0.112 (0.97)	-0.431 (1)
LOC108366609	**-0.672 (0.05)**	**-0.635 (0.1)**	-0.112 (0.96)	-0.075 (1)
LOC108374055	**-0.685 (0.1)**	**-0.891 (0.02)**	-0.057 (0.99)	-0.264 (1)
LOC108356217	**-0.695 (0.06)**	**-0.686 (0.09)**	0.163 (0.95)	0.172 (1)
LOC108360834	**-0.698 (0.02)**	**-0.789 (<0.01)**	0.208 (0.89)	0.117 (1)
LOC108355031	**-0.729 (0.1)**	**-0.839 (0.06)**	0.34 (0.82)	0.229 (1)
LOC108361065	**-0.734 (0.07)**	**-1.019 (<0.01)**	-0.141 (0.96)	-0.426 (1)
LOC108361673	**-0.737 (0.08)**	**-0.922 (0.02)**	-0.166 (0.95)	-0.352 (1)
LOC108360871	**-0.742 (<0.01)**	**-0.616 (0.01)**	-0.158 (0.91)	-0.032 (1)
LOC108382136	**-0.807 (<0.01)**	**-0.695 (<0.01)**	-0.184 (0.62)	-0.073 (1)
LOC108360000	**-0.854 (<0.01)**	**-0.699 (0.07)**	0.065 (0.99)	0.22 (1)
LOC108362473	**-0.884 (0.03)**	**-0.915 (0.03)**	-0.026 (1)	-0.057 (1)
LOC108363255	**-0.887 (0.03)**	**-0.814 (0.07)**	-0.127 (0.97)	-0.053 (1)
LOC108360773	**-0.897 (0.02)**	**-0.812 (0.07)**	-0.259 (0.9)	-0.173 (1)
LOC108367630	**-0.904 (<0.01)**	**-1.049 (<0.01)**	0.223 (0.91)	0.078 (1)
LOC108358264	**-0.911 (0.02)**	**-1.04 (<0.01)**	-0.1 (0.98)	-0.229 (1)
LOC108382768	**-0.913 (<0.01)**	**-0.569 (0.01)**	-0.396 (0.3)	-0.052 (1)

	Population effect (LFC relative to Whatcom)		Treatment effect (LFC relative to high treatment)	
LOC108367576	**-0.919 (<0.01)**	**-0.795 (0.02)**	-0.238 (0.88)	-0.114 (1)
LOC108373010	**-0.932 (<0.01)**	**-1.047 (<0.01)**	0.29 (0.85)	0.176 (1)
LOC108370170	**-0.956 (<0.01)**	**-0.947 (0.01)**	-0.052 (0.99)	-0.043 (1)
LOC108363417	**-0.99 (0.01)**	**-0.839 (0.06)**	-0.427 (0.69)	-0.276 (1)
LOC108366136	**-1.037 (<0.01)**	**-1.052 (<0.01)**	-0.231 (0.92)	-0.247 (1)
LOC108370288	**-1.475 (<0.01)**	**-0.936 (0.01)**	0.341 (0.79)	0.88 (0.13)
LOC108361583	0.234 (0.75)	**0.702 (0.07)**	**-0.753 (0.08)**	-0.285 (1)
LOC108377641	0.209 (0.76)	**0.739 (0.04)**	**-0.723 (0.08)**	-0.193 (1)
LOC108375590	0.047 (0.94)	**0.511 (0.01)**	**-0.6 (<0.01)**	-0.135 (1)
LOC108357977	-0.042 (0.93)	**-0.338 (0.09)**	**0.5 (<0.01)**	0.204 (1)
LOC108360848	-0.103 (0.92)	**-0.942 (0.01)**	**1.125 (<0.01)**	0.285 (1)
LOC108378257	-0.237 (0.37)	**-0.489 (0.01)**	**0.419 (0.08)**	0.167 (1)
LOC108362165	-0.33 (0.61)	**-0.794 (0.06)**	**0.79 (0.1)**	0.326 (1)
LOC108376929	-0.366 (0.26)	**-0.593 (0.02)**	**0.641 (0.02)**	0.414 (0.81)
LOC108355752	0.054 (0.96)	-0.133 (0.93)	**-0.95 (0.04)**	**-1.138 (0.02)**

Appendix Table 2. Log2 fold change of genes significantly differentially expressed (Benjamini-Hochberg (BH) p-value < 0.1) only between low treated (~43% RH) Yakima flies relative to low treated Whatcom flies. Positive log fold change values indicate upregulation and negative values indicate downregulation in Yakima flies.

Locus	Log2 fold change (BH p-value)
LOC108355255	1.329 (< 0.01)
LOC108374084	1.252 (< 0.01)
LOC108377955	1.247 (< 0.01)
LOC108382070	1.232 (< 0.01)
LOC108355671	1.19 (< 0.01)
LOC108370595	1.167 (< 0.01)
LOC108361623	1.158 (< 0.01)
LOC108357615	1.145 (< 0.01)
LOC108369388	1.126 (< 0.01)
LOC108366009	1.094 (< 0.01)
LOC108366134	1.063 (< 0.01)
LOC108381459	1.05 (< 0.01)
LOC108373350	1.041 (< 0.01)
LOC108372248	1.038 (< 0.01)
LOC108372770	1.03 (< 0.01)
LOC108366894	1 (< 0.01)
LOC108360096	0.989 (< 0.01)
LOC108375693	0.977 (0.01)
LOC108366055	0.97 (0.01)
LOC108376416	0.966 (< 0.01)
LOC108360383	0.965 (< 0.01)
LOC108373893	0.934 (0.02)
LOC108356202	0.928 (0.01)
LOC108379068	0.928 (0.02)
LOC108379636	0.926 (0.02)
LOC108367456	0.917 (0.02)
LOC108369970	0.912 (0.02)
LOC108365008	0.901 (< 0.01)
LOC108373050	0.899 (0.02)
LOC108378939	0.898 (0.02)
LOC108360382	0.894 (< 0.01)
LOC108367534	0.894 (0.01)
LOC108360232	0.894 (< 0.01)
LOC108375792	0.888 (0.02)
LOC108378940	0.887 (0.03)
LOC108355087	0.887 (0.03)
LOC108381395	0.887 (0.03)
LOC108377443	0.881 (0.03)
LOC108373465	0.877 (0.02)
LOC108370365	0.876 (0.02)
LOC108375031	0.873 (0.01)
LOC108365136	0.871 (< 0.01)
LOC108381127	0.862 (< 0.01)
LOC108372167	0.858 (0.02)
LOC108366277	0.857 (< 0.01)
LOC108362357	0.854 (0.03)
LOC108355016	0.842 (0.03)
LOC108367921	0.842 (0.03)
LOC108365913	0.839 (0.03)
LOC108369448	0.839 (0.04)
LOC108367920	0.835 (0.01)
LOC108366181	0.833 (0.04)
LOC108381968	0.828 (0.04)
LOC108363848	0.825 (0.01)
LOC108370451	0.825 (0.04)
LOC108380532	0.824 (0.04)
LOC108380159	0.815 (0.03)
LOC108374502	0.812 (0.02)
LOC108370191	0.81 (0.03)
LOC108366835	0.81 (0.05)
LOC108370671	0.808 (0.01)
LOC108367242	0.807 (0.05)
LOC108377397	0.806 (< 0.01)
LOC108358836	0.805 (0.05)
LOC108375411	0.805 (0.06)
LOC108382379	0.798 (0.04)
LOC108360474	0.797 (0.02)
LOC108357715	0.794 (0.03)
LOC108374850	0.792 (0.01)
LOC108358824	0.791 (0.01)
LOC108370163	0.787 (< 0.01)
LOC108361509	0.782 (0.05)
LOC108363139	0.781 (0.02)
LOC108371618	0.781 (0.02)

Locus	Log2 fold change (BH p-value)
LOC108379524	0.78 (0.04)
LOC108371493	0.78 (0.03)
LOC108365028	0.778 (0.07)
LOC108366574	0.775 (0.07)
LOC108368189	0.775 (0.05)
LOC108359127	0.774 (0.07)
LOC108354938	0.774 (0.02)
LOC108376128	0.774 (0.06)
LOC108354732	0.773 (0.07)
LOC108359737	0.772 (0.07)
LOC108374359	0.772 (0.07)
LOC108358226	0.768 (0.07)
LOC108369036	0.766 (< 0.01)
LOC108361510	0.766 (0.07)
LOC108375495	0.761 (0.06)
LOC108377017	0.759 (0.02)
LOC108371331	0.758 (0.08)
LOC108361466	0.758 (< 0.01)
LOC108359008	0.752 (0.08)
LOC108359292	0.75 (< 0.01)
LOC108354295	0.75 (0.08)
LOC108367707	0.749 (0.02)
LOC108374978	0.745 (< 0.01)
LOC108378774	0.744 (0.08)
LOC108373147	0.74 (0.05)
LOC108359738	0.737 (0.08)
LOC108376390	0.737 (0.04)
LOC108366831	0.734 (0.09)
LOC108381679	0.732 (0.02)
LOC108360755	0.732 (0.07)
LOC108383011	0.729 (0.1)
LOC108368050	0.728 (0.1)
LOC108358427	0.727 (0.07)
LOC108359543	0.725 (0.08)
LOC108362791	0.723 (0.09)
LOC108381264	0.721 (0.06)
LOC108373456	0.718 (0.1)
LOC108358219	0.717 (0.08)
LOC108372809	0.715 (0.04)
LOC108376295	0.714 (0.04)
LOC108370672	0.713 (0.07)
LOC108381662	0.712 (0.07)

Locus	Log2 fold change (BH p-value)
LOC108362386	0.71 (0.06)
LOC108374127	0.708 (< 0.01)
LOC108379429	0.704 (0.04)
LOC108375254	0.701 (0.08)
LOC108369212	0.701 (0.06)
LOC108365158	0.699 (0.05)
LOC108373577	0.697 (0.1)
LOC108371646	0.696 (0.05)
LOC108362347	0.696 (0.02)
LOC108363378	0.695 (0.03)
LOC108376888	0.694 (0.07)
LOC108366777	0.693 (< 0.01)
LOC108370355	0.692 (0.08)
LOC108377316	0.692 (0.05)
LOC108382878	0.692 (0.01)
LOC108362687	0.692 (0.07)
LOC108376273	0.686 (0.09)
LOC108366711	0.686 (0.05)
LOC108358909	0.677 (0.06)
LOC108357574	0.676 (0.07)
LOC108375469	0.675 (< 0.01)
LOC108362214	0.675 (0.04)
LOC108358741	0.675 (0.08)
LOC108374501	0.668 (0.05)
LOC108373613	0.665 (0.09)
LOC108362653	0.664 (0.08)
LOC108365633	0.66 (0.03)
LOC108359460	0.659 (0.09)
LOC108363802	0.655 (0.03)
LOC108380947	0.647 (0.08)
LOC108360533	0.645 (0.02)
LOC108365957	0.644 (0.02)
LOC108374503	0.643 (0.06)
LOC108381775	0.643 (0.03)
LOC108362667	0.642 (< 0.01)
LOC108362590	0.641 (0.08)
LOC108369490	0.638 (0.04)
LOC108354626	0.638 (0.03)
LOC108357407	0.637 (0.02)
LOC108359782	0.632 (0.01)
LOC108376497	0.63 (0.02)
LOC108373432	0.627 (0.09)

Locus	Log2 fold change (BH p-value)
LOC108366613	0.623 (0.08)
LOC108369528	0.623 (0.03)
LOC108363779	0.619 (0.02)
LOC108373348	0.617 (0.06)
LOC108368473	0.614 (0.05)
LOC108370218	0.61 (0.06)
LOC108381035	0.607 (0.08)
LOC108365788	0.602 (0.03)
LOC108369527	0.601 (0.05)
LOC108364318	0.593 (0.08)
LOC108363629	0.59 (< 0.01)
LOC108364949	0.587 (0.04)
LOC108374469	0.579 (0.07)
LOC108366133	0.577 (0.09)
LOC108371483	0.576 (< 0.01)
LOC108358206	0.574 (0.08)
LOC108365399	0.57 (0.05)
LOC108366718	0.566 (0.07)
LOC108358013	0.564 (< 0.01)
LOC108367088	0.564 (0.03)
LOC108379707	0.563 (0.04)
LOC108360525	0.562 (0.02)
LOC108365895	0.562 (0.05)
LOC108367905	0.557 (< 0.01)
LOC108358316	0.55 (0.06)
LOC108365471	0.549 (0.09)
LOC108367287	0.544 (0.02)
LOC108372615	0.544 (0.04)
LOC108363171	0.534 (0.03)
LOC108367292	0.531 (0.1)
LOC108374019	0.53 (0.04)
LOC108357712	0.529 (0.06)
LOC108358313	0.529 (0.04)
LOC108372050	0.527 (0.06)
LOC108365187	0.524 (< 0.01)
LOC108375817	0.522 (0.1)
LOC108381891	0.521 (< 0.01)
LOC108373181	0.52 (< 0.01)
LOC108363119	0.519 (0.03)
LOC108375471	0.518 (0.07)
LOC108375952	0.512 (0.05)
LOC108373055	0.511 (0.09)

Locus	Log2 fold change (BH p-value)
LOC108376339	0.509 (0.03)
LOC108365068	0.508 (0.01)
LOC108379610	0.502 (0.08)
LOC108368478	0.5 (0.06)
LOC108363266	0.498 (0.05)
LOC108360293	0.495 (< 0.01)
LOC108376967	0.493 (0.08)
LOC108378842	0.492 (0.02)
LOC108359096	0.491 (0.08)
LOC108381953	0.487 (0.02)
LOC108367840	0.471 (0.06)
LOC108364499	0.47 (0.08)
LOC108365034	0.469 (0.06)
LOC108369148	0.468 (< 0.01)
LOC108372290	0.468 (< 0.01)
LOC108371463	0.463 (0.04)
LOC108358342	0.462 (< 0.01)
LOC108357195	0.458 (< 0.01)
LOC108373476	0.452 (0.05)
LOC108377937	0.452 (0.05)
LOC108377311	0.451 (0.03)
LOC108377086	0.45 (0.08)
LOC108358332	0.447 (0.06)
LOC108380433	0.439 (0.06)
LOC108377378	0.438 (0.02)
LOC108363497	0.435 (< 0.01)
LOC108379724	0.43 (0.06)
LOC108371476	0.425 (0.09)
LOC108355684	0.421 (0.06)
LOC108377808	0.421 (0.02)
LOC108374880	0.42 (0.04)
LOC108355017	0.42 (0.1)
LOC108369894	0.419 (0.01)
LOC108372614	0.417 (0.08)
LOC108363028	0.415 (0.08)
LOC108359997	0.407 (0.05)
LOC108360938	0.405 (0.03)
LOC108370823	0.402 (0.06)
LOC108381173	0.392 (< 0.01)
LOC108375941	0.392 (0.04)
LOC108363732	0.392 (0.08)
LOC108361336	0.389 (< 0.01)

Locus	Log2 fold change (BH p-value)
LOC108368512	0.386 (0.09)
LOC108361260	0.385 (0.07)
LOC108366569	0.381 (0.05)
LOC108379504	0.379 (0.04)
LOC108366600	0.378 (0.08)
LOC108376734	0.377 (0.09)
LOC108371863	0.374 (< 0.01)
LOC108359555	0.372 (0.02)
LOC108377302	0.371 (0.08)
LOC108365435	0.369 (0.07)
LOC108370241	0.364 (0.07)
LOC108375518	0.364 (0.04)
LOC108368750	0.362 (0.03)
LOC108363991	0.362 (0.06)
LOC108377683	0.36 (0.01)
LOC108368731	0.358 (0.08)
LOC108379124	0.358 (0.1)
LOC108357954	0.349 (0.09)
LOC108358676	0.343 (0.04)
LOC108376458	0.342 (< 0.01)
LOC108375868	0.335 (0.07)
LOC108377001	0.334 (0.08)
LOC108361553	0.325 (0.02)
LOC108371626	0.325 (0.07)
LOC108378290	0.321 (0.07)
LOC108365797	0.32 (0.07)
LOC108371274	0.319 (0.01)
LOC108363986	0.317 (0.07)
LOC108365024	0.315 (< 0.01)
LOC108363131	0.315 (0.04)
LOC108360330	0.314 (0.09)
LOC108356846	0.313 (0.09)
LOC108359488	0.312 (0.04)
LOC108359442	0.302 (0.08)
LOC108364436	0.302 (0.03)
LOC108360144	0.301 (0.07)
LOC108358595	0.299 (0.08)
LOC108368146	0.295 (0.09)
LOC108381528	0.293 (0.04)
LOC108371759	0.293 (0.06)
LOC108362727	0.29 (0.05)
LOC108364171	0.284 (0.07)

Locus	Log2 fold change (BH p-value)
LOC108363531	0.282 (0.04)
LOC108369241	0.281 (0.08)
LOC108363170	0.28 (0.02)
LOC108382356	0.279 (0.05)
LOC108380998	0.27 (0.08)
LOC108360642	0.269 (0.07)
LOC108361964	0.265 (0.08)
LOC108363996	0.262 (0.09)
LOC108375053	0.261 (0.1)
LOC108366655	0.26 (0.09)
LOC108376800	0.254 (< 0.01)
LOC108365072	0.252 (0.09)
LOC108365357	0.247 (0.06)
LOC108365890	0.24 (0.03)
LOC108366517	0.239 (0.08)
LOC108366409	0.23 (0.05)
LOC108360490	0.225 (0.03)
LOC108372291	0.224 (0.01)
LOC108365924	0.219 (0.08)
LOC108361076	0.213 (0.09)
LOC108363150	0.194 (< 0.01)
LOC108365175	0.193 (< 0.01)
LOC108362043	0.18 (0.05)
LOC108361556	-0.18 (0.02)
LOC108369938	-0.19 (0.1)
LOC108371324	-0.208 (0.06)
LOC108365418	-0.227 (0.03)
LOC108371488	-0.23 (0.06)
LOC108376876	-0.231 (0.06)
LOC108369785	-0.231 (0.07)
LOC108366767	-0.232 (0.05)
LOC108359974	-0.239 (0.06)
LOC108371611	-0.249 (0.07)
LOC108361494	-0.26 (0.06)
LOC108365025	-0.27 (0.06)
LOC108375360	-0.272 (0.04)
LOC108373327	-0.274 (0.07)
LOC108375962	-0.275 (0.07)
LOC108359778	-0.277 (0.04)
LOC108368188	-0.279 (0.02)
LOC108380078	-0.28 (0.08)
LOC108372194	-0.283 (0.05)

Locus	Log2 fold change (BH p-value)
LOC108367158	-0.284 (0.05)
LOC108376629	-0.289 (0.04)
LOC108368854	-0.29 (0.08)
LOC108376401	-0.295 (< 0.01)
LOC108363614	-0.295 (0.08)
LOC108358969	-0.296 (0.04)
LOC108354882	-0.302 (0.09)
LOC108370186	-0.302 (0.06)
LOC108382898	-0.306 (0.08)
LOC108359083	-0.306 (0.07)
LOC108377809	-0.307 (0.04)
LOC108368540	-0.318 (0.08)
LOC108378995	-0.318 (0.09)
LOC108369966	-0.32 (0.03)
LOC108357907	-0.322 (0.01)
LOC108382778	-0.322 (0.04)
LOC108363273	-0.323 (0.03)
LOC108355901	-0.325 (0.1)
LOC108370290	-0.327 (0.09)
LOC108378988	-0.327 (0.04)
LOC108382735	-0.328 (0.09)
LOC108359271	-0.329 (0.07)
LOC108368708	-0.331 (0.02)
LOC108359524	-0.331 (0.02)
LOC108375419	-0.333 (0.06)
LOC108355246	-0.337 (0.06)
LOC108371156	-0.339 (< 0.01)
LOC108359370	-0.34 (0.06)
LOC108371133	-0.34 (0.04)
LOC108373935	-0.341 (0.01)
LOC108369075	-0.341 (< 0.01)
LOC108367068	-0.341 (0.04)
LOC108360214	-0.344 (0.1)
LOC108365757	-0.344 (0.02)
LOC108378504	-0.347 (0.08)
LOC108360124	-0.348 (0.03)
LOC108379589	-0.354 (0.02)
LOC108378445	-0.355 (0.06)
LOC108361998	-0.36 (0.07)
LOC108373434	-0.364 (0.08)
LOC108367281	-0.365 (0.02)
LOC108356360	-0.369 (0.05)
LOC108379263	-0.369 (0.08)
LOC108354943	-0.374 (0.04)
LOC108359565	-0.375 (0.09)
LOC108364054	-0.376 (0.09)
LOC108354162	-0.379 (0.02)
LOC108372378	-0.386 (0.03)
LOC108380013	-0.389 (0.09)
LOC108375483	-0.394 (0.03)
LOC108364760	-0.397 (< 0.01)
LOC108380631	-0.402 (< 0.01)
LOC108380722	-0.402 (0.1)
LOC108365026	-0.403 (< 0.01)
LOC108363833	-0.405 (0.03)
LOC108373433	-0.411 (0.08)
LOC108377241	-0.412 (0.04)
LOC108368204	-0.415 (0.03)
LOC108358911	-0.415 (0.07)
LOC108365758	-0.416 (< 0.01)
LOC108366065	-0.417 (0.05)
LOC108382511	-0.417 (0.07)
LOC108365361	-0.418 (0.03)
LOC108362546	-0.419 (0.08)
LOC108380813	-0.421 (< 0.01)
LOC108366511	-0.422 (0.08)
LOC108369418	-0.424 (< 0.01)
LOC108360874	-0.432 (0.07)
LOC108374000	-0.439 (0.02)
LOC108371827	-0.448 (0.08)
LOC108371875	-0.457 (0.09)
LOC108376422	-0.461 (0.09)
LOC108357175	-0.465 (0.01)
LOC108358516	-0.468 (< 0.01)
LOC108367733	-0.469 (0.03)
LOC108371369	-0.471 (0.05)
LOC108361372	-0.479 (0.02)
LOC108363580	-0.481 (0.04)
LOC108364785	-0.482 (0.08)
LOC108378581	-0.494 (< 0.01)
LOC108371240	-0.495 (0.02)
LOC108362798	-0.501 (0.06)
LOC108371902	-0.502 (0.04)
LOC108354506	-0.505 (0.08)

Locus	Log2 fold change (BH p-value)
LOC108368775	-0.508 (0.06)
LOC108363781	-0.51 (0.04)
LOC108360505	-0.51 (0.02)
LOC108371154	-0.513 (< 0.01)
LOC108380160	-0.515 (0.06)
LOC108370662	-0.518 (0.04)
LOC108374916	-0.522 (0.04)
LOC108356140	-0.53 (0.08)
LOC108376767	-0.537 (< 0.01)
LOC108375740	-0.539 (< 0.01)
LOC108366720	-0.539 (0.05)
LOC108365021	-0.544 (0.09)
LOC108354802	-0.55 (0.04)
LOC108363299	-0.559 (< 0.01)
LOC108366730	-0.559 (0.07)
LOC108366333	-0.559 (0.05)
LOC108370581	-0.56 (0.06)
LOC108370947	-0.56 (0.03)
LOC108376796	-0.562 (0.07)
LOC108360307	-0.574 (0.04)
LOC108366723	-0.578 (0.09)
LOC108376968	-0.581 (0.1)
LOC108367245	-0.584 (< 0.01)
LOC108374877	-0.59 (0.09)
LOC108375217	-0.594 (0.08)
LOC108358735	-0.594 (0.06)
LOC108364939	-0.595 (0.02)
LOC108373401	-0.596 (0.06)
LOC108366048	-0.597 (< 0.01)
LOC108360785	-0.598 (< 0.01)
LOC108361439	-0.604 (0.04)
LOC108374407	-0.604 (< 0.01)
LOC108358984	-0.604 (0.07)
LOC108376413	-0.608 (0.01)
LOC108372170	-0.611 (< 0.01)
LOC108369400	-0.619 (0.09)
LOC108373781	-0.627 (0.03)
LOC108361622	-0.628 (0.05)
LOC108377678	-0.629 (0.01)
LOC108374666	-0.635 (0.09)
LOC108358804	-0.64 (0.1)
LOC108369084	-0.643 (0.06)
LOC108360482	-0.644 (0.06)
LOC108366638	-0.65 (< 0.01)
LOC108366515	-0.651 (0.02)
LOC108369863	-0.655 (0.09)
LOC108360556	-0.658 (0.01)
LOC108361427	-0.66 (0.05)
LOC108382429	-0.663 (0.01)
LOC108363596	-0.664 (0.02)
LOC108366588	-0.67 (0.07)
LOC108369492	-0.676 (0.02)
LOC108367730	-0.678 (0.07)
LOC108371051	-0.682 (0.02)
LOC108375591	-0.684 (0.08)
LOC108361637	-0.684 (0.02)
LOC108358321	-0.686 (0.01)
LOC108369639	-0.687 (0.02)
LOC108369377	-0.69 (0.07)
LOC108356283	-0.691 (0.07)
LOC108370703	-0.698 (< 0.01)
LOC108376642	-0.699 (< 0.01)
LOC108368288	-0.707 (0.06)
LOC108370258	-0.709 (0.07)
LOC108364852	-0.71 (< 0.01)
LOC108356099	-0.711 (0.09)
LOC108374394	-0.712 (0.05)
LOC108372200	-0.713 (0.07)
LOC108368721	-0.713 (< 0.01)
LOC108360555	-0.714 (< 0.01)
LOC108376393	-0.714 (0.07)
LOC108376317	-0.715 (0.06)
LOC108375452	-0.715 (0.08)
LOC108380800	-0.716 (0.02)
LOC108360729	-0.718 (0.1)
LOC108356672	-0.724 (0.05)
LOC108370629	-0.725 (< 0.01)
LOC108367077	-0.726 (< 0.01)
LOC108361056	-0.727 (0.09)
LOC108373693	-0.727 (0.09)
LOC108363709	-0.731 (0.08)
LOC108364545	-0.736 (0.09)
LOC108377422	-0.737 (0.04)
LOC108367195	-0.739 (< 0.01)

Locus	Log2 fold change (BH p-value)
LOC108367036	-0.739 (0.07)
LOC108381685	-0.739 (0.08)
LOC108363821	-0.739 (0.09)
LOC108357445	-0.741 (0.04)
LOC108356790	-0.745 (0.04)
LOC108372850	-0.749 (0.08)
LOC108365518	-0.752 (0.04)
LOC108360727	-0.753 (0.06)
LOC108371795	-0.753 (0.06)
LOC108363263	-0.754 (0.06)
LOC108368252	-0.755 (0.08)
LOC108364307	-0.756 (0.01)
LOC108362439	-0.758 (0.08)
LOC108364536	-0.759 (0.06)
LOC108356872	-0.759 (< 0.01)
LOC108365569	-0.762 (0.06)
LOC108377634	-0.764 (0.08)
LOC108374079	-0.764 (0.01)
LOC108363401	-0.776 (0.02)
LOC108355572	-0.776 (0.07)
LOC108359001	-0.778 (0.04)
LOC108378485	-0.781 (< 0.01)
LOC108373628	-0.782 (< 0.01)
LOC108380407	-0.783 (0.01)
LOC108368173	-0.786 (0.05)
LOC108378949	-0.787 (0.05)
LOC108364588	-0.789 (< 0.01)
LOC108354534	-0.792 (0.06)
LOC108378102	-0.793 (0.04)
LOC108368277	-0.794 (0.06)
LOC108365570	-0.794 (0.04)
LOC108365347	-0.795 (< 0.01)
LOC108369453	-0.795 (0.06)
LOC108354856	-0.803 (0.05)
LOC108358762	-0.804 (0.05)
LOC108369903	-0.804 (< 0.01)
LOC108372790	-0.811 (0.05)
LOC108356213	-0.818 (0.01)
LOC108372599	-0.829 (0.04)
LOC108368766	-0.837 (0.03)
LOC108366587	-0.841 (0.03)
LOC108375376	-0.856 (0.03)
LOC108368550	-0.858 (0.02)
LOC108362845	-0.862 (0.03)
LOC108382193	-0.862 (0.03)
LOC108364773	-0.871 (0.02)
LOC108364716	-0.873 (0.02)
LOC108371427	-0.877 (0.02)
LOC108369807	-0.877 (0.02)
LOC108381153	-0.891 (0.01)
LOC108358985	-0.912 (0.02)
LOC108365140	-0.912 (0.02)
LOC108365179	-0.916 (< 0.01)
LOC108355890	-0.918 (< 0.01)
LOC108381402	-0.92 (0.02)
LOC108382112	-0.923 (< 0.01)
LOC108362376	-0.932 (0.02)
LOC108372334	-0.934 (< 0.01)
LOC108360719	-0.937 (< 0.01)
LOC108364774	-0.947 (< 0.01)
LOC108373963	-0.964 (0.01)
LOC108359898	-0.965 (0.01)
LOC108382192	-0.968 (0.01)
LOC108374915	-0.981 (< 0.01)
LOC108371553	-0.983 (< 0.01)
LOC108369901	-1.032 (< 0.01)
LOC108375790	-1.062 (< 0.01)
LOC108363787	-1.089 (< 0.01)
LOC108380477	-1.133 (< 0.01)
LOC108376609	-1.134 (< 0.01)
LOC108366614	-1.245 (< 0.01)
LOC108369817	-1.246 (< 0.01)
LOC108376920	-1.325 (< 0.01)
LOC108370410	-1.359 (< 0.01)
LOC108378596	-1.56 (< 0.01)

Appendix Table 3. Log2 fold change of genes significantly differentially expressed (Benjamini-Hochberg (BH) p-value < 0.1) only between high treated (~85% relative humidity) Yakima flies relative to low treated Whatcom flies. Positive log fold change values indicate upregulation and negative values indicate downregulation in Yakima flies.

Locus	Log2 fold change (BH p-value)
LOC108370720	1.579 (< 0.01)
LOC108381897	1.274 (< 0.01)
LOC108359744	1.225 (< 0.01)
LOC108383213	1.196 (< 0.01)
LOC108380723	1.158 (< 0.01)
LOC108369242	1.065 (< 0.01)
LOC108358220	1.055 (< 0.01)
LOC108378229	1.049 (< 0.01)
LOC108363040	1.019 (< 0.01)
LOC108369976	1.018 (< 0.01)
LOC108374586	1.014 (< 0.01)
LOC108360030	0.998 (0.01)
LOC108359562	0.96 (< 0.01)
LOC108372410	0.946 (0.02)
LOC108368338	0.907 (0.03)
LOC108361844	0.905 (0.02)
LOC108357773	0.903 (0.03)
LOC108371291	0.878 (< 0.01)
LOC108361842	0.87 (0.03)
LOC108373406	0.856 (0.02)
LOC108363784	0.847 (0.04)
LOC108366533	0.841 (0.01)
LOC108364535	0.841 (0.04)
LOC108355523	0.831 (0.06)
LOC108379988	0.825 (0.06)
LOC108365352	0.825 (< 0.01)
LOC108358024	0.816 (0.02)
LOC108363762	0.808 (0.06)
LOC108379705	0.804 (0.05)
LOC108359224	0.801 (0.07)
LOC108356280	0.794 (0.08)
LOC108362878	0.794 (0.04)
LOC108370320	0.774 (0.03)
LOC108363376	0.772 (0.08)
LOC108359250	0.767 (0.07)
LOC108372564	0.767 (0.07)
LOC108375403	0.764 (0.08)
LOC108355205	0.761 (0.02)
LOC108363851	0.761 (0.08)
LOC108371941	0.756 (0.1)
LOC108368108	0.747 (0.02)
LOC108361353	0.734 (0.09)
LOC108377879	0.728 (0.1)
LOC108358042	0.728 (0.1)
LOC108369240	0.724 (0.09)
LOC108374965	0.699 (0.09)
LOC108357367	0.684 (0.09)
LOC108364326	0.668 (0.08)
LOC108372871	0.665 (0.02)
LOC108354407	0.659 (0.06)
LOC108367228	0.645 (0.04)
LOC108374380	0.639 (0.02)
LOC108361736	0.625 (0.04)
LOC108365750	0.625 (0.1)
LOC108370529	0.62 (0.08)
LOC108369321	0.619 (< 0.01)
LOC108364709	0.595 (0.01)
LOC108369225	0.587 (0.09)
LOC108374381	0.578 (0.06)
LOC108366797	0.543 (0.01)
LOC108379305	0.539 (0.09)
LOC108359123	0.526 (0.07)
LOC108369263	0.524 (0.06)
LOC108362631	0.515 (0.09)
LOC108360003	0.508 (0.03)
LOC108362093	0.507 (0.03)
LOC108375966	0.506 (0.02)
LOC108355153	0.499 (0.03)
LOC108375042	0.49 (0.03)
LOC108378903	0.49 (0.04)
LOC108359788	0.486 (0.04)
LOC108374718	0.485 (0.05)

Locus	Log2 fold change (BH p-value)
LOC108360320	0.474 (0.09)
LOC108380524	0.471 (0.09)
LOC108369198	0.466 (0.08)
LOC108376779	0.466 (0.07)
LOC108369086	0.457 (0.07)
LOC108374171	0.451 (0.05)
LOC108366522	0.445 (0.08)
LOC108378670	0.442 (0.08)
LOC108367360	0.411 (0.03)
LOC108372224	0.411 (0.06)
LOC108355133	0.408 (0.07)
LOC108373980	0.407 (0.07)
LOC108372103	0.405 (0.1)
LOC108363007	0.4 (0.09)
LOC108361320	0.4 (0.03)
LOC108366450	0.398 (0.03)
LOC108375522	0.389 (0.01)
LOC108365975	0.362 (0.1)
LOC108366858	0.357 (0.06)
LOC108375778	0.354 (0.09)
LOC108367571	0.344 (0.07)
LOC108371130	0.341 (0.1)
LOC108360297	0.341 (0.1)
LOC108372399	0.336 (0.03)
LOC108362715	0.326 (0.04)
LOC108365341	0.326 (0.09)
LOC108381957	0.316 (0.1)
LOC108360858	0.309 (0.1)
LOC108361154	0.287 (0.04)
LOC108359084	0.287 (0.08)
LOC108360460	0.243 (0.02)
LOC108364923	0.22 (0.08)
LOC108363591	-0.234 (0.08)
LOC108379628	-0.267 (0.09)
LOC108374399	-0.28 (0.05)
LOC108371201	-0.281 (0.06)
LOC108356580	-0.293 (0.08)
LOC108358977	-0.304 (0.08)
LOC108363020	-0.305 (0.02)
LOC108374684	-0.328 (0.06)
LOC108375556	-0.329 (0.05)

Locus	Log2 fold change (BH p-value)
LOC108375162	-0.345 (0.02)
LOC108363148	-0.349 (0.04)
LOC108370546	-0.358 (0.09)
LOC108374088	-0.358 (0.05)
LOC108367136	-0.36 (0.01)
LOC108369431	-0.379 (0.09)
LOC108372852	-0.38 (0.09)
LOC108368628	-0.381 (0.02)
LOC108370322	-0.382 (0.09)
LOC108360377	-0.389 (0.08)
LOC108362141	-0.392 (< 0.01)
LOC108364915	-0.399 (0.01)
LOC108363862	-0.403 (0.09)
LOC108371392	-0.411 (0.06)
LOC108371504	-0.416 (0.02)
LOC108359538	-0.426 (0.01)
LOC108361086	-0.428 (0.05)
LOC108360565	-0.433 (0.08)
LOC108355486	-0.446 (0.1)
LOC108370711	-0.448 (0.08)
LOC108371647	-0.452 (0.06)
LOC108367139	-0.463 (0.02)
LOC108360532	-0.468 (0.07)
LOC108375248	-0.468 (0.09)
LOC108377349	-0.47 (< 0.01)
LOC108373448	-0.478 (0.03)
LOC108360698	-0.482 (0.05)
LOC108373149	-0.501 (0.02)
LOC108363280	-0.521 (0.06)
LOC108370317	-0.524 (0.06)
LOC108367530	-0.524 (0.02)
LOC108367910	-0.531 (0.02)
LOC108364271	-0.535 (0.02)
LOC108368356	-0.539 (0.08)
LOC108363798	-0.541 (0.08)
LOC108353943	-0.543 (0.08)
LOC108371005	-0.545 (< 0.01)
LOC108368151	-0.552 (< 0.01)
LOC108366164	-0.557 (0.02)
LOC108376966	-0.561 (0.08)
LOC108360414	-0.565 (0.09)

Locus	Log2 fold change (BH p-value)
LOC108377122	-0.574 (0.09)
LOC108369156	-0.588 (< 0.01)
LOC108372517	-0.594 (0.09)
LOC108363706	-0.597 (0.06)
LOC108380819	-0.61 (0.02)
LOC108357293	-0.623 (0.09)
LOC108379209	-0.628 (0.08)
LOC108371577	-0.629 (0.04)
LOC108365850	-0.638 (0.02)
LOC108370063	-0.641 (< 0.01)
LOC108372613	-0.656 (< 0.01)
LOC108355274	-0.679 (0.02)
LOC108372437	-0.697 (< 0.01)
LOC108368679	-0.701 (0.07)
LOC108373346	-0.701 (0.05)
LOC108372015	-0.707 (0.02)
LOC108373736	-0.712 (0.03)
LOC108369406	-0.72 (0.06)
LOC108377138	-0.722 (< 0.01)
LOC108377891	-0.725 (< 0.01)
LOC108367628	-0.725 (0.1)
LOC108376643	-0.728 (0.09)
LOC108359020	-0.729 (0.06)
LOC108360095	-0.732 (< 0.01)
LOC108355461	-0.735 (< 0.01)
LOC108369889	-0.747 (0.08)
LOC108361676	-0.753 (0.05)
LOC108371669	-0.753 (0.05)
LOC108354551	-0.756 (< 0.01)
LOC108371335	-0.76 (0.08)
LOC108371090	-0.76 (0.1)
LOC108365725	-0.77 (0.1)
LOC108359393	-0.776 (0.06)
LOC108369307	-0.779 (0.08)
LOC108353964	-0.78 (0.09)
LOC108374735	-0.785 (0.03)
LOC108374254	-0.785 (0.02)
LOC108372193	-0.789 (0.03)
LOC108373735	-0.79 (0.02)
LOC108374792	-0.791 (0.06)
LOC108356578	-0.795 (0.05)
LOC108373379	-0.801 (0.04)
LOC108356312	-0.802 (0.04)
LOC108361846	-0.816 (0.07)
LOC108358021	-0.822 (0.06)
LOC108366462	-0.823 (0.06)
LOC108358835	-0.829 (0.06)
LOC108368182	-0.83 (0.06)
LOC108373089	-0.834 (0.04)
LOC108372817	-0.844 (0.05)
LOC108355920	-0.848 (0.02)
LOC108359076	-0.849 (< 0.01)
LOC108366272	-0.85 (0.05)
LOC108380352	-0.851 (0.02)
LOC108381946	-0.86 (0.05)
LOC108360792	-0.863 (0.01)
LOC108354691	-0.871 (0.03)
LOC108360833	-0.874 (0.04)
LOC108358271	-0.878 (0.01)
LOC108375349	-0.886 (< 0.01)
LOC108361437	-0.888 (0.01)
LOC108377441	-0.889 (< 0.01)
LOC108376126	-0.904 (0.03)
LOC108370774	-0.905 (0.01)
LOC108379133	-0.916 (0.02)
LOC108355827	-0.949 (0.02)
LOC108354231	-0.964 (0.02)
LOC108369922	-0.969 (< 0.01)
LOC108360583	-0.975 (0.02)
LOC108371295	-0.977 (< 0.01)
LOC108375143	-0.98 (0.02)
LOC108374268	-0.991 (0.01)
LOC108365701	-1.005 (< 0.01)
LOC108381094	-1.02 (< 0.01)
LOC108364005	-1.033 (< 0.01)
LOC108369063	-1.045 (< 0.01)
LOC108368956	-1.085 (< 0.01)
LOC108376868	-1.272 (< 0.01)

Appendix Table 3. Log2 fold change of genes significantly differentially expressed (Benjamini-Hochberg (BH) p-value < 0.1) only between low treated (~43% RH) and high treated (~85% RH) flies from either Yakima or Whatcom. Positive log fold change values indicate upregulation and negative values indicate downregulation in low treated flies for the indicated population.

	Locus	Log2 fold change (BH p-value)
Whatcom	LOC108364005	-1.033 (< 0.01)
	LOC108369063	-1.045 (< 0.01)
	LOC108368956	-1.085 (< 0.01)
	LOC108376868	-1.272 (< 0.01)
Yakima	LOC108362368	1.263 (< 0.01)
	LOC108378911	1.171 (< 0.01)
	LOC108358104	1.076 (< 0.01)
	LOC108373887	1.012 (0.01)
	LOC108367143	0.987 (0.01)
	LOC108370009	0.968 (0.03)
	LOC108379025	0.962 (0.03)
	LOC108359377	0.927 (0.01)
	LOC108358106	0.925 (0.05)
	LOC108372702	0.913 (0.03)
	LOC108368817	0.912 (0.05)
	LOC108365809	0.895 (0.06)
	LOC108367732	0.883 (0.05)
	LOC108381411	0.872 (0.07)
	LOC108372959	0.868 (0.05)
	LOC108369902	0.867 (0.08)
	LOC108363807	0.852 (0.06)
	LOC108372459	0.838 (0.1)
	LOC108378354	0.838 (0.06)
	LOC108382710	0.82 (0.1)
	LOC108378523	0.806 (0.03)
	LOC108359507	0.791 (0.08)
	LOC108357128	0.786 (0.1)
	LOC108363257	0.783 (0.05)
	LOC108362002	0.778 (0.1)
	LOC108364295	0.719 (0.07)
	LOC108363197	0.707 (0.08)
	LOC108380864	0.706 (0.06)
	LOC108363675	0.688 (0.08)
	LOC108361682	0.665 (0.05)
	LOC108354081	0.663 (0.03)

	Locus	Log2 fold change (BH p-value)
Yakima	LOC108377878	0.654 (0.03)
	LOC108364165	0.623 (0.07)
	LOC108374270	0.617 (0.06)
	LOC108365505	0.603 (< 0.01)
	LOC108375555	0.592 (0.05)
	LOC108377820	0.587 (0.08)
	LOC108363117	0.583 (0.1)
	LOC108364830	0.568 (0.1)
	LOC108376093	0.564 (0.04)
	LOC108376834	0.494 (0.05)
	LOC108359933	0.489 (0.05)
	LOC108358099	0.463 (0.1)
	LOC108372920	0.419 (0.07)
	LOC108380185	0.41 (0.03)
	LOC108363774	0.383 (0.02)
	LOC108368076	0.36 (0.07)
	LOC108382473	0.34 (0.07)
	LOC108374930	0.336 (0.08)
	LOC108360092	0.311 (0.04)
	LOC108360292	0.31 (0.05)
	LOC108359936	0.296 (0.09)
	LOC108367106	0.259 (0.04)
	LOC108375560	-0.325 (0.02)
	LOC108372794	-0.347 (0.07)
	LOC108366759	-0.405 (0.09)
	LOC108361579	-0.428 (0.1)
	LOC108380467	-0.431 (0.03)
	LOC108376056	-0.436 (0.1)
	LOC108360876	-0.438 (0.03)
	LOC108364330	-0.458 (0.03)
	LOC108366028	-0.466 (0.09)
	LOC108371658	-0.482 (0.02)
	LOC108374812	-0.525 (0.09)
	LOC108374196	-0.542 (0.04)
	LOC108364500	-0.546 (0.03)
	LOC108381158	-0.567 (0.07)

	Locus	Log2 fold change (BH p-value)
Yakima	LOC108378194	-0.631 (0.03)
	LOC108371502	-0.647 (0.05)
	LOC108366971	-0.647 (0.02)
	LOC108375700	-0.703 (0.06)
	LOC108382957	-0.738 (0.1)
	LOC108360875	-0.74 (0.03)
	LOC108372204	-0.75 (0.05)
	LOC108378793	-0.783 (0.09)
	LOC108356782	-0.805 (< 0.01)
	LOC108357871	-0.805 (0.09)
	LOC108371624	-0.822 (0.08)
	LOC108360771	-0.823 (0.1)
	LOC108373770	-0.828 (0.08)
	LOC108356413	-0.828 (0.08)
	LOC108373105	-0.837 (0.1)
	LOC108380751	-0.843 (0.08)
	LOC108370442	-0.885 (0.04)
	LOC108376972	-0.891 (0.05)
	LOC108382748	-0.919 (< 0.01)
	LOC108382203	-0.92 (0.05)
	LOC108372709	-0.922 (0.02)
	LOC108376080	-0.941 (0.02)
	LOC108380861	-0.991 (0.02)
	LOC108369712	-1.008 (< 0.01)
	LOC108379837	-1.11 (< 0.01)