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Research

Distinct Adult Eclosion Traits of Sibling Species *Rhagoletis pomonella* and *Rhagoletis zephyria* (Diptera: Tephritidae) Under Laboratory Conditions

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Abstract

Closely related phytophagous insects that specialize on different host plants may have divergent responses to environmental factors. *Rhagoletis pomonella* (Walsh) and *Rhagoletis zephyria* Snow (Diptera: Tephritidae) are sibling, sympatric fly species found in western North America that attack and mate on plants of Rosaceae (~60 taxa) and Caprifoliaceae (three taxa), respectively, likely contributing to partial reproductive isolation. *Rhagoletis zephyria* evolved from *R. pomonella* and is native to western North America, whereas *R. pomonella* was introduced there. Given that key features of the flies' ecology, breeding compatibility, and evolution differ, we predicted that adult eclosion patterns of the two flies from Washington State, USA are also distinct. When puparia were chilled, eclosion of apple- and black hawthorn-origin *R. pomonella* was significantly more dispersed, with less pronounced peaks, than of snowberry-origin *R. zephyria* within sympatric and nonsympatric site comparisons. Percentages of chilled puparia that produced adults were ≥67% for both species. However, when puparia were not chilled, from 13.5 to 21.9% of apple-origin *R. pomonella* versus only 1.2% to 1.9% of *R. zephyria* eclosed. The distinct differences in eclosion traits of *R. pomonella* and *R. zephyria* could be due to greater genetic variation in *R. pomonella*, associated with its use of a wider range of host plants than *R. zephyria*.

Key words: apple maggot fly, apple, snowberry, dispersion, diapause

Phytophagous insects that are closely related but specialize on different hosts or that evolved in different regions may become reproductively isolated and have divergent responses to environmental factors. Documenting these responses can help us better understand how insect genetics, physiology, and ecology interact to produce observed adaptive morphological or ecological traits. They could also help us understand consequences of specialization on host plants and ultimately how insects speciate. Within frugivorous insects, flies in the genus *Rhagoletis* (Diptera: Tephritidae) are model organisms for understanding relationships among various factors and speciation processes. Key among these factors is synchronization of eclosion timing with host fruit phenology, a prerequisite for host race formation in the best studied *Rhagoletis* species, the apple maggot fly, *Rhagoletis pomonella* (Walsh) (e.g., Feder et al. 1993, 1997).

Rhagoletis pomonella and *Rhagoletis zephyria* Snow are sibling species (Bush 1966) that are found sympatrically in western North America (Yee and Klaus 2015). The two flies are very similar morphologically (Bush 1966) and genetically, as diagnostic genetic markers for distinguishing them remain elusive (Xie et al. 2008), although single nucleotide polymorphisms can distinguish the two species as distinct genotypic clusters (Doellman et al. 2020). Both *R. pomonella* and *R. zephyria* are univoltine, having one major generation per year and eclose as adults in summer (Dean and Chapman 1973, Tracewski and Brunner 1987).

Beyond these similarities, there are major ecological differences between the species that could affect their responses to environmental stimuli. One is that they are adapted to attack and mate on dissimilar plants with varying fruiting phenologies. Specifically, *R. pomonella* attacks ≥60 plant taxa only in the Rosaceae, mostly *Crataegus* and *Malus* spp. (Bush 1966, Yee and Norrbom 2017). In contrast, *R. zephyria* is known to attack only three members of Caprifoliaceae: the snowberries *Symphoricarpos albus* var. *laevigatus* (Fernald) S. F. Blake in western North America and *S. albus* var. *albus* (L.) S. F. Blake and *S. occidentalis* Hooker in eastern North America (Smith and Bush 2000, Gavrilovic et al. 2007). As far as known, the flies share no common hosts.

Host range is one key factor for understanding major aspects of *Rhagoletis* ecology. Fly use of many plant species could result in high genetic diversity (Berlocher 1995), or the reverse, in that high genetic diversity promotes use of many plant species. Although which scenario occurred is debatable, higher genetic diversity could be a reason *R. pomonella* in the eastern United States was able to shift from *Crataegus* to domestic apple ~160 yr ago, developing into a genetically distinct apple race (Feder et al. 2003). There are no known host races of *R. zephyria*, suggesting less genetic variation in this fly. Partial reproductive isolation between the species (Yee and Goughnour 2011, Hood et al. 2015) is likely one consequence of genetic variation and adaptation to different host plants for adult breeding and larval feeding. The hybridization rate between the species in the eastern United States is ~0.1% per generation (Feder et al. 1999); in Washington State, USA, ~1.44% (Arcella et al. 2015) or ~0.1% (Doellman et al. 2020).

A related factor for understanding ecological differences as well as reproductive isolation between *R. pomonella* and *R. zephyria* is that the flies evolved in different regions and climates. *Rhagoletis pomonella* was introduced into western North America, probably from the eastern United States before 1979 (AliNiazee and Penrose 1981). In contrast, *R. zephyria* is native to western North America (Bush 1966, Hood et al. 2013) and possibly the Great Lakes region in the central United States (Gavrilovic et al. 2007). Genetic evidence suggests that *R. zephyria* evolved from a subpopulation of *Crataegus*-infesting *R. pomonella* in Mexico with latitudinal diapause variation that moved into the colder (what would become) United States and there altered its host discrimination, shifting, and adapting to *Symphoricarpos*, giving rise to *R. zephyria* (Xie et al. 2008).

Rhagoletis pomonella and *R. zephyria* may also differ in their eclosion or diapause responses to warm temperatures. *Rhagoletis pomonella* from different populations across North America do not require chilling to eclose (e.g., Hall 1937, Neilson 1962, Baerwald and Mallory Boush 1967, Prokopy 1968, Dean and Chapman 1973). For example, 55–85% of nonchilled apple-origin *R. pomonella* puparia in Oregon, USA produced adults (AliNiazee 1988). In contrast, <1% of nonchilled *R. zephyria* puparia from Washington State produced adults (Tracewski and Brunner 1987), although 55–75% of nonchilled puparia of *R. zephyria* from Oregon did so (AliNiazee 1988), with reasons for different reported responses unknown. Verification of *R. zephyria* responses to no-chill conditions needs further study.

Given that key features of the flies' ecology, breeding compatibility, and evolution differ, it can be hypothesized that genetically programmed and environmentally triggered eclosion times of *R. pomonella* and *R. zephyria* have diverged during evolution. Eclosion traits of *R. pomonella* after chilling, as well as no-chill, controlled conditions have been documented (e.g., Neilson 1962, Feder et al. 1997, Lyons-Sobaski and Berlocher 2009, Rull et al. 2016), as have eclosion timing of *R. pomonella* from apple and hawthorn and *R. zephyria* in the eastern United States (Hood et al. 2015). Despite this, eclosion distributions as opposed to timing alone of *R. zephyria* have not been documented nor compared with those of *R. pomonella*. In this study, we characterize eclosion time distributions and diapause responses for *R. pomonella* and *R. zephyria* pupae exposed to different temperatures. Results generated here for eclosion traits under controlled laboratory conditions are compared and discussed in reference to known fly eclosion and activity periods in the field.

Materials and Methods

Study Sites and Fruit Collections

Fly puparia used in experiments originated in apple (*Malus domestica* Borkhausen), black hawthorn (*Crataegus douglasii* Lindley), and snowberry (*S. albus* var. *laevigatus*) fruit collected at sympatric and nonsympatric sites in Washington State in 2016, 2017, and 2018 in ecosystems ranging from sagebrush-bunchgrass to ponderosa pine to coast forest (Lyons and Merilees 1995) (Supp Table 1 [online only]). Sympatric sites were those where host plants of the two flies were <1.6 km apart. Apple sites were in backyards or in parks; hawthorn sites were in rural or urban areas; while snowberry sites were sympatric with either apple and/or hawthorn sites (Saint Cloud, Vancouver, Nile, and Ronald). Earlier (late July to early August) ripening apples were collected in Woodland and Vancouver, while later (late August to early September) ripening apples were collected in Centralia, BZ Corner, and Saint Cloud. Fallen apples were sampled from the ground beneath trees on dates from 28 July to 11 September (range) in all 3 yr. Black hawthorns ripened and were collected off trees from 8 to 17 August in Ronald and Nile in 2 yr. Depending on the site, snowberries were collected off bushes from 9 to 31 August in all 3 yr, with two exceptions: 5 September 2018 at Saint Cloud and 10 October 2018 at Vancouver, the two latest snowberry collections. Snowberries were more widespread and easier to collect than apples and hawthorns and produced more larvae than both, accounting for greater sample sizes of *R. zephyria* in the experiments.

Pupal Collections

All fruit types were placed on hardware cloth (1.3 × 1.3 cm openings) suspended on rubber tubs to allow fly larvae to emerge. For sympatric and nonsympatric site comparisons in 2017 and 2018 (see next section), apples were held outdoors for larval emergence in shaded facilities in mid-August to mid-September while there was ~15–12.5 h light (daylight + civil twilight). Black hawthorn and snowberries were held for larval emergence in the laboratory at 16 h light and 22–24°C. In 2016, for nonsympatric site comparisons (next section), apples and snowberries were simultaneously held outdoors under the same conditions, as were apples in 2017 and 2018. In all 3 yr, puparia on the bottom of tubs were collected every 1 or 2 d over an ~1 mo period. All puparia were immediately transferred to 16:8 L:D and 20–22°C. Puparia were held for 10–13 d in ~20% moist (wt:wt) sandy loam (2016, 2017) or aquarium sand (2018) (CaribSea Inc., Ft. Pierce, FL) inside semisealed (not airproof) 473-ml cups before the chill or no-chill treatments.

Treatments and Sympatric and Nonsympatric Comparisons

Three chill duration treatments of 180, 150, and 130 d (similar durations that resulted in lesser to greater eclosion spans for *R. pomonella* in Neilson [1962]) at 2–4°C and one no-chill treatment at 20–22°C were tested in 2016–2018. Subsamples of puparia in 2018 that formed every other day were selected for no-chill treatment. Different chill durations were tested to investigate for their possible effects on eclosion patterns of the two flies. Sympatric and nonsympatric comparisons were kept separate to take into account possible effects of fruit development timing in same or different environments on eclosion responses. Sympatric site comparisons of chilled puparia and no-chill puparia comprised six pairs and four pairs, respectively, each differing in site, *R. pomonella* host origin, or chill duration. Sympatric site comparisons

of chilled puparia were *R. pomonella* from apple versus *R. zephyria* from: Saint Cloud, chilled 150 d; Saint Cloud, chilled 130 d; Vancouver, chilled 130 d; *R. pomonella* from black hawthorn versus *R. zephyria* from: Nile, chilled 180 d; Nile, chilled 130 d; Ronald, chilled 130-d chill (38–596 puparia per species per comparison). The four sympatric no-chill pairs were *R. pomonella* from apple versus *R. zephyria* from Saint Cloud and from Vancouver; *R. pomonella* from black hawthorn versus *R. zephyria* from Nile and from Ronald (50–589 puparia).

Nonsympatric site comparisons of chilled puparia comprised three pairs of treatments. One was *R. pomonella* from apple from Centralia, Saint Cloud, and BZ Corner apple versus *R. zephyria* from Ronald, Cle Elum, Ellensburg, and Nile, 180-d chill. The second pair was *R. pomonella* from Woodland apple versus *R. zephyria* from Roslyn and Nile, 150-d chill. The third was *R. pomonella* from Woodland apple versus *R. zephyria* from Washougal, Sams Walker, Cle Elum, Ellensburg, Klickitat, Goldendale, and Yakima, 130-d chill (30–550 puparia). For the nonsympatric site comparison of no-chill puparia, the comparison was *R. pomonella* from Woodland apple versus *R. zephyria* from Ellensburg, Goldendale, Klickitat, Cle Elum, and Yakima (45–539 puparia).

Puparia for sympatric and nonsympatric comparisons were handled the same way. For chill treatments, cups with puparia of each species were placed adjacent to one another on shelves within the same incubators (0.57 m^3, Thermo Fisher Scientific, Asheville, NC) for 180, 150, or 130 d at 2–4°C without lighting. Incubators were set to 3°C, but actual mean readings ranged from 2.70 to 3.46°C across treatments (and years), as measured hourly using Hobo data loggers (Onset Corp, Bourne, MA). For the no-chill treatment, puparia were exposed to a constant 22.0°C in the dark. To determine eclosion times, cups with chilled puparia of both species were transferred after 180, 150, or 130 d to 22–24°C under 16:8 L:D. Cups with each species were placed side by side and checked daily for newly eclosing adult flies for a period of up to 120 d postremoval from chilling. Cups with no-chill puparia were checked daily for newly eclosing adults for a period of up to 120 d postpupal formation. Data loggers recorded temperatures throughout the 120 d. Water was added to cups every 2–4 wk to maintain humidity at ~100%.

To determine percent eclosion, we counted the numbers of eclosed flies, eclosed parasitoids, and parasitoids inside dissected puparia (except in the nonsympatric, 180-d chill test) at ~0.7–1.6 yr after the 120-d monitoring periods had ended. There were no live insects by these times. Parasitoids and any flies that eclosed after 120 d (none in chill treatments, few in the no-chill treatment; see Results) were excluded when calculating percent eclosion. All flies eclosing from apple and hawthorn were considered *R. pomonella* while all flies from snowberry were considered *R. zephyria*. As estimated hybridization rates between flies are only 0.1–1.44% (Feder et al. 1999, Arcella et al. 2015, Doellman et al. 2020) any hybrids were not considered in the analyses.

Statistics

First eclosion day, mean eclosion day, eclosion span (last eclosion day minus first eclosion day) and eclosion dispersion of flies or fly eclosion distributions using the interquartile range (IQR), lower quartile, and upper quartile (Whaley 2005, Arcidiacono 2019) were calculated. Dispersion is the scatter, spread, or variability of a distribution and measures the extent to which it is stretched or squeezed (Whaley 2005, NIST 2013). Data for females and males were combined ($P > 0.05$) for analyses. For sympatric comparisons, the six pairs were included in one analysis and compared using paired *t*-tests, pairing each site-chill duration combination (data were normal with homogenous variances). Dispersion of apple- and hawthorn-origin *R. pomonella* did not differ (mean IQRs were 14.1 and 15.1 [$P > 0.05$], respectively). For nonsympatric comparisons, the three pairs (180-, 150-, and 130-d chill treatments) were analyzed. Our sampling design was too unbalanced and uneven to conduct a single linear mixed effects model using site (random effect), temperature treatment (fixed effect), and geography (sympatric or allopatric; fixed effect) to compare eclosion differences. Specifically, we did not test all chill durations within all sites, some sites (for use in nonsympatric comparisons) had only *R. pomonella* or only *R. zephyria*, and nonsympatric site comparisons (in 2016) also included sites used for sympatric site comparisons (other study years). For the no-chill treatment within sympatric and nonsympatric comparisons, eclosion of *R. zephyria* was low (<2%), so dispersion measures were unreliable and not calculated. Relationships between the IQR and the upper quartile, lower quartile, mean eclosion day, and eclosion span within fly species were calculated using Pearson correlations by including data from sympatric and nonsympatric sites comparisons to increase observation points ($n = 9$).

Percent eclosion from puparia in the six pairs of sympatric, chilled treatments were analyzed using a Wilcoxon signed-rank test, as data were not normal; eclosion of apple- and hawthorn-origin *R. pomonella* did not differ ($P > 0.05$), so one analysis was performed. For sympatric no-chill groups and nonsympatric 150-d chill, 130-d chill, and no-chill groups, eclosion rates were compared using a test of two proportions (Zar 1999), due to data being available for only one or two sites for apple- or hawthorn-origin *R. pomonella*. SAS software (SAS Institute, Inc. 2009) was used for analyses, except for Wilcoxon signed-rank tests (Wilcoxon Signed-Ranks Test Calculator https://www.socscistatistics.com/tests/signedranks/default.aspx).

Results

Sympatric Site Comparisons of Eclosion Timing and Dispersion

For the sympatric site comparison, the mean first eclosion day and mean day of eclosion for *R. pomonella* and *R. zephyria* did not differ (Table 1). The eclosion span of *R. pomonella* was numerically greater than that of *R. zephyria*, although it did not differ significantly, due in part to one male *R. zephyria* that eclosed on day 93, causing the large variance. However, the mean IQR was significantly greater (= greater dispersion) in *R. pomonella* than *R. zephyria* (Table 1). The lower quartile did differ while the upper quartile in *R. pomonella* was nearly greater statistically ($P = 0.0593$). Within each of the six pairs, IQR s for *R. pomonella* from apple and black hawthorn were greater than for *R. zephyria*, whether puparia were chilled for 180, 150, or 130 d (Figs. 1 and 2). Within each pair of sympatric sites, eclosion peaks for *R. pomonella* were less defined or pronounced than those of *R. zephyria*.

Nonsympatric Site Comparisons of Eclosion Timing and Dispersion

In nonsympatric site comparisons, mean first eclosion day was earlier for *R. pomonella* than *R. zephyria* but the mean day of eclosion, the lower quartile, and the upper quartile did not differ. However, the eclosion span and mean IQR of *R. pomonella* were significantly greater than those of *R. zephyria* (Table 2). Within each of the three nonsympatric comparisons, the IQRs of *R. pomonella* from apple was greater than of *R. zephyria*, when puparia were chilled for 180,

Table 1. Paired *t*-test results of eclosion for sympatric *Rhagoletis pomonella* and *R. zephyria* populatons (males and females combined) from Washington State, USA

Variable	*R. pomonella*	*R. zephyria*	*t*-value	*P*-value
First eclosion day	29.8 ± 2.7	29.5 ± 1.6	–0.14	0.4479
Mean day eclosion	49.7 ± 3.3	47.9 ± 0.6	0.56	0.5978
Eclosion span	41.7 ± 4.4	35.8 ± 7.1[a]	0.85	0.4354
IQR	14.6 ± 2.1	6.1 ± 1.0	4.49	**0.0065**
LQ (days)	43.0 ± 4.2	45.0 ± 0.5	–0.51	0.6310
UQ (days)	57.5 ± 2.8	51.1 ± 0.9	2.43	0.0593

Six pairs of each species within (1) Saint Cloud, 150-d chill; (2) Saint Cloud, 130-d chill; (3) Vancouver, 130-d chill; (4) Nile, 180-d chill; (5) Nile, 130-d chill; (6) Ronald, 130-d chill treatments: mean first eclosion day, mean day of eclosion, eclosion span, interquartile range (IQR), lower quartile (LQ), upper quartile (UQ) ± SEM.

[a]One *R. zephyria* eclosed on day 93, resulting in an eclosion span of 71 d. Figs. 1 and 2 show numbers of flies that eclosed. Bold values highlight significant probabilities.

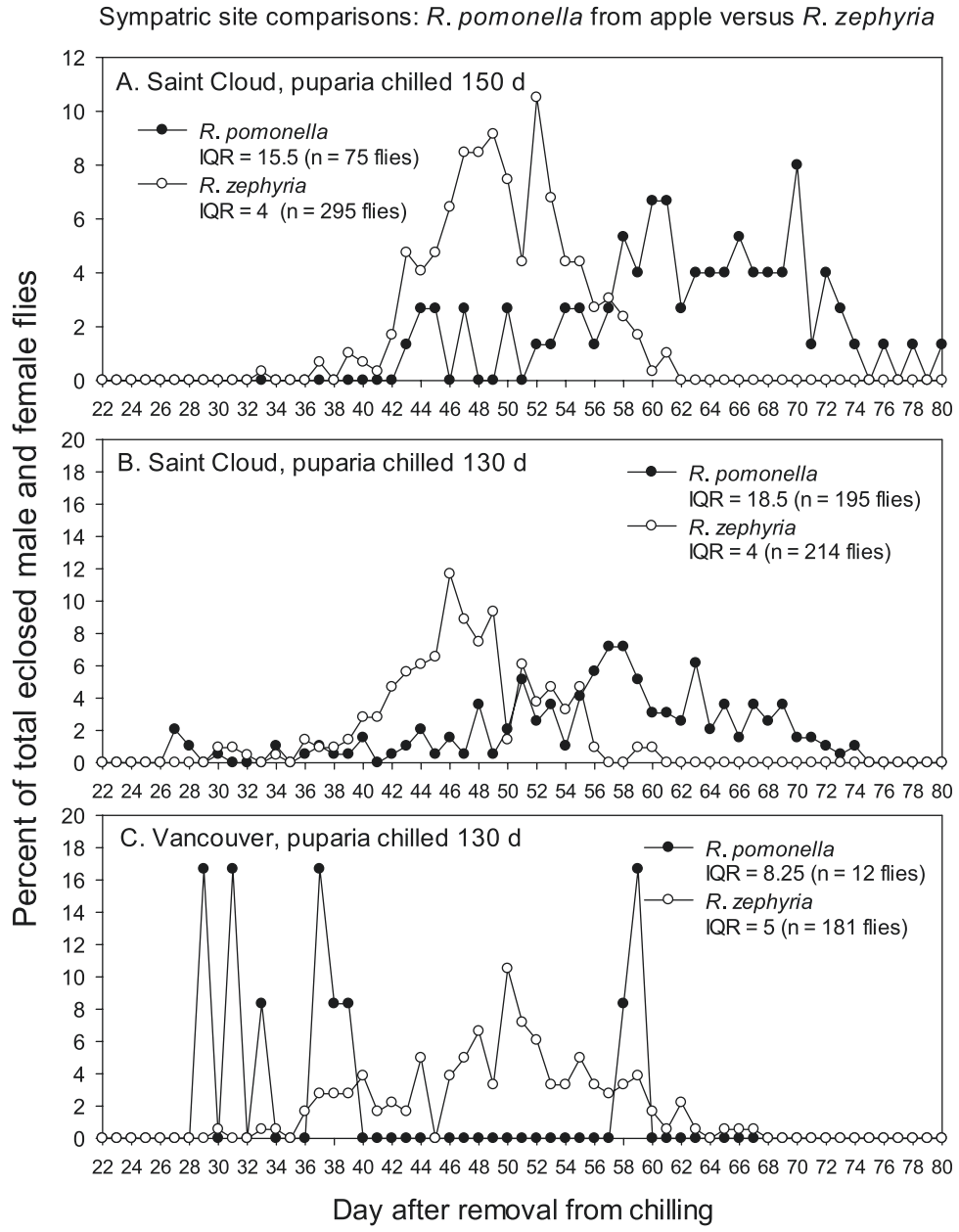

Fig. 1. Sympatric site comparisons of percent eclosion of apple-origin *Rhagoletis pomonella* and snowberry-origin *Rhagoletis zephyria* by individual site-chill duration treatment: (A) Saint Cloud, 150 d; (B) Saint Cloud, 130 d; and (C) Vancouver, 130 d. IQR = interquartile range; larger IQR indicates greater dispersion.

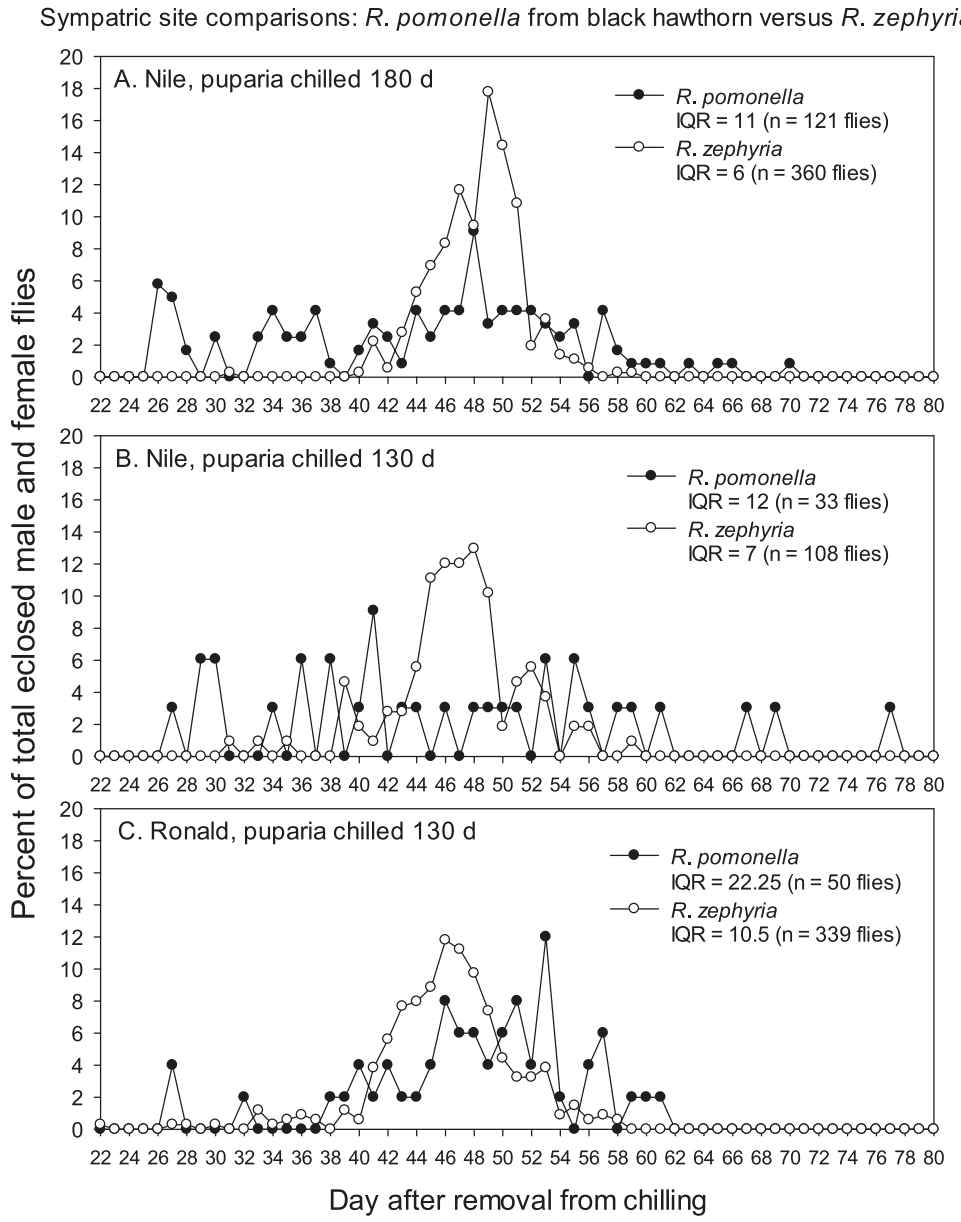

Fig. 2. Sympatric site comparisons of percent eclosion of black hawthorn-origin *Rhagoletis pomonella* and snowberry-origin *Rhagoletis zephyria* by individual site-chill duration treatment: (A) Nile, 180 d; (B) Nile, 130 d; and (C) Ronald, 130 d. IQR = interquartile range; larger IQR indicates greater dispersion.

Table 2. Paired *t*-test results of eclosion in nonsympatric *Rhagoletis pomonella* and *R. zephyria* (males and females combined) from Washington State, USA

Variable	*R. pomonella*	*R. zephyria*	*t*-value	*P*-value
First eclosion day	28.2 ± 2.7	37.4 ± 3.2	7.97	**0.0154**
Mean day of eclosion	47.9 ± 4.6	49.8 ± 2.1	−0.53	0.6484
Eclosion span (days)	41.2 ± 1.0	25.6 ± 1.3	10.79	**0.0085**
IQR	15.5 ± 1.5	5.8 ± 0.4	6.78	**0.0211**
LQ (days)	40.3 ± 5.3	47.0 ± 2.0	−1.39	0.2990
UQ (days)	55.8 ± 4.9	52.8 ± 2.2	0.84	0.4913

Three pairs of each species within 180-, 150-, and 130-d chill treatments: mean first eclosion day, mean day of eclosion, eclosion span, interquartile range (IQR), lower quartile (LQ), upper quartile (UQ) ± SEM. Figs. 3 and 4 show numbers of flies that enclosed. Bold values highlight significant probabilities.

150, or 130 d (Figs. 3 and 4). For all three comparisons, eclosion of *R. pomonella* lacked distinct peaks and was more protracted than that of *R. zephyria*.

The only significant ($P < 0.05$) correlation between IQR and other measures of eclosion was detected in *R. pomonella*, where the IQR and the lower quartile (value that marks where 25% of the data

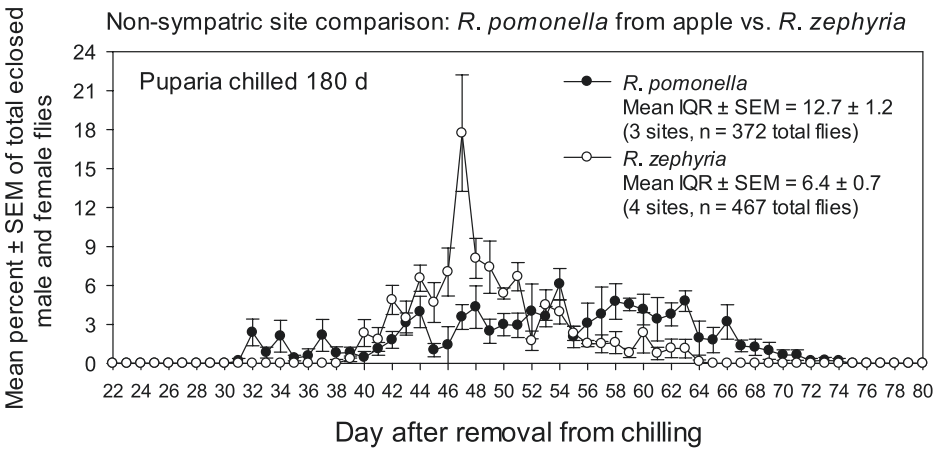

Fig. 3. Nonsympatric site comparison of mean percent eclosion ± SEM of apple-origin *Rhagoletis pomonella* and snowberry-origin *Rhagoletis zephyria* chilled for 180 d from three and four sites, respectively. IQR = interquartile range; larger IQR indicates greater dispersion.

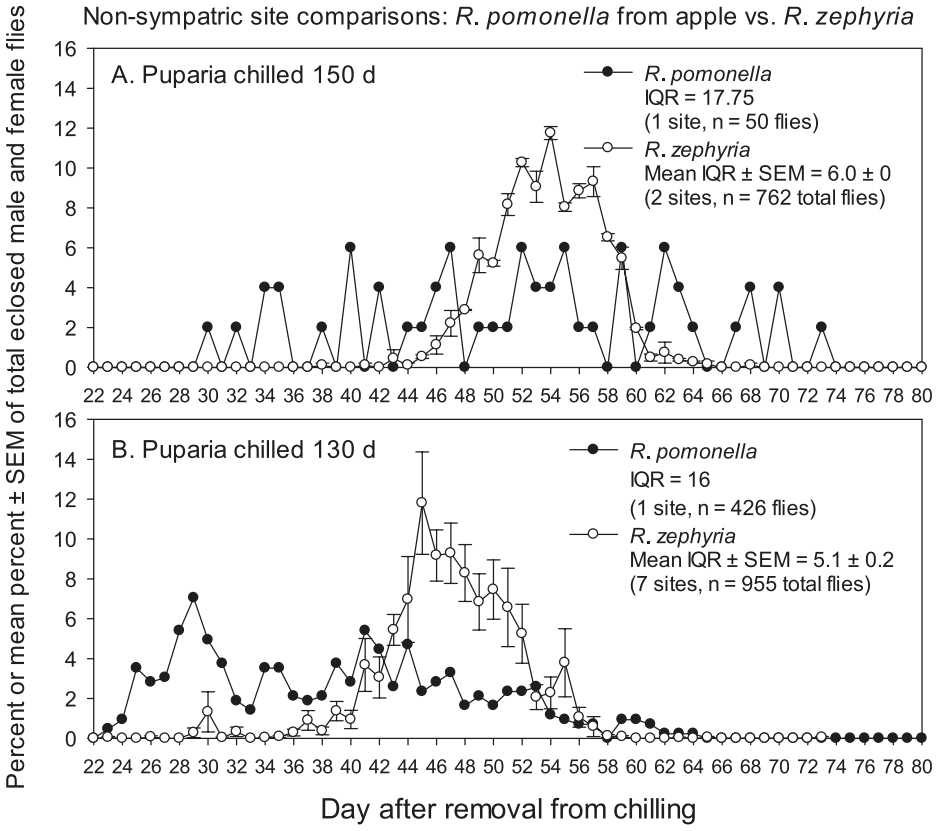

Fig. 4. Nonsympatric site comparisons of percent eclosion or mean percent eclosion ± SEM of apple-origin *Rhagoletis pomonella* and snowberry-origin *Rhagoletis zephyria* chilled for (A) 150 d and (B) 130 d. For both (A) and (B), the one site for *R. pomonella* was Woodland. IQR = interquartile range; larger IQR indicates greater dispersion. For (A), mean chill and adult rearing temperatures were 3.56°C and 22.69°C, respectively; for (B), they were 2.70°C and 23.89°C, respectively; colder chilling and warmer eclosion temperatures possibly account for earlier eclosion in (B) (Reid and Laing 1976, Jones at al. 1989).

are below it) were negatively correlated (r = –0.71053; P = 0.0319). This suggests that greater dispersion in *R. pomonella* is more related to flies eclosing earlier than later (based on the insignificant relationship with the upper quartile). However, low data points may affect this interpretation. For *R. zephyria*, the correlation between IQR and the lower quartile was also negative, but it was not significant (r = –0.16994; P = 0.6620).

Sympatric Site Comparisons of Percent Eclosion

The percentage of adults that eclosed from chilled *R. pomonella* and *R. zephyria* puparia from sympatric sites did not differ (Table 3). However, 7.5 times more adults eclosed from no-chill apple-origin *R. pomonella* than no-chill *R. zephyria* puparia (Table 3). No-chill hawthorn-origin *R. pomonella* eclosed at a greater although not statistically different rate than no-chill *R. zephyria* puparia.

Table 3. Mean percent eclosion ± SEM or percent eclosion from chill and no-chill *Rhagoletis pomonella* and *R. zephyria* puparia from unparasitized puparia (numbers inside parentheses) from sympatric and nonsympatric sites in Washington State, USA

Chill treatment, host plant of *R. pomonella*[a]	*R. pomonella*	*R. zephyria*	Statistics[b]	*P*-value
Sympatric site comparisons				
180, 150, 130 d, apple, black hawthorn (six pairs)	67.1 ± 12.5	66.7 ± 3.3	W = 8	>0.05
No chill, apple	13.5 (362)	1.8 (552)	χ^2 = 48.58	**<0.0001**
No chill, black hawthorn	4.31 (116)	1.9 (642)	χ^2 = 2.67	0.1022
Nonsympatric site comparisons				
150, 130 d, apple	77.0 (618)	71.2 (2,412)	χ^2 = 8.38	**0.0038**
No chill, apple	21.9 (539)	1.3 (1,205)	χ^2 = 222.04	**<0.0001**

[a]Host plant of all *R. zephyria* was snowberry. [b]W, from Wilcoxon signed-rank test; χ^2, from test of two proportions. Bold values highlight significant probabilities.

No adult flies eclosed from chill treatments after 120 d of daily monitoring, but in the no-chill groups combined, 5.5% of *R. pomonella* and 63.2% of *R. zephyria* eclosed after 120 d.

Nonsympatric Site Comparisons of Percent Eclosion

The percentage of adults that eclosed from chilled *R. pomonella* was significantly greater than for *R. zephyria* puparia from nonsympatric sites (Table 3). As was the case for the nonsympatric site comparison, the eclosion rate of no-chill *R. pomonella* was significantly greater (17.7 times) that of no-chill *R. zephyria* (Table 3). No nonsympatric black hawthorn flies were sampled to compare with results for sympatric sites.

Discussion

Our results highlight two major differences in adult eclosion between *R. pomonella* and *R. zephyria* in sympatric and nonsympatric populations in Washington State. The first is that eclosion dispersion of *R. pomonella* from apple and black hawthorn is consistently greater than that of *R. zephyria*, regardless of chill duration. In the nonsympatric site comparison, a significantly longer eclosion span of *R. pomonella* was also associated with greater dispersion. The second difference is that diapause in *R. zephyria* is more rigid than that in *R. pomonella*. Specifically, a greater proportion of *R. zephyria* pupae need chilling to terminate diapause and eclose as adults than *R. pomonella*. Moreover, the difference is more pronounced for apple than black hawthorn-infesting populations of *R. pomonella*, as more black hawthorn- than apple-origin flies appear to require chilling to break diapause.

The apparent lack of differences between results for sympatric and nonsympatric site comparisons suggests local environmental differences had little or no impact on eclosion dispersion of *R. pomonella* and *R. zephyria*. Whether our findings pertain only to the flies we collected at the specific sites sampled here or are generalizable to flies elsewhere remains to be determined. However, the pronounced differences we observed in the current study, especially for apple-origin flies, imply that our results represent a general difference among the different races and species of these *Rhagoletis* taxa.

None of the chill durations we tested decreased eclosion dispersion of *R. pomonella* to the levels seen in *R. zephyria*. Unlike *R. zephyria*, it appears that chilling for >180 d is needed to reduce eclosion dispersion of *R. pomonella*. Consistent with this hypothesis, eclosion of *R. pomonella* from a New Brunswick, Canada population (Neilson 1962) was more dispersed when puparia were chilled at 0°C for 70 d than 140 and 210 d (eclosion span of ~35–85, ~30–65, and ~30–55 d, respectively), with the eclosion curve for 140 d resembling our curves for flies from Washington State at 130 and 150 d. Synchronicity (a simultaneous occurrence of events; high synchronicity = low dispersion) of eclosion was greatest after 280 d of chilling for Canadian flies (span of ~28–45 d) (Neilson 1962). In addition, synchronicity of eclosion in eastern hawthorn and apple races of *R. pomonella* progressively increased after puparia were chilled at 4°C for 7–56, 63–133, and 154–245 d, with greatest synchronization after chilling for 280–756 d (Feder et al. 1997).

Optimal temperatures for diapause and postdiapause development of *R. pomonella* and *R. zephyria* pupae are also known to differ, optimal temperatures being defined as thermal conditions that minimize the number of days to eclosion and/or maximize eclosion synchronization (Brown and AliNiazee 1977). For Oregon fly populations, the optimum for diapause development of *R. pomonella* is between 0 and 3°C (range, −6 to 12°C) and for *R. zephyria* between 6 and 9°C (range, −2 to 12°C) (AliNiazee 1988). Diapause termination (lower developmental threshold) for *R. pomonella* is achieved at 6.4–6.7°C (Reissig et al. 1979, Laing and Heraty 1984, AliNiazee 1988, Jones et al. 1989) or 8.7°C (Reid and Laing 1976), while for *R. zephyria*, it is achieved at 8.1°C (AliNiazee 1988). Optimal postdiapause temperatures for *R. pomonella* are 18–24°C versus 16–22°C for *R. zephyria* (AliNiazee 1988). Our current results suggest differences in these optima between species do not affect the average times of eclosion as much as eclosion dispersion. Whether these optimal temperatures also maximize fly eclosion numbers was unclear based on the current study.

Comparisons of eclosion by *R. pomonella* and *R. zephyria* in the field are currently lacking, so how dispersion patterns seen here translate to those in nature remains to be determined. Eclosion from soil by apple- and black hawthorn-origin *R. pomonella* in southwestern Washington State under emergence cages has been documented (Mattsson et al. 2015), but curves shown were based on cumulative rather than daily eclosion, precluding comparisons with dispersion data here. However, although environmentally related factors in the field would be expected to modify patterns seen in the laboratory, studies of eclosion of apple-origin *R. pomonella* from soil in eastern North America (Porter 1928, Hall 1937, Dean and Chapman 1973, Laing and Heraty 1984) suggest there are similarities in eclosion dispersion in the current study and in the field. This appears true even taking into account greater genetic diversity in eastern than western U.S. populations of *R. pomonella* (Sim et al. 2017).

For eastern *R. pomonella* populations, field eclosion can be highly variable with different degrees of dispersion, as seen here. For example, in New York, USA, there are 'typical' *R. pomonella* eclosion curves, but also curves with a 'double peak', a 'prolonged with late peak', 'early peak and heavy postpeak', and 'late peak and short postpeak', spanning an ~51-d period from 20 June to 10 August (Dean and Chapman 1973). Eclosion curves of *R. pomonella* in Ontario, Canada resembled those in our study, in that there were multiple peaks and eclosion was dispersed, spanning the period from

5 July to 17 September or 74 d (Laing and Heraty 1984). Spans of field eclosion differed from our means of ~42 d possibly due to variable degree day accumulations in nature. Seasonal trap capture curves of *R. pomonella* do not measure eclosion directly but must be correlated and in some instances also resemble our eclosion curves, even when traps were checked only weekly (e.g., AliNiazee and Westcott 1987, Meck et al. 2008), spanning up to ~90 d.

Field eclosion data for *R. zephyria* from soil are lacking to compare with our data. However, limited seasonal *R. zephyria* trap capture data suggest dispersion of trap captures of this species is similar to that of the eclosion curves we report here for the fly. In British Columbia, flies were caught from ~1 July to 7 August or over ~37 d, distinctly peaking on 24 July (Madsen 1970). In Washington State, flies were caught over ~55 d, peaking in early August (Tracewski and Brunner 1987). Because flight continues after eclosion ends, eclosion dispersion of *R. zephyria* in the field must be less than trap catch dispersion. Pending more data, conclusions about similarities of laboratory and field eclosion curves are tentative, as field eclosion curves of both species must be affected by genetics modified by environment.

The comparisons reported are between native *R. zephyria*, which are widespread and abundant in Washington State, and introduced *R. pomonella*, which probably underwent some bottleneck and likely do not represent the range of variation in *R. pomonella* in North America (Sim et al. 2017). The eclosion characteristics of the native *R. zephyria* have presumably been shaped by evolution (perhaps stabilizing selection) over quite a long time period. Differences in eclosion patterns seen here between the two species are primarily genetic, because puparia of both were exposed to the same chill conditions. Whether host plant range affects genetic diversity or the reverse, eclosion patterns of *R. zephyria* versus *R. pomonella* are probably associated with either host fruit phenology or host fruit diversity.

Higher genetic diversity is a plausible explanation for the more variable, dispersed, and protracted eclosion times seen in *R. pomonella* than *R. zephyria* populations. Even though recent introduction may have resulted in less variation in western than eastern U.S. populations of *R. pomonella* (Sim et al. 2017), genetic polymorphisms in Washington *R. pomonella* appear greater than in Washington *R. zephyria* (Arcella et al. 2015). It may be that the genetic diversity of *R. pomonella* provides it greater opportunities to exploit more plants over a broad period, whereas lower genetic diversity of *R. zephyria* limits it to snowberries, coincident with a lower eclosion dispersion.

While eclosion dispersion of *R. pomonella* could be related in part to broad windows of host fruit availability, this may not be true for *R. zephyria*. In Washington State, *R. pomonella* larvae can be found in black hawthorn in July and in ornamental hawthorn (*Crataegus monogyna* Jacquin) in October (Tracewski et al. 1987; Yee and Goughnour 2008, 2019), encompassing a 4-month span in seasonal host use that could partially explain high eclosion dispersion levels. However, the fruiting phenology of snowberry suggests the temporal range of host availability may not be a reason for the relatively low dispersion eclosion of *R. zephyria*. In southwestern Washington, ripening of snowberry (turned white) first occurred 7–14 July, ≥50% of fruit were ripe by mid-August, and ripe fruit were retained on plants in September and October, remaining throughout fall and winter (Tracewski and Brunner 1987). Depending on location, snowberry continually fruit from July through August, as flowers, green fruit, and ripe fruit can all occur within a bush at the same time (W. L. Y., personal observations). Yet *R. zephyria* numbers on traps peak in early to mid-August and decline afterwards even when many berries are present and ripe (Tracewski and Brunner 1987), suggesting the fly does not maximally utilize the host resource or that only earlier ripening snowberries are optimal for larval development.

Despite the narrower eclosion periods of *R. zephyria* than *R. pomonella*, eclosion times of the two species overlap, such that eclosion differences might only weakly isolate the species. More likely, then, is that host fruit attractiveness and mating on fruit (Prokopy et al. 1971) play larger roles than eclosion timing in reproductive isolation between species. *Rhagoletis zephyria* is more attracted to odors isolated from snowberry than apple (Cha et al. 2017), suggesting fruit odors could serve an important role in keeping the species apart in most instances.

When puparia were not chilled, apple-origin *R. pomonella* eclosed at a greater rate than *R. zephyria*, while eclosion of no-chill black hawthorn-origin *R. pomonella* was numerically but not statistically greater. Nonchilled apple- origin flies in the field eclose at highly variable rates of 0–48.6%, depending on when puparia formed during the season (Hall 1937, Porter 1928), while rates of nonchilled apple- and hawthorn-origin flies in the laboratory range from 0.2% to 100% (Neilson 1962, Baerwald and Mallory Boush 1967, Prokopy 1968, AliNiazee 1988, Rull et al. 2016). As with eclosion responses after chilling, variable responses of *R. pomonella* to no-chill conditions including in Washington State may be due in part to its greater genetic diversity, as has been shown for the species in the eastern United States and Mexico (Doellman et al. 2018, 2019). Diapause responses of no-chill *R. zephyria* (here; Tracewski and Brunner 1987) appear more rigid, although data are geographically limited and more populations need to be studied to confirm reported higher eclosion rates (AliNiazee 1988). *Rhagoletis pomonella* can also diapause multiple years in the field (Dean and Chapman 1973), a phenomenon unstudied in field *R. zephyria*. Whether *R. pomonella* has high numbers of diapausers each year while *R. zephyria* has nearly none in the field in Washington State as suggested here remains unknown.

Maintenance at 20–22°C appears to stimulate eclosion hormone release (Truman et al. 1981) in more *R. pomonella* than *R. zephyria*, but the timing of its release did not seem to differ between the two species. Over the 120-d monitoring period, nonchilled *R. pomonella* and *R. zephyria* eclosed from 40 to 106 d (66-d range) and 37–94 d (57-d range), respectively, suggesting some physiological responses underlying eclosion in the species are similar when their puparia are not chilled. No such species comparisons in the field exists, but in Canada, eclosion spans of pre-winter *R. pomonella* were lower than here, at 24–32 d (between 18 September and 14 November) (Hall 1937), perhaps because declining field temperatures suppressed development of *R. pomonella* that otherwise would have eclosed.

The distinct eclosion traits of *R. pomonella* and *R. zephyria* indicate key physiological responses of the sibling species diverged during the evolution of *R. zephyria*. Differences in eclosion traits of the two species could be due to greater genetic variation in *R. pomonella* associated with it attacking the fruit of a wider taxonomic range of host plants than host plants of *R. zephyria* both now and in the past. Whether differences in eclosion patterns within other closely related insect species specializing on different host plants are common and similar to those we observed for *R. pomonella* and *R. zephyria* warrants further study.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

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