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Genetic structure of cherry fruit fly (*Rhagoletis cingulata*) populations across managed, unmanaged, and natural habitats

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Abstract

The cherry fruit fly (CFF), *Rhagoletis cingulata* Loew (Diptera: Tephritidae: Trypetini), is endemic to eastern North America and Mexico, where its primary native host is black cherry [*Prunus serotina* Ehrh. (Rosaceae)]. Cherry fruit fly is also a major economic pest of the fruit of cultivated sweet (*Prunus avium* L.) and tart (*Prunus cerasus* L.) cherries. Adult CFF that attack wild black cherry and introduced, domesticated cherries in commercial and abandoned orchards are active at different times of the summer, potentially generating allochronic isolation that could genetically differentiate native from sweet and tart CFF populations. Here, we test for host-related genetic differences among CFF populations in Michigan attacking cherries in managed, unmanaged, and native habitats by scoring flies for 10 microsatellite loci. Little evidence for genetic differentiation was found across the three habitats or between the northern and southern Michigan CFF populations surveyed in the study. Local gene flow between native black cherry, commercial, and abandoned orchards may therefore be sufficient to overcome seasonal differences in adult CFF activity and prevent differentiation for microsatellites not directly associated with (tightly linked to) genes affecting eclosion time. The results do not support the existence of host-associated races in CFF and imply that flies attacking native, managed, and unmanaged cherries should be considered to represent a single population for pest management purposes.

Introduction

The cherry fruit fly, *Rhagoletis cingulata* Loew (Diptera: Tephritidae: Trypetini; hereafter abbreviated CFF) is a native insect to North America that attacks the fruit of black cherry trees [*Prunus serotina* Ehrh. (Rosaceae)], primarily in the eastern United States (Glasgow, 1933). For over a century, CFF has also infested introduced and domesticated sweet cherry, *Prunus avium* L., and tart cherry, *Prunus cerasus* L. (Bush, 1966). Cherry fruit fly is considered a major economic pest of sweet and tart cherries because of the zero tolerance of fruit processors and consumers for fly larvae in cherries (Liburd et al., 2001; Pelz-Stelinski et al., 2005; Teixeira et al., 2007). Because larvae feeding within cherries are difficult to eradicate, CFF adults are targeted with insecticide sprays 2–3 times every season to limit oviposition into fruit, with the most prevalent insecticides presently in use being the organophosphates and phosmet (Wise et al., 2012).

Growers currently consider migrants from local native black cherry populations to be an important source of CFF in orchards. However, this hypothesis has not been tested explicitly and the population genetic structure of CFF is poorly characterized. In particular, it is not known whether the colonization of sweet and tart cherries by CFF (derived historically from black cherry-infesting populations) has led to host-associated population differentiation

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on these introduced hosts, a phenomenon that has been documented frequently within the genus *Rhagoletis* (reviewed in Berlocher & Feder, 2002). In this regard, many instances of host race formation in *Rhagoletis* involve shifts to novel plants having different fruiting times than the ancestral, native host (Feder et al., 1988, 1993, 1995; Bush, 1992). As a consequence, new host races of flies evolve to eclose as adults at different times in the season to match ripe fruit availability on the novel plant (Bush, 1969; Smith, 1988; Feder et al., 1997; Berlocher, 2000). Because adult *Rhagoletis* have a limited life expectancy in the field (about 1 month; Boller & Prokopy, 1976), the differences in eclosion time result in allochronic mating isolation between novel and ancestral host populations, generating genetic differentiation. *Prunus serotina*, *P. avium*, and *P. cerasus* fruit are available for oviposition at different times, with the native black cherries being available much later in the season than the introduced cultivated sweet and tart cherries (Teixeira et al., 2009). Moreover, adult CFF flies associated with black cherries and abandoned and managed orchards are active at different times of the season. Thus, the potential exists for host-associated differentiation among CFF flies.

From both pest management and evolutionary biology perspectives, it would be of interest to discern the origin of CFF that infest cultivated cherries and assess whether they represent a genetically diverged host race of flies formed on novel hosts. Specifically, have CFF formed resident races in managed and/or unmanaged orchards or do they alternatively represent immigrants from nearby native black cherry trees? With respect to the pattern of seasonal flight activity, evidence suggests that a degree of temporal isolation may exist among flies. Teixeira et al. (2007) determined flight periods for CFF populations by trapping adults in domesticated cherry trees in unmanaged (neglected) and managed orchards, as well as in native black cherries growing in nearby natural areas. Cherry fruit flies were captured earliest in neglected orchards (June–July) compared with managed orchards (peak immediately after harvest in late June–early August). Fruit infestation (as judged by larval presence) mirrored the flight period in the unmanaged vs. managed orchards. In comparison, the CFF flight period in black cherry extended over most of the season (June–September). However, fruit infestation in black cherry occurred only late in the season (late July–September; Teixeira et al., 2009).

Here, we investigate the genetic structure of CFF populations infesting native black cherry and introduced and domesticated cherries based on patterns of allelic variation at 10 microsatellite loci. Our study sample consisted of flies trapped as adults at two paired sets of unmanaged, managed, and native black cherry field sites located in the northern and southern parts of the cherry growing region in the state of Michigan in July 2007. We tested the hypothesis that *R. cingulata* in different habitats would display host-associated divergence or geographic variation based on overall levels of genetic differentiation at the 10 microsatellite loci examined. We report little evidence of genetic differentiation in the CFF samples examined and discuss the pest management and evolutionary implications of our findings.

Materials and methods

Insect collection and population sample

CFF adults were collected off of yellow cardboard Pherocon AM traps (Trece, Adair, OK, USA), or yellow plastic Rebell traps (Great Lakes Integrated Pest Management, Vestaburg, MI, USA), coated with Tanglefoot™ adhesive (VWR International, Poole, UK) that were positioned within the canopies of individual cherry trees in managed orchards, unmanaged orchards, and stands of black cherry in early- to mid-July 2007 as described in Teixeira et al. (2009). Collections from cultivated cherries were made from *P. cerasus* (tart cherry) in the southern part of lower Michigan's cherry producing region and from *P. avium* (sweet cherry) in the northern part (Table 1; Figure 1). Sweet cherries in Michigan ripen earlier than tart cherries at a given location, although the difference in fruiting time is not as pronounced as it is in Europe (Johannesen et al., 2013); the major tart cherry variety grown in Michigan (Montmorency) ripens at least 10 days before the major tart cherry variety in Germany (Schattenmorelle).

While managed cherries and native black cherries were both abundant in the vicinities of the collection sites, unmanaged cherries were rarer; abandoned, untended trees tend to die off within 3–5 years due to viral disease (Wise et al., 2012). Habitat definitions (managed, unmanaged, and black cherry) follow those of Teixeira et al. (2007). The location, host, sex, and time of collection for each fly was noted and recorded. Field trapped samples were immediately frozen at −20 °C prior to genetic analysis. We also included in the study 40 *R. pomonella* individuals reared from infested hawthorn (*Crataegus mollis* L.) fruit near Grant, MI (Michel et al., 2010) as a 'positive' control population for the 10 microsatellites to distinguish two phylogenetically recognized and diverged taxa (Berlocher et al., 1993; Smith & Bush, 1997).

DNA isolation

DNA from individual flies was isolated using PureGene DNA Isolation kits (Gentra Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Prior to DNA isolation, Tanglefoot adhering to CFF from the

Table 1 Collecting sites for *Rhagoletis cingulata* and *Rhagoletis pomonella* in the study

Species	Collection no.	County	Locality	Latitude, longitude	Host plant	Habitat type[1]	Geographic region[2]	No. individuals (n)
R. cingulata	1	Berrien	Coloma	42°09′32.30″N, 86°18′16.52″W	*Prunus cerasus*	M	S	37
	2	Allegan	Fennville	42°34′52.68″N, 86°08′54.33″W	*P. cerasus*	UM	S	42
	3	Allegan	Fennville	42°36′04.73″N, 86°09′22.49″W	*Prunus serotina*	BC	S	41
	4	Grand Traverse	Williamsburg	44°49′06.10″N, 85°26′10.12″W	*Prunus avium*	M	N	40
	5	Leelanau	Bingham	44°53′03.93″N, 85°41′03.30″W	*P. avium*	UM	N	40
	6	Leelanau	Cedar Lake	44°48′37.60″N, 85°39′27.34″W	*P. serotina*	BC	N	40
R. pomonella	7[3]	Newaygo	Grant	43°21′00.17″N, 85°53′21.98″W	*Crataegus mollis*	na	na	40

[1]M, Managed Orchard; UM, Unmanaged Orchard; BC, Black Cherry.
[2]S, South lower Michigan site; N, North lower Michigan site.
[3]Data from Michel et al. (2010) (Hawthorn site #1).

field traps was removed by incubation of flies for 1–2 min in Histoclear solution (VWR International, Poole, UK). Excess Histoclear was drained from the cleared flies, which were then treated with DNA isolation buffer. DNA was quantified using a BioTek Epoch Microplate Reader using Gen5 data analysis software (BioTek Instruments, Winooski, VT, USA).

Microsatellites

The microsatellite markers used in the study were a subset of those originally developed for *R. pomonella* by Velez et al. (2006). A pilot study identified 10 *R. pomonella* microsatellite loci that PCR-cross-amplify alleles in *R. cingulata* and these 10 loci were used in the present study. These microsatellite loci are designated P4, P18, P27, P36, P37, P45, P50, P54, P66, and P80 following Velez et al. (2006) and Michel et al. (2007, 2010). Table 2 provides summary information about the 10 microsatellite loci. Total genomic DNA isolated from individual flies were PCR-amplified for the 10 microsatellites using locus specific oligonucleotide primers in a total reaction volume of 25 μl containing GeneScript *Taq* polymerase (0.5 μl; 2.5 U), 0.5 mM Mg^{2+}, 0.2 mM dNTP mix (0.05 mM each), 0.5 μM of both forward and reverse primers, and 50–100 ng of template DNA. Reactions were run either on a RoboCycler 96 (Agilent Technologies, Clara, CA, USA) or a PE 9700 thermal cycler (Perkin Elmer, Waltham, MA, USA) using the following temperature profile: 5 min at 94 °C, 1 min at 50–58 °C, and 1 min at 68 °C for one cycle; 1 min at 94 °C, 1 min at 50–58 °C, and 1 min at 68 °C for 35 cycles; and 10 min at 68 °C for a final extension cycle. Two of the

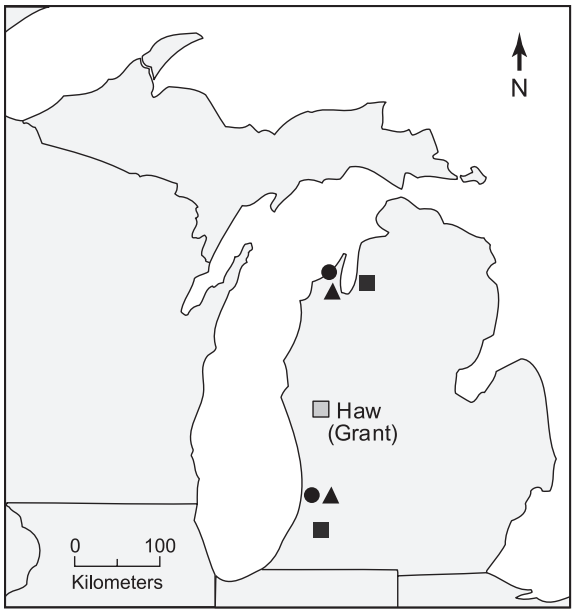

Figure 1 Map of cherry fruit fly collection sites. Three habitats were sampled at each of two geographically separated localities in Michigan. ■ = Managed orchard; ▲ = unmanaged orchard; ● = wild black cherries. □ Haw = location of hawthorns used for *Rhagoletis pomonella* control population (Michel et al., 2010).

10 microsatellites (P50 & P66) were scored at Michigan State University using a ABI3700 fragment analyzer and Applied Biosystems GeneScan 3.1 software (Life Technologies, Grand Island, NY, USA), while the remaining eight microsatellites were genotyped at the University of Notre Dame using a Beckman CEQ8000 and Genetic Analysis

Table 2 Ten microsatellite loci scored to test the degree of habitat-associated population differentiation in six populations of *Rhagoletis cingulata* and a reference population of *Rhagoletis pomonella*

	R. cingulata (populations 1–6)					*R. pomonella* (population 7)				
Locus[1] (GenBank ID)	Range (bp)	N_a	f	H_O	H_E	Range (bp)	N_a	f	H_O	H_E
P4 (AY734888)	156–162	3	0.614	0.451	0.477	165–201	14	0.216	0.900	0.891
P18 (AY734902)	277–307	9	0.645	0.420	0.519	289–317	13	0.398	0.821	0.780
P27 (AY734911)	112–145	5	0.605	0.549	0.541	144–168	10	0.274	0.774	0.821
P36 (AY734920)	256–284	6	0.947	0.106	0.102	263–310	2	0.846	0.308	0.265
P37 (AY734921)	216–255	11	0.911	0.165	0.167	200–224	14	0.243	0.743	0.900
P45 (AY734929)	144–146	2	0.514	0.405	0.501	161–183	10	0.348	0.696	0.837
P50 (AY734934)	154–177	15	0.363	0.707	0.805	149–168	17	0.216	0.946	0.910
P54 (AY734938)	206–220	7	0.850	0.246	0.262	214–235	17	0.176	0.811	0.913
P66 (AY734950)	203–223	6	0.674	0.338	0.457	218–246	11	0.297	0.730	0.854
P80 (AY734964)	205–213	7	0.688	0.435	0.475	197–215	10	0.243	0.892	0.861

N_a, number of alleles; f, frequency of most common allele; H_O, observed heterozygosity; H_E, expected heterozygosity.
[1]Refers to locus ID's as in Velez et al. (2006).

System 9.0 software (Beckman Coulter, Indianapolis, IN, USA).

Population genetics

Microsatellite Analyzer (MSA, vers. 4.05; Dieringer & Schlötterer, 2003) was used to estimate allele frequencies, number of alleles per locus, observed and expected heterozygosities, as well as to generate input files for STRUCTURE (vers. 2.3.3; Pritchard et al., 2000). Tests for the presence of null alleles were carried out using the program Micro-Checker (vers. 2.2.3; Van Oosterhout et al., 2004), with null allele frequencies estimated using the method of Dempster et al. (1977) as implemented in the computer program FreeNA (Chapuis & Estoup, 2007). Population deviations from Hardy-Weinberg equilibrium were assessed using the exact test of Guo & Thompson (1992), as implemented in Arlequin 3.11 (Excoffier et al., 2005) with 1 000 000 Markov chain steps and a burn in of 100 000 steps. Linkage disequilibrium was estimated between all pairs of loci using Arlequin with the EM algorithm and 10 000 permutations to assess significance ($\alpha = 0.05$).

To examine the effect of latitude (north vs. south) and habitat (black cherry, managed, and unmanaged) on patterns of overall population genetic structure in this system, we conducted a permutation-based multivariate analysis of variance in R 2.13.1 (R development core team, Vienna, Austria) using the function adonis in the vegan 2.0 package (Oksanen et al., 2012). This method partitions sum of squares for distance matrices in a manner similar to the more common AMOVA, but specifically allows for both nested and crossed factors. Here, we tested for the effect of latitude (north vs. south) and habitat (managed, unmanaged, and black cherry) as crossed factors on a matrix of Nei's D (Nei, 1972) for all pairwise combinations of populations using 1000 permutations to determine the percentage of variance explained by the factors. In addition, F_{ST} values were calculated between pooled northern and southern Michigan sites and across habitats within the northern and southern collections using Powermarker, with confidence intervals determined by 1 000 bootstrap replicates across loci (Liu & Muse, 2005). To examine overall patterns of microsatellite relatedness among cherry fly populations, a neighbor-joining dendrogram (network) was constructed based on Nei's D pairwise distance measures (Nei, 1972) using the program PowerMarker (Liu & Muse, 2005), with 10 000 bootstrap replicates carried out across loci.

The program STRUCTURE 2.3.3 (Pritchard et al., 2000) was used to test for evidence of multilocus genotypic clustering of cherry flies, potentially by host plant species. To avoid the potential confounding effects of latitude, as is observed for the apple maggot fly (Feder & Bush, 1989; Feder et al., 1990; Xie et al., 2007, 2008; Michel et al., 2007, 2010; Powell et al., 2013), we initially conducted two separate STRUCTURE analyses, one for the northern and one for the southern latitudinal regions in Michigan we surveyed. These tests were executed using a burn-in length of 100 000 for STRUCTURE followed by 250 000 MCMC repetitions for three replicates each of K = 1–4 under four models: (1) admixture and correlated allele frequencies; (2) admixture with independent allele frequencies; (3) no admixture with correlated allele frequencies; and (4) no admixture with independent allele frequencies. The results from all four models were congruent for both latitudinal regions. Thus, model 1 (admixture and correlated allele

frequencies) was used for all subsequent simulations. Seven additional replicates were performed for each of K = 1–4. Data from both regions were then combined and analyzed using a burn-in length of 250 000 and 500 000 MCMC repetitions for 10 replicates each with K = 1–6. Convergence on a common value of ln Pr(X/K) for replicate runs at each value of K was taken to indicate that our runtimes were sufficient to allow convergence of the independent Markov chains within each run.

G-heterogeneity tests as implemented in VassarStats (www.vassarstats.net) were used to test for population subdivision on an individual locus basis by habitat (2 × 3 contingency tables) or latitude (2 × 2 contingency tables). For all loci, alleles were pooled to create two allele classes, with the major allele at each locus representing one allele class, and all other alleles combined to form the second allele class (see Table S1).

Results

Rhagoletis cingulata microsatellite loci

The number of alleles per locus ranged from three (P4) to 15 (P50) among the 10 microsatellites, with the frequency of the most common allele varying from 0.363 (P50) to 0.947 (P36) (Table 2). Observed heterozygosities (H_O) in the trapped CFF individuals (Populations 1–6) varied from 0.106 (P36) to 0.707 (P50) (Table 2). Slight departures from Hardy–Weinberg (H–W) equilibrium were observed within the six CFF populations surveyed (Table S1). Likewise, pairwise linkage disequilibrium was observed between some pairs of microsatellite loci in some of the CFF populations (Table S2). Null alleles were found to be possible at two of the 10 microsatellite loci scored in the study (P18 & P66), with null allele frequencies estimated to be 0.055 for locus P18 and 0.082 for P66.

Population structure

The neighbor-joining network displayed no evidence for populations clustering by latitude or host/habitat type (Figure S1). No internal node in the network had greater than 50% bootstrap support, suggesting no strong pattern of genetic differentiation among sites.

STRUCTURE analysis revealed an unambiguous result of no genotypic clustering either within or between northern and southern geographic regions. For analyses within regions, a model of K = 1 had the highest log likelihood, indicating no genotypic structuring by habitat type. Similarly, STRUCTURE analysis of the overall dataset with all six CFF populations together had the highest likelihood under a model of K = 1, indicating no clustering by latitude (Figure S2). When STRUCTURE was run for CFF populations 1–6 with K>1, most individuals had an equal

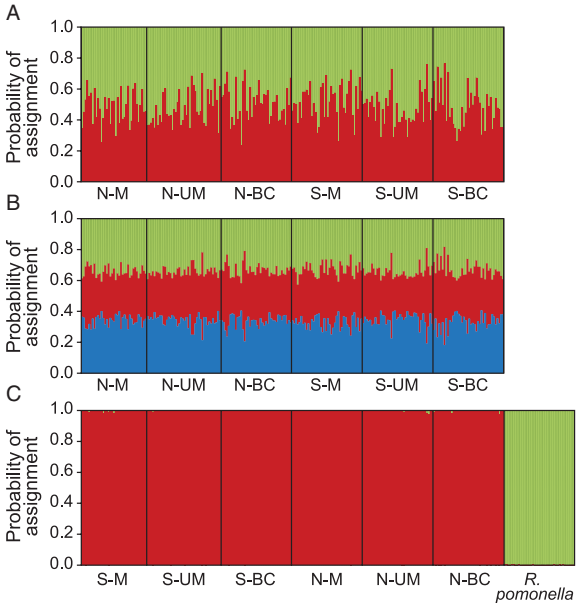

Figure 2 Barplots for STRUCTURE analyses of (A) six cherry fruit fly (CFF) populations with K = 2, (B) six CFF populations with K = 3, and (C) six CFF populations plus *Rhagoletis pomonella* control population with K = 2. In each plot, each individual fly is represented along the x-axis, with its probability of assignment to a cluster on the y-axis. Different colors within a vertical column represent the probability of assignment of an individual fly to each of the hypothesized genotype clusters. In (A) and (B), barplots show that CFF individuals have an equal probability of assignment to each of K = 2 genotype clusters, respectively. In (C), the 10 microsatellites differentiate *R. pomonella* and *R. cingulata*.

posterior probability of assignment to alternative genotype clusters regardless of habitat or geographic location (K = 2, K = 3; Figure 2A and B). Inclusion in the STRUCTURE analysis of an *R. pomonella* population from Grant, MI (Michel et al., 2010) as a control indicated that there was strong resolving power to distinguish the apple maggot fly from CFF populations based on the 10 microsatellites scored in the study. Setting K = 2 for populations 1–6 plus the *R. pomonella* control sample resulted in all *R. pomonella* and CFF individuals being assigned with essentially 100% posterior probability to alternate clusters (Figure 2C).

Analysis of variance

The permutation tests revealed no significant latitude or host/habitat effect on genetic distance among CFF populations (latitude: F = 1.16, r^2 = 0.32, P = 0.45; habitat type: F = 0.2, r^2 = 0.11, P = 0.9). Similarly, G-tests performed for individual microsatellites revealed no

significant differentiation for any locus as a function of habitat (Table S3). However, one locus (P4) showed significant differentiation with respect to latitude (north vs. south; Table S3).

Discussion

Analysis of 10 microsatellite loci indicated that CFF adults captured on yellow sticky traps at six localities in western Michigan in mid-July 2007 were not genetically differentiated with respect to habitat type. This was reflected in the overall F_{ST} value among managed orchard, unmanaged orchard, and native black cherry northern populations for the 10 microsatellite loci. These were 0.0025 (95% CI 0.0085–0) among the three northern sites and 0.0032 (95% CI 0.0068–0) among the three southern populations. One of the 10 microsatellites studied (P4) displayed significant allele frequency differences between northern and southern sites. Thus, there is some evidence for geographic variation among CFF populations. However, P4 was not significant on a table-wide basis and the level of geographic differentiation displayed by all other nine loci was low (Table S3). Thus, based on the analyses of allelic variation at the 10 microsatellite loci examined here, geographic differentiation for CFF in Michigan is not pronounced and is slight, at best; overall F_{ST} for the 10 microsatellites between northern and southern sites was 0.0153 (95% CI 0.0351–0.0002).

The lack of genetic differentiation observed in this set of CFF populations, while somewhat surprising, is not unprecedented. Johannesen et al. (2013) carried out a study of the invasion dynamics of *R. cingulata* in Europe involving analysis of allelic variation at 14 microsatellite loci developed by Maxwell et al. (2009), independent of our own set of 10 loci. These authors found no evidence for plant-related genetic structure in introduced CFF populations in Europe or in reference CFF populations from Michigan. The results of Johannesen et al. (2013) therefore are similar to our own in finding little evidence for population genetic structure for CFF.

There are two general possible explanations for the observed lack of genetic differentiation among CFF populations in Michigan. First, gene flow may be extensive among CFF populations, resulting in flies in the state representing a large, freely interbreeding met population with migration rates sufficient to minimize local genetic drift and isolation by distance. Black cherry (and hence *R. cingulata*) is common in western Michigan (Voss, 1985; Farrar, 1995) and the range of this native fly host plant is continuous from the Michigan border on the south to the straits of Mackinac to the north. Individual black cherry tree produce thousands of fruit annually that potentially can serve as a resource for CFF.

Second, it is conceivable that CFF is subdivided geographically and/or by host-association and that the 10 microsatellites analyzed in the study were simply not sufficient or sensitive enough to detect genetic structuring. For example, genes affecting adult eclosion, which may differ among populations in accord with host fruiting time and adult flight activity variation among habitats, as implied by previous work (Teixeira et al., 2007), might not have been tightly linked to or in linkage disequilibrium with any of the 10 microsatellites we scored. Also, CFF from different sources could mix in flight prior to sorting themselves via assortative mating and differential oviposition on the different hosts available in the black cherry, unmanaged, and managed orchard habitats. Thus, by sampling adults taken off traps in a narrow time window in the summer, we may have failed to detect host-associated differentiation when it was in fact present. A more definitive test of host-race formation in CFF therefore would require an analysis of individuals that are sampled and reared directly from infested host fruits in the different environments.

There are reasons to suspect, however, that the 10 microsatellites used in the study were sufficient to reveal large-scale genetic subdivision in CFF if it were present. First, the 10 microsatellites displayed a fair degree of polymorphism in CFF, and were far from monomorphic. With the number of alleles present, if extensive population structuring was present in CFF, then it should have been evident in the microsatellites. Second, the same 10 microsatellites have been effective in detecting host-related differentiation for other *Rhagoletis* taxa (Michel et al., 2010). Third, if CFF were sorting and mating in a habitat-specific manner, the genetic signature of this non-random mating should be evident even for flies captured in flight on sticky traps in the form of significant departures from H–W equilibrium at individual microsatellites and linkage disequilibrium among loci. The fact that most loci were in H–W (Table S1) and linkage equilibrium (Table S2) argues against habitat-specific mating. Finally, a degree of geographic differentiation would still be expected due to local drift even in the absence of microsatellite linkage to selected loci and despite the trapping sampling scheme used in the study. Nevertheless, we failed to detect spatial structuring in CFF.

Interestingly, compared with *R. pomonella*, the level of genetic polymorphism observed for CFF was low. For example, the single *R. pomonella* population of 40 individuals from Grant, MI that we used as a taxonomic control (Hawthorn site #1 from Michel et al., 2010) had a significantly higher: (1) mean number of alleles per locus (11.8 vs. 7.1; t-test: $P<0.01$); (2) mean observed heterozygosity (0.762 vs. 0.382; t-test: $P<0.0001$); and (3) mean expected heterozygosity (0.803 vs. 0.431; t-test: $P<0.0001$) than the

six pooled CFF sites from Michigan. Indeed, observed heterozygosities in the *R. pomonella* control population were higher than the observed heterozygosities for all 10 microsatellite loci (Table 2).

One possible explanation for the observed difference in genetic variation in *R. cingulata* vs. *R. pomonella* may be ascertainment bias (Ellegren et al., 1995), a phenomenon in which allelic variation is observed to be higher in the species in which microsatellites were originally developed. While the microsatellite markers employed in the present study were developed initially by Velez et al. (2006) for analysis of *R. pomonella*, our data do not support the hypothesis that ascertainment bias is responsible for the reduced genetic variability of *R. cingulata*. The levels of microsatellite variability that we observed are similar to those measured in *R. cingulata* using an independently derived microsatellite suite by both Maxwell et al. (2009) and Johannesen et al. (2013).

A more likely explanation for the difference in variation is the complex biogeographic history for *R. pomonella* involving several past cycles of geographic isolation, contact, and gene flow among different hawthorn-infesting demes of the flies (Feder et al., 2003, 2005). The result has been the creation of extensive adaptive latitudinal clines in *R. pomonella*, resulting in high levels of genetic polymorphism in the fly compared to other *Rhagoletis* species groups.

Implications for pest management and evolutionary biology

The results of the current study support the view of cherry growers in considering that many CFF found in their commercial orchards come from nearby local native black cherry and/or abandoned domesticated cherry orchard sources. Thus, the practice of spraying 2–3 times for CFF prior to harvest would appear warranted, because unless these outside host sources are eliminated (not feasible for *P. serotina*), flies will continually migrate into commercial orchards every year and require appropriate control measures. Nonetheless, the degree to which *P. serotina* and abandoned cherry sources can be removed from the immediate vicinity of commercial operations may still help to curtail the risk for infestation. It is also important to note that following cherry harvest, pesticide control of CFF is usually stopped. This allows fairly high infestation rates to occur in the cherries that remain in the orchards; we have observed larval infestation rates of up to 32% in samples collected in northern lower Michigan 4 weeks after the termination of spraying in 2010 (JJ Smith, pers. obs.). Consequently, it may be worthwhile to also control this residual, post-harvest CFF orchard population to guard against infestation of commercial crops the following year and to determine the relative contributions of these post-harvest resident CFF flies to the following year's infestation.

In conclusion, the current study suggests that in contrast to other *Rhagoletis* flies (Berlocher et al., 1993; Berlocher, 2000; Dambroski & Feder, 2007; Powell et al., 2013), CFF does not appear to have readily formed host races or sibling species associated with host plants differing in their fruiting times. Overlap in adult seasonal activity and local gene flow between native black cherry, commercial, and abandoned orchards may therefore be sufficient to prevent differentiation for alleles at microsatellite loci not directly associated with (tightly linked to) genes affecting eclosion time. Migration may be limited regionally in Michigan, allowing for a low level of geographic differentiation among CFF populations for certain loci. Our results indicate, however, that the level of this regional differentiation, if present, is low due to the generally high density of CFF flies and their cherry host plants, and an implied high migration rate. For the purposes of pest management, it would appear that CFF attacking native, managed, and unmanaged cherries should be considered to represent a single population.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Neighbor-joining dendrogram (network) of *Rhagoletis cingulata* populations based on overall Nei's D genetic distances for the 10 microsatellite loci. Northern sites are designated by circles and southern sites by squares. M: managed orchard; U: unmanaged orchard; B: wild black cherry. Percentages shown on branches are bootstrap support from 10 000 replicates across loci.

Figure S2. STRUCTURE analyses of the full data set, including all six *Rhagoletis cingulata* populations. Mean (± SEM) estimated ln likelihood of data for 10 replicates each of K = 1–6 using the admixture model 1 with correlated allele frequencies in STRUCTURE.

Table S1. Allele frequencies at 10 microsatellite loci in six populations of *Rhagoletis cingulata* (1–6) and *R. pomonella* (7).

Table S2. Pairwise linkage disequilibrium between pairs of CFF microsatellite loci in cherry fruit fly populations 1–6.

Table S3. G-tests for geographic and habitat-related allele frequency differences among cherry fruit fly populations.