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Host-adapted aphid populations differ in their migratory patterns and capacity to colonize crops

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Summary

1. Although phytophagous insects can vary genetically in host use and exhibit long-range movements, the combined implications of these phenomena for pest management have received limited attention.

2. To address this, we surveyed the genetic diversity of pea aphid *Acyrthosiphon pisum* using twelve microsatellite loci and assessed host association patterns and annual movement from a putative source region (Columbia River Basin) to the Palouse region of northern Idaho and western Washington, where the aphid is a pest of pea *Pisum sativum*.

3. A total of 320 identified unique genotypes clustered into four genetic groups, with two host plant associations: alfalfa *Medicago sativa* (three genetic groups), and pea *Pisum sativum* and vetch *Vicia villosa* (one genetic group). All four genetic groups occurred in the Columbia River Basin and in migrant aphids collected in pan traps during spring colonization in the Palouse during 2 years of this study. Patterns of group arrival on the Palouse were spatially structured early in the season, consistent with differing migration patterns. Despite genetic diversity of migrants, a single genetic group became predominant in pea crops each year.

4. Clonal laboratory colonies of pea aphids established from field-collected specimens and representing two predominant genetic groups exhibited reciprocal performance trade-offs, with alfalfa being a poor host for a pea-associated aphid genotype and vice versa.

5. *Synthesis and applications*. Annual spring migrants of pea aphids in the pea production region of the Palouse are genetically diverse, with different host plant affinities consistent with origination from source populations in the Columbia River Basin. As the season progresses, a single genetic group adapted to pea becomes predominant in the crop. Management of pea aphid in the Palouse will be improved by monitoring the temporal and spatial variation of specific genetic groups of the aphid arriving as immigrants during each crop season, providing this information to producers and adjusting estimates of risk of crop damage accordingly. The principle could apply to other pest species with host-adapted populations that colonize crops on an annual basis.

Key-words: agricultural ecology, biotypes, host adaptation, host races, migration, pea aphid *Acyrthosiphon pisum*, pea *Pisum sativum*, population genetics, regional movement

Introduction

Genetically distinct, sympatric populations of phytophagous insects (or 'host races') are well documented in natural populations, where they contribute to population genetic structure and potential speciation (Futuyma & Peterson 1985; Drès & Mallet 2002). For insect pests, genetically distinct populations, or cryptic species, can affect local abundance and potential severity of crop injury (Kennedy 1992; Claridge, Dawah & Wilson 1997; De Barro *et al.* 2011). Coupled with regional-scale movements, genetic structure in pests potentially complicates pest management, but if

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understood presents opportunities to improve it (e.g. Nagoshi, Meagher & Hay-Roe 2012). One of the most thoroughly studied examples of host race formation in a phytophagous insect occurs within the pea aphid complex, *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae). Following initial reports of local adaptation by pea aphid populations to different leguminous host plants (Via 1991a,b), it is now well-established that relatively stable genetic groups of pea aphid are maintained by genetic linkages among performance and habitat choice, and by ecological and post-zygotic barriers to hybridization in North America and Europe (Via 1999; Via, Bouck & Skillman 2000; Hawthorne & Via 2001; Ferrari *et al*. 2006; Ferrari, Via & Godfray 2008; Peccoud *et al*. 2009, 2014; Peccoud & Simon 2010). Despite this, the implications for management of pea aphid, a significant pest of legumes and vector of plant viruses world-wide (Blackman & Eastop 2007), have not been examined explicitly. In this study, we document host-associated genetic structure in populations of pea aphids, the movements of different genotypes of the aphid and colonization success in an agricultural region and its implications for pest and disease management.

In the Pacific Northwest, USA (PNW), pea aphid is a pest of legumes, especially in pea and lentil grown in northern Idaho and south-eastern Washington State (the Palouse region), one of the principal pulse-growing regions of the United States (NASS 2013). Intermittent outbreaks of pea aphid in the Palouse have been documented over decades (Clement 2006; Clement, Husebye & Eigenbrode 2010), and these are associated with significant reduction in yields and economic returns to farmers (Elbakidze, Lu & Eigenbrode 2011). Pea aphid recolonizes the Palouse region each spring following winter extirpation resulting from late drying of crops and severe winter conditions (Clement 2006; Clement, Husebye & Eigenbrode 2010). The putative source for these immigrants is the Columbia River Basin (*c*. 200 km away), which is situated upwind, to the west of the Palouse and *c*. 700 m lower in elevation (Clement 2006; Clement, Husebye & Eigenbrode 2010) (Fig. 1). Management approaches presume pea aphid is genetically uniform in the PNW, but risks posed to pulse crops by annual aphid migrations could differ depending upon which host races are prevalent, their capacity to colonize crops, and whether there are spatial or temporal patterns underlying their arrival (Homan, Stoltz & Schotzko 1991; Stokes 2012; Stokes, Bechinski & Eigenbrode 2013). To explore these possibilities, we surveyed the genetic diversity of possible source and immigrant pea aphids in the inland PNW, sampling established populations on crop plants in the Columbia River Basin and migrant aphids and established populations in the Palouse, asking three questions: (i) Is there evidence for host-associated population genetic structure in PNW pea aphid? (ii) Do genetic groups found on crops in the putative source region occur as migrants into the Palouse? (iii) Are there spatial or temporal patterns in the arrival of these genetic groups in the Palouse?

Materials and methods

FIELD SAMPLING

We sampled pea aphids from crops in the Columbia River Basin (hereafter, Basin), in April 2010–2012 and during the growing season in pea fields and pan traps in the Palouse in 2011 and 2012 (Fig. 1). In the Basin, aphids were sampled in alfalfa *Medicago sativa* L., vetch *Vicia villosa* L., red clover *Trifolium pratense* L. and crimson clover *T. incarnatum* L., and pea *Pisum sativum* L. by sweep netting (three 100-m transects of one hundred 180° sweeps). Most samples were taken from alfalfa, which is the predominant legume in the Basin with fourfold the cultivated area of pea (145 000 vs. 34 000 hectares), while vetch and clover are rare, comprising a total of just 200 ha (Han *et al*. 2012). At sampling time, many pea fields had not yet emerged, so alfalfa was *c*. 10-fold more available for sampling. In addition, other potential wild hosts for pea aphid, including silky *Lupinus sericeus* and silver lupine and *Lupinus argenteus* and feral vetch in perennial remnants and roadsides were inspected for aphids. In 2010, a total of ten sites were sampled (five in alfalfa crops, two in vetch fields, one pea field and one crimson clover field). In both 2011 and 2012, 20 sites were sampled. In 2011, these were 12 alfalfa fields, two clover fields, three vetch fields and three pea fields, and in 2012, these were 19 alfalfa fields and one vetch field. The original five alfalfa fields from 2010 were sampled in 2011, and all those sampled in 2011 were sampled in 2012.

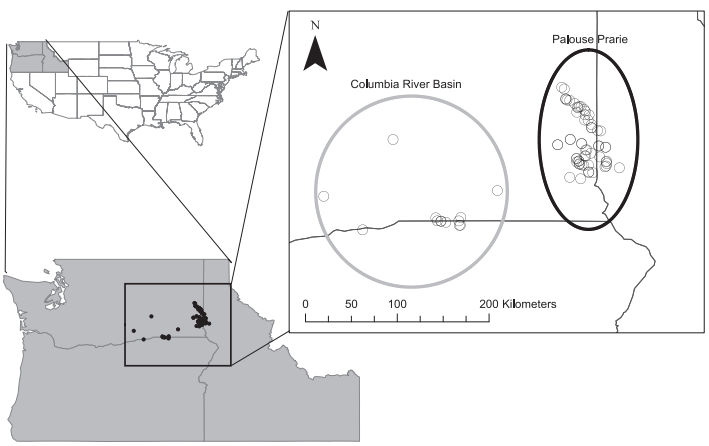

Fig. 1. A map of the study area; pea aphid sampling locations are denoted by open symbols (○). Ecoregions are denoted by shaded circles, with black representing the Palouse and grey representing the Columbia River Basin.

In the Palouse region, pea aphids were sampled by sweep netting at 3- to 4-day intervals throughout the 2011 and 2012 growing season in 34 commercial pea fields, and in a network of pan traps near these fields to ascertain regional phenology. At each of 34 pea fields, sampling consisted of all aphids collected in 100 sweeps (180°) of the crop in two transects. Yellow pan traps designed to capture winged aphids (alatae) (Coon & Pepper 1968) were also deployed in each sample location. Traps consisted of plastic yellow plates (23 cm diameter) secured on a platform 50 cm above the ground and filled with propylene glycol. Within each site, traps were positioned 10 m apart along field margins. Crops were planted during May each year, and traps were serviced twice weekly throughout the growing season (mid-May until late July or early August) by removing all pea aphids, then cleaning and refilling traps with propylene glycol. Pea aphids collected from traps or by sweep netting in the Basin and the Palouse were transferred to 95% ethanol. A subsample of no more than 12 of these aphids from each location and date was genotyped.

MICROSATELLITE GENOTYPING

DNA was extracted from individual pea aphids subsampled from Basin and Palouse samples (n = 1111) using the Qiagen DNeasy Blood and Tissue kit (Qiagen, Inc., Valencia, CA, USA). A negative control was included in each extraction to monitor for possible contamination of DNA extraction reagents. Twelve microsatellite loci, A1A09M, A1A12M, A1B07M, A1B08M, A1B12M, ApF08M, ApH08M, ApH10M, Ap-03, S23, S30 and Sm11, were multiplexed into one polymerase chain reaction (PCR). These markers were selected from a published set of 14 used to genotype pea aphid in previous studies (Peccoud *et al.* 2008, 2009; Table S1 in Supporting Information). This PCR multiplex consisted of 0·07 μM of A1A09M, A1B07M, A1B08M, S23, 0·10 μM of ApF08M, ApH10M, S30, 0·13 μM of A1B12M, ApH08M, A1A12M, Ap-03, 0·29 μM of Sm11, 1X Multiplex PCR Master Mix (Qiagen, Inc.) and 0·5X Q-solution (Qiagen, Inc.) in a 7 μL reaction volume (Simon *et al.* 1999; Caillaud *et al.* 2004; Kurokawa *et al.* 2004; Wilson *et al.* 2004). The thermal profile was an initial denaturation step of 95 °C for 15 min followed by 10 cycles of 94 °C for 30 s, 62 °C touchdown 0·50 °C per cycle for 90 s and 72 °C for 60 s, followed by 20 cycles of 94 °C for 30 s, 57 °C for 90 s and 72 °C for 60 s. PCR products were run on a 3130xl Genetic Analyzer (Applied Biosystems by Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's specifications. Results were visualized and allele sizes called using GENEMAPPER 3.7 (Applied Biosystems by Life Technologies). The probability of identity siblings was calculated for all loci in GENALEX 6.5 (Peakall & Smouse 2006). The results indicated genotypes containing seven loci or greater could be kept in the data set without raising the probability of identical genotypes by chance above 0·002. To determine the number of aphid clones sampled, genotypes were matched using the program GENALEX 6.5.

ASSIGNING INDIVIDUALS TO GENETIC GROUPS AND PUTATIVE HOST RACES

Identification of discrete groups of pea aphid genotypes, indicating candidate populations or host races, was done using STRUCTURE v2.3.4 (Pritchard, Stephens & Donnelly 2000; Falush, Stephens & Pritchard 2003). A burn-in of length 100 000 was used and values of K = 2–20 groups were tested. STRUCTURE runs were performed under an admixed model of ancestry and the correlated allele frequency model with 400 000 Monte Carlo Markov chain (MCMC) repetitions. STRUCTURE runs were replicated ten times for each value of K, and the value of K was inferred using the methods of Evanno, Regnaut & Goudet (2005) and the STRUCTURE HARVESTER program (Dent & von Holdt 2012). STRUCTURE was run first without replicate genotypes (clones; i.e. n = 320) and then rerun for all individuals (n = 1111) to corroborate the same best value of K. Pea aphid produces sexual forms in the fall and overwinters as eggs in the PNW (Cooke 1963), allowing recombination so that groups can be interpreted as true populations.

SPATIOTEMPORAL PATTERNS OF IMMIGRATION BY PEA APHID POPULATIONS

The abundances of aphids in each genetic group were analysed for spatial autocorrelation and shifts in the frequencies of genetic groups over the course of the pea-growing season. We used the R statistical programming language (R Core Team 2012) to implement the add-on package 'NCF' (Bjørnstad 2013) for construction of spatial correlograms using Mantel's statistic (Mantel 1967). The package calculates the significance of departures from random to greater similarity or dissimilarity between pairs. The patterns were similar for both years, so data from both were pooled for this analysis, although constrained to include pairwise comparisons within year. The trapping period was divided into early (May–June)- and late (July)-season collections, which were analysed separately.

We also performed standard population genetic analyses using co-dominant data with the software add-on GENALEX 6.5 (Peakall & Smouse 2006) to summarize and analyse allele frequencies and genetic diversity (observed [H_o] and expected [H_e] heterozygosities), and we tested whether alleles at each locus were in Hardy–Weinberg equilibrium using a reduced data set, omitting clonal duplicates. Rarefied allelic richness was computed using the R add-on package 'HIERFSTAT' (Goudet 2014) to correct for uneven sample sizes. To test the hypothesis that immigrants to the Palouse can originate in the Basin, we also assessed the number of individual genotypes that were common to the Basin and pan trap samples in the Palouse in each year.

RESPONSES OF APHID GENOTYPES TO HOST PLANTS

To confirm that host-associated aphid genotypes differ in their potential as pests on different crop species, as reported for pea aphids elsewhere (Via 1991a,b, 1999; Peccoud *et al.* 2008, 2009), we tested the performance of three field-collected pea aphid clones, two collected from alfalfa and one collected from pea. Aphid clones were collected via sweep net in pea and alfalfa fields in northern Idaho (46·7325°N 116·9992°W, 786 m a.s.l.). Asexual colonies were propagated from individual nymphs and maintained at the Manis Entomological Laboratory at the University of Idaho (Moscow, ID, USA) in multiple 60 × 60 × 60 cm-mesh tents (BugDorm 2120F; BioQuip, Rancho Dominguez, CA, USA) in a glasshouse: 20 ± 2 °C, L18:D6 photoperiod and 50% r.h. Colonies are maintained on potted broad bean *Vicia faba* L., a universal host for pea aphid (Peccoud *et al.* 2014). We sampled multiple individuals from each colony and confirmed that colonies were

separate genetic clones using the microsatellite genotyping and genetic group assignment methods described above.

Alfalfa (cv. 'Gunner') and pea (cv. 'Aragorn') seed was sown in 12-cm-diameter pots containing *c.* 330 g ± 7 g SD of soil (Sunshine mix no. 1; Lot no. S13-084; SunGro Horticulture, Agawam, MA, USA). Planting times were staggered to allow plants to attain comparable above-ground biomass (28 days for alfalfa and 14 days for pea) and grown in a glasshouse under the same conditions as above. Ten mature apterous aphids from each colony were placed on individually caged pea or alfalfa plants. After 10 days, cages were removed and all aphids on each plant were counted. Each genotype (n = 3)-by-plant species (n = 2) combination was replicated ten times, for a total of N = 60 experimental units. We compared final population sizes using two-way ANOVA, treating aphid genotype and host plant species as fixed effects. Statistical analysis was performed with the software JMP 10.0 (SAS Institute, Cary, NC, USA) and a type I error rate of α = 0·05.

Results

COLLECTIONS IN THE BASIN

Pea aphids were found on virtually all alfalfa fields sampled in the Basin during the study and on two vetch fields in 2010. No aphids were found on pea, or on potential wild hosts.

SEASONAL TRENDS IN APHID PHENOLOGY IN THE PALOUSE

The abundances of aphids sampled in pea fields on the Palouse in 2011 and 2012 exhibited similar temporal patterns, with mean abundances of aphids per 100 sweeps per site peaking in late June–early July (Fig. 2). In contrast, abundances of aphids collected from pan traps had different patterns in each year. In 2011, aphid abundance in traps generally increased through the season while in 2012 abundance peaked in late June (Fig. 2). In 2011 aphids were collected in pan traps 20 days before they were detectable in the crop, while in 2012 aphids were detected by both means on the first sample date. The sampling periods spanned the typical emergence to harvest interval for dry pea in the Palouse (USA Dry Pea and Lentil Council 2010).

ASSIGNING INDIVIDUALS TO INFERRED POPULATIONS AND PUTATIVE HOST RACES

In the 1111 aphids from the Basin and Palouse that were genotyped, 320 unique multilocus genotypes were detected, of which 252 were singletons. The remaining 58 genotypes accounted for 76·4% (n = 859 aphids) of the total aphid abundance. Likelihood values derived from replicate STRUCTURE runs and subsequent analysis of the second-order rate of change in posterior probabilities (ΔK; Evanno, Regnaut & Goudet 2005) provided clear support for a value of K = 4 genetic groups (Fig. 3).

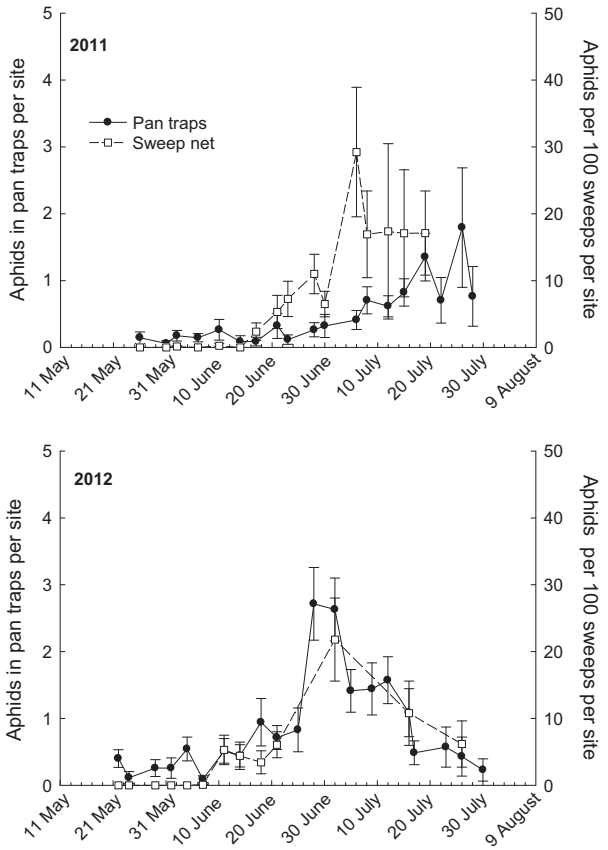

Fig. 2. Mean numbers of pea aphids collected in pea fields on the Palouse using sweep net sampling during 2011 and 2012 (100 sweeps per site and date), and in pan traps placed adjacent to pea fields during the same years (total catch for three traps per site). Bars are standard errors of the mean.

Aphids sampled from alfalfa in the Basin consisted of three genetic groups (coloured blue, yellow and green in Fig. 4). Aphids sampled from vetch in the Basin in 2010 and pea plants in the Palouse region were primarily from one group (coloured red).

SPATIOTEMPORAL PATTERNS OF IMMIGRATING PEA APHIDS

All four genetic groups found on plants in the Basin were also detected among the aphids collected in pan traps, although the frequencies differed. One genetic group (coloured blue in Fig. 4) that occurred infrequently in alfalfa fields in the Basin was relatively common among immigrants to the Palouse. In contrast, the genetic group coloured yellow in Fig. 4 exhibited the reverse pattern.

Pea aphid genetic groups sampled in pan traps on the Palouse showed evidence of spatial autocorrelation during the early portion of the pea-growing season, but not in the latter portion of the season (Fig. 5). Early in the season (May and June), sample sites less than 20 km apart yielded relatively similar compositions of genetic groups (Mantel's similarity index > 0; P = 0·002). The index

declined as distances between sites increased, becoming significantly negative (dissimilar) for geographic distances that exceeded 90 km ($P = 0{\cdot}046$), indicating overdispersion or a regular pattern of occurrence of genetic groups at that spatial scale. Later in the season (July and August), Mantel's similarity index was near zero at all spatial scales, indicating random spatial distribution of genetic groups sampled.

In 2011, the composition of genotypes captured in pan traps in the Palouse region was similar throughout the season (Fig. 4); in 2012, although pea-associated types were evident in May, abundances shifted from primarily alfalfa-associated genotypes early in the growing season to pea-associated genotypes late in the growing season (2011: $\chi^2 = 0{\cdot}380$, d.f. = 1, $n = 128$, $P = 0{\cdot}537$; 2012: $\chi^2 = 30{\cdot}940$, d.f. = 1, $n = 282$, $P < 0{\cdot}0001$; Table 1).

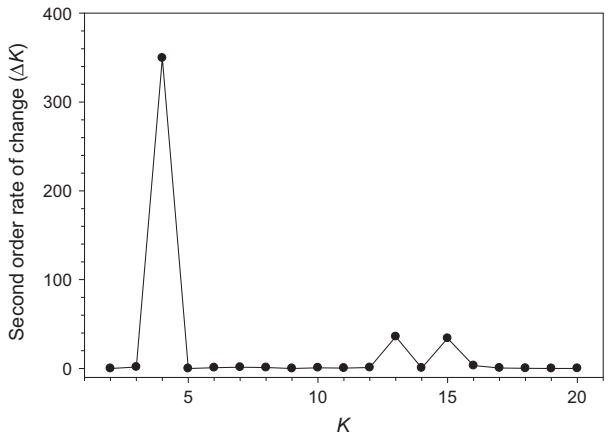

Fig. 3. The second-order rate of change (ΔK = [L" (K)]/STDEV) in the log-likelihood values of $K = 2$–20 hypothesized genetic groups of pea aphid *Acyrthosiphon pisum*. Likelihood values, L (K), were derived from ten replicate runs of STRUCTURE for each K, with a burn-in period of 100 000 runs and 400 000 Monte Carlo Markov chain (MCMC) repetitions.

Allele frequencies for all loci in both regions diverged significantly from Hardy–Weinberg equilibrium, consistent with host-associated genetic structure (Table S2). The mean number of alleles per locus was greater in the predominant genetic groups in the Basin (green and yellow, with 7·75 and 8·33 alleles per locus, respectively) compared with the predominant groups captured in pan traps on the Palouse (blue: 4·00 alleles per locus, red: 4·33 alleles per locus). Furthermore, the mean genetic diversity of red and blue clusters was higher (57·4–82·0% heterozygosity) than for the yellow and green clusters predominant on alfalfa (36·0–69·1% heterozygosity) (Table S3), with an average heterozygosity over all loci of 62·1%.

In both years, certain multilocus genotypes were present in both the Basin and the Palouse pan trap samples. In 2011, four such genotypes comprised 94 of the 324 individuals analysed (28·3%); in 2012, six such genotypes comprised 229 of the 660 individuals analysed (34·6%).

RESPONSES OF APHID GENOTYPES TO HOST PLANTS

Representative pea aphid genotypes exhibited a significant 'home field advantage' when feeding on the plant species from which they were collected. The two aphid colonies originating from alfalfa developed larger populations

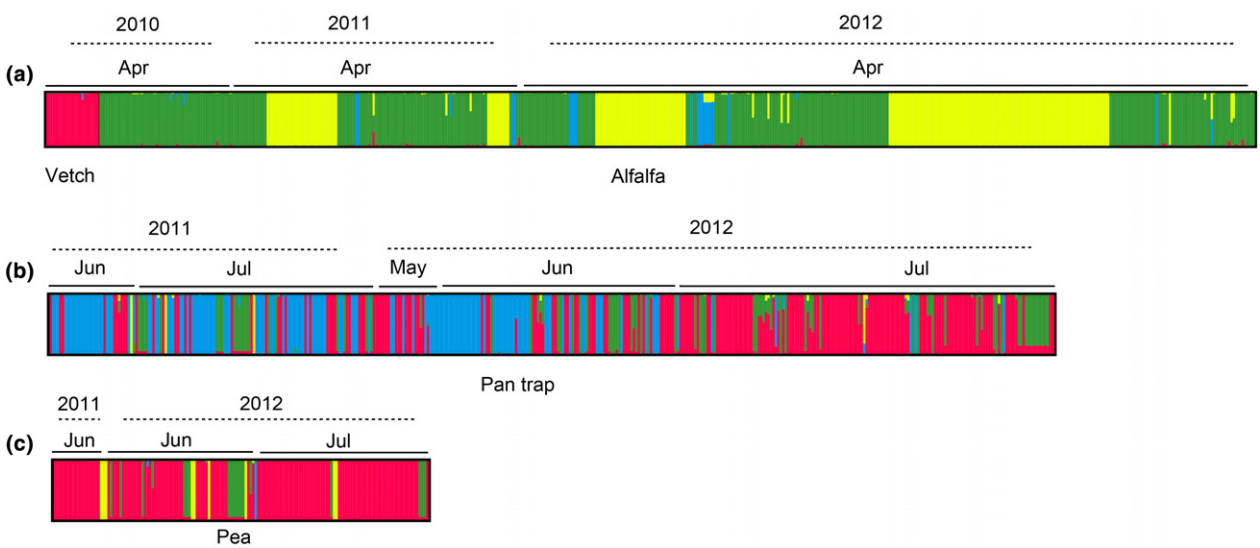

Fig. 4. STRUCTURE diagram showing genetic group identity of 1111 pea aphid *Acyrthosiphon pisum* individuals across three sample years, three field crops and two collection methods (sweep net and pan trap): (a) sweep net samples collected from populations present in commercial crop fields in the Columbia River Basin, (b) pan trap samples of migratory aphid phenotypes arriving near pea fields in the Palouse region and (c) sweep net samples collected from populations present in pea in the Palouse region. Aphid genotypes are arranged by ascending order of sample date within each combination of year, month and crop type.

when reared on alfalfa than when reared on pea ($F_{1,54}$ = 3·781, P = 0·057; and $F_{1,54}$ = 41·105, P < 0·0001, for Alfalfa 1 and Alfalfa 2, respectively), and the colony originating from pea developed larger populations when reared on pea than on alfalfa ($F_{1,54}$ = 214·690; P < 0·0001; Fig. 6). A significant aphid genotype × host plant species interaction for the variable 'population size after 10 days' ($F_{2,54}$ = 12·179; P < 0·0001) indicates that there are trade-offs in performance on the two host plants. The clone originating from pea performed especially poorly on alfalfa, exhibiting a 93% lower mean population size when reared on alfalfa than when reared on pea. Reduced performance on the non-natal host was less pronounced for the two clones originating from alfalfa, which exhibited 52% and 17% lower population sizes when reared on pea, than when reared on alfalfa. Based on the analysis with STRUCTURE, the two clones originating from alfalfa belong to a predominant genetic group associated with alfalfa (coloured green in Fig. 4), and the clone originating from pea belongs to the predominant group associated with pea (coloured red in Fig. 4).

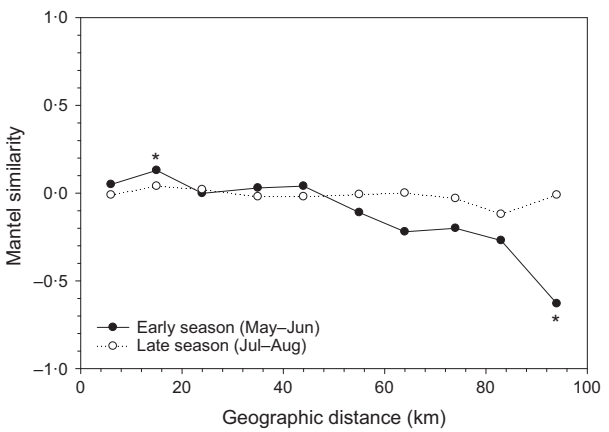

Fig. 5. The similarity of the composition of immigrant pea aphid genetic groups (n = 4 groups; Mantel's similarity index) in aphids collected in pan traps in the Palouse region, calculated as function of pairwise distances between trap sites, and separately for early- and late-season samples. Data were pooled for both study years (2011 and 2012). Asterisks denote significant (P < 0·05) departures from random for a given geographic distance.

Discussion

Host specialization among populations of agricultural pests can present unique challenges for pest management (Kennedy 1992). These are potentially compounded in landscapes in which pest dynamics depend jointly on the geographic heterogeneity of source and sink host plants and the spatial distributions and movements of the pests. Although genetic variability in host performance and preference has been documented for some pest species (Claridge, Dawah & Wilson 1997; De Barro *et al.* 2011), including aphids (Via 1991a,b; Lushai & Loxdale 2002), and genetic structure of pest populations has been used to infer geographic patterns of migration (Taylor, Shields & Davis 1995; Mun *et al.* 1999; Anderson & Congdon 2013), rarely have the two been coupled with a view to improving pest management (but see Lushai & Loxdale 2004; Nagoshi, Meagher & Hay-Roe 2012). Here, we address this using the pea aphid, an important model for host race formation (Via 1991a,b; Ferrari *et al.* 2006; Peccoud *et al.* 2009) but focusing on its long-distance dispersal and pest status in an important production region.

First, we confirmed that host-adapted genetic groups of pea aphid occur in the PNW. Although pea aphid host races are known in eastern North America (Via 1991a,b), Europe (Ferrari *et al.* 2006; Peccoud *et al.* 2009) and Chile (Peccoud *et al.* 2008), their existence in western North America was uncertain (Leonardo & Muiru 2003). Pea aphids collected from alfalfa, vetch and pea in both the Columbia River Basin and the Palouse region of eastern Washington and northern Idaho have distinct genetic structure based on a set of microsatellite markers. Furthermore, genotypes collected from pea and alfalfa fields in the PNW exhibit performance trade-offs similar to those documented for populations in other regions (Via 1991a,b, 1999; Hawthorne & Via 2001; Peccoud *et al.* 2008, 2009).

Secondly, we show annual movements of pea aphids into the Palouse, based on pan trap samples over 2 years, include individuals falling into the genetic groups associated with pea and alfalfa, but that those establishing on pea in the Palouse are almost entirely from a single group evidently specialized on pea. This is consistent with genetic winnowing favouring genotypes adapted to a specific host plant species (Via 1999). Presumably host-associated genetic groups we have detected are maintained

Table 1. Absolute and relative monthly abundances of migratory alfalfa- and pea-associated aphids captured via pan trap in the Palouse in 2011 and 2012

Year	Month	Alfalfa-associated genotypes	Pea-associated genotypes	% Alfalfa-associated	% Pea-associated
2011	June	25	6	80	20
	July	73	24	75	25
2012	May/June	68	40	63	37
	July	51	123	29	71

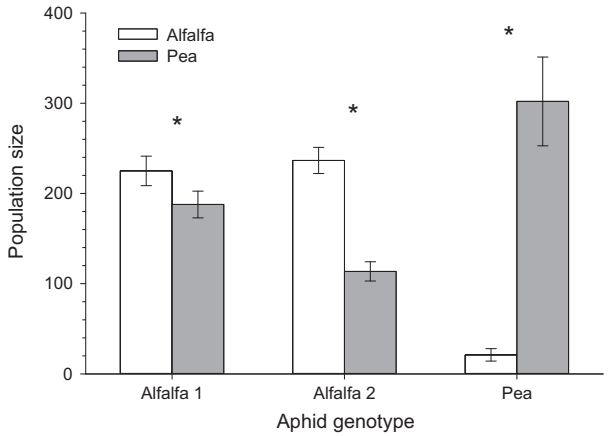

Fig. 6. Effect of aphid genotype × host plant species interaction on population size after 10 days. Bars represent one standard error, and asterisks show significant differences ($P < 0{\cdot}05$) in contrasts testing for differences in population size due to effects of host plant species for each biotype. Alfalfa 1 and Alfalfa 2 clones were collected from alfalfa, and the third clone was collected from pea.

in the Basin by a combination of differential habitat choice and selection against non-adapted migrants and hybrids on the host plant (Via 1999; Via, Bouck & Skillman 2000; Peccoud *et al.* 2014). Pea aphids are not known to overwinter in the Palouse (Clement, Husebye & Eigenbrode 2010) so the region likely does not contribute to maintenance of pea aphid genetic structure and host associations.

Co-occurrence of the same genetic groups, and *c.* 30% of the multilocus pea aphid genotypes, in the Basin and in Palouse pan traps is consistent with the long-held view by producers and scientists that pea aphids colonizing spring planted pea crops in the Palouse arrive on prevailing westerly winds from source populations in alfalfa or other perennial legumes in the lower elevations of the Basin, where winter conditions are mild (Eichmann 1940; McWhorter & Cooke 1958; Cooke 1963; Homan, Stoltz & Schotzko 1991; Clement, Husebye & Eigenbrode 2010). This corroborates aphid risk models in use that are based on early spring Basin weather data (http://www.cals.uidaho.edu/aphidtracker/). Still, our data are not definitive concerning the origins of pea aphid immigrants, since the detected genetic groups may occur elsewhere in the PNW. Although pea aphids were not found on wild and feral legumes in the Basin, more extensive sampling across the PNW could identify other potential sources of immigrants to the Palouse.

Spatial and temporal patterns in the arrival of genetic groups in the Palouse region were apparent in both years of this study. Early season was marked by genetic composition that was similar among trap sites at up to 20 km and dissimilar at distances greater than 90 km. This large-scale pattern suggests that aphids from particular source locations are deposited together across specific parts of the Palouse. Later in the season, this structure dissipates, possibly because of the increasing proportion of trapped aphids originating locally from pea fields, or because of changes in source populations and weather patterns. Longer term monitoring will help identify spatial variation in risk for pea aphid infestations in the Palouse.

These patterns have implications for pea aphid management as a direct pest and as a virus vector. Current action thresholds for pea aphid direct injury (e.g. Homan, Stoltz & Schotzko 1991; Stokes, Bechinski & Eigenbrode 2013) or assessments of potential risk of infestation based on monitoring of aphid movements could be too conservative, since these have been developed based on research with pea-adapted genotypes (Stokes 2012). The pea aphid is a vector of two injurious viruses affecting pulse production in the Palouse, *Pea enation mosaic virus* (PEMV) and *Bean leaf roll virus* (BLRV) (Clement, Husebye & Eigenbrode 2010). BLRV infects alfalfa and other legumes and is transmissible from these hosts by pea aphid (Cockbain & Gibbs 1973). BLRV was found in 17–50% of alfalfa fields in the Basin in prior years (2008 and 2010, H. Pappu and S. Eigenbrode, unpublished data). PEMV does not occur in PNW alfalfa, which is considered a non-host for this virus (Larsen, Kaiser & Klein 1996). Thus, aphids originating from vetch, pea and certain other hosts potentially carry PEMV or BLRV, while those originating from alfalfa can only carry BLRV, with implications for disease risk and epidemiology.

Specific recommendations to producers that could be developed based on this information include modifying thresholds for treatment based on the predominant genetic groups present in sentinel pan traps. Pan traps are deployed annually to report arrival patterns of aphids in the Palouse and to monitor virus in these aphids. If samples of these arriving aphids were genotyped, recommendations for treatment could be modified to account for different risks posed by different mixtures of arriving genotypes. This modification could influence decisions involving direct injury treatment thresholds and treatments based on risks of virus transmission. To realize this, empirical studies are needed to quantify economic impacts of different mixtures of colonists and cost-effective methods for genotyping developed.

The findings of this study have broader implications for management of aphids and other mobile pests and vectors. Large-scale pest movements or migrations coupled with genetic information can contribute important information about pest biology pertinent to management (Taylor, Shields & Davis 1995; Mun *et al.* 1999; Nagoshi, Meagher & Hay-Roe 2012; Anderson & Congdon 2013) and our study illustrates this potential for an important cosmopolitan pest of legumes. Similar effects could occur in other aphid–crop or herbivore–crop systems in which distance migration is known to occur (Bommarco & Ekbom 1995; Irwin 1999; Klueken *et al.* 2009; Harrington & Clark 2010) and migrants have been genotyped (Guillemaud, Mieuzet & Simon 2003; Lushai & Loxdale 2004). The repercussions of aphid host race associations for disease management

has not been examined in any system, to our knowledge, and should be explored further.

In conclusion, this study answered our three motivating questions affirmatively: (i) there is clear evidence for host-associated population genetic structure in PNW pea aphid. (ii) The same genetic groups found on crops in the putative source region occur as migrants into the Palouse. (iii) The genetic structure of migrant populations varies temporally and spatially. Experimental evidence suggests that many migrants may be incapable of establishing populations, and genetic surveys indicate that aphids colonizing pea in the Palouse are predominantly of one genetic group, despite the genetic diversity of incoming alates. These findings corroborate long-held assumptions about pea aphid movements in the region. These patterns are also germane to improving pest management techniques, since pea aphid populations differ in their propensity to colonize different crops, and for phytovirus dynamics, since potential source species differ as hosts of these viruses. Finally, these results provide evidence of movement of multiple pea aphid host-associated genotypes at the landscape scale, with importance for understanding the mechanisms that contribute to the occurrence and maintenance of host races in this species.

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Data accessibility

The data upon which this paper is based are archived and can be accessed at https://www.reacchpna.org/portal and also from Dryad Digital Repository http://dx.doi.org/10.5061/dryad.4kj53 (Eigenbrode *et al*. 2016).

References

Anderson, K.L. & Congdon, B.C. (2013) Population genetics suggest that multiple invasion processes need to be addressed in the management plan of a plant disease vector. *Evolutionary Applications*, **6**, 660–672.

Bjørnstad, O. (2013) Ncf: spatial nonparametric covariance functions. R package version 1.1-5. UWL http://CRAN.R-project.org/package = ncf.

Blackman, R.L. & Eastop, V.F. (2007) *Aphids on the Worlds Herbaceous Plants and Shrubs*. John Wiley and Sons Inc, New York.

Bommarco, R. & Ekbom, B. (1995) Phenology and prediction of pea aphid infestations on peas. *International Journal of Pest Management*, **41**, 109–113.

Caillaud, M.C., Mondor-Genson, G., Levine-Wilkinson, S., Mieuzet, L., Frantz, A. & Couer D'Acier, A. (2004) Microsatellite DNA markers for the pea aphid *Acyrthosiphon pisum*. *Molecular Ecology Notes*, **4**, 446–448.

Claridge, M.F., Dawah, H.A. & Wilson, M.R. (1997) Species in insect herbivores and parasitoids – sibling species, host races and biotypes. *Species: The Units of Biodiversity*. *Systematics Association Special Volume Series 54* (eds M.F. Claridge, H.A. Dawah & M.R. Wilson), pp. 247–272. Chapman & Hall, London.

Clement, S.L. (2006) Pea aphid outbreaks and virus epidemics on peas in the US Pacific Northwest: histories, mysteries, and challenges. *Online Plant Health Progress*, doi: 10.1094/PHP-2006-1018-01-RV.

Clement, S.L., Husebye, D.S. & Eigenbrode, S.D. (2010) Ecological factors influencing pea aphid outbreaks in the US Pacific Northwest. *Aphid Biodiversity Under Environmental Change: Patterns and Processes* (eds P. Kindlmann, A.F.G. Dixon & J.P. Michaud), pp. 108–128. Springer, The Netherlands.

Cockbain, A.J. & Gibbs, A.J. (1973) Host range and overwintering sources of bean leaf roll and pea enation mosaic viruses in England. *Annals of Applied Biology*, **73**, 177–187.

Cooke, W.C. (1963) *Ecology of the Pea Aphid in the Blue Mountain Area of Eastern Washington and Oregon*. U.S. Department of Agriculture, Agricultural Research Service, Technical Bulletin 1287, Washington, DC.

Coon, B.F. & Pepper, J.O. (1968) Alate aphid species captured in yellow pans. *Journal of Economic Entomology*, **61**, 1472–1473.

De Barro, P.J., Liu, S., Boykin, L.M. & Dinsdale, A.B. (2011) *Bemisia tabaci*: a statement of species status. *Annual Review of Entomology*, **56**, 1–19.

Dent, E.A. & von Holdt, B.M. (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359–361.

Drès, M. & Mallet, J. (2002) Host races in plant-feeding insects and their importance in sympatric speciation. *Philosophical Transactions of the Royal Society Series B*, **357**, 471–492.

Eichmann, R.D. (1940) The pea aphid on canning peas in eastern Washington as influenced by alfalfa plantings. *Journal of Economic Entomology*, **33**, 137–139.

Eigenbrode, S.D., Davis, T.S., Adams, J.R., Husebye, D., Waits, L.P. & Hawthorne, D. (2016) Data from: Host-adapted aphid populations differ in their migratory patterns and capacity to colonize crops. *Dryad Digital Repository*, http://dx.doi.org/10.5061/dryad.4kj53.

Elbakidze, L., Lu, L. & Eigenbrode, S.D. (2011) Evaluating vector-virus-yield interactions for peas and lentils under climatic variability: a limited dependent variable analysis. *Journal of Agricultural and Resource Economics*, **36**, 504–520.

Evanno, G., Regnaut, S. & Goudet, J. (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Falush, D., Stephens, M. & Pritchard, J.K. (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, **164**, 1567–1587.

Ferrari, J., Via, S. & Godfray, H.C.J. (2008) Population differentiation and genetic variation in performance on eight hosts in the pea aphid complex. *Evolution*, **62**, 2508–2524.

Ferrari, J., Godfray, H.C.J., Faulconbridge, A.S., Prior, K. & Via, S. (2006) Population differentiation and genetic variation in host choice among pea aphids from eight host plant genera. *Evolution*, **60**, 1574–1584.

Futuyma, D.J. & Peterson, J.C. (1985) Genetic variation in the use of resources by insects. *Annual Review of Entomology*, **30**, 217–238.

Goudet, J. 2014. Package 'hierfstat'. URL: http://www.r-project.org,http://www.unil.ch/popgen/softwares/hierfstat.htm. Accessed 15 August 2014.

Guillemaud, T., Mieuzet, L. & Simon, J.-C. (2003) Spatial and temporal genetic variability in French populations of the peach-potato aphid, *Myzus persicae*. *Heredity*, **91**, 143–152.

Han, W., Yang, Z., Di, L. & Mueller, R. (2012) CropScape: a Web service based application for exploring and disseminating US conterminous geospatial cropland data products for decision support. *Computers and Electronics in Agriculture*, **84**, 111–123.

Harrington, R. & Clark, S. (2010) Trends in the timings of the start and end of annual flight periods. *Aphid Biodiversity Under Environmental Change: Patterns and Processes* (eds P. Kindlmann, A.F.G. Dixon & J.P. Michaud), pp. 41–54 Springer, The Netherlands

Hawthorne, D.J. & Via, S. (2001) Genetic linkage of ecological specialization and reproductive isolation in pea aphids. *Nature*, **412**, 904–907.

Homan, H.W., Stoltz, R.L. & Schotzko, D.J. (1991) *Aphids on Peas and Lentils and their Control*, Current Information Series, No. 748, Bulletin of University of Idaho Cooperative Extension System.

Irwin, M.E. (1999) Implications of movement in developing and deploying integrated pest management strategies. *Agricultural and Forest Meteorology*, **97**, 235–248.

Kennedy, G.G. (1992) Impact of intraspecific variation on insect pest management. *Evolution of Insect Pests* (eds K.C. Kim & B.A. McPheron), pp. 425–451. John Wiley and Sons Inc, New York.
Klueken, A.M., Hau, B., Ulber, B. & Poehling, H.M. (2009) Forecasting migration of cereal aphids (Hemiptera: Aphididae) in autumn and spring. *Journal of Applied Entomology*, **133**, 328–344.
Kurokawa, T., Yao, I., Akimoto, S.-I. & Hasegawa, E. (2004) Isolation of six microsatellite markers from the pea aphid, *Acyrthosiphon pisum* (Homoptera, Aphididae). *Molecular Ecology Notes*, **4**, 523–524.
Larsen, R.C., Kaiser, W.J. & Klein, R.E. (1996) Alfalfa, a non-host of pea enation mosaic virus in Washington State. *Canadian Journal of Plant Science*, **76**, 521–524.
Leonardo, T.E. & Muiru, G.T. (2003) Facultative symbionts are associated with host plant specialization in pea aphid populations. *Proceedings of the Royal Society B: Biological Sciences*, **270**, S209–S212.
Lushai, G. & Loxdale, H.D. (2002) The biological improbability of a clone. *Genetical Research*, **79**, 1–9.
Lushai, G. & Loxdale, H.D. (2004) Tracking movement in small insect pests, with special reference to aphid populations. *International Journal of Pest Management*, **50**, 307–315.
Mantel, N. (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.
McWhorter, F.P. & Cooke, W.C. (1958) The hosts and strains of *Pea enation mosaic virus*. *Plant Disease Reporter*, **42**, 51–60.
Mun, J.H., Song, Y.H., Heong, K.L. & Roderick, G.K. (1999) Genetic variation among Asian populations of rice planthoppers, *Nilaparvata lugens* and *Sogatella furcifera* (Hemiptera: Delphacidae): mitochondrial DNA sequences. *Bulletin of Entomological Research*, **89**, 245–253.
Nagoshi, R.N., Meagher, R.L. & Hay-Roe, M. (2012) Inferring the annual migration patterns of fall armyworm (Lepidoptera: Noctuidae) in the United States from mitochondrial haplotypes. *Ecology and Evolution*, **2**, 1458–1467.
NASS (2013) *Crop Production*: ISSN: 1936–3737. National Agricultural Statistics Service of the USDA, Washington, DC.
Peakall, R. & Smouse, P.E. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.
Peccoud, J. & Simon, J.-C. (2010) The pea aphid complex as a model of ecological speciation. *Ecological Entomology*, **35**, 119–130.
Peccoud, J., Figueroa, C.C., Silva, A.X., Ramirez, C.C., Mieuzet, L., Bonhomme, J., Stoeckel, S., Plantegenest, M. & Simon, J.-C. (2008) Host range expansion through multiple colonizations of specialized clones. *Molecular Ecology*, **17**, 4608–4618.
Peccoud, J., Ollivier, A., Plantegenest, M. & Simon, J.-C. (2009) A continuum of genetic divergence from sympatric host races to species in the pea aphid complex. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 7495–7500.
Peccoud, J., de la Huerta, M., Bonhomme, J., Laurence, C., Outreman, Y., Smadja, C.M. & Simon, J.-C. (2014) Widespread host-dependent hybrid unfitness in the pea aphid species complex. *Evolution*, **68**, 2983–2995.
Pritchard, J., Stephens, M. & Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 949–959.
R Core Team (2012) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-9000051-07-0. URL: http//222.R-project.org/.
Simon, J.-C., Baumann, S., Sunnucks, P., Hebert, P., Pierre, J., Le Gallic, J. & Dedryver, C. (1999) Reproductive mode and population genetic structure of the cereal aphid *Sitobion avenae* studied using phenotypic and microsatellite markers. *Molecular Ecology*, **8**, 531–545.
Stokes, B.S. (2012) Bionomics of pea aphids on commercial dry peas. M.S. Thesis, University of Idaho.
Stokes, B.S., Bechinski, E.J. & Eigenbrode, S.D. (2013) Derivation and validation of a binomial sequential decision plan for managing pea aphids (Hemiptera: Aphididae) as direct pests of dry pea (Fabales: Fabaceae) in the Pacific Northwest. *Journal of Economic Entomology*, **106**, 2613–2620.
Taylor, P.S., Shields, E.J. & Davis, J.I. (1995) *Empoasca fabae* (Harris) (Homoptera: Cicadellidae) identification and population studies using allozyme electrophoresis. *Environmental Entomology*, **24**, 1109–1114.
USA Dry Pea and Lentil Council (2010) *USA Dry Peas, Lentils & Chickpeas, Processing Information & Technical Manual*. USA Dry Pea and Lentil Council, Moscow, ID.
Via, S. (1991a) The genetic structure of host plant adaptation in a spatial patchwork: demographic variability among reciprocally transplanted pea aphid clones. *Evolution*, **45**, 827–852.
Via, S. (1991b) Specialized host plant performance of pea aphid clones is not altered by experience. *Ecology*, **72**, 1420–1427.
Via, S. (1999) Reproductive isolation between sympatric races of pea aphids. I. Gene flow restriction and habitat choice. *Evolution*, **53**, 1446–1457.
Via, S., Bouck, A.C. & Skillman, S. (2000) Reproductive isolation between divergent races of pea aphids on two hosts. II. Selection against migrants and hybrids in the parental environments. *Evolution*, **54**, 1626–1637.
Wilson, A.C., Massonnet, B., Simon, J.-C., Prunier-Leterme, N., Dolatti, L., Llewellyn, K.S. *et al.* (2004) Cross-species amplification of microsatellite loci in aphids: assessments and application. *Molecular Ecology Notes*, **4**, 104–109.

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Supporting Information

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

Table S1. Microsatellite marker data.

Table S2. Tests of Hardy–Weinberg equilibrium data.

Table S3. Allele frequency data.