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Measuring Local Genetic Variability in Populations of Codling Moth (Lepidoptera: Tortricidae) Across an Unmanaged and Commercial Orchard Interface

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ABSTRACT The genetic structure of adult codling moth, *Cydia pomonella* (L.), populations was characterized both inside a managed apple, *Malus domestica* Borkdhausen, orchard and in surrounding unmanaged hosts and nonhost trees in central Chile during 2006–2007. Adult males were collected using an array of sex pheromone-baited traps. Five microsatellite genetic markers were used to study the population genetic structure across both spatial (1–100 ha) and temporal (generations within a season) gradients. Analysis of molecular variance (AMOVA) found a significant, but weak, association in both the spatial and temporal genetic structures. Discriminant analysis also found significant differentiation between the first and second generation for traps located either inside or outside the managed orchard. The Bayesian assignment test detected three genetic clusters during each of the two generations, which corresponded to different areas within the unmanaged and managed apple orchard interface. The lack of a strong spatial structure at a local scale was hypothesized to be because of active adult movement between the managed and unmanaged hosts and the asymmetry in the insecticide selection pressure inside and outside the managed habitats. These data highlight the importance of developing area-wide management programs that incorporate management tactics effective at the landscape level for successful codling moth control.

KEY WORDS *Cydia pomonella*, genetic structure, microsatellite, Bayesian assignment, TESS

Agricultural and forest landscapes are heterogeneous environments characterized by an array of disparate quality host-plant patches distributed within a background matrix of unsuitable habitats for herbivorous insect pests. Based on the specificity of the herbivore and the distribution of the host, this pronounced landscape heterogeneity can produce barriers to dispersal and gene flow for rather specialist herbivore pests (Van Dongen et al. 1998, Schroeder and Degen 2008, Lavandero et al. 2009, Lavandero et al. 2011). In addition, agricultural landscapes are also characterized by frequent environmental disturbances associated with cultural practices and spraying of pesticides, creating local extinction and colonization that continually restrict and expand the spatial distribution of the insect pest species among host plants belonging either to the cultivated or to the uncultivated areas (Carrière et al. 2004, Vialatte et al. 2005, Mazzi and Dorn 2012). One relevant effect of management practices on pest populations is the selection for genes conferring resistance to pesticides (Whalon et al. 2008). This selection pressure has both an economic and genetic impact and is a dominant factor affecting the genetic population structure of resistance genes of pests at the landscape scale (Caprio et al. 2004, Carrière et al. 2004, Ricci et al. 2009, Franck and Timm 2010).

Management of moderately dispersive pests has been shown to be more effective through area-wide practices that include all of the potential hosts within the program (Vreysen et al. 2007, Koul et al. 2008). Creating area-wide pest management programs based on information on the genetic structure (e.g., subpopulations, migration rates, and gene flow) of these pest species at the landscape scale could increase the success of these programs (Manel et al. 2003). For instance, the identification of boundaries to gene flow between pest populations based on their genetic characteristics could improve the identification of pest recruitment zones that should be under area-wide management. Such area-wide management units should include both managed and unmanaged populations, to prevent recurrent pest immigration from unmanaged sites that produce pest resurgence in

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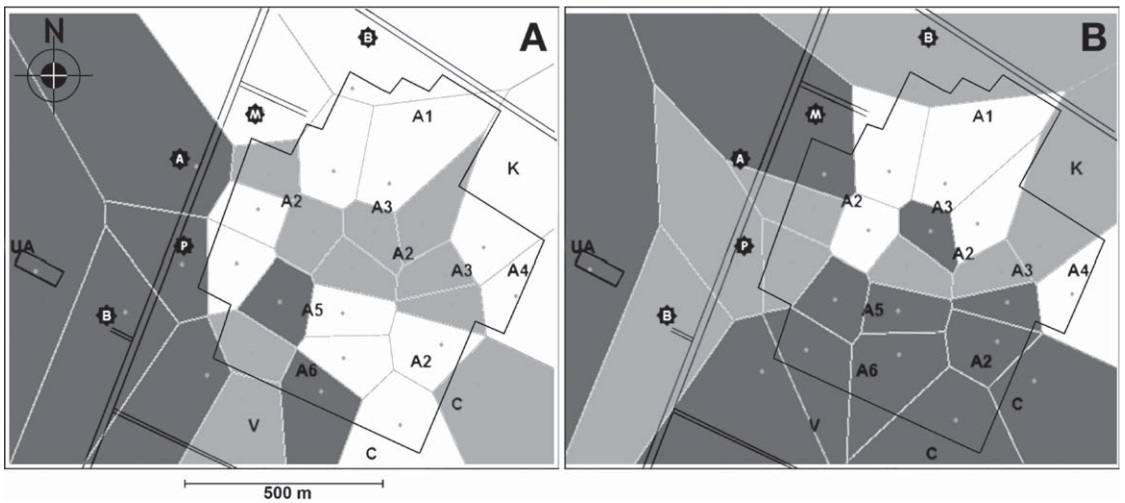

Fig. 1. Voronoi tessellations of adult male codling moth multilocus genotype probability distribution across space, obtained by TESS applied to: (A) first, and (B) second generation during 2006–2007.

managed populations (Kovaleski and Mumford 2007, Franck and Timm 2010, Franck et al. 2011).

Codling moth, *Cydia pomonella* L., is a lepidopteran pest of key importance for apple (*Malus domestica* Borkhausen), pear (*Pyrus communis* L.), and walnut (*Juglans regia* L.) production in temperate areas worldwide (Barnes 1991). Although it can occasionally develop on a few other Rosaceae species, it is regarded as an oligophagous pest with moderate dispersive capabilities (Keil et al. 2001, Gu et al. 2006). The use of area-wide management of codling moth has been applied in some pome-producing areas with different levels of success (Bloem et al. 2007, Kovaleski and Mumford 2007, Knight 2008). This variability has been associated with the presence of codling moth population sources in uncultivated areas (Kovaleski and Mumford 2007). Understanding the population genetic structure of this pest may benefit the identification and discovery of codling moth populations in unmanaged areas. Such populations can be sources of codling moth immigration through effective dispersal and gene flow toward managed orchards (Franck et al. 2011, Margaritopoulos et al. 2012). Therefore, the implementation of area-wide management can be improved by considering the impact of the use of insecticide sprays, mating disruption, and sterile insect technique among other components on the codling moth population genetic structure.

Landscape characteristics at a local scale, that is 1–100 ha, might have important influence on the spatial structure and dynamics of the populations of the codling moth (Trematerra et al. 2004; Ricci et al. 2009, 2011). Furthermore, dispersal of codling moth adults across the orchard boundaries seems to be a significant component of population dynamics for codling moth (Tyson et al. 2007). In particular, significant dispersal between population sources and sinks has been studied previously based on mark and capture and geostatistical analyses showing that immigration of codling moth males and females from the unmanaged surroundings to the insecticide managed orchard, is probably the main variable affecting the spatial population dynamics of this pest (Basoalto et al. 2010).

Here, we describe the genetic structure of a codling moth population at the local scale in a typical agroecosystem of central Chile, dominated by apple production for export markets. We analyzed microsatellite genotypes of adult males captured in a grid of pheromone traps in a large apple orchard and its surroundings, which included unmanaged apple and pear hosts scattered in a matrix of unsuitable habitats. We describe the influence of trap location inside or outside the managed area on the genetic variability and population structure of the codling moth during two consecutive generations in one growing season. We found a significant, but weak, differentiation between populations in both the spatial and temporal genetic structure supported by three population genetics analyses.

Materials and Methods

Study Area. The study site was the Quechereguas orchard located 5 km north of Molina (35° 5′37″ S, 71° 16′6″ W) in the central valley of the Maule region, Chile (Basoalto et al. 2010; see Fig. 1). It was located in a relatively flat landscape of the central valley (150–250 m above sea level). This site was composed of a large commercial orchard (≈100 ha) with plots of different apple varieties and other fruit species such as kiwi fruit (*Actinidia deliciosa* (Chevalier) Liang & Ferguson), cherry (*Prunus avium* (L.)), and grape vine (*Vitis vinifera* L.). The surrounding area was a heterogeneous rural landscape with a low-density of human settlement. Packing facilities and harvest bin piles were located near the apple fruit plots. A small plot (0.2 ha) with unmanaged apple trees (UA), and scattered unsprayed pear, quince, and walnut trees

Table 1. Cumulative catch of male codling moth in sex pheromone-baited traps placed both inside a commercial managed orchard and a number of unmanaged hosts and nonhosts outside the orchard for each generation in 2006–2007 and the number of individuals genotyped

Trap location	Management	Trap no.	First generation		Second generation	
			Captured	Genotyped	Captured	Genotyped
Inside orchard	Managed	20	685	176	879	102
Outside orchard	Unmanaged	9	252	44	279	27
Total		29	937	220	1,158	129

were located 100–300 m west of the commercial orchard in the low-density human settlement area (Basoalto et al. 2010; see Fig. 1).

Insecticides applied by the grower included chlorpyrifos 480 g/liter EC, chlorpyrifos 750 g/kg WG, phosmet 700 g/kg WP, azinphos-methyl 350 g/kg WS, and methoxyfenozide 240 g/liter SC. The treatment concentrations of active ingredients (a.i.) per liter were 0.5 g for chlorpyrifos, 0.5 g for phosmet, 0.4 g for azinphos-methyl, and 0.05 g for methoxyfenozide. In addition, fruit thinning with carbaryl 850 g/kg WP at a rate of 0.85 g/liter (a.i.) was performed at the beginning of the sampling period. No mating disruption or other sex pheromone-based control methods were applied during the study.

Data Collection. The activity of codling moth adult males was monitored using off-white diamond-shaped traps (Pherocon IIB, Trécé Inc., Adair, OK) baited with 1.0 mg (*E, E*)-8,10-dodecadien-1-ol (codlemone) wax septa (Exosect Ltd., Southampton, United Kingdom). Pheromone traps were placed in the field on 4 October 2006. Traps were placed in the tree canopy at a height of 2.5–3 m. Moths were removed and counted weekly. Lures and sticky liners were replaced at 6- and 2- to 4-wk intervals, respectively, during the season until March 2007. In total, 50 traps were spaced in an irregular grid with an intertrap spacing ranging from 60 to 70 m. The trap grids were designed to include plots of all commercial fruit species (Table 1; and see Basoalto et al. 2010 for further details). Males caught in the 29 traps with the highest catches were selected for the genetic analyses. These traps were separated in two groups based on their location inside or outside the managed area, that is, apple plots with insecticide sprays (20 traps) versus all the other locations without insecticide sprays (nine traps; Table 1). The traps placed outside the managed area were distributed as follows: one in the unmanaged apple plot, one in a single pear tree, and seven on different nonhost trees. The density of male moths caught per trap outside the managed area was lower and more variable (mean ± SE = 59.0 ± 14.5) than inside the managed area (mean ± SE = 78.2 ± 6.2). The capture of males in sex pheromone-baited traps in this site was previously shown with geostatistic and immunomarking techniques to be influenced by distance (≈150–300 m between traps), and not by host-plant species (Basoalto et al. 2010). Therefore, adult males while searching for sources of sex pheromone were regarded as individuals affected by pheromone trap location in space rather than by pheromone trap location on a particular host-plant species.

Microsatellite Analysis. In total, 349 out of 2,095 individuals captured during the season were genotyped (Table 1). Randomly selected individuals (1–21 per trap) were genotyped during both each codling moth generations. Because a larger population of males was collected with sex pheromone-baited traps placed inside the managed area than in the unmanaged area, sample size was subsampled to adjust the number of analyses to fall between 15–18% of the collected individuals of each area. Five microsatellite markers (*Cp2.39*, *Cp3.169*, *Cp3.180*, *Cp3.56*, and *Cp3.K*) were chosen based on their polymorphism (Franck et al. 2005, 2007) to differentiate Chilean codling moth populations (Fuentes-Contreras et al. 2008). DNA extraction was performed using the abdomens of adult males following the "salting out" procedure (Sunnucks and Hales 1996). The tissue was homogenized inside plastic tubes provided with TNES buffer (Tris-HCl 50 mM, pH 7.5, NaCl 400 mM, EDTA 20 mM, and SDS 0.5%). The samples were incubated overnight at 37°C with proteinase K (10 mg/ml). DNA was precipitated with 5M NaCl, followed by centrifugation at 10,000 rpm. The supernatant was washed twice with 350 μl cold ethanol, dried, and then suspended in 15 μl ultrapure distilled water. Polymerase chain reaction (PCR) amplifications were carried out using a final volume of 10 μl, containing Tris-HCl 10 mM, pH 9.0, KCl 50 mM, $MgCl_2$ 1.5 mM, 50 μM each dNTP, 0.4 μM each primer, 0.5 U of *Taq*DNA polymerase (Invitrogen, Sao Paulo, Brazil), and 2 μl of DNA template (≈10 ng/μl). Amplifications were performed with a Mastercycler gradient Eppendorf thermocycler (Eppendorf International, Hauppauge, NY) under the following conditions: 2 min at 94°C, 30 cycles of 30 s at 94°C, 40 s at annealing temperature of 55°C for primers *Cp3.169* and *Cp3.K*, and 61°C for primers *Cp2.39*, *Cp3.56*, and *Cp3.180*, and 40 s at 72°C, with a final extension at 72°C for 3 min. PCR products were separated in 6% polyacrylamide gels using a BIO-RAD Sequi-Gen GT Electrophoresis Cell. Band sizes were visually estimated after silver nitrate staining using the protocol from Silver Sequence kit (Promega Biosciences, Madison, WI) with pGEM-3Zf (+) Vector (Promega Biosciences) as a reference in the same gel.

Data Analysis. Individuals were grouped as spatial and temporal samples based on trap location (inside or outside insecticide managed area), and generation (first and second flight). Individuals were grouped for each of the two generations based on the accumulation of degree-days (DD; lower threshold of 10°C) from first sustained moth catch to the completion of

the first (456 DD) and second generation (1,044 DD) for the season (Knight 2007). However, the timing of the first and the second generation of the codling moth overlap, and to avoid wrong generation assignments, individuals trapped between 400 and 600 DD (approximately last 10 d of December 2006 and first 10 d of January 2007) were excluded from the analysis.

The microsatellite data were analyzed for evidence of null alleles and technical artifacts like "stuttering" and large allele "dropout" using MICROCHEKER version 2.2.3 (Van Oosterhout et al. 2004). Deviations from the Hardy-Weinberg equilibrium were tested using GENEPOP version 4.0.10 (Raymond and Rousset 1995). The frequency of null alleles (*Na*) for each locus was estimated using FREENA (Chapuis and Estoup 2007). The number of alleles, allelic richness corrected for sample size using rarefaction (*A*), Nei's gene diversity (H_e), and fixation index (F_{IS}), were calculated for each locus with FSTAT version 2.9.3 (Goudet 2001).

Hierarchical analyses of the molecular variance (AMOVA) of codling moth samples among trap location (inside and outside the managed area) or generation (first and second generations) were performed using the software ARLEQUIN version 3.11 (Schneider et al. 2000). The genetic differentiation among groups and samples was estimated with the Fixation index, F_{ST} (Weir and Cockerham 1984) and tested using 1,023 permutations. ARLEQUIN was also used to test for the presence of significant association between pairs of loci, based on an exact test of linkage disequilibrium including a Bonferroni correction. In addition, a linear discriminant analysis using the *lda* function in the package MASS of R software (R Development Core Team 2013) was performed to differentiate the genetic variability from microsatellite loci between samples according to trap location and generation (four groups: first generation-inside orchard, first generation-outside orchard, second generation-inside orchard, and second generation-outside orchard).

The number of genetic clusters (K) was estimated using different Bayesian-based methods implemented in STRUCTURE version 2.3.2 (Pritchard et al. 2000), in TESS version 2.3.1 (Chen et al. 2007), and in GENELAND version 3.2.4 (Guillot et al. 2005). Five independent runs assuming values of K from 1 to 10 with 600,000 Markov chain Monte Carlo (MCMC) repetitions and a burn-in period of 60,000 were performed with STRUCTURE, assuming population admixture and correlated allele frequencies. The posterior probability (probability of K given the data) was then calculated for each mean value of K using the mean estimated log likelihood of K to choose the optimal K describing the genetic data set. Subsequently, the most probable number of populations was taken using the method proposed by Evanno et al. (2005) based on the rate of change in the log probability of data between successive K values. Furthermore, the proportion of the genome derived from each of these K genetic clusters (proportion of ancestry) was estimated for each codling moth individual.

The TESS algorithm was run with 10,000 sweeps, discarding the first 5,000 with 20 independent iterations for each model for maximum number of clusters (Kmax) varying from 2 to 10 and the interaction parameter set as $\psi = 0.6$. It was used with and without admixis, both assuming correlated frequencies of alleles. The highest likelihood runs were selected based on the Deviance Information Criterion (DIC) and graphed against Kmax as suggested by Chen et al. (2007), allowing us to select the number of clusters (K) that best explained the genetic data set. Subsequently, the most probable number of populations was taken using the method proposed by Evanno et al. (2005) based on the rate of change in the log probability of data between successive K values. Then the program was run 100 times for the selected Kmax with 50,000 sweeps discarding the first 10,000. Then the 10 highest likelihood runs were averaged. Finally, a Voronoi tessellation obtained from TESS for codling moth population genetic structure in space was plotted for visual assessment.

Five independent runs with K values from 1 to 10, with 100,000 MCMC iterations, 100 thinning, and a true spatial model combined with the correlated frequency model were performed with GENELAND. Postprocessing MCMC outputs were analyzed with 100 pixels for horizontal and vertical discretization and a burn-in of 200 saved iterations.

TESS uses the same algorithm as STRUCTURE except that it takes into account the spatial coordinates of the genotyped individuals to infer population genetic structure among the samples. GENELAND uses also geo-referenced individual and multilocus genotypes for the inference of the number of populations, but its philosophy was to identify genetic discontinuities between those populations, assuming that populations are continuously distributed in the sampled spatial domain. Thus, the most likely genetic structure should be revealed by these independent tests with spatial and nonspatial clustering models.

Results

Total trap catches were higher inside than outside the managed apple orchard during both the first and second generation (Table 1). The genetic variability observed during each generation for population samples inside and outside the managed orchard is reported for each microsatellite locus in Table 2. The number of alleles ranged from 2 for locus *Cp3.180* to 22 for locus *Cp3.169.* Evaluations of Hardy-Weinberg equilibrium showed a significant ($P < 0.05$) excess of homozygotes for loci *Cp2.39*, *Cp3.169*, and *Cp3.180*, and deficit for locus *Cp3.56* in the whole study pooled sample. The H_e for all samples pooled ranged from 0.095 for locus *Cp3.180* to 0.865 for locus *Cp2.39.* F_{IS} was variable between loci and samples analyzed. Null alleles were found at low frequencies for loci *Cp3.180*, *Cp3.56*, and *Cp3.K* (<1%). The highest frequency of null alleles (11%) was obtained in locus *Cp3.169.* No significant linkage disequilibrium, after a Bonferroni correction, was detected between any pair of loci.

Table 2. Measurements of the genetic diversity: number of alleles (Alleles), allelic richness (*A*), Nei's gene diversity (H_e), fixation index (F_{IS}), and frequency of null alleles (N_a) for selected loci for adult moths sampled inside and outside the commercial apple orchard during both generations separately and combined during 2006–2007

Locus	First generation		Second generation		Both
	Inside	Outside	Inside	Outside	
Cp2.39[a]					
Alleles	14	13	14	11	16
A	11.812	11.865	10.838	10.625	15.867
H_e	0.869	0.888	0.857	0.801	0.865
F_{IS}	0.209	0.189	0.140	0.029	0.175
N_a	0.099	0.075	0.045	0.0001	0.055
Cp3.169[a]					
Alleles	20	17	18	12	22
A	12.154	13.158	11.844	11.786	21.939
H_e	0.833	0.772	0.849	0.838	0.835
F_{IS}	0.252	0.242	0.219	0.358	0.253
N_a	0.111	0.090	0.087	0.143	0.108
Cp3.180[a]					
Alleles	2	2	2	2	2
A	1.846	1.923	1.983	2.000	2.000
H_e	0.066	0.067	0.128	0.201	0.095
F_{IS}	0.657	−0.024	−0.069	−0.106	0.189
N_a	0.094	0.000	0.000	0.000	0.002
Cp3.56[a]					
Alleles	10	8	8	6	10
A	6.432	6.309	6.118	6.000	10.000
H_e	0.650	0.630	0.690	0.695	0.662
F_{IS}	−0.540	−0.586	−0.433	−0.439	−0.505
N_a	0.000	0.001	0.000	0.000	0.000
Cp3.K					
Alleles	12	10	10	9	13
A	9.034	9.123	8.722	9.000	12.994
H_e	0.825	0.862	0.805	0.808	0.826
F_{IS}	0.006	0.034	−0.047	−0.039	−0.004
N_a	0.000	0.017	0.000	0.000	0.004

[a] Significant departure from Hardy-Weinberg equilibrium, $P < 0.05$, Fisher's exact test.

The hierarchical AMOVA showed that almost all genetic variability occurred at the individual level (98.5%) regardless of the first hierarchical level considered (trap location or generation; Table 3). The covariance components because of differences among groups (trap location or generation) were not significant (Table 3). However, the covariance component because of difference between generations within the trap location groups, although small, was significant. Indeed, the highest genetic differentiation (F_{ST} = 1.6%) was observed between the first and the second generation samples outside the orchard (Table 4). Pairwise genetic differentiation between population samples from different generations and different trap location inside and outside the managed orchard were also significant (F_{ST} <1.2%; Table 4).

Table 4. Pairwise F_{ST} (above the diagonal) and their significance levels (below the diagonal) for groups of samples according to generation (first or second) or trap location (inside or outside orchard) of codling moth

Sample	First generation		Second generation	
	Inside	Outside	Inside	Outside
First generation—inside		0.00387	0.00041	0.01201
First generation—outside	0.08		0.00839	0.01612
Second generation—inside	0.23	0.001		−0.00067
Second generation—outside	0.009	0.001	0.36	

Discriminant analysis found significant linear discriminant functions (Wilks' $\lambda = 0.78$; $F_{(30,778)} = 2.23$; $P < 0.0002$). The first and second root explained 95% of the genetic variability. The comparison between samples based on trap location showed no significant differences for both generations (first generation: squared Mahalanobis distance (SMD) = 0.26; $F_{(10,265)} = 0.74$; $P = 0.69$; second generation: SMD = 0.68; $F_{(10,265)} = 1.11$; $P = 0.35$), while significant differences were found between the first and the second generation for traps located either inside or outside the orchard (inside orchard: SMD = 0.87; $F_{(10,265)} = 4.10$; $P < 0.001$; outside orchard: SMD = 1.40; $F_{(10,265)} = 1.85$; $P = 0.05$).

Bayesian population genetic structure analyses with STRUCTURE showed K = 4 (log likelihood = −5,343.2; posterior probability ≈ 1) without correspondence with any groups of samples based on trap location or generation. TESS runs with spatially explicit sampling locations showed for the first generation K = 3 (DIC = 6,443.4), with some correspondence between samples across space, that is, between

Table 3. Results of AMOVA according to trap location (inside or outside treated orchard) or generation (first or second) for codling moth

Source of variation	df	ss	Variance components	Percentage of variation	*F* statistics
Among groups (trap location)	1	2.463	0.00013	0.08	$F_{CT} = 0.00008^{NS}$
Among populations within groups (between generations within trap location)	2	5.502	0.00700	0.43	$F_{SC} = 0.00426^{*}$
Among individuals within populations	345	544.696	0.01681	1.02	$F_{IS} = 0.01027^{NS}$
Within individuals	349	534.500	1.62026	98.54	$F_{IT} = 0.01456^{NS}$
Total	697	1087.161	1.64420	100.0	
Among groups (generation)	1	3.990	0.00540	0.33	$F_{CT} = 0.00328^{NS}$
Among populations within groups (between trap locations within generation)	2	3.974	0.00315	0.19	$F_{SC} = 0.00192^{NS}$
Among individuals within populations	345	544.696	0.01681	1.02	$F_{IS} = 0.01027^{NS}$
Within individuals	349	534.500	1.62026	98.46	$F_{IT} = 0.01541^{NS}$
Total	697	1087.161	1.64562	100.0	

ss, sum of squares; NS, nonsignificant.
*, $P < 0.05$.

individuals trapped inside or outside the orchard (Fig. 1A). For instance, during the first codling moth generation, the dark gray genetic cluster was more associated with the unmanaged apple orchard and the unmanaged surrounding area, while the light gray and white genetic clusters were more associated with the managed apple orchard (Fig. 1A). For the second generation, TESS also showed $K = 3$ (DIC = 3,717.7), but without any clear spatial correspondence of the genetic clusters (Fig. 1B). Thus, a change in spatial distribution of the genetic clusters between the first and second codling moth generation was observed. Finally, GENELAND produced a mode of one population in both generations.

Discussion

A weak spatial and temporal genetic structure of codling moth was found at a local scale in a typical apple orchard of the pome fruit production region in central Chile. Our population genetic data do not provide evidence about the predominant direction of codling moth movement. However, based on previous studies on mark and capture techniques the predominant dispersal was the seasonal flight of codling moth adults from the unmanaged (population source) into the managed apple orchard (population sink; Basoalto et al. 2010). After insecticide sprays against the first codling moth generation, the population from the managed orchard was likely largely reduced. Thus, the genetic contribution of the immigrating codling moth individuals from the unmanaged plot population to the second generation in the managed orchard was probably higher (Basoalto et al. 2010). However, this latter result could also be because of the relatively small sample size of adults sampled outside the orchard relative to the larger proportion of adults analyzed from inside the orchard in the second generation (Table 1).

A source-sink dispersal pattern has been previously shown with mark and capture of adult codling moth during the second generation (Basoalto et al. 2010). In this study a large proportion of fruits in the unmanaged apple plot dropped at mid-season owing to codling moth damage after the development of the first generation (Basoalto et al. 2010). This likely reduced the availability of fruit resources for larval development during the second generation in the unmanaged apple plot, and could have influenced adult immigration toward the managed orchard with its higher fruit availability. A similar effect was found at a local scale in orchards from southern France and Greece, using sibship assignments of microsatellite data (Franck et al. 2011, Margaritopoulos et al. 2012). Unfortunately, genetic results in our study were obtained from adult males only; hence, no direct information on female dispersal or successful contribution to the next generation can be obtained. Further improvement of traps containing ethyl (*E, Z*)-2,4-decadienoate (pear ester) and acetic acid will improve the catch of female codling moth adults (Knight 2010a,b,; Barros-Parada et al. 2013), and allow the inclusion of female adult dispersal data and a more complete population genetic studies in the future.

Because insecticide sprays can also affect adult flight behavior of the codling moth, the dispersal of the population from the managed apple orchard could be influenced by this management practice. For instance, azinphosmethyl residues produce an increase in flight activity of codling moth males (Abivardi et al. 1998, Dorn and Gu 1999), but male codling moths exposed to methoxyfenozide were not as responsive to calling females as were the untreated males (Hoelscher and Barrett 2003). Therefore, changes in male flight behavior or attraction to sex pheromone-baited traps could have been associated with insecticide sprays with azinphosmethyl (first generation) or methoxyfenozide (second generation) during the season. Finally, the use of highly attractive sex pheromone-baited traps could have influenced or biased the low levels of spatial genetic structure found in our study (Basoalto et al. 2010). It is possible that some individuals trapped inside the orchard originated and dispersed from the unmanaged insecticide-free areas outside the orchard. Such source–sink population dynamics has been proposed to explain the population dynamics of this pest species across space (Trematerra et al. 2004; Ricci et al. 2009, 2011; Basoalto et al. 2010).

Adult dispersal across orchard boundaries has been shown to influence spatial population dynamics of the codling moth (Tyson et al. 2007). Such dispersal can lead to successful mating and genetic contribution to the next generation, thus establishing gene flow between populations in unmanaged and managed areas (Franck et al. 2011, Margaritopoulos et al. 2012). The maintenance of gene flow between unmanaged and managed populations has been proposed as a significant mechanism that counteracts the increase of insecticide resistance frequencies in codling moth populations (Fuentes-Contreras et al. 2007). In particular, any selection pressure for resistance imposed by insecticide sprays within managed orchards could be reduced by the immigration and reproduction with susceptible moths from unmanaged host plants common in apple production areas of central Chile (Basoalto et al. 2010).

The limited genetic structure observed among trap locations in the same generation, suggests that historical and recent gene flow could be significant across the studied unmanaged and commercial orchard interface. Our results suggest that spatial connectivity between surrounding host plants and other neighboring orchards needs to be more carefully addressed to develop area-wide programs for codling moth control. In contrast, such connectivity could mitigate the building-up of insecticide resistance of this pest by conserving susceptibility.

Finally, it is worth to mention that we performed a nonreplicated study, which is not unusual at this spatial scale of genetic studies with codling moth (Franck et al. 2011, Margaritopoulos et al. 2012), but further analyses in other locations are necessary to extrapolate our main findings to the main pome fruit production area in central Chile.

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