Patterns of Mitochondrial Haplotype Diversity in the Invasive Pest *Epiphyas postvittana* (Lepidoptera: Tortricidae)

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ABSTRACT The light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), is a horticultural pest of Australia and New Zealand that has more recently invaded Hawaii, Europe, and California. A 2,216-bp region of the mitochondrial genome containing the *cytochrome oxidase I* and *II* genes was sequenced from 752 individuals. Haplotype network analyses revealed a major split between a predominantly Western Australian clade and all other samples, suggestive of either a deep genetic divergence or a cryptic species. Nucleotide and haplotype diversity were highest in the country of origin, Australia, and in New Zealand populations, with evidence of haplotype sharing between New Zealand and Tasmania. Nucleotide and haplotype diversity were higher in California than within the British Isles or Hawaii. From the total of 96 haplotypes, seven were found in California, of which four were private. Within California, there have been at least two introductions; based on genetic diversity we were unable to assign a likely source for a single moth found and eradicated in Los Angeles in 2007; however, our data suggest it is unlikely that Hawaii and the British Isles are sources of the major *E. postvittana* population found throughout the rest of the state since 2006.

KEY WORDS *Epiphyas postvittana*, mitochondrial population genetics, haplotype, invasive species, origin

Epiphyas postvittana (Walker) (Lepidoptera: Tortricidae), commonly known as the light brown apple moth, is an Australian native tortricid moth that is now globally recognized as a horticultural pest, most notably damaging fruit crops (see Brown et al. 2010, and Suckling and Brockerhoff 2010 for a thorough recent review of the biology and management of the pest). In New Zealand and Australia, the most costly damage is to grapes (*Vitis* spp.) and pipfruit (primarily growers of apples, pears, and nashi), where larvae cause either esthetic or internal injury, with adults also able to act as a vector for pathogens, such as *Botrytis cinerea* that causes gray mold on grapes (Bailey et al. 1997). More importantly, however, for fresh fruit-exporting countries, the presence of the pest has become a major market access issue. Identification of a single larva during inspections may delay an entire shipload of fruit from entering certain countries until appropriate sanitation measures have been conducted (Varela et al. 2010).

E. postvittana has a history of range expansion through invasion. This ability to invade new territories is probably due to the moth displaying many enabling traits, including multivoltinism, high fecundity, multiple mating ability, wide temperature tolerance, reduced susceptibility to Allee effects and lag phase, and a wide host range (Kolar and Lodge 2001). One of the more recent invasions has been into the United States. Two individuals were captured near Berkeley, CA, in 2006, and the species was officially confirmed as present in the state by March 2007 (Varela et al. 2008). As of December 2007, >16,800 moths had been caught in sex pheromone traps distributed throughout California (Varela et al. 2008, Brown et al. 2010). Extensive surveys for moths in central coastal California over the past 40 yr failed to detect *E. postvittana* (Brown et al. 2010), suggesting a relatively recent introduction of the pest; however, debate over the exact length of time that *E. postvittana* has been in California continues (Chen 2010). The Californian invasion is the latest of many recorded invasions by *E. postvittana*, which has established populations in New Zealand (Danthanarayana 1975, Dugdale 1988), England (Dan-

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thanarayana 1975), Hawaii (Chong 1961), Ireland (Bond 1998), and most recently, The Netherlands (Brown et al. 2010) and Sweden (Suckling and Brockerhoff 2010). Various sources (Baker 1968, Bradley et al. 1973, Danthanarayana 1975, Varela et al. 2008) cite the occurrence of *E. postvittana* in New Caledonia. However, we were unable to find any primary reference positively identifying the moth and to date efforts to pheromone trap *E. postvittana* in the country, instigated by us, have been unsuccessful.

As a first step toward understanding the global population structuring and likely pathways and history of invasion of *E. postvittana*, we have sequenced a 2.2-kb region of the mitochondrial genome from >600 individuals to investigate patterns of mitochondrial haplotype diversity and structure throughout the range of the moth. Mitochondrial DNA markers are commonly used for such purposes (Grapputo et al. 2005, Puillandre et al. 2008, Barr 2009, Carter et al. 2009), and although mitochondrial DNA can be problematic for assessing variability after colonization (Davies et al. 1999), its relatively low effective population size can facilitate divergence in this marker among reproductively isolated source populations and thus provide useful diagnostic information for invasive populations in the short term (Avise 2004). In the current study, we have addressed three questions. First, what is the state of haplotype diversity in populations of *E. postvittana* and how are they distributed, both among the global regions and among populations within these regions. Second, do levels of haplotype diversity among the regions reflect the known history of invasions of *E. postvittana*? Third, can this single molecular marker alone suggest hypotheses of possible invasion pathways used by *E. postvittana;* and more particularly, suggest likely source(s) of the recent Californian populations.

Materials and Methods

Population Sampling. In total, 681 *E. postvittana* were sampled from 188 locations (considered as 60 populations) and three laboratory colonies from within five countries (Australia, New Zealand, Republic of Ireland, United Kingdom, and the United States). The Australian samples came from 21 wild locations (field collected) and a laboratory colony at the South Australian Research and Development Institute, which originated from a population collected in Victoria. The New Zealand samples came from 18 wild locations and a laboratory colony at Plant and Food Research, which originated from Nelson. The Irish and United Kingdom samples (collectively British Isles) came from five wild locations. The U.S. samples comprised three locations from Hawaii (the largest island in the Hawaiian chain), including an F2 generation of a laboratory colony originating from collection of 50 larvae from Hawaii made in 2007 (ARS colony of P. Follett). The remaining 33 samples from mainland United States were from locations within 13 counties in California. Samples from five of the Californian counties were represented by a single individual. Figure 1 and Supp Table 2 [online only] provide details concerning collection locations. Samples were collected mainly by sex pheromone trapping (adult males), but larvae also were caught by hand from 2007 to 2009. Individual moths were removed from pheromone traps and stored separately in 100% ethanol until being analyzed. Barr et al. (2009) provide further details on sampling within California.

Molecular Biology. The majority of Australian, New Zealand, Irish, British, and the Hawaiian samples were extracted and analyzed at Plant and Food Research, Auckland, New Zealand. DNA from whole moths or larvae was extracted with the DNeasy kit (QIAGEN, Hilden, Germany) using the animal tissue protocol, with the addition of a 3-min incubation with 0.02 mg of RNase A after the digestion step and then centrifugation for 3 min at 12,100 $\times$ *g* to remove any remaining undigested body parts and wing scales. Samples were eluted in 200 μl of the supplied elution buffer (QIAGEN). Additional samples from California, laboratory colonies (New Zealand, Australia, and Hawaii), and field collections from Southsea, United Kingdom, and Hawaii were extracted according to Barr et al. (2009).

A 2,216-bp region of the mitochondrial genome containing the *cytochrome oxidase I* and *II* genes (*COI-COII*) was amplified using the primer pair TY-J-1490 and TK-N-3785 (Simon et al. 1994) or in two fragments by using primer pairs TY-J-1460 and tort4, and tort2 and TK-N-3785. Polymerase chain reactions (PCRs) in 50 μl contained ≈300 ng of genomic DNA as template, 2.5 mM $MgCl_2$, 0.2 mM dNTP, 10 pmol of each primer, 1× PCR buffer (Invitrogen, Carlsbad, CA), 2.5 U of Platinum *Taq* polymerase (Invitrogen), and 5 μl of cresol red dye. The thermocycling conditions were 94°C for 2 min followed by 40 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 2 min, with a final extension of 72°C for 10 min. Alternatively, PCRs were performed using Ex *Taq* polymerase (Takara Mirus Bio, Otsu, Shiga, Japan) and modified thermal cycling conditions (e.g., cycle number reduced to 35 and extension time to 60 s for shorter PCR fragments). PCR products were sequenced directly on both strands by using the primers TY-J-1490, mt8, mt11, tort1, tort2, tort3, tort4, tort5, tort7, and TK-N-3785 (Simon et al. 1994, Gleeson et al. 2000). Dye terminator cycle sequencing reactions were conducted according to manufacturer's instructions (Applied Biosystems, Foster City, CA) and resolved on an ABI3730 (Massey University Sequencing Facility, Palmerston North, New Zealand; or at Davis Sequencing Facility, Davis, CA).

Data Analyses. Sequences were visually verified and assembled into contigs using Sequencher 4.8 (Gene Codes, Ann Arbor, MI). An alignment of the 681 resulting haplotypes was produced using MacClade 4.06 (Maddison and Maddison 1992). Population diversity statistics and neutrality tests were conducted using ARLEQUIN 3.1 (Excoffier et al. 2005) and MEGA 4.0 (Tamura et al. 2007). Modeltest version 3.7 (Posada and Crandall 1998), executed within PAUP* version 4.0b10 (Swofford 2003), was used to determine the best fitting model of the data; the Tamura and Nei genetic distance model with allowance for invariant sites and a gamma correction of 0.997 (TN+G+I).

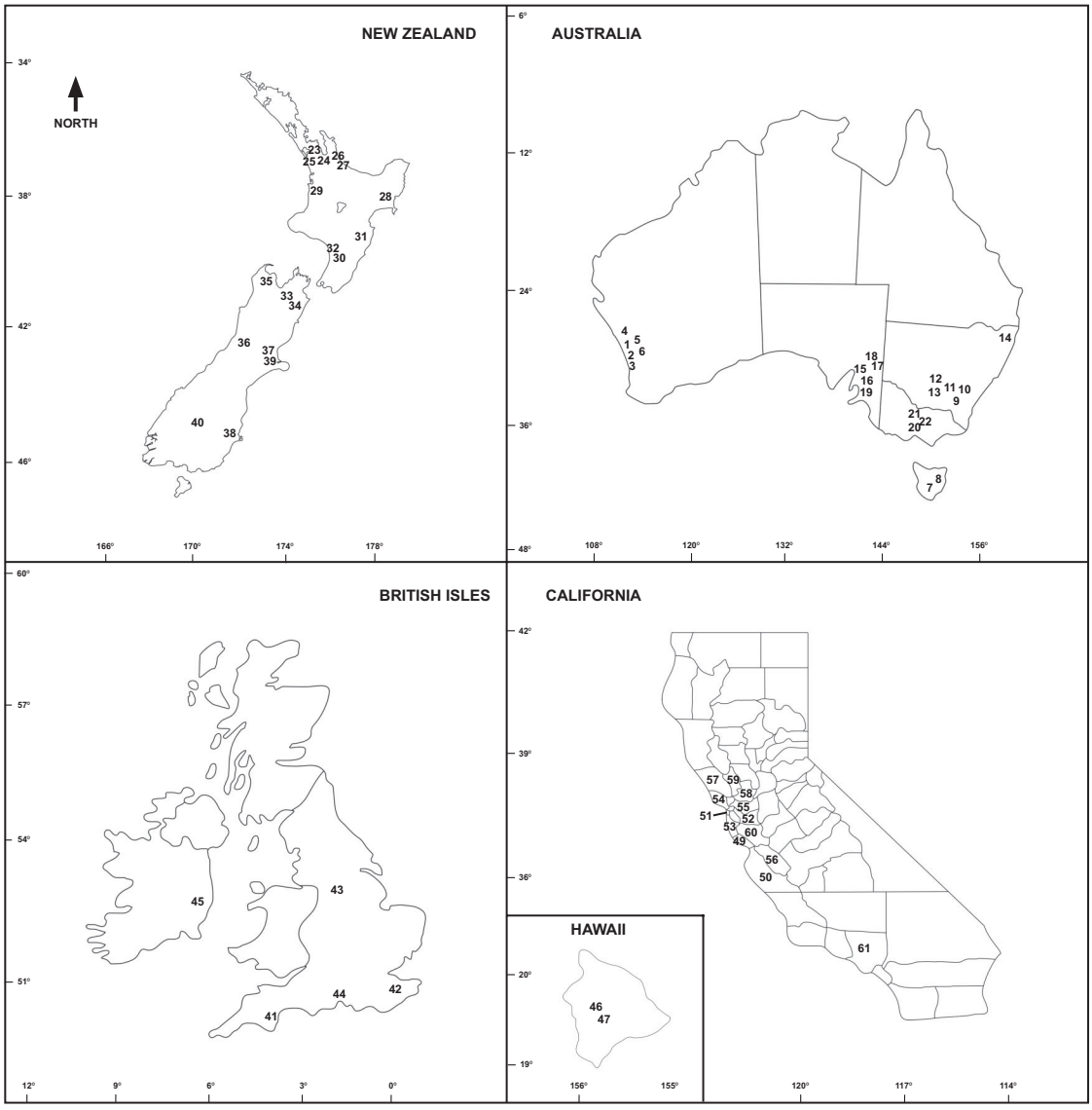

Fig. 1. Maps of the five areas in which *E. postvittana* were sampled. Numbers indicate the location of the various sampling sites of the different populations of wild caught individuals. Population names that correspond with each number and the exact latitude/longitude information can be found in Supp Table 2 [online only]. 1, Dalkeith; 2, Applecross; 3, Harvey; 4, Gingin; 5, West Swan; 6, Kensington; 7, Grove; 8, Woodstock; 9, Flynn; 10, Hawker; 11, Macquarie; 12, Leeton; 13, Waggawagga; 14, Chatsworth Island; 15, Nuriootpa; 16, Gumeracha; 17, Loxton; 18, Berrie; 19, Urrbrae; 20, Parkville; 21, Heathcote; 22, Yarra Valley; 23, Manurewa; 24, New Lynn; 25, Titirangi; 26, Te Puke; 27, Maketu; 28, Gisborne; 29, Kawhia; 30, Palmerston North; 31, Hawke's Bay; 32, Wanganui; 33, Marlborough; 34, Awatere; 35, Nelson; 36, Paroa; 37, Chaney's Forest; 38, Dunedin; 39, Christchurch; 40, Clyde; 41, Exeter; 42, Kent; 43, Nottingham; 44, Southsea; 45, Tinahely; 46, Volcano 1; 47, Volcano 2; 49, Santa Cruz; 50, Monterey; 51, San Francisco; 52, Alameda; 53, San Mateo; 54, Marin; 55, Contra Costa; 56, San Benito; 57, Sonoma; 58, Solano; 59, Napa; 60, Santa Clara; 61, Los Angeles County.

Tajima's D test was used with 1,000 simulations to assess population demography to test for signatures of bottlenecks and expanding populations.

Analyses of molecular variance (AMOVAs) were performed to test for the presence of structure among and within the five major sampled areas: Australia, New Zealand, the British Isles, Hawaii, and California and to test for further structure within the regions that contained additional levels of structure, e.g., areas (states) within Australia, areas separated by a geographic barrier in New Zealand, and Ireland and England within the British Isles. Within Australia, the Australian Capital Territories (ACT) population was incorporated as a population of New South Wales due to its geographical position within New South Wales. The Hawaiian and Californian areas were analyzed

Table 1. Results of AMOVA analyses for all populations of *E. postvittana*

Area	Among region						Among pop within regions					
	% variance	df	SSD	Variance	Φ_{CT}	*P* value	% variance	df	SSD	Variance	Φ_{SC}	*P* value
Global	16.7	4	455.5	0.756	0.167	<0.00001	17.31	46	573.5	0.787	0.21	<0.00001
Australia	24.51	4	107.925	0.93	0.245	0.00089	8.43	8	42.39	0.32	0.112	0.0001
New Zealand	22.48	4	213	1.003	0.225	<0.00001	11.72	12	114.6	0.524	0.151	<0.0001
British Isles	20.74	1	16.3	0.388	0.207	0.19453	5.56	3	7.1	0.104	0.07	0.0362
California							2.84	6	45.83	0.129		
Hawaii							4.56	2	0.363	0.005		

	Within pop						Total		
	% variance	df	SSD	Variance	Φ_{ST}	*P* value			
Global	65.99	617	1852.55	3.003	0.34	<0.00001	667	2881.6	4.55
Australia	67.07	107	272.39	2.546	0.329	<0.00001	119	422.7	3.8
New Zealand	65.79	208	611.31	2.939	0.342	<0.00001	224	938.8	4.47
British Isles	73.69	63	86.9	1.378	0.263	<0.00001	67	110.1	1.87
California	97.16	200	877.69	4.389	0.029	0.09158	206	923.5	4.52
Hawaii	95.44	38	4.38	0.115	0.046	0.13978	40	4.743	0.12

with no structure above the population level. Because only a single individual was sampled from the southern part of California (Los Angeles), a northern (all Californian populations other than Los Angeles) v. southern California analysis was not possible. Other Californian counties (i.e., populations) that were represented by only one or two individuals were removed from the Φ_{ST} analysis. Some modifications to population groupings were made in an attempt to increase the statistical power of tests. Within New Zealand and Australia, any population that had fewer than five individuals was grouped with a nearby population if one was located within 20 km. If no nearby population was found, a population with fewer than five individuals was removed from the Φ_{ST} analysis. Under these criteria the following samples were removed from all AMOVA: Cooks Beach, New Zealand; Urrbrae, South Australia; Heathcote, Victoria; Dalkeith, Western Australia; Applecross, Western Australia and three individuals from Chatsworth Island, New South Wales, whereas two individuals from Maketu, New Zealand, were grouped with the Te Puke population; four moths from Woodstock, Tasmania, were grouped with the only other Tasmanian population; and the three samples from within the ACT were considered as one population because of their proximity. The laboratory-reared individuals from New Zealand and Australia were removed from population structure analyses but retained for haplotype network analyses, whereas the laboratory-reared Hawaiian material was used in all analyses because of the shorter time in captivity (F2 generation) compared with other laboratory material (>1 yr) and the overall paucity of samples from Hawaii. In addition, pairwise Φ_{ST} values were calculated to compare variability among regions within Australia and New Zealand. The Bonferroni correction was used to adjust *P* values for multiple comparisons (Lowe et al. 2004).

A maximum likelihood tree was constructed from all haplotypes, plus two outgroup species from the *Epiphyas* genus [*Epiphyas ashworthana* (Newman) and *Epiphyas sobrina* (Turner)] by using PHYML (Guindon and Gascuel 2003) executed in Geneious 4.8.4 (Drummond et al. 2009). Haplotype network analysis was conducted according to the method of Templeton et al. (1992) by using TCS 2.1 (Clement et al. 2000) to build networks at the 95% confidence level. The Australian and New Zealand laboratory samples were retained for the global haplotype network but were omitted for the Australia and New Zealand-only haplotype network, along with samples containing a divergent set of haplotypes predominantly from Western Australia. The samples that were removed or grouped from the AMOVAs were retained for the haplotype analyses.

Results

Sequence Variation. A 2,216-bp region of the mitochondrial genome, including the *COI-COII* genes, was obtained from 752 individual *E. postvittana* (GenBank accessions HM346380–HM346475). Mean base pair frequencies were A, 0.33; C, 0.13; G, 0.13; and T, 0.39. The aligned sequences contained no indels with 116 variable sites, 40 singleton sites, and 75 parsimony informative sites, 69 of which were two-fold variable sites and six of which were three-fold variable. Of the mutational events, 99 were transitions and 17 transversions. Across the data set, there were 86 synonymous and 30 nonsynonymous mutations, with only a single substitution within the tRNA-Leu that occurred in an individual from Tasmania. Overall, 96 haplotypes were identified, eight of which were shared between at least two areas. The overall haplotype diversity was 0.89, and the average nucleotide diversity over the entire data set was 0.0046, with uncorrected pairwise *p*-distances between any two haplotypes ranging from 0.0005 to 0.0145.

Population Genetic Analyses. For the global comparison, variation was significantly structured among areas (Australia, New Zealand, British Isles, Hawaii, and California), among populations within each area, and within populations (Table 1). Statistically significant structuring was also observed among Australian (Table 2) and New Zealand regions (Table 3), but not the British Isles, where structuring was only detected within populations. Levels of structuring were low in

Table 2. Pairwise Φ_{ST} values for comparisons between Australian states of *E. postvittana*

	Tasmania	New South Wales	Western Australia	South Australia
New South Wales	0.403			
Western Australia	0.416[a]	0.137[a]		
South Australia	0.554[a]	0.131[a]	0.157[a]	
Victoria	0.531[a]	0.178[a]	0.165[a]	0.062

[a] Value is significant at 95% level, using Tamura and Nei model with γ correction.

California and Hawaii (Φ_{ST} <0.05) in comparison with other regions and were also not significant ($P >$ 0.05). Comparisons of pairwise Φ_{ST} values across the Australian states revealed that Tasmania was distinct from other states (Table 2). Φ_{ST} values derived from a pair that includes Tasmania range from 0.403 (Tasmania vs. New South Wales) to 0.554 (Tasmania vs. South Australia), compared with 0.062 (Victoria vs. South Australia) to 0.178 (New South Wales vs. Victoria) for mainland Australian comparisons.

Haplotype and nucleotide diversity statistics for all the populations that contained more than ten individuals are given in Table 4. Of all the global areas, haplotype diversity was greatest in Australia followed by the remaining areas, in decreasing order New Zealand, California, the British Isles, and Hawaii. Nucleotide diversity did not follow the same ranking. Instead, California had the highest overall nucleotide diversity followed then by New Zealand, Australia, the British Isles, and Hawaii. Of the Australian populations, Waggawagga, New South Wales, had the highest haplotype diversity followed closely by the ACT (see Supp Table 2 [online only] for locations of all populations). The lowest haplotype diversity in Australia was found in a hand-collected population from Nuriootpa, South Australia, which also had the lowest nucleotide diversity value of any population in Australia. The highest nucleotide diversity value belonged to the state of Tasmania. The New Zealand samples had a much wider range of haplotype diversity values than Australia, ranging from 0.20 to 0.81, and the overall nucleotide diversity for New Zealand was also marginally higher than for Australia. Palmerston North in the North Island of New Zealand had the highest nucleotide diversity of any population or area. The Californian samples had fairly high haplotype diversity values that did not cover the large range seen in either New Zealand or Australia. The nucleotide diversity of 0.0045 for Marin County was the highest for any Californian population. Haplotype and nucleotide diversity values for populations within both the British Isles and Hawaii were extremely low.

Tajima's D test was conducted on the five global areas (Table 5). A significant deviation from neutral expectations was observed in Australia, with a negative D value being indicative of an expanding population, balancing selection or low level population structuring. No other area showed statistically significant deviations from neutral expectations, although Hawaii was almost significant.

Haplotype Analyses. Haplotype networks were produced for the 96 haplotypes found in the overall data set

Table 3. Pairwise Φ_{ST} values for comparisons between New Zealand geographic regions of *E. postvittana*

	Upper North Island	Lower North Island	Upper South Island	Western South Island
Lower North Island	0.467[a]			
Upper South Island	0.404[a]	0.112[a]		
Western South Island	0.128[a]	0.401[a]	0.294[a]	
Eastern South Island	0.153[a]	0.225[a]	0.154[a]	0.126[a]

[a] Value is significant at 95% level, using Tamura and Nei model with γ correction.

Table 4. Diversity statistics for areas (countries) and populations of *E. postvittana* containing 10 or more individuals

Area/Pop	Nucleotide diversity (π)	Haplotype diversity (H)	No. haplotypes (K)	No. samples (n)
Australia	0.0031	0.95	56	127
ACT	0.0027	0.96	11	13
Waggawagga, New South Wales	0.0021	0.97	10	12
Tasmania	0.0042	0.92	10	14
Leeton, New South Wales	0.0025	0.89	11	18
Nuriootpa, South Australia	0.0006	0.51	3	10
New Zealand	0.0037	0.82	32	231
Chaney's Forest	0.0033	0.81	6	27
Palmerston North	0.0053	0.81	8	16
Dunedin	0.0030	0.80	4	10
Awatere	0.0047	0.78	7	20
Nelson	0.0021	0.63	4	12
New Lynn	0.0021	0.63	4	14
Titirangi	0.0012	0.60	6	19
Te Puke	0.0016	0.57	4	20
Kawhia	0.0018	0.55	2	11
Hawke's Bay	0.0027	0.51	3	10
Clyde	0.0013	0.32	3	12
Paroa	0.0007	0.20	2	10
Wanganui	0.0002	0.20	2	10
California	0.0040	0.67	7	214
San Mateo	0.0043	0.71	3	12
Monterrey	0.0042	0.71	6	38
Santa Cruz	0.0036	0.70	5	93
Marin	0.0045	0.62	3	11
San Francisco	0.0042	0.60	3	22
Alameda	0.0043	0.52	2	23
Hawaii	0.0001	0.23	4	41
Hawaii	0.0001	0.25	4	23
British Isles	0.0014	0.55	6	68
Tinahely, Ireland	0.0020	0.55	3	27
Kent, United Kingdom	0.0009	0.47	3	17
Southsea, United Kingdom	<0.0001	0.14	2	14

Table 5. Tests of neutrality for areas of *E. postvittana*

Statistic	Australia	New Zealand	Hawaii	California	British Isles
Tajima's D	−1.7574	−0.0371	−1.4354	2.7448	−0.9104
P value	0.0117	0.5761	0.0511	0.9936	0.1954

(global network; Fig. 2) and the 84 haplotypes represented in just the Australian and New Zealand populations (Australia and New Zealand network; Fig. 3). The Australian haplotypes were the most broadly distributed throughout the global network. Unique singletons account for 60% of the 96 haplotypes, and only seven of the shared haplotypes occurred in Australia and another location. There was no geographical clustering observed in the Australian haplotypes except for those labeled H71–H77, all from Tasmania. The New Zealand haplotypes also showed a wide distribution throughout the network but had many singletons radiating from a few high frequency haplotypes. A similar pattern was seen in the haplotypes from the British Isles. The Hawaiian samples showed a star-like pattern of three haplotypes arising from the most common haplotype shared by 36 individuals.

The Californian samples included seven haplotypes (H1, H2, H88, H93, H94, H95, and H96). Six of these haplotypes were between one and eight mutational steps from another Californian haplotype in the network. Although H1 and H95 are separated by one mutational step and H94 and H96 are separated by two mutational steps, the six haplotypes do not form a single strong genetic cluster in the network. The seventh haplotype (H93) was >15 mutational steps away from another Californian haplotype. The H2 haplotype, commonly found in samples from New Zealand, the British Isles, and Hawaii, was uncommon in California and was only found once, in a single moth detected in southern California (Los Angeles). The northern California populations include six haplotypes of which H1 (44%) and H93 (35%) were the predominant types. The tally of haplotypes per county is presented in Supp Table 3 [online only]. Although Santa Cruz and Monterey counties exhibit the largest number of different haplotypes (K = 6 and 5, respectively), most counties with more than one moth had relatively high haplotype diversities (Table 4). The northern California counties represented by a single moth always exhibited one of the two common haplotypes (H1 or H93).

The unattached network in the bottom left corner of the global haplotype network represents a subset of four haplotypes found mainly in Western Australia. These haplotypes would be most parsimoniously attached to either H3 or H4 through 21 missing nodes, which at the chosen level of significance (5%) precluded their attachment. This genetically distinct group was restricted to Australia only and represented a substantial portion of the Western Australia samples (see Supp Table 2 [online only]), with other individuals in New South Wales and Victoria (n = 3 and 1, respectively). Haplotype diversity within this subsample was 0.54, and nucleotide diversity was 0.0009, consisting of four transitions and two transversions. The average pairwise p-distance between any of these four haplotypes in the subgroup and any of the remaining 92 haplotypes was 0.0117. The haplotypes from this group (h20, h21, h42, and h46) were removed from AMOVAs, and Φ_{ST} analyses because of the possibility that they represented a cryptic species. This removal also is supported by phylogenetic analysis using outgroups (Fig. 4), which illustrates that this group is sister to all the other *E. postvittana* haplotypes. Even if our assertion that this group represents a cryptic species is incorrect, addition of these haplotypes into an AMOVA of Australian populations does not significantly alter inferences concerning gene flow throughout the country, with most of the variation remaining within populations and a large overall Φ_{ST} (data not shown). Also, because these haplotypes were only found in Australia, they have no bearing on hypotheses of likely colonization scenarios for *E. postvittana*.

For the Australasian haplotype network, haplotypes were grouped by region as used in the AMOVAs (Fig. 3). The Australian haplotypes, for the most part, did not show any clustering related to states and were spread across the entire network. Again, the Tasmanian haplotypes are the exception to this. Some *trans*-Tasman clustering within the network appeared with the haplotypes H15 and H47 shared by individuals in both Tasmania and New Zealand. New Zealand showed some country-wide clustering, but lacked any distinctive geographic clustering that would suggest long-term isolation.

Discussion

Patterns of Mitochondrial Haplotype Diversity and Distribution. This study represents the first major population genetic analysis of the invasive lepidopteran pest *E. postvittana* and reveals that the species is genetically diverse. Of the 96 mitochondrial haplotypes sampled in the study four, predominantly from Western Australia, form a genetic cluster divergent from the other haplotypes by 23 mutational steps (1% sequence divergence). Although benchmarks for detecting species limits have been proposed based on mitochondrial variation (Hebert et al. 2003, 2004), empirical data do not always agree with this approach (Roe and Sperling 2007, Virgilio et al. 2008, Glover et al. 2010). The level of divergence within our mitochondrial gene region for *E. postvittana* is consistent with previous findings between sibling species of Lepidoptera (Sperling and Hickey 1994). Although this group of individuals with divergent haplotypes might represent a new species within *Epiphyas*, this assertion needs to await further molecular, morphological, and sex pheromone analyses, and it remains possible that we have simply not sampled sufficiently to find intermediate haplotypes between the two genetically divergent clusters or that they are now extinct.

Patterns of mitochondrial haplotype diversity and association vary greatly between regions for this species. Only eight of the 96 haplotypes were shared between any two regions. Sharing of haplotypes among populations within those regions was more frequently observed in the nonnative areas than in Australia, partly due to a high number of singletons in all Australian populations. Overall diversity statistics, numbers of haplotypes and their genetic relationship to one another were diverse and complex but gener-

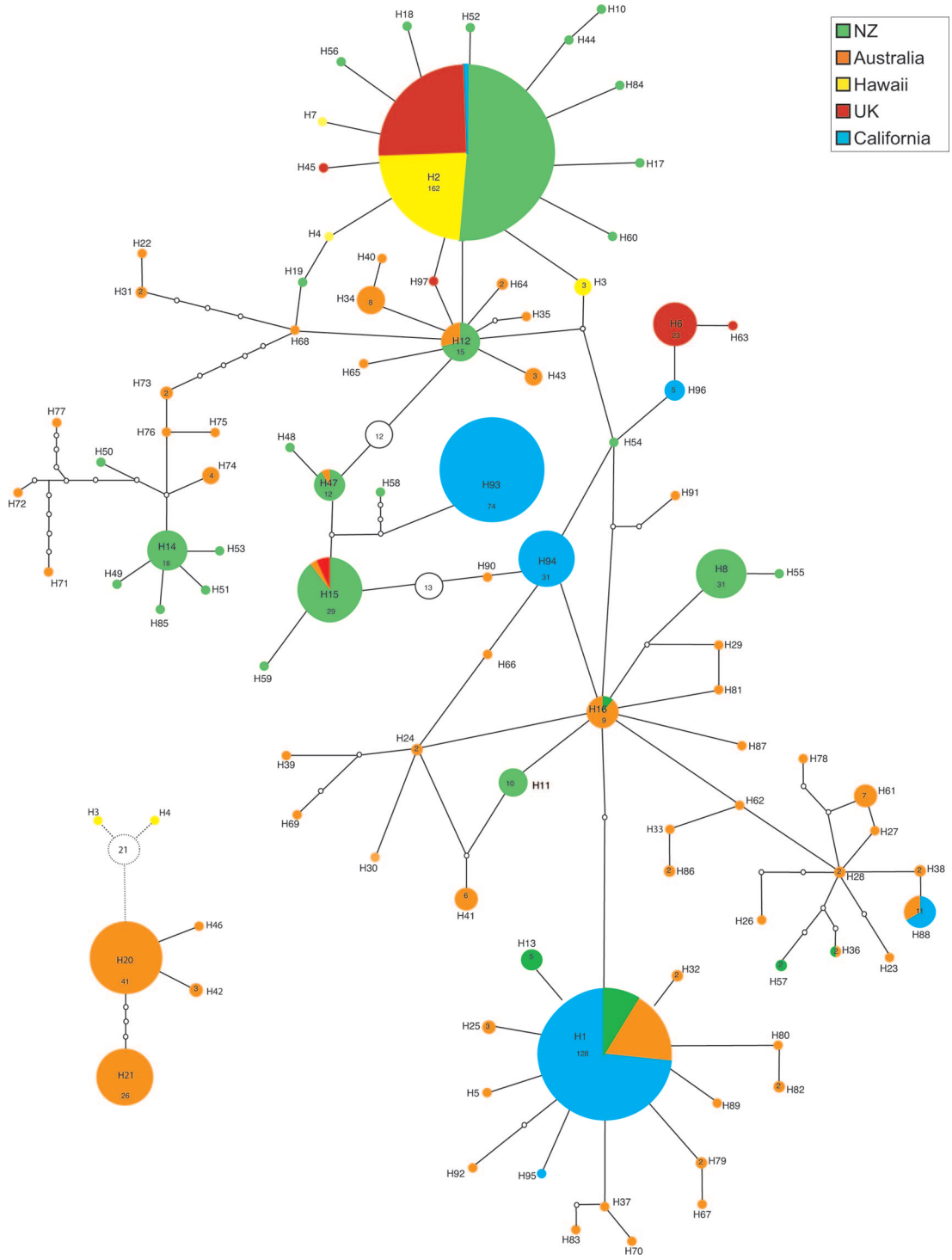

Fig. 2. Parsimony network of global *E. postvittana COI-COII* haplotypes at the 95% level. Colors indicate areas (countries) according to the key. The size of the colored circles is proportional to the number of individuals carrying the haplotype and the slice of the pie indicates the proportion of individuals from each area. Unfilled small circles (nodes) between colored haplotypes represent a missing haplotype or single mutational change. Two large uncolored circles were used in place of very long connections that would have required 12 or 13 small circles. Haplotype numbers are indicated with the prefix H, whereas the other number inside the circle is the number of individuals found with that haplotype. The unattached smaller network on the left represents the genetically distinct group found in Australia. (Online figure in color.)

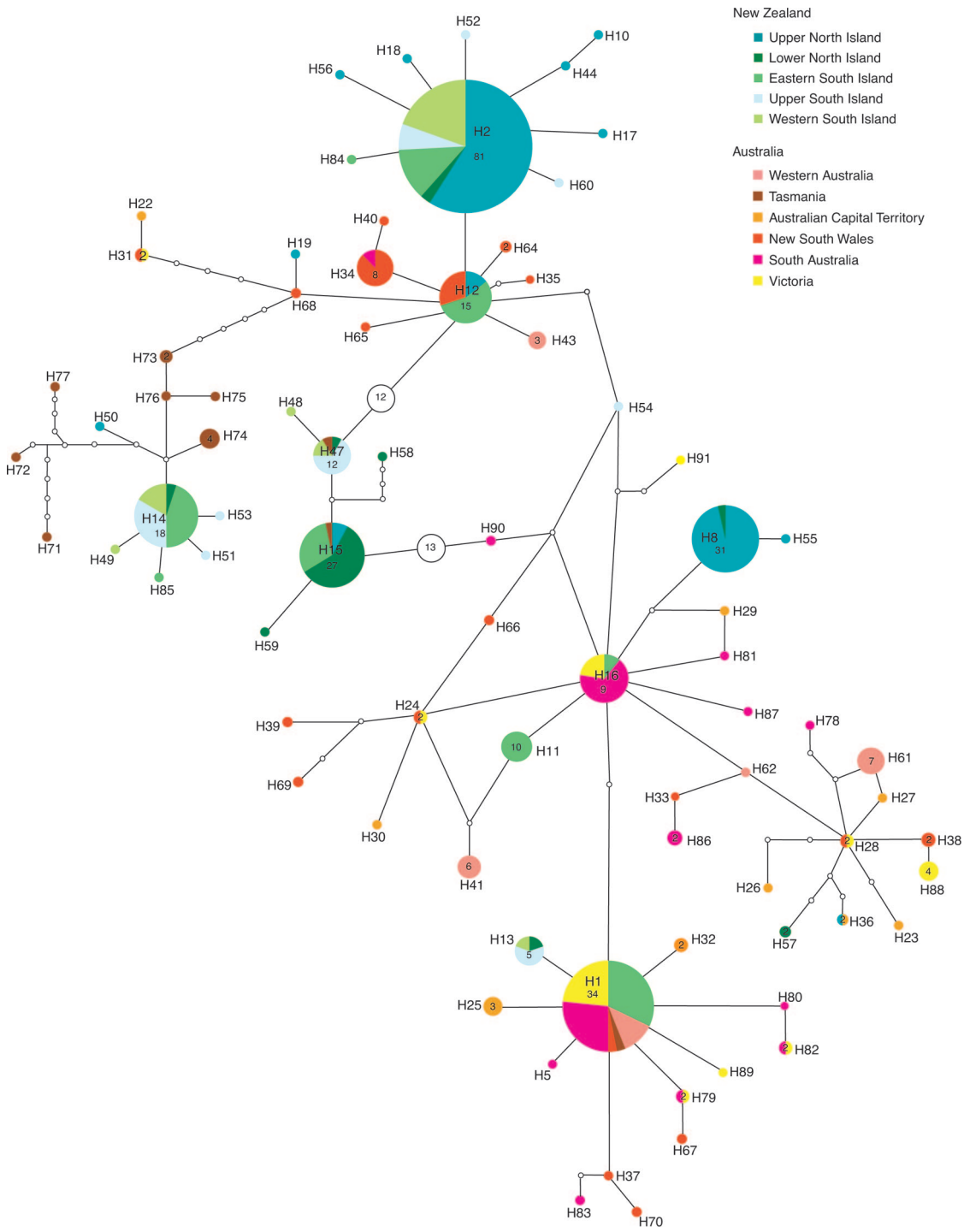

Fig. 3. Parsimony network of Australasian *E. postvittana COI-COII* haplotypes at the 95% level. Colors indicate regions, states within Australia and geographical regions within New Zealand according to the key. The size of the colored circles is proportional to the number of individuals carrying the haplotype and the slice of the pie indicates the proportion of individuals from each region. Unfilled smaller circles represent a single mutational change, whereas unfilled larger circles more mutational changes with the number indicated inside the circle. Haplotype numbers are indicated with the prefix H, whereas the other number inside the circle is the number of individuals possessing the haplotype. (Online figure in color.)

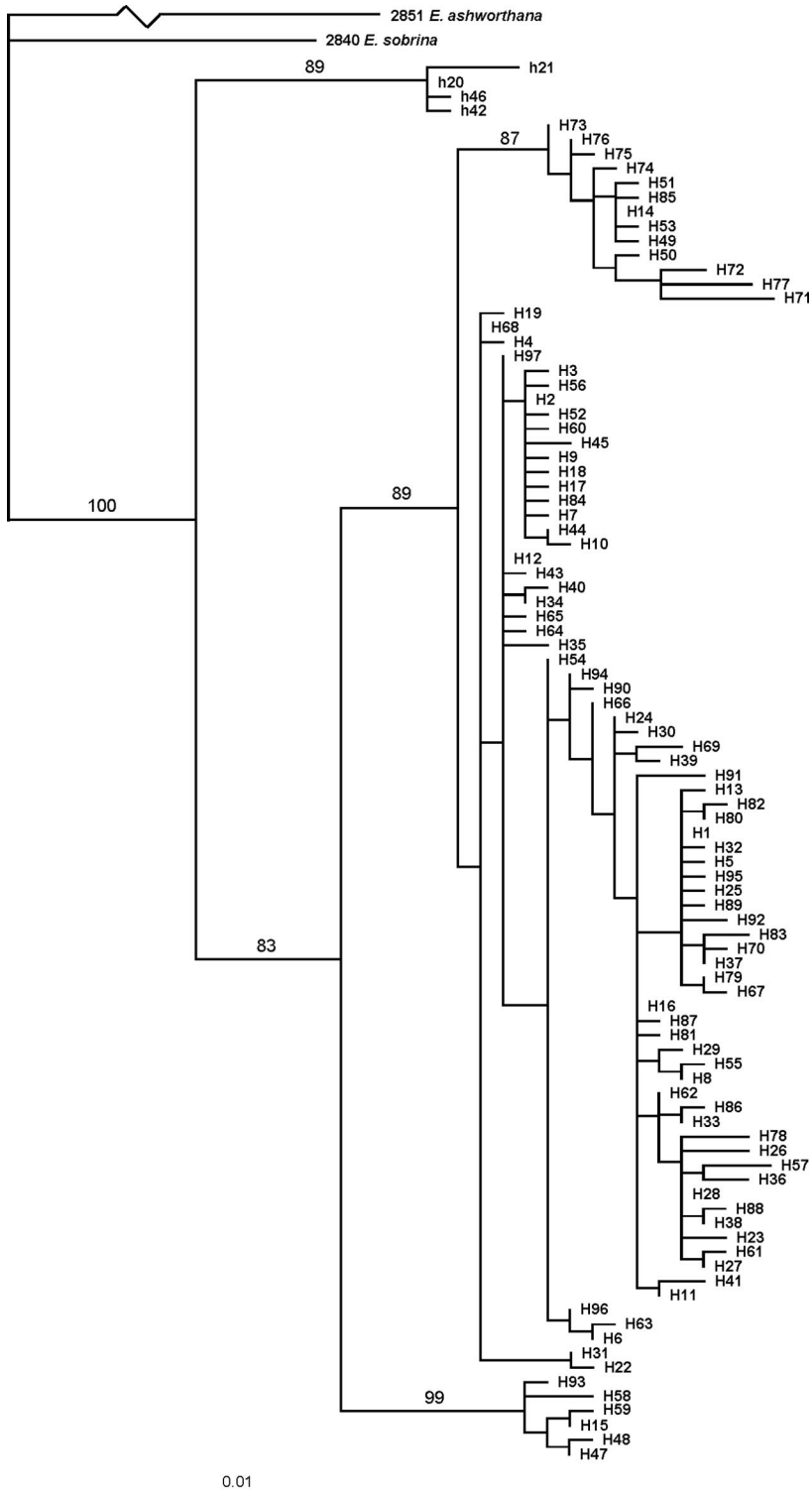

Fig. 4. Maximum likelihood tree of all *E. postvittana COI-COII* haplotypes, with *E. ashworthana* and *E. sobrina* used as outgroups. Bootstrap values derived from 1,000 bootstrap replicates are given above the relevant edge for all basal nodes. Haplotypes from the predominantly Western Australian clade are indicated with a lowercase h, whereas all others have an H prefix. The branch leading to *E. ashworthana* is actually 10 times longer than shown, hence the broken symbol on the branch. Haplotype occurrences in each location can be found in Supp Table 1 [online only].

ally do conform to expectations for a species that is moving out from a home range over time (Avise 2000). Most diversity was observed in the older populations found in Australia and New Zealand with, except for California, diversity declining for increasingly younger invasions.

We observed regional structuring among the Australian states, with populations further structured within those states. The 32 singleton haplotypes, like most of those found in Australia, were not the nearest neighbor to others from the same population or state. Only Tasmania showed haplotype clustering, with the haplotypes there being most similar to one another with between four and 13 nucleotide substitutions separating them from the nearest mainland Australian haplotype. The pairwise Φ_{ST} values between Tasmania and the other states (Table 2) illustrate the influence of this region on the overall values; e.g., the Φ_{CT}. An AMOVA of only the mainland states (data not shown) confirms the impact of the Tasmanian samples with a reduction of the Φ_{CT} from 0.245 to 0.104 and the majority of the variance being accounted for within populations (77% of the overall variance, $\Phi_{ST} = 0.226$ [$P < 0.0001$]). The range of pairwise Φ_{ST} values also was reduced to between 0.062 and 0.178. That Tasmania is an island, isolated from the rest of Australia, is likely to have played a role in the increased genetic distance from the mainland populations. The lack of structuring seen in the mainland states is probably related to the ability of the moth to move, or be moved, across the landscape. Commercial orchards and vineyards provide ideal conditions for *E. postvittana* population sizes to expand dramatically, thus increasing the opportunity for accidental anthropogenic transport. We are also aware that reduced diversity in the hand collected samples is probably due to the collection method potentially providing samples consisting of closely related male and female larvae.

Within New Zealand there was evidence of geographic structuring (Tables 1 and 3). However, there was no strong evidence for structuring by island (North vs. South) nor an influence of geographic proximity. Pairwise Φ_{ST} values between all five geographic areas (Table 3) showed that the lower North Island and the upper South Island were the least different (0.112) but that these regions were the only two where a low pairwise value corresponds to physical proximity (still separated by at least 23 km of open water). The pairwise Φ_{ST} value between the upper and lower North Island (0.467) was the highest of the relevant comparisons. Haplotype patterns suggestive of population expansion were also observed, particularly in the upper North Island (around H2); however, it is not possible to tell whether these patterns are reflective of demography or are the result of genetic drift.

Based on our data, Ireland and England do not represent significantly different gene pools (Table 1). Private haplotypes found in the sample may be the result of insufficient sampling within countries of likely origin (see discussion below). The potential singletons (H45 and H63) may be hidden elsewhere in the population, especially considering the low sample sizes for two populations (Exeter, $n = 5$; Nottingham, $n = 6$). The low levels of overall and population-specific haplotype diversity within the British Isles are indicative of a recently colonized area.

California is the most recent introduction where we have extensive sampling and as such presents an interesting case. Nucleotide diversity in California was the highest of any region and had the greatest number of populations with values in the upper ranges of nucleotide diversity levels (Table 3). In California, seven haplotypes were recovered. A single specimen, found in Los Angeles, was the only case of haplotype 2 in the state. This individual was trapped, and thus eradicated, in 2007. No other moths were trapped in the surrounding counties in southern California at that time. However since then, a large number of moths have been trapped in the city, all with one of the six haplotypes already found in Northern California (data not shown), indicating a rapid population expansion throughout the state. Apart from the 2007 Los Angeles sample, the counties within California are genetically similar. Two haplotypes (H1 and H93) were the most frequently sampled within all the counties in northern California and the counties are genetically interchangeable based on Φ_{ST} statistics. Excluding H1 and H88, the haplotypes sampled from northern California have not been sampled from any other geographic areas. The mutational distances between the "private" Californian haplotypes and others nearby in the network does not allow us to speculate on any areas as potential sources due to the overall complexity of the haplotype network. The genetic similarity of the Californian populations and the recent spread of *E. postvittana* from the north into the Los Angeles area suggests that the population is one large panmictic group.

Haplotype Diversity and the History of Invasions. Based on expectations for founding populations (Nei et al. 1975), only a proportion of the haplotypes present in the native range of *E. postvittana* should be present in nonnative regions after colonization (assuming the native range is genetically diverse). However, several factors can influence the genetic diversity of a colonization event, including the geographic source or sources of the invasion (Davies et al. 1999), the number of colonizers or colonization events (Tobin et al. 2009), selection pressure in the new habitat (Lee 2002), and generation of new mutations in the population (directly related to generation time and population size). Of the nonnative regions included in our study, New Zealand, represents an interesting situation and problem for evaluating colonization because of its proximity and geographical position relative to Australia (downstream of the prevailing wind). The position of the New Zealand haplotypes throughout the haplotype network supports a multiple invasion scenario for New Zealand. Fox (1978) found several species of Australian butterfly in Taranaki, on the western side of the North Island, after prolonged northwesterly winds and *E. postvittana* has been

shown to be capable of using atmospheric winds for long-distance dispersal (Danthanarayana 1976).

Unlike movement between Australia and New Zealand, we might expect propagule pressure to be lower between Australasia and the more geographically distant areas. Genetic variability within the British Isles is low compared with Australia and New Zealand, but the haplotypes sampled from the area do not all form evolutionary clusters when analyzed in the network. This suggests that the populations from the British Isles are the product of at least three female lines, conceivably derived from a single invasion event. Hawaii also shows low genetic diversity based on both haplotype and nucleotide diversity estimates (Table 4). Patterns seen in Hawaii are indicative of post invasion population growth, quite likely given that *E. postvittana* has been recorded in the islands for at least 50 yr (Chong 1961). Assuming that our samples are indicative of variation across the entire island chain, a single-colonization event model for the invasion into Hawaii is the most parsimonious explanation. An introduction resulting from a single female-line is plausible if new mutations could have been generated since that event. Mutation rates for the insect mitochondrial genome can vary for different lineages (Crozier et al. 1989, Zakharov et al. 2004), but, in general, rates are expected to be near 0.01–0.02 substitutions per site per million years (Brower 1994). Therefore, unless our models of invasion include very old colonization events, we should not expect the significant variation to have been generated within Hawaii.

The Californian Invasion. The situation in California is particularly interesting because the patterns of genetic diversity do not conform to classical expectations for a recent colonization. In fact, the levels of genetic diversity are high compared with other populations. This makes identification of the likely source population difficult. Although there are numerous examples of invasion source identification using only mitochondrial haplotypes (Grapputo et al. 2005, Puillandre et al. 2008, Barr 2009, Carter et al. 2009), the levels of genetic diversity observed for the colonizing populations in these studies were not at the level seen here for California. Despite this, some initial inferences can be made concerning likely invasion scenarios that can be tested with further sampling and more molecular markers. First, there have probably been two initial invasions into California, one invasion in central/northern California and a very minor invasion into Los Angeles. The H2 haplotype found in the single moth from Los Angeles in 2007 is also commonly found in Hawaii, the British Isles, and New Zealand but was not detected in any of the other Californian counties. The Los Angeles moth could have been derived from any of the global geographic source areas but the populations within northern California (six haplotypes) do not share haplotypes with Hawaii or the British Isles and therefore are not likely to be the result of invasions from those areas. Similarly, although the occurrence of H2 in Hawaii suggests the British Isles and New Zealand as sources (particularly the upper North island), it is premature to rule out Australia as a potential source because the populations there are very diverse and insufficiently sampled (see below). We can, however, argue against Ireland being a possible source because established populations were not recorded there until ≈13 yr ago (Bond 1998).

As for the remaining Californian haplotypes, if an introduction occurred in the distant past and the effective population size was very low (too low to detect moths), then we would expect a severe bottlenecking of genetic diversity under neutral evolution models because diversity is lost through subsampling during the introduction event and the colonization process (Nei et al. 1975). Given the large amount of variation within the northern Californian samples and the lack of statistically significant demographic values (Tajima's D), this scenario is not likely based on our data. The large genetic distance among the Californian individuals suggests a few possible scenarios, namely, that there was an introduction from a very diverse source population, or multiple invasions from different sources. The mitochondrial data cannot distinguish between these scenarios. The high levels of diversity in many areas in Australia and New Zealand suggest that a single invasion event for the northern haplotypes is possible. However, the failure to find these haplotypes in any of the populations so far prevents us from supporting this scenario alone. Although our genetic data indicate that Santa Cruz county and Monterey county harbor the greatest number of haplotypes and could be the location of the introduction, these values are based on greater sample sizes than in other counties, resulting from the availability of samples collected by the Californian Department of Food and Agriculture for delimitation purposes only. Consequently, our county-level sampling within California was not designed to track population dynamics of the pest specifically or test intercounty movement. Additional work is required to address issues of finer level population structure, insect movement, clines, and the estimation of effective population sizes. In addition, geographic records for horticultural commerce and transport pathways, insect dispersal abilities, and weather conditions could be integrated into future work. One important result of our genetic data are that dispersal calculations for the northern Californian introduction should not include Los Angeles as part of the dispersal range during 2006–2008 because it very probably represents a distinct introduction event. Unfortunately, the high haplotype diversity values seen in the potential source populations (Table 4) and the lack of evolutionary patterns (networks) to explain distributions of those haplotypes hinders our ability to evaluate the likelihood of different sources for an invasion event into California or to rule out a single-source invasion model.

Although our sampling effort was extensive, the presence of private haplotypes outside the native range suggests we have not sampled all the haplotype diversity present in the global populations of the moth. This issue is further supported by saturation curve analysis (Supp Fig. 1 [online only]), which suggests

that all populations except for California are yet to be sufficiently sampled. These observations highlight the challenges of sampling associated with studies that aim to determine the origin of invasive populations by sampling from source and founding regions (Muirhead et al. 2008). The population sizes of *E. postvittana* in the source populations may well be very high, able to harbor high levels of haplotype diversity. Without reasonable levels of sampling, to the point where at least the founding population haplotypes are also observed in source populations, producing hypotheses of likely origins and pathways of introductions of the pest is difficult. Also, using the longer 2.2-kb fragment has increased the number of haplotypes we have observed in our samples and although this has diminished the chances of deriving incorrect scenarios, it also has made finding matching haplotypes more difficult. Suggestions to improve our ability to determine the origins of colonizing populations for *E. postvittana* include increasing the levels of sampling of the moth, particularly in the more likely source populations of Australia and New Zealand and increasing the number and type of molecular markers used.

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