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The effects of local selection versus dispersal on insecticide resistance patterns: longitudinal evidence from diamondback moth (*Plutella xylostella* (Lepidoptera: Plutellidae)) in Australia evolving resistance to pyrethroids

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Abstract

When strong directional selection acts on a trait, the spatial distribution of phenotypes may reflect effects of selection, as well as the spread of favoured genotypes by gene flow. Here we investigate the relative impact of these factors by assessing resistance to synthetic pyrethroids in a 12-year study of diamondback moth, *Plutella xylostella*, from southern Australia. We estimated resistance levels in populations from brassicaceous weeds, canola, forage crops and vegetables. Differences in resistance among local populations sampled repeatedly were stable over several years. Levels were lowest in samples from weeds and highest in vegetables. Resistance in canola samples increased over time as insecticide use increased. There was no evidence that selection in one area influenced resistance in adjacent areas. Microsatellite variation from 13 populations showed a low level of genetic variation among populations, with an AMOVA indicating that population only accounted for 0.25% of the molecular variation. This compared to an estimate of 13.8% of variation accounted for by the resistance trait. Results suggest that local selection rather than gene flow of resistance alleles dictated variation in resistance across populations. Therefore, regional resistance management strategies may not limit resistance evolution.

Keywords: selection patterns, *Plutella xylostella*, insecticide resistance, synthetic pyrethroids, canola, *Brassica*, permethrin

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Introduction

The evolution of insecticide resistance provides many classic examples of rapid evolutionary change in populations (Georghiou, 1972; Tabashnik, 1994). Because resistance is often based on a simple genetic change involving one or a few loci (Roush & McKenzie, 1987), the genes and processes involved in resistance evolution can be followed relatively easily. Yet the pattern of spread of resistance remains poorly documented. In most cases, resistance has been identified from a limited number of populations or strains, and both longitudinal and spatial data on changes in resistance are relatively rare.

Longitudinal data on resistance patterns are available in some situations where resistance management strategies have been implemented for pest species. For example, Forrester *et al*. (1993) conducted an intensive, long-term monitoring program to evaluate the impact of a pyrethroid resistance management strategy in cotton for *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in Australia. They observed a steady increase in pyrethroid resistance over time in both sprayed cotton areas and an unsprayed refugium. Similarly, in the housefly, *Musca domestica* L., Denholm *et al*. (1985) tracked pyrethroid resistance alleles selected in treated populations in enclosed farm buildings to untreated populations outdoors and on neighbouring farms. These studies suggest that resistance can evolve locally but also spread to adjacent areas through gene flow. In the case of the mosquito, *Culex pipiens* L., gene flow appears to have been very widespread, in that a resistance allele derived from a single mutation event now has a worldwide distribution (Labbe *et al*., 2005).

When gene flow spreads resistance alleles from areas where selection occurs, resistance is expected to be high in areas adjacent to those where chemicals are applied, as well as in areas where selection is taking place. Eventually, resistance levels should increase in all areas unless there is selection against the resistance alleles in the absence of chemical applications. However, very high levels of gene flow may disrupt this spatial structure and lead to high levels of resistance only in areas directly affected by spraying and an increase in resistance levels over time in all areas where a pest occurs (Caprio & Tabashnik, 1992). Conversely, very low levels of gene flow could produce sharp changes in resistance levels between adjacent areas dictated by local selection pressures and only a very slow overall increase in resistance in unsprayed areas.

In this paper, we consider patterns of resistance in multiple populations of the diamondback moth, *Plutella xylostella* (L.), an insect renowned for developing resistance to insecticides (Talekar & Shelton, 1993), which has damaged *Brassica* vegetable crops throughout the world. We address the development of resistance to pyrethroids. Previous research on *P. xylostella* has indicated that resistance to insecticides in this species can vary over short distances (< 10 km) within islands (Tabashnik *et al*., 1987) and also among mainland populations (Shelton *et al*., 1993). Microsatellite data pertaining to *P. xylostella* suggest high levels of gene flow in this species (Endersby *et al*., 2006), and there is also direct evidence of long distance movement (Chapman *et al*., 2002). This suggests the potential for resistance to spread relatively rapidly and to affect region-specific levels of resistance.

Our sampling extends over a 12-year period in southern Australia, where vegetable growers started using low cost, synthetic pyrethroid insecticides in the 1980s. In many horticultural districts, synthetic pyrethroids, particularly permethrin, became the sole means of controlling Lepidoptera in *Brassica* vegetables. Growers first experienced difficulty in controlling *P. xylostella* in the early 1980s; and, by 1985, insecticide control failures and ploughing of damaged crops were common. Resistance to a range of insecticide chemical groups, including the synthetic pyrethroids, was documented at this time (Wilcox, 1986; Altmann, 1988; Hargreaves, 1996). The initial response by the growers to control failure of synthetic pyrethroids was to increase the rates and frequency of insecticide applications and revert to use of organophosphate insecticides.

Brassica vegetables account for a very small proportion of the host plants available for *P. xylostella* in southern Australia but receive the most intensive use of insecticide. Vast areas of brassicaceous weeds occur throughout southern Australia, particularly in Western Australia, where wild radish, *Raphanus raphanistrum*, has developed resistance to multiple herbicide modes of action (Rieger *et al*., 1999; Walsh *et al*., 2004) and in New South Wales, where wild turnip, *Brassica tournefortii*, and turnip weed, *Rapistrum rugosum*, are particularly widespread. In high rainfall areas of southern Victoria and Tasmania, about 70% of dairy farmers grow large areas of forage brassicas such as turnips (*Brassica rapa*) in late spring to early summer to fill a gap in summer feed (Moate *et al*., 1999). Moreover, recent expansion of canola growing areas in southern Australia has provided a new host for *P. xylostella* and insecticide application to control outbreaks of the pest on this crop has started relatively recently.

Samples across these hosts since 1993 provide a resource for investigating spatial and longitudinal changes in resistance patterns. We consider four issues. First, to what extent are patterns of resistance stable over time and differences among populations maintained? We examine several populations where repeated sampling has taken place. Second, is resistance associated with different types of host plant? We address the association between resistance and host type, focusing particularly on whether resistance in samples from weeds has remained lower than in other crops and whether resistance on samples from canola has changed over time as pesticide applications have started to be applied to this crop. Third, is there evidence of a change in spatial resistance patterns, as gene flow has spread resistance alleles to populations adjacent to sprayed areas? We test for a spatial pattern across all hosts and on specific host types using Mantel tests. Finally, is there an association between genetic variation and population differences in resistance patterns? We use genetic variation at microsatellite loci to compare patterns of variation and also consider levels of genetic and phenotypic differentiation among populations. Answers to these questions highlight the importance of local selection for resistance in determining patterns across populations, despite the spread of resistance alleles to unsprayed hosts. They also show a recent history of selection in canola increasing levels of resistance. Implications for the management of *P. xylostella* resistance across regional areas are discussed.

Materials and methods

Insect collection

One hundred and fifty samples of eggs, larvae or pupae of *P. xylostella* were collected from 104 locations in southern

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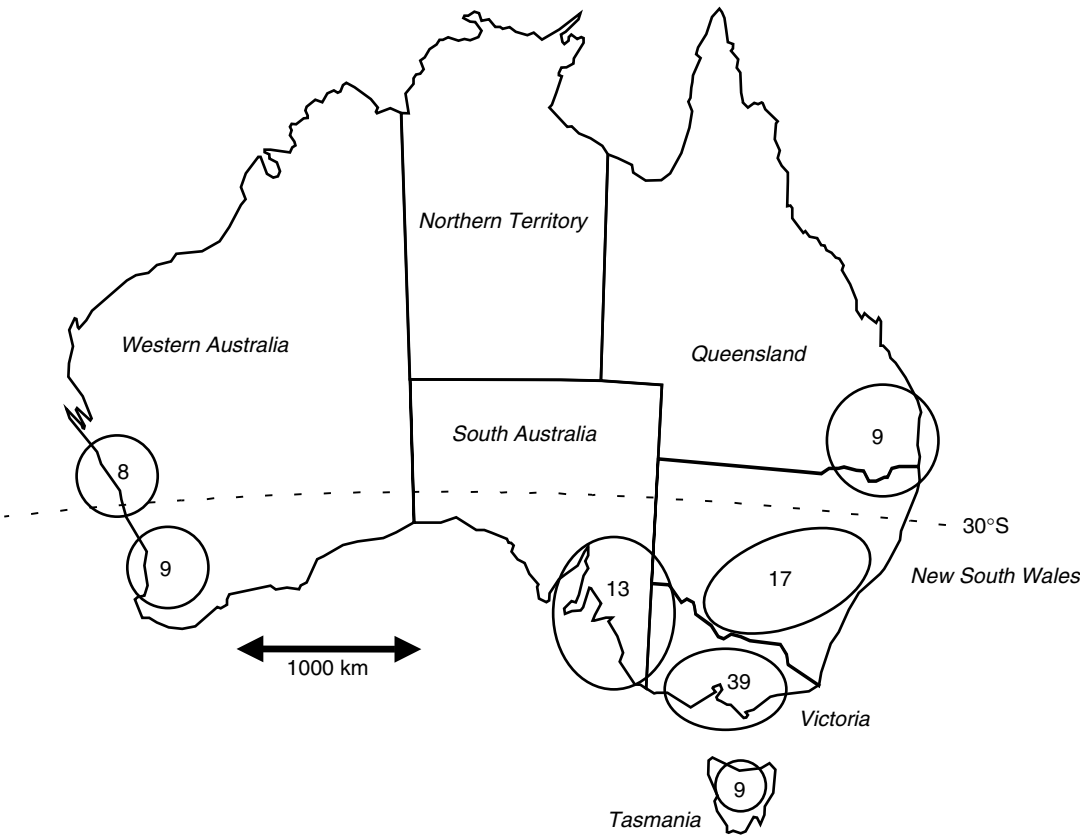

Fig. 1. Sampling locations of *Plutella xylostella* (L.) in southern Australia. Numbers within circles signify the number of unique sites within the circled region from which a sample was tested for resistance to permethrin.

Australia from April 1993 to September 2005 (fig. 1, table 1). Larvae were reared on seedling leaves of cabbage (*Brassica oleracea* var. *capitata* cv. Green Coronet) at 25°C (16 h : 8 h, L : D) at ambient relative humidity for one to nine generations. A population (Waite) of *P. xylostella* that is susceptible to synthetic pyrethroid insecticides was collected in an organic vegetable garden *ca*. 1987 (Baker & Kovaliski, 1999) and has been maintained in the laboratory for use as a reference in each assay since 1996.

Bioassay

Third instar larvae of *P. xylostella* were tested for susceptibility to permethrin using a leaf dip bioassay (after Tabashnik & Cushing (1987)). Cabbage leaf discs of 4.5 cm diameter were dipped for 5 s in distilled water solutions of formulated insecticide (Ambush® Emulsifiable Concentrate Insecticide–Crop Care Australasia Pty Ltd) and hung vertically to dry in a fume hood for 2 h. Control discs were dipped in distilled water. No wetting agents were used. Discs were placed into Gelman® 50 mm diameter × 9 mm plastic Petri dishes. Ten larvae were placed on each disc and four replicates of seven or eight concentrations were set up for the field populations tested and for the susceptible laboratory population. From 1996 onwards, the susceptible population was tested at the same time as every field population. Mortality was assessed after 48 h at 28°C. Larvae were considered dead if they did not move when touched with a paintbrush. In all, we tested 41 populations of *P. xylostella* from canola, 23 from weeds, 10 from forage crops and 76 from vegetables.

Analysis

Concentration-mortality data were analysed using the probit analysis program, POLO-PC (LeOra Software) (Russell *et al*., 1977). The lethal concentration expected to cause 50% mortality (LC_{50}) of each insecticide for each sample of *P. xylostella*, the 95% confidence intervals for these concentrations and the slope (+standard error) of the probit line was estimated. χ^2 tests for goodness-of-fit of the data to the probit model were run using POLO-PC. If the probit model did not fit (χ^2 test), the LC_{50} value for the particular sample may not have been reliably estimated and was adjusted by POLO-PC with the heterogeneity factor (χ^2/df). The index of significance for potency estimation (g) was used to calculate 95% confidence intervals for potency (relative potency is equivalent to resistance ratio) and if parallel slopes could not be fitted for a particular assay, then the ratio was calculated at LC_{50} (Robertson & Preisler, 1992).

Log transformed values of LC_{50} were used throughout the analyses to ensure normality. To test for differences between 11 locations sampled repeatedly over time for both the LC_{50s} and the slopes, a univariate analysis of covariance (ANCOVA), using the Generalised Linear Model in SPSS (Version 13.0), was undertaken that included location as a

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Table 1. Resistance ratio (RR), LC_{50} and 95% confidence intervals (C.I.) for permethrin tested on populations of *Plutella xylostella* (L.) collected on *Brassica* vegetables, canola, weeds and forage *Brassica* crops from southern Australia. RR was computed from comparison with bioassay data from the standard laboratory population (Waite) and tested on the same date as the field population.

State	Population	Location	Date	Gen	*n*	Slope ± s.e.	LC_{50} ppm (95% C.I.)	RR
Vegetables								
VIC	Werribee South	37°58′S 144°41′E	Apr-93	F3	185	1.61 ± 0.31	140.7 (89.7–253.5)	13.6
	Werribee South	37°58′S 144°41′E	Jan-94	F1	268	2.58 ± 0.31	172.7 (132.0–231.8)	18.4
	Werribee South	37°58′S 144°41′E	Feb-97	F5	284	2.75 ± 0.32	32.3 (25.5–40.1)	1.1
	Werribee South	37°58′S 144°41′E	May-98	F1	277	1.7 ± 0.19	156.5 (116.8–218.5)	6.1
	Werribee South	37°57′S 144°41′E	Dec-98	F1	119	2.38 ± 0.41	52.9 (34.1–73.3)	2.6
	Werribee South	37°57′S 144°41′E	Feb-99	F1	280	1.65 ± 0.20	33.2 (22.7–44.7)	3.4*
	Werribee South	37°53′S 144°43′E	Mar-99	F1	281	1.71 ± 0.27	9.6 (5.0–14.3)	1.9*
	Werribee South	37°58′S 144°41′E	Sep-99	F1	281	1.68 ± 0.20	25.5 (17.3–34.6)	3.6
	Werribee South	37°53′S 144°43′E	Mar-00	F1	280	1.50 ± 0.18	34.1 (22.2–48.6)	14.4
	Werribee South	37°55′S 144°43′E	Feb-01	F1	280	1.46 ± 0.21	72.9 (52.5–99.3)	7.9*
	Werribee South	37°56′S 144°42′E	Dec-02	F0	280	1.62 ± 0.21	169.8 (129.7–243.1)	8.2
	Werribee South	37°58′S 144°41′E	Dec-03	F1	280	1.70 ± 0.18	106.7 (66.1–157.6)	14.4
	Werribee	37°56′S 144°41′E	May-03	F1	280	2.33 ± 0.23	63.0 (52.6–75.9)	8.0
	Lysterfield	37°54′S 145°18′E	May-93	F2	178	3.39 ± 1.00	311.5 (118.6–433.6)	31.5*
	Lysterfield	37°54′S 145°18′E	Mar-94	F1	277	1.38 ± 0.16	120.0 (79.1–189.6)	12.1*
	Seaford	38°07′S 145°07′E	May-93	F4	193	2.82 ± 0.57	61.5 (41.4–81.0)	6.1
	Knoxfield	37°53′S 145°15′E	Dec-93	F7	182	1.75 ± 0.30	96.1 (61.2–187.9)	9.3
	Cranbourne	38°07′S 145°17′E	Feb-94	F4	192	2.61 ± 0.57	257.3 (132.0–408.0)	26.7
	Dandenong	38°02′S 145°13′E	Feb-94	F3	284	1.57 ± 0.23	184.3 (129.9–257.8)	17.8
	Lang Lang	38°16′S 145°34′E	Feb-94	F3	282	1.38 ± 0.22	18.5 (8.6–29.5)	2.2
	Mildura	34°11′S 142°09′E	Mar-94	F4	435	3.67 ± 0.39	260.2 (217.7–301.5)	26.3*
	Boisdale	37°53′S 146°59′E	Mar-94	F3	160	2.74 ± 0.38	121.2 (80.4–177.4)	12.5
	Lindenow	37°48′S 147°28′E	Feb-94	F1	276	2.39 ± 0.35	139.7 (94.2–193.5)	14.3
	Bairnsdale	37°48′S 147°28′E	Sep-99	F1	282	1.58 ± 0.19	125.9 (97.7–172.7)	13.0
	Lindenow	37°48′S 147°28′E	Sep-99	F1	279	1.57 ± 0.18	52.5 (39.9–68.5)	5.8
	Lindenow	37°48′S 147°28′E	Mar-02	F1	279	1.70 ± 0.19	93.7 (72.2–126.0)	2.8*
	Lindenow	37°48′S 147°28′E	May-03	F1	282	1.32 ± 0.17	85.6 (62.6–124.1)	15.7*
	Geelong	38°10′S 144°21′E	Dec-94	F4	360	1.66 ± 0.20	498.4 (312.7–780.6)	39.4*
	Yarra Junction	37°47′S 145°37′E	Jan-95	F4	285	1.87 ± 0.23	57.3 (36.1–80.9)	5.8
	Keysborough	38°00′S 145°10′E	Feb-95	F4	270	1.22 ± 0.17	20.6 (9.2–34.4)	2.1*
	Myrtleford	36°34′S 146°44′E	Feb-95	F4	278	1.55 ± 0.16	168.3 (121.4–231.3)	16.4*
	Stratford	37°58′S 147°05′E	Oct-98	F2	280	1.76 ± 0.23	19.4 (13.0–26.0)	1.8
	Rosebud	38°24′S 144°54′E	Feb-01	F1	280	1.88 ± 0.21	96.3 (73.4–131.5)	5.9
	Berwick	38°02′S 145°21′E	Mar-01	F1	281	1.64 ± 0.18	68.3 (54.2–87.3)	8.0
	Mooroopna	36°24′S 145°22′E	Nov-03	F1	280	1.89 ± 0.20	91.0 (73.0–113.7)	13.0*
QLD	Tenthill	27°34′S 152°15′E	Jul-96	F1	336	1.77 ± 0.25	811.2 (558.7–1119.7)	64.1
	Tenthill	27°34′S 152°15′E	Mar-99	F1	276	1.98 ± 0.23	321.1 (231.1–504.4)	69.5
	Tenthill	27°45′S 152°10′E	Jul & Aug-04	F1	280	1.58 ± 0.21	312.9 (188.2–481.3)	63.1*
	Glenore Grove	27°33′S 152°25′E	Sep-96	F4	235	1.53 ± 0.20	388.0 (225.6–578.6)	16.2*
	Glenore Grove	27°31′S 154°24′E	May-97	F6	239	1.36 ± 0.20	230.4 (127.1–339.5)	26.5
	Helidon	27°33′S 152°08′E	Oct-98	F2	282	3.22 ± 0.31	510.1 (427.8–611.7)	33.3
	Mt Sylvia	27°44′S 152°13′E	Oct-98	F2	281	2.09 ± 0.19	352.9 (269.2–468.1)	24.4
	Grantham	27°38′S 152°15′E	Sep-00	F1	279	1.37 ± 0.25	574.7 (296.9–2947.9)	47.6*
	Gatton	27°32′S 152°16′E	Oct-02	F2	280	1.72 ± 0.21	126.9 (94.6–185.0)	3.8*
	Gatton Res Stn	27°34′S 152°19′E	May-01	F3	281	1.81 ± 0.26	115.5 (83.8–160.6)	6.6
	Gatton Res Stn	27°34′S 152°19′E	May-02	F1	280	2.50 ± 0.27	120.0 (95.9–155.4)	4.4
	Gatton Res Stn	27°34′S 152°19′E	Jun-03	F1	280	0.93 ± 0.16	53.5 (30.1–93.2)	10.1*
	Gatton Res Stn	27°34′S 152°19′E	Jul & Aug-04	F1	280	1.66 ± 0.20	28.6 (20.3–37.7)	5.8*
	Stanthorpe	28°32′S 151°53′E	Dec-04	F1	319	3.45 ± 0.38	180.8 (150.5–215.1)	25.1
NSW	Castlereagh	33°40′S 150°41′E	May-00	F1	281	1.39 ± 0.19	144.8 (96.6–269.7)	21.6*
	Castlereagh	33°40′S 150°41′E	Nov-00	F1	280	0.94 ± 0.17	204.8 (126.9–476.7)	20.8*
	Cowra	33°51′S 148°39′E	May-02	F1	280	1.48 ± 0.22	250.2 (178.8–415.8)	9.2*
TAS	Wesleyvale	41°12′S 146°27′E	May-95	F2	354	2.03 ± 0.19	379.2 (217.6–801.0)	29.2
	Wesleyvale	41°12′S 146°27′E	Nov-02	F1	280	2.38 ± 0.34	245.3 (190.5–358.3)	10.3
	Devonport	41°11′S 146°21′E	Mar-98	F2	279	1.64 ± 0.20	75.6 (50.3–107.6)	2.3*
	Devonport	41°11′S 146°21′E	Dec-98	F2	280	1.56 ± 0.20	33.5 (21.6–46.6)	3.1*
	Devonport	41°11′S 146°21′E	Nov-01	F1	280	1.45 ± 0.22	287.4 (199.9–512.4)	8.7*
	Gawler	41°11′S 146°10′E	Jan-01	F2	280	1.78 ± 0.19	56.4 (41.3–77.3)	6.5
	Gawler	41°11′S 146°10′E	Feb-01	F1	280	2.13 ± 0.28	47.9 (33.4–63.1)	4.8
	Lillico	41°10′S 146°17′E	Mar-01	F2	228	1.37 ± 0.20	34.7 (24.2–46.9)	8.3

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Table 1 (Continued)

State	Population	Location	Date	Gen	*n*	Slope ± s.e.	LC_{50} ppm (95% C.I.)	RR
SA	Koppamurra	37°04′S 140°48′E	Nov-95	F1	275	1.70 ± 0.30	9.4 (3.6–15.7)	1.0
	Naracoorte	36°58′S 140°45′E	Nov-95	F1	267	1.44 ± 0.20	15.5 (5.8–27.3)	1.8
	Nairne	35°02′S 138°55′E	Jan-99	F2	281	1.44 ± 0.22	11.8 (6.2–17.7)	1.5
	Nairne	35°02′S 138°55′E	Nov-99	F1	280	1.30 ± 0.17	124.3 (82.1–177.6)	10.7*
	Nairne	35°02′S 138°55′E	Feb-03	F1	280	2.96 ± 0.44	12.8 (8.3–16.6)	2.0
	St Kilda	34°44′S 138°32′E	Mar-01	F1	280	1.92 ± 0.28	112.3 (73.0–170.1)	11.1
	Virginia	34°39′S 138°32′E	Apr-02	F1	280	3.71 ± 0.56	146.9 (120.5–175.8)	5.1
WA	Manjimup	34°15′S 116°09′E	Mar-95	F3	275	2.40 ± 0.28	254.5 (191.2–336.3)	29.7
	Manjimup	34°15′S 116°09′E	Oct-99	F1	280	1.25 ± 0.19	108.5 (72.7–177.7)	5.2*
	Perth	31°57′S 115°51′E	Mar-95	F2	362	2.19 ± 0.27	397.6 (293.6–527.8)	34.3
	Perth	31°57′S 115°51′E	Dec-98	F2	200	1.99 ± 0.30	58.0 (40.1–75.8)	5.8
	Albany	35°00′S 117°52′E	Feb-01	F4	280	1.57 ± 0.20	25.4 (17.5–33.9)	6.5
	Wanneroo	31°46′S 115°48′E	Feb-02	F2	280	1.85 ± 0.23	168.6 (124.6–256.8)	5.1*
	Wanneroo	31°46′S 115°48′E	May-02	F1	280	4.31 ± 0.58	185.6 (162.3–214.2)	6.8*
	Wanneroo	31°46′S 115°48′E	Jan-03	F1	280	1.95 ± 0.22	127.2 (98.6–173.6)	20.4*
	Mandogalup	32°11′S 115°50′E	Dec-02	F1	281	3.51 ± 0.44	164.1 (141.5–193.7)	6.0
Canola								
VIC	Balliang East	37°52′S 144°27′E	Oct-98	F2	280	2.45 ± 0.36	45.0 (31.7–-7.8)	2.2
	Balliang East	37°47′S 144°24′E	Oct-99	F1	280	0.92 ± 0.19	7.3 (0.4–17.3)	0.5
	Balliang	37°50′S 144°21′E	Sep-99	F1	280	1.19 ± 0.21	7.8 (3.2–12.8)	1.3*
	Woodhouse	33°50′S 148°40′E	Nov-02	F1	280	1.79 ± 0.20	87.1 (67.7–115.6)	2.9*
NSW	Billimari	33°41′S 148°37′E	Oct-02	F1	280	1.56 ± 0.20	142.3 (104.9–214.7)	7.2*
	Brocklesby	35°49′S 146°41′E	Oct-02	F1	281	1.69 ± 0.22	183.2 (133.0–292.8)	9.3*
	Cowra West	37°48′S 142°26′E	Oct-02	F1	280	2.70 ± 0.44	302.8 (226.5–517.1)	12.4
	Forbes	33°22′S 147°54′E	Sep-02	F1	280	2.35 ± 0.24	99.3 (82.8–121.1)	4.6
	Greenethorpe 1	34°00′S 148°25′E	Sep-02	F1	280	2.74 ± 0.27	81.8 (69.3–97.0)	3.7
	Greenethorpe 2	33°58′S 148°15′E	Oct-02	F1	279	2.36 ± 0.29	180.6 (123.8–331.6)	6.6
	Grenfell	33°54′S 148°10′E	Oct-02	F1	280	1.67 ± 0.32	178.4 (117.4–300.0)	5.4*
	Moree	29°14′S 150°02′E	Sep-03	F2	280	1.30 ± 0.17	123.0 (67.1–215.3)	12.9*
	Morongla	34°06′S 148°40′E	Oct-02	F1	280	2.18 ± 0.24	112.0 (82.8–161.9)	4.0
	Rand	35°33′S 146°38′E	Oct-02	F1	280	1.81 ± 0.30	376.8 (249.0–840.7)	12.3
	Temora	34°27′S 147°32′E	Oct-02	F1	280	1.59 ± 0.19	104.5 (79.3–145.3)	4.2*
	Young	34°15′S 148°15′E	Oct-02	F1	280	1.89 ± 0.22	140.3 (103.9–208.8)	6.4*
SA	Arthurton	34°16′S 137°45′E	Oct-99	F1	279	1.90 ± 0.22	26.1 (19.7–32.9)	2.2
	Minlaton	34°46′S 137°36′E	Oct-99	F1	280	1.84 ± 0.21	26.7 (18.6–35.5)	4.3
	Urania	34°41′S 137°36′E	Oct-99	F1	282	1.84 ± 0.20	34.7 (26.3–44.3)	3.0
	Wauraltee	34°34′S 137°33′E	Oct-99	F1	280	1.44 ± 0.19	27.1 (14.9–41.1)	3.5
	Yeelanna	34°09′S 135°44′E	Oct-99	F1	280	1.53 ± 0.27	7.2 (2.9–11.4)	1.0
	Millicent	37°36′S 140°20′E	Jan-01	F1	280	2.19 ± 0.34	126.2 (94.4–168.5)	6.2
	Millicent	37°29′S 140°16′E	Nov-02	F1	280	1.70 ± 0.20	132.3 (99.1–192.2)	4.5*
WA	Burabadji	31°12′S 116°49′E	Oct-99	F1	280	1.99 ± 0.26	49.2 (33.0–67.1)	6.7
	Geraldton	27°52′S 114°45′E	Sep-00	F1	280	1.94 ± 0.22	125.2 (94.1–180.2)	10.6
	Geraldton #1	27°52′S 114°45′E	Sep-01	F1	280	1.73 ± 0.20	128.8 (95.9–188.2)	15.1
	Geraldton #2	27°52′S 114°45′E	Sep-01	F1	281	1.72 ± 0.21	146.1 (112.1–205.8)	17.2
	Geraldton	27°52′S 114°45′E	Oct-02	F2	280	1.96 ± 0.28	248.6 (178.3–426.6)	9.9
	Geraldton	27°52′S 114°45′E	Aug-03	F1	320	2.06 ± 0.21	98.2 (75.9–130.6)	18.8*
	Geraldton A	27°52′S 114°45′E	Sep-04	F2	319	1.93 ± 0.18	140.4 (113.4–174.0)	12.6*
	Geraldton B	27°52′S 114°45′E	Sep-04	F2	322	2.58 ± 0.26	156.2 (120.4–198.8)	14.0
	Geraldton C	27°52′S 114°45′E	Sep-04	F2	322	1.51 ± 0.16	71.4 (46.2–101.7)	6.4*
	Geraldton D	27°52′S 114°45′E	Sep-04	F2	319	2.84 ± 0.51	92.6 (60.6–120.0)	8.3
	Ballidu	30°36′S 116°46′E	Oct-01	F1	281	1.51 ± 0.21	200.2 (140.1–346.6)	15.0*
	Mullewa	28°30′S 115°32′E	Jun-00	F1	280	1.75 ± 0.19	55.2 (44.1–69.1)	8.9
	White Peak	28°37′S 114°39′E	Sep-05	F1	360	2.41 ± 0.25	58.4 (46.0–71.7)	15.5
	Wongan Hills	30°54′S 116°43′E	Oct-99	F1	280	1.58 ± 0.19	39.1 (27.2–53.7)	5.1*
	Wongan Hills	30°54′S 116°43′E	Nov-01	F1	280	1.52 ± 0.18	145.6 (113.2–191.6)	11.5*
	Yuna	28°20′S 115°00′E	Sep-03	F2	280	2.10 ± 0.21	272.2 (208.2–368.3)	28.5*
	Yuna	28°20′S 115°00′E	Sep-03	F4	281	1.80 ± 0.19	115.7 (70.0–178.4)	8.3*
	Three Springs	29°24′S 115°47′E	Sep-04	F1	320	1.24 ± 0.16	41.7 (23.2–62.6)	9.0*
Forage *Brassica* crops								
VIC	Curdie Vale	38°31′S 142°49′E	Dec-99	F1	280	1.17 ± 0.17	39.3 (25.1–57.5)	6.0*
	Ayrford	38°25′S 142°51′E	Dec-99	F2	280	2.23 ± 0.27	50.4 (38.9–62.9)	4.4
	Garvoc	38°17′S 142°50′E	Jan-03	F1	280	1.36 ± 0.18	56.7 (33.7–91.7)	9.1*
	Hamilton	37°45′S 142°02′E	Jan-03	F1	280	1.63 ± 0.19	41.4 (27.2–59.4)	6.6*

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Table 1 (Continued)

State	Population	Location	Date	Gen	*n*	Slope ± s.e.	LC_{50} ppm (95% C.I.)	RR
TAS	Devonport	41°11′S 146°21′E	Feb-95	F2	279	1.97 ± 0.21	22.6 (16.5–30.3)	1.2
	Forth	41°12′S 146°15′E	Mar-95	F9	197	1.79 ± 0.21	70.6 (48.8–100.9)	5.6
	Woolnorth	40°39′S 144°43′E	Dec-99	F1	280	1.25 ± 0.18	43.6 (28.1–63.1)	5.1*
	Woolnorth	40°39′S 144°43′E	Mar-00	F2	280	1.91 ± 0.24	67.7 (48.5–91.5)	17.3
	Penguin	41°01′S 146°05′E	Nov-02	F1	280	2.08 ± 0.25	147.4 (116.2–199.1)	6.1
	Montagu	40°47′S 144°57′E	Jan-03	F1	280	2.17 ± 0.32	82.4 (54.6–112.8)	5.6*
Brassicaceous weeds								
VIC	Balliang East	37°50′S 144°21′E	Oct-98	F1	280	1.02 ± 0.16	18.3 (6.7–32.1)	2.2*
	Balliang East	37°52′S 144°27′E	Oct-98	F2	280	1.82 ± 0.21	44.5 (29.4–61.6)	2.5
	Loch	38°22′S 145°43′E	Sep-99	F1	280	1.21 ± 0.36	1.5 (0.0–4.9)	0.10*
	Clunes	37°18′S 143°47′E	Oct-99	F1	280	2.36 ± 0.41	9.7 (2.3–15.8)	0.50
	Cranbourne	38°07′S 145°17′E	Oct-99	F2	280	1.63 ± 0.21	20.3 (11.9–29.3)	2.1*
	Dalmore	38°10′S 145°25′E	Oct-99	F4	280	2.31 ± 0.34	12.4 (8.5–15.9)	2.0
	Springhurst	36°11′S 146°28′E	Oct-99	F1	280	1.23 ± 0.17	36.0 (22.1–53.2)	1.8*
	Thomastown	37°40′S 145°01′E	Oct-99	F4	280	1.44 ± 0.30	4.1 (0.9–7.9)	0.5*
	Stratford	37°58′S 147°05′E	Nov-99	F2	280	1.21 ± 0.17	25.3 (15.5–35.9)	1.3*
	Horsham	36°43′S 142°12′E	Nov-99	F3	280	1.17 ± 0.19	11.0 (5.0–17.3)	1.3*
	Derrinallum	37°57′S 143°14′E	Dec-99	F1	280	1.46 ± 0.20	17.8 (10.2–25.7)	3.0*
	Werribee South	37°58′S 144°41′E	Nov-99	F2	280	1.25 ± 0.17	48.1 (34.6–65.6)	4.1*
	Shoreham	38°26′S 145°03′E	Dec-02	F0	280	1.67 ± 0.21	110.4 (75.4–177.9)	5.5
	Garvoc	38°17′S 142°50′E	Nov-03	F1	281	1.70 ± 0.18	89.5 (68.5–116.5)	11.2*
	Garvoc	38°17′S 142°50′E	Dec-04	F1	280	1.42 ± 0.22	29.2 (12.9–48.5)	3.2*
ACT	Canberra	35°17′S 149°13′E	Oct-99	F1	280	0.79 ± 0.16	45.2 (19.7–87.2)	4.9*
NSW	Finley	35°39′S 145°34′E	Oct-99	F1	280	1.07 ± 0.17	37.0 (20.9–57.5)	4.0*
	Jugiong	34°50′S 148°19′E	Oct-99	F1	279	1.18 ± 0.17	45.2 (30.7–63.9)	4.9*
WA	Balingup	33°47′S 115°59′E	Oct-99	F1	280	1.12 ± 0.16	55.8 (34.2–89.8)	6.9*
	Bunbury	33°20′S 115°38′E	Oct-99	F1	280	1.99 ± 0.22	111.4 (90.6–141.2)	6.6
	Bridgetown	33°58′S 116°08′E	Oct-99	F1	280	1.58 ± 0.18	57.5 (42.0–79.4)	4.8*
	Deadman's Gully	34°12′S 116°02′E	Oct-99	F2	280	1.49 ± 0.18	82.9 (64.4–110.1)	6.9*
	Walkaway	28°56′S 114°48′E	Sep-03	F2	270	1.80 ± 0.18	117.5 (92.8–149.4)	12.3*

Date, field collection date; Gen, generation tested since field collection; *, RR calculated at LC_{50} due to nonparallel slopes; *n*, number of individuals exposed to permethrin; s.e., standard error of slope.

random factor and time since sampling started as a covariate. The LC_{50} and slope of the laboratory susceptible population (Waite) was also included in this analysis as a covariate but did not significantly influence values from the field populations. The variance component due to location was estimated with restricted maximum likelihood.

The effects of host, time since start of sampling and host by time interactions on LC_{50} and slope of the probit line were assessed using ANCOVA on one sample taken randomly from each location from the entire dataset, with LC_{50} of the laboratory susceptible population and generation in the laboratory at time of testing as covariates. An association was found with the LC_{50} of the covariate (see below), so subsequent analyses looking at separate hosts over time were made using unstandardized residuals derived from the regression.

Mantel tests (Mantel, 1967) in PopTools (Hood, 2002) with 10,000 iterations were used to look for spatial structure within the data by comparing population pairwise matrices of geographic distance (km) and difference between LC_{50}. Mantel tests were made on a host by host basis (canola, vegetables, weeds and forage) to exclude any confounding host effect. Effects of time were not controlled. The Mantel tests were conducted on differences between the residuals from the regression of geographic distance and LC_{50} after controlling for variation in LC_{50} of the susceptible laboratory population used as a comparison. To look for effects of potential laboratory adaptation, Mantel tests were repeated using samples that had only been reared through one generation prior to bioassay.

Thirteen of the samples tested for insecticide resistance (table 2) were also screened for the six microsatellite loci described by Endersby *et al.* (2005). Nei's measure of genetic distance (*D*) (Nei, 1978) for the microsatellite data was estimated with GDA (Lewis & Zaykin, 2001) and compared with distance measured in terms of the residuals from the regression with LC_{50}. Mantel tests (10,000 permutations in PopTools) were used to determine the significance of associations between these variables.

Comparing variation among populations

To compare levels of genetic variation among populations for the microsatellite markers with variation in resistance, we undertook a comparison of molecular variation and quantitative variation within and among populations. For the molecular comparison, we undertook an AMOVA with Arlequin (Schneider *et al.*, 2000) on the microsatellite data and computed the variance among populations. We then compared the proportion of variance accounted for by the population term for the molecular variation relative to the quantitative variation. F_{ST} values were also computed with Arlequin.

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Table 2. Australian samples of *Plutella xylostella* screened for both insecticide resistance and microsatellite loci.

Collected	Generation tested for resistance	Sample	Latitude/Longitude	Host category	*n*
Mar-02	F_1	Lindenow, Vic	37°48′S 147°28′E	Vegetable	30
May-02	F_1	Cowra, NSW	33°51′S 148°39′E	Vegetable	26
Sept-02	F_1	Forbes, NSW	33°22′S 147°54′E	Canola	60
Oct-02	F_1	Young, NSW	34°15′S 148°15′E	Canola	24
Oct-02	F_1	Rand, NSW	35°33′S 146°38′E	Canola	20
Nov-02	F_1	Millicent-H, SA	37°29′S 140°16′E	Canola	43
Dec-02	F_0	Werribee-N, Vic	37°58′S 144°41′E	Vegetable	50
Jan-03	F_1	Garvoc, Vic	38°17′S 142°50′E	Forage	48
May-03	F_1	Lindenow, Vic	37°48′S 147°28′E	Vegetable	25
May-03	F_1	Werribee-X, Vic	37°56′S 144°41′E	Vegetable	50
Jun-03	F_1	Gatton Res Stn, Qld	27°34′S 152°19′E	Vegetable	18
Nov-03	F_1	Garvoc, Vic	38°17′S 142°50′E	Weeds	50
Jul–Aug-04	F_1	Tenthill, Qld	27°45′S 152°10′E	Vegetable	53

n = number screened with microsatellites.

Table 3. Australian populations of *Plutella xylostella* (L.) susceptible to permethrin. Populations were classified as susceptible according to the method of Robertson & Preisler (1992), in which the 95% confidence intervals of the resistance ratio with a designated susceptible population include the value 1.0.

Population	State	Latitude/Longitude	Collected	Host	RR (95% C.I.)	Gen
Loch	VIC	38°22′S 145°43′E	Sep-99	Weeds	0.1 (0.0–0.7)*	F_1
Clunes	VIC	37°18′S 143°47′E	Oct-99	Weeds	0.5 (0.3–0.7)	F_1
Thomastown	VIC	37°40′S 145°01′E	Oct-99	Weeds	0.5 (0.2–1.2)*	F_4
Balliang East	VIC	37°47′S 144°24′E	Oct-99	Canola	0.5 (0.2–1.3)*	F_1
Werribee South	VIC	37°58′S 144°41′E	Sep-99	Vegetable	0.9 (0.4–2.0)*	F_2
Koppamurra	SA	37°04′S 140°48′E	Nov-95	Vegetable	1.0 (0.5–1.7)	F_1
Yeelanna	SA	34°09′S 135°44′E	Oct-99	Canola	1.0 (0.6–1.5)	F_1
Werribee South	VIC	37°58′S 144°41′E	Feb-97	Vegetable	1.1 (0.8–1.4)	F_5
Devonport	TAS	41°11′S 146°21′E	Feb-95	Forage	1.2 (0.7–1.7)	F_2
Stratford	VIC	37°58′S 147°05′E	Nov-99	Weeds	1.3 (0.3–6.2)*	F_2
Balliang	VIC	37°50′S 144°21′E	Sep-99	Canola	1.3 (0.6–2.6)*	F_1
Horsham	VIC	36°43′S 142°12′E	Nov-99	Weeds	1.3 (0.7–2.3)*	F_3
Werribee South	VIC	37°53′S 144°43′E	Mar-99	Vegetable	1.5 (0.8–2.7)*	F_2
Nairne	SA	35°02′S 138°55′E	Jan-99	Vegetable	1.5 (1.0–2.3)	F_2
Springhurst	VIC	36°11′S 146°28′E	Oct-99	Weeds	1.8 (0.4–8.7)*	F_1
Naracoorte	SA	36°58′S 140°45′E	Nov-95	Vegetable	1.8 (0.8–3.3)	F_1

RR, resistance ratio; *, calculated at LC_{50}.

For the quantitative analysis, we generated a distribution of concentrations at which individuals would have died based on the raw data and for the same number of individuals as scored in the microsatellite analysis. These data consist of a series of concentrations and survival of individual larvae at each concentration (four groups of larvae tested in groups of ten) and were used to generate a distribution of concentrations at which the individual larvae died. For instance, if 10 larvae died at 56.2 ppm and 12 larvae died at 100 ppm, we gave 10 larvae a score of 56.2 and 2 larvae a score of 100 and so on. Where a few larvae survived the highest concentration tested, we gave these larvae the value of the next highest concentration. We then undertook an analysis of covariance on log transforms of concentrations to compare the populations, with LC_{50}s for control values as a covariate.

Results

Resistance to the synthetic pyrethroid, permethrin, was confirmed in laboratory-reared *P. xylostella* from a range of host plants in Australia. For the overall study, LC_{50} values ranged from 2 to 1113 ppm and the resistance ratio varied from 0.1 to 69.5 in the field samples. However, 14 samples of *P. xylostella* out of 150 tested were equivalent in susceptibility to the laboratory population used as a control and two were more susceptible (table 3). Between 1995 and 1999, six of these samples were collected from weeds, six from vegetables, three from canola and one from a forage crop. LC_{50} values of the susceptible populations ranged from 1.5 to 36.0 ppm.

Location differences over time

The study of *P. xylostella* from 11 locations over time showed that resistance levels (measured as LC_{50}) varied between locations ($F_{9,23} = 4.37$, $P = 0.002$) (fig. 2), but changes over time within a site were not observed ($F_{1,23} = 0.71$, $P = 0.410$). There was a significant interaction between location and time ($F_{9,23} = 2.55$, $P = 0.034$). LC_{50} was not significantly affected by the number of generations the population had been in the laboratory at the time of testing ($F_{1,23} = 2.57$, $P = 0.122$). Resistance ratios in samples of

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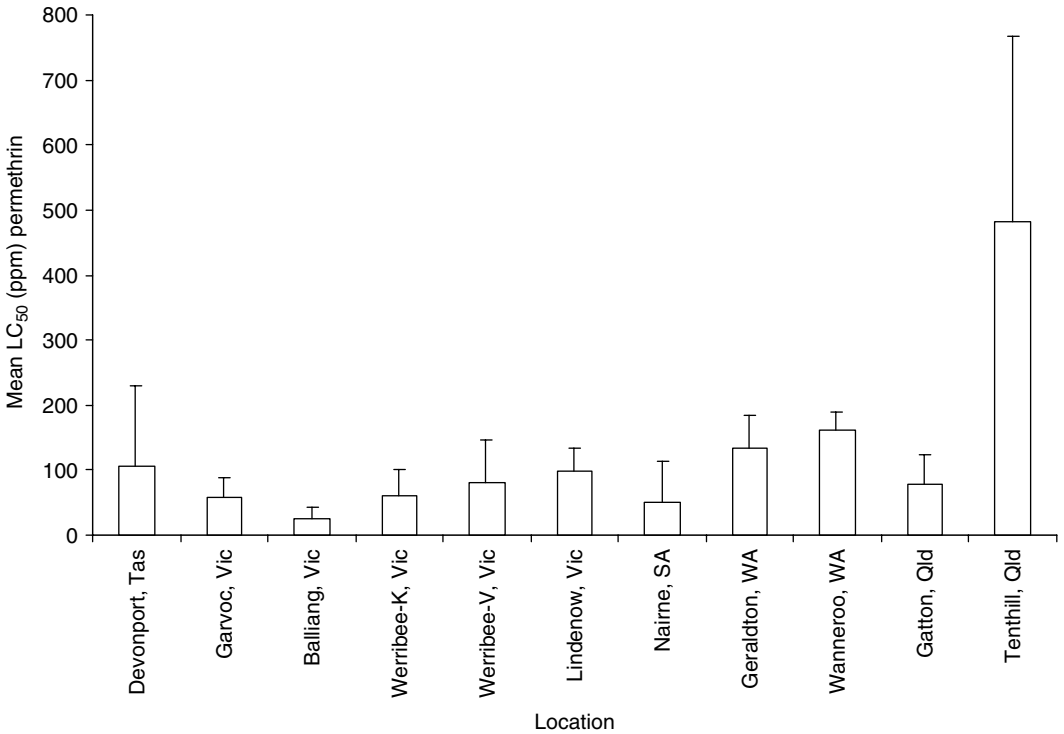

Fig. 2. Mean LC_{50} of permethrin (ppm) and standard deviation of *Plutella xylostella* (L.) sampled on multiple occasions from 11 locations in Australia.

P. xylostella collected from Gatton, Queensland, on four occasions between 2001 and 2004 have remained consistently low (mean = 6.7). In contrast, at Tenthill, 25 km from Gatton, resistance ratios have been consistently very high (mean = 65.6) each time a sample from this location has been tested (1996, 1999, 2004). The slope of the probit line also varied between locations ($F_{9,23} = 2.50$, $P = 0.037$), but not over time ($F_{1,23} = 0.61$, $P = 0.443$), and showed an interaction between time and location ($F_{9,23} = 2.53$, $P = 0.035$), indicating that the shape of the dose-response relationship changed over time in some locations. The number of generations a population had been in the laboratory at time of testing showed no significant effect on slope ($F_{1,23} = 0.85$, $P = 0.366$).

Resistance levels across regions, time and host

When considering all regions and using one sample per site, LC_{50} values were affected significantly by host type and there was a sampling time effect, an interaction between host and time and an interaction between location and time (table 4). The LC_{50} values for controls which were entered as a covariate in the analysis were also significant. To examine the effect of host on resistance further, we carried out an ANOVA on residuals after accounting for LC_{50} values for controls in a linear regression and then undertook *post hoc* tests. These indicated that the mean residuals for weeds were significantly lower by a *post hoc* test (Tukey b) than for the other hosts, as evident when the residuals for host are plotted (fig. 3a). The host by sampling time interaction reflected the fact that the effect of sampling time was significant for canola ($P < 0.001$) but not for any of the other hosts. The positive regression coefficient (0.00074 ± 0.00017) indicates that resistance on canola increased linearly at later sampling times (fig. 3b).

Table 4. ANCOVA for effects of host, time since sampling started, host by time interaction, LC_{50} for control samples and generation in laboratory at time of testing (labtime) on LC_{50} of samples of *Plutella xylostella* from 104 locations in Australia. LC_{50} values were log transformed before analysis.

	Source	df	Mean square	F	*P*
LC_{50}	Host	3	4.0710	5.0190	0.0029
	Days since sampling started	1	6.4510	7.9531	0.0059
	Host * Days since start	3	2.2539	2.7787	0.0454
	ln(LC_{50})control	1	11.5319	14.2170	0.0003
	labtime	1	0.0837	0.1032	0.7488
	Error	94	0.8111		
Slope	Host	3	0.2782	0.8807	0.4540
	Days since sampling started	1	0.6114	1.9355	0.1674
	Host * Days since start	3	0.1102	0.3488	0.7901
	labtime	1	0.4999	1.5826	0.2115
	Error	95	0.3159		

In contrast to the LC_{50} results, there was no significant effect of host, time or interaction between these factors on the slope of the probit lines (table 4). Note that control values for slope were not included in this analysis as a covariate because the slope of the controls and samples were not correlated.

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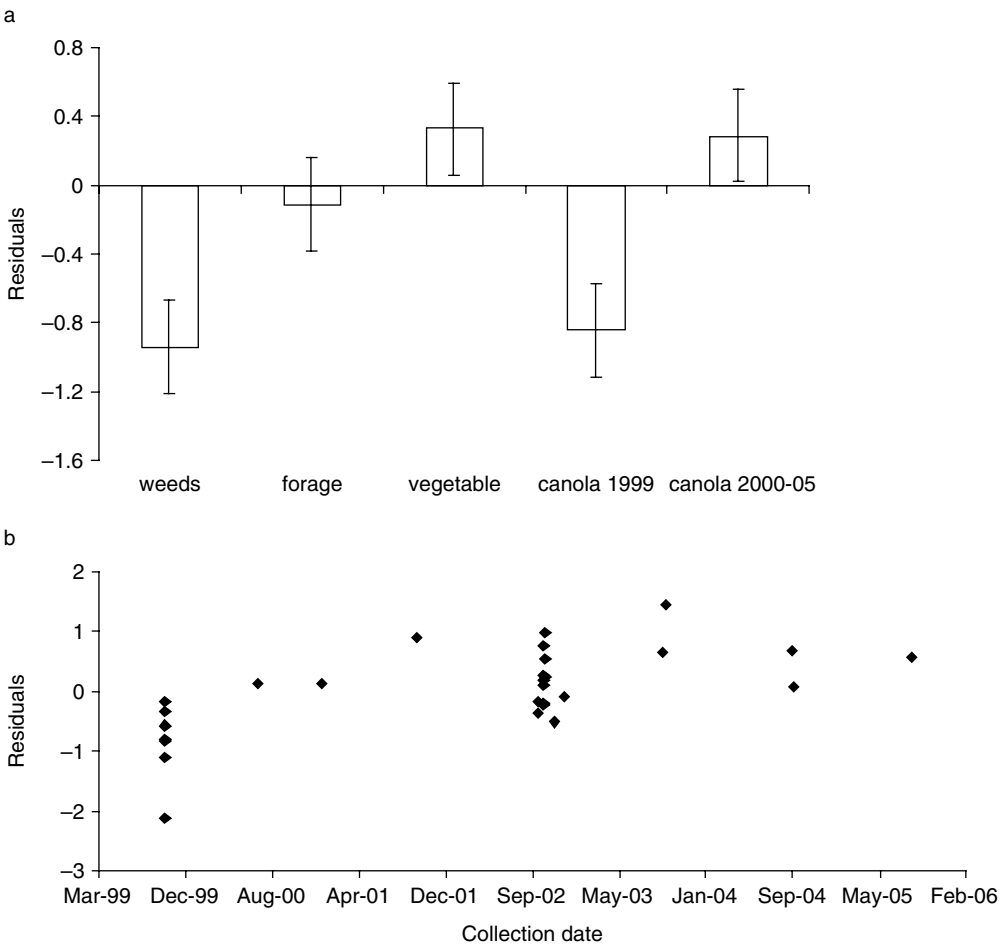

Fig. 3. (a) Mean residuals (±standard error) of regression of ln LC_{50} of permethrin against time (after correction for LC_{50} of control) of *Plutella xylostella* (L.) sampled from four host plant types in Australia. (b) Plot of residuals from regression of ln LC_{50} of permethrin against time (after correction for LC_{50} of control) for samples of *P. xylostella* collected from canola.

Spatial structure in resistance levels

No spatial structure was observed for resistance measured as LC_{50} residuals after accounting for control values (table 5) when considered on a host by host basis against geographic distance (km) using Mantel tests. Using data from samples that had only been reared through one generation prior to bioassay had little effect on the *P*-values for the Mantel tests (table 5). There was no pattern regardless of whether data were considered separately for eastern and western Australia or considered across the entire continent. Locations that were close together were, therefore, no more likely to show similar resistance levels than those far apart from each other, despite the clustered nature of the sampling sites (fig.1). There was also no spatial structure for slope values of the probit lines (results not presented).

Genetic distance versus difference in resistance

A comparison of Nei's genetic distance (Nei, 1978) between samples with differences in level of resistance measured in terms of the residuals of the regression showed

Table 5. Statistics for Mantel tests for spatial structure in permethrin resistance levels in *Plutella xylostella* (L.) from Australia from four categories of host plant. Samples from eastern and Western Australia were tested both joint and separately with 10,000 iterations.

Host plant	Mantel *r*	*P*	*P* (F_0–F_1)
Canola	0.02304	0.4103	0.2784
Canola East	−0.45933	0.9999	0.9997
Canola West	−0.28845	0.9421	0.4903
Vegetables	−0.04997	0.7794	0.3579
Vegetables East	0.08728	0.0612	0.0500
Vegetables West	−0.40748	0.8814	0.4982
Weeds	0.11140	0.1628	0.1671
Weeds East	0.11857	0.2325	0.5839
Weeds West	−0.82198	0.8868	0.1673
Forage	0.15101	0.1989	0.2451

Matrix variable 1 = difference in residuals of ln LC_{50}. Matrix variable 2 = geographic distance (km). *P*(F_0–F_1) = *P*-values for Mantel tests on samples that had been through a maximum of one generation in the laboratory before bioassay.

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no association (Mantel $r = 0.17$, $P = 0.1434$). Genetic distance among the populations, therefore, did not associate with pesticide resistance. The AMOVA to compare levels of molecular variation measured by microsatellites relative to the quantitative variation of insecticide resistance indicated that 0.25% of the variance was accounted for by molecular variation among the 13 population samples. The F_{ST} computed among all samples (table 2) across loci was 0.002, which did not differ significantly from a random value by permutation ($P = 0.12$). These values are similar to those reported by Endersby *et al*. (2006) for a wider range of populations of *P. xylostella* in Australia. In contrast, the analysis of covariance indicated a significant effect of population on the concentrations at which larvae died ($F_{(12,503)} = 7.29$, $P < 0.001$), and differences among population samples accounted for 13.9% of the variance in concentration at death. Population differences in resistance are, therefore, much larger than differences in molecular variation.

Discussion

Patterns of resistance in populations

The results indicate that resistance in *P. xylostella* is localized and associated with the host crops from which the samples were obtained. There was no evidence of spatial structure for resistance even though our sampling involved clusters of sites where broadacre crops and vegetables were grown. Locations either had persistently high or low resistance levels over several years, likely to be related to continued selection for resistance because of pyrethroid applications. Although *P. xylostella* may be transported on vegetable transplants leading to resistance problems in other localities (Shelton *et al*., 1996), no obvious patterns attributable to this phenomenon were observed in this study, though the origin of vegetable seedlings at particular locations was not traced in detail.

The extent of gene flow in *P. xylostella* in southern Australia is high enough to homogenise microsatellite allele frequencies (Endersby *et al*., 2006), but localized selection for insecticide resistance is strong enough to generate differences among populations without being reflected in neutral microsatellite allele frequency distributions. In a direct test of the association between resistance and genetic distance, we found no association between these variables. Phenotypic differentiation among populations was substantial in the absence of genetic population structure confirming that the neutral markers are not linked with resistance traits. Therefore, local patterns of selection are unrelated to genetic distance and are important in determining resistance patterns of *P. xylostella* across locations in Australia.

In a simulation model of a finite population of *P. xylostella* presented by Caprio and Tabashnik (1992), moderate levels of gene flow promoted local adaptation or evolution of resistance when genetic variation (i.e. presence of resistance alleles) was constrained in some fields but not in others. In contrast, if the background frequency of resistance alleles is low, in an infinite population, then high gene flow can impede the development of resistance (Georghiou, 1983; Lenormand & Raymond, 1998) but concurrently allow resistance alleles to spread to untreated areas, resulting in homogenisation of resistance (Caprio & Tabashnik, 1992).

Our study suggests that the evolution of resistance is not constrained by gene flow, but that gene flow does result in the spread of resistance alleles in Australian production districts to initiate and maintain resistance to permethrin in *P. xylostella*. The high LC_{50} values in some weed crops reflect movement of resistance alleles into these unsprayed hosts. Moreover, the gradual linear increase in resistance in *P. xylostella* in canola over several years is consistent with the fact that these crops were not sprayed for control of this pest prior to 2000 but have experienced increased selection pressure for pyrethroid resistance in recent years as the pest status of *P. xylostella* in canola has increased (Endersby *et al*., 2004). With the expansion in use of pyrethroids throughout the vast area of canola plantings, there may eventually be an increase in the background frequency of resistance alleles so that resistance homogenisation occurs across locations. In contrast to the situation in canola, the lack of increase in LC_{50} over time in vegetable crops may reflect a general reduction in use of synthetic pyrethroid insecticides in vegetable production regions that occurred after widespread control failures, when insecticides with new modes of action were registered for use.

Local selection also appears to be important in evolution of insecticide resistance in the pear psylla, *Psylla pyricola* Foerster, in orchards (Tabashnik *et al*., 1990), where a number of pyrethroid treatments explained a significant proportion of the variance in resistance over sites within regions. In this species, gene flow among populations (mean $F_{ST} = 0.08$), estimated using allozymes (Unruh, 1990), is thought to be too low to influence the response to selection (Tabashnik *et al*., 1990), and population differentiation was greater at a local level than between regions. Moreover, management of insecticide resistance within an individual orchard may achieve a decrease in the rate of development of resistance, even if neighbouring orchards are sprayed more frequently (Tabashnik *et al*., 1990).

Managing resistance

Strategies to delay evolution of resistance in *P. xylostella*, in which use of particular insecticide groups is restricted to particular times of the year, have been implemented to varying degrees in Australian vegetable crops (Deuter, 1989; Vickers *et al*., 2001). Within these restrictions on timing as well as a restriction on the number of applications made per planting, the strategies allow some flexibility in choice of insecticide mode of action at the level of the individual farmer. Despite the fact that the strategies were designed prior to studies of population structure in Australian *P. xylostella*, management on individual farms would seem to be appropriate when local selection is the major determinant of evolution of resistance (Tabashnik *et al*., 1990).

However, resistance to spinosad in *P. xylostella* developed in Hawaii within 2.5 years of use, despite general adherence to resistance management guidelines (restriction on number of applications) on individual properties by individual farmers (Zhao *et al*., 2002). This phenomenon occurred within an intensive *Brassica* production region with contiguous farms practising continuous, sequential planting of cabbage, which resulted in a common moth population being exposed to around 50 applications of spinosad in one year (Zhao *et al*., 2002). Intensive systems, such as this, are common in the production of vegetables and need to be

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taken into consideration in strategies to mitigate or delay resistance to insecticides. Regionally focused resistance management of new chemistries subsequently was implemented in Hawaii and use of spinosad was withdrawn until susceptibility was restored (Mau & Gusukuma-Minuto, 2004). Unfortunately, resistance then developed rapidly to indoxacarb (Zhao *et al*., 2006), one of the two new compounds that were used in rotation as replacements for spinosad.

Should tactics to delay resistance be implemented at the level of the individual farm, throughout an intensive production district, within an industry (i.e. vegetables vs. canola) or as an area-wide management system? Although we sampled widely in space and time and compared sites across distances ranging from one to 3800 km, there will still be unsampled locations for which the resistance status of *P. xylostella* is unknown, making it difficult to determine the boundaries of localized units of selection. In cases such as that described in Hawaii (Zhao *et al*., 2002), it would appear that resistance management should occur across the whole intensive production area, though a different approach from monthly rotations of two to three modes of action seems to be required. Similar tactics may apply wherever intensive production of vegetables takes place and where movement of moths between sequential plantings (Mo *et al*., 2003; Schellhorn *et al*., 2004) is prevalent.

It will be important to consider all host plant types and the consequences of different patterns of insecticide use that occur within the vegetable, canola and dairy industries for control of *P. xylostella*. In particular, how do our findings relate to future use of insecticides in canola and vegetables? The theoretical frequency of any allele before selection in its favour is estimated to range from 10^{-2} to 10^{-13} (Roush & McKenzie, 1987). With local selection influencing evolution of resistance to insecticides, it may be that compounds with new modes of action and, therefore, a low frequency of resistance alleles, could be used in canola during sporadic outbreaks of *P. xylostella* (for example, once every three years on average) without exacerbating resistance levels in the pest in vegetable crops.

One approach unlikely to be useful in the management of resistance in *P. xylostella* involves the use of neutral genetic markers. It has been proposed that such markers could be useful in identifying regions for area-wide control of resistance in *Helicoverpa armigera* (Hübner) (Scott *et al*., 2003, 2005). However, given that Australian populations of *P. xylostella* are only weakly differentiated genetically and given the lack of association between genetic distance and resistance variation, this approach would appear to be of little use in resistance management.

Because resistance is an evolutionary response to an environmental stress, the best that management strategies can hope to achieve is to delay the process (Hoy, 1998). The longitudinal data presented here indicate pyrethroid resistance develops locally on hosts that are sprayed regularly. Resistance then spreads to other areas, but local selection pressures, rather than gene flow, dictate levels of resistance.

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