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Inheritance and fitness costs of resistance to *Bacillus thuringiensis* toxin Cry2Ad in laboratory strains of the diamondback moth, *Plutella xylostella* (L.)

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The diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae), is one of the main pests of *Brassica* crops worldwide. Management of *P. xylostella* is particularly challenging, as different field populations have readily acquired resistance to a wide range of insecticides, including *Bacillus thuringiensis* (Bt) toxins. In this study, a novel strain of *P. xylostella* (Fuzhou-R2Ad) with 120-fold resistance to Bt Cry2Ad was selected in the laboratory, after screening for 66 generations from the susceptible strain Fuzhou-S. In the absence of Bt Cry2Ad toxin, the Fuzhou-R2Ad had significantly lower fitness as compared to the susceptible strain, which might be related to induced genetic changes to Bt toxins. We used several models to measure the dominance levels of insecticide resistance among different strains and found an incompletely recessive inheritance pattern of the Fuzhou-R2Ad resistance, which might be controlled by multiple genes. This study constitutes the first report of laboratory-acquired resistance to Cry2Ad toxin in *P. xylostella*. Our work presents further insights into the mechanism of Bt resistance and has immediate implications for the integrated pest management of *P. xylostella* globally.

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is one of the world's most destructive pests of *Brassica* crops, and causes an estimated cost of US\$4-5 billion annually in direct damage and pest management globally^{1,2}. Although there are multiple tactics for DBM management, chemically-synthesized insecticides remain the most common and widely-used approach. As insecticide-based management has caused substantial resistance problems in DBM³⁻⁷, biological pesticides are increasingly promoted as sustainable and environmentally-friendly alternatives. More specifically, the use of *Bacillus thuringiensis*, a soil-dwelling bacterium, offers durable and effective pest control without negative side effects on humans, vertebrates and most beneficial organisms^{8,9}. This also has led to the development of genetically-modified (GM) crops, using Bt genes that biosynthesize the toxic crystalline (Cry) protein. However, given the ability of DBM to rapidly develop resistance to insecticides, there is significant concern that this pest could equally inherit and sustain resistance to Bt toxins.

A lot of research has been conducted on the genetic basis of insect resistance to Bt toxins¹⁰⁻¹⁴. The work has shown that a high level of resistance is primarily conferred through one or several autosomal genes, which are either recessive or incompletely recessive^{6,15,16}. In contrast, the relatively low resistance is acquired through dominant inheritance mechanisms^{17,18}. Four different models have been defined for insecticide resistance and dominance, based on phenotypic traits. First, a D_{LC} model was applied for insecticide resistance, centred on LC_{50} values of dose-mortality curves¹⁹⁻²¹. Next, Roush and McKenzie developed an effective dominance D_{ML} model by assessing mortality at a particular dose of a given insecticide²². Third, the relative fitness of dominance D_{WT} was

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calculated based upon the fitness of particular genotypes in insecticide-treated areas^{23,24}. Last, a general formula has been proposed for dominance levels in relation to insecticide resistance²⁵. Overall, dominance levels can be calculated for different traits, including insect fitness in insecticide-treated or untreated area D_{WNT} . Although the dominance level could be estimated by D_{LC} , D_{ML} , D_{WT} and D_{WNT} , it may still be varied by environmental influences, genetic information and the selection of an insecticide resistance allele. Using D_{LC} and D_{ML} models, it has been shown that the resistance in Cry1Ac-selected strains was incompletely recessive in a field-derived population of DBM⁶. Pereira *et al.* has demonstrated a recessive inheritance of Cry1F resistance in European corn borer *Ostrinia nubilalis*, which was indicated by a dominance level D_{LC} less than 0.11¹⁶. However, to our knowledge, there are no published studies that utilize the various models, especially D_{WNT} , to fully evaluate the degree of the dominance.

In the present study, we evaluate, for the first time, the inheritance properties of a laboratory DBM strain with high resistance to Bt Cry2Ad, by comparing dominance of insecticide resistance between the susceptible strain, the positive and negative cross of the resistant strain and the backcross. Furthermore, we investigate levels of dominance and inheritance of resistance to Cry2Ad toxin in the hybrid, resistant and susceptible strains without selection pressure. Additionally, we estimate whether inheritance of Bt Cry2Ad resistance in *P. xylostella* is controlled by a single-gene or multiple genes. The results of this research have direct implication for resistance management of DBM to Cry2Ad, and can provide further information to advance the effective control of DBM globally.

Materials and Methods

Cry Toxin. Cry2Ad toxin was obtained from a Bt strain, BRC-HZP10, which was supplied by the Key Laboratory of Biopesticide and Chemical Biology, Fujian Agriculture and Forestry University (Fuzhou, China). The purity of the extracted Cry2Ad protein reached 88.34%²⁶. Prior to its use in the experiments, Cry2Ad toxin was prepared in 0.2% Triton X-100.

Insect strains. A susceptible strain of *P. xylostella*, Fuzhou-S, was collected in 2004 from fields of cabbage (*Brassica oleracea* var. capitata) in Fuzhou (Fujian, China; 26.08°N, 119.28°E). Whole-genome sequencing was applied to characterize the full genomic mapping²⁷. The Fuzhou-S strain has been kept for over 150 generations under greenhouse conditions without exposure to insecticides, with individuals reared on potted radish seedlings (*Raphanus sativus* L. var. sativus) under the condition of 25 ± 1 °C, 65 ± 5 % RH and 16 L:8D photoperiod.

A resistant strain was derived from the Fuzhou-S strain, by exposing the 3rd instar larvae of DBM to *R. sativus* leaves treated with Cry2Ad toxin. Fresh and untreated *R. sativus* leaves were dipped into the Cry2Ad toxin protein solution at LC_{75} concentration for 10 s, and excess solution was wiped off with filter paper. After 48 h, the surviving larvae were then selected, allowed to pupate and chosen for production of further progeny²⁸. Similar to the Fuzhou-S strain, the resistant Fuzhou-R2Ad strain has been maintained for about 70 generations in the laboratory without any exposure to insecticides except for Cry2Ad.

Bioassay. Following the procedures as outlined above, *R. sativus* leaves (ca. 10 mm diameter) were treated with five gradient concentrations of Cry2Ad solution. After drying, leaves were fed to the 3rd-instar *P. xylostella* larvae that had previously been starved in clear plastic cups (78 mm (top) and 51 mm (bottom) in diameter, 82 mm height) for 2 h^{29–31}. Each concentration was tested for a batch of 12 DBM larvae, and the experiments were independently repeated three times with 10 leaves in each replicate. In a control group, larvae were fed with leaf disks (ca. 10 mm diameter) that had been treated with distilled water containing 0.2% Triton X-100.

The treated larvae were then transferred to a climate chamber at 25 ± 1 °C, 65 ± 5 % RH, and a 16 L:8D cycle. After 48 h, fresh untreated *R. sativus* leaves were added. Mortality of larvae was recorded after 72 h, and a toxicity regression curve was developed to estimate the value of LC_{50} with 95% confidence intervals.

Hybridization. After pupation, each pupa was transferred individually into a collection tube for further eclosion. Emerged adults were sexed, and used for production of a F1 generation through reciprocal mass crosses. For one cross, 30 Fuzhou-R2Ad females were allowed to mate with 30 Fuzhou-S males in one laying cage (100 mm diameter and 80 mm height). For a second cross, 30 Fuzhou-S females were paired with 30 Fuzhou-R2Ad males³², larvae from the two parental colonies were defined as F1 (Fuzhou-R2Ad♀ × Fuzhou-S♂) and F1' (Fuzhou-R2Ad♂ × Fuzhou-S♀), and subject to the above bioassays. Subsequently, F2 progeny was obtained through single-pair crosses between F1 progeny, and a backcross (BC) was produced by pairing a F1 hybrid with the Fuzhou-S strain (F1 × Fuzhou-S). Lastly, 20 susceptible adults (i.e., 10 females and 10 males) were mixed with 20 resistant adults (10:10 sex ratio) for a pooled hybrid (R × S). Dominance of Cry2Ad toxin resistance in F1, F1' and BC hybrids were determined based on the probit analysis (visualised by slopes of log dose–probit line (LD-P line)), LC_{50} value and corresponding 95% confidence limits.

Fitness tests. Newly-hatched larvae from Fuzhou-S, Fuzhou-R2Ad, F1 and F1' hybrid populations were randomly chosen, and individualized on potted turnip sprouts (ca. 40 mm diameter). On a daily basis, development of *P. xylostella* was monitored and the relevant biological parameters, including mortality, pupation rate, eclosion rate, and adult sex ratio, were recorded. Single-pair crosses of *P. xylostella* adults were conducted in 60 mm Petri dishes lined with moist filter paper, and mated females were allowed to lay eggs on the moist filter paper. Mated females were fed with 10% honey solution, and fecundity of each strain was recorded until all moths died.

Eggs were individually collected and incubated in Petri dishes, and egg eclosion rates were computed. Net population growth rate (R_0) was determined, defined as the ratio of new larvae (N_{n+1}) to the initial number (N_n). The relative fitness of the resistant strain was calculated by:

$$\text{Relative fitness} = R_0(\text{resistant or hybrid strain})/R_0(\text{susceptible strain})$$

Data analysis. For each bioassay, LD-P line, LC₅₀ value, 95% confidence limits and the relative standard deviation were assessed. Two LC₅₀ values are considered to be significantly different ($P < 0.05$) if their 95% confidence intervals do not overlap³³.

Based on the LC₅₀, the resistance ratio was defined as the ratio between the LC₅₀ value of Fuzhou-R2Ad, F1 or BC and that of the susceptible strain (i.e., Fuzhou-S). Degree of dominance (D) at LC₅₀ was calculated by:

$$D = (2\log LC_{RS} - \log LC_R - \log LC_S) / (\log LC_R - \log LC_S)$$

where LC_R, LC_{RS} and LC_S represent lethal concentrations for resistant homozygotes, heterozygotes, and susceptible homozygotes, respectively. The value of D ranges from -1 to 1 , representing a complete recessive towards an absolute dominance. Furthermore, D_{LC} was calculated by:

$$D_{LC} = (\log LC_{RS} - \log LC_S) / (\log LC_R - \log LC_S)$$

which is equal to $(D + 1)/2$ ³⁴. Hence, the D_{LC} value varies between 0 (recessive resistance) and 1 (dominant resistance).

We equally applied the D_{WT} model to evaluate relative fitness of dominance under Bt insecticide selection. D_{WT} was calculated by:

$$D_{WT} = (W_{TRS} - W_{TSS}) / (W_{TRR} - W_{TSS})$$

where W_{TSS} , W_{TRS} and W_{TRR} represent the relative fitness at a specific insecticide concentration for susceptible homozygotes, heterozygotes, and resistant homozygotes, respectively. If susceptible and resistant strains are considered as homozygous genotypes, D_{WT} will be taken as h ^{23,35}. In a similar fashion as D_{LC} , the h value ranges from 0 to 1 (i.e., from completely recessive to completely dominant resistance).

Another approach was used to assess dominance. For instance, D_{WNT} value was calculated by:

$$D_{WNT} = (W_{NTRS} - W_{NTSS}) / (W_{NTRR} - W_{NTSS})$$

where W_{NTSS} , W_{NTRS} and W_{NTRR} represent relative fitness in the absence of insecticide for susceptible homozygotes, heterozygotes, and resistant homozygotes, respectively²⁵. When the D_{WNT} value is 0.5 , resistance is called co-dominant. D_{WNT} values ranging from 0 to 0.5 demonstrate partial recessive, while D_{WNT} values between 0.5 to 1 refer to partial dominance.

To test the genetic mode of inheritance, the expected mortality (E) of BC and F2 under a certain concentration of insecticide was estimated according to Georghiou's method³⁶.

$$E_{BC} = (W_1 + W_2) \times 0.5$$

$$E_{F2} = (W_1 + W_2 + W_3) \times 0.25$$

in which W_1 , W_2 , W_3 represent the actual mortality of Fuzhou-S, Fuzhou-R2Ad, and F1, respectively, for a given dose of insecticide. Chi-square test was employed to compare observed and expected mortality of BC and F2³⁷. All of the above analyses, including one-way ANOVA with post-hoc Tukey's honestly significant difference, were performed by using data processing system (DPS) V9.01, while figures were developed using Prism Graphpad 6.

Results

Cry2Ad resistance ratio. The resistance to Cry2Ad developed slow, and increased 1.04 times at the 12th generation as compared to the susceptible strain (Table 1). Resistance gradually increased over subsequent generations and by generation 37 a 8.70-fold increase was observed over the susceptible strain. In the 66th generation, the relative resistance ratio was 120.59 (Table 1).

Biological fitness parameters. In the Fuzhou-S strain, survival rates (% ± standard error) of the 1st, 2nd, 3rd and 4th instar larvae were 90.30 ± 0.50, 57.80 ± 0.77, 93.23 ± 1.63, and 91.00 ± 1.59, respectively (Table 2). For the resistant Fuzhou-R2Ad strain, corresponding survival rates (%) were 67.25 ± 0.59, 58.71 ± 0.19, 100.00 ± 0.12 and 74.90 ± 1.97, respectively. Survival rates of 1st and 4th instar larvae of the Fuzhou-R2Ad strain were significantly lower than those of the Fuzhou-S strain, and the relative fitness (D_{WT}) of the Fuzhou-R2Ad strain was 0.29.

Other fitness parameters, such as egg hatch rate, survival rate of the 2nd-instar larvae, pupation rate, and female fecundity were significantly higher in F1 hybrid compared to F1'. And the relative fitness values of the positive cross F1 and negative cross F1' were 0.89 and 0.65, respectively.

Inheritance properties. All experimental strains proved susceptible to Cry2Ad, and no significant difference was recorded in LC₅₀ values between F1 and F1' strains (Table 3). In the pooled hybrid (R × S), the LC₅₀ value was significantly lower than that of Fuzhou-R2Ad strain. Also, the overlap in 95% confidence limits of LC₅₀ between F1 and F1' strains confirmed that Cry2Ad resistance was autosomally inherited, without maternal effects and sex linkage.

Estimation of dominance. Upon testing five different Cry2Ad toxin concentrations, LC₅₀ values for F1 and F1' progenies yielded $D_{F1} = -0.73$, $D_{F1'} = -0.44$, $D_{LC-F1} = 0.13$, $D_{LC-F1'} = 0.28$. The effective dominance (h) varied

Generation	number of insects tested	Slope \pm SE	LC ₅₀ (95% fiducial limits) (ng/mL)	RR*	P (df = 3)
0	216	4.34 \pm 0.50	6.65(5.58–8.28)	1.00	0.8812
12	216	1.78 \pm 0.26	6.92(4.83–9.11)	1.04	0.9964
16	216	4.54 \pm 0.38	32.35(26.43–37.92)	4.86	0.6909
27	216	1.68 \pm 0.27	51.53(32.94–70.37)	7.76	0.9999
37	216	2.35 \pm 0.32	57.79(41.96–73.00)	8.70	0.9998
41	216	2.13 \pm 0.28	120.20(96.79–157.82)	18.10	0.9973
52	216	2.35 \pm 0.32	154.45(123.84–200.47)	23.26	0.9058
66	216	1.26 \pm 0.31	800.73(372.94–6142.62)	120.59	0.9633

Table 1. Resistance ratio of *P. xylostella* to Cry2Ad over multiple generation selection as compared to the susceptible Fuzhou-S strain. *RR (resistance ratio) is calculated as LC₅₀ (Fuzhou-R2Ad, F1 or BC)/LC₅₀ (Fuzhou-S). LC₅₀(Fuzhou-S) is expressed as 6.65 ng/mL. Each LC₅₀ value represents the average of 8 independent measurements.

Biological characteristics	Fuzhou-S	Fuzhou-R2Ad	F1	F1'	F value	P
Initial amount of eggs	140	186	118	87		
Egg hatch (%)	80.72 \pm 1.22 ^{bb}	87.08 \pm 0.36 ^{aa}	82.26 \pm 0.57 ^{baB}	74.57 \pm 0.97 ^{cC}	36.82	0.0001
Survival rate 1st instar (%)	90.30 \pm 0.5 ^{aa}	67.25 \pm 0.59 ^{cC}	82.82 \pm 0.89 ^{bb}	85.01 \pm 1.27 ^{baB}	127.19	0.0001
Survival rate 2nd instar (%)	57.80 \pm 0.77 ^{cC}	58.71 \pm 0.19 ^{cC}	97.57 \pm 1.22 ^{aa}	85.74 \pm 1.01 ^{bb}	498.22	0.0001
Survival rate 3rd instar (%)	93.23 \pm 1.63 ^{bb}	100.00 \pm 0.12 ^{aa}	96.30 \pm 0.15 ^{abAB}	100.00 \pm 1.03 ^{aa}	15.99	0.0010
Survival rate 4th instar (%)	91.00 \pm 1.59 ^{aa}	74.90 \pm 1.97 ^{cC}	84.22 \pm 0.54 ^{baB}	76.59 \pm 1.05 ^{cBC}	28.22	0.0001
Number of pupae	17.00 \pm 0.67 ^{abAB}	16.00 \pm 1.00 ^{abAB}	21.00 \pm 1.00 ^{aa}	12.00 \pm 2.08 ^{bb}	8.02	0.0085
Pupation rate (%)	33.42 \pm 1.62 ^{cC}	25.77 \pm 1.02 ^{cC}	53.71 \pm 1.47 ^{bb}	83.98 \pm 2.46 ^{aa}	227.33	0.0001
Adult number	14 \pm 0.67 ^{abAB}	12 \pm 0.58 ^{abAB}	18.00 \pm 1.00 ^{aa}	10 \pm 1.53 ^{bb}	11.49	0.0029
Emergence rate (%)	86.11 \pm 3.87 ^{aa}	75.18 \pm 2.43 ^{aa}	86.42 \pm 0.72 ^{aa}	83.99 \pm 2.46 ^{aa}	4.06	0.0502
Sexual ratio (female:male)	1.17 ^{aa}	1.00 ^{aa}	1.31 ^{aa}	1.50 ^{aa}		
Fecundity/female	102 \pm 3.67 ^{abAB}	91 \pm 5.29 ^{abAB}	129 \pm 7.21 ^{aa}	82 \pm 8.97 ^{bb}	9.65	0.0049
Number of offspring eggs	1414	546	1062	574	—	—
R0	10.10	2.93	9.00	6.60	—	—
Relative fitness	1.00*	0.29	0.89	0.65		

Table 2. Population growth parameters of different *P. xylostella* strains. According to one-way with post-hoc Tukey's honestly significant difference, the same superscript letter following the numbers between rows of a given column indicates no significant difference between the strains at $P > 0.05$. The different upper and lower case letters stand for the significance with $P < 0.01$, and $P < 0.05$, respectively. *Relative fitness of the susceptible Fuzhou-S strain is defined as 1.

between 0.33 up to 0.71, and negatively correlated with the Cry2Ad protein concentration (Table 4). Based on the relative DBM fitness (Table 2), the respective fitness values of F1 and F1' in insecticide-treated areas D_{WNT} were 0.15 and 0.49. Hence, D , D_{LC} , and D_{WNT} parameters indicate that the genes conferring resistance to Cry2Ad in DBM selected strain was incompletely recessive. However, when subject to Cry2Ad at 25.32–202.60 μ g/ml, DBM larvae had relatively high h values (0.56–0.71), suggesting an incomplete dominant inheritance of the Cry2Ad resistance.

Genetic mode of inheritance. LD-P lines and expected values were distinguishable for both BC and F2 crosses (Figs 1 and 2). A plateau was not reached neither after the 50% mortality of BC progeny nor at 25% or 75% mortality levels of F2 hybrids. Chi-square analysis showed that the resistance heredity in experimental DBM strains may be controlled by multiple genes (Tables 5 and 6).

Discussion

A thorough understanding of pesticide resistance development in *P. xylostella* is crucial for an effective and sustainable management of this globally-important pest. Past research has shown that the development of Bt resistance depends on the particular Bt strain and the type of Bt toxin³⁸. Induced by Bt subspecies *kurstaki*, the resistance ratio of *P. xylostella* strain NO was 30 times³. Another *P. xylostella* strain NO-95 selected with high resistance to Bt subspecies *kurstaki* has very low resistance to Bt subspecies *aizawai*⁵. In 2014, a Cry1Ie susceptible *Ostrinia furnacalis* strain of ACB-BtS was found to have cross resistance to Cry1Ab, Cry1Ac and Cry1F toxins³⁹. Other work has shown that a given Bt toxin produced by the same Bt species may exhibit different impacts on a DBM strains/populations, due to the differential modes of action of the Bt toxins^{40,41}. In this study, we determine that DBM resistance development to the Bt Cry2Ad toxin is possible, after laboratory-based screening for 5 years

Strain or cross	Number of insects tested	Slope \pm SE	LC ₅₀ (95% fiducial limits) (ng/mL)	RR*	P (df = 3)
Fuzhou-S	216	1.44 \pm 0.25	9.84 (6.98–13.61)	1.00	0.8874
Fuzhou-R2Ad	216	1.26 \pm 0.31	800.73 (372.94–6142.62)	81.37	0.9633
F1 (Fuzhou-R2Ad♀ \times Fuzhou-S♂)	216	1.39 \pm 0.22	230.27 (155.81–457.35)	23.40	0.9737
F1' (Fuzhou-R2Ad♂ \times Fuzhou-S♀)	216	1.15 \pm 0.25	116.91 (77.44–187.60)	11.88	0.8206
R \times S (pooled)	432	1.27 \pm 0.23	173.59 (116.62–322.47)	17.64	—
S \times F1 (F1♀ \times S♂)	216	0.83 \pm 0.24	297.84 (160.45–1591.57)	30.27	0.9696
F2 (F1 \times F1)	216	1.14 \pm 0.25	77.71 (53.83–107.38)	7.90	0.8943

Table 3. Susceptibility to Cry2Ad toxin in a susceptible strain (Fuzhou-S), resistant strain (Fuzhou-R2Ad), and different reciprocal crosses of the *P. xylostella* strains. Resistance ratio is presented by LC50 of a given strain or cross divided by LC50 of the susceptible Fuzhou-S strain.

Concentration of Cry2Ad (ng/ml)	Strain or cross	Survival (%)	Fitness	<i>h</i>
25.32	Fuzhou-S	27.80 \pm 1.66	0.28	
	Fuzhou-R2Ad	97.22 \pm 1.60	1.00	
	F1(Fuzhou-R2Ad♀ \times Fuzhou-S♂)	77.14 \pm 1.19	0.79	0.71
50.65	Fuzhou-S	15.30 \pm 1.48	0.16	
	Fuzhou-R2Ad	94.44 \pm 0.00	1.00	
	F1(Fuzhou-R2Ad♀ \times Fuzhou-S♂)	65.71 \pm 2.83	0.69	0.64
101.30	Fuzhou-S	7.30 \pm 1.42	0.08	
	Fuzhou-R2Ad	86.11 \pm 2.78	1.00	
	F1(Fuzhou-R2Ad♀ \times Fuzhou-S♂)	51.43 \pm 2.89	0.60	0.56
202.60	Fuzhou-S	3.00 \pm 1.52	0.04	
	Fuzhou-R2Ad	75.00 \pm 2.78	1.00	
	F1(Fuzhou-R2Ad♀ \times Fuzhou-S♂)	45.71 \pm 2.03	0.61	0.59
405.21	Fuzhou-S	1.01 \pm 0.71	0.01	
	Fuzhou-R2Ad	66.67 \pm 3.21	1.00	
	F1(Fuzhou-R2Ad♀ \times Fuzhou-S♂)	22.86 \pm 1.28	0.34	0.33

Table 4. Effective dominance (*h*) of resistance to Cry2Ad in different strains of *P. xylostella*, as compared to Fuzhou-R2Ad. Mortality (%) is calibrated before fitness calculation, and it is calculated as $(W_{RR} - W_{RS}) / (W_{RR} - W_{SS})$, where W_{RR} , W_{RS} , and W_{SS} represent fitness values at a specific toxin concentration.

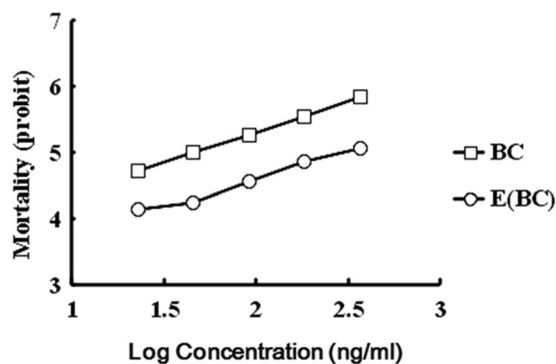


Figure 1. The slopes of log dose–probit lines (LD–P lines) for BC and the expected LD–P line of BC progeny (E_{BC}). Expected mortality at concentration *x* ng/ml is calculated as $0.5 \times (\text{mortality of F1 at } x \text{ ng/ml} + \text{mortality of Fuzhou-S at } x \text{ ng/ml})$, obtained from regression lines of parental strains.

and 66 generations. The resulting Fuzhou-R2Ad resistant strain had 120.59 times higher levels of resistance than the susceptible Fuzhou-S strain.

When unexposed to Bt Cry2Ad toxin, the Fuzhou-R2Ad has significantly lower fitness as compared to the susceptible strain. Similar findings has been made with DBM populations in Hawaii, where Dipel 2X[®] (a wettable powder formulation of *B. thuringiensis* subsp. *kurstaki* strain HD-1) resistant strain NO-QA exhibited reduced survival, egg hatching and mating rates⁴². Such reduction in fitness is possibly related to induced genetic changes

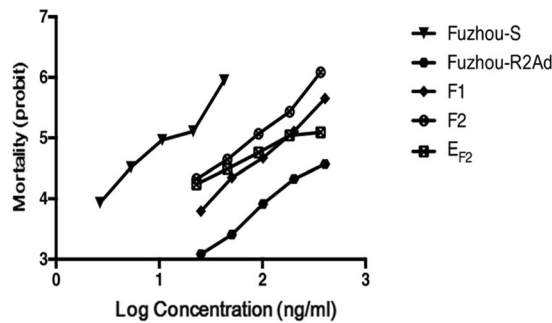


Figure 2. LD-P lines for susceptible (Fuzhou-S) and resistant parents (Fuzhou-R2Ad), F1, F2 and expected LD-P line of F2 progeny. Expected mortality at concentration x ng/ml is calculated as $0.25 \times (\text{Fuzhou-S mortality} + \text{Fuzhou-R2Ad mortality} + \text{F1 mortality})$, obtained from regression lines of parental strains.

Concentration of Cry2Ad (ng/ml)	Observed		Expected		χ^2	P
	Dead	Alive	Dead	Alive		
22.93	7	29	28	44	3.30	0.0692
45.86	8	28	36	36	6.56	0.0104
91.72	12	24	43	29	5.67	0.0172
183.44	16	20	51	21	6.02	0.0141
366.88	19	17	58	14	7.74	0.0054
$\Sigma\chi^2$					29.29	

Table 5. Observed and expected mortality of the BC strain of *P. xylostella* treated with Cry2Ad, as evaluated with a Chi square test (χ^2). The single gene conferring Cry2Ad resistance is defined as the Chi-square hypothesis.

Concentration of Cry2Ad (ng/ml)	Observed		Expected		χ^2	P
	Dead	Alive	Dead	Alive		
22.93	9	27	16	56	0.01	0.9357
45.86	13	23	22	50	0.13	0.5663
91.72	19	17	29	43	1.05	0.2222
183.44	24	12	37	35	1.70	0.1923
366.88	31	5	46	26	4.76	0.0131
$\Sigma\chi^2$					7.65	

Table 6. Observed and expected mortality of F2 strain of *P. xylostella* treated with Cry2Ad.

to Bt toxins, which may remain even in the absence of selection pressure^{24,43}. Hence, it is possible that effective DBM pest control can still be attained for resistant populations by discontinuing Bt Cry2Ad applications.

Inheritance of Bt resistance in the diamondback moth is considered to occur autosomally^{14,28,44}, and similar inheritance models have been recorded for the Asian corn borer *Ostrinia furnacalis*³⁸, the southern house mosquito *Culex quinquefasciatus*⁴⁵, and the cotton bollworm *Helicoverpa armigera*^{46,47}. As one notable exception, Malaysian populations of *P. xylostella* exhibited maternal effects on Cry1Ac resistance development³. In the current research, we detect susceptibility to Cry2Ad in all experimental strains or crosses, and confirm this to be autosomal resistance to Cry2Ad, without maternal effects or sex linkage (Table 3).

Our work also show that the resistance inheritance to Cry2Ad toxin in DBM strains is incompletely recessive. This is clearly shown by the following parameters: D_{F1} values of 0.73 and 0.13, D_{LC} values of 0.44 and 0.28, D_{WNT} values of 0.15 and 0.49 for F1 and F1' respectively. D , D_{LC} and D_{WNT} values indicate that resistance to Cry2Ad in the Fuzhou strains of *P. xylostella* is partially recessive. Secondly, the effective dominance is negatively regulated by concentrations of the Bt toxin^{48,49}, namely an incomplete recessivity of resistance at a high Cry2Ad level and an incomplete dominance at low concentrations of Cry2Ad protein. However, when DBM populations are treated with a low dose of toxin, the reduced selection pressure may cause bias because of the increased survival rate in the susceptible strain.

Our work constitutes the first report of Cry2Ad resistance in *P. xylostella*, sheds light upon Bt resistance development, and could guide further pest management interventions against a globally-relevant lepidopteran pest. Caution needs to be taken when extrapolating our findings, as our research is conducted under highly-artificial conditions with laboratory-reared individuals. Hence, one could still encounter an incompletely coincident resistance to Cry2Ad due to variations in DBM field populations⁵⁰. Further, we postulate that resistant heredity in local

diamondback moth populations is conferred by multiple genes (Figs 1 and 2; Table 5). All of the above provide fundamental insights into the mechanism and evolution of Bt resistance, according to the neo-Darwinian theory⁵¹. Further investigation of Bt resistance genes through molecular biology approaches, including molecular marker selection, would be a great help for the genetic manipulation of the diamondback moth. Moreover, the knowledge obtained from this research could boost the effectiveness of pest management interventions and enable sustainable DBM control globally.

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Additional Information

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