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Genetic characterization of field-evolved resistance to phosphine in the rusty grain beetle, *Cryptolestes ferrugineus* (Laemophloeidae: Coleoptera)

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Running Title: Genetic basis of phosphine resistance in *C. ferrugineus*
Abstract

Inheritance of resistance to phosphine fumigant was investigated in three field-collected strains of rusty grain beetle, Cryptolestes ferrugineus, Susceptible (S-strain), Weakly Resistant (Weak-R) and Strongly Resistant (Strong-R). The strains were purified for susceptibility, weak resistance and strong resistance to phosphine, respectively, to ensure homozygosity of resistance genotype. Crosses were established between S-strain x Weak-R, S-strain x Strong-R and Weak-R x Strong-R, and the dose mortality responses to phosphine of these strains and their F1, F2 and F1-backcross progeny were obtained. The fumigations were undertaken at 25°C and 55% RH for 72 hours.

Weak-R and Strong-R showed resistance factors of 6.3× and 505× compared with S-strain at the LC50. Both weak and strong resistances were expressed as incompletely recessive with degrees of dominance of -0.48 and -0.43 at the LC50, respectively. Responses of F2 and F1-backcross progeny indicated the existence of one major gene in Weak-R, and at least two major genes in Strong-R, one of which was allelic with the major factor in Weak-R. Phenotypic variance analyses also estimated that the number of independently segregating genes conferring weak resistance was 1 (nE = 0.89) whereas there were two genes controlling strong resistance (nE = 1.2). The second gene, unique to Strong-R, interacted synergistically with the first gene to confer a very high level of resistance (~80×).

Neither of the two major resistance genes was sex linked. Despite the similarity of the genetics of resistance to that previously observed in other pest species, a significant proportion (~15 to 30%) of F1 individuals survived at phosphine concentrations higher than predicted. Thus it is likely that additional dominant heritable factors, present in some individuals in the population, also influenced the resistance phenotype.

Our results will help in understanding the process of selection for phosphine resistance in the field which will inform resistance management strategies. In addition, this information will provide a basis for the identification of the resistance genes.

**Keywords:** genetics, dominance, gene interactions, selection pressure, management
1. Introduction

The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) is a cosmopolitan insect pest that infests a wide range of stored cereals and processed commodities [1, 2]. Until recently, phosphine (PH₃) fumigation has been effective in controlling this species in Australia, however, strongly resistant populations of *C. ferrugineus* have now been detected that threaten market access of infested commodities [3]. Resistant populations detected in Australia are capable of developing very high levels of resistance to phosphine, up to 1300×, significantly higher than levels reported in other grain insect pests [4].

The development of resistance is an evolutionary process in which a heritable change occurs within an insect population as an intrinsic response to selection imposed by humans [5]. An understanding of the genetics of resistance in *C. ferrugineus* may help us identify the factors driving the development of strong resistance to phosphine in this species. This information is crucial to the development of rational and sustainable resistance management. Strong resistance to phosphine in the lesser grain borer, *Rhyzopertha dominica* (Fabricius)[6], the red flour beetle, *Tribolium castaneum* (Herbst)[7], and the rice weevil, *Sitophilus oryzae* (Linnaeus) [8], is mediated by two major autosomal recessive genes, *rph1* and *rph2*. In homozygous isolation, each of these genes confers only weak resistance, ~4-30×, however, when they occur together in one individual, they interact synergistically and provide a very high level of resistance, up to ~600× [6-8]. Genes *rph1* and *rph2* are expressed as incompletely recessive in all the three species, irrespective of the phenotypes, and are not sex linked [6-9]. This genetic information together with well-established efficacy data against *R. dominica* [10], *T. castaneum* [11] and *S. oryzae* [12-14] facilitated the development and implementation of a resistance management strategy that has been effective in containing the resistance problem [15] or at least delaying further evolution of high level of resistance in these pest species in Australia [16].
No prior information is available on the genetics of phosphine resistance in *C. ferrugineus* despite the extraordinary levels of resistance detected in this species [4, 17]. To support the development of a rational, effective resistance management strategy, our aim was to determine the inheritance of resistance to phosphine in this species. We investigated key determinants in the selection of resistance including the number of resistance genes, their mode of inheritance, their relative dominance and gene interactions, if any, in two field-evolved resistant strains.

2. Materials and Methods

2.1 Insect strains

Three field-derived strains of *C. ferrugineus* were used in this study; phosphine-susceptible, QCF31, weakly resistant, QCF37 and strongly resistant, QCF73 [4]. Throughout this report we refer to these strains as S-strain, Weak-R and Strong-R, respectively. Before the commencement of genetic crosses, both Weak-R and Strong-R were fumigated at 0.04 mg L\(^{-1}\) over 48 hours and 1.0 mg L\(^{-1}\) over 144 hours, respectively, for three successive generations to promote homozygosity within the strains. All insects were cultured on a standard dietary medium of rolled oats + cracked sorghum + yeast (75:20:5%) at 30°C and 65% RH [18].

2.2 Inheritance of phosphine resistance

2.2.1 Genetic crosses

2.2.1.1 Multiply mated intercrosses (MIC)

To determine the mode of inheritance of phosphine resistance in *C. ferrugineus* three multiply mated (mass) intercrosses (MIC) were set up: S-strain x Weak-R; S-strain x Strong-R and Weak-R x Strong-R. Each cross consisted of 100 virgin adult males (♂) of one parent and 100 virgin adult females (♀) of the other parent. The resulting F\(_1\) hybrids were used to produce segregating F\(_2\) intercross and backcross progeny (F\(_2\)-BC). F\(_2\) insects were obtained by allowing F\(_1\) progeny to randomly mate with each other for two weeks. In the case of backcross, two F\(_2\)-BC populations were
obtained from each parental cross; S-strain x Weak-R; S-strain x Strong-R and Weak-R x Strong-R, by crossing virgin F₁ females from each cross, back to virgins males of each parental strain [(F₁♀ X Weak-R♂ and F₁♀ x S♂); (F₁♀ x Strong-R♂ and F₁♀ x S♂); (F₁♀ X Strong-R♂ and F₁♀ x Weak-R♂)]. In F₁, F₂ and F₁-BC, reciprocals (F₁’, F₂’ and F₁-BC’) were also established as a replicate to the original cross and their dose mortality response was compared and pooled with originals for analysis, if the responses were not significantly different.

2.2.1.2 Single pair intercrosses (SIC)

Three single pair intercrosses (SIC) (one ♀ + one ♂) were also established with the virgin parents (S-strain x Weak-R; S-strain x Strong-R and Weak-R x Strong-R) to investigate the expression of resistance at higher concentrations of phosphine in the F₁ generation. The mortality response data of three F₁ hybrid populations obtained from single pair intercrossing and multiply mated intercrossing were compared to control for skewness or errors associated with sexing, multiple mating or other strain based genetic background variation unrelated to phosphine resistance.

2.3 Phosphine susceptibility tests

Phosphine was generated from aluminium phosphide and its concentration measured using a gas chromatograph (Perkin-Elmer Clarus 580) according to a procedure described previously by Daglish et al [14]. Phosphine susceptibility in adults of the parental strains and the crosses (MIC and SIC) was assessed using the FAO recommended bioassay method at a range of phosphine concentrations (0.005 to 8.0 mg L⁻¹) in gas-tight desiccators (4 to 6 L) for 72 hours at 25 °C and 55% RH [4]. Mortality was assessed 7 days after the completion of the exposure period. The entire experiment was replicated twice with each test concentration for each replicate consisting of three batches of 50 adult beetles.

The parents and F₁ hybrids from each of the three MIC were also fumigated at exposure periods of 48 and 144 hours, in addition to the standard 72 hour exposure, to detect any variation in
the expression of resistance (degree of dominance) associated with length of exposure period, especially at the higher concentrations of phosphine. The estimated LC$_{50}$ values under the respective exposure periods were fitted to the standard equation $C^t=k$, where $k$ is constant and $n$ is the toxicity index that decides the effect of exposure period over concentrations [13]. A value of $n$ less than 1.0 is expected in the case that the expression of resistance factors vary significantly with exposure period on these strains/hybrids [14].

2.4 Data analysis

2.4.1 Resistance factor, dominance and sex linkage

The response of parental strains and reciprocal F$_1$ progeny in each cross were analysed and fitted to log dose-probit mortality response curves [19] using Genstat software version 16.0 [20]. Resistance factors were calculated as the ratio between LC$_{50}$ value of resistant parental strains or F$_1$ hybrids with LC$_{50}$ value of S-strain or Weak-R [21], depending on the crossing scheme. Sex linkage (influence of maternal factors) [19] was tested by overlap of 95% fiducial limits at LC$_{50}$ and relative potency analysis in the response of reciprocal F$_1$ hybrids. The degree of dominance was estimated from the response of parental strains and reciprocal F$_1$ hybrids in each cross, based on LC$_{50}$ and LC$_{99.9}$, according to the method of Stone [22] to reveal variations (if any) in the expression of the phenotype in F$_1$ hybrids against concentration. A high number of test insects ($n = 600$) were used for each concentration in the probit analysis for estimating LC$_{99.9}$, so that the estimates were reliable and closely representative of the observed data.

2.4.2 Number of genes conferring resistance

Three methods were used to determine the number of genes conferring resistance to phosphine in C. ferrugineus. First, the observed response of the F$_2$ and F$_1$-BC progeny to phosphine was compared visually to an expected response assuming monogenic inheritance. According to Tsukumoto et al. [23], if resistance is conferred by a single recessive gene, then a plateau or point of
inflection should occur in the log dose-probit response line of the F₂ at around 75% and in the F₁-BC response line at around 50%. Second, the null hypothesis of monogenic inheritance of resistance was tested on the basis of goodness-of-fit [24] between observed mortality and the theoretical expectations of F₂ and F₁-BC curves according to Georgiou et al. [25] using a modified chi-square [26], that incorporates the heterogeneity factor of the parental strains. Since, the observed response of F₁ hybrids in the three crosses differed significantly at high concentrations from the expected response for incompletely recessive inheritance, a theoretical F₁ curve was established based on incomplete recessive inheritance model and used in predicting the expectations for monogenic F₂ and F₁-BC response curves. The third test was based on the procedure outlined by Lande et al. [27] to approximate the number of freely segregating genetic factors (nE) associated with a heritable phenotype. The nE was estimated by comparing the genotypic and phenotypic variances that contribute quantitative trait difference between two populations. Thus, \( nE = \frac{(\mu_{p2} - \mu_{p1})^2}{8 \sigma_S^2} < n \), where \( \mu_{p1} \) and \( \mu_{p2} \) are the log₁₀ of the LC₅₀ values of the resistant and susceptible or weakly resistant strains, respectively, depending on the crossing scheme, and n was the actual number of genes. The additional genetic variance (\( \sigma^2_{S-Var} \)) associated with the F₁-BC generation was estimated as:

\[
\sigma^2_{S-Var} = \sigma^2_{B1} + \sigma^2_{B2} + [\sigma^2_{F1} + 0.5*(\sigma^2_{P1}) + 0.5*(\sigma^2_{P2})],
\]

where, \( \sigma^2_{B1} \), \( \sigma^2_{B2} \), \( \sigma^2_{F1} \), \( \sigma^2_{P1} \) and \( \sigma^2_{P2} \) refer to the phenotypic variances of the backcross to susceptible, backcross to resistant, the F₁ hybrid, homozygous resistant and susceptible insects, respectively. The phenotypic variance in each case was estimated from the slope of the probit regression [27].

3 Results

3.1 Inheritance of weak resistance to phosphine (S-strain x Weak-R)

3.1.1 Resistance levels, maternal effects and degree of dominance

The response of the parental strains, S-strain and Weak-R, and their reciprocal F₁ hybrids (F₁ and F₁'), were highly homogenous with a low heterogeneity and non-significant chi-square values, indicating good fit to the probit model (Table 1). The resistance ratio (RR) of Weak-R was 6.3× compared with the S-strain at the LC₅₀, whereas it was 1.6× and 2.0× for the F₁ and F₁', respectively.
The responses of reciprocal F₁ hybrids were very close to each other, as evident from the significant overlap of their fiducial limits at LC₅₀ (0.007-0.012) (Table 1). In addition, the estimated relative potency value of F₁ was 0.8 relative to the response of F₁ with fiducial limits [0.68-1.0], which include the value 1.0, confirming that there was no significant difference between the responses of the reciprocal F₁ hybrids. These results suggest that weak resistance was autosomal and maternal factors were absent and therefore the data of reciprocal F₁ hybrids was pooled for further interpretation. At the LC₅₀ the degree of dominance (DD) was -0.48, indicating the resistance is expressed as an incompletely recessive trait. However, a significant proportion (~15%) of individuals survived at higher doses (0.01 to 0.1 mg L⁻¹) suggesting that there may be a dose-dependent variation in expression of resistance trait in F₁ or additional resistant factors present at low frequency in the parental strains (S-strain or in Weak-R), perhaps a variant of the weak resistance gene (Figure 1). This is clearly evident as the DD approaches 1.0 when the LC₉₉.₉ value was used as a reference instead of the LC₅₀ (Table 1).

3.1.2 Number of genes conferring weak resistance

Visual examination of the observed F₂ and F₁-BC progeny response curves showed plateaus at ~75% (0.008 to 0.025 mg L⁻¹) and ~50% (0.018 to 0.035 mg L⁻¹) mortality levels conforming with the expected response from single, recessive gene inheritance (Figure 1A and B). However, modified chi-square analysis of individual concentrations of the observed F₂ and F₁-BC response curves identified significantly higher mortalities than expected at lower concentrations from 0.004 to 0.008 mg L⁻¹ (P<0.001, df = 1) for F₂ and 0.01 to 0.014 mg L⁻¹ (P = 0.01, df = 1) for F₁-BC (Figure 1A and B). These differences were reflected in the overall chi-square analysis of the F₂ (χ² = 32.3, df = 14, P = 0.001) and F₁-BC (χ² = 29.3, df = 14, P= 0.04) curves, allowing us to reject the null hypothesis of single gene inheritance (Tables S1 and S2). Analysis of the number of effective segregating factors between the S-strain and Weak-R gave nE = 0.8, indicating the presence of a single major gene between the two strains (Table 2). Thus, while both visual inspection and phenotypic variance analysis showed strong conformity to a single major gene being responsible for inheritance of the
Weak-R phenotype, the chi-square analyses and the response of $F_1$ hybrids at higher concentrations suggested the possible presence of additional factors. Based on these results, we hypothesise that resistance in Weak-R may be mediated by single major gene and one or more additional minor genes.

3.2 Inheritance of strong resistance to phosphine (S-strain x Strong-R)

3.2.1 Resistance levels, maternal effects and degree of dominance

Compared with the response of the S-strain, Strong-R and reciprocal $F_1$ hybrids were 505× and 6.0× more resistant, respectively, at the LC$_{50}$ (Table 1). The 95% confidence intervals of the responses of $F_1$ and $F_1'$ hybrids overlapped at all concentrations tested, including at the LC$_{50}$ [0.026-0.041 and 0.024-0.042], and were statistically indistinguishable (Table 1). The relative potency analysis of the responses of $F_1$ and $F_1'$ yielded a value of 0.95 with the fiducial limits [0.69-1.33] including the value 1.0, confirming the absence of sex-linked inheritance of strong resistance genes. Thus, the response data of $F_1$ reciprocal crosses were pooled for further analysis. The degree of dominance of the pooled $F_1$ was -0.43, showing that strong resistance was expressed as an incompletely recessive trait. However, as observed with the $F_1$ hybrids of the cross S-strain x Weak-R, a significant proportion (~25%) of the individuals within the $F_1$ cohort survived at higher concentrations of phosphine (0.06 to 1.0 mg L$^{-1}$) than expected (Figure 2). The calculated degree of dominance of the pooled $F_1$ at LC$_{99.9}$ was 0.39, indicating, a variation in expression of resistance in $F_1$, possibly contributed by additional resistance factors present in the S-strain or Strong-R at low frequency.

3.2.2 Number of genes conferring strong resistance

The observed $F_2$ curve exhibited a small point of inflection at 75% mortality at 0.08 to 0.1 mg L$^{-1}$ and a significant plateauing response at 94% mortality at 0.3 to 2.0 mg L$^{-1}$ (Figure 2A). The expected response for monogenic inheritance would show a distinct plateau at 75% mortality (Figure 2A). Therefore we can formally reject the null hypothesis of monogenic inheritance. The response lines more closely resembled those expected from resistance controlled by two completely
recessive genes, where the F₂ population consists of four distinct phenotypes, in the proportion 9:3:3:1. In this model of inheritance, three distinct plateaus are expected at approximately at 56%, 75% and 94% mortality. The first plateau occurs once all of the homozygous susceptible insects have died (9/16), the second, once insects homozygous resistant for gene_1 alone have also died (12/16). The third plateau is reached once insects homozygous resistant for gene_2 alone have died (15/16). Insects that are homozygous resistant for both gene_1 and gene_2 (1/16) do not begin to die until they are exposed to more than 0.6 mg L⁻¹. Lack of a plateau at 56% mortality in the observed F₂ response (Figure 2A) may be because insect heterozygous for gene_1 cannot be distinguished from susceptible insects at these concentrations. Although, the overall experimental data were insufficient to allow a firm conclusion, they were consistent with an inheritance pattern of two major incompletely recessive genes with the possible involvement of other factors.

Visual examination of the observed F₁-BC curve, indicated two plateaus at ~25 and ~75% mortality levels, at concentrations of 0.08 to 0.1 mg L⁻¹ and 0.8 to 5.0 mg L⁻¹, respectively, instead of a major plateau at 50% (Figure 2B) as would be expected from the single gene model. Resistance controlled by two recessive genes would be expected to produce three significant plateaus in the response line of F₁-BC at 25%, 50% and 75% mortality, resulting from the segregation of four different genotypes (25% heterozygous for both genes, 25% each homozygous resistant for either gene_1 or gene_2 but not both, and 25% homozygous resistant for both genes. However, the curve did not exhibit a plateau at 50% mortality, indicating that the phenotypic response of the two individual homozygote genotypes were similar.

The chi-square analysis of the observed responses of the F₂ and F₁-BC at each concentration showed significant deviations at high concentrations, 0.6 to 4.0 mg L⁻¹ for F₂ (χ² = 4.58, df = 1, P = 0.032) and at both lower, 0.06 and 0.08 mg L⁻¹ and higher concentrations, 0.6 to 3.0 mg L⁻¹ for F₁-BC (χ² = 16.33, df = 1, P < 0.001) curves (Table S3 and S4). The overall chi-square values of the F₂ (χ² = 25.2, df = 14, P = 0.014) and F₁-BC (χ² = 76.4, df = 14, P < 0.001) also support rejection of the null hypothesis of single gene inheritance. The analysis of phenotypic variance estimated the number of
freely segregating factors ($nE$) to be greater than 1. This is consistent with the results of the chi-square analysis of monogenic inheritance, and confirms the likely involvement of two major genes in the expression of resistance in Strong-R (Table 2).

3.3 Interactions between weak and strong resistance genes (Weak-R x Strong-R)

3.3.1 Resistance levels, maternal effects and degree of dominance

Mortality testing showed that Strong-R was 80-fold more resistant to phosphine than Weak-R (Table 1). There was a significant overlap between the dose response curves of the reciprocal $F_1$ hybrids at almost all concentrations and at the $LC_{50}$ (0.17-0.24 and 0.20-0.36), indicating that there was no significant difference in the responses of the reciprocal hybrids. Relative potency analysis of the reciprocal $F_1$ hybrids resulted in 95% confidence interval of 0.54-1.01, which included the value 1.0, confirming the absence of maternal factors contributing to the trait. Thus, the response data of $F_1$ reciprocal crosses were pooled for further analyses and interpretation. Comparison of degree of dominance estimated at the $LC_{50}$ (-0.14) and $LC_{99.9}$ (0.78) from the pooled $F_1$ mortality curve confirmed a change in expression of dominance in heterozygotes in response to the increased phosphine concentrations, as was also observed with $F_1$ hybrids of other crosses, S-strain x Weak-R and S-strain x Strong-R (Figures 1-3). Together, these results support the hypothesis that both Weak-R and Strong-R may have (possibly dominant) resistant factors at low frequency additional to the major incompletely recessive factors.

3.3.2 Number of genes shared between weak and strong resistance phenotypes

The observed $F_2$ response curve exhibited a significant plateau at ~75% mortality, at 0.3 to 1.0 mg L$^{-1}$, conforming to the pattern expected for monogenic inheritance (Figure 3A). These results were reflected in the modified chi-square analysis for individual concentrations as well as in the overall deviation in the response of the observed $F_2$ ($\chi^2 = 9.6$, df = 14, P = 0.65) in comparison to the expected $F_2$ (Table S5).

The $F_1$-BC response curve showed a significant plateau at ~50% mortality, at phosphine concentrations from 0.3 to 1.0 mg L$^{-1}$, and followed the pattern expected for monogenic inheritance
at all the concentrations, except at 0.15 and 0.2 mg L$^{-1}$ (Figure 3B). At these two doses, the observed mortality responses were lower ($P = 0.021$ and 0.014) than the theoretical expectations, suggesting the possible involvement of minor genes, in addition to a major gene in the response of the F$_2$-BC. However, the observed differences were not reflected in the overall deviation response of the observed F$_2$-BC ($\chi^2 = 18.3$, df = 14, $P = 0.1$) curves (Table S6), thus null hypothesis of monogenic inheritance was accepted on this basis.

The comparison of differences in the phenotypic and genotypic variances between Weak-R and Strong-R, and their progeny, estimated the number of freely segregating factors ($nE$) equal to 0.94, that is, one major factor. This was consistent with evidence from the response lines and chi-square analysis and therefore the null hypothesis of monogenic inheritance was accepted.

Our results confirm the existence of a unique factor in Strong-R, which accounts for 80× resistance (relative to the Weak-R), in addition to gene(s) present in Weak-R. It is evident that the single gene expressed in Strong-R (80×) interacts synergistically with the weak resistance gene(s) (6.3×) conferring a very high level of resistance, up to 505×.

### 3.4 Expression of dominant factors in heterozygotes

Comparison of mortality responses of all the pooled F$_1$ hybrids, produced from both single pair (SIC) and multiply mated intercrossing (MIC) of the parentals (Table S7), S-strain x Weak-R, S-strain x Strong-R and Weak-R x Strong-R, indicated an increase in the proportion of survivors, ~15, 25 and 30%, respectively, at high concentrations, confirming the existence of dominant resistance factor at low frequency in F$_1$ hybrids in addition to the major recessive genes. The responses of F$_1$ hybrids obtained from single pair and multiply mated intercrossing methods were compared to each other using relative potency analysis. The results showed that the response of F$_1$ hybrids remained the same, irrespective of the crossing methods. These results rejected possible background genetic errors associated with sexing and multiple mating within the parental cohorts, and confirm that the observed dominance in F$_1$ hybrids varied with concentration (Table 1 and S7).
3.4.1 Effect of exposure period on the expression of dominant factors in heterozygotes at high concentrations

Mortality data of parental strains, S-strain, Weak-R and Strong-R and their F₁ hybrids from three multiply mated intercrosses, S-strain x Weak-R, S-strain x Strong-R and Weak-R x Strong-R were compared at three exposure periods, 48, 72 and 144 hours. These results indicated small differences in the overall response of parental strains and F₁ hybrids to phosphine with time, specifically between 48 and 144 hours exposure periods (Table 3 and 4) and suggested that increase of exposure period improved the degree of linearity and homogeneity. With regard to the specific proportion of survivors of F₁ at higher concentrations in each cross, the response was evident in all the tested exposure periods, confirming the expression of dominant heritable factors at low frequency in addition to the major recessive factors for weak and strong resistance phenotypes. Grouped regression analysis on the mortality data and exposure periods showed that within each of the parent strains and F₁ hybrids, the concentration required to achieve 50% mortality (LC₅₀) could be best described by parallel curves (R²=0.993, F (6,17) = P<0.001), indicating that the observed changes in LC₅₀ are proportionate to the amount of increase in the exposure period. Thus neither the resistance nor the dominance factors were influenced by exposure period (Table 5 and Figure 4). The concentration (C) and time (t) relationship analysis yielded a toxicity index (n), value of 1.23, which is >1, suggesting that the effect of exposure period was not more significant than concentration on the expression of resistance factors in these strains/hybrids in this bioassay system.

In addition, the estimated degree of dominance at two mortality reference points, LC₅₀ and LC₉₉.₉, over three different exposure periods, 48, 72 and 144 hours, indicated that the expression of resistance in F₁ hybrids of all the crosses, varied from incomplete recessivity (-1) to complete dominance (+1). The latter occurring at higher concentrations of phosphine (Table 1 and Figures 1-3), confirming that the heterozygotes of weakly and strongly resistant C. ferrugineus have an additional resistance gene (s) expressing a dominant phenotype at low frequency, which provides a survival advantage at very high concentrations, irrespective of the length of the exposure period.
Another possible explanation is that the observed change of dominance level in heterozygotes of *C. ferrugineus* could be due to dose dependent phenotypic plasticity, without involving additional heritable factors. To investigate this hypothesis, heterozygotes surviving at higher concentrations in each cross, S-strain x Weak-R (0.008-0.08 mg L\(^{-1}\)), S-strain x Strong-R (0.035-1.5 mg L\(^{-1}\)) and Weak-R x Strong-R (0.2-3.0 mg L\(^{-1}\)) were inbred and their progeny (similar to the generation F\(_3\)) mortality responses analysed. If dominance is plastic in heterozygotes, then the response of progeny of selected F\(_1\) should resemble the response of F\(_2\). However, the results in each cross indicated a significant shift in response of the selected F\(_1\) progeny lines towards resistant parents (Figure S1-S3) indicating an increase in the level of resistance with selection and therefore that the observed change in the expression of resistance in heterozygotes is due to heritable factors (possibly minor genes from various parts of the genome) rather than phenotypic plasticity.

4 Discussion

Resistance factors of 6.3× and 505× for weak and strong resistances, respectively in *C. ferrugeniuss* resemble those reported for *R. dominica* (23.4×and 600×) [6] and *T. castaneum* (3.2× and 431×) [7]. Analysis of reciprocal F\(_1\) hybrids from the three crosses, S-strain x Weak-R, S-strain x Strong-R and Weak-R x Strong-R, failed to detect any maternal influence, thus the genes in both the weak and strong resistance phenotypes are autosomal, as was also observed with weak and strong resistance in *R. dominica*, *T. castaneum* and *S. oryzae* [6-8]. These results indicate that phosphine resistance is not due to genetic changes within the mitochondrial genome, even though key mitochondrial proteins have been identified as important for phosphine toxicity [28] or resistance [29, 30]. The level of resistance observed with the Strong-R strain in the current study was similar to levels measured in a range of field-derived strains with strong resistance to phosphine [4].

Our analysis of degree of dominance indicated that both weak and strong resistance phenotypes were expressed as incompletely recessive traits. However, survival of a significant proportion of F\(_1\) hybrids (~15-30) in the three crosses at higher concentrations (Figure1-3),
irrespective of the length of exposure period, indicated the presence of other heritable factors (possibly dominant in expression) contributing to the resistance phenotype, in addition to the major incompletely recessive resistance genes. The selected F₁ hybrids from the three crosses also revealed the existence of additional heritable factors, corroborating this hypothesis (Figures S1-S3). On the other hand it is possible that the S-strain carried a factor, possibly a variant of a resistance gene present in Weak-R, that contributed to higher resistance in the F₁s of the S-strain x Weak-R and S-strain x Strong-R crosses. However, probit analysis indicated a homogenous phenotypic response to phosphine in S-strain (Table 1), making this explanation less likely. In addition, survival at higher concentrations was also observed in the F₁ progeny of Weak-R x Strong-R cross (Figure 3A and 3B), an effect that cannot be explained by an S-strain genotype. The most parsimonious explanation, therefore, is that at least one additional dominant resistance factor is present at a low frequency in each of Weak-R and Strong-R. The possibility of an additional dominant factor contributing to phosphine resistance was also observed in R. dominica [6] in heterozygotes from a cross of Weak-R x Strong-R. However, this effect was not observed either in T. castaneum [7] or S. oryzae [8, 9], indicating the existence of differences between species in background genetic factors contributing to the expression of the resistance phenotype. Similarly, higher survival of F₁ (R x S) hybrids than expected in permethrin-resistant tobacco budworm, Heliothis virescens (F.), was attributed to the presence of minor dominant factors in addition to a major recessive factor, regulating the overall expression of resistance phenotype in this pest [31]. The practical significance of our results is that resistant heterozygotes of C. ferrugineus that carry a dominant allele have a greater chance than expected of surviving field fumigations. This may be a factor in the rapid development and selection of high level resistance in this species.

The novel resistance factors observed in C. ferrugineus could be new copies of already described weak or strong resistance genes resulting from gene duplication [32], or multiple mutations within the same gene as has occurred in several field insect pests [33-35], or they could be unique minor factors [31, 36]. These additional factors may contribute to the ability of C.
*ferrugineus* to withstand very high concentrations of phosphine over lengthy exposure periods (1 mg L⁻¹ for 168 h) [4], a rate that would readily kill strongly resistant individuals of other species.

The mortality responses of the F₂ and F₁-BC progeny and the additive phenotypic variance analyses from S-strain x Weak-R and Weak-R x Strong-R crosses provided strong evidence for a single major gene being responsible for each stepwise increase in resistance. It is also clear that full expression of the strong resistance phenotype requires that individuals are homozygous for each of these major, recessive resistance factors. The results from the S-strain x Strong-R cross were broadly consistent with this interpretation. However, consistent with the results from the analysis of the F₁ responses, the results from the F₂ and F₁-BC progeny indicate the possible contribution of other minor factors to the resistance trait. As with other phosphine resistant pest species, the very high level of resistance exhibited by the Strong-R (505×) appears to be the result of strong synergistic interactions between two major genes. These results are consistent with inheritance of weak and strong resistance to phosphine in *R. dominica, T. castaneum* and *S. oryzae*, where the weak resistance phenotype was predominantly controlled by a single major gene, *rph1*, and strong resistance was governed by the synergistic interaction of two major genes, *rph1* and *rph2* [6-8, 37].

Based on the overall genetic analyses, we propose that repeated fumigation of commercial grain stores under conditions designed to control other pest insect species, rapidly selected strongly resistant heterozygotes of *C. ferrugineus* that could then breed to produce strongly resistant homozygotes. More research on this aspect is needed to develop resistance management strategies [38] that can effectively eliminate resistant heterozygotes in the field.

5 Conclusion

While it is not possible to control strongly resistant *C. ferrugineus* using current registered phosphine dosages [39], the similarity in the resistance genetics across the four key grain insect pests, *R. dominica, T. castaneum, S. oryzae* and *C. ferrugineus*, indicates that a highly conserved genetic response to phosphine selection exists in these insects. In this context, a suitable alternative
fumigant with a different mode of action can effectively be used to curtail the selection of resistant individuals of *C. ferrugineus* and other grain insect species [40]. In addition, the results of this work will assist with identification of the *rph1* gene, which may provide new control opportunities to manage resistance to phosphine in grain insect pests. The similarity to the resistance in other species suggests that it will be straightforward to develop PCR-based rapid diagnostics [41] to support management of resistance to phosphine in *C. ferrugineus*.

**Acknowledgements**

The authors thank Dr Greg Daglish for his valuable comments on the manuscript and Hervoika Pavic, Virgine Tenshia Singarayan and Linda Bond for technical assistance throughout this study. We wish to acknowledge the support of Australia-India Grand Challenge Research Fund (GCF010006), jointly supported by Department of Industry and Science (DIS), Australia (http://www.industry.gov.au) and Department of Science and Technology (DST), India (http://www.dst.gov.in) for their support of this research.

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[27] R. Lande, The minimum number of genes contributing to quantititative variation between and within populations, Genetics, 99 (1981) 541-553.


Figure captions

**Figure 1**: Probit mortality response of S, Weak-R, F₁, F₂ (A) and F₁-BC progeny (B) of the cross S-strain x Weak-R (*Cryptolestes ferrugineus*) to phosphine over 72 hour exposure period at 25°C and 55% RH

**Figure 2**: Probit mortality response of S-strain, Strong-R and F₁, F₂ (A) and F₁-BC progeny (B) of the cross S-strain x Strong-R (*Cryptolestes ferrugineus*) to phosphine over 72 hour exposure period at 25°C and 55% RH

**Figure 3**: Probit mortality response of Weak-R, Strong-R and F₁, F₂ (A) and F₁-BC progeny (B) of the cross Weak-R x Strong-R (*Cryptolestes ferrugineus*) to phosphine over 72 hour exposure period at 25°C and 55% RH

**Figure 4**: The graph illustrating parallel type relationship between phosphine concentrations and exposure periods on the mortality of susceptible and phosphine-resistant strains and their F₁ hybrids of *Cryptolestes ferrugineus* obtained from three multiply mated inter crosses.
Fig. 1A
Fig. 2A
Fig. 2B
Fig. 3A
Fig. 3B
Fig. 4

Exposure period (hours)

Phosphine (mg L$^{-1}$)

- S-strain
- $F_1$ (S x Weak-R)
- Weak-R
- $F_1$ (S x Strong-R)
- $F_1$ (Weak-R x Strong-R)
- Strong-R
Table 1. Probit analysis of the response to phosphine of susceptible and phosphine-resistant strains of *Cryptolestes ferrugineus* and their reciprocal F\(_1\) progenies, obtained from single pair inter-crosses (SIC).

<table>
<thead>
<tr>
<th>Strain/Hybrid</th>
<th>n(^a)</th>
<th>Slope ± SE (mg L(^{-1}))</th>
<th>LC(_{50}) (95% FL)</th>
<th>LC(_{99})</th>
<th>HF(_b) Modifie d (\chi^2) df</th>
<th>P Value (CI(_d))</th>
<th>RR(^c)</th>
<th>DD(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-strain (QCF31)</td>
<td>316</td>
<td>6.8 ± 0.056 (0.005</td>
<td>0.02</td>
<td>6.4</td>
<td>7.9</td>
<td>1</td>
<td>0.16</td>
<td>-</td>
</tr>
<tr>
<td>Weak-R (QCF37)</td>
<td>271</td>
<td>6.09 ± 0.035 (0.033</td>
<td>0.11</td>
<td>2.8</td>
<td>6.2</td>
<td>8</td>
<td>0.62</td>
<td>6.3</td>
</tr>
<tr>
<td>Strong-R (QCF73)</td>
<td>200</td>
<td>4.88 ± 0.037 (2.53</td>
<td>0.36</td>
<td>0.53</td>
<td>15.8*</td>
<td>7</td>
<td>0.02</td>
<td>505</td>
</tr>
<tr>
<td>F(_1) (S-Strain ♀ X Weak-R ♂)</td>
<td>250</td>
<td>2.80 ± 0.008 (0.007-</td>
<td>0.11</td>
<td>2.7</td>
<td>19.5*</td>
<td>1</td>
<td>0.05</td>
<td>1.6</td>
</tr>
<tr>
<td>F(_1) (Weak-R ♀ X S-Strain ♂)</td>
<td>249</td>
<td>2.61 ± 0.009 (0.009-</td>
<td>0.16</td>
<td>3.2</td>
<td>11.6</td>
<td>1</td>
<td>0.4</td>
<td>2.0</td>
</tr>
<tr>
<td>F(_1) Pooled</td>
<td>499</td>
<td>2.67 ± 0.009 (0.008-</td>
<td>0.13</td>
<td>4.8</td>
<td>17.1</td>
<td>1</td>
<td>0.10</td>
<td>1.8</td>
</tr>
<tr>
<td>F(_1) (S-Strain ♀ X Strong- ♂)</td>
<td>249</td>
<td>1.94 ± 0.033 (0.026-</td>
<td>1.3</td>
<td>5.3</td>
<td>24.8**</td>
<td>1</td>
<td>0.01</td>
<td>6.0</td>
</tr>
</tbody>
</table>

\(\chi^2\) df P Value (CI\(_d\)) | LC\(_{50}\) | LC\(_{99}\) | LC\(_{5}\) | LC\(_{95}\) |
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>S-strain (QCF31)</td>
<td>316</td>
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</tr>
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<td>Weak-R (QCF37)</td>
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<td>2.8</td>
</tr>
<tr>
<td>Strong-R (QCF73)</td>
<td>200</td>
<td>4.88 ± 0.037 (2.53</td>
<td>0.36</td>
<td>0.53</td>
</tr>
<tr>
<td>F(_1) (S-Strain ♀ X Weak-R ♂)</td>
<td>250</td>
<td>2.80 ± 0.008 (0.007-</td>
<td>0.11</td>
<td>2.7</td>
</tr>
<tr>
<td>F(_1) (Weak-R ♀ X S-Strain ♂)</td>
<td>249</td>
<td>2.61 ± 0.009 (0.009-</td>
<td>0.16</td>
<td>3.2</td>
</tr>
<tr>
<td>F(_1) Pooled</td>
<td>499</td>
<td>2.67 ± 0.009 (0.008-</td>
<td>0.13</td>
<td>4.8</td>
</tr>
<tr>
<td>F(_1) (S-Strain ♀ X Strong- ♂)</td>
<td>249</td>
<td>1.94 ± 0.033 (0.026-</td>
<td>1.3</td>
<td>5.3</td>
</tr>
</tbody>
</table>

* SIC: Single pair inter-crosses | \(\chi^2\): Chi-square test | P: Probability | CI\(_d\): Confidence interval | RR: Risk ratio | DD: Decision difference
<p>| | | | | | | |</p>
<table>
<thead>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R♂</td>
<td>8</td>
<td>0.17</td>
<td>0.041</td>
<td></td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>F₁ (Strong-R)</td>
<td>252</td>
<td>1.71</td>
<td>0.033</td>
<td>(0.024-2.1)</td>
<td>7.4</td>
<td>33.4***</td>
</tr>
<tr>
<td>♂ X S-Strain</td>
<td>4</td>
<td>±</td>
<td>0.042</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.17</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>F₁ Pooled</td>
<td>502</td>
<td>1.81</td>
<td>0.033</td>
<td>(0.025-1.7)</td>
<td>11.</td>
<td>31.4**</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>±</td>
<td>0.041</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.16</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>F₁ (Weak-R)</td>
<td>250</td>
<td>2.14</td>
<td>0.20</td>
<td>(0.17-5.6)</td>
<td>3.8</td>
<td>16.2</td>
</tr>
<tr>
<td>♂ X Strong-</td>
<td>1</td>
<td>±</td>
<td>0.24</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>R♂</td>
<td>0.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>F₁ (Strong-R)</td>
<td>250</td>
<td>2.05</td>
<td>0.27</td>
<td>(0.2-8.6)</td>
<td>11.</td>
<td>18.9</td>
</tr>
<tr>
<td>♂ X Weak-</td>
<td>5</td>
<td>±</td>
<td>0.36</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>R♂</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>F₁ Pooled</td>
<td>500</td>
<td>2.07</td>
<td>0.23</td>
<td>(0.19-7.3)</td>
<td>12.</td>
<td>27.9*</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>±</td>
<td>0.29</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.17</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

* Number of insects subjected to phosphine bioassay, excluding control

b Heterogeneity factor (HF) = Regression mean deviance / Total mean deviance (in responding to series of concentrations of phosphine)

c Resistance Ratio (RR) = Resistance Ratio (LC₅₀ of resistant or F₁ Hybrid / LC₅₀ of susceptible/ Weakly resistant strain)

d Confidence Interval (CI) of the resistance ratio, e Degree of Dominance (DD) = (2log LC₅₀ of resistant or F₁ Hybrid / LC₅₀ of susceptible/ Weakly resistant strain) / (Log LC₉₉₉-Log LC₅₀), where f and g represent estimated dominance levels for mortality reference points at LC₅₀ and LC₉₉₉

* Significant (P<0.05); ** Significant (P<0.01); *** Significant (P<0.001)
Table 2 Estimated number of segregating factors ($nE$) conferring resistance to phosphine in *Cryptolestes ferrugineus* using Lande’s method (1981)

<table>
<thead>
<tr>
<th>Strain/Cross</th>
<th>$\sigma^2_{B1}$</th>
<th>$\sigma^2_{B2}$</th>
<th>$\sigma^2_{F1}$</th>
<th>$\sigma^2_{P1}$</th>
<th>$\sigma^2_{P2}$</th>
<th>$\mu_{P1}$</th>
<th>$\mu_{P2}$</th>
<th>$\sigma^2_s$</th>
<th>$nE$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak-R</td>
<td>0.091</td>
<td>0.133</td>
<td>0.102</td>
<td>0.022</td>
<td>0.027</td>
<td>-1.454</td>
<td>-2.251</td>
<td>0.098</td>
<td>0.80</td>
</tr>
<tr>
<td>Strong-R</td>
<td>0.261</td>
<td>0.881</td>
<td>0.344</td>
<td>0.022</td>
<td>0.042</td>
<td>0.452</td>
<td>-2.251</td>
<td>0.766</td>
<td>1.20</td>
</tr>
<tr>
<td>Weak-R x Strong-R</td>
<td>0.164</td>
<td>0.588</td>
<td>0.234</td>
<td>0.027</td>
<td>0.042</td>
<td>0.452</td>
<td>-1.454</td>
<td>0.484</td>
<td>0.94</td>
</tr>
</tbody>
</table>

$nE = (\mu_{P2} - \mu_{P1})^2 / 8 \sigma^2_s \leq n$, where $\mu_{P1}$ and $\mu_{P2}$ were the log$_{10}$ of the LC$_{50}$ values of the resistant and susceptible or weakly resistant strains, respectively.

$\sigma^2_s (\text{Variance}) = \sigma^2_{B1} + \sigma^2_{B2} + \left[ \sigma^2_{F1} + 0.5* (\sigma^2_{P1}) + 0.5* (\sigma^2_{P2}) \right]$, where, $\sigma^2_{B1}$, $\sigma^2_{B2}$, $\sigma^2_{F1}$, $\sigma^2_{P1}$ and $\sigma^2_{P2}$ were the phenotypic variances of the $F_1$S, $F_1$R, $F_1$ hybrid (pooled), homozygous resistant (R) and susceptible (S) insects, respectively.
Table 3 Response of susceptible (S) and phosphine-resistant (Weak-R and Strong-R) strains of Cryptolestes ferrugineus to phosphine at three different exposure periods

<table>
<thead>
<tr>
<th>Strain</th>
<th>Exposure (h)</th>
<th>Slope ± SE</th>
<th>LC\textsubscript{50} (95% FL) (mg l\textsuperscript{-1})</th>
<th>LC\textsubscript{99.9} (mg l\textsuperscript{-1})</th>
<th>HF\textsuperscript{a}</th>
<th>Modified χ\textsuperscript{2}</th>
<th>df</th>
<th>P Value</th>
<th>RR\textsuperscript{b} (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-strain</td>
<td>48</td>
<td>5.7 ± 0.1</td>
<td>0.007 (0.0026-0.00175)</td>
<td>0.02</td>
<td>2.9</td>
<td>4.28</td>
<td>6</td>
<td>0.64</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>6.8 ± 0.1</td>
<td>0.0056 (0.0053-0.0059)</td>
<td>0.02</td>
<td>6.4</td>
<td>7.9</td>
<td>11</td>
<td>0.16</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>144</td>
<td>5.53 ± 1.58</td>
<td>0.003 (0.0015-0.0041)</td>
<td>0.01</td>
<td>17.3</td>
<td>13.4</td>
<td>7</td>
<td>0.06</td>
<td>-</td>
</tr>
<tr>
<td>Weak-R</td>
<td>48</td>
<td>4.50 ± 0.234</td>
<td>0.063 (0.06-0.066)</td>
<td>0.31</td>
<td>1</td>
<td>2.61</td>
<td>6</td>
<td>0.85</td>
<td>9 (2.57-32.76)</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>6.09 ± 0.36</td>
<td>0.093 (0.033-0.037)</td>
<td>0.11</td>
<td>2.8</td>
<td>6.2</td>
<td>8</td>
<td>0.62</td>
<td>6.3 (5.37-7.3)</td>
</tr>
<tr>
<td></td>
<td>144</td>
<td>7.33 ± 0.75</td>
<td>0.037 (0.033-0.042)</td>
<td>0.06</td>
<td>1.01</td>
<td>7.24</td>
<td>6</td>
<td>0.29</td>
<td>7 (3.83-13.67)</td>
</tr>
<tr>
<td>Strong-R</td>
<td>48</td>
<td>5.04 ± 0.85</td>
<td>4.81 (3.71-6.16)</td>
<td>19.8</td>
<td>6.7</td>
<td>5.53</td>
<td>6</td>
<td>0.59</td>
<td>705 (2.54-6.50)</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>4.88 ± 0.53</td>
<td>2.83 (2.53-3.17)</td>
<td>12.2</td>
<td>7.87</td>
<td>6.3</td>
<td>6</td>
<td>0.39</td>
<td>505 (165-1554)</td>
</tr>
<tr>
<td></td>
<td>144</td>
<td>8.38 ± 1.63</td>
<td>1.63 (1.5-1.8)</td>
<td>3.81</td>
<td>2.35</td>
<td>5.81</td>
<td>7</td>
<td>0.562</td>
<td>539 (37.89-7660)</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Heterogeneity factor (HF) = Regression mean deviance / Total mean deviance (in responding to series of concentrations of phosphine)

\textsuperscript{b} Resistance Ratio (RR) = Resistance Ratio (LC\textsubscript{50} of resistant / LC\textsubscript{50} of susceptible)
Confidence Interval (CI) of the resistance ratio

* Significant ($P<0.05$); ** Significant ($P<0.01$); *** Significant ($P<0.001$)
Table 4 Probit mortality response of $F_1$ progeny (pooled), obtained from multiply mated inter crosses (MIC) between strains of *Cryptolestes ferrugineus* to phosphine at three different exposure periods

<table>
<thead>
<tr>
<th>Cross</th>
<th>Exposure (h)</th>
<th>Slope ± SE</th>
<th>$\text{LC}_{50}$ (mg l$^{-1}$)</th>
<th>$\text{LC}_{99.9}$ (mg l$^{-1}$)</th>
<th>$\text{HF}^a$</th>
<th>$\text{DD}^b$</th>
<th>Modified $\chi^2$</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_1$ Pooled (S-Strain X Weak-R)</td>
<td>48</td>
<td>6.53 ± 0.0066</td>
<td>0.02</td>
<td>11.8</td>
<td>-</td>
<td>-1.00</td>
<td>84.8***</td>
<td>6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>3.13 ± 0.0083</td>
<td>0.08</td>
<td>13.6</td>
<td>-</td>
<td>0.62</td>
<td>10.3</td>
<td>8</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>144</td>
<td>3.17 ± 0.0066</td>
<td>0.06</td>
<td>4.2</td>
<td>-</td>
<td>1.00</td>
<td>24.9**</td>
<td>10</td>
<td>0.005</td>
</tr>
<tr>
<td>$F_1$ Pooled (S-Strain X Strong-R)</td>
<td>48</td>
<td>2.04 ± 0.036</td>
<td>1.18</td>
<td>4.5</td>
<td>-</td>
<td>0.16</td>
<td>5.09</td>
<td>6</td>
<td>0.532</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>1.7 ± 0.028</td>
<td>1.80</td>
<td>23.2</td>
<td>-</td>
<td>0.40</td>
<td>6.9</td>
<td>8</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>144</td>
<td>1.66 ± 0.022</td>
<td>1.60</td>
<td>6.7</td>
<td>-</td>
<td>0.70</td>
<td>25.9*</td>
<td>9</td>
<td>0.002</td>
</tr>
<tr>
<td>$F_1$ Pooled (Weak-R X Strong-R)</td>
<td>48</td>
<td>1.70 ± 0.42</td>
<td>27.8</td>
<td>5.4</td>
<td>-</td>
<td>1.00</td>
<td>4.97</td>
<td>6</td>
<td>0.547</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>1.42 ± 0.23</td>
<td>34.6</td>
<td>13.4</td>
<td>-</td>
<td>1.00</td>
<td>7.4</td>
<td>9</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>0.33</td>
<td>1.82</td>
<td>0.12</td>
<td>5.8</td>
<td>4.1</td>
<td>-</td>
<td>0.41</td>
<td>9.1</td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>± (0.095-0.14)</td>
<td>0.13</td>
<td>0.14</td>
<td>0.38</td>
<td>0.41</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ \text{Heterogeneity factor (HF)} = \frac{\text{Regression mean deviance}}{\text{Total mean deviance}} \text{ (in responding to series of concentrations of phosphine)} \]

\[ \text{Degree of Dominance (DD)} = \frac{(2 \log \text{LC}_{50} - \log \text{LC}_c - \log \text{LC}_d)}{(\log \text{LC}_c - \log \text{LC}_d)}, \] where \( c \) and \( d \) represent estimated dominance levels for mortality reference points at \( \text{LC}_{50} \) and \( \text{LC}_{99.9} \)

* Significant \((P<0.05)\); ** Significant \((P<0.01)\); *** Significant \((P<0.001)\)
Table 5 Relationship between phosphine concentration (C) exposure period (T) on mortality of strains of Cryptolestes ferrugineus and their F₁ hybrids at LC₅₀

<table>
<thead>
<tr>
<th>Parentals/Hybrids</th>
<th>CXT relationship</th>
<th>Toxicity index (n) †</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-strain</td>
<td>LC₅₀ = 0.0965t⁻⁰.⁶⁸₁ or C⁻¹.⁴⁶⁸ᵗ = 0.032</td>
<td>1.468</td>
</tr>
<tr>
<td>Weak-R</td>
<td>LC₅₀ = 0.854t⁻⁰.⁶⁸₁ or C⁻¹.⁴⁶⁸ᵗ = 0.793</td>
<td></td>
</tr>
<tr>
<td>Strong-R</td>
<td>LC₅₀ = 55.335t⁻⁰.⁶⁸₁ or C⁻¹.⁴⁶⁸ᵗ = 362.013</td>
<td></td>
</tr>
<tr>
<td>F₁ (S X Weak-R)</td>
<td>LC₅₀ = 0.1368t⁻⁰.⁶⁸₁ or C⁻¹.⁴⁶⁸ᵗ = 0.0539</td>
<td></td>
</tr>
<tr>
<td>F₁ (SX Strong-R)</td>
<td>LC₅₀ = 0.568t⁻⁰.⁶⁸₁ or C⁻¹.⁴⁶⁸ᵗ = 0.4358</td>
<td></td>
</tr>
<tr>
<td>F₁ (Weak-R X Strong-R)</td>
<td>LC₅₀ = 4.456t⁻⁰.⁶⁸₁ or C⁻¹.⁴⁶⁸ᵗ = 8.967</td>
<td></td>
</tr>
</tbody>
</table>

R² = 0.993, F(6,17) = P < 0.001

† the value of n estimated by inverting the time factor (=-0.681) and indicates the relative contribution of concentration and exposure period to toxicity, where n <1, refers that exposure period is an important variable than concentration.
Graphical Abstract

Inheritance of phosphine resistance in rusty grain beetle, *Cryptolestes ferrugineus* (Stephens)

F₁-backcross progeny response

S x Weak-R

S x Strong-R

Weak-R x Strong-R

*Weak R*: One major gene ~6.3x

*Strong R*: two major genes ~50x

Weak –R and Strong R

- differ in one major gene
- share weak resistance gene
Research Highlights

- Inheritance of weak (Weak-R) and strong (Strong-R) resistance phenotypes was investigated
- A single major, incompletely recessive, autosomal gene confers weak resistance
- Two major incompletely recessive, autosomal genes, one allelic with Weak-R gene, confer Strong-R
- Expression of F₁ hybrids suggested presence of additional dominant factor at low frequency
- Expression of Strong-R requires interaction of both weak and strong resistance genes