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# The molecular genetics of phosphine resistance in the lesser grain borer and implications for management

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**Abstract.** We analysed high-level resistance to phosphine in *Rhyzopertha dominica* (F.), the lesser grain borer (LGB), using both classical and advanced molecular genetic techniques. We found that two major genes primarily control resistance. The first gene has been present in LGB populations for many years and is responsible for 'weak' resistance, i.e. resistance that can be controlled with a properly applied fumigation. Insects with a stronger level of resistance were detected in 1997. These insects have the gene responsible for 'weak' resistance plus another gene. The second gene has little effect on its own but strongly enhances the effect of the first gene. We also found that neither gene has any measurable fitness effect over 16 generations. Our analyses show that, to combat resistance, fumigations must be fully effective. If eradication is not complete, resistance will rapidly regenerate in an insect population.

## Two genes confer high-level phosphine resistance

In 1971, the Queensland Department of Primary Industries collected a line (QRD14) of the lesser grain borer, *Rhyzopertha dominica* (F.), that displayed an extremely low tolerance to phosphine. As low-level resistance began to become widespread, a second line (QRD369) was collected in 1991. With the discovery of high-level resistance in Australia in 1997, a third line (QRD569) was collected (Collins et al. 2002). Classical genetic analysis revealed that at least two genes were responsible for high-level resistance. We initiated a DNA fingerprinting procedure (Figure 1a) (Schlipalius et al. 2001) that allowed the inheritance of chromosomal fragments to be determined directly (Figure 1b). This procedure identified nine genetic linkage groups equivalent to the nine chromosomes found in *R. dominica*. Two genes were found to be responsible for the high-level resistance trait, including one gene in the weakly resistant strain collected in 1991 and that gene plus an additional gene in the strain collected in 1997 (Figure 1c) (Schlipalius et al. 2002). The DNA marker linked to the 1997 gene has proven to be a 99.99% accurate predictor of resistance in laboratory studies. The DNA marker linked to the 1991 gene is still under study, but is at least 99.9% accurate. A Grains Research and Development Corporation (GRDC)-funded project to develop these markers for rapid testing of resistance in field-collected samples is in progress.

## Overview of resistance genetics

The 'resistance genes' referred to in this paper are actually found in two forms: a sensitive form and a resistant form (allele). The phosphine-sensitive allele of the gene existed before the mutation that resulted in the resistance allele. In addition, *R. dominica*, like us, are diploid, i.e. they contain two copies of every gene, one inherited from their mother and the other from their father. Thus, fully sensitive beetles like QRD14, collected in 1971, can be represented as 1991 (S/S): 1997 (S/S) to indicate that they have none of the resistant forms of the genes subsequently identified. Similarly, the weakly resistant beetles collected in 1991 can be represented as 1991 (R/R): 1997 (S/S) to indicate that they have no resistance allele of the 1997 gene. The highly resistant beetles collected in Millmerran in 1997 are 1991 (R/R): 1997 (R/R). The strains will subsequently

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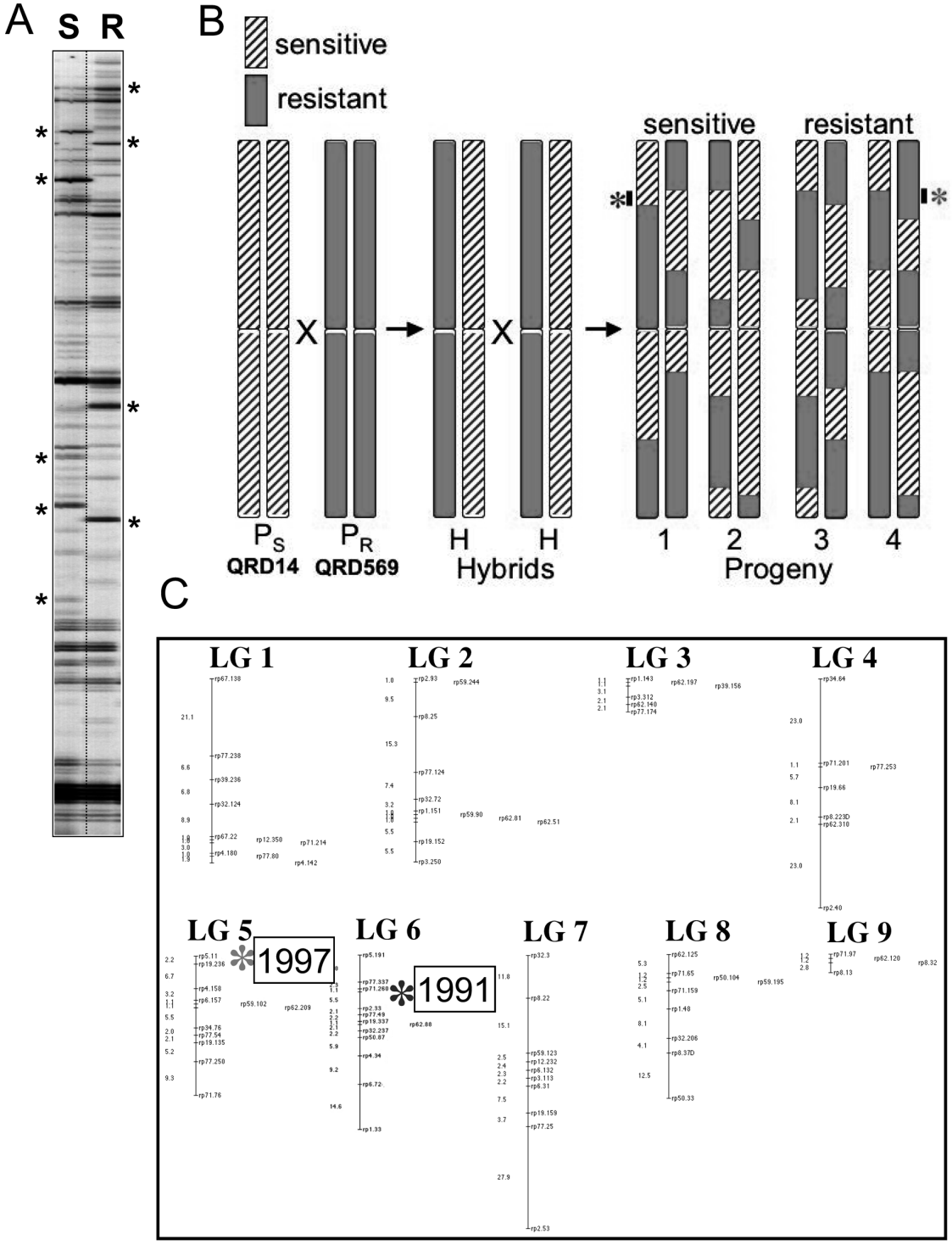


**Figure 1**. Identification of two resistance genes responsible for high-level resistance to phosphine in *Rhyzopertha dominica* strain QRD569. Representative DNA fingerprints (panel A) from sensitive (S) and resistant (R) beetles are used for the genetic mapping of the resistance genes. The diagram in panel B shows the mapping scheme for a single chromosome pair as representative of the full chromosomal complement of nine pairs. The sensitive ($P_S$) and resistant ($P_R$) parental strains were crossed to produce $F_1$ hybrids. The inheritance of every chromosomal segment in each of 92 $F_2$ progeny determined as is represented by the $F_2$ progeny which contain solid (resistant) or hatched (sensitive) chromosomal segments. Resistance testing of the progeny allowed them to be separated according to phosphine resistance or sensitivity. The asterisks (*) indicate specific chromosomal segments inherited from either the sensitive or resistant parent that are absolutely co-inherited with the sensitivity or resistance trait, respectively. Panel C shows the summary genetic map that was produced from the 92 progeny. Indicated on the map are the sites of the two genes responsible for high-level phosphine resistance.

be referred to as either sensitive, weakly resistant or highly resistant, or S/S:S/S, R/R:S/S, R/R:R/R.

## Gene interactions and recurring resistance problems

The availability of DNA markers allowed us to rapidly and accurately determine how the two genes and their allelic forms interact with each other to provide the observed levels of resistance. We were particularly interested in mimicking the situation in the field, in which the resistance allele from 1991 is already widespread and therefore likely to be abundant in any population of beetles (at least in the eastern half of the country). The 1997 resistance gene has been resident in the Darling Downs region of south-eastern Queensland for the past 6 years but has been subjected to strict control measures to limit its dispersal. As such, the background population should consist primarily of the R/R:S/S genotype (allele combination) present in the region before 1997. Control failures are occasionally found to contain highly resistant beetles, presumably of the R/R:R/R genotype. Even with extreme efforts to eradicate highly resistant beetles at the site of a control failure, any survivors would mate with the still most abundant genotype existing in the field, i.e. R/R:S/S. The offspring of such beetles would be of an intermediate type, R/R:R/S. Such intermediate beetles exhibit significantly enhanced resistance compared with weakly resistant insects, R/R:S/S (Table 1). Fumigations that do not completely eliminate this intermediate class of beetle following an initial resistance outbreak are likely to result in interbreeding of R/R:R/S individuals. This is a dangerous situation in which highly resistant R/R:R/R offspring are extremely likely to result.

## Persistence of resistance genes in the field

Given the hazard associated with even low levels of the 1997 resistance allele in *R. dominica* populations in grain storages, we undertook to estimate the persistence of this gene in the field. To mimic the situation that might occur in the field, we established a genetically heterogeneous population of beetles, according to the cross shown in Figure 1b. This population contained every possible combination of resistance genotypes (three of which are shown in Table 1) with respect to the 1991 and 1997 genes. This population was maintained for 16 discrete generations by serial transfer of at least 100 adult beetles from each generation into fresh jars of wheat at 30°C, 55% relative humidity. The frequency of the resistance form of the 1997 gene in the population was found not to vary over the 16 generations of the experiment ($\chi^2$ = 0.408, $P > 0.523$, df = 1), suggesting that the resistance allele does not affect the health of the beetles in the absence of phosphine. Therefore, the allele will probably persist indefinitely in the field in the absence of eradication measures.

## Implications

DNA markers have been located that identify two resistance genes in highly resistant *R. dominica* found in Millmerran, Queensland. We have determined that a single genetic change led to the development of high-level resistance from the previously weakly resistant beetle population. The ease of this transition is consistent with the documentation of multiple, widely scattered, independent outbreaks of high-level resistance in eastern Australia. All situations that result in the generation of large insect populations, such as poor hygiene around storage sites or ineffective fumigation procedures, are likely to facilitate the development of new outbreaks of high-level resistance. In particular, repeated fumigations, especially if they are only partially effective, are likely to select for genotypes of intermediate resistance when they occur (i.e. R/R:R/S) resulting in the generation of highly resistant progeny (i.e. R/R:R/R). Re-selection of high-level resistance is particularly likely if a control failure due to genetically resistant beetles does not adequately control individuals of intermediate resistance (beetles homozygous for the weak resistance gene and heterozygous for the strong resistance gene, i.e. R/R:R/S).

Our analysis, together with that of Greg Daglish (unpublished data), indicates that the resistance alleles that we have studied will be completely persistent in the field, even without the selective pressure of phosphine fumigation. Thus, rotation of control chemicals will not,

**Table 1.** Phosphine resistance thresholds of *Rhyzopertha dominica* of weakly resistant (R/R:S/S), intermediate (R/R:R/S) and highly resistant (R/R:R/R) genotypes. The fraction of survivors of each genotype is shown as a percentage for each of the applied phosphine dosages. Treatments were for 48 hours at 25°C.

| Genotype | | Dose of phosphine (mg/L) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1991 gene | 1997 gene | 0.0 | 0.05 | 0.1 | 0.2 | 0.3 | 0.4 | 1.0 |
| R/R | S/S | 20.0 | 16.6 | 0 | 0 | 0 | 0 | 0 |
| R/R | R/S | 66.7 | 66.7 | 58.9 | 0 | 0 | 0 | 0 |
| R/R | R/R | 13.4 | 16.6 | 41.1 | 100 | 100 | 100 | 100 |

of itself, lead to a reduction in the frequency of phosphine resistance alleles in the field.

Our molecular diagnostic procedure is extremely accurate at predicting phosphine resistance levels in laboratory studies of *R. dominica*. Validation of the test is currently under way. The speed and accuracy of the test should make it useful in general phosphine resistance monitoring as well as specific monitoring of farms and central storages that have been the subject of control failures.

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