



Significant population structure in Australian *Cryptolestes ferrugineus* and interpreting the potential spread of phosphine resistance

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ARTICLE INFO

Article history:

Received 20 February 2018

Received in revised form

3 May 2018

Accepted 3 May 2018

Available online 22 May 2018

Keywords:

Dispersal

Phosphine resistance

Microsatellite markers

Grain beetle

Phylogeography

ABSTRACT

An analysis of the population genetics of *Cryptolestes ferrugineus*, across all major regions in Australia where grain is grown and stored in bulk, provides an initial step in investigating the movement of these insects and implications for the spread of phosphine resistance. Microsatellite data revealed two levels of genetic structure. Across Australia, two clusters were detected, one in eastern Australia (Queensland, New South Wales, Victoria and Tasmania) and Western Australia (Cluster 1) and one in South Australia (Cluster 2). Intermediate between them, in eastern South Australia, are a couple of populations that are equally admixed for the two clusters. Populations in Western Australia and western SA belong to different clusters suggesting that the extensive and arid Nullarbor Plain restricts the natural dispersal of these beetles. Isolation-by-distance and the detection of clustering among local populations suggests there is considerable gene flow at a local scale in *C. ferrugineus*, and we infer this is by flight. Limited natural gene flow between eastern and western Australia might, in part, explain why extremely high resistance has not been detected in Western Australia.

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1. Introduction

The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), is a cosmopolitan pest of bulk-stored grain. Its pest status has increased significantly through the recent development of extremely high resistance levels to the fumigant phosphine (resistance factor >1000) (Nayak et al., 2013). A major issue in dealing with phosphine resistance in this species is understanding the movement of the insects relative to the spatio-temporal distribution of bulk-stored grain and any other breeding habitats. With respect to the latter, *C. ferrugineus* has been recorded under bark on occasions (Champion, 1902; Donisthorpe, 1946) and local, seasonal movement up to a kilometre away from grain storage has been reported (Holloway et al., 2018) but little else is known about its life away from bulk grain storage.

Interpreting the movement of such tiny insects requires a diversity of approaches, but the genetic analysis of population structure across space does allow inferences to be drawn (e.g., Ridley et al., 2011, 2016) and these, in turn, suggest hypotheses about their movement that can be tested by other means (e.g., Ahmad et al., 2013; Rafter et al., 2015). We thus examined the potential, in *C. ferrugineus*, for the spread of phosphine resistance genes by estimating population structure at the continent level, to infer gene flow within and between Australian grain growing regions. We previously reported a deep (~8%) divergence between two mitochondrial DNA (mtDNA) lineages that co-occur in this species in Australia (and elsewhere), and demonstrated that, despite the depth of this divergence, beetles from the two lineages mate at random with one another (Toon et al., 2016). Thus, insects from both lineages comprise a single gene pool and species.

Of more than 40 described species of *Cryptolestes*, five are considered to be stored grain pests world-wide (Hagstrum et al., 2013), and three of these have been recorded in Australia, i.e. *C. ferrugineus*, *C. pusillus* (Schonherr) and *C. pusilloides* (Steel and

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Howe) (Sinclair and Bengston, 1980). *Cryptolestes ferrugineus* infests grain stores in all grain growing regions in Australia (see Fig. 1) and was, until recently, effectively managed with phosphine fumigation, with only weak resistance to the fumigant detected (Nayak et al., 2010). Extremely high phosphine resistance has since evolved in some populations in eastern Australia (Nayak et al., 2013). Weak phosphine resistance is considered to be associated with homozygous resistance alleles at a single locus, and high resistance with the simultaneous presence of homozygous resistance alleles at a second locus (Jagadeesan et al., 2016). Weak resistance to the fumigant phosphine has been detected in all grain growing regions but extremely high resistance has not yet been detected in the Western Australian or Tasmanian grain growing regions.

Flight by *C. ferrugineus* is likely to be influenced by ambient temperature, with little activity noted at less than 20 °C in the laboratory (Cox and Dolder, 1995) and movement away from stored grain detected only when maximum temperatures during the day were above 20 °C (Throne and Cline, 1994; Holloway et al., 2018). At lower temperatures, passive movement could still occur with the transport of bulk grain between storage areas, or with infested residual grain overlooked in machinery and other equipment as it is moved to other, relatively distant, localities (Sinclair and White, 1980). Movement of grain between farms is not commonly

practiced in Australia. Nevertheless, flight between populations that are part of a relatively continuous grain growing belt is likely, because climatic conditions are often favourable (long summers with minima even above 20 °C at times) and distances among on-farm grain storage sites are relatively short. Flight between some of the grain producing areas of Australia is assumed to be restricted; Tasmania is isolated from the mainland by Bass Strait, and the arid Nullarbor Plain separates eastern and western grain growing regions (Fig. 1). Our aim is, therefore, to estimate the potential for the spread of phosphine resistance genes among populations of *C. ferrugineus*. To achieve this, we estimated the genetic structure of *C. ferrugineus* populations within and between Australian grain growing regions using variation at microsatellite markers.

2. Materials and methods

2.1. Study area and sampling

Beetles were sampled in the summer and autumn of 2014 from a number of locations (see below) with bulk stored grain in six states in Australia. For the purposes of this study we grouped samples into three regions, namely western mainland (Western Australia), eastern mainland (South Australia, Victoria, New South Wales and Queensland) and the island state of Tasmania in south eastern

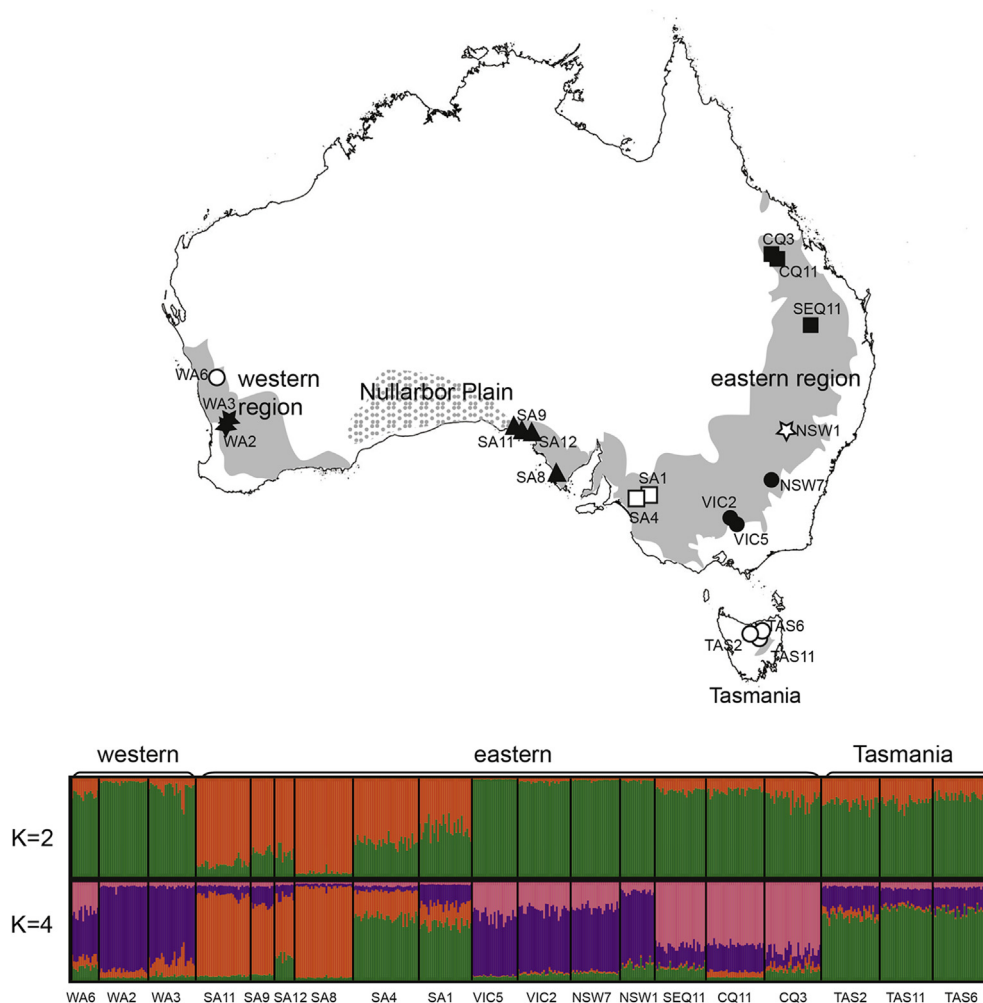


Fig. 1. Grain growing regions of Australia, sites where *Cryptolestes ferrugineus* were sampled and cluster analysis results from microsatellite data. Cluster membership for GENELAND analysis is shown as icons on map. Below the map are STRUCTURE plots showing two (top) or four (bottom) clusters. Each vertical bar shows the proportion of admixture for each individual.

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