Flight of *Rhyzopertha dominica* (Coleoptera: Bostrichidae)—a Spatio-Temporal Analysis With Pheromone Trapping and Population Genetics

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Abstract

The flight of the lesser grain borer, *Rhyzopertha dominica* (F.), near grain storages and at distances from them, was investigated to assess the potential of these beetles to infest grain and spread insecticide resistance genes. We caught *R. dominica* in pheromone-baited flight traps (and blank controls) set at storages, in fields away from storages, and in native vegetation across a 12-mo period. A functional set of highly polymorphic microsatellite markers was developed, enabling population genetic analyses on the trapped beetles. Pheromone-baited traps caught just as many *R. dominica* adults at least 1 km from grain storages as were caught adjacent to grain storages. Samples of beetles caught were genetically homogeneous across the study area (over 7,000 km2) in South Queensland, Australia. However, a change in genetic structure was detected at one bulk storage site. Subsequent analysis detected a heterozygous excess, which indicated a population bottleneck. Only a few beetles were caught during the winter months of June and July. To assess the mating status and potential fecundity of dispersing *R. dominica* females, we captured beetles as they left grain storages and quantified offspring production and life span in the laboratory. Nearly all (95%) of these dispersing females had mated and these produced an average of 242 offspring. We demonstrated that *R. dominica* populations in the study area display a high degree of connectivity and this is a result of the active dispersal of mated individuals of high potential fecundity.

Key words: lesser grain borer, microsatellite, dispersal

The ecology of *Rhyzopertha dominica* (F.), the lesser grain borer, has been extensively studied because it is a major pest of stored grain globally, from the tropics to temperate areas (Hagstrum and Subramanyam 2009). The flight of these insects has been well documented (see below), but the mechanistic link between active dispersal and population genetic connectivity has yet to be tested. *Rhyzopertha dominica* is resistant to a range of chemical treatments in many countries (Lorini and Galley 1999, Collins et al. 2002, Lorini et al. 2007, Opit et al. 2012, Daglish et al. 2013), and active dispersal of this pest not only serves as a mechanism for the infestation of newly harvested grain but also has the potential to disperse resistance genes.

*Rhyzopertha dominica* may reproduce outside of grain storage systems for it has been shown to complete development on oak seeds in the laboratory (Wright et al. 1990, Edde and Philips 2006, Jia et al. 2008), and small numbers have been reared from field-collected seeds (Jia et al. 2008). It has been suggested that in certain parts of the USA, *R. dominica* overwinters in wooded areas and reinfests grain from these refugia (Mahroof and Philips 2007), but supportive data are sparse. The mating status of females dispersing from infestations in the field is not known, so the infestation potential of single females remains to be determined. We do know, however, that female *R. dominica* mate readily under laboratory conditions and can store sperm for at least 4 wk (Ridley et al. 2012).

Visual tracking of *R. dominica* is difficult because of their small size, so traps are invariably used to investigate aspects of their ecology. When food grain or other resources are used in traps, catches have been typically low (e.g. Sinclair and Haddrell 1985, Ahmad et al. 2013). Pheromone lures for *R. dominica* were developed after the isolation and identification of the pheromones by Williams et al.
(1981). By contrast to food-baited traps, pheromone-baited traps capture much larger numbers of beetles and, in North America, trap catches may even be significantly higher in wooded areas than near bulk storage (e.g. Mahroof et al. 2010). Rhyzopertha dominica is reported to disperse widely and beetles have been caught close to grain storage (Dowdy and McGaughey 1994, Toews et al. 2006), and also away from storage in open fields and wooded areas (Vela-Coiffier et al. 1997, Edde et al. 2005, Toews et al. 2006). Flight traps baited with pheromone lures have enabled the seasonal flight activity and geographical distribution of R. dominica to be quantified in Canada (Fields et al. 1993), and seasonal flight activity to be quantified in Kansas and Oklahoma in the USA (Edde et al. 2006, Toews et al. 2006).

Adults of R. dominica are likely moving between habitats, but what that movement means in terms of gene flow and the corresponding movement of insecticide resistance genes has not been determined. We therefore used pheromone traps along with control traps to expand our perspective on active dispersal in R. dominica.

Population genetics analyses on R. dominica dispersal have been reported using allozymes (Guedes et al. 1997) and dominant markers, for example AFLPs (Xuhong et al. 2011). The former generally have a low number of alleles per locus and the latter preclude population genetic analyses that rely on codominant markers. We developed a functional set of highly polymorphic microsatellite markers, using two different next-generation sequencing technologies. These newly developed markers enabled us to assess the effect of movement on the genetic structure of R. dominica across the study area, test for evidence of recent bottlenecks, and infer which individual beetles in samples were likely recent immigrants at each site by means of assignment tests. We could thus combine a comprehensive population genetics analysis with the direct trapping of flying beetles (using pheromones) and then add information on the mating status and potential fecundity of dispersing females to assess the potential consequences of movement of R. dominica adults.

Materials and Methods

Trapping Program

Traps were deployed across an agricultural landscape in a grain-growing district in southern Queensland, about 350 km west of Brisbane on the Darling Downs. Most of the traps were deployed across farmland, on which grain is stored in bulk after harvest and progressively moved off the farm as it is sold. Fig. 1 displays the spatial layout of the traps. The details of the trapping methods have been presented by Ridley et al. (2011b) so just the key elements are reiterated here. The traps were deployed in a split block design, replicated 15 times. Each block was a single farm split into a storage site and a field site, usually along a fence or tree line, at least 1 km from the storage site and any other grain storage. At each such site Lindgren funnel traps (Contech Inc., Victoria, BC, Canada) were suspended from a steel support so that the trap was 1.5 m above the ground. Three traps were set at each site and spaced at least 20 m apart at field sites and as far apart as feasible at storage sites (generally 10–20 m depending on the size of the storage complex). One trap at each site was baited with a R. dominica lure (Trécé Inc., Oklahoma, USA). One was a control with no lure and a third was baited with a lure designed to attract beetles from the genus Tribolium (Trécé Inc.). This paper concerns only R. dominica data, so the Tribolium traps effectively make up another control for R. dominica. (Ridley et al. (2011b) reported the results of the Tribolium trapping).

Statistical analyses were carried out combining the data from the traps baited with Tribolium lure with the data from the blank (no lure) traps and also with these data kept separate. No change in inference was warranted across these two analyses, so the results of the combined data set are presented. Traps were set for 7-d periods at intervals of 4–6 wk from November 2008 to October 2009. Sets of three traps were also located in three native vegetation reserves (two in Kumbarilla State Forest and one in Southbrook National Park), and at two commercial grain depots (see Fig. 1). Insects caught in the traps were sorted and sexed based on their genitalia (Potter 1935) and stored in 100% ethyl alcohol.

Spatio-Temporal Pattern of Trap Catches

We compared the number of beetles caught at farm storages with the number caught in farm field sites using a linear mixed-effects model (Zuur et al. 2009). Beetle abundances were log (n + 1) transformed prior to analyses. Farm was treated as a blocking random effect, with trap type nested within trap location and within sampling time. Trap type, trap location, and sampling time were treated as fixed effects. Data were analysed using R 3.2.4 (R Core Team 2016). Post hoc pairwise comparisons across factors of interest were made using Tukey’s HSD tests.

To investigate the relationship between the geographical distribution of the traps and numbers of beetles caught, a Mantel test (Mantel 1967, Sokal 1979) evaluated whether sites that were closer to each other were also similar in the number of beetles trapped through time. We computed a standardized Mantel statistic ( r) (Sokal and Rohlf 1995) relating a matrix of Relative Sorensen distances (Faith et al. 1987) of sites, based on the numbers of trapped R. dominica beetles over time, to a matrix of geographic distance between sites (Greater Circle Distance computed based on latitude and longitude information collected using a hand-held GPS unit). These analyses were run in PC-Ord 5 (McCune and Mefford 1999).

Sex ratio differences in trap catches were summarized by trap location * trap type, after averaging trap catches across sampling times. Deviations from a sex ratio of 50:50 (males:females) in trap catches were assessed using confidence intervals of percent of trap catch made up of female beetles; deviations were considered significantly different from unity if the confidence interval did not include 50%. Data from the two winter trapping periods, June and July, were removed from the sex ratio analysis because of the low numbers of individuals (<50 in total) caught during those periods. The spearman’s rank correlation coefficient was calculated in GenStat (2013) to assess the relationship between the mean number of beetles caught each month in control traps and lure-baited traps at farm storage and field sites.

Mating Status and Potential Fecundity of Emigrating Females

All beetles that emerged from the opening at the base of two infested silos of wheat during a 2-h period of continuous observation at dusk on a day in mid spring (March 2009) were individually collected and immediately placed alone into diet cups filled with 10 g of wheat (12% moisture content). Beetles were transported to the laboratory where each beetle was (together with the original wheat) transferred into jars containing 10 g of wheat (12% moisture content) and stored at 25 °C and 65% RH. Each week, the cups were checked for dead beetles, and live beetles were transferred to fresh wheat. All beetles were treated as potential females because of the difficulty of conclusively sexing live R. dominica. The study was concluded after 40 wk. All cups of grain were held for 10 wk for offspring to
develop into adults, when they were counted. The originally captured beetles and their offspring were sexed after death based on their genitalia (Potter 1935). A total of 57 R. dominica was collected from one silo (35 % 22.5°) and 13 from the other silo (11 % 2.5°). Data from these silos were pooled because of the small number of beetles collected from the second silo.
Microsatellite Isolation and Development

Microsatellites were isolated and optimized from two separate next-generation sequencing runs (a 1/16 of a 454 run and a 90 bp PE Illumina lane) to ensure a full set of microsatellite loci for analysis. Both sequencing runs were performed on a group of beetles. For the 454 run, 50 individuals were pooled and extracted from strain QRD14 (Collins et al. 2002) using DNEasy columns (Qiagen). For the Illumina run, individuals from both the phosphine-susceptible strain QRD14 and phosphine-resistant individuals from the strain QRD569 (Collins et al. 2002) were sequenced. The Illumina data were assembled with SOAPdenovo (Luo et al. 2012) using k-mers from 17 to 83 to assess the relationship between kmer used and microsatellite length recovered. This analysis was performed because many of the 454 derived markers were monomorphic in initial screening, and loci with higher repeat number are generally more variable. Based on this analysis an optimal assembly using a kmer length of 73 was used for the identification of microsatellites. For both the 454 and Illumina datasets microsatellites were identified and primers designed using the QDD algorithm (Meglécz et al. 2010), which uses Primer3 for primer design (Untergasser et al. 2012). We selected only trinucleotide and tetranucleotide repeats to allow for more accurate binning, and all sequences were searched against the nonredundant nucleotide database at Genbank to ensure that loci were not in protein-coding regions.

The primers selected were synthesised (IDT, Australia) with an M13 tail added to the forward primer (Schuelke 2000) and a 5’-‘pigtail’ added to the reverse primer to ensure consistency in amplicon size (Brownstein et al. 1996). Primers were initially screened against eight individuals from strain QRD14, with microsatellite loci that showed strong amplification and no evidence of nonspecific binding subsequently screened against 32 individuals from QRD14. Fluorescent dye was added during the PCR reaction using the M13 tailing method (Schuelke 2000). Loci were amplified in 12 μl reaction mixtures containing 0.03 units of My Taq (Bioline), 1 × buffer, 0.1 μM of forward primer, 0.2 μM fluorescently labeled M13 primers and 0.2 μM of reverse primer. Amplification conditions were as follows: 10 min initial denaturation (95°C) followed by a two-step protocol with 25 cycles of 95°C for 25 s, annealing of 57°C for 30 s and 72°C for 45 s and 10 cycles of 95°C for 30 s, annealing of 54°C for 30 s and 72°C for 45 s; and final extension of 72°C for 10 min. PCR products were cleaned using one unit each of Exonuclease I and Antarctic Phosphatase (New England Biolabs, Ipswich, USA) and pooled such that only one locus per fluorescent dye was present in each pool. Microsatellite amplicons were then detected on an ABI3730 (Macrogen Inc. Seoul, South Korea). Peaks were confirmed and binned using the microsatellite plugin in Geneious version 8 (Kearse et al. 2012).

The number of alleles (Na) per locus, and the observed (Ho) and expected (He) heterozygosities were calculated in GENALEX 6 (Peakall and Smouse 2012). Null allele frequencies and locus specific global Fis’s were estimated using the expectation maximization algorithm of Dempster et al. (1977) as implemented in FreeNA (Chapuis and Estoup 2007). Hardy–Weinberg exact tests were performed in Genepop v. 4.1 (Rousset 2008).

Microsatellite Genotyping

Fifteen microsatellite loci were genotyped across 19 population samples derived from nine sites within our study area. Samples from the same sites but taken at different times were included to assess temporal changes in genetic structure over one year. These sites represented two depots (where grain is collected from multiple farms), two silo sites (where produced grain is stored on site), two field sites (on farm but away from silos), and two native vegetation sites (away from farms in natural bushland). DNA was extracted using a DIY spin column protocol using 96-well silica filter plates from Epoch life sciences (Missouri City, TX, USA). The full protocol is provided in supplementary material (Supp. Data 1 [online only]). Microsatellites were genotyped and scored as described in the previous section; details of the loci used, dyes, and pooling regime are given in Supp. Table 1 [online only]. Individuals that failed to amplify at more than six of the 15 loci were considered low quality DNA extractions and were discarded. This left 592 individuals in the final dataset.

Microsatellite Analyses, Genetic Structure, Bottleneck Testing, and Immigrant Detection

Summary statistics were derived in the same way as for the laboratory culture QRD14. We then used the Bayesian clustering algorithm implemented in the program Structure 2.2.4 (Pritchard et al. 2000) to assess genetic structure across all samples. All analyses were conducted using the admixture model with allele frequencies correlated, and sampling location was not used as a prior. We conducted 10 replicate analyses for each value of K (the number of population clusters specified) from K = 1 to K = 10 using different starting seeds, each analysis comprised 100,000 burn-in iterations followed by 1 million iterations. The most likely value of K was inferred post hoc using the deltaK method (Evanno et al. 2005) implemented in Structure Harvester (Earl and von Holdt 2012). The results for K = 2 and K = 3 were then permuted and plotted using CLUMPP (Jakobsson and Rosenberg 2007) and Distruct (Rosenberg 2004) as implemented in CLUMPAK (Kopelman et al. 2015).

We calculated pairwise Fst’s in FreeNA using the uncorrected allele frequencies, and performed G-tests for genotypic differentiation in GenePop, applying Bonferroni correction to the p-values. A principal components analysis (PCA) was performed on these pairwise FST values in GENALEX 6 (Peakall and Smouse 2006). Genetic structure in storage may be fumigated with phosphine to kill pests, especially in grain depots, which can cause localised population bottlenecks. We performed the standard differences test and the Wilcoxon test, implemented in the program Bottleneck (Piry et al. 1999), to detect any recent population bottlenecks by testing for excess heterozygosity. Simulations of mutation drift equilibrium were performed using the Two-Phase mutation model. We also calculated Fs using GenePop v. 4.1 (Rousset 2008).

We performed genetic assignment tests (in GeneClass2 (Piry et al. 2004)), for each of the sampling dates for which four or more populations had been genotyped (Nov 2008, Apr 2009, and Oct 2009). These tests use allele frequency data to assess the probability of an individual belonging to each population sampled. We applied the Bayesian criteria of Rannala and Mountain (1997) to estimate the likelihood of each such individual being a first generation migrant based on the ratio between the likelihood that the sample belongs to the source population and the highest likelihood value for all populations sampled. Probability computation (Monte-Carlo resampling) was performed using the method of Paetkau et al. (2004) with 1,000 individuals and the alpha value set to 0.01.

Results

Trap Catch

The temporal pattern of R. dominica catches in pheromone-baited traps at grain storages and in fields is depicted in Fig. 2. Over
40,000 *R. dominica* adults were caught during the 12-mo study, most of which (94.4%) were caught in *R. dominica* lure-baited traps (Fig. 2). The control traps (no lure) caught 2.8% of the total and the traps baited with *Tribolium* lure caught 2.8% of all the *R. dominica* captured. There was a strong positive correlation between the number of beetles caught in lure baited traps and control traps at storage sites ($r = 0.927, n = 11, P < 0.001$; Figs. 2 and 3). A moderate correlation was detected between the number of beetles caught in lure-baited traps and control traps at the farm field sites ($r = 0.591, n = 11, P = 0.056$). *Rhyzopertha dominica* were caught at all sites including the three native vegetation sites.

Unsurprisingly, significant variation in the numbers of *R. dominica* trapped on farms was attributable to the random effect of site ($F = 8.66; df = 14, 138; P < 0.01$). Sampling time ($F = 34.09; df = 10, 138; P < 0.01$), trap location ($F = 10.93; df = 1, 152; P < 0.01$), and trap type ($F = 3218.17; df = 1, 301; P < 0.01$) were major influences on the number of *R. dominica* trapped, but their effects were not independent of one another (trap type*sampling time: $F = 65.55; df = 10, 301; P < 0.01$; trap type*trap location: $F = 66.29; df = 1, 301; P < 0.01$; trap type*sampling time*trap location: $F = 2.20; df = 10, 301; P = 0.02$). Examination of the three-way interaction showed that, in general, the traps baited with *R. dominica* lure caught significantly more beetles than the two control traps (*Tribolium* lure and no lure). At any given sampling time, the *R. dominica* lure-baited traps caught an equivalent number of beetles at both storage and field sites (Fig. 2). The highest numbers of beetles were trapped in the austral spring and summer (August–March), with trap catches declining in April. Significantly fewer beetles were trapped in June and July (austral winter) relative to the other months (Fig. 2).

Data from traps baited with lures set within the farming landscape (farm and depot traps), when analysed by the Mantel test...
Only when native vegetation sites are included in the analysis do geographically closer sites show more similarity to one another in trap catch \((r = 0.21; P = 0.02)\). Trap catch in the native vegetation was relatively low for the duration of the trapping period, except for the December 2008 trap catch of 887 beetles in one week for NV3 (Supp. Data 1 [online only], Fig. 1). The native vegetation traps did not have a rebounding response following winter as was the case with the storage and field sites (Supp. Data 1 [online only], Fig. 1).

The bulk of beetles caught in control traps on farms were caught around storages (Fig. 3). Control traps set in the field on farms caught only 10% of the total trap catch of control traps set on farms. Only ten R. dominica were caught in the four control traps across two depot sites and only two beetles were caught across the entire trapping period in the six control traps at native vegetation sites.

The sex ratio (defined as percentage of females) of R. dominica trapped in pheromone baited traps was significantly skewed toward females. The mean percentage of females trapped (with 95% confidence intervals) was 62.6% (58.0–67.2%) in field traps and 59.5% (56.3–62.6%) in storage traps. The sex ratio of beetles in control traps was more variable and not significantly different from 50%. The mean percentage of females trapped was 55.9% (44.8–67.1%) in field traps and 59.5% (45.0–66.7%) in storage traps.

Mating Status and Potential Fecundity of Emigrating Females

Forty-six female beetles were captured as they left infested silos and were transferred individually to wheat for lifespan and fecundity tests in the laboratory. All but two females produced offspring, one of which died within the first week of collection. One female produced 518 offspring without access to males over a 20-wk period. More than half of living females were still ovipositing viable eggs 9 wk after collection and without access to males (Fig. 4). Mated females produced, on average, a total of 242 offspring. Most oviposition was completed in the first 10 wk (Fig. 4). The combined sex ratio of the offspring produced by dispersing females did not differ statistically from unity except for the first week of oviposition, when more males were produced, week seven, when more females were produced and week 14 when more males were produced. Median time to death of the emigrant beetles was 12 wk post-capture, and this period was similar for males and females (13 and 12 wk, respectively) as were survivorship curves. Three beetles, all male, survived for the duration of the 40-wk study.

Microsatellite Isolation and Development

The 454 and PE 90 Illumina sequencing data sets provided a similar distribution of repeat types, with dinucleotides being most common (Supp. Data 1 [online only], Fig. 2). When we assembled the Illumina data the size of microsatellite (in both absolute base pairs and in repeat count) increased with increasing kmer size up to the point at which the kmer became too long and few contigs were assembled (Supp. Data 1 [online only], Fig. 3). An optimal kmer size of 73 was identified; this assembly yielded 8,338 microsatellites over 5 bp for which primers could be made. We optimized 21 of these loci based on the laboratory strain QRD14 (for details see Supp. Table 1 [online only]), the number of alleles per locus ranged from one to five in the 32 individuals genotyped from QRD14 (mean = 2.5). When we applied 15 of these markers to field collected beetles the number of alleles was much higher, ranging from three to 31 across all 592 individuals (mean = 12.5) and the mean number of alleles per population in the wild caught beetles was 6.5 (range 2.6–13.2; Supp. Table 1 [online only]). We also provide primer options for an additional 8,317 loci (Supp. Table 2 [online only]).

Microsatellite Analyses of Field-Collected Beetles

Three of the markers (RL12, RL15, and RL43) showed evidence of a high occurrence of null alleles (>16%) when genotyped in wild caught beetles (but not in beetles from the laboratory culture). All analyses were performed with and without these three loci. The structure results were identical regardless of the exclusion or inclusion of these loci, as was the bottleneck analysis. Fewer individuals were detected as recent immigrants (by assignment testing) in the reduced dataset (26 cf 34), but all individuals were assigned to the same source population in both analyses. All results presented are therefore based on the full dataset of 15 loci.

The delta K method indicated that K = 2 was the most likely number of population clusters in the total dataset, although K = 3 also had some support (Supp. Data 1 [online only] Fig. 3). When the plots of K = 2 and K = 3 are compared (Fig. 1) it is apparent that K = 3 does not further resolve additional clusters, but rather splits populations evenly into the second two clusters. Therefore K = 2 is the most appropriate inference of population structure in this dataset. In both the K = 2 and K = 3 plots the only population samples that cluster differently are the M Depot (see Fig. 1 for locality) samples from April 2009, October 2009, and November 2009, although the individuals at these sites assigned to the second (yellow) cluster become less differentiated from individuals from other populations over time. Apart from these three samples the remainder essentially represent a single population cluster with some individuals not being clearly assigned to either cluster. Genetic differentiation was low across all sites (global FST = 0.028 with and without null allele frequency correction), and the structure algorithm is generally less able to assign individuals fully when differentiation is low. The highest pairwise FST was 0.082, between M Depot April 2009 and the Farm 8 Silo April 2009 sample. Only 71 out of the 361 pairwise G tests of genetic differentiation were significant after Bonferroni correction, and most of these were in comparisons between the later three M Depot samples and all other population samples (Supp. Table 3 [online only]). The Principal components analysis of these pairwise FST’s also separated these three M Depot sites from all other samples (Fig. 5). The tests for recent population bottlenecks returned significant heterozygote excess \(P < 0.05\) at two sites, M Depot Apr 2009 and Farm 8 Silo Nov 2009. The one tailed Wilcoxon test for heterozygote excess was highly significant for M Depot Apr 2009 \(P = 0.006\;\text{Table 1}\). The FIS values were all positive (indicating some degree of inbreeding) with the exception of M Depot Apr 2009, which had a negative FIS as a result of the heterozygous excess.

The population assignment tests indicated which individuals were more likely to have come from a sample other than the one with which they were collected. The test indicated that 13 beetles were likely to have been recent immigrants across the seven sites sampled in November 2008, 11 across the four sites sampled in April 2009, and 10 across the four sites sampled in October 2009, when the alpha level of significance was set to 0.01 (Supp. Table 4 [online only]). Within each of these three separate analyses there was no discernible pattern in the assignment of the 34 putative immigrant individuals to the alternative source populations. The putative populations of origin were separated by as much as 115 km from the population tested.
Fig. 4. Survival and reproduction of individual *R. dominica* females (*n = 46*) in the laboratory (25 °C and 65% RH) after being captured leaving bulk stored wheat. Female survival (▲) and the percentage of living females producing offspring (■) are displayed in the top plot. The mean offspring produced by females that produced at least one offspring in the week is displayed in the middle plot and the percentage of female offspring is displayed in the lower plot. Bars represent 95% confidence intervals. Only one female produced offspring in week 15 post collection, so there is no confidence interval for this data point.
Discussion

We conclude that *R. dominica* disperses widely and at relatively high frequency through active flight. The large numbers involved and the fact that most females tend to be mated explains the genetic homogeneity across the 7,000 km² of our study area. Our trapping results indicate that dispersal flights of more than 1 km are common, and the assignment tests indicate such flights can cover more than 100 km in some cases. Transportation of infested grain could be hypothesized to mediate gene flow of stored grain insects, but anthropogenic movement between farms appears to have a negligible influence on gene flow at the spatial scale investigated here, because transportation of grain between farms in our study area is rare.

*Fig. 5.* Principal components analysis of pairwise FST’s calculated using the uncorrected allele frequencies in FreeNA. The 19 population samples derived from nine sites over five collection periods in the Darling Downs, Queensland study area.

**Table 1.** Results of the standard difference test and Wilcoxon test for excess heterozygosity, and FIS values for the 19 population samples derived from nine sites over five collection periods in the Darling Downs, Queensland study area

<table>
<thead>
<tr>
<th>Population</th>
<th>Standard differences test</th>
<th>Wilcoxon test one tail H excess</th>
<th>Wilcoxon test two tail</th>
<th>FIS</th>
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<tbody>
<tr>
<td>Mdepot-Nov08</td>
<td>0.373412</td>
<td>0.619568</td>
<td>0.807739</td>
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<td>Farm1 Field-Nov08</td>
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<tr>
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<td>TDepot-Oct09</td>
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<td>0.678772</td>
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<td>NV1-Oct09</td>
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<td>0.389404</td>
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<td>Mdepot-Nov09</td>
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<tr>
<td>Farm15 Silo-Nov09</td>
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<tr>
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<td>0.094276</td>
<td>0.052002</td>
<td>0.104004</td>
<td>0.1421</td>
</tr>
</tbody>
</table>

Bold values are below 0.05.
around grain storage and this is probably because stored grain is producing the majority of new individuals, otherwise, we would expect to see more beetles in control traps in open fields and in native vegetation. Only a few beetles were caught in control traps at depots but this probably represents the demand to control large infestations at these locations whereas some on-farm grain storage sites, particularly when grain is stored for feed, can be heavily infested without commercial repercussions. We set traps at farms storages to collect a representative sample from the storage site, as opposed to sampling from grain itself, to assess the movement of these beetles and their general spatial distribution. We assume that the beetles caught at the storage site are dispersing from the storages but given the large numbers of beetles caught in traps away from storages this assumption may not be valid in all cases.

Movement and Gene Flow

Our combined results demonstrate that genetic homogeneity of *R. dominica* in our study area is primarily the result of active dispersal. The large individual flight distances inferred through assignment tests indicate that genetic homogeneity would likely extend over much larger spatial scales. This interpretation is consistent with the tests indicate that genetic homogeneity of *R. dominica* reported by Guedes et al. (1997), where FsT’s were low and no isolation by distance was detected across the states of Minas Gerais and Sao Paulo.

There are several methods available to infer migration from genetic data, but genetic assignment tests based on microsatellite allele frequencies are the most suitable for detecting recent immigration when differentiation (FsT) is relatively low. Where assignment tests have been compared to mark-recapture data they have given consistent results (Adams and Hutchings 2003, Lowe et al. 2006, Berry et al. 2004). The accuracy of assignment tests is related to the number of markers used and the genetic differentiation between the populations assessed, with accuracy reaching 100% at around FsT = 0.06 to 0.08 (Berry et al. 2004). In particular, at low pairwise FsT it is more difficult to determine whether an individual should be assigned to the home population or an alternative one. We used a high number of markers in this study but the pairwise FST’s were low, for example in the Nov08 samples the mean pairwise FsT was 0.01. The assignment of individuals to alternative source populations should therefore be considered an inference rather than strong evidence, and the number of recent immigrants detected (34) should be considered an underestimate of recent immigration.

We detected strong evidence for a recent bottleneck at the M Depot site in April 2009 (Table 1), this bottleneck was significant enough to change the allele structure at this site and alter the population assignment in the structure analysis (Fig. 1). Such a bottleneck at a grain depot is most likely the result of insecticide treatment, and it is likely that this was a selection event removing susceptible individuals as well as a reduction in population size. Following the bottleneck in April the same site remained differentiated but not as strongly (fewer individuals have a high posterior probability of belonging to the yellow cluster in Fig. 1) when sampled again in October and November 2009. This, presumably, represents the homogenising effect of movement and gene flow.

Seasonal Flight Activity of *R. dominica*

Flying beetles were caught in traps during all months of the year, but trap catch was reduced to almost nil during the winter months of June and July when mean maxima were 20.5 and 21.5 °C respectively. The reduced flight activity is in agreement with reported flight threshold temperatures for *R. dominica* (Dowdy 1994, Toews et al. 2006, Cox et al. 2007). Beetle abundance likely remained stable through the winter months because trap catches rebounded in August, suggesting that beetles had been developing in grain or grain residues and flew when conditions again became suitable for their flight. Grain is an excellent insulator and grain bulks on the Darling Downs typically remain at a suitable temperature for the development of *R. dominica* through the winter period (Ridley et al. 2011). The limited flight of stored pests, for at least part of the year, provides one of the few opportunities to manage these pests using their biology against them. Grain storage managers and farmers can implement disinfection procedures during cool periods when the number of dispersing *R. dominica* is relatively low and reinfection from neighbouring grain bulks is unlikely.

Mating Status and Potential Fecundity of Emigrating Beetles

Nearly all females caught leaving infested grain had mated (95%) and mated females were able to use the stored sperm for many weeks to produce offspring. Mated females are, therefore, capable of initiating an infestation on their own and are also moving more genetic material than unmated females and this presumably adds to gene flow. *Rhizopertha dominica* mates readily in the laboratory (Potter 1935), and the more males a dispersing female mates with prior to flight, the greater the likelihood is that she is carrying insecticide resistance genes. We did not quantify the number of males the females had mated with and this ecologically significant parameter remains unknown. The life span and offspring production of the females collected in our study are consistent with figures reported for adults reared under laboratory conditions for their whole life (Birch 1945, 1953; Howe 1950), indicating that the beetles we collected were young. Young adult *R. dominica* are more likely to initiate flight (Barrer et al. 1993, Dowdy 1994), which also supports the hypothesis that younger beetles are more likely to disperse.

Implications for Insecticide Resistance

*Rhizopertha dominica* has developed resistance to the fumigant phosphine (Collins et al. 2002, Lorini et al. 2007, Opit et al. 2012) and grain protectant insecticides (Lorini and Galley 1999, Daglish et al. 2013). Our work highlights the potential for rapid transfer of resistance genes across the landscape but movement of susceptible individuals may also be significant in terms of insecticide resistance. The dilution of resistance genes with susceptible individuals moving into sites where selection has taken place may prevent the fixation of resistance (Caprio and Tabashnik 1992). The shift in population assignment at the M Depot site following the bottleneck event is likely an example of this process occurring in the field. Dilution of phosphine resistance by immigrating beetles has been shown to have a dramatic effect on the ability to control resistant populations in simulations (Lilford 2009). Modelling studies report that intermediate levels of movement delay resistance development more than low or high levels of movement (Caprio 2001, Kang et al. 2014); further research to empirically test this prediction, in light of the levels of movement recorded in this study, is needed.

A significant practical implication from this analysis is that, at least in Australia the impact of native vegetation in terms of production of pests and as a refuge from insecticide resistance is small at best. Nontreated grain parcels such as waste grain, residues in machinery, and grain used for stock feed seem far more likely to influence the spatio-temporal dynamics of these pests and their resistance to insecticides such as phosphine.
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References