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RESEARCH ARTICLE

Mating behaviour and reproductive output in insecticide-resistant and -susceptible strains of the maize weevil (*Sitophilus zeamais*)

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Keywords

Cost of insecticide resistance; female mating preference; pyrethroid resistance; stored grain insects.

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Abstract

Insecticide resistance is a broadly recognised and well-studied management problem resulting from intensive insecticide use, which also provides useful evolutionary models of newly adapted phenotypes to changing environments. Two common assumptions in such models are the existence of fitness costs associated with insecticide resistance, which will place the resistant individuals at a disadvantage in insecticide-free environments, and the prevalence of random mating among insecticide-resistant and -susceptible individuals. However, cases of insecticide resistance lacking apparent fitness disadvantages do exist impacting the evolution and management of insecticide resistance. Assortative mating, although rarely considered, may also favour the evolution and spread of insecticide resistance. Thus, the possible existence of both conditions in the maize weevil (*Sitophilus zeamais*), a key pest of stored cereals, led to the assessment of the mating behaviour and reproductive fitness of insecticide-resistant and -susceptible weevil strains and their reciprocal crosses. The patterns of female and male mating choice also were assessed. Although mating behaviour within and between weevil strains was similar without mate choice, mating within the resistant strain led to higher reproductive output than within the susceptible strain; inter-strain matings led to even higher fertility. Thus, no apparent fitness cost associated with resistance seems to exist in these weevils, favouring the evolution of this phenotype that is further aided by the higher fertility of inter-strain matings. Mate choice reduced latency to mate and no inter-strain preference was detected, but female weevils were consistent in their mate selection between 1st and 2nd matings indicating existence of female mating preference among maize weevils. Therefore, if female mate selection comes to favour trait(s) associated with insecticide resistance, higher reproductive fitness will be the outcome of such matings favouring the evolution and spread of insecticide resistance among maize weevil populations reverting into a management concern.

Introduction

Insecticide resistance is a widely-recognised and well-studied management problem resulting from the long-term use of insecticides for managing arthropod pest species (Pickett, 1949; Ripper, 1956; Metcalf, 1980; Guedes *et al.*, 2016). A range of genetic, biological and

operational factors influence the evolution of insecticide resistance (Georghiou & Taylor, 1977*a,b*). They include the number of genes involved, their dominance and initial frequency, the species generation time and migration, and the insecticide structure and pattern of use, among others (Georghiou & Taylor, 1977*a,b*; Roush & McKenzie, 1987). These factors have been

incorporated into population-based models of insecticide resistance evolution, which pool individuals into groups disregarding their individual differences (e.g. Georghiou & Taylor, 1977*a,b*; Roush & McKenzie, 1987; Mallet, 1989; McKenzie, 1996), and also into the more recent individual-based models that consider such individual variation (e.g. Renton, 2013; Stratonovitch *et al.*, 2014).

Two frequent assumptions in the above-mentioned models of evolution of insecticide resistance are the existence of fitness costs associated with insecticide resistance and the prevalence of random mating among insecticide-resistant and -susceptible individuals (Georghiou & Taylor, 1977*a,b*; Roush & McKenzie, 1987; Mallet, 1989; Renton, 2013; Stratonovitch *et al.*, 2014). The fitness cost assumption recognises the resistant individuals as being at a disadvantage under insecticide-free environments and provides support for the momentary interruption of insecticide use as a potential management tactic allowing the resistance to recede (Roush & McKenzie, 1987; McKenzie, 1996; Coustau *et al.*, 2000; Kliot & Ghanim, 2012). This is one of the bases of insecticide resistance management recommendations suggested for aphids, budworms and grain weevils, among others (e.g. Roush & McKenzie, 1987; McKenzie, 1996; Coustau *et al.*, 2000; Foster *et al.*, 2003; Fragoso *et al.*, 2005).

In fact, fitness costs are expected whenever an organism adapts to a new environment facing distinct environmental stressors, including new host plants and their toxic secondary metabolites and synthetic agrochemicals like insecticides. The strong selection pressure imposed by these environmental stressors usually leads to drastic phenotypic changes and pleiotropic effects demanding resource and energy allocation from the selected individuals, thus impairing their competitive potential without this selection (i.e. without the stressor; Foster *et al.*, 2003; Guedes *et al.*, 2006; Kliot & Ghanim, 2012). Nonetheless, although common enough, fitness costs associated with insecticide resistance are far from universal in occurrence among insect species and insecticides, and vary among homozygous and heterozygous individuals, again with management consequences like compromising the recommendation of momentary interruption of insecticide use to allow susceptibility to reestablish (Kliot & Ghanim, 2012).

The second assumption, that of random mating among insecticide-resistant and -susceptible populations, is also incorrect in a number of instances. Fitness cost associated with insecticide resistance itself may lead to mating disadvantage while compromising mating competitiveness (Rowland, 1991; Berticat *et al.*, 2002; Platt *et al.*, 2015). Curiously, instances of insecticide resistance favouring mating competitiveness also have been

recorded (Arnaud & Haubruge, 2002; Smith *et al.*, 2011). Indeed sex-specific selection differences, including subtle traits and behavioural peculiarities, may affect mating behaviour and evolution of insecticide resistance presenting a marked contrast with asexual organisms (Jaffe *et al.*, 1997; Crowder *et al.*, 2010; Morrow & Connallon, 2013).

Extended and intensive selection for insecticide resistance is a condition in which the expected assumptions of fitness cost occurrence and random mating may be compromised. Long-standing environmental stress imposed by insecticide applications in genetically variable arthropod pest populations may lead to epistasis while selecting certain genes able to ameliorate the fitness cost usually associated with insecticide resistance (McKenzie & O'Farrell, 1993; McKenzie & Batterham, 1994; Clarke *et al.*, 2000). Such modifier genes may minimise potential developmental instability, reflected in reduced fluctuating asymmetry (i.e. small random deviations from perfect bilateral symmetry) (Markow, 1995; Møller & Swaddle, 1997), associated with insecticide resistance enhancing the performance of the resistant phenotype (Ribeiro *et al.*, 2007), and these modifier genes may alter the energy metabolism in resistant individuals (Clarke *et al.*, 2000; Guedes *et al.*, 2006). As a consequence, modifier genes may allow the maintenance of the resistant phenotype without compromising potentially conflicting physiological processes involved with insect development, maintenance and reproduction (Guedes *et al.*, 2006; Ribeiro *et al.*, 2007; Araújo *et al.*, 2008*a*; Lopes *et al.*, 2010).

Insecticide resistant strains of the maize weevil, *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae), a key worldwide pest of stored cereals, are a suitable model to assess the possible occurrence of assortative mating associated with lack of fitness cost in insecticide-resistant weevils. This is so because a Brazilian strain of this species was recognised as lacking apparent fitness costs associated with pyrethroid resistance in heavier (and larger) insects with altered energy metabolism and reduced fluctuating asymmetry (and thus developmental instability) (Guedes *et al.*, 2006; Ribeiro *et al.*, 2007; Araújo *et al.*, 2008*a,b*; Lopes *et al.*, 2010; Silva *et al.*, 2010*a,b*). Inter-strain differences in body mass (and size), together with reduced fluctuating asymmetry, are suggestive of potential sexual selection and assortative mating (Markow, 1995; Møller & Swaddle, 1997; Dongen, 2006; Jiang *et al.*, 2013; Crespi, 2014). Furthermore, recent studies of mating behaviour among grain weevils, particularly the related rice weevil (Campbell, 2005; Benelli *et al.*, 2016; Romano *et al.*, 2016), reinforce the usefulness of the maize weevil for the intended study.

The peculiarities reported above in the maize weevil led to the assessment of the mating behaviour and reproductive fitness of insecticide-resistant and -susceptible weevil

strains and their reciprocal crosses to test whether any particular cross is favoured and results in high progeny production. The patterns of female and male mating choice also were assessed to test whether random mating indeed prevails among maize weevils. Lack of fitness cost was expected for the pyrethroid-resistant strain, but inter-strain matings with the susceptible weevils may lead to higher fertility favouring the spread of the resistant alleles in the population, which is also a management concern. Furthermore, the lower fluctuating asymmetry and higher body mass of the resistant weevils may favour their sexual selection further favouring the spread of the resistant alleles in maize weevil populations.

Materials and methods

Insects

Two strains of the maize weevil were used, one susceptible and another resistant to pyrethroid insecticides. The susceptible population was initially established in the late 1980s from field-collected insects from Sete Lagoas County (state of Minas Gerais, Brazil), while the resistant strain was field-collected by the early 1990s from Jacarezinho County (state of Paraná, Brazil). The latter strain exhibits high levels of dichlorodiphenyltrichloroethane (DDT) and pyrethroid resistance ($>100\times$), which are periodically checked, mainly due to altered target site sensitivity with secondary involvement of enhanced glutathione-*S* transferase activity (Fragoso *et al.*, 2003, 2007; Ribeiro *et al.*, 2003; Araújo *et al.*, 2011), but may not exhibit apparent fitness cost associated with this trait (Fragoso *et al.*, 2005; Guedes *et al.*, 2006; Oliveira *et al.*, 2007). Subsequent bidirectional selection experiments and studies of fluctuating asymmetry are suggestive of the existence of secondary genes able to mitigate the fitness costs usually associated with insecticide resistance in this weevil strain (Ribeiro *et al.*, 2007; Corrêa *et al.*, 2011). The insecticide-resistant weevils exhibit higher body mass and altered energy metabolism compared with susceptible weevils (Araújo *et al.*, 2008*a,b*; Lopes *et al.*, 2010; Silva *et al.*, 2010*a,b*). Both strains are maintained in large numbers (to minimise potential effects of genetic drift) in whole maize kernels free of insecticide residues under controlled conditions of temperature ($25 \pm 2^\circ\text{C}$), relative humidity ($70 \pm 5\%$), and photoperiod (LD 12:12 h).

General procedure for mating experiments

Newly-emerged virgin female and male weevils (1 day old) were obtained from stock cultures; sexed using their pattern of rostrum length, texture and punctuation (Halstead, 1965; Tolpo & Morrison, 1965); and individually

isolated in maize-containing (transparent) glass tubes (100 mm high \times 25 mm diameter) for 3 days allowing sexual maturation. The insects were code-marked on the thorax using coloured nail polish to allow recognition of sex and strain; the marking material and procedure was preliminarily tested to avoid interfering with the study and no significant interference was observed. Each adult weevil was weighed on a microelectronic balance (Metler-Toledo UMT-2, Columbus, OH, USA), and the lengths of the rostrum, thorax and elytra were determined using the software ImageJ (NIH, Bethesda, MD, USA) from digitalised images captured using a CCD camera connected to a stereomicroscope (Nikon SMZ-10A, Melville, NY, USA). The insects were mated in experimental arenas consisting of open Petri dishes (3 cm diameter) with their inner walls coated with Teflon[®] PTFE (DuPont, Wilmington, DE, USA) to prevent the insects from escaping.

Single female–single male mating behaviour and fertility

Females and males of each maize weevil strain were paired on the Petri dish arenas and allowed to mate. Individual pairs were established between females and males of the same strain, and different strains in reciprocal mating. Mating behaviour was recorded using a digital video recorder (DSR-M810, Sanyo, Japan) connected to a video camcorder (Model wv-BP330, Panasonic, Japan) with a 3.5–8 mm 1:1.4 lens placed inside a microclimate chamber (model I-30B, Percival Scientific, Perry, IA, USA) with the controlled environmental conditions of the colony rearing chambers, as specified above. The individual behaviours were classified using a set of behaviours simplified from that described by Walgenbach & Burkholder (1987). This set of behaviours was recorded using the software JWatcher (Blumstein & Daniel, 2007) and included: walking, interacting (including initial and subsequent contacts and mount attempts), mounting (including correct mount, male leg elevation, male grasping of female and male stroking female with hindlegs), and copulation (i.e. intromission, abdominal flexing, until dismounting). Twenty couples were followed for each of the four types of mating (i.e. resistant female and male [$\text{♀R} \times \text{♂R}$], susceptible female and male [$\text{♀S} \times \text{♂S}$], resistant female and susceptible male [$\text{♀R} \times \text{♂S}$], and susceptible female and resistant male [$\text{♀S} \times \text{♂R}$]). After the end of the copulatory period, the females were collected and transferred to glass tubes (100 mm high \times 25 mm diameter) containing 30 g of maize for egg-laying. The maize grains were replaced weekly, and the egg-containing maize grains were periodically inspected using an MX-20 Specimen Radiography System (Faxitron X-Ray Corp., Wheeling, IL, USA) to

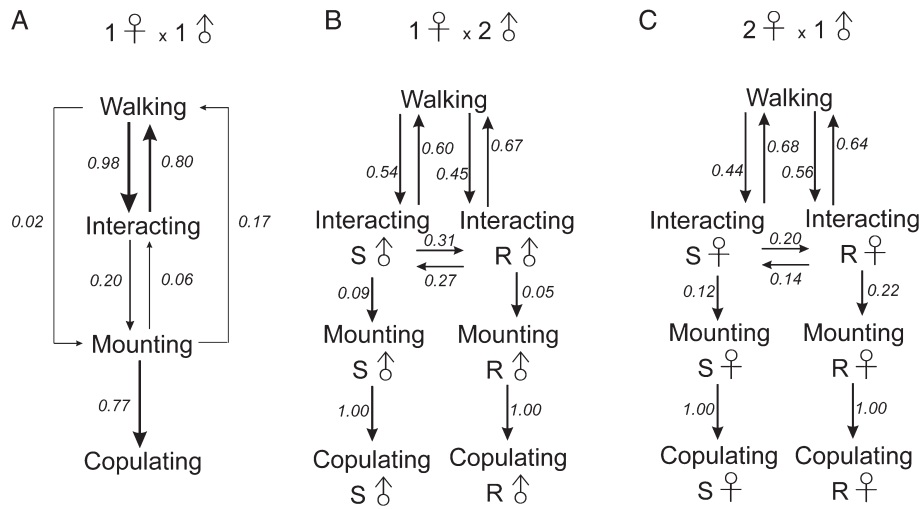


Figure 1 Ethograms of the mating behaviour of the maize weevil (*Sitophilus zeamais*) without mating choice (A) and with either female choice (B) or male choice (C) represented as 1st order transition diagrams. The solid arrows indicate each behavioural transition. The relative thickness of each arrow represents the frequency of each behavioural transition (A: $n = 80$; B: $n = 56$; C: $n = 64$).

record the offspring produced (number of hatched larvae per week).

Mate choice

Inter-strain male and female mating preferences of insecticide-resistant and -susceptible maize weevils also were recorded following the same methods described above, but allowing mate choice between same-sex individuals of both strains. Therefore, for each female (or male) of each strain (either insecticide-resistant or -susceptible) placed in the mating arena, two members of the opposite sex and from different strains were provided. The mating behaviour was recorded as previously described, and the mating preference was registered for each weevil mating trio (one chooser and two partner options for choice) on two successive days assessing the mate choice for the 1st and 2nd matings; the same individuals were used for these two successive matings. The body mass of each individual insect was recorded as previously described, as were the lengths of the rostrum, thorax and elytra.

Statistical analyses

Simplified ethograms depicting the sequence and frequency of events were manually constructed based on 1st order behavioural transitions. Eventual differences in latency to mate and mating duration among matings were subjected to time-failure analyses using Cox regression with weevil body mass as covariate (PROC PHREG; SAS software, SAS Institute, Cary, NC, USA).

Weekly weevil fertility (no. of hatched larvae/female) was subjected to regression analysis against female life-time using the curve-fitting procedure of TableCurve 2D (Systat, San Jose, CA, USA). The significant regression models ($P < 0.05$) were tested from the simplest (linear and quadratic) to more complex peak models, and model selection was based on parsimony, high F -values (and mean squares), and steep increases in R^2 with model complexity. Residual distribution was also checked for each analysis to validate parametric assumptions. The dichotomous results from the free-choice mating bioassays were subjected to the randomness G test using William’s procedure to correct the G values (Sokal & Rohlf, 1995). Logistic regressions were subsequently used to test whether the respective body mass and lengths of the rostrum, thorax and elytra from the choosing individual, the chosen, and the not-chosen ones may be playing a role in mating selection.

Results

Single female–single male mating behaviour and fertility

The simplified ethograms representing the 1st order of behavioural transitions of no-choice mate pairs of maize weevil were similar regardless of the mating type (i.e. if $R \times R$, $S \times S$, $R \times S$, or $S \times R$), and therefore a single general diagram was used for representation (Fig. 1A). The time budget for the described transitions was also similar among different mating types ($\chi^2 < 2.71$, d.f. = 3, $P > 0.44$), even using adult weevil body mass as covariate, and the results were represented in a single general

bar graph (Fig. 2A). Nonetheless, the latency to mate was negatively correlated with mating duration ($r = -0.32$, $P = 0.0008$, $n = 106$), and mating duration was positively correlated with total fertility ($r = 0.57$, $P < 0.0001$, $n = 106$).

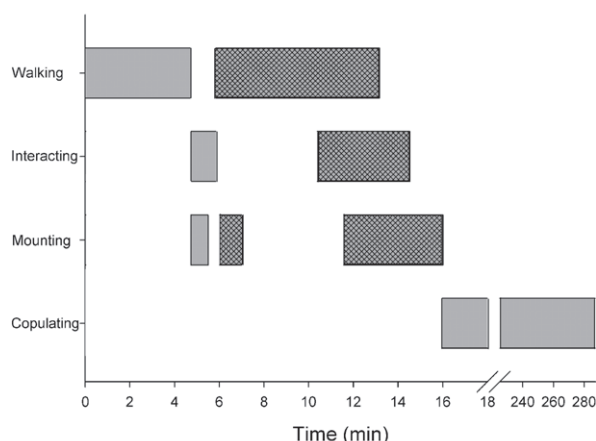
The weekly fertility was significantly different among matings mainly due to offspring production (Fig. 3, Table 1). The peak of fertility was roughly similar among matings with no more than a half-week delay among them (Fig. 3, Table 1). However, the number of offspring larvae produced varied significantly among matings with the inter-strain matings exhibiting higher fertility than the susceptible couples ($F_{3,103} = 2.74$, $P = 0.04$; Fig. 3). The insecticide-susceptible couples produced 19.14 ± 4.26 hatched larvae/female, while the S × R and R × S matings led to the production of 36.69 ± 5.32 and 32.36 ± 5.69 hatched larvae/female, respectively. The insecticide-resistant couples exhibited intermediate total fertility of 25.58 ± 6.28 hatched larvae/female.

Mate choice

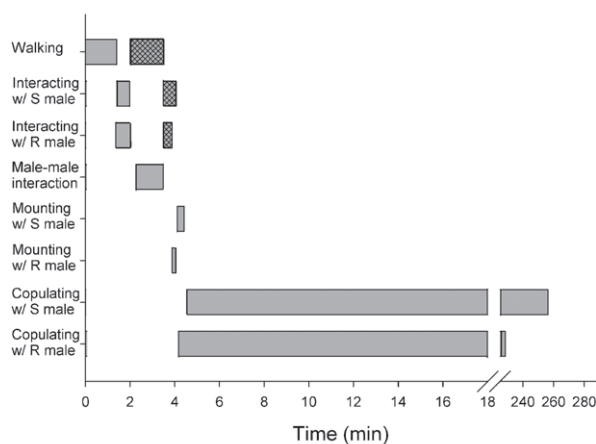
The simplified ethograms representing the 1st order behavioural transitions of free-choice matings of females and males of both strains, each simultaneously provided with a member of the opposite sex belonging to each strain, were similar and are exhibited in Fig. 1B and 1C. The time budgets for the described transitions were also similar (Fig. 2B and 2C), except for the latency to mate, which was significantly longer when the female was given a choice of two males, than when the male was the choosing sex ($\chi^2 = 10.10$, d.f. = 4, $P = 0.001$; Fig. 4A), regardless of the choosing strain ($\chi^2 = 0.49$, d.f. = 1, $P = 0.87$). Mating duration was significantly different only when 1st matings were compared with 2nd matings ($\chi^2 = 11.48$, d.f. = 1, $P < 0.001$) with the former lasting over twice as long (Fig. 4B).

Females of both strains did not exhibit significant preference between males from both strains, resistant and susceptible to insecticides, in the 1st nor in the 2nd mating ($G_{adj} < 0.85$, d.f. = 1, $P > 0.05$). The same trend was observed regarding male mating preferences, again regardless of male strain and whether 1st or 2nd mating ($G_{adj} < 2.60$, d.f. = 1, $P > 0.05$). Curiously, although males did not exhibit consistency when choosing their respective female mating partner in successive matings ($G_{adj} < 2.61$, d.f. = 1, $P > 0.05$), females did exhibit consistency in their mate selection, which was independent of the female and male strains involved ($G_{adj} > 210.41$, d.f. = 1, $P < 0.05$; Fig. 5). Logistic regressions were subsequently used to test whether the respective body mass and lengths of the rostrum, thorax and elytra from the choosing individual, the chosen, and the not-chosen ones may

A 1 Female x 1 Male



B 1 Female x 2 Males



C 2 Females x 1 Male

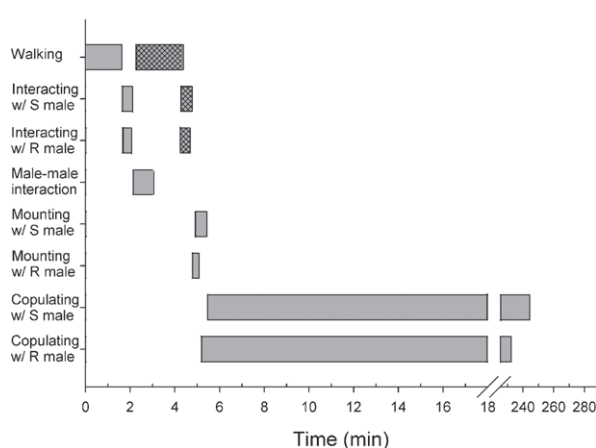


Figure 2 Schematic representation of time budgets of the mating behaviour of the maize weevil (*Sitophilus zeamais*) without mating choice (A) and with either female mate choice (B) or male choice (C). The horizontal histogram bars indicate the average duration of each behaviour. The dashed bars indicate events that were repeated before copulation eventually took place, as indicated in the transition diagrams of Fig. 1.

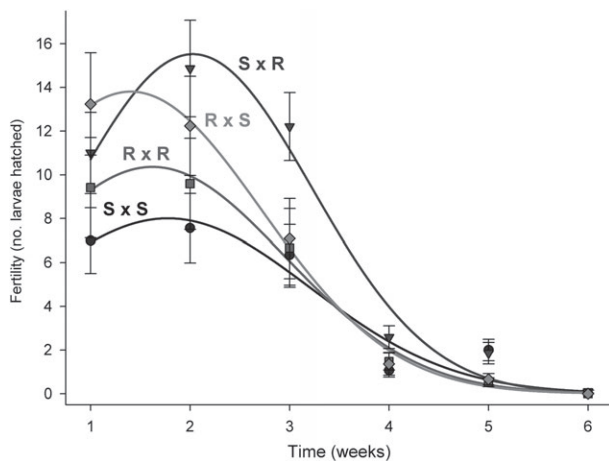


Figure 3 Weekly fertility of females of the maize weevil (*Sitophilus zeamais*) for each mating type (i.e. resistant female and male [R x R], susceptible female and male [S x S], resistant female and susceptible male [R x S], and susceptible female and resistant male [S x R]). Each symbol indicates the mean (\pm SE) observed values.

be playing a role in mating selection. However, despite the significance of the logistic model tested ($\chi^2 = 52.56$, d.f. = 14, $P < 0.001$), none of the traits assessed provided significant explanation for the pattern of mate choices observed ($\chi^2 < 0.02$, d.f. = 1, $P > 0.90$).

Discussion

Two common assumptions in models of insecticide resistance evolution, associated fitness cost and random mating, have important implications for insecticide resistance management (Rowland, 1991; Coustau *et al.*, 2000; Berticat *et al.*, 2002; Kliot & Ghanim, 2012; Platt *et al.*, 2015). This is because they potentially influence the spread of the resistance alleles in the population and they may compromise the management tactic of momentary suppression and eventual re-use of the insecticide under scrutiny (Roush & McKenzie, 1987; Mallet, 1989; McKenzie, 1996). The reported association of pyrethroid resistance in the maize weevil with lack of fitness cost and

reduced fluctuating asymmetry in locomotor appendages (Fragoso *et al.*, 2005; Guedes *et al.*, 2006; Oliveira *et al.*, 2007; Ribeiro *et al.*, 2007), as well as higher body mass likely due to differential energy metabolism (Araújo *et al.*, 2008a,b; Lopes *et al.*, 2010; Silva *et al.*, 2010a,b), led us to assess the mating behaviour and reproductive fitness of these insects, in addition to the patterns of female and male mating choice in the maize weevil. However, there was no direct association between insecticide resistance and mating behaviour.

The mating behaviour between individual weevil pairs was similar regardless of the strains involved, but within the range of results reported for this species and also for the related rice weevil (Walgenbach & Burkholder, 1987; Campbell, 2005). Higher fertility was correlated with mating duration, as expected based on earlier findings by Walgenbach & Burkholder (1987) and Campbell (2005) with the maize and rice weevil, respectively. Mating duration was negatively correlated with latency to mate indicating that couples that took longer to mate, mated for less time and exhibited reduced fertility, regardless of the maize weevil strain; the reproductive period was similar among the different crosses. However, insecticide-resistant weevils did not exhibit associated fitness costs, as previously reported (Fragoso *et al.*, 2005; Oliveira *et al.*, 2007; Ribeiro *et al.*, 2007).

A more curious finding though was the higher fertility of matings between insecticide-resistant weevils and particularly among inter-strain matings. This fact favours the maintenance and spread of the insecticide resistance alleles in the population particularly if the heterozygous individuals with dominant or co-dominant resistance alleles do not exhibit a fitness disadvantage either (Roush & McKenzie, 1987; McKenzie 1996; Kliot & Ghanim, 2012). The latter condition may also be favoured by hybrid vigour (i.e. heterosis, or the enhanced quality of hybrid offspring), which has been reported in insects since the late 1920s and is sometimes associated with insecticide resistance (Muir, 1928; Davidson, 1958; Newton & Dixon, 1988; Gassmann *et al.*, 2009). Regardless, the different genetic background between the two maize weevil

Table 1 Summary statistics of non-linear regression curves of reproductive output for each mating type (i.e. resistant female and male [R x R], susceptible female and male [S x S], resistant female and susceptible male [R x S], and susceptible female and resistant male [S x R])^a

Model	Mating Type	Parameter (\pm SEM)			F ₂	Degrees of Freedom (reg; error)	P	R ²
		a	b	C				
Gaussian (3-parameters) $y = a \exp(-0.5((x-c)/b)^2)$	S x S	8.01 \pm 0.98	1.43 \pm 0.33	1.78 \pm 0.31	8.64	3; 65	<0.001	0.21
	S x R	15.52 \pm 1.12	1.19 \pm 0.13	2.03 \pm 0.11	44.45	3; 109	<0.001	0.45
	R x R	10.36 \pm 0.46	1.33 \pm 0.12	1.62 \pm 0.11	37.87	3; 58	<0.001	0.57
	R x S	13.80 \pm 0.42	1.32 \pm 0.10	1.40 \pm 0.10	45.54	3; 80	<0.001	0.53

^aAll of the equation parameters are significant at $P < 0.05$ by Student's *t* test.

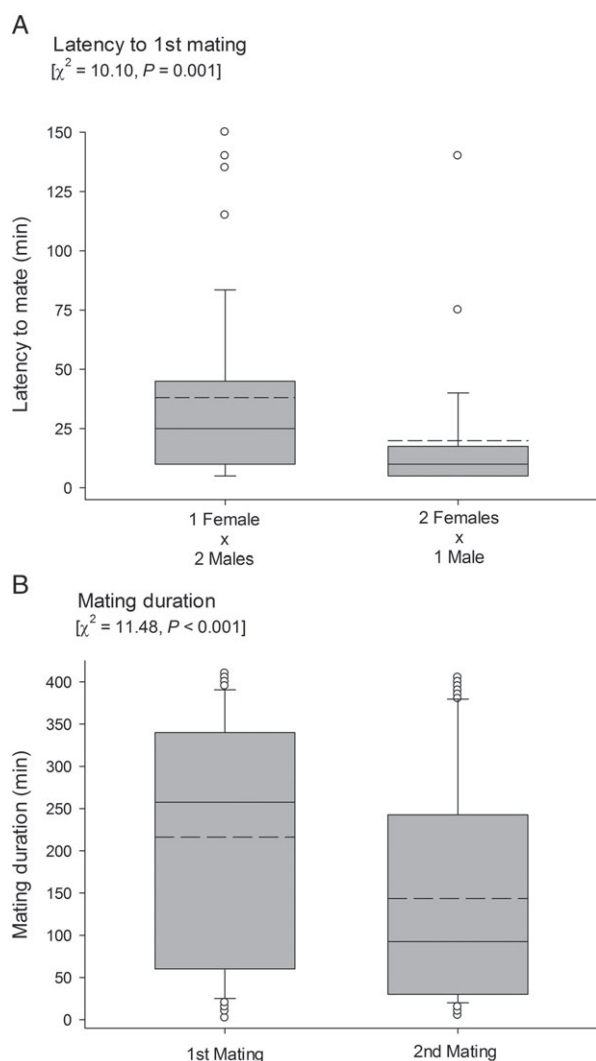


Figure 4 Latency to mate (A) and mating duration (B) in maize weevils (*Sitophilus zeamais*) with mate choice. The box plots indicate the median (solid line), mean (dashed line) and range of dispersion (lower and upper quartiles, and outliers) of the results.

strains and their pleiotropic consequences are potential causes for the higher fertility observed in inter-strain matings with potential impacts on the spread of insecticide resistance since heterozygous offspring will be carriers of the resistance allele(s). This is likely so because the differing genetic background and endosymbiont (*SPE* and *Wolbachia*) load of maize weevil populations do affect the distinct pattern of energy metabolism observed in these populations, as well as their behaviour and reproduction (Fragoso *et al.*, 2005; Guedes *et al.*, 2006; Araújo *et al.*, 2008a,b; Lopes *et al.*, 2010; Silva *et al.*, 2010a,b; Carvalho *et al.*, 2014)

The mate choice experiments with two successive matings on following days indicated smaller latency to mate

when the males were provided with a choice of females than when females were provided with a choice of males. The former situation likely facilitates mate searching and interaction/mounting reducing the latency to mate. In contrast, when a choice of males is provided to the female, potential female choice and male–male competition may take place delaying copulation in the maize weevil (Walgenbach & Burkholder, 1987). The rather long period of copulation among maize weevil couples also was expected, as already reported in this species and the rice weevil (Walgenbach & Burkholder, 1987; Campbell, 2005), and likely results from male guarding to delay subsequent matings enhancing the sexual fitness of the 1st male to mate with the female (Walgenbach & Burkholder, 1987; Miller, 2013). However, mating duration was not affected by strain.

We hypothesised that strain assortative mating, particularly due to female choice, may take place among maize weevils based on strain differences in fluctuating asymmetry and body mass, which were respectively lower and higher in the insecticide-resistant males (resistant females were also heavier than susceptible ones; Guedes *et al.*, 2006; Oliveira *et al.*, 2007; Ribeiro *et al.*, 2007). However, no strain preference was detected for female and male weevils, and therefore insecticide resistance is not directly favoured in mate selection, which would favour the maintenance and spread of insecticide resistance alleles. Curiously though, we obtained evidence that female weevils, but not males, do exhibit mate choice. The evidence came from the consistency exhibited by female weevils in choosing their male partners in two successive matings, which did not take place when the males were the choosing partner selecting between alternative females. Although we were not able to recognise any key morphological trait guiding the selection among the ones tested (i.e. body mass, and length of the rostrum, thorax and elytra), female mate choice does take place among maize weevils, presumably as a result of untested morphological traits, chemical cues or behavioural traits.

Female mate preferences are bound to guide assortative mating in weevils. Although insecticide-resistant males were not favoured in these selections, an as yet unrecognised trait or set of traits is likely used by the female weevils when choosing their mates. Such trait or set of traits may still favour the maintenance and spread of insecticide resistance in genotypes combining resistance with the female-favoured trait(s). This is more likely to take place in intensively and extensively insecticide-selected strains, which are more likely to contain either less costly insecticide resistance alleles or modifier alleles able to ameliorate the fitness costs usually associated with insecticide resistance (Clarke *et al.*, 2000; Coustau *et al.*, 2000; Guedes

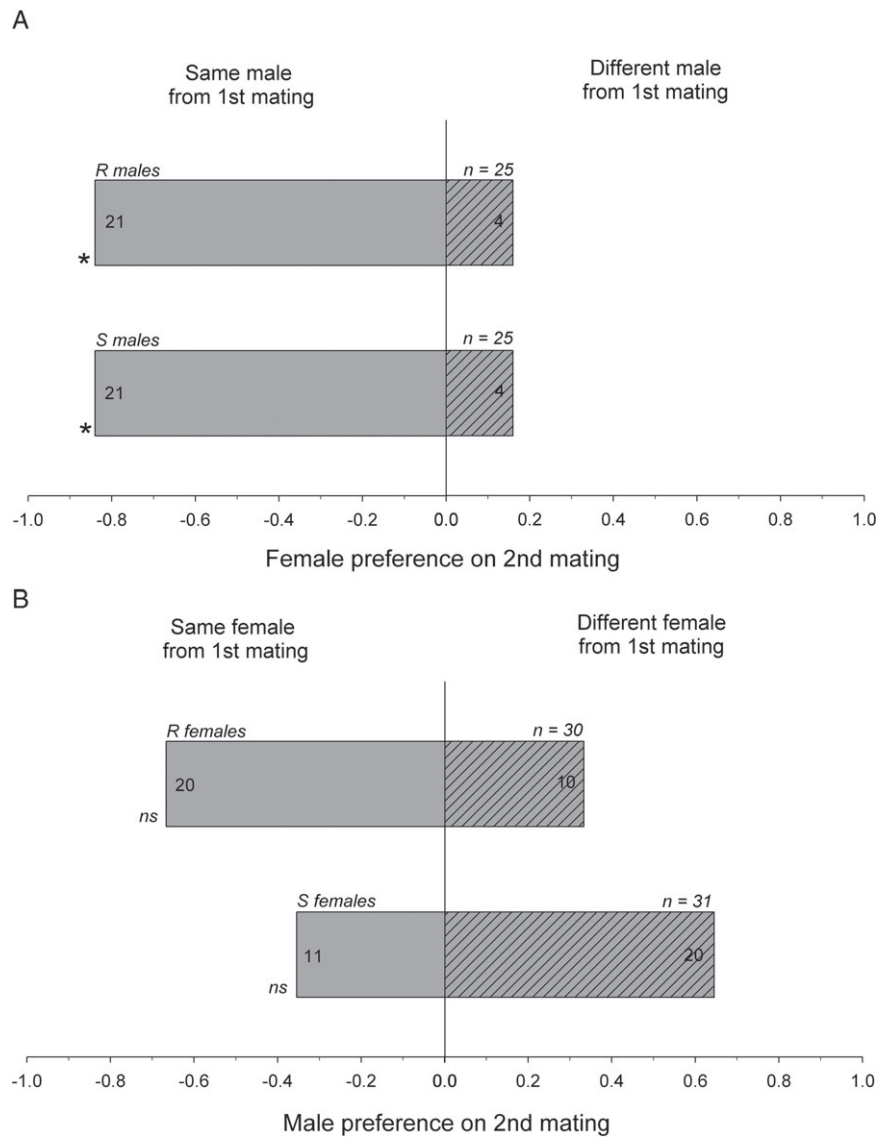


Figure 5 Consistency of female (A) and male (B) dichotomous mating preference of maize weevils (*Sitophilus zeamais*) from insecticide-susceptible (S) and -resistant (R) strains in two successive matings on following days. The number of trials (*n*) is indicated, as well as the preference responses in each set of trials (number is indicated in each bar and proportions are indicated on the X-axis). Asterisks indicate significant departure from random expectation, and *ns* indicates lack of significant departure from randomness, based on the adjusted *G* test ($P < 0.05$).

et al., 2006; Kliot & Ghanim, 2012). Further studies assessing different maize weevil strains with a broader range of trait variation and focusing on traits more frequently associated with assortative mating in arthropods are likely to shed light on the issue.

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