1   **Evolutionary Genetics of Insecticide Resistance and the Effects of Chemical Rotation**

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7

1 **Abstract**

2 Repeated use of the same class of pesticides to control a target pest is a form of artificial selection

3 that leads to pesticide resistance. We studied insecticide resistance and cross-resistance to five

4 commercial insecticides in each of six populations of the red flour beetle, *Tribolium castaneum*.

5 We estimated the dosage response curves for lethality in each parent population for each

6 insecticide and found an 800-fold difference among populations in resistance to insecticides. As

7 expected, a naïve laboratory population was among the most sensitive of populations to most

8 insecticides. We then used inbred lines derived from five of these populations to estimate the

9 heritability (h^2) of resistance for each pesticide and the genetic correlation (r_G) of resistance among

10 pesticides in each population. These quantitative genetic parameters allow insight into the adaptive

11 potential of populations to further evolve insecticide resistance. Lastly, we use our estimates of the

12 genetic variance and covariance of resistance and stochastic simulations to evaluate the efficacy

13 of "windowing" as an insecticide resistance management strategy, where the application of several

14 insecticides is rotated on a periodic basis.

15

1   **Introduction**

2   The adaptive response of pest arthropods to insecticide application is a striking example of rapid

3   Darwinian evolution, occurring over a few generations in response to a strong man-made selection

4   pressure. The ability of arthropods to evolve insecticide resistance has led in extreme cases to crop

5   failures or resurgence of vector-borne diseases (Mallet 1989; Kranthi et al 2002). A better

6   understanding of the phenotypic and genetic basis of the evolution of insecticide resistance and

7   cross-resistance is necessary to slow resistance evolution and maintain control over pest insects.

8   Repeated use of the same class of pesticides to control a target pest is a form of artificial

9   selection that leads to pesticide resistance. Worldwide, more than 500 species of insects, mites,

10   and spiders have developed some level of pesticide resistance. The majority of currently

11   commercialized synthetic insecticidal chemistries can be grouped into four modes of action: nerve

12   and muscle function disruption, growth inhibition, respiration inhibition, and midgut disruption.

13   Of the insecticides approved for use, the vast majority act on an organism's neuro-musculature.

14   The common physiological target of theses insecticides suggests that a pest's response to treatment

15   with one insecticide will be genetically correlated with its susceptibility to other functionally

16   related insecticides. There is a large body of evolutionary genetic literature describing how to

17   estimate such genetic correlations and how to use them to predict the correlated response of traits

18   in populations, be it positive or negative, to bouts of selection (e.g., Lynch and Walsh 1998).

19   Here, we report our phenotypic and genetic estimates of resistance and cross-resistance to

20   five commercial insecticides in each of six populations of the flour beetle, *Tribolium castaneum*.

21   We use inbred lines derived from five of these populations to estimate two quantitative genetic

22   parameters, the heritability (h^2) and genetic correlation (r_G) of resistance between pesticides in

23   each population. These quantitative genetic parameters allow insight into the adaptive potential of

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1   populations to evolve insecticide resistance. Heritability determines how rapidly a population

2   responds to a single selective agent, while genetic correlations determine how adaptation to one

3   selective agent indirectly affects adaptation to other selective agents (Sih et al. 2004). In particular,

4   positive genetic correlations imply that the evolution of resistance to one insecticide results in the

5   evolution of cross-resistance to one or more other insecticides. Negative genetic correlations imply

6   that the evolution of resistance to one insecticide results in an evolutionary increase in the

7   sensitivity to one or more other insecticides. By estimating the genetic variance and covariance of

8   resistance to insecticides with different modes of action across several populations, we are able to

9   detect geographic variation in the capacity to evolve resistance. As we report, the data suggest that

10   some populations have a past history of strong selection for resistance to some insecticides but not

11   others. We use our estimates of the genetic variance and covariance of resistance to evaluate the

12   efficacy of the insecticide resistance management strategy of "windowing," wherein the

13   application of several insecticides is rotated on a periodic basis to maintain pest-control efficacy

14   and to delay the evolution of resistance.

15   Insecticide resistance tends to be polygenic when studied in laboratory selection

16   experiments but monogenic when studied in pest insects isolated from the field (Roush and

17   McKenzie 1987; ffrench-Constant et al. 2004). This finding has been attributed to the difference

18   in the strength of selection imposed in the laboratory relative to that in the field. Because selection

19   is generally weaker in laboratory evolution studies, they may favor resistance based on many

20   genes, each of small effect, segregating within the experimental population. Field selection, in

21   contrast, consists of applying much higher concentrations of insecticide and therefore stronger

22   selection. As a result, the field response tends to be based on rare mutations of large effect at single

23   genes (see Figure 1 in ffrench-Constant et al. 2004). Laboratory selection occasionally produces

1   monogenic resistance, most often when genetic material is incorporated into the laboratory strain

2   from field populations that have already had extensive exposure to the specific test insecticide or

3   to an insecticide with cross resistance to the test insecticide. Although this dichotomy between

4   monogenic and polygenic response may be an artifact of genetic interference (see below), we

5   estimated the genetic variance and covariance of our populations for resistance at both the median

6   lethal dose (LD_{50}) and lethal dose, 90% (LD_{90}) concentrations of all insecticides. This allowed us

7   to compare the capacity for an evolutionary response at two different strengths of selection.

8   In general, the initial response of a population to strong selection depends upon either the

9   advent of new mutations or upon pre-existing alleles of major effect segregating within a target

10   population (Olson-Manning et al. 2012). When dependent on new mutations, an adaptive response

11   to strong selection is slowed by the waiting time necessary for those mutations to occur. Because

12   the evolution of insecticide resistance has been so rapid, it is likely based on existing allelic

13   variation, which in theory facilitates the most rapid adaptive response to strong selection (Olson-

14   Manning et al. 2012). However, when two independently acting genes are subjected

15   simultaneously to strong selection, 'interference' occurs between them, slowing the response to

16   selection (Barton 1995). Interference occurs between simultaneously favored alleles at two loci

17   because linked beneficial mutations arising in different individuals compete when the favored

18   allele at one locus is found by chance in a linked genetic background with the non-favored allele

19   at the other locus. Increasing the recombination rate between the two loci increases the likelihood

20   that both favorable alleles will become fixed by selection. However, with strong selection even

21   unlinked loci exhibit interference with one another (Barton 1994). Thus, when selection is strong,

22   as it is for insecticide resistance in the field, interference occurs regardless of the chromosomal

23   distribution of the selected alleles across a genome, because strong selection creates associations

1   even between unlinked loci. Moreover, interference is asymmetric: genes of larger effect exert

2   greater interference on genes of smaller effect. Thus, very strong selection may result in a selection

3   response based on one gene of large effect even when other genes capable of contributing to the

4   adaptive response are segregating in a population. In addition, interference is relatively more

5   important to the response to strong selection in spatially structured populations, like agricultural

6   fields, because local associations between loci created by selection are broken down more slowly

7   because recombination is reduced by the deviations from random mating imposed by population

8   structure.

9   We report our studies of resistance and cross-resistance to five commercial insecticides

10   (Malathion, Sevin, Spinosad, Pyrethrin, and Imidacloprid) in the flour beetle, *T. castaneum*. In our

11   studies, we used a global distribution of populations (South America, North America, Europe,

12   India, and Africa) and a laboratory strain (c-SM), sequestered in the laboratory before 1940, i.e.,

13   before any of the tested commercial insecticides were developed. From all populations except that

14   from Peru, we also derived a population of inbred lines by twelve generations of brother-sister

15   mating. It is unlikely that flour beetles, which are common pests of stored products harvested for

16   human consumption, have been targeted by direct application of any of the insecticides we studied.

17   However, empty grain bins are routinely treated with insecticides, like methoxychlor, malathion

18   or methoprene, prior to grain storage as a deterrent to flour beetle colonization. In addition, one of

19   our experimental populations was collected in Bhopal, India, a few years after the explosion of a

20   carbaryl insecticide manufacturing plant. In another population (Peru), a decades' long mosquito

21   abatement campaign, involving repeated application of pesticides, may have inadvertently affected

22   *T. castaneum* (Griffing et al. 2013).

23   In this report, we first characterize the dosage response curves for lethality in each parent

6

1. population for each insecticide. Notably, we discovered nearly an 800-fold difference among

2. populations in resistance to some insecticides and, as expected, the naïve laboratory population

3. was among the most sensitive of populations to most insecticides. Second, we report our estimates

4. of the heritability (h^2) and genetic correlation (r_G) of resistance and cross-resistance, respectively,

5. within populations (e.g., Jackson et al. 2007; Tabashnik et al. 2009). These estimates were obtained

6. by testing the insecticide sensitivity of inbred lines around the LD_{50} and LD_{90} of the parent

7. populations from which the lines were derived by inbreeding. Lastly, we use our estimates of the

8. genetic variance and covariance of resistance to evaluate the efficacy of "windowing" as an

9. insecticide resistance management strategy, where the application of several insecticides is rotated

10. on a periodic basis.

11.

12. **Materials and Methods**

13. *Commercial Insecticides*

14. We tested the response of populations of *T. castaneum* to commercial formulations of five

15. insecticides encompassing five different classes of compounds. We purchased insecticides directly

16. from distributors (see Table 1 for specifications of each formulation). All formulations act to

17. disrupt neural transmission with the primary physiological targets being enzymes within the

18. cholinergic pathway, except for pyrethrin which targets voltage-gated ion channels (ffrench-

19. Constant et al 2004). Acetylcholine is a neurotransmitter that functions to send impulses between

20. cells at muscular junctions. Nerve transmission is terminated by the cleaving of acetylcholine by

21. acetylcholinesterase (AChE). Organophosphate and carbamate insecticides bind AChE

22. irreversibly, preventing the termination of nerve transmission and resulting in death by hyper

23. excitation and paralysis (ffrench-Constant et al 2004). Newer insecticides, such as spinosad and

7

1   imidacloprid, act as antagonists of the nicotinic acetylcholine receptor (nACR), also resulting in

2   death by hyper excitation and paralysis. The acetylcholine receptor is composed of 11 subunits,

3   which are bound differently by each insecticide (Perry et al 2011). Newer insecticide formulations

4   achieve their specificity by binding one or more of these subunits. Overall, these chemicals have

5   rapid and robust insecticidal effects prior to the evolution of resistance.

6

Table 1. Insecticides used in this study

Insecticide	Compound Class	Target Enzyme	Year Approved	Brand name
Malathion	Organophosphate	AChE	1956	Malathion
Carbaryl	Carbamate	AChE	1959	Sevin
Pyrethrin	Botanical (*Chrysanthemum*)	Sodium gated ion channels	1970	Pyrethrin Garden Insect Spray
Imidacloprid	Neonicotinoid	nACR	1994	Dominion 21
Spinosad	Microbial (*S. spinosa*)	nACR	1997	Monterey Garden Insect Spray

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8

9   *Species Description*

10   *Tribolium castaneum*, a major global pest of grain and stored products, is a model

11   laboratory organism used to develop and test important ecological and evolutionary concepts (e.g.,

12   Wade 2016). This species is believed to colonize grain stores through anthropogenic movement

13   because its own dispersal abilities are limited (Drury et al. 2016; but see Ridley 2011). The

14   combination of near obligate commensalism and limited dispersal results in a globally distributed

15   species composed of a multitude of genetically differentiated populations with an average,

16   pairwise, genomic F_{ST} of 0.180 (Demuth and Wade, 2007 a and b; Drury et al., 2009).

17   We used beetles from six populations: five populations have a wide global distribution

18   (South America, North America, Europe, India, and Africa) and one is a long-held laboratory strain

1 (c-SM). The origin of the outbred laboratory population, c-SM, is described in Wade (1977). The

2 other four populations were collected from various types of stored products over the last several

3 decades (Table 2). All wild caught collections originated from more than 50 non-virgin adults.

4 Subsequent stocks were maintained at a population size of > 500 individuals on a standard medium

5 (20:1, flour: brewer's yeast, by weight) and in 24 h darkness.

6

Table 2. *Tribolium castaneum* wild Population Information

Population	Collection Date	Collection Location
Bhopal, India	1989	Flour Granary
Chicago, Illinois (c-SM)	1938	Laboratory Strain
Dar es Salaam, Tanzania (Des)	1989	Market
Jerez, Spain	1998	Livestock Feed
Purdue, Indiana, USA	2008	Livestock Feed
Lima, Peru	1996	Market

7

8

9 *Inter-population Covariation Bioassays*

10 We assessed the resistance of adult beetles from each population in Table 2 to each of the five

11 insecticides in Table 1. We tested the lethality of Malathion and carbaryl at 12 concentrations of

12 each insecticide: 0.00005%, 0.00015%, 0.00050%, 0.001500%, 0.005000%, 0.01500%,

13 0.05000%, 0.1500%, 0.50000%, 1.500%, 5.000% and 15.000%. Since lethality was essentially

14 0.00 at the five lowest concentrations, we eliminated the four lowest concentrations from our tests

15 of pyrethrin, imidacloprid and Spinosad. Since commercial concentrations of Spinosad and

16 pyrethrin were 0.50% and 1.0%, respectively, we did not investigate the higher concentrations for

17 either insecticide.

18 We administered an insecticide by pipetting 2 mL of a solution at the desired concentration

19 onto 400 mg of whole grain flour in a small, 20 mL weigh boat and allowed the medium to dry

1   overnight into a chip. For each population and each dilution, we presented a chip to each of eight

2   replicate groups of 20 adults (6 populations x 8.2 dilutions on average x 6 treatments (5 insecticides

3   + control) x 8 replicates x 20 adults = 44,928 exposed beetles). Control media consisted of one

4   chip with 2 mL of distilled water, which we considered a 0.00 ppm dilution in our analysis (see

5   Analysis Section below). We recorded the number of dead adults in each replicate 18 and 65 hours

6   (139 hours for pyrethrin) after the adult beetles were introduced to a treated chip. From the

7   mortality data of these dilution series, we estimated the LD_{50} and LD_{90} for each population to each

8   insecticide (see Analysis Section below).

9

10 *Intra-population Covariation Bioassays*

11 From all populations but Peru, we created dozens of inbred lines by imposing twelve generations

12 of brother-sister mating. We assessed the levels of genetic variation (heritability) and covariation

13 (genetic correlations) of insecticide resistance by treating adult beetles from 12 inbred lines from

14 each population with each insecticide at the LD_{50} concentration of the corresponding, outbred

15 parent population. As above, we pipetted 2 mL of insecticide solution onto 400 mg of whole grain

16 flour in a weigh boat and allowed the medium to dry overnight into a chip. We presented one chip

17 to each of eight replicate groups of 20 adults (5 populations x 12 inbred lines x 6 treatments (5

18 insecticides + control) x 8 replicates x 20 adults = 57,600 exposed beetles). Controls for each line

19 consisted of one chip with 2 mL of distilled water, considered a 0.00 ppm dilution as above. For a

20 set of inbred lines, we recorded the numbers of dead adults in each replicate at the same time point

21 used to calculate the LD_{50} of their respective outbred parent population.

22 For the Purdue and Bhopal populations, we estimated the genetic variation (heritability)

23 and covariation (genetic correlations) of insecticide resistance using the inbred lines as above but

10

1   at the LD_{90} concentration of each outbred population. This study allowed us to compare the

2   heritabilities and genetic correlations of the LD_{50} and LD_{90} environments for these two

3   populations.

4

5   *Analysis*

6   For each insecticide at each observation time, we used a 4 parameter logistic regression to find the

7   LD_{50} or LD_{90} and its corresponding standard error for each population. The duration of exposure

8   in subsequent analyses was determined from observing the highest concentration that killed all

9   treated individuals and the lowest concentration that killed no individuals. This ensured the LD_{50}

10   would fall between these concentrations.

11

12   *Statistical Methods: Variance Components and Heritabilities*

13   We estimated heritability of resistance for each population from the inbred line means using

14   standard quantitative genetic methods (Lynch and Walsh 1998). When a sample of inbred lines is

15   large and chosen randomly with respect to the character to be analyzed, then differences among

16   line means provide an unbiased estimate of the genetic differences among lines for the characters.

17   Under the assumption that the inbred lines are homozygous at the contributing loci, strain

18   differences must be related to additive genetic variance and not to dominance interactions within

19   loci. The nature of the relationship of strain differences to additive genetic variance can be deduced

20   from the discussion of Crow and Kimura (1970, p. 100) concerning the effects of inbreeding on

21   character variance. The additive genetic variance among inbred lines [$V_a(i)$] is related to the

22   additive genetic variance in the randomly mating population [$V_a(r)$] from which they were derived

23   by the coefficient of inbreeding, f: $V_a(i) = V_a(r)(1+f)$. Thus, for high levels of inbreeding (i.e., f ~

1   1), the additive genetic variance in the population from which the inbred lines were derived can be

2   estimated as half the variance among the inbred line means. Narrow sense heritability (h^2) is the

3   fraction of the total variance due to additive genetic effects. We estimated the narrow sense

4   heritability from the inbred line values as $h^2 = $ ½Var(Among Inbred Line Means)/ [½Var(Among

5   Lines)+ Var(Within Lines)]. The within-line component of variance is a repeated measure,

6   estimated from multiple, near genetically identical, individuals for each trait.

7   We calculated the heritabilities from the observed among-line variance components

8   estimated from a generalized linear model with a binomial fit, using restricted maximum likelihood

9   (REML), followed by a delete-one-line jackknife. The jackknife has been shown to perform well

10   for estimating variance components, heritabilities and genetic correlations (Knapp et al., 1989;

11   Simons and Roff, 1994). In the delete-one jackknife, all observations from one line are deleted and

12   a 'pseudo- value' of the desired statistic is calculated. This process is repeated, until a pseudo-

13   value has been created for the set of inbred lines for each population. The estimate of the statistic

14   and its standard error are calculated from the mean and standard error of the full set of pseudo-

15   values (Roff, 2006).

16

17   *Statistical Methods: Genetic Correlations for Resistance*

18   We used standard analysis of variance procedures to partition the variance of measured resistance

19   to different insecticides within and between inbred lines. The same standard procedures will yield

20   a partitioning of the covariance for two traits if a synthetic variable, the sum of values for two

21   characters, is formed for each animal in every line and subjected to the same analysis. The

22   component of variance among lines for this synthetic variable contains the component of variance

23   among lines for each of the two characters singly plus twice their among-lines component of

12

1   covariance. This component of covariance is the bivariate analogue of the component of variance

2   among strains.

3

4   *Matrix Stability*

5   The genetic variance-covariance matrix (the 'g-matrix') summarizes the inheritance of multiple,

6   phenotypic traits. The stability of this summarizing parameter is important as it affects our ability

7   to predict how the phenotypic traits evolve by selection.   To determine the stability of the

8   covariance matrices among our populations, we compared our matrixes by the method of random

9   skewers (Cheverud and Marroig 2007; Calsbeek and Goodnight 2009; Pitchers et al. 2013). After

10 a set of random selection vectors is applied to each matrix in a pair of covariance matrices, the

11 vector correlation between the altered matrixes is measured. The strength of this correlation,

12 compared to the correlation of the random vectors, is a measure of the similarity of the matrices.

13 In addition to estimating the stability of g-matrices among populations, we also estimated the

14 stability of the LD_{50} and LD_{90} matrixes within populations.

15

16 *Truncation selection*

17 Response (R) to short-term selection is the function of heritability (h^2) and the selection differential

18 (S): $R = h^2 \cdot S$. This relationship is often called the breeders' equation. The between-generation

19 change, (the response to selection) R, is the change in means between the population before

20 selection and the population in the next generation. S is the within-generation change in the mean

21 due to selection as $S = \mu^* - \mu$ where μ is the population mean before selection and μ^* the mean of

22 the parents that reproduce (the population mean after selection). Thus, S is the difference between

23 selected parents and the population as whole (within generation). In the case of pesticide

13

1   application, S can be related back to the dose of insecticide applied. Assuming a normally

2   distributed polygenic basis for resistance, susceptibility should range from 0-1 with a mean of

3   0.50. If the population is treated at its LD_{50} concentration, the mean before selection will be 0.50

4   and the phenotypic mean of the individuals remaining post exposure will be the mean of the normal

5   distribution as if it was truncated at 0.50 and 1. Thus, we can calculate the phenotypic mean of

6   the post exposure individuals using the truncated normal distribution. Let u be the phenotypic

7   mean of the population, sigma be the calculated phenotypic standard deviation, a be the lower

8   bound of the distribution, the truncation point, and let b be the upper bound (in this case 1). The

9   Z-scores associated with the distribution are:

10
$$Z_a \frac{a - u}{\sigma}$$

11
$$Z_b \frac{b - u}{\sigma}$$

12   And the estimated phenotypic mean of the surviving individuals is then:

13
$$E(u_z) = u + \sigma \frac{\varphi(Z_a) - \varphi(Z_b)}{\Phi(Z_b) - \Phi(Z_a)}$$

14   where ϕ is the probability density function of the standard normal distribution, and Φ is the

15   cumulative distribution function of the standard normal distribution. Thus the selection differential

16   S is equal to the difference between phenotypic mean of the selected population ($E[u_z]$)) and the

17   unselected phenotypic mean (u), to give

18
$$S = u_s - u$$

19
$$R = u_o - u$$

20
$$R = h^2 S$$

21
$$u_o = h^2 \sigma \frac{\varphi(Z_a) - \varphi(Z_b)}{\Phi(Z_b) - \Phi(Z_a)} = u$$

1   From this, we can estimate u_o the between generational change in LD_{50}.

2

3   *Change in Correlated Characters*

4   The extent to which traits are genetically correlated allows us to predict the genetic change in trait

5   *Y* when truncation selection is applied a different but correlated targeted trait *X*. In this case, let

6   *Xp* be the population phenotypic mean for lethality to a particular pesticide, and let *Yp* be the

7   population mean for lethality to a second pesticide to which this population, this generation is not

8   exposed. We calculate *Xo* using the equations above. To determine what effect the pesticide

9   application will have on the population's resistance to the unapplied pesticides we calculate the

10   correlated change, where σ_{Ax} and σ_{Ay} are the additive genetic standard deviations of the applied

11   and the untreated pesticides, respectively. And, pG_{xy} is the additive genetic correlation of

12   resistance phenotypes between the two chemicals:

13
$$pG_{xy} = \frac{(X_o - X_p)(Y_o - Y_p)}{\sigma_{Ax}\sigma_{Ay}}$$

14

15

16

17   *Stochastic simulations*

18   We ran evolutionary projections with the equations above and the observed quantitative genetic

19   parameters estimated from our breeding experiments. The additive genetic variance,

20   environmental variance and heritabilities were generated at random from a truncated normal

21   distribution with a mean equal to the empirically estimated variance or heritability, a standard

22   deviation equal to standard deviation of the jackknifed among-line pseudo-values of the inbred

23   lines, and a truncation point at 0 to prevent the sampled variances from becoming negative.

15

1   Phenotypic variances for the simulation were the sum of the sampled additive and environmental

2   variance values. Phenotypic means for the first generation of simulations were the measured means

3   from our population work. In generations beyond 1, the phenotypic mean from the previous

4   generation (n-1) was used. For simulations in which only one insecticide is used, each generation,

5   a new random draw from the distribution of possible V_A, V_E, h^2 is used, the series of new means

6   are followed for a total of five generations. To produce 95% predictive intervals around these

7   estimates, the simulations are run 100,000 times and the bottom 2.50% and top 97.50% are used

8   as the range of the 95% predictive interval.

9   We also ran simulations to estimate evolutionary trajectories when insecticides with known

10   genetic correlations are rotated among generations. We used the same procedure as above, except

11   two trait means were followed simultaneously. Each with its own V_A, V_E, and h^2 distributions,

12   however, truncation selection is only applied to one trait each generation. The change in the

13   alternative trait is estimated using genetic change in correlated characters equations. As with the

14   single trait simulations, we use the genetic correlation and its error and the additive genetic

15   variance in the alternative trait as the sampling distributions for these estimates.

16

17   **Results**

18   *Variation among populations*

19   We discovered a wide range of resistance profiles among our populations and among insecticides.

20   Populations varied nearly 800-fold in susceptibility to Malathion with the Bhopal population

21   (LD_{50}: 4.441, SE +/- 0.166, n = 1920) and the Peru population (LD_{50}: 5.576, SE +/- 0.168, n =

22   1920) exhibiting the highest resistance. The Jerez (LD_{50}: 0.007, SE +/- 3.72 x 10^{-4}, n = 1920) and

23   the laboratory strain c-SM (LD_{50}: 0.011, SE +/- 4.26 x 10^{-4}, n = 1920) had the lowest resistance.

16

1   The differences among populations in their resistance to Sevin showed a similar but less extreme

2   pattern, with the Bhopal population (LD$_{50}$: 1.417, SE +/- 0.088, n = 1540) and the Peru population

3   (LD$_{50}$: 2.633, SE +/- 0.139, n = 960) again on the high end of the resistance profiles and Jerez

4   (LD$_{50}$: 0.786, SE +/- 0.051, n = 1700) and the laboratory strain c-SM (LD$_{50}$: 0.956, SE +/- 0.050,

5   n = 1680) on the susceptible end.

6   For Imidacloprid, the most recently introduced of the insecticides tested, we observed

7   intermediate variation in resistance among populations with a 5-fold difference between the

8   highest and lowest resistance profiles. Again, we found that Bhopal (LD$_{50}$: 0.945, SE +/- 0.063, n

9   = 1280) and Peru (LD$_{50}$: 0.898, SE +/- 0.049, n = 1280) were the most resistant populations but

10   Tanzania (LD$_{50}$: 0.161, SE +/- 0.011, n = 1280) and Purdue (LD$_{50}$: 0.222, SE +/- 0.013, n = 1280)

11   were the two most susceptible populations, more susceptible than the sheltered laboratory

12   population c-SM (LD$_{50}$: 0.395, SE +/- 0.022, n 1280).

13   All populations were highly sensitive to the certified organic insecticides and there was

14   little variation among-populations in LD$_{50}$. For pyrethrin, the LD$_{50}$ concentration showed a 2.6-

15   fold difference among populations with Peru (LD$_{50}$: 0.039, SE +/- 0.002, n = 600) and c-SM (LD$_{50}$:

16   0.033, SE +/- 0.002, n = 600) being the most resistant and Tanzania (LD$_{50}$: 0.016, SE +/- 0.001, n

17   = 600) and Jerez (LD$_{50}$: 0.015, SE +/- 0.001, n = 600) being the most susceptible. Spinosad, a

18   natural product of the soil bacterium *Saccharopolyspora spinosa* and relatively recently approved

19   as an insecticide (DATE), exhibited the lowest variation in LD$_{50}$ among populations with Peru

20   (LD$_{50}$: 0.149, SE +/- 0.009, n = 800) and Purdue (LD$_{50}$: 0.096, SE +/- 0.006, n = 800) being the

21   most resistance and Jerez (LD$_{50}$: 0.069, SE +/- 0.004, n = 800) and c-SM (LD$_{50}$: 0.070, SE +/-

22   0.006, n = 800) being the most susceptible.

23

1   *Genetic variation segregating within populations*

2   We detected substantial variation among populations for resistance to all pesticides, the largest

3   difference being the 796-fold difference in Malathion LD_{50} between the Bhopal and Jerez

4   populations. This variability suggests that the different histories of exposure to insecticides have

5   resulted in different levels of evolved insecticide resistance. We then turned to the question of the

6   extent the observed phenotypic variation around the population mean estimates (represented above

7   by their respective standard errors) was a result of genetic variation segregating among individuals

8   within a population. To estimate the genetic component of resistance variation, we used a set of

9   12 inbred lines. The variation among line means provides an estimate of the standing genetic

10   variation segregating with the original population and the variation among individuals within lines

11   is an estimate of the environmental or experimental variation. Each inbred line was exposed, in

12   replicate, to the population-by-insecticide specific LD_{50} to determine whether genetic variation

13   was segregating in the population for susceptibility/resistance to each insecticide.

14   We detected significant heritable genetic variation in all 25 insecticide-by-population

15   combinations with one exception, Tanzania treated with Sevin ($\sigma_A^2 = 0.0027$, SE +/- 0.0019, $p =$

16   0.1857; $h^2 = 0.4070$, SE +/- 0.2514, $p = 0.100$). Despite having the longest history of past selection

17   and some of the highest values of LD_{50}, Malathion heritabilities were extremely high (> 0.9) in

18   Purdue ($\sigma_A^2 = 0.0749$, SE +/- 0.0350, $p = 0.0554$; $h^2 = 0.9790$, SE +/- 0.0187, $p << 0.0001$),

19   Tanzania ($\sigma_A^2 = 0.1012$, SE +/- 0.0208, $p < 0.0005$; $h^2 = 0.9625$, SE +/- 0.0268, $p << 0.0001$), and

20   c-SM ($\sigma_A^2 = 0.1024$, SE +/- 0.0294, $p = 0.0025$; $h^2 = 0.9633$, SE +/- 0.0426, $p << 0.0001$). The

21   insecticide with the largest range of heritabilities was the recently introduced Imidacloprid with

22   Bhopal having the highest heritability ($\sigma_A^2 = 0.0201$, SE +/- 0.0096, $p = 0.0596$; $h^2 = 0.8113$, SE

23   +/- 0.0978, $p << 0.0000$) and Jerez ($\sigma_A^2 = 0.0014$, SE +/- 0.0006, $p = 0.0396$; $h^2 = 0.2927$, SE +/-

18

1   0.0708, $p = 0.0017$) the lowest. We found that h^2 was not correlated with a population's mean

2   resistance, measured by its LD_{50} ($r[LD_{50}, h^2] = 0.0405$, n = 25, N.S.). Thus, no matter how high

3   the mean resistance of a population to an insecticide, genetic variation for further resistance

4   remained within it.

5

6   *Genetic correlations among resistance phenotypes*

7   Estimation of a genetic correlation between two traits generally requires measuring the two

8   phenotypes on the same individual. This is not possible when the phenotypes are deaths on two

9   insecticides. However, because individuals from the same inbred line are nearly genetically

10   identical, we measured the response to two separate insecticides on different individuals from the

11   same set of inbred lines, whose degree of genetic relatedness is known. In this way, the correlation

12   between inbred line means can be related quantitatively to the genetic variation and covariation of

13   individual traits in the population from which the inbred lines were derived. Using this method,

14   we estimated the genetic correlations reported in Tables 3 and 4.

15   Genetic correlations estimated near the parent population LD_{50} ranged from a high of

16   +0.840 to Sevin and Imidacloprid sensitivity for the Jerez population to a low of -0.628 for the

17   Purdue population between sensitivity to Sevin and Malathion (Table 3). Genetic correlations

18   between the same two insecticides varied widely among populations. In the most extreme case,

19   resistance to Malathion and Sevin were highly positively correlated (+0.796) in the Tanzania

20   population but highly negatively correlated (-0.620) in the Jerez population. Thirty-eight percent

21   of the 50 correlations estimated were significant. Among the 19 significant genetic correlations,

22   positive genetic correlations outnumbered negative correlations 16 to 3.

23

19

1   *Stability of correlations among populations*

2   A random skewers test (see above) showed that the genetic variance-covariance matrixes for the

3   Purdue, Bhopal and Tanzania populations were significantly similar to one another (Table 6). Jerez

4   was also quantitatively similar to these but not significantly so, while the genetics of the laboratory

5   c-SM population were the least similar to those of the four wild populations.

6

7   *Stability of correlations among concentrations*

8   Only two populations, Purdue and Bhopal, were investigated for genetic correlations in the LD_{90}

9   response of inbred lines to multiple insecticides (Table 4). The correlation between inbred line

10   mean responses to an insecticide at its LD_{50} and LD_{90} tended to be high and positive (Table 5):

11   five of the eight measured correlations were significantly positive, while another was borderline

12   significant (p = 0.052). A random skewers test (see above) showed that genetic variance-

13   covariance matrixes for the Purdue population estimated at the LD_{50} and LD_{90} were essentially

14   identical (r = +0.95, p = 0,008). Those of Bhopal were similar but not significantly so (Table 6).

15

16   *Projection and predictive intervals*

17   We used the estimated narrow sense heritabilities, h^2 (Tables 1 and 2), and the genetic correlations

18   (Tables 3 and 4) to project the evolution of insecticide resistance in response to the application of

19   one insecticide or a rotation between two pesticides at the population's LD_{50}. We report

20   evolutionary change due to insecticide treatment as the fractional change in the LD_{50} in Figure 3.

21   We used the genetic correlations with heritabilities to estimate the expected change in LD_{50} of one

22   insecticide due to the treatment of the population with a different insecticide. As resistance

23   evolves, application of an LD_{50} insecticide concentration becomes increasingly futile (Table 9).

20

1   From the stochastic projections, using parameters estimated from the Purdue population,

2   Malathion resistance would more than double (1.011-fold increase, 95% CI: 0.83-1.14) in just 5

3   generations of exposure at its LD_{50}. Similarly, the two most recently approved insecticides would

4   have a greater that 50% increase in their resistance profiles after 5 generations of treatment

5   (Imidacloprid: 0.563-fold increase, 95% CI: 0.32-0.82; Spinosad: 0.691-fold increase, 95% CI:

6   0.32- 1.07). We also estimated h^2 and r_G for two populations at the LD_{90} concentration. We find

7   that heritabilities and genetic correlations between treatments to remain constant across

8   concentrations (Table 5) as well as between populations (Table 6).

9   To illustrate how the efficacy of windowing depends jointly on the heritability (h^2) and the

10   genetic correlation (r_G), we explored 4 different insecticide application strategies for controlling

11   the Purdue population (see Figure 3). Resistance evolves most rapidly when an insecticide of high

12   heritability is applied continuously, generation after generation (Table 9). When the same

13   insecticide is applied periodically, resistance evolves at a fraction of the continuous rate. However,

14   the evolution of resistance is nearly halted by windowing or rotating every generation between two

15   insecticides with a negative genetic correlation of similar magnitude to the heritability. For

16   example, in simulations, rotating the insecticides Sevin and Malathion results in significantly

17   impeding the evolution of resistance to both chemicals. By generation 5, the population has been

18   exposed to Malathion 3 times and resistance to the chemical has only increased 12.8% (95% CI: -

19   0.19-0.46). Whereas, if the population was treated with Malathion in 3 sequential generations the

20   resistance profile would increase 60.6% (95% CI 0.46-0.70). A slowing of the evolution of

21   resistance by 47.5%. The population would have been exposed to Sevin twice, and because of the

22   negative correlation between the two insecticides, the population actually becomes more

23   susceptible to Sevin treatment (-0.076-fold change, 95% CI -0.37-0.22).

1   However, windowing between insecticides with a positive genetic correlation is

2   comparable to continually applying a single insecticide. In the case where the genetic correlation

3   is positive and larger than the heritability, windowing could accelerate the rate at which resistance

4   evolves. For example, rotating Imidacloprid with Sevin actually hastens the evolution of resistance

5   to Sevin. After five generations of rotation (3 Imidacloprid, 2 Sevin) there is a 0.248-fold increase

6   (95% CI 0.03-0.47) in resistance to Sevin when just 2 sequential treatments of Sevin by itself only

7   produces a 0.092-fold increase (95% CI -0.07-0.27). This change in resistance to Sevin is higher

8   that of 5 sequential Sevin treatments alone (0.230-fold increase, 95% CI-0.03-0.51).

9

10   **Discussion**

11   Current agricultural practices rely heavily on insecticides to control most pest species. As every

12   economically significant insect order evolves resistance to these chemicals, it is imperative to

13   develop novel genetic tools for pest control to preserve crop yields and limit disease transmission.

14   Newer strategies of pest control, such as transgenic crops and endonuclease and microorganism

15   mediated gene drives (Esvelt et al. 2014), aim to decrease dependence on insecticides, which are

16   becoming less effective and can have negative side effects on particular ecological communities

17   (Whitehorn et al. 2012). While these technologies are in the process of development, insecticide-

18   usage strategies like windowing may prolong the efficacy of the current pest control methods,

19   allowing for more time for innovation while still maintaining economic yields.

20   We find that populations are highly variable from one another in their sensitivity to

21   insecticides and the data suggest that some of this variation is an evolutionary product of their

22   history of exposure to insecticides. Specifically, two of our wild populations, Bhopal and Peru,

23   have had a history of a brief but intense exposure (Bhopal) or long term exposure (Peru) to

22

1   Malathion and chemically related insecticides. Beetles from both populations are much more

2   resistant to pesticides overall than are beetles from c-SM, the naïve laboratory population. Another

3   aspect of our findings also indicates the importance of a population's history of exposure to

4   insecticides. In general, among the five wild populations, we observed significantly greater

5   resistance to the older insecticides, Malathion and Sevin, and much lower resistance to the more

6   recently introduced insecticides, Imidacloprid and Spinosad. Moreover, the variation among

7   populations in resistance is greater for the older insecticides and much lower for those more

8   recently introduced.

9   The large differences in resistance between and within populations allowed us to estimate

10   the heritability of resistance to each insecticide as well as the genetic correlation of resistance

11   across insecticides. These genetic estimates allowed us to test the efficacy of the pest control

12   strategy of 'windowing,' whose efficacy depends upon rotating the application of negatively

13   genetically correlated insecticides. Surprisingly, we found instances of negative genetic

14   correlations in resistance to insecticides sharing the same mechanism of action (Purdue population)

15   as well as the converse, namely, positively correlated insecticides acting on entirely different

16   pathways (Spinosad and Pyrethrin resistance in the Tanzania population). Although integrated pest

17   management strategies have been designed to insure rotation of insecticides with different modes

18   of action, the evolution of insecticide resistance depends critically on the sign and magnitude of

19   the genetic correlation of resistance between the rotated insecticides. As we showed in Figure 7, a

20   negative genetic correlation is critical to limiting or preventing the evolution of insecticide

21   resistance. Rotation between insecticides with a positive genetic correlation of pest resistance can

22   lead to more rapid evolution of resistance to both pesticides than would the application of only a

23   single pesticide. Unfortunately, our data indicate that the sign and magnitude of a genetic

23

1 correlation depends not only on the pair of insecticides to be rotated but also on the specific host

2 population targeted for control. That is, rotating between Malathion and Sevin would limit the

3 evolution of resistance in the Purdue population, where the genetic correlation of resistance is

4 negative, but rotation of the same two insecticides would accelerate the evolution of resistance in

5 the Tanzania population, where the genetic correlation is positive.

6 We estimated heritability and genetic correlations at both the LD_{90} as well as the LD_{50}

7 concentration for all insecticides for two pest populations and found that the genetic correlations

8 of resistance were nearly constant across the two concentrations. This finding is important, if it

9 proves to be general. Constancy of the genetic correlations of resistance across insecticide

10 concentrations permits the development of a management strategy that remains effective even as

11 resistance profiles evolve. Our findings support insecticide rotation as an efficacious strategy for

12 limiting or halting the evolution of insecticide resistance when the genetic correlations among the

13 rotated insecticides are negative. Therefore, we recommend the estimation of the heritability and

14 genetic correlations of resistance between insecticides and to develop application strategies based

15 on negatively correlated genetic responses while avoiding simultaneous or sequential use of

16 insecticides for which pest resistance is positively genetically correlated.

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19 *Acknowledgments*

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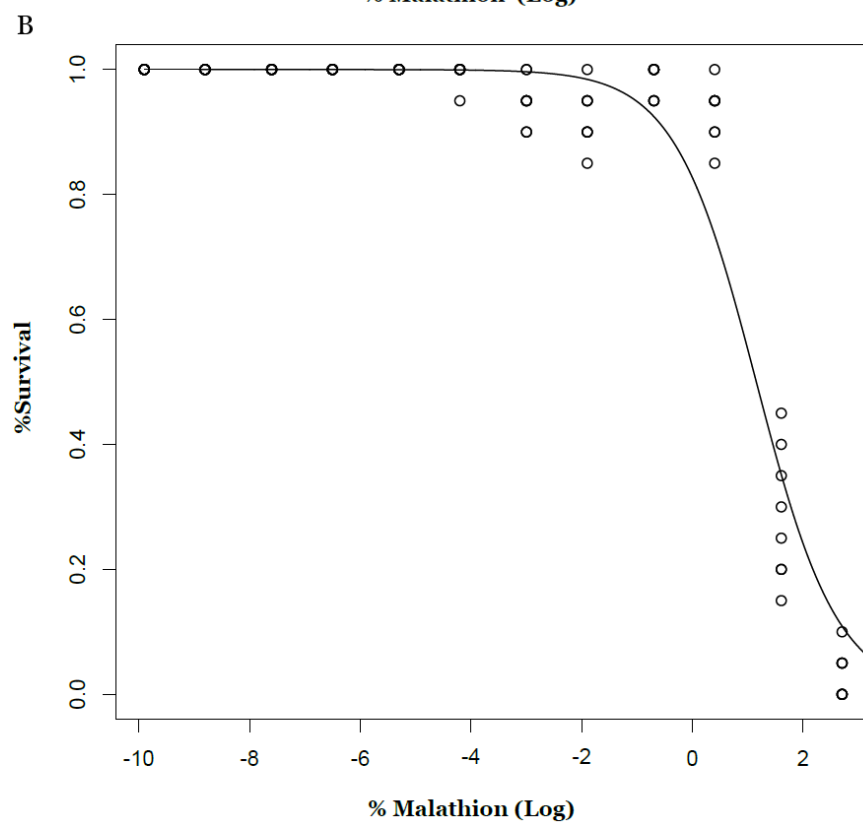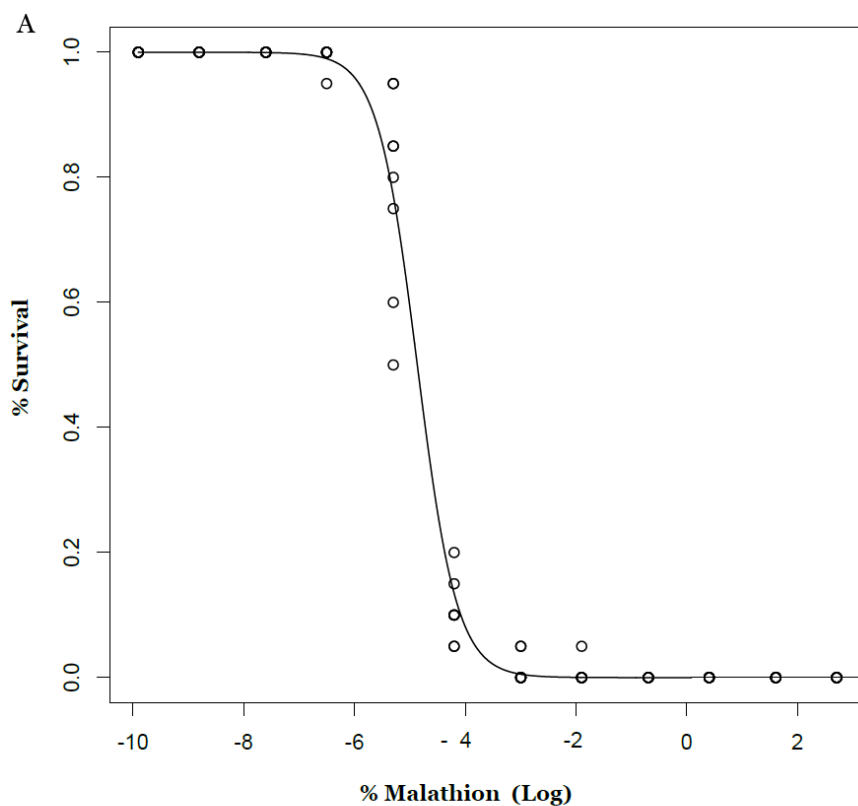
1   **Figure 1.** Estimation of the Malathion LD50 for the c-SM laboratory population (A) and Bhopal population (B). At the

2   dosage of 0.0106 (SE: 0.0004) and 4.441 (SE: 0.166), there is 50% survivorship of c-SM and Bhopal adults respectively.

3   A curve, as shown above, was generated for each population on each insecticide at 2 different time points, 18 and

4   65 hours. See supplementary material for all 60 curves.

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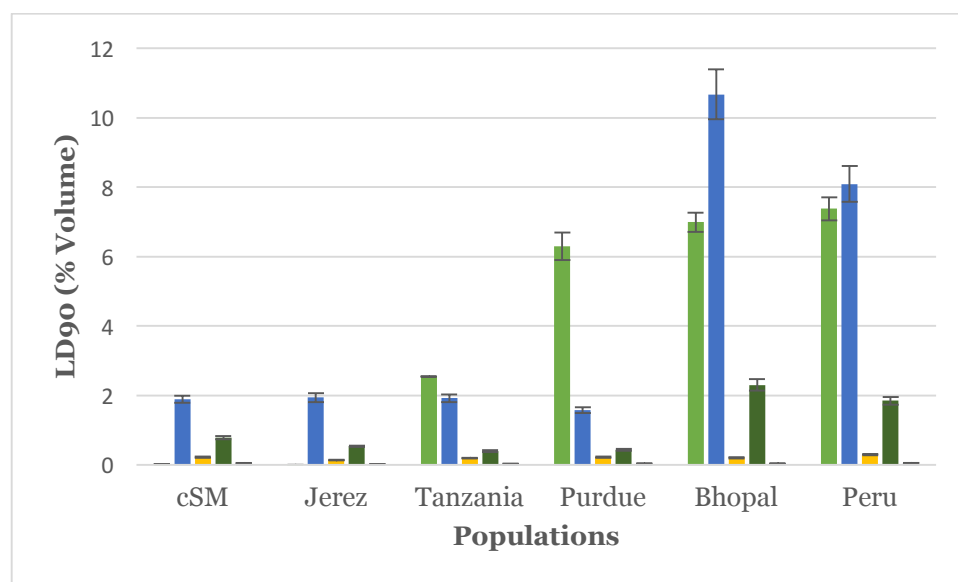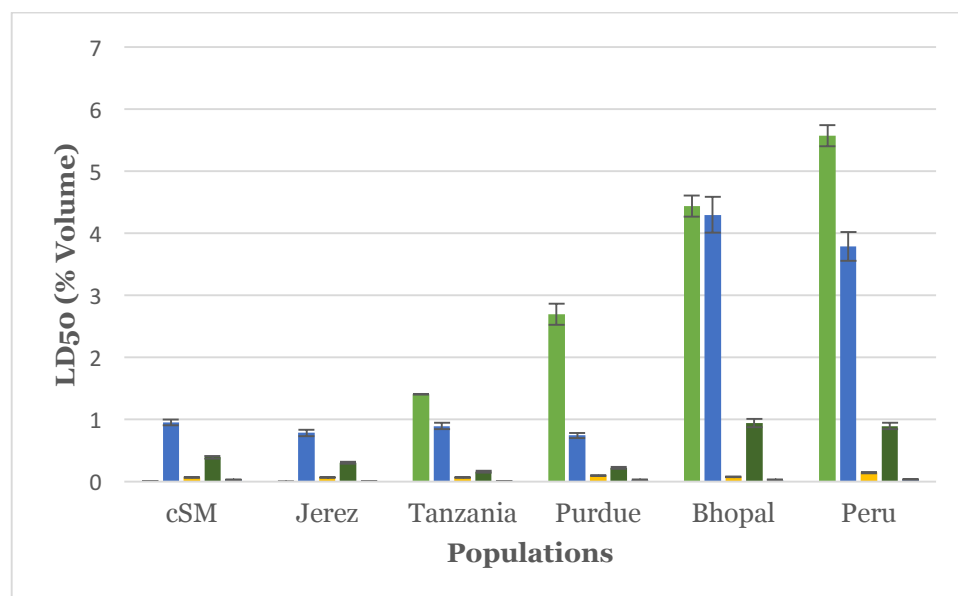
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5   **Figure 2.** The average concentrations in percent by volume of the insecticides, reading from left to right Malathion

6   (light green), Sevin (blue), Spinosad (yellow), Imidacloprid (dark green), Pyrethrin (dark blue), required to kill 50%

7   (LD_{50}, A) or 90% (LD_{90}, B) of adult beetles from the six populations listed along the x-axis.

8

Table 3. The additive variance (σ_A^2), the environmental or error variance (σ_E^2) and the heritability (h^2) of the resistance phenotype for each insecticide applied to each population at the LD50 of that population.

	Purdue			Bhopal			DES			Jerez			cSM		
	σ_A^2	σ_E^2	h^2	σ_A^2	σ_E^2	h^2	σ_A^2	σ_E^2	h^2	σ_A^2	σ_E^2	h^2	σ_A^2	σ_E^2	h^2
Malathion	0.075	0.002	**0.979**	0.043	0.017	**0.715**	0.101	0.004	**0.962**	0.024	0.005	**0.834**	0.075	0.002	**0.979**
SE	0.035	0.001	0.019	0.01	0.005	0.087	0.021	0.003	0.027	0.009	0.002	0.031	0.035	0.001	0.019
p-value	0.055	0.043	<0.001	0.001	0.003	<0.001	<0.001	0.144	<0.001	0.027	0.058	<0.001	0.055	0.043	0
Sevin	0.004	0.006	**0.362**	0.01	0.012	**0.518**	0.003	0.004	0.407	0.01	0.005	**0.682**	0.004	0.006	**0.362**
SE	0.001	0.002	0.138	0.007	0.002	0.229	0.002	0.002	0.251	0.006	0.003	0.062	0.001	0.002	0.138
p-value	0.014	0.007	0.026	0.176	<0.001	0.045	0.186	0.041	0.1	0.125	0.13	<0.001	0.014	0.007	0.026
Imidacloprid	0.017	0.009	**0.67**	0.02	0.005	**0.811**	0.014	0.01	**0.595**	0.001	0.001	**0.293**	0.017	0.009	**0.67**
SE	0.005	0.001	0.094	0.01	0.002	0.098	0.005	0.002	0.1	0.001	0.001	0.071	0.005	0.001	0.094
p-value	0.063	<0.001	<0.001	0.002	0.001	0.001	0.015	<0.001	<0.001	0.004	<0.001	0.002	0.063	<0.001	<0.001
Spinosad	0.02	0.008	**0.772**	0.017	0.019	**0.466**	0.017	0.013	**0.556**	0.028	0.008	**0.766**	0.02	0.008	**0.729**
SE	0.01	0.001	0.136	0.004	0.004	0.097	0.006	0.003	0.117	0.008	0.001	0.05	0.01	0.001	0.136
p-value	0.008	0.008	<0.001	<0.001	0.013	<0.001	0.015	0.003	<0.001	<0.001	0.001	<0.001	0.008	0.008	<0.001
Pyrethrin	0.008	0.006	**0.588**	0.059	0.024	**0.713**	0.046	0.009	**0.829**	0.032	0.017	**0.657**	0.008	0.006	**0.588**
SE	0.005	0.002	0.13	0.011	0.006	0.086	0.012	0.004	0.076	0.01	0.005	0.106	0.005	0.002	0.13
p-value	0.13	0.023	0.001	<0.001	0.003	<0.001	0.002	0.028	<0.001	0.01	0.004	<0.001	0.13	0.023	0.001

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Table 4. The additive variance ($\sigma A2$), the environmental or error variance ($\sigma E2$) and the heritability ($h2$) of the resistance phenotype for each insecticide applied to each population at the LD90 of that population.

	Purdue			Bhopal		
	$\sigma A2$	$\sigma E2$	h^2	$\sigma A2$	$\sigma E2$	h^2
Imidacloprid	0.015	0.014	**0.522**	0.002	0.003	**0.391**
SE	0.007	0.004	0.144	0.001	0.001	0.103
p-value	0.027	0.005	0.001	0.009	0.002	<0.001
Malathion	0.072	0.015	**0.824**	0.033	0.012	**0.73**
SE	0.03	0.005	0.053	0.008	0.004	0.103
p-value	0.025	0.022	<0.001	0.002	0.012	<0.001
Sevin	0.003	0.006	*0.341*	0.008	0.011	**0.402**
SE	0.002	0.002	0.154	0.004	0.003	0.118
p-value	0.098	0.005	0.055	0.095	0.005	<0.001
Spinosad	0.018	0.011	**0.625**	0.015	0.019	**0.443**
SE	0.011	0.003	0.195	0.004	0.004	0.11
p-value	0.144	0.003	0.004	0.002	0.001	<0.001

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Table 5. Genetic correlations (rG) between insecticides for each population at the LD_{50} below the diagonal and the significance level (p) above the diagonal.

Purdue		Imidacloprid	Malathion	Pyrethrin	Sevin	Spinosad
	Imidacloprid		0.762	0.002	0.002	0.407
	Malathion	-0.052		0.029	0.036	0.077
	Pyrethrin	**0.570**	**0.384**		0.291	0.095
	Sevin	**0.631**	**-0.628**	0.553		0.618
	Spinosad	0.361	0.570	0.337	-0.190	
Bhopal		Imidacloprid	Malathion	Pyrethrin	Sevin	Spinosad
	Imidacloprid		0.002	<0.001	0.900	0.005
	Malathion	**0.622**		0.218	0.665	0.160
	Pyrethrin	**0.698**	0.350		0.280	0.121
	Sevin	0.163	-0.057	0.268		0.329
	Spinosad	**0.576**	0.440	0.576	0.236	
Tanzania (DES)		Imidacloprid	Malathion	Pyrethrin	Sevin	Spinosad
	Imidacloprid		0.196	0.096	0.680	0.001
	Malathion	0.414		0.012	0.420	<0.001
	Pyrethrin	0.581	**0.712**		0.353	<0.001
	Sevin	0.219	0.181	-0.231		0.304
	Spinosad	**0.756**	**0.796**	**0.796**	0.225	
Jerez		Imidacloprid	Malathion	Pyrethrin	Sevin	Spinosad
	Imidacloprid		0.172	0.682	<0.001	0.337
	Malathion	0.538		0.664	0.010	0.003
	Pyrethrin	0.159	-0.114		0.741	0.035
	Sevin	**0.840**	**0.780**	-0.057		0.017
	Spinosad	-0.275	**-0.620**	**0.440**	**-0.532**	
cSM		Imidacloprid	Malathion	Pyrethrin	Sevin	Spinosad
	Imidacloprid		0.016	0.455	0.013	0.555
	Malathion	**0.556**		0.116	0.016	0.741
	Pyrethrin	-0.246	-0.446		0.370	0.999
	Sevin	**0.547**	**0.558**	0.260		0.393
	Spinosad	0.217	0.170	0.007	0.353	

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Table 6. Genetic correlations (rG) between insecticides for each population at the LD90 below the diagonal and the significance level (p) above the diagonal. (No values for Pyrethrin are included due to timing constraints)

Purdue		Imidacloprid	Malathion	Sevin	Spinosad
	Imidacloprid		0.801	0.082	0.839
	Malathion	0.121		0.749	0.065
	Sevin	0.480	-0.121		0.590
	Spinosad	-0.090	0.676	-0.182	
Bhopal		**Imidacloprid**	**Malathion**	**Sevin**	**Spinosad**
	Imidacloprid		0.263	0.502	0.386
	Malathion	-0.313		0.021	<0.001
	Sevin	0.229	**0.480**		0.708
	Spinosad	0.308	**-0.835**	-0.103	

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Table 7. Correlation between response at LD50 and response at LD90. (No values for Pyrethrin are included because at the higher dosage it was lethal to all beetles from all populations)

	Purdue		Bhopal	
	Covariance	Correlation	Covariance	Correlation
Imidacloprid	0.008	**0.510**	0.004	0.571
SE	0.004	0.213	0.002	0.234
p-value	0.093	0.043	0.843	0.052
Malathion	0.052	**0.697**	-0.016	-0.428
SE	0.023	0.167	0.009	0.245
p-value	0.060	0.002	0.137	0.165
Sevin	0.002	**0.606**	0.007	**0.852**
SE	0.001	0.279	0.005	0.291
p-value	0.267	0.048	0.048	0.004
Spinosad	0.002	0.114	0.013	**0.900**
SE	0.006	0.319	0.004	0.051
p-value	0.724	0.863	0.205	<0.001

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Table 8. Similarity among genetic varience-covariance matrices with respect to their predicted evolutionary responses estimated by the method of random skewers.

G-matrix Similarity Among Populations

	Purdue	Bhopal	Des	Jerez	cSM
Purdue		0.064	0.031	0.214	0.182
Bhopal	**0.695**		0.026	0.081	0.212
Des	**0.795**	**0.815**		0.125	0.224
Jerez	0.397	0.657	0.55		0.179
cSM	0.451	0.409	0.372	0.456	

G-matrix Consistancy Among Lethal Doses (50:90)

	r	p
Purdue	**0.95**	0.008
Bhopal	0.53	0.17

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Table 9. Means and 95% predictive intervals for stocastic simulations of the fractional increase of insecticide resistence when the Purdue populations is exposed to the chemical at its median lethal dose.

	Generation of Exposure				
	1st	**2nd**	**3rd**	**4th**	**5th**
Imidacloprid	0.112	0.225	0.338	0.450	0.563
2.50%	0.01	0.08	0.15	0.24	0.32
97.50%	0.23	0.39	0.54	0.68	0.82
Malathion	0.202	0.404	0.606	0.808	1.011
2.50%	0.10	0.28	0.46	0.65	0.83
97.50%	0.25	0.48	0.70	0.92	1.14
Pyrethrin	0.090	0.179	0.268	0.358	0.448
2.50%	-0.04	-0.01	0.03	0.08	0.14
97.50%	0.24	0.39	0.52	0.65	0.77
Sevin	0.046	0.092	0.138	0.184	0.230
2.50%	-0.07	-0.07	-0.06	-0.05	-0.03
97.50%	0.18	0.27	0.35	0.43	0.51
Spinosad	0.138	0.276	0.415	0.553	0.691
2.50%	-0.02	0.05	0.13	0.23	0.32
97.50%	0.32	0.52	0.71	0.90	1.07

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Table 10. Means and 95% predictive intervals for stocastic simulations of the fractional increase of insecticide resistence when the Purdue populations is exposed to differing chemicals with know genetic correlations at its median lethal dose. Negative Correlation: Malathion-Seven, Positive Correlation: Imidacloprid-Sevin, No Correlation, Malathion-Spinosad

	Generation of Exposure				
	1st	2nd	3rd	4th	5th
	Malathion	**Sevin**	**Malathion**	**Sevin**	**Malathion**
Malathion	0.112	0.092	0.120	0.066	0.128
2.50%	0.01	-0.12	-0.12	-0.21	-0.19
97.50%	0.23	0.24	-0.12	0.35	0.46
Sevin	-0.054	-0.011	-0.065	-0.022	-0.076
2.50%	-0.20	-0.19	-0.30	-0.28	-0.37
97.50%	0.09	0.18	0.17	0.24	0.22
	Imidacloprid	**Sevin**	**Imidacloprid**	**Sevin**	**Imidacloprid**
Imidacloprid	0.113	0.167	0.228	0.282	0.344
2.50%	0.01	0.03	0.02	0.06	0.07
97.50%	0.23	0.31	0.45	0.52	0.63
Sevin	0.054	0.097	0.151	0.194	0.248
2.50%	-0.03	-0.04	-0.01	0.00	0.03
97.50%	0.14	0.24	0.32	0.40	0.47
	Malathion	**Spinosad**	**Malathion**	**Spinosad**	**Malathion**
Malathion	0.202	0.310	0.481	0.588	0.760
2.50%	0.10	-0.07	0.05	0.01	0.15
97.50%	0.25	0.69	0.94	1.17	1.41
Spinosad	0.108	0.246	0.353	0.491	0.599
2.50%	-0.27	-0.16	-0.20	-0.09	-0.09
97.50%	0.48	0.66	0.91	1.07	1.28

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Supp. Table 1. Covariation of each insecticide pair for each population at the LD50. Below the diagnal: Estimate of the additive genetic covarience between the traits; Above the daignal: *p*-value of one sample, two tailed t-test for difference from 0.

Purdue		Imidacloprid	Malathion	Pyrethrin	Sevin	Spinosad
	Imidacloprid		0.848	0.079	0.023	0.378
	Malathion	-0.002		0.118	0.139	0.168
	Pyrethrin	0.007	0.011		0.436	0.202
	Sevin	0.005	-0.010	0.002		0.661
	Spinosad	0.007	0.022	0.004	-0.001	
Bhopal		Imidacloprid	Malathion	Pyrethrin	Sevin	Spinosad
	Imidacloprid		0.033	0.030	0.677	0.047
	Malathion	0.018		0.253	0.859	0.157
	Pyrethrin	0.024	0.017		0.422	0.101
	Sevin	0.002	-0.001	0.006		0.427
	Spinosad	0.011	0.012	0.018	0.003	
Des		Imidacloprid	Malathion	Pyrethrin	Sevin	Spinosad
	Imidacloprid		0.223	0.122	0.334	0.084
	Malathion	0.016		0.030	0.458	0.024
	Pyrethrin	0.015	0.048		0.444	0.024
	Sevin	0.001	0.003	-0.003		0.327
	Spinosad	0.011	0.022	0.022	0.002	
Jerez		Imidacloprid	Malathion	Pyrethrin	Sevin	Spinosad
	Imidacloprid		0.274	0.591	0.151	0.446
	Malathion	0.003		0.628	0.135	0.066
	Pyrethrin	0.001	-0.003		0.783	0.080
	Sevin	0.003	0.012	-0.001		0.168
	Spinosad	-0.002	-0.016	0.013	-0.009	
cSM		Imidacloprid	Malathion	Pyrethrin	Sevin	Spinosad
	Imidacloprid		0.093	0.492	0.056	0.485
	Malathion	0.023		0.089	0.084	0.662
	Pyrethrin	-0.007	-0.033		0.420	0.994
	Sevin	0.014	0.036	0.011		0.303
	Spinosad	0.004	0.008	0.000	0.010	

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Supp. Table 2. covariation of each insecticide pair for each population at the LD $_{90}$. Below the diagnal: Estimate of the additive genetic covarience between the traits; Above the daignal: p-value of one sample, two tailed t-test for difference from 0.

Purdue		Imidacloprid	Malathion	Sevin	Spinosad
	Imidacloprid		0.742	0.081	0.691
	Malathion	0.004		0.793	0.274
	Sevin	0.003	-0.001		0.598
	Spinosad	-0.001	0.024	-0.001	
Bhopal		**Imidacloprid**	**Malathion**	**Sevin**	**Spinosad**
	Imidacloprid		0.359	0.482	0.377
	Malathion	-0.003		0.142	0.007
	Sevin	0.001	0.009		0.570
	Spinosad	0.002	-0.019	-0.002	

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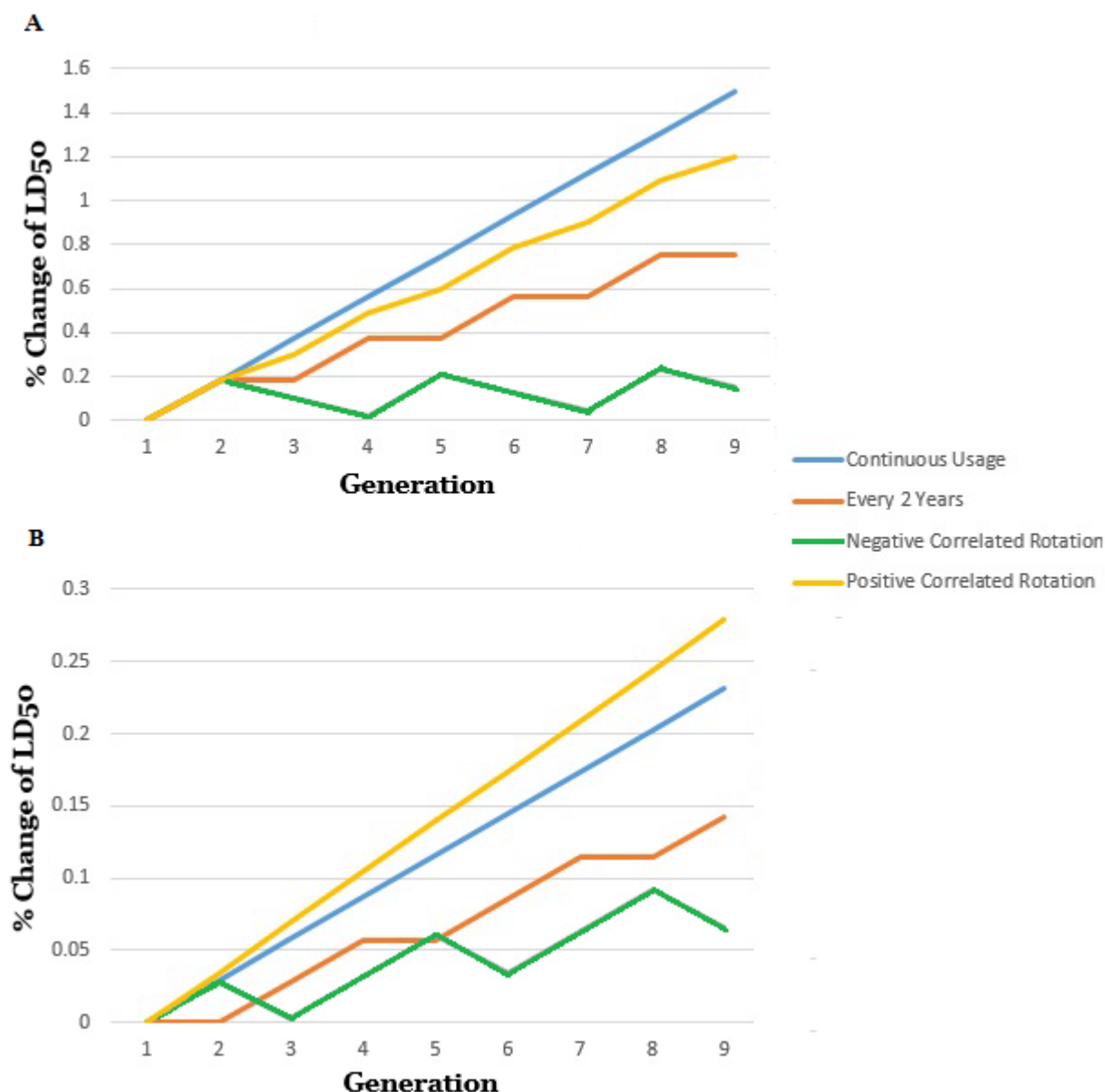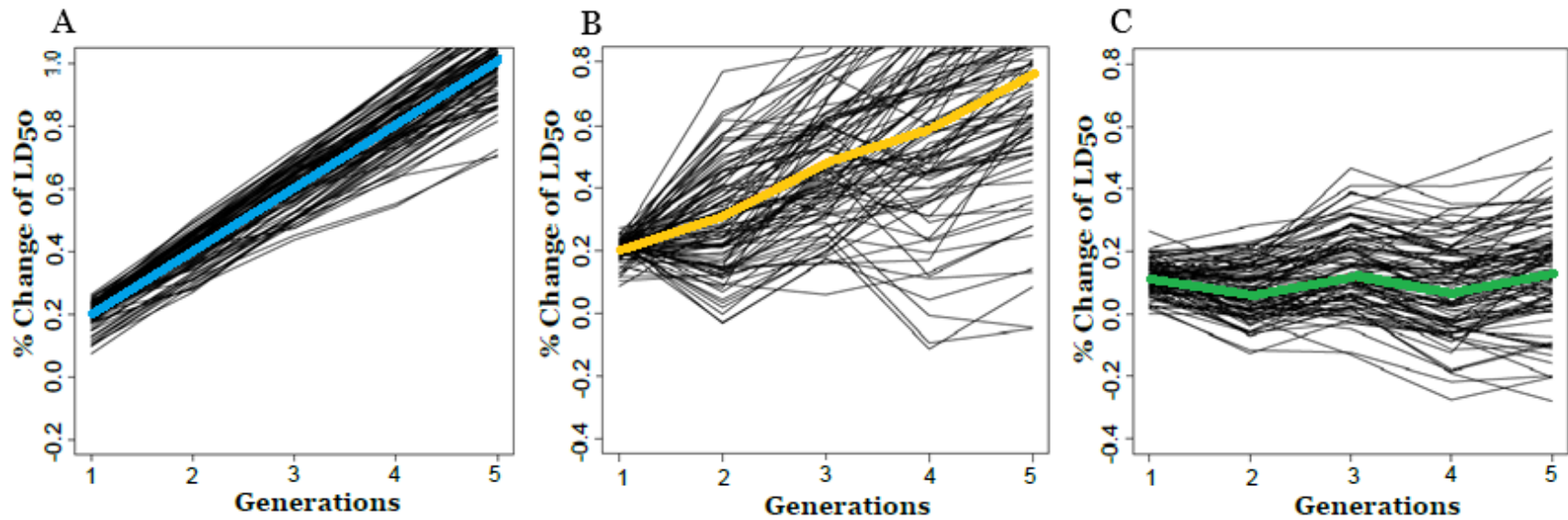11

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3   **Figure 3.** The estimated effects of four separate insecticide rotation strategies on the evolution of resistance towards

4   Malathion (A) and Sevin (B). 1) Continuous spraying with no rotation (blue). 2) Spraying one insecticide every two

5   years with no rotation (orange). 3) Yearly rotation between negatively correlated insecticides (gray). 4) Yearly

6   rotation between positively correlated insecticides.

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3   **Supp Figure 1.** The variance about the estimated effects of four separate insecticide rotation strategies on the evolution of resistance

4   towards Malathion (A) and Sevin (B). 1) Continuous application of Malathion with no rotation (blue). 2) application of one insecticide,

5   Malathion, every other year with no rotation (orange). 3) Yearly rotation between negatively correlated insecticides, Malthion and

6   Sevin (gray).