ABSTRACT

ZILNIK, GABRIEL LEON. The Evolution of Fitness Modifiers and Their Impact on Insecticide Resistance. (Under the direction of Fred Gould)

Insecticide resistance has been recognized as an important issue among scientists, agrochemical companies, and government policy makers. A large body of empirical and theoretical research has demonstrated the importance of product stewardship through monitoring for resistance. While a great deal about the molecular genetic basis of resistance has been explored theoretically an understudied area is the reduction of fitness costs to resistance. We developed a population genetic model that explores epistatic interactions in a two-locus system with one locus conferring insecticide resistance with deleterious fitness costs in the absence of spraying and the second locus modifying fitness costs of the resistance locus. While only a single empirical study has found modifiers present in wild populations we demonstrate a range of conditions where modifiers could evolve. Modifiers reduce the fitness costs through epistatic interactions with resistance alleles. In the presence of modifiers resistant genotypes have equal fitness to susceptible genotypes in periods of no spraying. We find modifiers spread readily through a population in periods of relaxed insecticide selection helping to preserve resistance alleles at high frequencies. Once modifiers enter the population resistance alleles cannot be removed from the population even after hundreds of generations without insecticide selection. We find that minor fitness costs associated with the modifier slows the spread of modifiers in a population because they negate any fitness advantage imparted by their interaction with resistance alleles. We believe modifiers could have a negative impact on rotational strategies for product stewardship as they prevent the rapid decrease of resistance alleles during periods of rotation. Modifiers have evolved under situations where resistance has been detected and product use has

continued for prolonged periods of time. Modifiers are difficult to detect by traditional screens as they only impact the population during periods of relaxed pesticide selection and thus can evolve quietly and quickly spread through a population. We discuss approaches to detect and mitigate modifier evolution in pest populations.

The Evolution of Fitness Modifiers and Their Impact on Insecticide Resistance

by
Gabriel Leon Zilnik

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APPROVED BY:

Fred Gould Committee Chair	Matthew Booker
R. Brian Langerhans	Alun Lloyd

DEDICATION

For Elizabeth and Raymond. Thank you for your unwavering support.

BIOGRAPHY

Gabriel Zilnik was born in Pittsburg, California and grew up in the Phoenix area of Arizona. He attended Arizona State University where he obtained a Bachelors degree in Anthropology from the School of Human Evolution and Social Change. While at ASU he worked with Dr. James Hagler at the United States Department of Agriculture. There he found he had an acute interest in agricultural pest management.

After obtaining a Fellowship with the Genetic Engineering and Society Center at North Carolina State University Gabriel continued to grow his interest in pest management. Here he worked as part of an interdisciplinary team to develop a chapter on genetic engineering approaches to vector management of the Dengue virus. Gabriel has increasingly become interested in agricultural regulations and policy. He plans to pursue a career as a regulatory affairs professional after the completion of his graduate work.

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Lastly I want to extend a special thanks to Fred Gould my committee chair. He pushed me to succeed and had faith while I struggled. Fred was an unending source of support and encouragement in times of defeat. This thesis would not have happened without his presence.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

THE EVOLUTION OF FITNESS MODIFIERS AND THEIR IMPACT ON INSECTICIDE RESISTANCE

APPENIX A

THE EVOLUTION OF FITNESS MODIFIERS AND THEIR IMPACT ON INSECTICIDE RESISTANCE

Introduction

Insecticide resistance, the ability for an insect to survive exposure to application of chemical toxins, has grown rapidly in prevalence in the last 50 years despite being first recognized over 100 years ago (Melander 1914). Increasingly, as the discovery of novel pesticides (particularly those with more positive environmental profiles) has become more capital intensive entomologists have turned their attention to detecting and mitigating insecticide resistance (Labbé et al. 2007; Roush 1989; Gould 2009). One aspect of insecticide resistance management is the tendency for resistance alleles to carry fitness costs that, in the absence of an insecticide, reduce one or more life history traits leading to lower contributions to subsequent generations (Kliot and Ghanim 2012; Gould et al. 2006; Roush 1989). Fitness costs allow strategies to slow or eliminate resistance over time, such as rotating chemistries or the planting of non-transgenic refuges. Yet empirical data shows that fitness costs can vary widely across populations and species and depends on the specific molecular mechanism by which resistance has evolved (Kliot and Ghanim 2012; Feyereisen, Dermauw, and Van Leeuwen 2015). Particularly disconcerting has been evidence of resistance fitness costs that are small or decrease over time (Gould et al. 2006; Feyereisen, Dermauw, and Van Leeuwen 2015). A low fitness cost of resistance encourages maintenance of resistance alleles in populations, even when resistance-generating pesticides are no longer used. For example, in the Colorado potato beetle, *Leptinotarsa decemlineata*, resistance alleles associated with

pyrethroids persist in localities where their use has discontinued in some cases for over a decade (Alyokhin et al. 2008; Piiroinen et al. 2013; Grapputo et al. 2005) .

The reasons why some resistance alleles have small or no fitness costs have received comparatively little attention over the last several decades. Several empirical papers on Australian sheep blowfly, *Lucilia cuprina*, in the 1980s demonstrated the evolution of an allele at a separate locus that improved the fitness of organophosphate-resistant flies, leading to the maintenance of resistance alleles in wild populations when insecticide selection was absent (Clarke and McKenzie 1987; McKenzie and Clarke 1988). We refer to this secondary allele as a modifier (herein: modifier allele or modifiers) whose function is to reduce or eliminate fitness costs associated with resistance to an insecticide. Beyond one experimental demonstration of evolution of a modifier allele, other data on the evolution of lower costs are based on observation of temporal changes of fitness costs of resistance (Prabhaker, Toscano, and Henneberry 1998; Feyereisen, Dermauw, and Van Leeuwen 2015; Tabashnik 1989). Furthermore, only one theoretical paper has touched on the topic of epistatic modifier evolution by providing a heuristic model and calling for future simulation studies (Uyenoyama 1986). We believe the evolution of modifier alleles deserves increased attention, as empirical evidence has already shown this type of interaction to evolve in at least one pestiferous species.

Under conditions where resistance evolves but chemical usage continues for many years or generations, modifiers may evolve and spread through a population (Clarke and McKenzie 1987; McKenzie 1996; McKenzie and Clarke 1988). The best-studied system in

which fitness costs and associated modifier loci have been measured is *L. cuprina*. (Kliot and Ghanim 2012; McKenzie and Clarke 1988; Feyereisen, Dermauw, and Van Leeuwen 2015; Clarke and McKenzie 1987). To determine if reduced fitness cost of resistance was an inherent trait of the resistance locus, McKenzie and Clarke (1988) crossed resistant and susceptible flies, and then subjected them to Diazinon (an organophosphate). Survivors were backcrossed 9 times to susceptible lines. The backcrossing eliminated the genetic background of the resistance locus, isolating the resistant (R) allele in a susceptible genetic background. They then repeated their competition experiment and found that susceptible flies out-competed backcrossed individuals (Clarke and McKenzie 1987; McKenzie and Clarke 1988; McKenzie 1996). Thus, a modifier locus that increased resistant individual fitness, and segregated independently of the resistant locus, was identified (McKenzie and Game 1987). This system shows that resistant populations of *L. cuprina* can maintain Diazinon resistance when competed against susceptible lines (McKenzie and Clarke 1988).

We theoretically address the evolution of epistatic interactions between modifier and resistance alleles that lead to reduced fitness costs on resistant alleles. Epistasis is the non-additive genetic effects of alleles at multiple loci, where an allele at one locus impacts the phenotypic expression of an allele at a second locus. Although, these loci can be genetically linked we assume that they are independently assorting in our model (see **Methods**). Epistasis has been implicated in the expression of complex phenotypes such as height or behavior (Labbé et al. 2007; Phillips 2008; Roush 1989; Mackay 2013; Gould 2009). In these cases alleles at multiple loci interact in a non-additive way to produce the phenotype of an

individual. That is to say, the final phenotype is not just the sum of the toxin is not present in the environment. Fitness is typically measured via life history traits (Roush and McKenzie 1987) yet resistant individuals may have lower fitness in some life history traits such as growth rate while having equal or higher fitness in other traits such as sexual competitiveness or lifespan (Roush and McKenzie 1987; Smith et al. 2011). Further, fitness can be modulated by other biological factors, such as sex (Kliot and Ghanim 2012; Smith et al. 2011; Gould et al. 2006; Roush 1989).

The sheep blowfly modifier remains the only empirical evidence of modifiers evolving in the wild. From a theoretical perspective, only a single heuristic model of modifiers has been developed, but not investigated thoroughly (Uyenoyama 1986). Uyenoyama's (1986) model explored the possibility of a detoxification locus (e.g. Cytochrome P-450, mixed function oxidase) metabolizing the insecticide. A mutation in the detoxification locus led to a resistant and susceptible locus within the population, wherein resistant individuals had much higher survivorship in the presence of the insecticide. Effectively, this metabolic process co-opts a necessary biological function but leads to reduced fitness in the absence of the insecticide. To overcome this fitness disadvantage when insecticide selection is low or absent Uyenoyama (1986) proposes the duplication of the locus leading to overexpression of detoxification enzymes. Further selection on the loci leads to specialization of the detoxification locus while the wild-type locus returns to serving the original metabolic function. Full or marginally reduced fitness would then be restored. Even if fitness is marginally reduced the resistant phenotype could persist in the environment as

has been demonstrated through models of low fitness costs in resistant genotypes (Gould et al. 2006). While this remains a plausible biological model of how reduced fitness costs could evolve Uyenoyama (1986) did not explore the model further, but called for future simulation studies to investigate the dynamics of such a system evolving. Our approach differs in that we assume a modifier locus that directly acts on the resistance locus, but we believe the same outcomes we observe to be applicable to Uyenoyama's system as it involves multiple interacting loci.

Here, we use simple population genetics models to explore the expected evolutionary trajectory of these so-called "modifier alleles" in the presence of resistance alleles. We first describe a two-locus model with a resistance locus and a modifier locus with varying degrees of dominance as described in the Results. We then quantitatively assess the impact of decreased fitness costs on resistance allele frequency to observe the impact on the evolution of resistance alleles. Finally, we qualitatively describe the modifier loci. This work has the potential to inform insecticide resistance management under conditions of long-term pesticide use. Furthermore, we hope this sparks interest in expanding the theoretical landscape of epistatic genetic interactions with respect to insecticide resistance evolution and resistance management.

Methods

We employ a bi-allelic, two-locus frequency model with selection (Hartl and Clark 2007). We assume a closed population, and do not consider population dynamics such as overlapping generations, age cohorts. We assume loci are unlinked and therefore,

independently assort. Following convention, R and S at the resistance locus represent resistant and susceptible alleles, respectively. The efficacy of the insecticide at lowering fitness is *k* (see Table 1 for variable definitions), so the fitness of the SS genotypes equals 1-*k*. We assume an insecticide efficacy (*k)* of 80%. The dominance of resistance (i.e., how much resistance a single R allele causes in a heterozygous individual relative to a homozygous resistant individual), is denoted by *r*. Thus, an SR individual has a fitness value of 1-*kr*. For simplicity, we assume RR genotypes are fully resistant to an insecticide. Thus, genotype frequencies at the resistance locus under insecticide selection are

$$S^2(1-k):2RS(1-kr):R^2$$

We explore resistance allele fitness costs that range from very low (0.05) to extremely high (0.5) (Tabashnik 1989; Roush 1989). We use a standard population genetic notation of s_R to denote the fitness cost of homozygous resistant individuals, and h_R to denote the dominance of that fitness cost. Modifying the initial frequencies with insecticide selection, we obtain:

$$S^2(1-k):2RS(1-kr)(1-s_Rh_R):R^2(1-s_R).$$

For the fitness modifying locus, we have a neutral (N) allele and a modifier allele (M). The N allele has no affect on fitness. The M allele itself can carry a fitness cost (s_2) and corresponding dominance (h_2). In addition, the M allele modifies the fitness of resistance allele carriers (z). For M heterozygotes, we include a dominance coefficient (h_3). Thus, we obtain 9 genotypes with selection as follows:

$$S^2N^2(1\text{-}k) : 2S^2NM(1\text{-}k)(1\text{-}s_Mh_M) : S^2M^2(1\text{-}k)(1\text{-}s_M) : 2SRN^2(1\text{-}kr)(1\text{-}s_Rh_R) :$$

$$4SRNM(1-kr)((1-s_R h_1(1-zh_z)))(1-s_M h_M) : 2SRM^2(1-kr)((1-s_R h_R(1-z)))(1-s_M) :$$

$$R^2N^2(1-s_R) : 2R^2NM((1-s_R(1-zh_z)))(1-s_M h_M) : R^2M^2((1-s_R(1-z)))(1-s_M).$$

When the population is released from insecticide selection, the two insecticide selection variables (k, r) go to zero, yielding genotype frequencies and fitness costs as:

$$S^2N^2 : 2S^2NM(1-s_M h_M) : S^2M^2(1-s_M) : 2SRN^2(1-s_R h_R) :$$

$$4SRNM((1-s_R h_R(1-zh_z)))(1-s_M h_M) : 2SRM^2((1-s_R h_R(1-z)))(1-s_M) :R^2N^2(1-s_R) :$$

$$2R^2NM((1-s_R(1-zh_z)))(1-s_M h_M) : R^2M^2((1-s_R(1-z)))(1-s_M).$$

We calculate allele frequencies for each generation. Again, we assume a closed, panmictic population, with alleles independently assorting. For simplicity of representing the fitness of both loci we follow Gillespie (2004) and Hartl and Clarke (2007), we substitute w_{ij} for relative fitness where i = 1, 2, 3 for SS, SR, RR and j = 1, 2, 3 for NN, NM, MM, respectively (Table 2). So, in generation t, we see that frequencies of genotypes before selection are:

Eq. 1

$$1 = S^2N^2 + 2S^2NM + S^2M^2 + 2SRN^2 + 4SRNM + 2SRM^2 + R^2N^2 + 2R^2NM + R^2M^2$$

Incorporating selection we obtain:

Eq. 2

$$\bar{w} = S^2N^2w_{11} + 2S^2NMw_{12} + S^2M^2w_{13} + 2SRN^2w_{21} + 4SRNMw_{22} + 2SRM^2w_{23} +$$

$$R^2N^2w_{31} + 2R^2NMw_{32} + R^2M^2w_{33}$$

We then determine allele frequencies in generation t + 1. Following the R allele we have:

Eq. 3

$$R' = \frac{2SRN^2w_{21} + 4SRNMw_{22} + 2SRM^2w_{23} + R^2N^2w_{31} + 2R^2NMw_{32} + R^2M^2w_{33}}{\acute{w}}$$

We can do the same for all alleles, which can be found in Supplementary Equations 1-3.

Taking the above formulae we built a simulation in R (version 3.3.0) to explore the impact of parameters on the evolution of R and M alleles (R Core Team, 2016). For brevity, we included a subset of simulated parameters that would likely interest resistance researchers (**Table 1**). Our R program is available upon request.

We ran simulations for a maximum of 500 generations. To explore specific resistance scenarios we included an end condition where the simulation was ended (included in figure captions when present). For example, we simulated the cessation of spraying and ran the simulation for 500 generations or until the resistance allele reached 10^{-3} in the population. Except where noted, we use initial modifier and resistance allele frequencies of 10^{-5}. We include a rotation schedule where an insecticidal spray is used for 20 generations and then stopped for 30 generations (except where noted), and is then repeated.

We anticipate that linkage disequilibrium (LD) may affect simulated outcomes. As stated earlier both alleles were independently segregating on separate chromosomes. To assess the level of LD we used the parameter D where D = SN*RM – SM*RN. We then used D to determine what impact, if any, LD had on our results by capturing haplotype frequencies at major time points where the rounds of spraying ended and began. For instance, if a rotation included 20 generations of spraying and then 30 generations of no spray we captured time points 21, 51, 71, 101, and 121 (time point 1 is the initial model condition).

The model was then seeded with those haplotype frequencies and allele frequencies were compared for differences.

Results

Modifier evolution is tied directly to resistance alleles through indirect selection. Modifiers increase the fitness of resistant genotypes (SR & RR). Under strong insecticide selection, genotypes carrying modifier and resistance alleles have higher fitness than genotypes not carrying modifiers. Thus, the rate of increase of M directly correlates to the fitness cost (s_R) of the R allele (**Figure 1**). When the R allele has zero fitness cost ($s_R = 0$) the M allele does not increase in frequency. The initial round of spraying only causes an increase of a single order of magnitude in M allele frequency in all cases explored. However this small increase sets the stage for further increases during the second round of spraying.

Unsurprisingly, M prevents the loss of the R allele by alleviating the fitness cost of resistance. In our simulation, insecticide selection follows 20 generations of spraying then 30 generations of no sprays (**Figure 2**). As expected, a neutral M allele maintains a high frequency throughout. As R allele fitness costs increase, however, we see a concomitant drop in the frequency of R allele during unsprayed time periods. This increase in s_R coupled with the neutral M allele leads to the R allele staying at a relatively high frequency in rotation periods (**Figure 2**). We see a decrease in the R allele during rotation when $s_R > 0$. That decrease in the R allele increases in amplitude as s_R gets higher, but we also see the M allele begin to increase in frequency much more rapidly as well. This mirrors what we might expect to see when rotation of an insecticide continues despite resistance evolving in the field. The

only thing that increases the fluctuation of M and R alleles during periods of no spray is when $s_M > 0$ (**Figure 3**). Fundamentally, once the M allele enters into the population rotation becomes an ineffective method to remove R alleles from the population. Under conditions where M alleles increase above 10^{-3}, R frequency never falls below 10^{-3}, despite hundreds of generations without insecticide use (**Supplementary Figure 4**). We observed an increase in the time to loss of R alleles when only 50% of the fitness cost of resistance is removed (**Supplementary Figure 4**), indicating that modifiers can have an impact even if they do not remove the full fitness cost of resistance alleles. During spraying, the M allele only impacts R allele frequency when $s_M > 0$. This impact is due to the fact that, as s_M increases, the fitness of RM haplotypes begins to approach the fitness of RN haplotypes. Effectively, $s_M > 0$ decreases the size of z below 1. Eventually, when $s_M > s_R$ RN haplotypes end up with a higher fitness overall (note that the left columns of **Figure 3** remain nearly identical for the first two rounds of selection). However, our results show that the impact of M remains low, since the intensity of insecticide selection ($k = 0.8$) greatly outweighs s_M. We observe a limited set of scenarios where the R allele could be eliminated from the population. The M allele would have to carry a substantial fitness cost that reduces RM and SM haplotypes to a lower fitness than RN haplotypes. The R allele would have to have a fitness cost as well to keep RN haplotypes at a lower fitness cost than SN haplotypes. Even then, it would require the cessation of insecticide spray early after detecting resistant phenotypes.

As modifiers arise *de novo*, their evolution is impacted by relatively low fitness costs (e.g. $s_M < 0.2$) on the modifiers themselves. If selection for resistance is low either through

intermittent sprays (e.g. only spraying once economic thresholds—taking action after the cost of application is less than the damage a pest would cause—are reached) or a low level of insecticide efficacy ($k < 0.2$), M alleles with fitness costs could be lost or kept at low frequencies ($< 10^{-5}$) before selection on RM genotypes can increase the frequency of M. (The latter situation would be unrealistic as an insecticide with only 20% efficacy would likely be abandoned.) Above $s_M = 0.2$, no realistic circumstances were observed (even when $s_R = 0.5$) where the intensity of selection against resistance could increase the frequency of the modifier prior to cessation of pesticide usage, unless the resistance allele becomes fixed in the population. Furthermore, the dominance of the modifier effect has a large impact on selection in its favor (**Supplemental Figure 1**). RM genotypes might increase in the population under high modifier costs ($s_M \geq 0.15$) and under intense selection against R alleles ($s_R \geq 0.2$). Once resistance becomes fixed in a population, modifiers will reach an equilibrium, but because of the indirect selection for the modifier allele, due to insecticide selection on R alleles, this equilibrium will only reach fixation when $s_M = 0$.

We did not observe an appreciable impact of LD in our simulations. After seeding the model from time points where spraying began (see **Methods**) we found very little qualitative difference between time points when we reran the model (**Figure 5**). We observe that when LD is set to 0 before each round of spraying there is a small delay in the increase of both R and M alleles while under a spray regime. There is also a decrease of R alleles during no spray periods. That said, by the end of the simulation R and M alleles converged to or near the same frequency as the simulations that began under initial conditions. Therefore, we

believe LD has a minor impact on final allele frequencies, but LD is greatly outweighed by the strength of selection on both alleles. This demonstrates that initial allele frequencies have a greater impact on model outcome than LD.

Discussion

While we show modifiers impact resistance evolution, other factors do explain the maintenance of resistance alleles at high levels. Cross-resistance, where a locus confers resistance to multiple insecticides, has been shown to lead to such maintenance. In Colorado potato beetle the maintenance of DDT resistance due to continued pyrethroid use has been observed in the US and Europe (Grapputo et al. 2005). Pyrethroids have a similar mode of action to DDT, such that mutations conferring resistance to DDT also act against Pyrethroids, helping maintain resistance (Grapputo et al. 2005; Piiroinen et al. 2013; Pittendrigh, Reenan, and Ganetzky 1997). Thus, even if large-scale use of DDT were required due to the failure of currently registered products, its efficacy would be short lived. The Insecticide Resistance Action Committee (IRAC) recommends rotating modes of action to delay resistance, with the assumption that mutations that confer resistance to multiple modes of pesticide action are less likely to arise than mutations that act on a larger spectrum of insecticides within a single mode of action While a mode-of-action-rotation approach may delay resistance, we show here that modifiers can still maintain R alleles at high frequencies, and that *de novo* modifiers actually increase when insecticide selection relaxes.

Recessive fitness costs can also foster maintenance of resistance in large populations. When at high frequencies, cessation of spraying leads to a rapid decline in R frequency. Yet

this decline leads to an increase of RS heterozygotes, such that R alleles are hidden from selection. Yet this period of relaxed insecticide selection also leads to increased selection on RM haplotypes, and on the M allele in general. This increased selection on RM haplotypes becomes especially relevant if modifiers carry a fitness cost. With non-zero fitness costs ($s_M > 0$), modifiers replace the fitness cost of R alleles when the interaction reduces s_R. Although M alleles may reduce enough of the fitness costs to allow for stochastic effects to fix R alleles in the population, a thorough exploration of stochastic effects were outside the scope of this study and should be investigated further.

In general fitness costs tend to be recessive (Bourguet and Raymond 1998). Because of this we discover an interesting dynamic: initial rounds of spraying cause large increases in R alleles as compared to M alleles. As M allele frequencies rise, selection in favor of M-carrying individuals rises to match the rate of R alleles. This increase appears to be caused by M alleles "hiding from selection". Further, this effect is magnified when modifiers act additively or recessively. While empirical data is lacking, we suggest that modifiers are most likely to have an impact on resistance evolution under recessive fitness costs and a dominant modifier effect. This seemed to be the case for Diazinon resistance in *L. cuprina* (Clarke and McKenzie 1987; McKenzie and Clarke 1988).

The most likely pathways for fitness modifiers to evolve and emerge are under long-term, intense insecticide selection. Under these circumstances, the fitness of R-carrying individuals may be optimized through the secondary evolution of fitness modifiers. Further, the evolution of multiple R alleles with varying fitness costs is not precluded under such

circumstances. Selection can become relaxed under an integrated management program where pest numbers do not reach economic thresholds and thus insecticidal sprays are not used. Under periods of relaxed selection (e.g. no spraying) modifiers increase in frequency. After crossing a low threshold M frequency (between 10^{-5} and 10^{-4}), we see a rapid rise due to hitchhiking when selection intensity increases (**Figure 2**). Another approach to managing resistance has been rotation of modes of action. Rotation of modes of action has support as a resistance management strategy despite mixed evidence of effectiveness (Gould et al. 2006; Prabhaker, Toscano, and Henneberry 1998; Tabashnik 1989). If modifiers are present, this makes rotational strategies dangerous as selection intensifies on RM haplotypes in rotational periods. Thus rotation may actually decrease the time to fixation of resistance as modifiers ameliorate any fitness costs of R alleles.

One major implication of our work is the importance of ongoing monitoring of competitive fitness between resistant and susceptible populations. Early in spray regimes, we predict that resistant genotypes will have reduced fitness compared with susceptible individuals (**Figures 1** & **2**). As selection continues, we expect M alleles to arise (though here we modeled them as present at low frequencies). Eventually, the proportion of RM haplotypes increases, with M-carrying genotypes yielding a greater average fitness over time of RN genotypes. As is the case with *L. cuprina,* Diazinon resistance carried a non-trivial fitness cost and resistance modifiers evolved and spread over a decade (McKenzie and Clarke 1988). This makes competitive fitness trials paramount to revealing and monitoring pesticide resistance. These trials would pit known susceptible populations against resistant

populations without pesticide pressure. This competitive approach effectively encapsulates all fitness parameters with the goal of revealing the overall fitness cost of resistance for a particular pesticide. Additionally, backcrossing of resistant populations to susceptible populations while selecting for pesticide resistance could reveal the presence of modifiers. However, we must keep in mind that modifiers would likely only be present at high frequencies after long-term pesticide selection. Early in a pesticide's life cycle we believe modifiers would be at such a low frequency ($< 10^{-5}$) that random sampling of populations would likely miss modifiers. We accept that competitive fitness trials can be more time consuming and laborious, as they require multiple generations of both susceptible and resistant populations and many pests may only have a single generation per year or prolong generation times. However, we do not believe it to be any more labor intensive than attempting to choose and monitor specific life history traits as these competitive trials encapsulate all fitness parameters such as mating competitiveness, life span, and fecundity, among others.

Selection on standing genetic variation or on novel mutations are the two main avenues for organisms to adapt to novel environments such as novel pesticides (Barrett and Schluter 2008). Rapid adaptation to novel pesticides can and has come from standing genetic variation that leads a base level of resistance in many pest species (Busi, Neve, and Powles 2012; Neve 2007; Gould, Anderson, and Jones 1997). The presence of resistance alleles in natural populations leads to rapid loss of chemistries due to the often-widespread adoption and intense usage of pesticides in modern agriculture. Our model treats resistance and

modifier alleles as standing genetic variation occurring together in nature. Due to the intense selection of the pesticide spray we observe rapid adaptation to the insecticide and a concomitant increase in the modifier allele when spraying is stopped. Further exploration of the introduction of novel mutations after pesticide sprays have started could be useful, but we do not believe that those results would differ much from our model's predictions.

Here, we assume modifiers completely alleviate fitness costs, but if fitness costs are only low or moderate, alleviating some of the cost may lead to maintenance of resistance alleles in a population for many generations. Using stochastic simulations, others have shown resistance alleles with small fitness costs can be maintained in large populations (Gould 2008). Modifiers can evolve even when fitness costs are relatively low. We believe this combination of low fitness costs and stochasticity could lead to modifiers being much more widespread than currently documented, but more work will be needed.

Fundamentally, we do not find modifiers are a widespread occurrence in nature as Clarke and McKenzie's research (1987; 1988) remains the only empirical evidence of modifiers evolving in the wild. Our results illustrated a number of scenarios under which modifiers may be favored by selection and increase in frequency. Furthermore, researchers have observed instances where multiple alleles, even multiple mutations effecting similar genes evolve with varying fitness costs (2007; Feyereisen, Dermauw, and Van Leeuwen 2015). One plausible scenario for a modifier to evolve, not modeled here, would be a gene duplication event during pesticide selection wherein a resistance allele metabolizes a pesticide substrate, but the original gene is also responsible for another biological function

(Uyenoyama 1986). After duplication, the homologous genes could then specialize in function, one returning to the original function and the other activating only in the presence of a pesticide.

We have outlined how an epistatic modifier allele could evolve under insecticide selection. While the empirical evidence of modifier alleles evolving remains limited to a single case we demonstrate a wide set of criteria where they can impact resistance allele evolution. so more investigation is needed. The current usage of *Bacillus thuringensis* (Bt) crops in the United States and across the globe would be an ideal scenario for modifier alleles to evolve. Bt efficacy varies from species to species and several prominent pests have evolved resistance to various Bt toxins (Gould et al. 1992; Gould, Anderson, and Jones 1997). Thus, modifier alleles could be present at low frequencies in the field and we would not observe their impact until the use of Bt toxins ceased. We believe that competitive fitness assays as were done with *L. cuprina* (Grapputo et al. 2005; Clarke and McKenzie 1987; Piiroinen et al. 2013; Pittendrigh, Reenan, and Ganetzky 1997) could reveal whether or not modifiers are present in pest populations currently controlled by Bt toxins. Furthermore, the theoretical underpinnings we establish in this paper should be used to continue to explore the impact of modifiers under various conditions. These conditions include stochastic simulations that incorporate migration and mutation, population dynamics, and population structure. Additionally, many approaches to insecticide resistance management have been developed over the last three decades that should be simulated with the presence of modifiers.

FIGURES

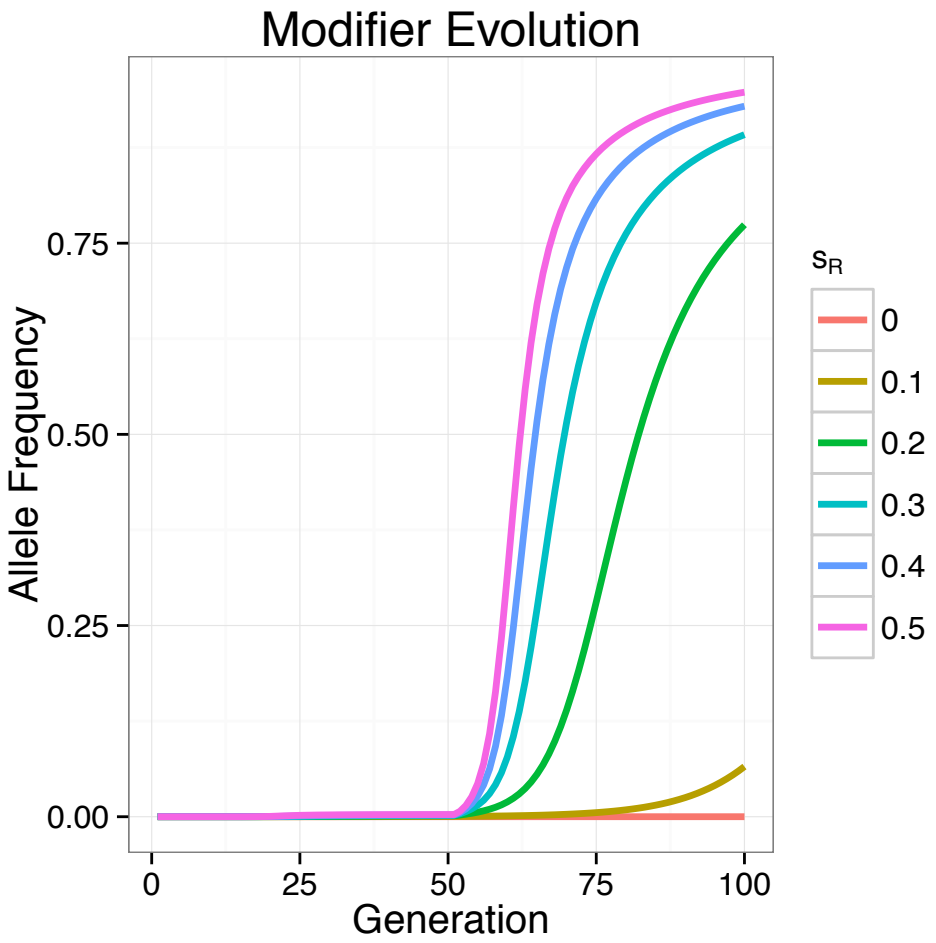

Figure 1. Change in M allele frequency based on the fitness cost of R (s_R) over 100 generations. Both M and R allele begin at 10^{-5} with no fitness cost for the M allele. Insecticide selection begins at generation 1 for 20 generations, then again at 51 for 30 generations. We see a concomitant increase in the frequency of M as the fitness cost for R increases. Surprisingly, relatively modest costs of R ($s_R > 0.1$) lead to rapid increase in M during the second round of selection.

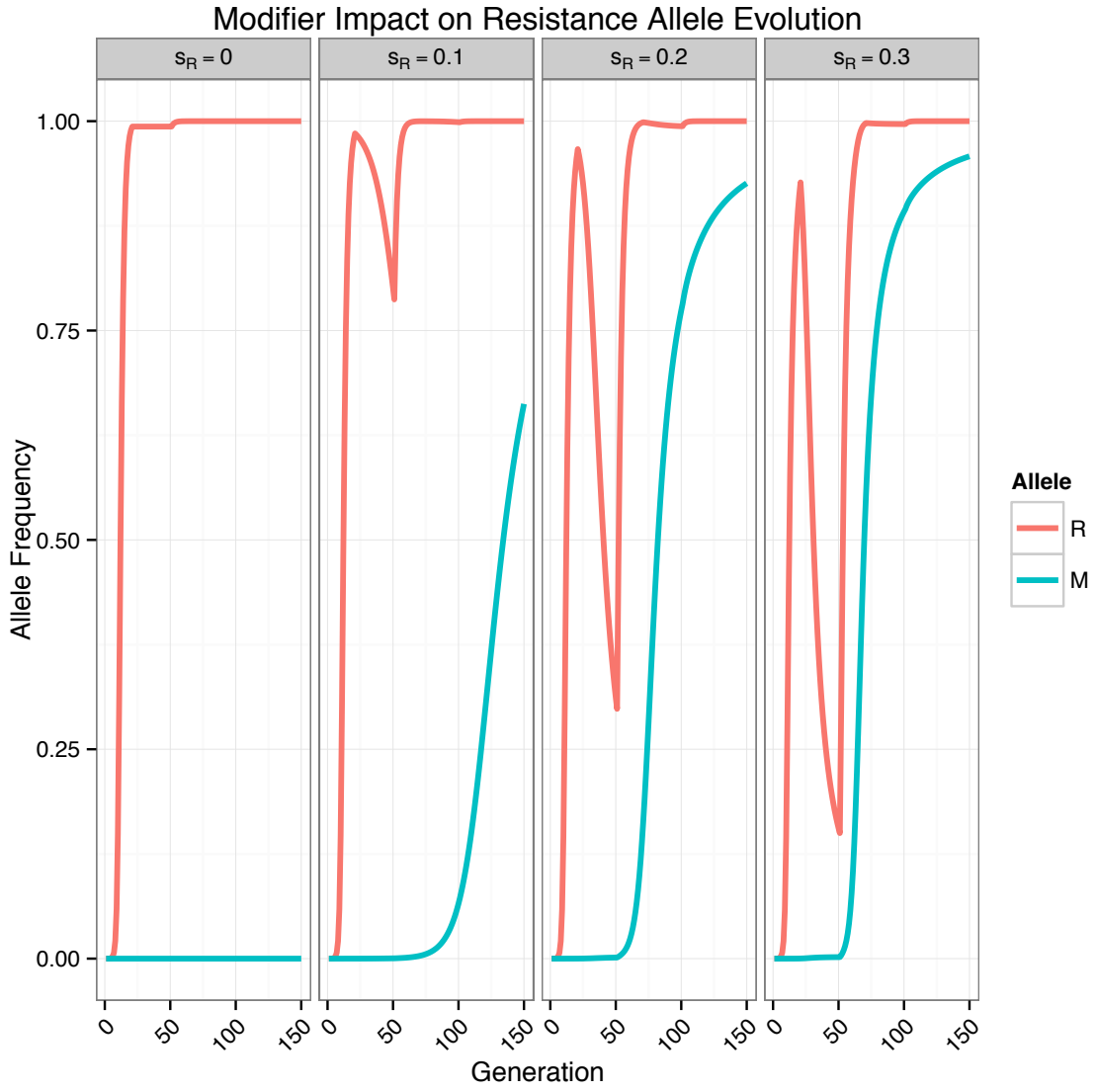

Figure 2. Change in allele frequency over time for R & M locus. Three rotations of spray (20 generations) and no spray (30 generations) occurred. Fitness cost (s_R) of R alleles listed across the columns. Initial frequency of M and R alleles = 10^{-5}. Insecticide had 80% efficacy. All effects of fitness costs were additive ($h_M = h_R = 0.5$) and modifier effect was dominant ($h_z = 1$). We observe a rapid rise of the M allele as s_R increases. The impact of M alleles requires several rounds of selection on the R allele through use of insecticide sprays.

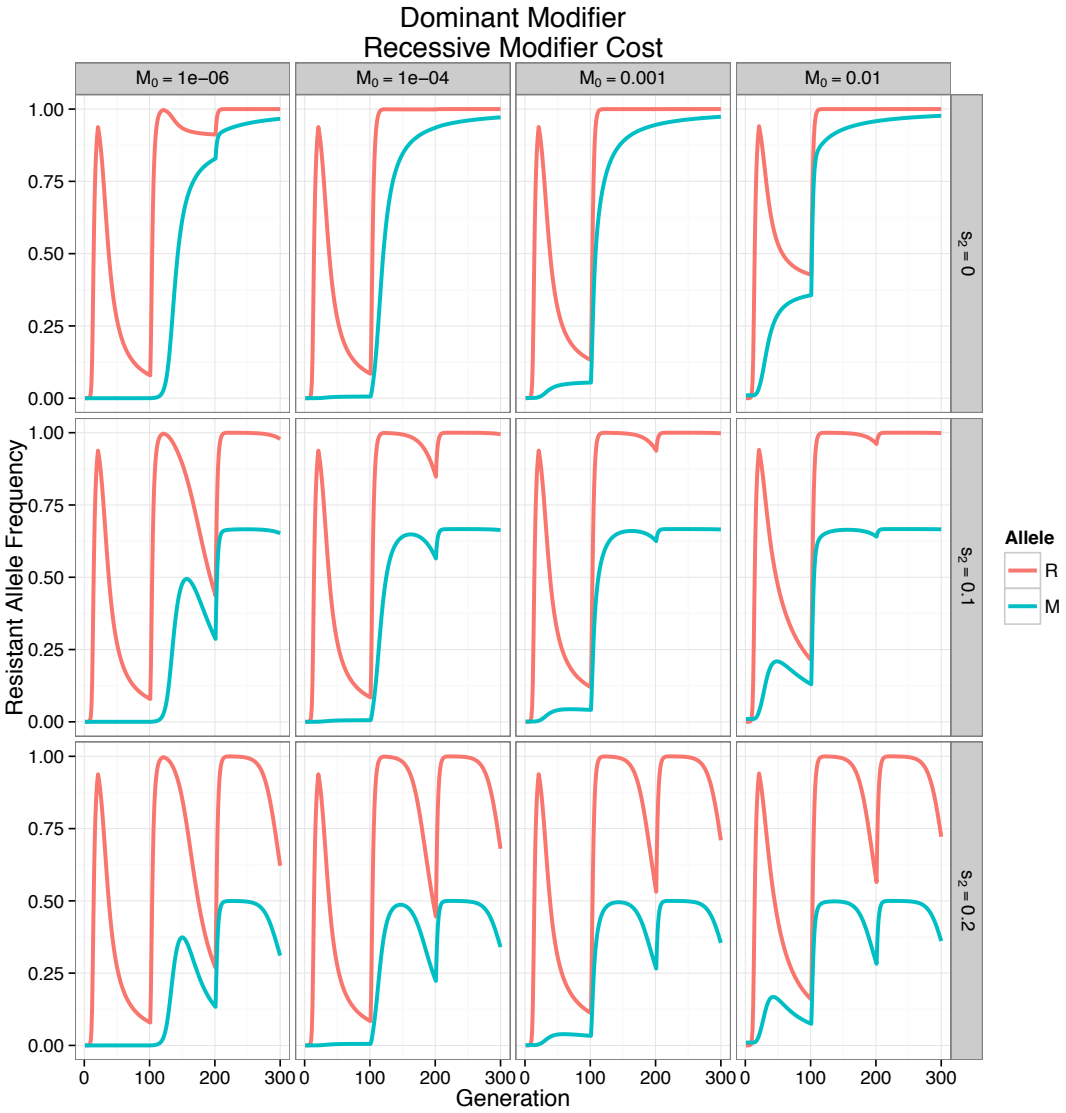

Figure 3. Change in allele frequency over time for R & M locus, three rotations of spray (20 generations) and no spray (80 generations) occurred. Initial frequency for R is 10^{-5}. Initial frequency of M is depicted across the columns. Insecticide had 80% efficacy. We used a moderate additive fitness cost for R ($s_R = 0.2$, $h_R = 0.5$) and the rows indicate recessive fitness costs for M (s_M, $h_M = 0$). The modifier effect is dominant ($z = 1$). As s_M increases we see larger fluctuations in the frequency of R (most prevalent in bottom row). The R allele fails to reach fixation after three rounds of selection when $s_M = 0.2$, but does so after two rounds of selection when $M_0 > 10^{-4}$ and $s_M = 0$, as well as after three rounds of selection when $M_0 > 10^{-3}$ and $s_M = 0.1$. Once R reaches fixation selection pressure does not increase M above 0.675 and 0.5 when $s_M = 0.1$ and $s_M = 0.2$ respectively. Of interest is that increasing duration of the rotation (no spray) period does not lead to loss of the resistance allele due to the modifier even at low frequency.

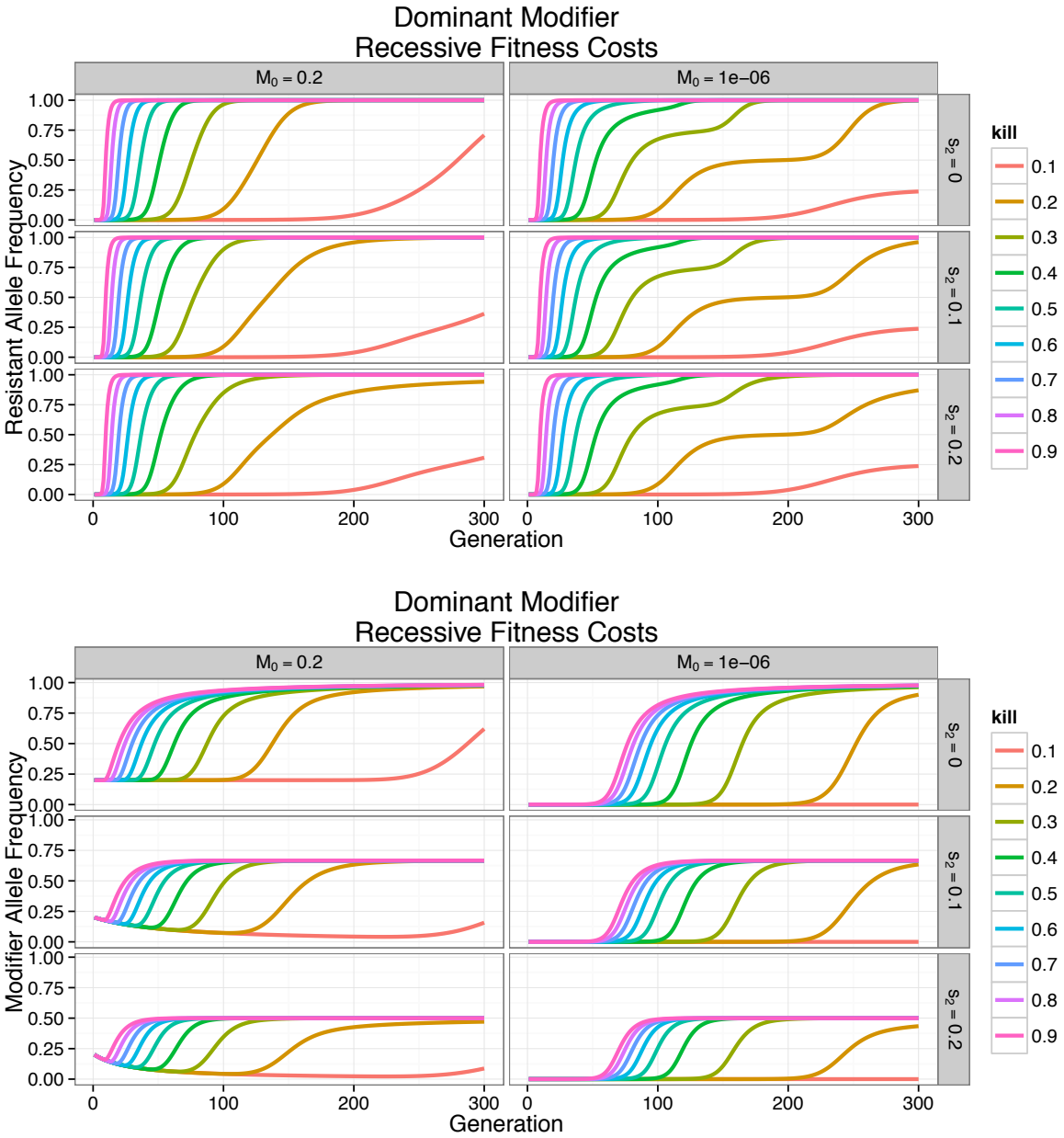

Figure 4. Allele frequency for R and M alleles when impacted by insecticide efficacy and initial frequency of M alleles. See Table 1 for initial conditions not listed in figure. Insecticide intensity impacts R and M allele frequency positively. As M allele frequency increases this allows R alleles to overcome plateaus due to downward pressure from s_R.

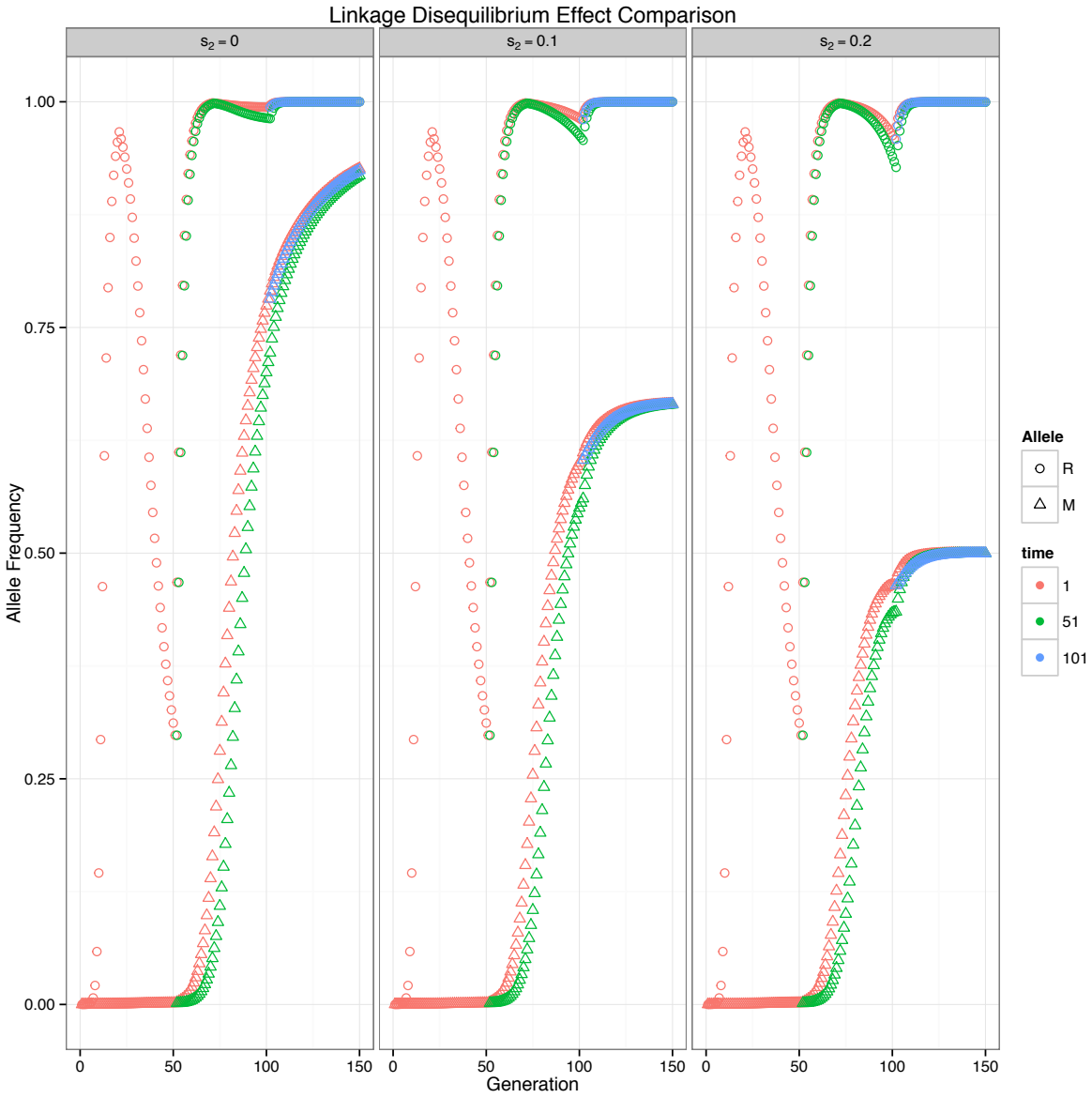

Figure 5. Impact of linkage disequilibrium (LD) on R & M allele evolution. Three rotations of spray (20 generations) and no spray (30 generations) occurred. Red circles and triangles are the original simulation, while green and blue circles and triangles depict the reseeding of the model at the second and third rounds of spraying (respectively). Fitness cost of M alleles listed across columns while s_R was held constant (0.2). LD appears to only off set the increase in R and M alleles. LD appreas to also partially intensify the decrease in R alleles during times of no spray.

TABLES

Table 1.1 Variables and parameters modeled.

Variable	Definition	Initial Conditions
R, S	Resistant, Susceptible	10^{-5}; 1-10^{-5}
M, N	Modifier, Neutral	10^{-5}; 1-10^{-5}
k	Insecticide efficacy	0.8
r	level of resistance	1
s_i	fitness cost, where i = R Susceptible/Resistant Locus; i = M Neutral/Modifier Locus	$s_R = 0.2$; $s_M = 0$
h_i	dominance coefficient, where i = R Susceptible/Resistant Locus; i = M Neutral/Modifier Locus; i = Z Modifier effect	$h_R = 1$; $h_M = 1$; $h_z = 1$
z	modifier coefficient	1

Table 1.2. Relative fitness values for change in allele frequency with and without insecticide selection.

Genotype	Relative Fitness	With Insecticide	Without Insecticide
SSNN	w_{11}	$(1-k)$	1
SSNM	w_{12}	$(1-k)(1-s_2h_2)$	$(1-s_2h_2)$
SSMM	w_{13}	$(1-k)(1-s_2)$	$(1-s_2)$
SRNN	w_{21}	$(1-kr)(1-sRh_1)$	$(1-sRh_1)$
SRNM	w_{22}	$(1-kr)((1-sRh_1(1-zh_3)))(1-s_2h_2)$	$((1-sRh_1(1-zh_3)))(1-s_2h_2)$
SRMM	w_{23}	$(1-kr)((1-sRh_1(1-z)))(1-s_2)$	$((1-sRh_1(1-z)))(1-s_2)$
RRNN	w_{31}	$(1-sR)$	$(1-sR)$
RRNM	w_{32}	$((1-sR(1-zh_3)))(1-s_2h_2)$	$((1-sR(1-zh_3)))(1-s_2h_2)$
RRMM	w_{33}	$((1-sR(1-z)))(1-s_2)$	$((1-sR(1-z)))(1-s_2)$

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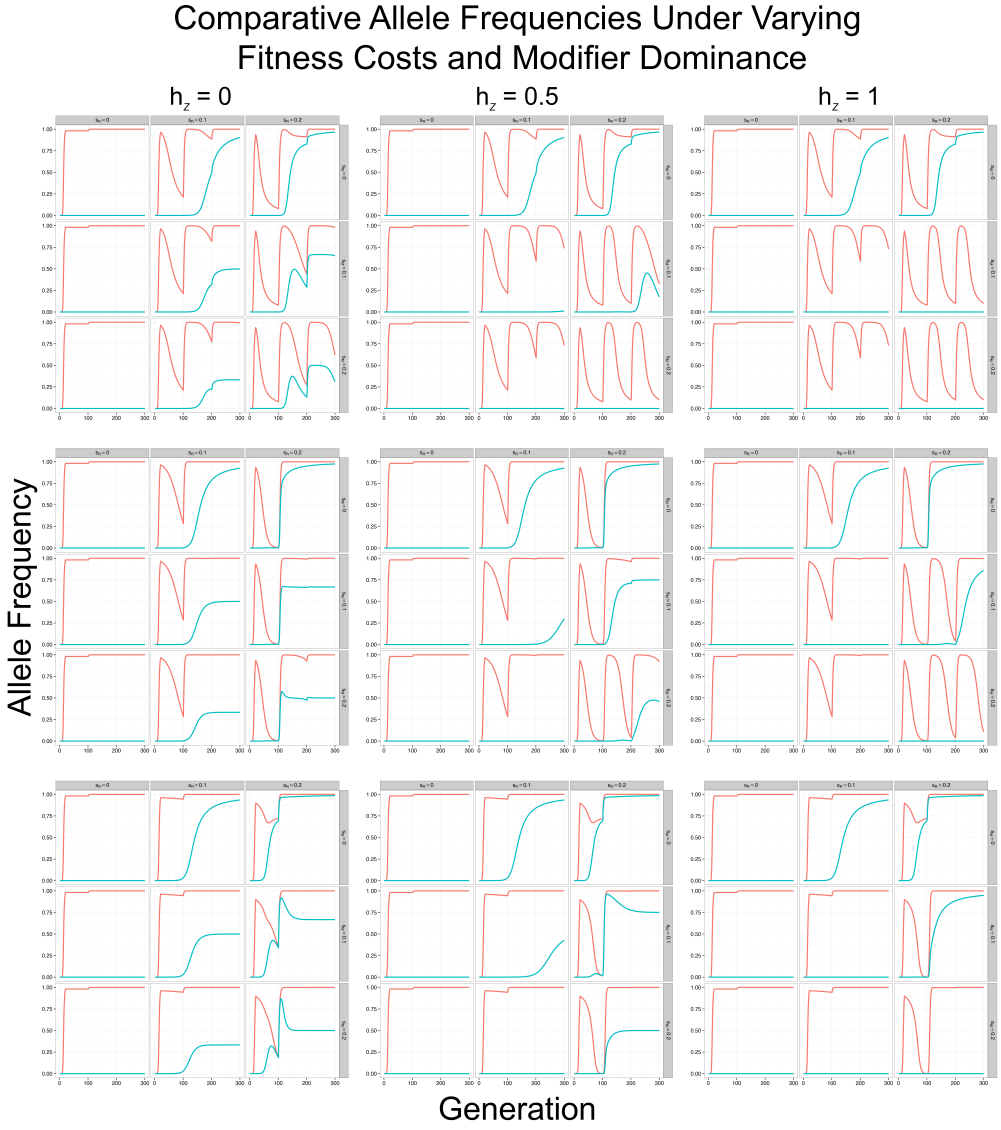
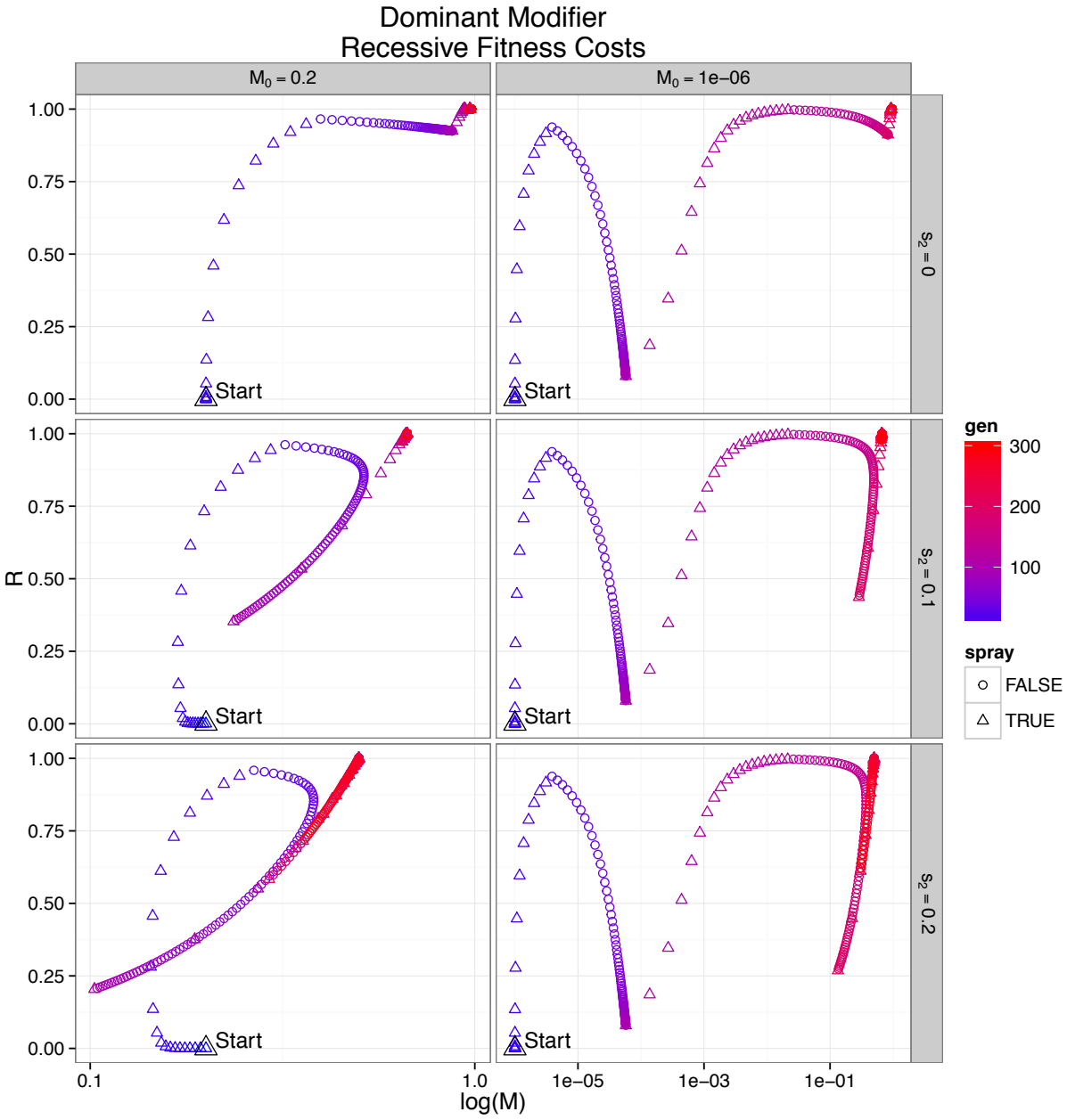
APPENDICES

APPENDIX A

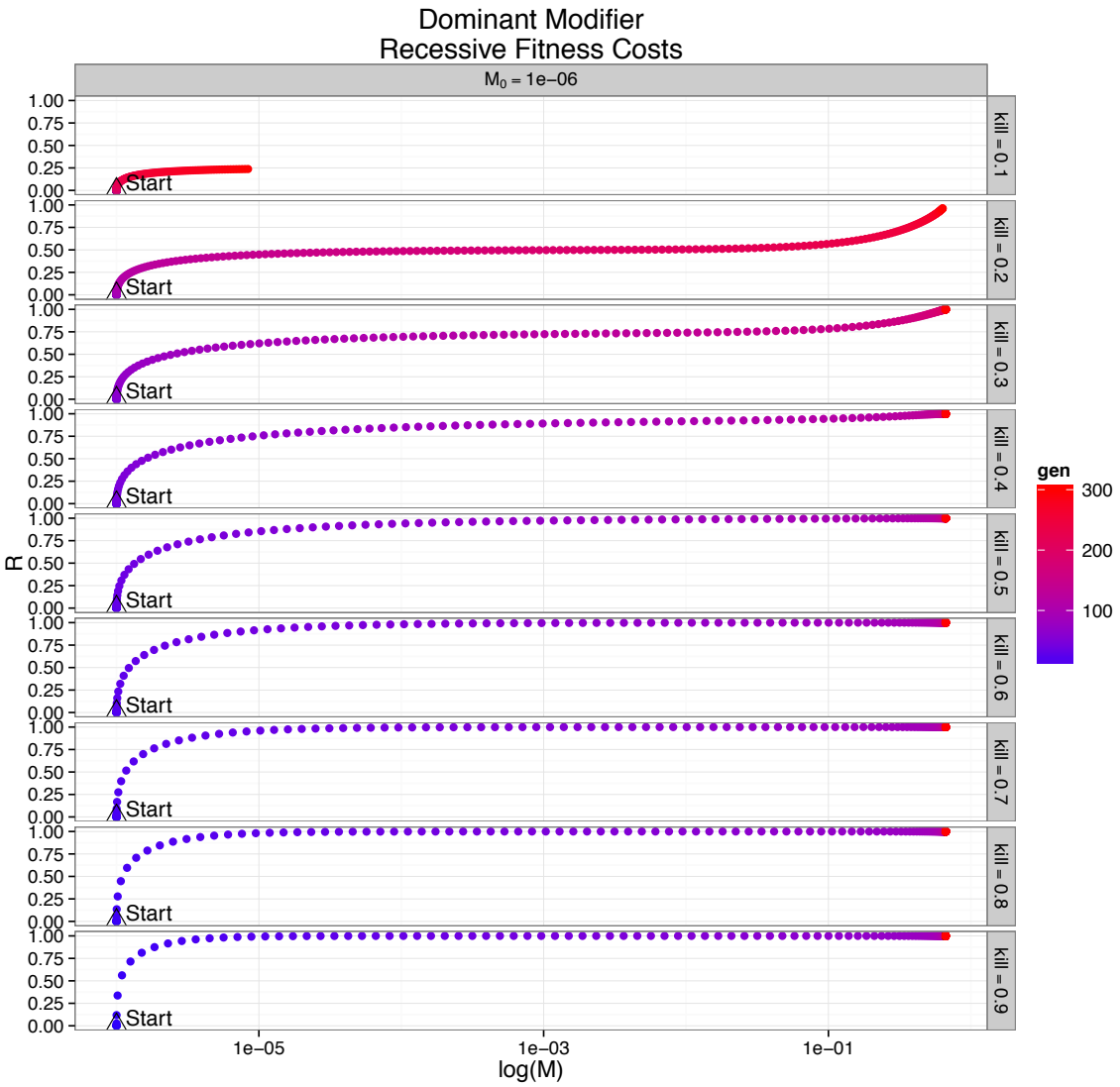
SUPPLEMENTARY FIGURES



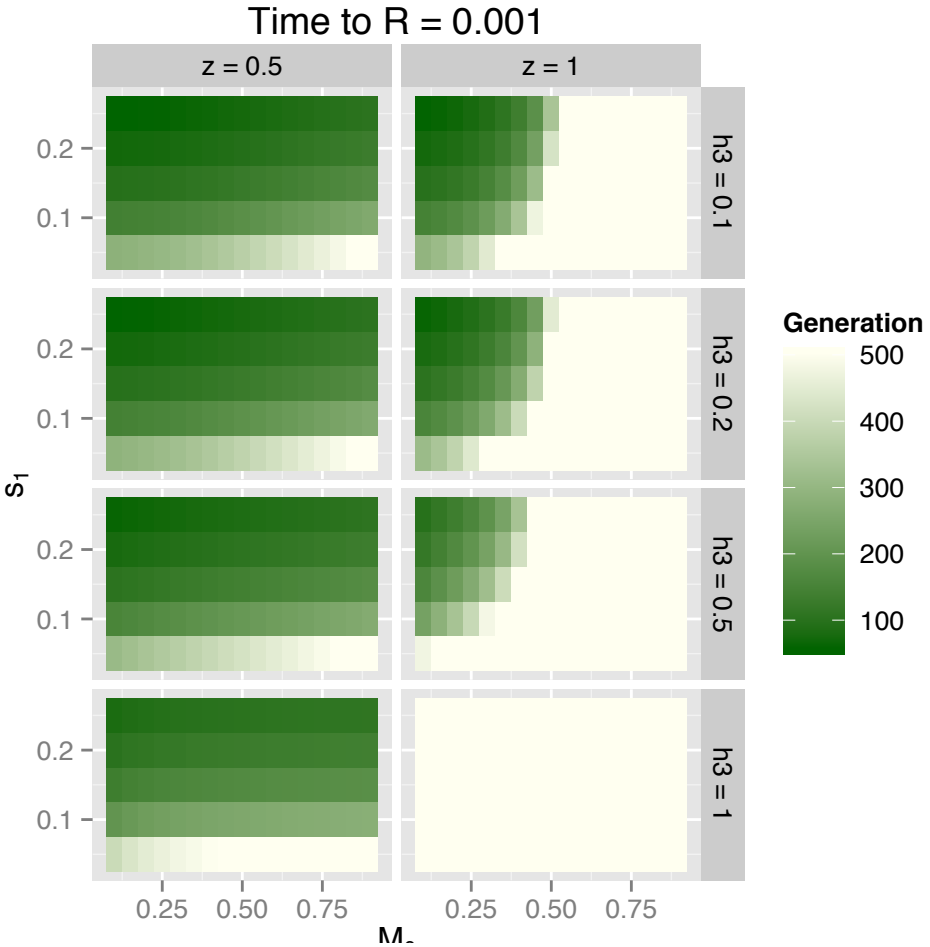
Supplementary Figure 1. Atlas of all combinations of fitness costs and modifier dominance modeled. Fitness costs were always modeled as recessive. For all conditions see supplementary table 1.



Supplementary Figure 2. Modified phase plot of R and M allele frequencies over 300 generations. See supplementary table 1 for modeling conditions. Positive relationship exists between M allele frequency and R allele frequency. R allele frequency increases (distance between time steps) much more quickly over M allele frequency. However, minor increases in M allele appear to have a significant contribution to maintaining R allele frequency.



Supplementary Figure 3. Modified phase plot of R and M allele frequencies with varying insecticide efficacies. As would be expected the more intense the selection the more quickly both R and M alleles increase over time. Most notable is the increase in R allele frequency at low selection (k = 0.2 and 0.3) where R allele frequency appears to plateau until M allele frequency reaches approximately 0.1 then begins to rapidly increase until the end of the simulation.



Supplementary Figure 4. Time to "loss" of R allele under no spray conditions. We observe that when modifiers reach a high frequency in the population and act either additively or dominantly a large area of the parameter space has the R allele maintained in the population.

APPENDIX B

SUPPLEMENTARY EQUATIONS

SEq. 1

$$S' = \frac{S^2N^2w_{11} + 2S^2NMw_{12} + S^2M^2w_{13} + 2SRN^2w_{21} + 4SRNMw_{22} + 2SRM^2w_{23}}{\overline{w}}$$

SEq. 2

$$N' = \frac{S^2N^2w_{11} + 2S^2NMw_{12} + 2SRN^2w_{21} + 4SRNMw_{22} + R^2N^2w_{31} + 2R^2NMw_{32}}{\overline{w}}$$

SEq. 3

$$M' = \frac{2S^2NMw_{12} + S^2M^2w_{13} + 4SRNMw_{22} + 2SRM^2w_{23} + 2R^2NMw_{32} + R^2M^2w_{33}}{\overline{w}}$$