Pesticide Biochemistry and Physiology 97 (2010) 123–128

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

A review of the interactions between multiple insecticide resistance loci

Melissa C. Hardstone, Jeffrey G. Scott *

Department of Entomology, Comstock Hall, Cornell University, Ithaca, NY 14853-0901, USA

ARTICLE INFO

Article history:
Received 8 October 2008
Accepted 31 July 2009
Available online 5 August 2009

Keywords:
Insecticide resistance
Epistasis
Evolution
Gene interaction
Insecta

ABSTRACT

Insecticide resistance is an ever escalating problem worldwide in many pest populations and numerous cases of insecticide resistance are polygenic. Therefore, it is important to investigate the types of interactions that occur between insecticide resistance loci as this will dictate the level of resistance (and effectiveness of a chemical control strategy). Interactions also play a role in the evolution and/or maintenance of multigenic resistance in the field. Given that a limited number of mechanisms confer resistance, it might be possible to establish general rules for interactions between mechanisms. Several variables might dictate the type of interaction, such as the nature of the resistance mechanisms, genotype, etc. Interactions can be synergistic, antagonistic or additive. Based on this literature review, the most common interaction of multiple homozygous resistance loci is synergistic and additive when loci are heterozygous. When one locus is homozygous and the other locus is heterozygous the most common interaction was synergistic, although very few studies have examined this type of interaction. Possible factors that drive these interactions, exceptions to the trends, and future research needs are discussed.

1. Introduction

Insecticide resistance is an ever growing, complicated, and global problem in many pest species. Resistant pest populations can decrease crop yields (and profitability), and result in the re-emergence or epidemic levels of diseases that affect humans or animals. In order to combat resistant pest populations many alternate tactics are pursued including (but not limited to) increasing the frequency of insecticide application, increasing the concentration of insecticide, and using multiple insecticides with different modes of action either in a mixture or rotation [1]. Resistance management is greatly facilitated by understanding the resistance; including the number of loci involved and whether the loci interact in a synergistic, antagonistic or additive manner [2].

The use of insecticides can provide a strong selective force in a pest population such that high levels of resistance are achieved, and insects can evolve more than one resistance mechanism (multigenic resistance). In an individual animal or a pest population, interactions between multiple insecticide resistance loci can shape the effectiveness of chemical control efforts. In multigenic resistance, mechanisms can interact in an additive fashion such that overall resistance follows the model $RR_{Additive} = RR_1 + RR_2 + \ldots RR_n - (n - 1)$, where RR is the resistance ratio conferred by an individual locus and n is the total number of resistance loci [3,4]. Insecticide resistance loci interact synergistically (or antagonistically) when the product of the resistance ratios is greater than (or less than) the resistance ratio expected from an additive type interaction ($RR_{Additive}$) [5–9]. Low resistance levels make it difficult to discriminate between the types of interactions. For example, when using the additive model if $RR_1 = 1.5$ and $RR_2 = 3$, the resulting resistance will be $1.5 + 3 - 1 = 3.5$. If the observed resistance is 4.2-fold, it is difficult or impossible to determine if the interaction is additive or synergistic. An additional complication to understanding the dynamics of interactions between resistance mechanisms is that homozygous and heterozygous resistant genotypes exist in field populations. However, few studies have analyzed resistance loci in multiple genotypic states or genotype combinations.

The goal of this paper is to summarize what is known about interactions between multiple insecticide resistance loci, examine if trends or patterns of interactions can be identified, and offer suggestions for future studies. The guidelines we used to assess the type of interaction occurring between loci were as follows: (1) cases where insecticide resistance ratios were $\leqslant 1.0$ were not included (since they did not confer resistance), (2) in studies that used factorial analysis, resistance/linkage group interactions were not included in cases where one of the mechanisms within the combination did not alone significantly contribute to the resistance, and (3) given that there is uncertainty in determination of LD_{50} (or LC_{50}) values (usually given by 95% confidence intervals), we needed an estimate of the uncertainty of $RR_{Additive}$ to be able to determine if data fit the model. A survey of several papers reporting LD_{50} and LC_{50} values suggest the 95% confidence intervals were frequently ±5–15% of the LD_{50} (or LC_{50}) value for homozygous strains. Thus, we estimated that the variance in

* Corresponding author. Fax: +1 607 255 0939.
E-mail address: jgs5@cornell.edu (J.G. Scott).

0048-3575/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.pestbp.2009.07.010

$RR_{Additive}$ was approximately 20%. If the observed RRs were within $RR_{Additive}$ ± 20%, we concluded the interaction between resistance loci was additive.

2. House fly (*Musca domestica*)

Most studies that have examined interactions between multiple insecticide resistance loci were done using house flies. This pest is capable of transmitting a variety of human and veterinary diseases, is frequently the direct target of chemical control efforts [10–12] and has rapidly developed resistance to virtually all insecticides used against it. House flies are also a useful system to study insecticide resistance since many strains with visible markers have been isolated and can be used in genetic crosses. Two major methods of linkage analysis of resistance factors have been employed. The crossing procedure developed by Tsukamoto [13], in which an unmarked (usually resistant) fly is crossed to a multi-chromosomally marked (usually susceptible) fly, can detect either dominant or recessive resistance factors (depending on the crosses set up). The other method, developed by Sawicki [14], crosses an unmarked resistant strain to a mutant marker susceptible strain, and backcrosses the F_1 male progeny to marker carrying females. With repeated selection (phenotype and insecticide) of the F_2 and subsequent generations, a homozygous strain can be established having a resistance locus on one or more identified autosomes [14]. However, it is occasionally possible to lose a resistance locus using this method if the selection pressure is not strong enough and there is a large fitness cost associated with the resistance locus [15,16].

2.1. Interactions between multiple homozygous resistance loci

The consensus interaction (32 of a total 44 results) between two or more homozygous resistance loci in house flies, is synergistic (Table 1). This trend holds true for studies that include a wide range of insecticide classes in both topical and residual bioassays

Table 1
Summary of interactions between multiple homozygous insecticide resistance loci.

Insect species	Bioassay method[a]	Life stage[b]	Strain name	Resistance loci/chromosome	Insecticide tested	Number of observed interactions				Citation
						Antagonistic	Additive	Synergistic		
M. domestica	T	A		E0.39 and *Ace*	Trichorfon			1		[17]
					Malathion			1		
					Fenitrothion			1		
					Parathion			1		
					Dimethoate			1		
					Azamethiphos			1		
M. domestica	R	A		*Deh* and *kdr*	DDT			1		[18]
M. domestica	R	A		*pen*[c] and *para* (Cal P-R)	Parathion		1			[19]
					Tributyltin chloride	1				
				pen[c] and *para* (Para-clw)	Parathion			1		
M. domestica	T	A		3 > 4 = 2	Gamma-BHC		4			[13]
M. domestica	T	A	Strain 1673	3 (*pen*) and 5 (gene *a*)	Chlorthion-ethyl			1		[20]
					Diazinon			1		
M. domestica	T	A		2, 3 and 5	Carbaryl			4		[21]
				2, 3 and 5	Ronnel		2	2		
M. domestica	T	A		2 and 5	Diazinon			1		[22]
					Diazoxon			1		
					Malaoxon ethyl			1		
					Malaoxon			1		
					Chloroxon ethyl		1			
					Chloroxon			1		
					Chlorthion		1			
					Malathion ethyl		1			
					Parathion			1		
M. domestica	T	A	LPR	5 ⩾ 3 > 1 > 2	Permethrin			1		[23]
M. domestica	T	A	LPR	1, 2 (P450) and 3 (*kdr*)	Permethrin	1		7		[15]
M. domestica	T	A	ALHF	3 (*kdr*) and 5 (P450)	Permethrin			1		[24]
M. domestica	T	A	SeALHF	P450s, hydrolases, L1014H (*kdr*)	Permethrin			1		[25]
				M. domestica	**Totals**	**2**	**10**	**32**	**=**	**44**
Cx. p. quinquefasciatus	S	L4	JPAL	P450 and kdr	Permethrin			1		[16]
				Cx. p. quinquefasciatus	Totals	0	0	1	=	1
M. persicae	T	A		R_2 (esterase) and *kdr*	Deltamethrin			1		[40]
					DDT			1		
				R_3 (esterase) and *kdr*	Deltamethrin			2		
				M. persicae	**Totals**	**0**	**0**	**4**	**=**	**4**
D. melanogaster	R	A	SyS-1002	X, 2 and 3	DDT		3			[41]
			SyS-102	X, 2 and 3	DDT		3			
D. melanogaster	T	A		1 = 2 > 3	Deltamethrin		3	1		[42]
				D. melanogaster	**Totals**	**0**	**9**	**1**	**=**	**10**
					Grand totals	**2**	**19**	**38**	**=**	**59**

[a] Topical (T), residual (R), feeding (F) or submersion (S).
[b] Egg (E), larval instars (L1, L2, etc.), pupae (P) or adult (A).
[c] Originally described as *tin* [46].

where adults are analyzed [13,15,17–25]. There were exceptions (5 studies), however, as 10 of 44 results showed an additive interaction between homozygous resistance loci and 2 of 44 results showed antagonistic interactions (Table 1). There were no unique features (strains, insecticide(s), methods, etc.) for the studies that showed additive or antagonistic interactions, making it unclear why these studies varied from the general trend of synergistic interactions.

In the SKA house fly strain organophosphate resistance was linked to chromosomes 2 (GST and gene *a*), 3 (*Pen*) and 5 (microsomal detoxification factor *Ses*) [22]. Resistance loci on chromosomes 2 + 5 conferring resistance to diazinon, diazoxon, malaoxon, malaoxon ethyl, chloroxon, and parathion interacted synergistically [22] (Table 1). However, these same two resistance loci (2 + 5) interacted in an additive fashion in response to chloroxon ethyl, chlorthion, and malathion ethyl (Table 1). From the Sawicki [22] study, as well as that of Georgiou [21], it can be seen that the type of interaction can differ when the same resistance factors are challenged with different insecticides. Hoyer and Plapp [19] also observed a variable interaction between two (*tin* and *para*) homozygous resistance loci (Table 1) depending on the strain the *para* locus originated from. When *tin* was in combination with the *para* locus derived from the Para-clw strain, the interaction was synergistic [19]. Conversely, when *tin* was in combination with *para* derived from the Cal P-R strain, the interaction was additive [19]. This study shows that even when strains are treated with the same insecticide and contain the same resistance mechanisms, the interaction can differ if the resistance locus was derived from different parental strains. This might be due to different *para* alleles between the two strains, although this is unknown.

2.2. Interactions between multiple heterozygous resistance loci

Based on studies using house flies, when multiple heterozygous resistance mechanisms are present, the most common interaction (53 of 67 results, 9 of 10 studies) is additive [5,20,26–32]. However, there were 8 cases of synergistic and 6 cases of antagonistic interactions (Table 2). There is no correlation between the insecticide used, resistance loci involved, house fly strain, or bioassay methodology and the type of interaction that is observed when loci are heterozygous (Table 2).

Synergistic interactions were found in 4 of the 10 house fly cases examining multiple resistance loci in the heterozygous condition. Linkage of the pyraclofos resistant house fly strain, YBOL, indicated that factors in the heterozygous state (in order from greatest effect to least) on chromosomes 2, 5 and 4 were significantly linked to resistance. Synergistic interactions were found for the combinations of loci on autosomes 2 + 5 and 2 + 4 + 5 [28]. Synergistic interactions (between linkage groups 2 + 4, 2 + 5 and 2 + 3 + 5) were also found in the P-Pro strain resistant to profenofos. In P-Pro, chromosome 2 had the greatest positive effect and therefore was probably a major resistance factor [29]. Examination of SKA (with resistance loci on autosomes 2, 3 and 5) [14], YBOL, and P-Pro strains suggested that synergistic interactions might be correlated to having more than two loci involved in resistance. However, the highly permethrin resistant Miyakonojo strain exhibited a synergistic interaction between resistance loci on autosomes 3 + 5, while linkage group combinations 2 + 3, 2 + 5 and 2 + 3 + 5 were not significantly different from additive [27]. Additionally, of the seven studies that have more than two heterozygous resistance loci, three studies showed additive interactions [5,30,31] (Table 2).

While antagonistic interactions were observed between multiple heterozygous loci in 6 of the total 67 published results, there does not seem to be a distinct variable that drives this result (Table 2). Two of the three studies that contain strains resistant to permethrin [5,31] exhibit at least one antagonistic interaction, but there is also this type of interaction to the organophosphates profenofos [29] and diazinon [20]. First order interactions of the permethrin resistant LPR strain were not significantly different from additive except for loci on autosomes 2 + 3 which had an antagonist interaction. Also, the second order interaction 1 + 2 + 3 was additive [5].

Studies on the Miyokonojo strain with 13-fold total resistance to permethrin that was linked to three chromosomes (2, 3 and 5) revealed one first order chromosome combination 3 + 5 with a synergistic interaction, while the other chromosome combinations were additive [27]. In the >18,400-fold permethrin resistant strain

Table 2
Summary of interactions between multiple heterozygous insecticide resistance loci.

Insect species	Bioassay method[a]	Life stage[b]	Strain name	Resistance loci/chromosome	Insecticide tested	Number of observed interactions				Citation
						Antagonistic	Additive	Synergistic		
M. domestica	n/a	A	SKA	2, 3 (*pen*), and 5 (gene *a*)	Diazinon			1		[14]
					Parathion			1		
M. domestica	n/a	A		2 and 3	Isolan		1			[26]
M. domestica	T	A	Strain 1673	3 (*pen*) and 5 (gene *a*)	Chlorthion-ethyl		1			[20]
					Diazinon	1				
M. domestica	T	A	LPR	3 (*kdr*) > 1 > 2	Permethrin	1	4			[5]
M. domestica	T	A	Miyakonojo	2, 3, and 5	Permethrin		3	1		[27]
M. domestica	T	A	YBOL	2 > 5 > 4	Pyraclofos		2	2		[28]
M. domestica	T	A	P-Pro	2 > 5 > 1 ⩾ 3 ⩾ 4	Profenofos	3	20	3		[29]
M. domestica	F	L3	YPPF	2 > 1 = 3 > 5	Pyriproxyfen		11			[30]
M. domestica	T	A	YPER	2 > 3 (super-*kdr*) > 5 > 1	Permethrin	1	10			[31]
M. domestica	T	A	NYINDR	4 > 3	Indoxacarb		1			[32]
				M. domestica	**Totals**	**6**	**53**	**8**	**=**	**67**
Culex spp.	S	L4		Esterases, AChE	Chlorpyrifos			1		[4]
					Temephos			1		
Cx. p. quinquefasciatus	S	L4	JPAL × SLAB F_1	P450 and *kdr*	Permethrin			1		[16]
				***Culex* spp.**	**Totals**	**0**	**0**	**3**	**=**	**3**
D. melanogaster	R	A	91R	2 and 3	DDT		1			[43]
			91R	1, 2 and 3	DDT		3	1		
				D. melanogaster	**Totals**	**0**	**4**	**1**	**=**	**5**
					Grand totals	**6**	**57**	**12**	**=**	**75**

[a] Topical (T), residual (R), feeding (F) or submersion (S).
[b] Egg (E), larval instars (L1, L2, etc.), pupae (P) or adult (A).

YPER, resistance was linked to four chromosomes (1, 2, 3 and 5) and all combinations resulted in additive interactions (except 2 + 3 which was antagonistic) [31].

2.3. Interactions between resistance loci when one is homozygous and other is heterozygous

Only one house fly study, using the strain 1673 [20] (with resistance loci derived from SKA) contained information on the interaction between resistance loci when the genotype of one was homozygous and the other was heterozygous (Table 3). When either resistance locus (*Pen* or gene *a*) was heterozygous (and the other locus was homozygous) and challenged with chlorthion, the result was a synergistic interaction. Conversely, when challenged with diazinon, *Pen*/*Pen*;+/gene *a* exhibited an antagonistic interaction and +/*Pen*;gene *a*/gene *a* interacted additively [20]. Thus, based on these limited number of studies, no consistent trend for the interaction of homozygous and heterozygous resistance loci was observed.

3. Mosquito (*Culex* spp.)

Mosquitoes are vectors of pathogens to humans and other animals. They are one of the major pests targeted for chemical control campaigns since often insecticides are the only tools that can be used during a vector-borne disease outbreak. Pyrethroid and carbamate insecticides are used as adulticides for aerial sprays as well as to treat bed nets [33,34], while organophosphate insecticides and insect growth regulators are used as larvicides [35]. However, resistance to insecticides has become a worldwide problem [36,37]. Despite the importance of insecticide resistance in mosquito control, very few studies have examined the interaction between resistance mechanisms. Due to the limited number of studies, no patterns of interactions could be discerned. The available studies on interactions between resistance mechanisms in mosquitoes are reviewed below.

Currently there are only two studies [4,16] that have looked at the interaction between resistance mechanisms in mosquitoes. Raymond et al. [4] used two organophosphate resistant mosquito strains; Tem-R, a strain of *Cx. p. quinquefasciatus* that has a B1 esterase detoxification mechanism and an unknown mechanism, and MSE, a strain of *Cx. p. pipiens* with insensitive acetylcholinesterase (Ace^R), and the susceptible SLAB strain. When crosses of these strains were treated with an esterase inhibitor (DEF), low levels of resistance (1.53-fold to temephos, 1.88-fold to chlorpyrifos) remained and were attributed to the unknown mechanism. The levels of resistance to temephos conferred by each heterozygous locus were $RR_1 = 1.53$, $RR_3 = 135$ and $RR_4 = 4.24$ with the observed $RR_{1\&3\&4} = 279$. The levels of resistance to chlorpyrifos conferred by each heterozygous locus were $RR_1 = 1.88$, $RR_3 = 21.5$ and $RR_4 = 58.4$ with the observed $RR_{1\&3\&4} = 165$. Thus, these resistance loci interact synergistically (Table 2). However, using a mathematical model that considered pharmacokinetics of the particular resistance mechanisms present in the Tem-R and MSE strains of mosquito, Raymond et al. [4] determined that the interaction was best described by a mixture of additive and synergistic interactions, whereby $RR_{1\&3\&4} = RR_1 \times (RR_3 + RR_4 - 1)$.

Hardstone et al. [16] studied mosquito strains resistant to permethrin, and examined interactions between both homozygous and heterozygous resistance loci (Table 3). The strains used were JPAL [38], a strain of *Cx. p. quinquefasciatus* with 29,000-fold permethrin resistance due to target site insensitivity (*kdr*) and cytochrome P450 monooxygenase-mediated detoxification, and ISOP450, a strain of *Cx. p. quinquefasciatus* lacking *kdr*, with 1300-fold permethrin resistance conferred solely by the same P450 mechanism found in JPAL [39]. When both mechanisms (P450 and *kdr*) were heterozygous (Table 2), a synergistic interaction resulted [16]. Synergism was also observed for the interaction of homozygous loci, P450 + *kdr* (Table 1), which agrees with the trend observed in the house fly studies.

While the two studies on mosquitoes both show synergistic interactions (by the $RR_{Additive}$ model), more studies are clearly needed. In addition, models based on pharmacokinetics should continue to be developed as a predictor of interactions between resistance loci.

4. Aphid (*Myzus persicae*)

There have been numerous studies on insecticide resistance in the peach-potato aphid, *M. persicae*, however very few have investigated the interaction between resistance loci. Martinez-Torrez et al. [40] were able to isolate the resistance contribution of each resistance locus. In this study, E4 and FE4 esterases were combined into one mechanism and separated by phenotype (esterase activity level) since genotype information on this resistance mechanism is difficult to obtain. While different from the type of data available in the house fly and mosquito literature, determining interactions in the aphid could provide some insight into overall patterns of interacting insecticide resistance loci. The aphids used in the Martinez-Torres et al. study were clones and were homozygous for all resistance loci they possessed (Table 1). The DDT resistance conferred by *kdr* alone was 62-fold, while the resistance phenotype of the esterases was small with 1.5-fold for esterase R_2 and 0.7-fold for esterase R_3 (not included in Table 1). Aphid clones treated with deltamethrin showed 35-fold resistance with *kdr* alone, and 3.2- and 3.8-fold resistance with esterase R_2 alone and esterase R_3 alone, respectively. The *kdr* + esterase R_2 clones treated with DDT and deltamethrin and the *kdr* + esterase R_3 clones treated with deltamethrin all interacted synergistically [40]. These results agree with patterns observed in the house fly and mosquito (Table 1), although more studies with aphids would be valuable.

Table 3
Summary of interactions between insecticide resistance loci when one (or more) is homozygous and the remaining loci are heterozygous.

Insect species	Bioassay method[a]	Life stage treated [b]	Strain name	Resistance loci/mechanism	Insecticide tested	Number of observed interactions			Citation
						Antagonistic	Additive	Synergistic	
M. domestica	T	A	Strain 1673	3 (*pen*) and 5 (gene *a*)	Chlorthion-ethyl			2	[20]
					Diazinon	1	1		
				M. domestica	**Totals**	**1**	**1**	**2**	**= 4**
Cx. p. quinquefasciatus	S	L4	ISOP450 × JPAL F_1	P450 and *kdr*	Permethrin			1	[16]
				Cx. p. quinquefasciatus	**Totals**	**0**	**0**	**1**	**= 1**
					Grand totals	**1**	**1**	**3**	**= 5**

[a] Topical (T), residual (R), feeding (F) or submersion (S).
[b] Egg (E), larval instars (L1, L2, etc.), pupae (P) or adult (A).

5. *Drosophila melanogaster*

When multiple resistance loci are homozygous in *D. melanogaster*, they tend to interact additively (Table 1) which is different from the other insects analyzed in this review (house fly, mosquito, and aphid). King and Somme [41] determined that in the SyS-1002 strain the 20-fold DDT resistance contribution was greatest for chromosome 2, but was also significant for X and chromosome 3. For this strain, all first order interactions (X + 2, X + 3, 2 + 3) and the second order interaction (X + 2 + 3) were not significantly different from that expected under additivity (Table 1) [41]. For the DDT resistant *D. melanogaster* strain SyS-102, the major contribution to resistance was linked to X, with smaller, but significant factors on chromosome 2 and 3. Similar to SyS-1002, all first order and second order interactions of resistance loci were not significantly different from additive (Table 1) [41]. Double homozygotes of a deltamethrin resistant strain of *D. melanogaster* (Table 1) had significant resistance contributions linked to chromosomes 1, 2 and 3 [42]. When resistance factors were combined (1 + 3, 2 + 3 and 1 + 2 + 3), all interactions were additive, except 1 + 2 which interacted synergistically (Table 1) [42].

Dapkus and Merrell [43] analyzed *D. melanogaster* chromosome linkage of DDT resistance. They separated the factorial analysis of heterozygous chromosomes into two series; where series I examined recessive effects and series II examined dominant effects. For series I, it was observed that chromosome 2 and 3 have important resistance factors and that the first order combination of 2 + 3 results in additivity (Table 2). In the series II data, chromosome 2 had the greatest effect with a large factor also on chromosome 3 and a significant but minor factor on chromosome 1. Combinations of 1 + 2, 1 + 3 and 1 + 2 + 3 resulted in additive interactions (Table 2). A synergistic interaction was found between loci on autosomes 2 + 3 (Table 2) [43].

With the available studies on *D. melanogaster*, the general trend of interactions between multiple resistance linkage groups that are all either homozygous or heterozygous is of the additive type. No information is currently available about the type of interaction between resistance conferred by multiple mechanisms in differing genotypic states (*i.e.*, one is homozygous and one is heterozygous).

6. Discussion

Interactions between resistance mechanisms are varied, but given that there are a limited number of mechanisms responsible for insecticide resistance there do seem to be some broad trends. Generally, when multiple resistance loci were homozygous synergistic interactions resulted, while additivity was observed when resistance loci were heterozygous. There were many exceptions to these generalizations, particularly in studies using *D. melanogaster*, making it unclear which variables (type of resistance mechanisms/loci, fitness costs and benefits associated with each mechanism, insecticide used, species, life stage, etc.) could alter the resulting interaction.

The literature is deficient in studies that include interactions between mechanisms that are in different genotypic states (*i.e.*, homozygous locus A interacting with heterozygous locus B) as most studies examined interactions between loci in the same genotypic state (*i.e.*, locus A is heterozygous and interacting with a heterozygous locus B). While the most common interaction between homozygous and heterozygous resistance loci was synergistic, more studies are needed to confirm this trend.

For an animal in a treated environment, overall, it would be most beneficial (higher probability of survival) to have an interaction (except for a cancelling interaction) between resistance mechanisms, so that the resulting resistance would be higher than the level conferred by any of the mechanisms individually. In fact, antagonistic interactions would not necessarily be detrimental for animal to possess, since this interaction could provide higher levels of resistance than either resistance mechanism alone and therefore could still be selected [44]. For example, resistance to an insecticide conferred by locus A is 50-fold and locus B is 20-fold. If the observed level of resistance when both loci are homozygous is 60-fold, the level of resistance is higher than if only locus A or B was present.

In an untreated environment, the balance between the interaction of resistance loci, the fitness cost of each resistance allele, and the possible interaction the resistance loci have on the overall fitness, can dictate the evolutionary outcome of the loci in nature. If a resistance locus has a high fitness cost, often the allele frequency will decrease in an untreated environment [45]. In the presence of an additional resistance locus (with a different fitness cost) and a possible interaction between the two fitness costs, it becomes complicated to assess the ultimate evolutionary outcome of the resistance mechanisms and the level of resistance that they together will provide. It is possible that the frequency of a costly mechanism may still decrease in the population, but just at a slower rate than if it was present alone. It is also possible that the interaction between the two mechanisms could counter a fitness cost associated with one of the mechanisms allowing the allele to be maintained in a population. Determining the fitness costs associated with resistance loci/alleles are difficult to quantify and for most loci is unknown. No study looking at interactions between resistance loci has incorporated this information into observed resistance levels or into models of interactions. Most studies have evaluated resistance loci interactions in the presence of insecticides using strains with known resistance mechanisms or chromosomal linkage and insecticide bioassays.

In future studies that determine how multiple insecticide resistance mechanisms interact, it is important and necessary to analyze each locus individually. This includes determining the resistance level contributions of each mechanism in each genotypic state (homozygous and heterozygous) and analyzing the interactions between all possible genotype combinations. It would also be beneficial to assess how the fitness costs/benefits of the mechanisms can influence the observed interactions and resistance phenotype. With a larger body of literature that includes more underrepresented data points, we may then be able to construct a comprehensive model that can accurately predict the evolutionary outcome of multiple insecticide resistance loci in a resistant insect pest population. Based on the patterns found in the literature, it might be possible to establish some general rules for interactions between mechanisms that would be useful in the development of resistance management strategies.

References

[1] G.P. Georghiou, Management of resistance in arthropods, in: G.P. Georghiou, T. Saito (Eds.), Pest Resistance to Pesticides, Plenum Press, New York, 1983, pp. 769–792.
[2] NRC, Executive summary, pesticide resistance strategies and tactics for management, in: NRC (Ed.), Pesticide Resistance Strategies and Tactics for Management, National Academy Press, Washington, DC, 1986, pp. 1–9.
[3] B.J.M. Bohannan, M. Travisano, R.E. Lenski, Epistatic interactions can lower the cost of resistance to multiple consumers, Evolution 53 (1999) 292–295.
[4] M. Raymond, D.G. Heckel, J.G. Scott, Interactions between pesticide resistance genes: model and experiment, Genetics 123 (1989) 543–551.
[5] J.G. Scott, T. Shono, G.P. Georghiou, Genetic analysis of permethrin resistance in the house fly, *Musca domestica* L., Experientia 40 (1984) 1416–1418.
[6] R.M. Sawicki, Interactions between different factors or mechanisms of resistance to insecticides in insects, in: F.C.F. Korte (Ed.), Environmental Quality & Safety Supplement, Keorg Thieme Publishers, 1975, pp. 429–436.
[7] D.L. Hartl, A.G. Clark, Principles of Population Genetics, Sinauer Associates, Sunderland, Mass, 2007.

[8] J.H. Moore, S.M. Williams, Traversing the conceptual divide between biological and statistical epistasis: systems biology and a more modern synthesis, BioEssays 27 (2005) 637–646.
[9] J.M. Cheverud, E.J. Routman, Epistasis and its contribution to genetic variance components, Genetics 139 (1995) 1455–1461.
[10] L.O. Howard, F.C. Bishopp, The House Fly and How to Suppress It, US Department of Agriculture, Washington, DC, 1924.
[11] A.W.A. Brown, Insect Control by Chemicals, John Wiley & Sons Inc., New York, 1951.
[12] J. Keiding, The house fly – biology and control World Health Organization (WHO), Vector Biology and Control Division, WHO/VBC/86.937, 1986.
[13] M. Tsukamoto, Methods for linkage-group determination of insecticide-resistance factors in the housefly, Botyu-Kagaku 29 (1964) 51–59.
[14] R.M. Sawicki, A.W. Farnham, The use of visible mutant markers in the study of resistance of house flies to insecticides, in: Fourth British Insecticide and Fungicide Conference, 1967, p. 1.
[15] N. Liu, J.G. Scott, Genetics of resistance to pyrethroid insecticides in the house fly, *Musca domestica*, Pest. Biochem. Physiol. 52 (1995) 116–124.
[16] M.C. Hardstone, C.A. Leichter, J.G. Scott, Multiplicative interaction between the two major mechanisms of permethrin resistance, *kdr* and cytochrome P450-monooxygenase detoxification, in mosquitoes, J. Evol. Biol. 22 (2008) 416–423.
[17] I. Denholm, A. Devonshire, K. Gorman, G. Moores, Use of biochemical markers to study the interaction of insecticide resistance genes, in: D. Otto, B. Weber (Eds.), Insecticides: Mechanism of Action and Resistance, Intercept Ltd., Andover, 1992, pp. 293–304.
[18] R.F. Hoyer, F.W. Plapp Jr., A gross genetic analysis of two DDT-resistant house fly strains, J. Econ. Entomol. 59 (1966) 495–501.
[19] R.F. Hoyer, F.W. Plapp Jr., Insecticide resistance in the house fly: identification of a gene that confers resistance to organotin insecticides and acts as an intensifier of parathion resistance, J. Econ. Entomol. 61 (1968) 1269–1276.
[20] R.M. Sawicki, Interaction between the factor delaying penetration of insecticides and the desethylation mechanism of resistance in organophosphorus-resistant houseflies, Pest. Sci. 1 (1970) 84–87.
[21] G.P. Georghiou, Isolation, characterization and re-synthesis of insecticide resistance factors in the housefly, *Musca domestica*, in: Proc. 2nd Int. Cong. Pest. Chem., vol. 2, 1971, pp. 77–94.
[22] R.M. Sawicki, Resynthesis of multiple resistance to organophosphorus insecticides from strains with factors of resistance isolated from the SKA strain of house flies, Pest. Sci. 4 (1973) 171–180.
[23] J.G. Scott, G.P. Georghiou, The biochemical genetics of permethrin resistance in the Learn-PyR strain of house fly, Biochem. Genet. 24 (1986) 25–37.
[24] N. Liu, X. Yue, Genetics of pyrethroid resistance in a strain (ALHF) of house flies (Diptera: Muscidae), Pest. Biochem. Physiol. 70 (2001) 151–158.
[25] N. Liu, J.W. Pridgeon, Metabolic detoxication and the *kdr* mutation in pyrethroid resistant house flies, *Musca domestica* (L.), Pest. Biochem. Physiol. 73 (2002) 157–163.
[26] G.P. Georghiou, Genetics of resistance to insecticides in houseflies and mosquitoes, Exp. Parasitol. 26 (1969) 224–255.
[27] Y. Takada, T. Hiroyoshi, M. Hirano, Linkage group analysis of permethrin resistance in the Miyakonojo colony of the housefly, *Musca domestica* L. (Diptera: Muscidae), Appl. Entomol. Zool. 23 (1988) 122–126.
[28] S. Lee, T. Shono, Linkage group analysis of pyraclofos resistance in the housefly, *Musca domestica* L., Appl. Entomol. Zool. 31 (1996) 127–134.
[29] C. Park, T. Shono, Y. Ahn, Linkage group analysis of profenofos resistance in the housefly (Diptera: Muscidae), Korean J. Appl. Entomol. 35 (1996) 159–163.
[30] L. Zhang, K. Harada, T. Shono, Genetic analysis of pyriproxyfen resistance in the housefly, *Musca domestica* L., Appl. Entomol. Zool. 32 (1997) 217–226.
[31] T. Shono, S. Kasai, E. Kamiya, Y. Kono, J.G. Scott, Genetics and mechanisms of permethrin resistance in the YPER strain of house fly, Pest. Biochem. Physiol. 73 (2002) 27–36.
[32] T. Shono, L. Zhang, J.G. Scott, Indoxacarb resistance in the house fly, *Musca domestica*, Pest. Biochem. Physiol. 80 (2004) 106–112.
[33] M. Zaim, A. Aitio, N. Nakashima, Safety of pyrethroid-treated mosquito nets, Med. Vet. Entomol. 14 (2000) 1–5.
[34] F. Cui, L.-F. Lin, C.-L. Qiao, Y. Xu, M. Marquine, M. Weill, M. Raymond, Insecticide resistance in Chinese populations of the *Culex pipiens* complex through esterase overproduction, Entomol. Exp. Appl. 120 (2006) 211–220.
[35] WHO, Pesticides and their Application for the Control of Vectors and Pests of Public Health Importance, 2006, p. 12.
[36] J. Hemingway, H. Ranson, Insecticide resistance in insect vectors of human disease, Annu. Rev. Entomol. 45 (2000) 371–391.
[37] W.G. Brogdon, J.C. McAllister, Insecticide resistance and vector control, J. Agromed. 6 (1999) 41–58.
[38] A.M. Amin, J. Hemingway, Preliminary investigation of the mechanisms of DDT and pyrethroid resistance in *Culex quinquefasciatus* Say (Diptera: Culicidae) from Saudi Arabia, Bull. Entomol. Res. 79 (1989) 361–366.
[39] M.C. Hardstone, C.A. Leichter, L.C. Harrington, S. Kasai, T. Tomita, J.G. Scott, Cytochrome P450 monooxygenase-mediated permethrin resistance confers limited and larval specific cross-resistance in the southern house mosquito, *Culex pipiens quinquefasciatus*, Pest. Biochem. Physiol. 89 (2007) 175–184.
[40] D. Martinez-Torres, S.P. Foster, L.M. Field, A.L. Devonshire, M.S. Williamson, A sodium channel point mutation is associated with resistance to DDT and pyrethroid insecticides in the peach-potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), Insect Mol. Biol. 8 (1999) 339–346.
[41] J.C. King, L. Somme, Chromosomal analysis of the genetic factors for resistance to DDT in two resistant lines of *Drosophila melanogaster*, Genetics 43 (1958) 577–593.
[42] O. Peyronnet, Y. Pichon, Y. Carton, R. Delorme, Genetic analysis of deltamethrin resistance in laboratory-selected strains of *Drosophila melanogaster* MEIG, Pest. Biochem. Physiol. 50 (1994) 207–218.
[43] D. Dapkus, D.J. Merrell, Chromosomal analysis of DDT-resistance in a long-term selected population of *Drosophila melanogaster*, Genetics 87 (1977) 685–697.
[44] J.F. Crow, M. Kimura, Evolution in sexual and asexual populations, Am. Nat. 909 (1965) 439–450.
[45] G.P. Georghiou, The evolution of resistance to pesticides, Annu. Rev. Ecol. Syst. 3 (1972) 133–168.
[46] F.W. Plapp, Biochemical genetics of insecticide resistance, Annu. Rev. Entomol. 21 (1976) 179–197.