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The Avoided Target: The *Ceratitis capitata* Cellular Encapsulation Response

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Abstract

The three most common and most successful methods for controlling fruit-fly pest species (particularly *Ceratitis capitata*) are the sterile-insect technique, insecticide use, and biological control. Yet while innovative research in the first two have meant significant improvement in the efficiency of these techniques over the past two decades, by comparison, improvements in the efficiency of biological-control techniques have lagged. It is asserted that such will continue to be the case until more researchers systematically address how to overcome, evade, deactivate the immune systems of target host species, in particular the cellular encapsulation response. The encapsulation response to wasp parasitization in both Drosophila and Ceratitis are reviewed. It is suggested that the past four decades of cellular, molecular and genetic research in Drosophila immunity and defense against parasitoid wasps can serve as a springboard for rapid significant improvement of our present, nearly non-existent model of Ceratitis immunity.

Keywords: Medfly; Ceratitis; Drosophila; Encapsulation; Cellular immunity; Hemocyte

Introduction

Because no one technique is successful, the present best approach to the control of economic fruit-fly pests (*Ceratitis capitata* and others) in any one region requires choosing from among a suite of methods. Most prominent and effective among these methods are the Sterile-Insect Technique (SIT), the application of insecticides, and biological control by natural or fortuitous enemies of a given target pest insect.

SIT involves the release into wild area radiation-sterilized males of a target pest species [1]. Sterilized males, when released in large enough numbers, will numerically displace local fertile males in competition for available fertile females. Any one Dipteran female can produce a limited number of eggs during a short lifetime: an average *C. capitata* female lays 759 eggs in her lifetime [2] and is polyandrous [3]; thus females that have mated with a sterile male will invest their limited physical resources in producing eggs that do not contain viable embryos, leaving fewer resources for a subsequent mating with a fertile male. However, SIT is not ideal-sterile males that are raised in artificial environments are less sexually competitive in the wild than their fertile counterparts [4] and SIT is not useful against wild populations of sufficiently high densities [5].

The use of pesticides is also useful against target pest species. There are two major classes of insecticide used against *C. capitata*: organophosphates (e.g. malathion) and carbamate esters (e.g. carbaryl), both of which target the function of the Acetylcholinesterase (AChE) enzyme. AChE is synthesized in post-synaptic cells (e.g. neurons in the brain, muscles in the soma), and contributes to the regulation of the activity of neurotransmitter Acetylcholine (ACh) by hydrolyzing it. When malathion interacts with AChE, a key serine residue on the enzyme's active-site surface is stably covalently attached to a substituted phosphate group that is provided by malathion. Interaction with carbaryl results in the covalent attachment of a substituted carbamate group to the same serine residue. Both acts result in the steric blockage of the active site and the inactivation of AChE. Failure of AChE to act results in a statistical increase in Ach in the synapse, and thus a statistical increase in the stimulation of the post-synaptic cell: i.e., tetany [6]. However, *C. capitata* populations rapidly evolve resistance and cross-resistance to insecticides [7-10]. Additionally, long-term exposure to organophosphates and carbamate esters (including malathion and carbamyl) has been linked to significantly increased likelihood of sister-chromatid exchange events in people [11-13].

Biological control involves the release of large numbers of organisms to which a pest species is a target: predators, infectious agents, and parasites. The most widely used form of biological control of fruit-fly pests are parasitoid wasps, females of which will inject an egg into (or deposit an egg on) an immature stage of a developing fruit fly. The egg will hatch and release a wasp larva that develops inside the soma of the fly larva. When the fly pupates, the wasp larva will then consume the body of the fly and commandeer the puparium that was built by the fly. The wasp then pupates and emerges from the fly puparium [14]. Ideally, the new generation of wasps is able to repeat the process. One major limitation of biological control is that it is against the interest of an obligate parasitoid population to eliminate the host population. Ideally, local host and parasitoid populations maintain co-evolutionary dynamic equilibria of survival so as to allow both species to continue to propagate themselves. Additionally, target hosts such as *C. capitata* possess immune systems of varying efficacies that can successfully defend the fly host against a wasp invader.

While these three approaches are effective, it is in our economic interest for researchers to improve them. For example, recent advances in SIT include addressing known weaknesses in the system, such as improving the vitality and sexual competitiveness of sterile males. Males are sterilized by exposure to radiation as pupae. However, it was recently determined that such radiation damages fly tissue and kills many of the fly's natural gut biota [15], resulting in smaller, weaker, less competitive males. Subsequent research addressed this problem by

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feeding larvae (which would subsequently be irradiated) an anticipatory excess of beneficial gut bacteria. Ecclosed treated adults exhibited mild but significant increases in mean head, thorax, and abdomen lengths, but a greater response to treatment was observed in mating behavior: copulatory activity increased by nearly four fold over that of controls [16]. Another possible solution is ginger root oil: exposure of irradiated males to aromatherapy in the form of ginger root oil vapors resulted in a twofold increase in mating frequency, as well as greater survival time (as indicated by recapture experiments) in the wild [17].

Researchers have also addressed drawbacks in the insecticide approach. Recent work in identifying alternative, less toxic, pesticides has yielded striking outcomes. "Natural" insecticides like spinosad are at least as effective as malathion in combating fruit-fly pests [18,19] while exhibiting very low toxicity to people, though it can kill beneficial wasp parasitoids that target fruit flies [20]. Furthermore, existing attract-and-kill methods have been improved: removal of putrescine from BioLure, an attractant bait, had no significant effect on the number of *C. capitata* that were drawn in, but overall reduced the attraction of non-target insects by about 20% (though there was considerable variability by species [21]).

Yet the most effective component of the larger biological-control approach, the use of parasitoid wasps, has not made such significant advances. Researchers continue to seek alternative parasitoid species that are viable in a given region and that can further reduce target host populations, using methods that have not changed in decades. Very little is being done to better understand host-parasitoid interactions, and the genetic controls thereof, in a way that can improve parasitoid control. Thus it is the purpose of this review to compare what is already known about host cellular immune responses in Drosophila to that of pest species among the Tephritidae.

The Encapsulation Response in Drosophila

At the interface between Dipteran host and Hymnopteran endoparasitoid is the host immune system. The Drosophila immune system has been studied for over three decades, not because Drosophila species are pests (all but one member of this genus target economically invisible dead and rotting fruit), but because it is a fortuitously useful model for vertebrate innate (non-adaptive) immunity. There are both humoral (non-cellular) and cellular aspects to the immune system of any host. In *D. melanogaster*, humoral immunity comprises the synthesis and release of immune-effector molecules (such as anti-microbial peptides) by the fat body, a large organ that is the functional analog of the mammalian liver. The agents of cellular immunity, however, are the "blood" cells, or *hemocytes*. Circulating hemocytes and the fluid matrix in which they exist are collectively referred to as *hemolymph*. In *D. melanogaster* larvae and adults, the dorsal heart pumps hemolymph and causes it to circulate through the *hemocoel*, the body cavity that serves as the open circulatory system of insects. While the humoral and cellular immune systems do interact and overlap in function, it is the hemocytes that are the agents of encapsulation of metazoan invaders such as the eggs of parasitoid wasps [22].

D. melanogaster, though separated from *C. capitata* by more than 125 million years of evolution [23], nonetheless exhibits an identical life cycle: egg, three larval instars (designated L1-L3), pupa, adult. At 25°C, the elapsed time from egg lay to ecclosion is about 10 days. Hemocytes differentiate during an early embryonic stage, and continue to divide and differentiate for the duration of the life of the fly (hemocytes are not destroyed during metamorphosis, as most non-imaginal cells are). While hemocytes serve more than one function, their primary functions are not the transport of gases and nutrients, but rather the defense of the host. Thus, *D. melanogaster* hemocytes are analogous in function to some mammalian non-lymphocytic leukocytes, rather than erythrocytes. In healthy unparasitized *D. melanogaster* larvae, hemocytes can be found in three interconnected compartments: (1) circulating hemocytes that are carried through the hemocoel by the hemolymph; (2) sessile hemocytes that adhere to the walls of the hemocoel; and (3) the lymph gland, a hematopoietic organ that is composed of four to six pairs of lobes that are arranged symmetrically about the dorsal aorta [24]. The lymph gland is composed of hundreds to thousands of developing hemocytes at various stages of maturity, while the circulating and sessile hemocytes are mature [25]. Mature hemocytes consist of two types of cell. Plasmatocytes, which constitute c. 95% of mature circulating and sessile hemocytes, are phagocytic. They are thus comparable to the mammalian monocyte/macrophage lineage. The remaining c. 5% of mature hemocytes is crystal cells, so named because they carry crystals of Prophenol Oxidase (proPO), the inactive form of an enzyme that catalyzes melanin synthesis. Crystal cells contribute to the clotting mechanism, so they are best compared to the mammalian megakaryocyte/platelet lineage. With each larval molt, the hematopoietic system changes: the lymph gland comprises more cells, and contains more differentiated cells; the circulating and sessile hemocyte populations also increase. As Drosophila approach pupation, the lymph gland disperses, releasing thousands of hemocytes into circulation, presumably to consume larval tissue while imaginal tissues develop into adult structures [24].

However, if a parasitoid wasp, such as *Leptopilina boulardi*, injects an egg into the hemocoel of a *D. melanogaster* larva, circulating plasmatocytes will adhere to the egg [26], and shortly thereafter, a third class of hemocyte, the lamellocyte, differentiates from the plasmatocyte lineage in the lymph gland [24]. Lamellocytes are large, flat adhesive cells that will leave the lymph gland (thus causing the lymph gland to disperse [27]), enter the hemocoel, and adhere to the wasp egg [26]. A sufficient number of lamellocytes will form a multilayered cellular capsule around the wasp egg [28] (this response is evocative of the behavior of mammalian eosinophils). Crystal cells can also be part of the capsule, and will rupture and release proPO which will then be proteolytically activated, and catalyze the formation of melanin. The wasp egg dies, likely from multiple causes-suffocation, starvation, and molecular damage due to reactive oxygen species that are produced by cells in the capsule. This series of events is collectively referred to as the encapsulation response. In *D. melanogaster*, encapsulation is the primary cellular immune response to endoparasitization. It is worth noting that this response is not functional throughout the larval period - lymph-gland lamellocytes in *D. melanogaster* cannot differentiate until L3. Thus, parasitoid eggs that are present in the hemocoel of an L2 larva can develop without defending against encapsulation until the host molts into L3 [27], by which time a wasp larva, which is much better able to avoid encapsulation, may already have hatched.

Encapsulation responses to endoparasitization have been observed in many holometabolous insect hosts. Among the Lepidoptera, larvae of pest species tobacco budworm moth (*Heliothis viriscens*) and soybean looper (*Chrysodeixis includens*), can encapsulate eggs of parasitoid wasps [29,30]. In larvae of the heart-shaped scale insect [*Protopulvinaria pyriformis* (Cockerell)], a Hemipteran pest species, vigorous encapsulation responses protect hosts from parasitoid wasps [31]. Encapsulation has been extensively studied in the Diptera. Larvae of the mosquito *Aedes aegyptii*, the vector for yellow fever, and housefly larvae (*Musca domestica*) can both defend themselves against parasitic nematodes by means of an encapsulation response

[32,33]. Additionally, Tephritid fruit-fly pests such as *C. capitata* and *Bactrocera cucurbitae* are capable of combating parasitoid wasp infestation via encapsulation [34,35]; (the Ceratitis response will be discussed in the next section). However, the encapsulation response has been most extensively studied in Drosophila. Multiple species of Drosophila have been tested for their ability to encapsulate the eggs of various parasitoid wasps [36-38].

Importantly, encapsulation responses are variably successful. Monconduit and Prévost [39] demonstrated that larvae of the sister species *D. melanogaster* and *D. simulans* exhibited significantly different encapsulation rates (10% and c. 50% respectively) when parasitized by *Asobara tabida*. Two years later, Eslin and Prévost demonstrated that CHC (circulating hemocyte concentration) was almost four times greater in healthy unparasitized *D. simulans* larvae than in those of *D. melanogaster*, suggesting a correlation between CHC and encapsulation capacity [40]. This correlation was strongly supported when six closely related species of the *D. melanogaster* subgroup were assayed for CHC and encapsulation capacity against *A. tabida* [38]. Finally, a strong case was made for causality when it was observed that four Drosophila lines that were experimentally selected for increased encapsulation rates against *A. tabida* (an average of four times the encapsulation rate as that of four non-selected control lines) also exhibited CHC values that were about twice those of controls [41].

Additionally, there is evidently a dissectible genetic component to the encapsulation response. Encapsulation rates by isofemale lines of *D. melanogaster* were stable over 15 generations [42]. Subsequent work allowed the isolation of two strains of *D. melanogaster*: a "susceptible" (S) strain that exhibited an encapsulation rate of less than 3% against *L. boulardi*, and a "resistant" (R) strain that encapsulated *L. boulardi* over 80% of the time [43,44]. These encapsulation rates were stable and heritable, indicating a genetic component to resistance to parasitoids. Results indicated that resistance to *L. boulardi* was conferred by a single gene-the R strain possessed two copies of a dominant allele, while the S strain was homozygous recessive at the same locus [43]. Strikingly, both the S and R strains exhibited strong encapsulation rates of at least 90% against a different wasp, *A. tabida*, suggesting that there may be different genetic mechanisms for different wasp parasitoids [44]. Resistance to *A. tabida* was inferred to be the result of another single gene [45]. Subsequent research was able to quickly generate a genetic recombination map of these two putative genes, Rlb (Resistance to L. boulardi) and Rat (Resistance to A. tabida) [46,47]. Strikingly, the *Rlb* gene, whose existence has been inferred by genetic and phenotypic analyses, may have been identified with a gene whose molecular existence has been characterized: *edl* (*Ets-domain lacking* [48]), which encodes a transcription factor that modifies the activity of another transcription factor, Pointed [49]. Pointed is known to contribute to the regulation of the MAPK signaling pathway, and, strikingly, when overexpressed in *D. melanogaster* larval plasmatocytes, results in a significant c. fourfold increase in CHC, a c. threefold increase in circulating lamellocytes, and melanized encapsulations of self-tissue [50]. While conclusive evidence identifying *Rlb* with *edl* remains to be obtained, it is worth noting that most of the work in this promising direction was obtained with cost-effective low-technology techniques-genetic mapping, cell counting, and microscopy.

The upshot of such research is clear: (1) encapsulation rates are heritable; (2) encapsulation rates vary measurably between and within different species of Drosophilid host; and (3) CHC correlates with encapsulation rate.

The Encapsulation Response in Ceratitis is Not Well Described

While the encapsulation response is well understood in the Drosophila system, the same cannot be said about our understanding of the Ceratitis mechanism. Researchers of *C. capitata* and the Tephritidae have made some effort to address the host cellular immune response to parasitization, but apparently many of those reports that address encapsulation do so pursuant to goals other than that of describing and understanding the cellular encapsulation response mechanism. A list of recent publications that include at least some encapsulation data with respect to Tephritid hosts is presented in (Table 1).

The most common objective among those reports that address encapsulation is that of determining the host specificity of a chosen wasp species. It stands to reason that a potential target pest-Tephritid host should exhibit a low enough encapsulation rate so as to permit the success of a parasitoid wasp. For example, five species of Ceratitis (including *C. capitata*) and *Bactrocera cucurbitae* ware screened for their "acceptability and suitability" to serve as hosts for the parasitoid wasp *Psyttalia concolor*. Researchers found that of these six species, wasps were not able to ecclose at all from four of these potential host species, and that failure was qualitatively linked to the 100% success rate of the host encapsulation response. However, while wasps successfully ecclosed from *C. capitata* 9.1 times out of every 20 parasitizations, no observations were made as to whether those wasp that failed to ecclose from *C. capitata* hosts did so because of a successful encapsulation response or not [51]

A recent report described how the parasitoid *Diachasmimorpha longicaudata* was introduced to six different fruit-fly species: *B. invadens*, and five Ceratitis species, including *C. capitata*. In this study, *C. capitata* was not assayed for an encapsulation response because the wasp ecclosion rate was just over 50%, and thus making *C. capitata* "suitable" for the rearing of wasps. Yet the four host species that exhibited very low wasp ecclosion rates were examined for encapsulation and consistently, exhibited high encapsulation rates (c. 25% to c. 50% [34]). While these observations were informative with respect to the stated objective of the investigation, a pre-existing understanding of the host encapsulation response mechanism would have allowed researchers to make testable predictions as to what host physiological phenomena might explain the variations in encapsulation responses, thereby steering subsequent research in a profitable direction.

Interestingly, in another study, the wasp *Coptera occidentalis* (which, unlike most wasp species used for biological control, is a pupal parasitoid) is quite successful: wasps emerged from almost 80% of fly puparia. This could be consistent with observations in *D. melanogaster* of the dispersal of the larval lymph gland at onset of metamorphosis [24] which could be accompanied by a putative act of differentiation that prevents hemocytes from becoming lamellocytes. Importantly, the researchers observed no encapsulation among parasitized hosts, but that is the extent of any investigation into encapsulation [52]. A question worth asking would have been whether encapsulation responses had been attempted and failed (in which case lamellocytes, if they appear in *C. capitata*, could be found in circulation), or had not been attempted (in which case lamellocytes would have been absent).

Furthermore, *Bactrocera cucurbitae* and five species of Ceratitis (including *C. capitata*) were tested for whether they could serve as good breeding hosts for another potential biological control agent, *Psyttalia cosyrae*. No wasps ecclosed from pupariae of *B. cucurbitae*, *C. anonae*, *C. fasciventris*, and *C. rosa*. Importantly, the researchers

Tephritid host		Parasitoid wasp		References
Genus	**species**	**Genus**	**species**	
Ceratitis	*capitata*	*Coptera* *Diachasmimorpha* *Fopius* *Psyttalia*	*occidentalis* *longicaudata* *arisanus* *ceratitivorus* *concolor* *cosyrae* *lounsburyii*	[52] [34] [35,55,58] [55] [51,57] [57] [57]
	anonae	*Diachasmimorpha* *Psyttalia*	*longicaudata* *concolor* *cosyrae*	[34] [51] [53]
	catoiriii	*Fopius*	*arisanus*	[35]
	cosyra	*Diachasmimorpha* *Psyttalia*	*longicaudata* *concolor* *cosyrae*	[34] [51] [53]
	fasciventris	*Diachasmimorpha* *Psyttalia*	*longicaudata* *concolor* *cosyrae*	[34] [51] [53]
	rosa	*Diachasmimorpha* *Psyttalia*	*longicaudata* *concolor* *cosyrae*	[34] [51] [53]
Bactrocera	*cacuminata*	*Diachasmimorpha*	*krausii*	[72]
	cucumis	*Diachasmimorpha*	*krausii*	[72]
	cucurbitae	*Fopius* *Psyttalia*	*arisanus* *ceratitivorus* *concolor* *fletcheri* *cosyrae*	[35,73] [54] [50] [73] [52]
	dorsalis	*Diachasmimorpha* *Fopius*	*longicaudata* *tryoni* *ceratitivorus*	[74] [74] [54]
	invadens	*Diachasmimorpha*	*longicaudata*	[34]
	jarvisi	*Diachasmimorpha*	*krausii*	[72]
	latifrons	*Fopius*	*ceratitivorus*	[54]
	oleae	*Psyttalia*	*concolor* *lounsburyii*	[75] [76]
	tryoni	*Diachasmimorpha*	*krausii*	[72]
	zonata	*Fopius*	*arisanus*	[35]
Anastrepha	*ludens*	*Fopius*	*arisanus*	[63]
	obliqua	*Fopius*	*arisanus*	[63]
	serpentina	*Fopius*	*arisanus*	[63]
Dacus	*ciliatus*	*Fopius*	*arisanus*	[35]
	demmerezi	*Fopius*	*arisanus*	[35]
Neoceratitis	*cyanescens*	*Fopius*	*arisanus*	[35]
Bactrocera	*cacuminata*	*Diachasmimorpha*	*krausii*	[72]
	cucumis	*Diachasmimorpha*	*krausii*	[72]

Table 1: Publications that address encapsulation responses.

then dissected sample host larvae and found that every dissected larva contained an encapsulated wasp egg. The correlation between CHC and encapsulation rates in Drosophila species was recognized in the discussion, and "The same cellular defense mechanism likely occurs in the system studied here but has not been as thoroughly investigated for tephritid hosts." [53]. While the stated scope of the experimentation would not necessarily include an investigation of the encapsulation response *per se*, it is not scientifically satisfactory at this late date to leave the encapsulation response in the realm of likelihood.

A five-year survey of fruit-fly pests, performed in Tucumán Province, Argentina, revealed that five species of local wasp that parasitized wild populations of *C. capitata* and *Anastrepha fraterculus*. Furthermore, it added to previous data that supports the idea that local indigenous wasp populations are unable to successfully parasitize *C. capitata*, which has only been in the Americas since the early 1900s. However, the authors do not go on to address why this might be so, suffice it to say: "Two possible mechanisms would be encapsulation or antibiosis, but unless experimentally tested, these scenarios will remain hypothetical." [54]. Again, the encapsulation response is invoked, only to be left unexplained.

Other investigations also presented descriptions of encapsulation responses in *C. capitata*, but again, did not examine the structure of the response [55-57]. Worth noting is that not all recent investigations dealt with screen for hosts-a few others examined the effects of particular host environments on the development of parasitoid wasps [52,58].

Though there are few more recent publications that experimentally address encapsulation, it is clear that researchers of *C. capitata* and the Tephritidae have made some effort to address the host cellular immune response to parasitization. However, these efforts are too often presented incidentally, as if understanding the primary host defense against wasp parasitoids were somehow peripheral to any effort to make biological control a more effective tool. But what remains is the conclusion that no one has described the *C. capitata* hematopoietic system, or its encapsulation-response mechanism.

What Can be Done?

Between a wasps egg in the hemocoel of its host and successful ecclosion of a parasitoid wasp stands the host encapsulation response, the efficacy of which is in large part the product of genetic background. Thus any attempts to control or eradicate host pest Dipteran populations must address the host genetics of hemocyte development and behavior. Yet though larval CHC is apparently the principal index of Drosopholid defense against parasitization, and though many Ceratitis researchers do recognize and cite at least some research on Drosophila-wasp immune interactions, very little has been documented on hematopoiesis and cellular immunity in *C. capitata* or any other Tephritid pest species. Most investigators, though they dissect parasitized host larvae, go no farther than confirming the presence of live or dead parasitoids and then counting them. A few do go a bit farther and make note of whether parasitoid eggs/larvae have been encapsulated. So too, for any measurements of genetic heritability of encapsulation, and for CHC. There is little reason to expect an improvement in the biological control approach, if we continue to ignore the cellular and molecular aspects of the host/parasitoid competition.

There are useful protocols for the measurement of CHC and encapsulation rate that have been developed and used successfully for research into Drosophila cellular immunity. It is suggested that these protocols, adapted as is necessary to accommodate developmental differences in Ceratitis and other pest Tephritid species, will prove useful in characterizing the Ceratitis encapsulation response:

CHC

It might prove beneficial to Ceratitis researchers to employ a tested, economical, low-technology protocol for measuring CHC, such as the one that is described by Sorrentino and Schultz [59]. Importantly, there is strong evidence to support the idea that CHC values are not normally distributed, but rather consistent with a log-normal distribution. Thus, statistical comparisons of CHC values among treatment/background groups should make use of transformed mean *ln* CHC values, which are the means of the natural logarithms of individual CHC values [60].

Encapsulation rate

The measurement of encapsulation rate is a protocol that again would require very little additional effort to incorporate into investigations of

Ceratitis immune responses. A commonly used protocol, as well as the necessity of transformation of values for statistical analysis, is described by Carton and Boulétreau [61].

It could be that part of the problem is a "cultural" one - while Drosophila researchers are more concerned with understanding and manipulating the host, Ceratitis researchers are (understandably) more concerned with understanding and manipulating the parasitoid. Yet perhaps it is worth considering the ongoing response to the unusual case of *Drosophila suzukii*, which, unlike most Drosopholids, attacks healthy farmed fruits, and is thus an agricultural pest species. Invasive populations of *D. suzukii* were first found in the Americas and the European subcontinent in 2008 [62,63]. While many researchers predictably investigated the utility of pesticides and bait against *D. suzukii*, there are nonetheless some researchers who examined the host cellular immune response to parasitoids. It was determined that *D. suzukii* larvae not only have CHC values approximately four times greater than those of *D. melanogaster* larvae, they also *resultantly* exhibit a very robust cellular encapsulation response against multiple wasp species from four genera. Parasitization by thirteen of the tested wasp strains was met with successful encapsulation rates in *D. suzukii*, while *D. melanogaster* exhibited zero encapsulations when parasitized by those same strains [64]. A report in the next year confirmed the significantly higher CHC, as well as significant basal levels of circulating lamellocytes, in five strains of unparasitized *D. suzukii* larvae. Additionally, while those five strains of *D. suzukii* also exhibited variably high (59-87%) encapsulation rates against *Leptopilina heterotoma*, they were less successful against *Asobara tabida* (6-26%). Control wild-type *D. melanogaster* exhibited zero encapsulation rates. Lastly, a strong correlation between CHC and encapsulation was confirmed in *D. suzukii* [65]. The upshot of this clear: Drosophila researchers who investigate host-parasitoid interactions are in the habit of asking about correlations between encapsulation rates and CHC. At this point in time, it may be advantageous to Ceratitis researchers to consider adopting a standardized approach to systematically quantifying encapsulation, CHC, and parasitization-induced hematopoietic changes, modelled on the existing tested system used by Drosophila researchers. Such choices will likely lead to improvements in constructing improved strategies for the control or eradication of pest Tephritid populations.

Additionally, it will be important to actively address the genetic contribution to CHC and encapsulation in *C. capitata* via transgenic techniques. Evidence for genetic components to CHC and the encapsulation response is abundant. Larvae of *D. subobscura* have hemocytes but cannot produce lamellocytes or encapsulate parasitoid wasp eggs [66]. Geographically different natural populations of *D. melanogaster* exhibit variable CHC values and encapsulation rates of *Asobara tabida* [67]. The egg-larval parasitoid wasp *Fopius arisanus* (which is used to control *C. capitata*) at best emerges from 9% of parasitized *C. capitata*, but cannot ecclose at all from parasitized *Anastrepha obliqua* [58]. Homozygous recessive alleles at the *Rlb* and *Rat* genetic loci (which still have to be positively identified molecularly) in *D. melanogaster* abrogate the host larval ability to encapsulate *L. boulardi* and *A. tabida*, respectively [47]. A *D. melanogaster* larva that possesses a mutant genotype of the gene *serpent* (*srp*), srp^{3} / srp^{neo45}, has no circulating hemocytes [68]. Loss-of-function alleles of the *D. melanogaster hopscotch* gene (a member of the JAK family of non-receptor tyrosine kinases) reduce CHC and encapsulation capacity [69], while gain-of-function alleles have the opposite effect [27]. It would be a simple matter to identify and confirm experimentally the functions of *C. capitata* homologues of these and other functionally important genes. Key gene products would serve as targets for disabling host immune responses. For instance, an exogenous compound, introduced in bait or carried by the air, could be designed to inhibit the function of a key protein in the encapsulation response. Alternatively, introduction into the wild of large numbers of female and male *C. capitata* that are homozygous for recessive alleles that disable the cellular encapsulation response would not operate under the handicap of reduced viability and competitiveness that plague sterile males, and might stand a better chance of weakening the encapsulation response of an entire local population of flies, rendering them more susceptible to wasp parasitization [70]. Additionally, a laboratory population of *C. capitata* whose members carry in their genomes a transgene that would conditionally overexpress an identified suppressor of the encapsulation response could also ensure greater populational susceptibility to wasp parasitization [71].

Of course, the forgoing in no way should be taken to mean that continued analysis of parasitoids themselves cannot continue to yield useful data. Most parasitoids wasps have evolved ways of combating host cellular defenses. Some rely on evasion techniques such as sequestration of eggs in locations that are relatively inaccessible to host hemocytes, or molecular mimicry. Most wasps examined thus far, though, make use of suppressive tactics-toxins, or viruses/virus-like particles (VLPs) that target hemocytes, particularly lamellocytes, for inactivation or destruction. However, a discussion of this equally important other side to the host-parasitoid struggle is beyond the scope of this review [72-76].

Summary

Parasitoid control of Tephritid pest species is approaching the limit of its potential, yet is alone usually insufficient to control or possibly eradicate a local pest Tephritid population. In order to improve biological control of *C. capitata* with parasitoid wasps, it will be necessary to construct a genetic/physiological model of the *C. capitata* cellular immune system. For this to happen, researchers will have to expand their repertoires to systematically include simple, effective, inexpensive techniques that have already been pioneered by Drosophila researchers.

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