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Aggression in invertebrates

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Invertebrates are outstanding model systems for the study of aggression. Recent advances and promising new research approaches are bringing investigators closer to the goal of integrating behavioral findings with those from other disciplines of the neurosciences. The presence of highly structured, easily evoked behavioral systems offer unique opportunities to quantify the aggressive state of individuals, to explore the mechanisms underlying the formation and maintenance of dominance relationships, to investigate the dynamic properties of hierarchy formation, and to explore the significance of neural, neurochemical and genetic mechanisms in these behavioral phenomena.

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Current Opinion in Neurobiology 2003, **13**:736–743

This review comes from a themed issue on
Neurobiology of behaviour
Edited by Mark Konishi and Randolph Menzel

0959-4388/\$ – see front matter

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DOI 10.1016/j.conb.2003.10.003

Introduction

This review describes several invertebrate models in which intraspecific aggression is readily evoked in dyadic interactions between animals; these models enable studies to be performed at levels ranging from the behavioral through the physiological and ultimately to the molecular and genetic levels. Elegant models of interspecific aggression in invertebrates also exist [1], but these will not be dealt with here. In the species described here, agonistic behavior patterns appear to be pre-wired in the nervous system, as animals with no previous social experience can engage conspecifics in normal agonistic encounters. During such fights, paired animals exchange highly stereotypical behaviors that escalate through different intensity levels and that, ultimately, result in a decision with behavioral consequences for both winners and losers. A common theme in these studies is that amines, peptides and steroid hormones, substances that function as neuromodulators and/or as neurohormones, serve as important modulators of aggression.

Aggression in social insects: bees, ants, wasps and termites

It may be surprising that aggression is seen in social insects, considering that selfish behavior is rare in groups with shared reproductive interests, such as honey bee colonies; however, stereotyped agonistic behavior within a hive [2] is common during worker policing [3,4]. Moreover, aggression in the context of nestmate recognition has been explored in ants [5,6], bees [7], wasps [8] and termites [9] where the determination of self versus non-self is frequently based on the expression of cuticular hydrocarbon profiles [10,11]. With pheromonal commands reflecting the collective needs of the colony [12], many aspects of social behavior are under endocrine and genetic control, including the reproductive division of labor [13–15], investments in reproductive individuals [16], drone assassinations, queen execution by workers [17,18] or queen duels [19]. As in many other systems, agonistic success is fostered by physical superiority [20], promotes reproductive opportunities [21] and correlates with amine function [22].

Aggression in other invertebrates: spiders and dragon flies

Ritualized displays and cues that are predictive of agonistic success enable the assessment of a rival's relative fighting ability, in particular, in species with dangerous weapons, such as spiders [23]; the strategies that underlie aggression and intraspecific, intersexual cannibalism in this group [24–26] are shaped by the structure of the population [27]. Dominance enhances feeding opportunities in dragon flies [28] but few physiological studies that relate specifically to aggression have been carried out using these models.

Aggression in non-social insects: crickets

Detailed electrophysiological studies have been carried out in crickets, particularly looking into acoustic signaling. (Singing is used in mating behavior and in aggression in crickets and other insects [29].) Amine neuron systems (serotonin, octopamine, dopamine and histamine) have been fully mapped in cricket nervous systems, including those systems present in the brain and the ventral nerve cord (reviewed in [30]). Depletion of nervous system amines, either globally using reserpine or selectively with blockers of synthesis specific for serotonin or for octopamine/dopamine, produces alterations in aggression, but these effects are subtle [31]. Although reserpine depletes the nervous system of all amines and produces lethargic behavior, crickets are able to fight at different levels of intensity. Selective depletion of serotonin had no effects on fighting behavior, whereas octopamine/dopamine

depletion lowered the intensity of fights. The authors conclude that octopamine and/or dopamine are not required for the function of motor circuits involved in aggression, but that they do seem to play a role in releasing the behavior in response to appropriate stimuli. Other studies report that brain levels of serotonin are lowered in losing males during fights, but only if the wings that are important in singing behavior are intact [32]. Removal of the wings results in lowering of brain serotonin in both winners and losers of fights. Injections of the opioid antagonist naloxone enhanced aggression in losing male crickets and in females [33], while injection of a vertebrate μ -opioid agonist reduced aggression in male winners. These results suggest that status-specific effects must be considered when injecting drugs into winning and losing animals for this kind of study. Interestingly, it has been demonstrated that forcing losing male crickets to fly after agonistic encounters rapidly restores their willingness to fight [34]. This fact, well known to gamblers involved in cricket-fighting, requires an intact ventral nerve cord between the brain and the thoracic segments of the nerve cord.

Crustacean models of aggression

The first anatomical and physiological studies with crustacean species were performed more than 100 years ago. Indeed, the eminent figures TH Huxley, S Freud and G Retzius conducted extensive early anatomical studies on the nervous systems of these organisms. In the mid-twentieth century, fundamental questions of synaptic physiology were answered by B Katz, SW Kuffler, P Fatt, CAG Wiersma and others, due to the anatomical simplicity of their peripheral nervous systems of the crustacean models. These same systems now provide exciting information on neuronal function at a 'systems' level; thus, important studies with the crustacean stomatogastric ganglion describe, at the level of identified neurons, how modulation affects the output of a neural network [35]. A more recent frontier in which, once again, crustacean models offer opportunities that are not readily available with other species, is in the study of social behavior. Crustaceans such as crayfish and lobsters appear to be ideal for exploration of the neural basis of aggression because: (1) their structurally elegant, modular neural systems feature relatively few, large aminergic neurons, whose distribution has been mapped and whose physiological properties have been defined [36[•],37^{••}]; (2) the behaviorally relevant neural circuits have also been mapped [38[•]–40] and socially modulated changes in these circuits that relate to amine neuron function can be observed [39,41^{••}]; (3) amine levels can be both monitored [42] and experimentally altered [43–46]; (4) stereotyped behavioral acts can be represented by quantitative measures in many contexts [47] and finally; (5) crustacean individuals maintain a fundamentally solitary existence, with dominance resulting largely from physical superiority. By contrast, fighting success in other systems is

often determined by an ability to form coalitions or by differential treatment of kin.

Agonistic meetings between crayfish or lobsters in controlled laboratory situations feature a series of highly structured behavioral acts, with escalation being governed by strict rules. Fights progress through ritualized visual displays, antennae whipping, claw lock, wrestling and, if physical asymmetries are only minor, brief periods of unbridled claw use [48–50]. The expression of particular fighting strategies varies with hunger states [51], body size [52] and previous agonistic success [53]. Although fighting frequently serves to obtain or defend resources, such as shelter [54] or mates [55], its occurrence, particularly in the absence of a resource, suggests an inherent predisposition towards agonism [56,57].

As in other groups, amine neuron systems (serotonin and octopamine) are implicated as key physiological regulators of agonistic behavior and social dominance in crustaceans [37^{••},42], but controversy surrounds the experimental results in this area and their interpretation by different authors. Acute, experimental injections of serotonin and octopamine in lobsters (*Homarus americanus*) produced postures resembling those seen in dominant (serotonin-like posture) and subordinate (octopamine-like posture) animals during and after agonistic encounters [58]. It was these observations that inspired the detailed examination of the roles of amines in aggression in crustaceans by many authors, using a variety of species. Postural changes and enhancement of aggression were recently reported in a second lobster species (*Munida quadrispina*) [59]. Acute and constant infusion of serotonin in crayfish (*Astacus astacus*) produces aggression with a unique specificity: after a delay of 10–30 min, treated individuals engage larger opponents in prolonged bouts of fighting, even in instances that carry substantial risk of injury [46,50,60[•]–62]. Conversely, in a different species of crayfish (*Procambarus clarkii*), serotonin injections produced postural changes that did not resemble those seen during agonistic encounters; serotonin injections also reduced levels of aggression in agonistic encounters, whereas a serotonin analogue enhanced aggression [43]. In interpreting these observations, other authors do not focus on a direct role for serotonin in decapod aggression; instead, they suggest that serotonin treatment in *H. americanus* might indirectly affect social interactions through an inhibition of retreat in the losing animals [63]. Crayfish with lowered serotonin levels (*Orconectes rusticus*) are indistinguishable from controls in terms of fighting behavior [45], but serotonin-depleted lobsters showed enhanced levels of aggression [44], similar to those initially reported when serotonin levels were raised in lobsters and crayfish [60[•]]. One interpretation of such apparently contradictory results focuses on the possibility that levels of serotonin within a narrow window of concentration might have to be released at the correct time and place in the nervous

system for normal behavioral modulation. If this is so, then pharmacologically elevated or lowered levels of serotonin (or other modulators of behavior) could produce similar or different behavioral phenotypes, depending on the species examined and the multiplicity of effects mediated by the pharmacologically altered substance.

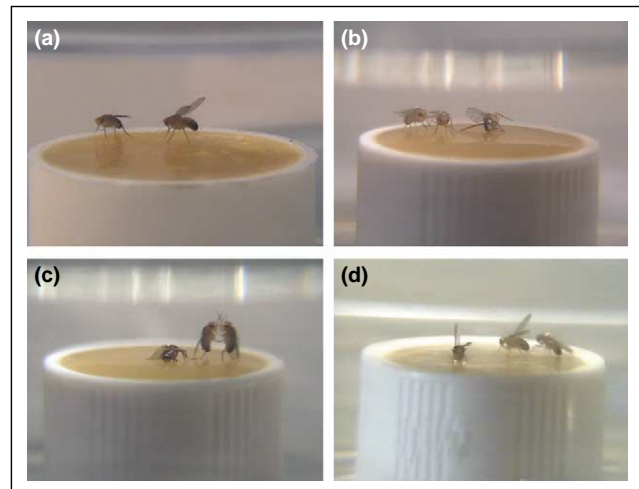
A novel model system: aggression in male fruit flies

Despite the wealth of information gathered from invertebrate models of aggression and that of the roles of amines such as serotonin and octopamine in this behavior, little, if any, information exists on exactly how amine neurons function during fighting. Do their firing rates change? If so, are the changes seen before, during or after fights? What are the consequences of such changes? The major difficulty that is shared by invertebrate and vertebrate models, is that the physiological activity in these neurons cannot easily be monitored while animals are fighting; thus, although hypotheses are abundant, we are left with the unsatisfactory conclusion that amines clearly are instrumental in aggression, but how they serve this role remains unknown.

One experimental approach that is not readily available in most invertebrate models, is the use of genetics and the accompanying powerful genetic experimental methods; thus, there are no inbred lines of lobsters, crickets or crayfish, and their genomes have not been sequenced. Molecular approaches, including RNAi, probably can be used with these species but it would be on an individual animal basis and would not involve the generation of experimental lines of, for example, highly aggressive animals. In all respects, other than the size of their neurons, fruit flies would be an ideal species to use, providing that robust patterns of aggression can be triggered in these animals under ethologically acceptable experimental conditions.

Aggression and territoriality are well-known in certain Hawaiian species of fruit flies [64,65]; what is less well-known, however, is that aggression also exists in common highly inbred species of fruit flies (*Drosophila melanogaster*). Originally described by Sturtevant in 1915 [66], other investigators have also reported an aggression in fruit flies during the period 1960–1990 [67–70]. This early work described many of the comprising components of the behavior, but in experimentally difficult scenarios involving many individuals. This was simplified recently to only a pair of male flies in a chamber with a small food cup and a headless mated female, illuminated from above. The food, potential mate and light serve to attract males to the surface, and within a few minutes both flies move onto the food cup, where they commence a series of encounters [71••]. The flies used in these fights are isolated as soon as they emerge as adults, and kept for 3–4 days in small test tubes containing food. Using these

Figure 1



The components of fighting behavior in adult male fruit flies: (a) wing threat, in which both wings are elevated for a sustained period of time. This is often seen early in fights; (b) fencing, in which animals push off with one of their legs in a sideward or forward direction; (c) boxing, in which both flies rise on their hind legs and thrash at each other with their forelegs; (d) chase, seen when a hierarchical relationship has been established, with the winner pursuing the loser who will either fly from the food surface or retreat to the edge of the food dish. Reprinted from [69], in a slightly modified form, with permission.

experimental conditions, an ethogram of the behavior was assembled, transition matrices were constructed and a Markov Chain analysis produced a quantitative analysis of the behavior. Flies meet and engage in encounters about once a minute. These encounters show varying intensity levels (Figure 1) and an average duration of 11 s; this varies proportionately with the intensity level. A hierarchical relationship is established relatively early in fights, but losers can continue to re-engage winners for several hours. With the behavior well characterized in a relatively simple experimental paradigm, one can now begin to take advantage of the powerful genetic methods that are available.

Mutant studies of aggression in flies

A few publications have explored the effects of selected classical mutations and chemical modifications of neurotransmitter levels on fighting behavior of flies. *Ebony* flies fail to incorporate β -alanine into their cuticles and *black* mutants show decreased synthesis of the same amino acid. These defects, in normal cuticular tanning, result in an early onset of courtship and enhanced territoriality [68]. A more recent study [72] has corroborated the results of earlier studies, which used the β -alanine mutants, and has explored the roles of dopamine and serotonin (using precursors of the amine or blockers of amine synthesis), octopamine (using a null mutant) and mushroom bodies (examined using transgenic animals expressing tetanus

toxin in output neurons of the mushroom bodies). The experimental protocol that was used here was complicated, with six males and three mated females in a chamber at the same time. The scoring system used was also complicated; however, the results successfully indicated that octopamine null mutants and flies with reduced synaptic output from the mushroom bodies showed reduced aggression, whereas, flies with elevated dopamine showed somewhat reduced aggression. Alteration of serotonin levels had no effect on aggression. It should be noted that many important controls were not included in these studies, which, according to the authors, were preliminary. Repetition of these studies, using a more simplified experimental protocol, with conditional mutant lines of flies would greatly help to interpret the observed results.

Much more powerful genetic methods are available for behavioral studies on aggression with flies, however, than have been reported thus far. These include conditional expression of mutations in the fly brain, whenever and wherever desired. One example is the GAL4/UAS system, originally described by Brand and Perrimon [73]. In this method, a cross between two transgenic lines of flies (one expressing the transcription factor GAL4 in subtypes of neurons, the other expressing the binding site for GAL4, driving expression of any desired gene) yields progeny in which desired genes are expressed in subtypes of neurons in the fly brain. In preliminary studies, we have expressed GAL4 in dopamine and serotonin neurons or selectively in dopamine neurons to drive expression of a temperature-sensitive mutant form of the protein dynamin in those cells [74**]. This protein behaves normally at 25°, becoming mutated at 30° and leading to a rapid block of vesicle recycling and hence of synaptic transmission at the elevated temperature [75*]. In preliminary studies (unpublished observations), we have observed decreased numbers of encounters between flies at the elevated temperature, using both GAL4 lines. Further experiments with this and related technology should yield valuable information about the role of subtypes of neurons in aggression in flies.

Aggression in female flies

As with male flies, Sturtevant was the first to mention aggression in female flies, in this case, directed towards males: ‘occasionally a female seems to frighten off a male by spreading her wings and moving quickly towards him’ [66]. A second mention of aggression in females came in a study comparing mating success in ebony and light males of freshly isolated *D. melanogaster* [65]. A more complete study of aggression in females was published last year, however, by Ueda and Kidokoro, using the common Canton-S strain of *D. melanogaster* [76**]. They observed and scored three patterns of female aggression, including ‘approach’, ‘lunge’ and ‘wings erect’. The authors report that these patterns ‘are similar to those of male aggression

in this species’ but that they differ from the patterns shown in female rejection behavior during courtship. Females show enhanced fighting if live yeast are growing on the surface of the food dish; conditioning female flies to the yeast food source ~12 hours before their fights, reduced the extent of fighting. This was interpreted as a possible adaptation to the enriched food source. Higher levels of aggression were seen when newly emerged females were held in isolation before the fights, in comparison with females held in groups of ten in a vial. Thus, both housing conditions and the quality of the food source influence fighting behavior in female flies, and, as with males, newly emerged females began to show aggression only a day after their emergence as adults.

Recently, we confirmed those behavioral patterns reported by Ueda and Kidokoro [76**], but also noted that specific differences are seen between the behavioral patterns in male and female *D. melanogaster* (unpublished observations). Certain patterns are seen predominantly in male flies, not in females (extended-duration wing-threat, boxing, tussling, holding and chasing), whereas, other patterns are seen mainly in females and not males (lunging with head butting, front limb fencing in an elevated posture). The remaining offensive and defensive patterns beyond these (for ethograms of male fighting behavior see [69]) are shared and seen in both males and females.

Of particular relevance here, is a report by Lee and Hall of a new behavioral phenotype seen in *fruitless* (*fru*) mutant male flies [77]: enhanced head interactions. *Fruitless* male flies court other males, forming long courtship chains, and show abnormal patterns of wing vibration during courtship, but otherwise appear capable of mating with females, depending on the *fru* mutant subtype [78]. In addition to enhanced head interactions, *fru* mutant males reportedly do not display boxing, which is a common high intensity component of male fruit fly fighting behavior. The head–head interactions are not seen during the first day after eclosion; their occurrence increases to a maximum 4–5 days later, in parallel with the chaining behavior.

These results raise an interesting question: does the *fru* mutation, with its well-studied effects on mating behavior, also direct the expression of female patterns of fighting behavior in the brains of male *fru* mutants? Are male- and female-specific patterns of fighting behavior specified in the brains of fruit flies after gender has been defined by the sex-determination hierarchy of genes? Is there an aggression-determination hierarchy of genes? Are male- and female-specific patterns of aggression defined as units in the brains of flies or can components of the behavior (like head butting in females and tussling in males) be transferred as individual modules into male and female brains, using methods of genetic manipulation? The fruit fly model offers exciting

Table 1

Aggression studies in various species of invertebrates (a partial listing, including only articles cited in this publication)

	Ethology	Physiology	Amines	References
INSECTS (social)				
Bees				
		*		
<i>Apis mellifera</i>	+		+	[2,13,14,15*,19]
<i>Apis florea</i>	+			[3]
<i>Apis cerana</i>	+			[4]
<i>Andrena scotica</i>	+			[7]
<i>Panurgus calcaratus</i>	+			[7]
<i>Bombus terrestris</i>	+		+	[22]
Ants				
<i>Formica pratensis</i>	+			[5]
<i>Formica exsecta</i>	+			[12]
<i>Linepithema humile</i>	+			[6,10,11,17]
<i>Solenopsis invicta</i>	+			[16,18]
<i>Cataglyphis niger</i>	+			[20]
<i>Odontomachus brunneus</i>	+			[21]
Termites				
<i>Reticulitermes</i> spp	+			[9]
Wasps				
<i>Vespa crabro</i>	+			[8]
INSECTS (non-social)				
Dragon flies				
<i>Pachidiplax longipennis</i>	+			[28]
Crickets				
		*		
<i>Gryllus bimaculatus</i>	+		+	[29–34]
Fruit flies				
		*		
<i>Drosophila melanogaster</i>	+	†	+	[67–70,71**,72,76**,77,78]
<i>Drosophila simulans</i>	+			[70]
<i>Drosophila sylvestris</i>	+			[64,65]
<i>Drosophila heteroneura</i>	+			[64,65]
<i>Drosophila ampelophila</i>	+			[66]
CRUSTACEANS				
Lobsters				
		*		
<i>Homarus americanus</i>	+	+	+	[36*,37**,44,49,58,60*,61,63]
<i>Munida quadrispina</i>	+		+	[59]
Crabs				
<i>Carcinus maenas</i>	+	*	+	[42]
Crayfish				
		*		
<i>Orconectes rusticus</i>	+		+	[45,46,48,50–52,55]
<i>Astacus astacus</i>	+	*	+	[60*,62]
<i>Procambarus clarkii</i>	+	+	+	[39,41**,43,56]
<i>Pacifastacus leniusculus</i>	+	+	+	[40,54]
OTHER ARTHROPODS				
Spiders				
		*		
<i>Dolomedes triton</i>	+			[26]
<i>Misumena vatia</i>	+			[27]
<i>Portia</i> spp.	+			[23]
<i>Latrodectus hasselti</i>	+			[24]

The table lists species in which: (i) ethological studies on aggression have been conducted (ethology); (ii) physiological and/or anatomical studies directly relating to aggression have been conducted (physiology); a role of amines in aggression has been demonstrated (amines).

* Anatomical and/or electrophysiological studies using intracellular and/or extracellular recording methods have been conducted in these species, but these are not directly related to aggression.

† Genetic studies related to aggression have been conducted.

possibilities for the identification of genes involved in producing patterns of a complex behavior like aggression in nervous systems. The shaping of these patterns, by experience and by hormones, throughout the lives of such organisms can then be fully elucidated.

Conclusions

Aggression is seen and has been studied behaviorally in many invertebrate species (Table 1), including social and non-social insects, other arthropods and crustaceans. In many of these species, detailed physiological and anatomical studies are now possible; they have been performed in relatively few of the insect species (mainly crickets and bees), but more widely among the crustaceans. Crustacean species are exciting because they enable the study of aggression to the level of the individual synapses, neurons and circuits that are key to this behavior. In all invertebrate systems examined thus far, altering the levels or function of amine neurons causes important changes in aggression; in one particular model, changes in status are accompanied by alterations in the function of certain synaptic regions relating to amines. Despite this progress, which goes far beyond our knowledge of vertebrate systems, no clear picture has yet emerged of exactly how amines modulate or alter the behavior. Recent experiments using fruit flies as a model for aggression have introduced the possibility of adding powerful genetic methods to the armamentarium of tools that are available for the study of aggression in invertebrates. Ultimately, this will allow investigators more insight into how this complex pattern of behavior is assembled in the nervous system and should shed further light on the role of modulators in the behavior.

Acknowledgements

It is difficult to summarize as large a field as aggression in invertebrates in a short review of this type, particularly one that tries to concentrate on recent literature. Moreover, studies in this large field use many species of animals and range from purely ethological examinations of agonistic behavior to physiological, molecular and genetic studies viewed from a neuroethological perspective. With the space constraints facing us, we recognized in advance that we could not do justice to the studies of all the excellent investigators who work in this field, and apologize to those of our colleagues who feel slighted by our somewhat amine-centric review of the field. EA Kravitz was supported by grants from NSF (IBN 90730), NIGMS (GM65595) and a starter grant from the Gund Fund of the Mind, Brain Behavior Program at Harvard University. R Huber was supported by grants from NSF (IBN 9874608) and NIMH (62557). Mostly, we acknowledge the outstanding contributions of the dedicated young undergraduate and graduate students and post-doctoral fellows who have worked with us over the years on the studies discussed here.

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