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Genetics of Aggression

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Abstract

Aggression mediates competition for food, mating partners, and habitats and, among social animals, establishes stable dominance hierarchies. In humans, abnormal aggression is a hallmark of neuropsychiatric disorders and can be elicited by environmental factors acting on an underlying genetic susceptibility. Identifying the genetic architecture that predisposes to aggressive behavior in people is challenging because of difficulties in quantifying the phenotype, genetic heterogeneity, and uncontrolled environmental conditions. Studies on mice have identified single gene mutations that result in hyperaggression, contingent on genetic background. These studies can be complemented by systems genetics approaches in *Drosophila melanogaster*, in which mutational analyses together with genome-wide transcript analyses, artificial selection studies, and genome-wide analysis of epistasis have revealed that a large segment of the genome contributes to the manifestation of aggressive behavior with widespread epistatic interactions. Comparative genomic analyses based on the principle of evolutionarily conservation are needed to enable a complete dissection of the neurogenetic underpinnings of this universal fitness trait.

"It is better to be feared than loved, if you cannot be both."

Niccoló Machiavelli (1469–1527)

Sexual selection: a type of natural selection in which members of one sex choose mates with particular features

Stabilizing selection: selection that selects against extreme phenotypic values and favors intermediate values of a trait, thereby reducing phenotypic variation

Heritability: the proportion of phenotypic variation that is explained by genetic variation

AGGRESSION: A UNIVERSAL FITNESS TRAIT

Aggression as a Quantitative Trait

Aggressive behavior enables individuals to survive by allowing them to compete for limited resources. Among social animals, aggressive displays serve to establish hierarchies in which the most dominant individuals have priority access to food and mating partners. Because dominance status reflects fitness, females are expected to select dominant males as preferred mating partners. Morphological characteristics, such as body armor, size of antlers, and intricate visual and/or vocal displays, can evolve as secondary characteristics that serve as proxies for fitness assessment by mating partners (67). Indeed, aggressive behavior is often under sexual selection (67). The establishment of social hierarchies prevents the need for continued aggression and associated risk of injury. Whereas low levels of aggression may be detrimental to survival and/or procreation, excessive levels of aggression are also harmful, as they divert energy from other essential activities, such as foraging; and they may carry a reproductive cost (86) and a high risk for injury or death. Thus, one can postulate that aggression is a trait under stabilizing selection (**Figure 1**), a hypothesis that has been corroborated by studies on species as diverse as water striders (39) and baboons (86) (See sidebar, Group Aggression).

From a genetics perspective, aggression is a quantitative trait, the manifestation of which is attributable to multiple segregating genes that are sensitive to the environment (40). Heritability estimates for aggressive behavior in people are generally high, ranging from 0.51–0.72 in a study of 3–10-year-old Dutch twins (57) and 0.37–0.57 in a study of adult twins (124). It should be noted, however, that nonadditive genetic variation from dominance and epistasis, as well as shared or correlated environments or shared experiences, are likely to inflate estimates of heritability in humans by unknown amounts (40). High heritability estimates for aggressive behavior have also been observed for other species, including monkeys (41), dogs (98), mice (115), and birds (33). These heritability estimates show that a substantial proportion of phenotypic variation in aggressive behavior is due to genetic variation. Consequently, response to artificial selection for either increased or decreased aggressive behavior is generally rapid and forms the basis for successful domestication. This is illustrated by the classic example of Belyaev's Russian silver foxes, which after selection for tameness retained their docile characteristics when selection was no longer applied, indicating the removal from the population of alleles that predispose to aggression (9).

Environmental effects on aggression include internal effects, i.e., regulation by hormones, and external effects, determined by the physical environment (e.g., stress) and, in the case of aggression, other individuals in the society (See sidebar, Hormonal Regulation of Aggression). Effects that arise when the genotype of one individual modifies the expression of traits in another individual have been coined indirect genetic effects (77). Previous experiences and

GROUP AGGRESSION

Organized aggression among social insects, in which groups work together to defend or occupy territory, is reminiscent of human warfare (or its more benevolent surrogate, team sports), in which coordinated action against outsiders is shaped by socially acceptable cultural norms imposed on an underlying susceptibility for aggression. Social insects, such as ants and honeybees, form societies in which only the queen is reproductively active. Such societies, often considered a super organism, have divisions of labor in which certain individuals are dedicated to defending the queen and prepared to sacrifice themselves to ensure the survival of their closely related siblings or work together to aggressively invade new territories. Self and nonself discrimination in ant warfare is mediated by pheromonal cues composed of cuticular hydrocarbons (49, 53). This represents an example of a mechanism for indirect genetic effects.

developmental conditions can also affect the expression of aggression.

The significant heritability of aggressive behavior presents a favorable scenario for identifying quantitative trait loci (QTLs) that affect aggression by linkage mapping and association analyses. To understand the genetic architecture of aggression, it is necessary to address the following questions. What are the genes that contribute to aggressive behavior? How do these genes interact in functional ensembles? How do polymorphisms within a subset of these genes contribute to phenotypic variation? How do genetic ensembles that mediate aggressive behavior within an individual respond to changes in environmental conditions (phenotypic plasticity), and how and to what extent do different genotypes vary in their responses to different environments (genotype-by-environment interactions)? In this review, we argue that it is difficult (if not impossible) to obtain answers to these complex questions by exclusively studying human populations, and we advocate a comparative genomics approach that integrates human genetics studies with studies on model organisms.

Pathological Aggression in People

Abnormal expression of aggressive behavior is a common consequence of traumatic brain injury, neuropsychiatric disorders, alcohol and substance abuse, and neurodegenerative diseases. Studies on the relationship between genetic variation and neurodegeneration-related aggression have focused primarily on Alzheimer's disease. Physical aggression and agitation occur in a variable portion (20%–65%) of patients diagnosed with probable Alzheimer's disease and are emotionally stressful for family members and caregivers. The genetic factors that determine comorbidity of aggression with neurodegeneration remain largely unknown. Difficulty in obtaining sufficiently large sample sizes and phenotypic heterogeneity have prevented large-scale linkage studies and genome-wide association studies and have limited genetic studies to

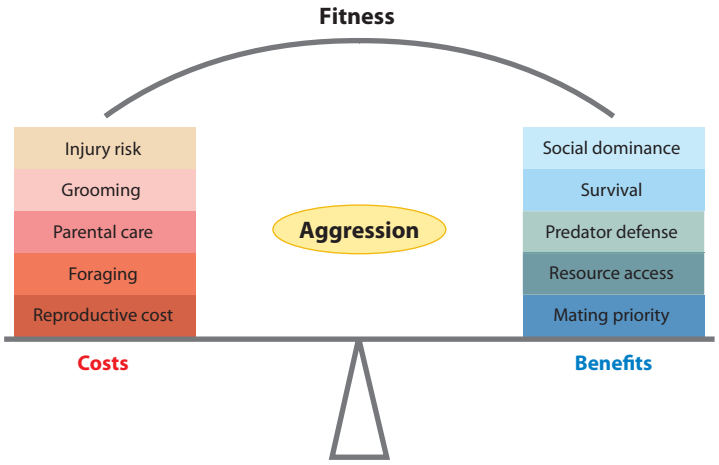


Figure 1

Schematic representation of the balance between costs and benefits for aggressive behavior as it relates to fitness.

HORMONAL REGULATION OF AGGRESSION

The relationship between aggression and testosterone—or its counterpart in fish, 11-ketotestosterone—is well established. In mice, aromatization of testosterone into estrogen in the brain results in territorial behaviors (121). In rats, the estrogen receptor β has been associated with aggressive behavior (87), whereas experiments employing virally delivered shRNA show some evidence for increased aggressiveness against juveniles mediated, in part, by the estrogen receptor α (102). Estrogen receptor–mediated mechanisms for aggression in mice display sexual dimorphisms; studies on estrogen receptor knockout mice have shown that estrogen receptor α increases male aggression but decreases female aggression, whereas the opposite pattern is observed for the estrogen receptor β (23). Additional hormonal systems that contribute to aggression have been implicated by studies on voles and cichlid fishes. Among prairie voles, a variable length microsatellite in the promoter region of the vasopressin 1a receptor regulates receptor level expression and is associated with anxiety-related and social behaviors (50). However, this correlation does not hold up across the vole phylogeny (44). In the African cichlid fish *Astatotilapia burtoni*, administration of a somatostatin antagonist increased aggression, whereas an agonist reduced aggression (110). Somatostatin receptor gene expression was negatively correlated with aggressive displays (111).

association analyses of single candidate genes. Such studies have focused on a small number of genes either previously known to be associated with Alzheimer's disease or implicated in aggression. Alzheimer's disease patients homozygous for the *apolipoprotein E e4* allele were reported to be more susceptible to aggressive episodes (25, 114), but this observation was not replicated in a population with a larger sample size (1,120 patients versus ∼100–400 patients) (54). Several studies have examined association of Alzheimer's disease–related aggression with polymorphisms in tryptophan hydroxylase (24), serotonin 5HT-2A and 5HT-2C receptors (5, 91), and the serotonin transporter (5, 92, 106, 113), but these studies have been controversial because of low statistical power.

Studies on the *catechol-O-methyl transferase* (*COMT*) gene have identified an association between the Val158Met polymorphism and aggression in schizophrenics (109). The Met/Met genotype has lower enzyme activity and is associated with higher levels of aggression and incidence of suicide attempts in schizophrenics and alcoholics (80, 85). These studies should be viewed with caution, however, because of the small sample sizes, differences in gender, patient history, environmental conditions, and heterogeneity of psychiatric manifestations. Furthermore, interpretations of studies on polymorphisms in candidate genes for neuropathology-related aggression are complex, as it is difficult to discriminate correlations with disease status from causality on the aggressive phenotype.

THE GENETICS OF AGGRESSION IN HUMAN POPULATIONS

Implication of Bioamines

The first major breakthrough to shed light on the neurogenetic basis of human aggression came in 1993 from a study on a large Dutch family (11). Several males in this family showed borderline mental retardation and abnormal violent behavior, which included impulsive aggression, arson, attempted rape, and exhibitionism. Affected individuals were deficient in the activity of monoamine oxidase A (MAOA), a central enzyme in the catabolism of biogenic amines, because of a point mutation in the eighth exon of the *MAOA* gene, which changed a glutamine to a termination codon. This was the first demonstration that bioamine metabolism may be critical in the regulation of impulsive aggression. Subsequent studies have corroborated the association between *MAOA* polymorphisms and aggression in humans (56, 71), mice (12), and rhesus monkeys (83).

The discovery that bioamine signaling may play a critical role in modulating aggressive behavior directed attention to serotonin as a central neurotransmitter involved in aggression, as lower levels of serotonin were related to increased aggression. A polymorphism in the promoter region (referred to as the short versus long allele) of the serotonin transporter gene (*SLC6A4*) has been associated with aggression and a wide range of neuropsychiatric disorders (79).

In addition to serotonin, genetic analyses have implicated dopamine, norepinephrine, and GABA neurotransmitters in aggression (72, 75). A study based on self reports of 298 15-year-old adolescents and assessments from 296 primary caregivers and 253 teachers identified an association of externalizing behavior with a variable number tandem repeat (VNTR) polymorphism in the third exon of the dopamine D4 receptor (*DRD4*) gene. Individuals homozygous for the short allele of the serotonin transporter and carrying the *DRD47r* variant had higher scores for aggression and/or delinquent behavior than other combinations of these loci (51). This is a rare example of a demonstration of epistasis in a human association study, in which the effect of the short allele of the serotonin transporter is modified by a polymorphic site in the *DRD4* gene (See sidebar, Epistasis).

Genotype-by-Environment Interactions

Aggression, like most behaviors, is plastic, and its manifestation depends on the environment.

Quantitative trait locus (QTL): a chromosomal region that harbors one or more genes with alleles that contribute to phenotypic variation

Linkage mapping: locating quantitative trait loci by linkage to polymorphic marker loci within a family or a mapping population derived from crosses between inbred lines

Association analysis: identification of quantitative trait loci associated with a statistical difference in phenotype between genotypes at polymorphic markers that segregate in a natural population

Tryptophan hydroxylase: the rate-limiting enzyme in the biosynthetic pathway of serotonin that attaches a hydroxyl group to the amino acid tryptophan

Catechol-*O*-methyl transferase: a methyl group–transferring enzyme involved in the breakdown of catecholamine neurotransmitters, including dopamine, epinephrine, and norepinephrine

Monoamine oxidase (MAOA): an enzyme that inactivates monoamines, such as norepinephrine and serotonin, by catalyzing their oxidation

Furthermore, this phenotypic plasticity may depend on the genotype (**Figure 2**). Following the identification of risk alleles for aggressive behavior of the *MAOA* and serotonin transporter genes, it became clear that the effects of these alleles were dependent on previous experience. A study on a large sample of boys with a history of childhood maltreatment showed that the VNTR at the promoter of the *MAOA* gene is associated with violent behavior only in individuals who were abused as children (13, 60). Maltreated children with high levels of this enzyme were less likely to develop antisocial problems than maltreated children with low levels of MAOA expression (35, 56). Further studies reported that this genotype-by-environment interaction is gender specific (56) and that extreme cases of maltreatment overshadowed the differences between alternate *MAOA* alleles (120). In addition, parental care could mitigate the effects of stress on behavioral outcomes in female carriers of the *MAOA* allele with low monoamine oxidase activity (61). A study on rhesus monkeys mirrored these observations. Rhesus monkeys that were reared under adverse social conditions (small social groups versus large interactive groups) were more aggressive, especially when they carried the *MAOA* allele corresponding to low levels of enzyme activity (59).

A similar genotype-by-environment interaction effect has been reported for the short and long allele polymorphism of the serotonin transporter gene. Here, people with one or two copies of the short allele exhibited more depressive symptoms as a result of stressful life experiences than did individuals homozygous for the long allele (14, 62). Associations of promoter polymorphisms of the serotonin transporter gene with aggressive behavior have been reported in some cases (48, 74, 107), but associations with suicidal behavior (which can be considered a form of self-aggression) have been controversial (10, 96, 112). Also, cocaine dependence did not affect differences in aggressive behavior among African-American individuals with alternative alleles (88).

EPISTASIS

Epistasis occurs when the action of one gene is modified by one or several other genes, resulting in nonadditive phenotypic effects. Such modifiers can be enhancers or suppressors. The analysis of epistatic interactions, especially higher-order interactions, is statistically challenging under conditions in which the genetic background cannot be precisely controlled, as is the case for most human genetics studies. Consequently, human genetics studies have mostly been restricted to the resolution of additive effects. In *Drosophila melanogaster*, the genetic background can be controlled precisely, and large numbers of individuals can be reared under controlled environmental conditions. Analysis of double heterozygous *P*-element insertion lines has shown extensive epistasis for olfactory behavior (42, 99), startle behavior (123), and aggression (129), and suppressing epistasis of naturally segregating modifiers of transposon-tagged genes that affect startle behavior (122), olfactory behavior, sleep phenotypes, and waking activity has been demonstrated (105). It is likely that widespread epistasis is a general feature across phyla, including humans, in which it may be an underlying cause for the missing heritability conundrum, the observation that the addition of the effects of many loci that contribute to complex trait variation falls short of explaining the total observed genetic variation (70, 128). Thus, it is reasonable to predict that in people, as in flies, the genetic underpinnings of aggressive behavior involve epistatic networks of pleiotropic genes.

Genotype-by-environment interactions of the *MAOA* and *SLC6A4* alleles on aggression have not been universally replicated. This is likely because of the low statistical power of some studies; differences in ethnicity, age, and sex of the study subjects; and different environmental factors analyzed to determine genotype-by-environment effects. Confounding factors of other neuropsychiatric influences of allelic variants at these loci introduce a further confounding element in the interpretation of genotype-by-environment interactions. Genotype-by-environment interactions together with genetic background effects present major complicating factors for larger-scale genome-wide association studies in human populations.

Externalizing behavior: behavior that directs problematic energy outward and is expressed as aggression, defiance, bullying, vandalism, theft, and other socially unacceptable actions

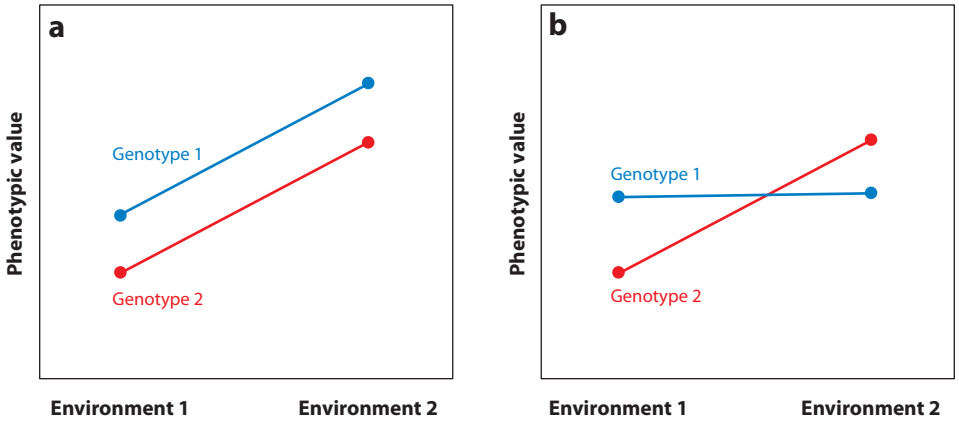


Figure 2

Interactions between the genome and the environment. The blue and red colors represent different genotypes. (*a*) Phenotypic plasticity. Phenotypic values shift for both genotypes in the two different environments but to the same extent, resulting in parallel reaction norms. (*b*) Genotype-by-environment interaction. Phenotypic values are affected differently for both genotypes in the two environments. The genotype indicated by the blue line has identical phenotypic values in environments 1 and 2, whereas the phenotypic value of the genotype indicated by the red line is substantially higher in environment 2 than in environment 1, resulting in crossing of reaction norms.

Challenges for Genome-Wide Association Studies in Human Populations

Whereas polymorphisms in genes encoding the serotonin transporter and MAOA have been definitively implicated in predisposition to antisocial and violent behavior, it is clear that they represent only the tip of the iceberg of a complex neurogenetic architecture that determines aggressive behavior. Human studies on the genetics of violent behavior have centered mostly on excessive aggression rather than on the genetic underpinnings that contribute to phenotypic variation in the normal range of aggression in the human population, with phenotypes encompassing the full spectrum of shyness, assertiveness, chronic suppressed or overt anger, intimidating behavior, and inclination toward violence. As with most studies of neuropsychiatric disorders, quantification of aggression relies on questionnaires or, in the case of studies on children, interviews with parents or teachers. Nevertheless, the phenotypic complexity of aggression, with its many manifestations and multiplicity of triggers, makes it difficult to quantify this trait at a level of specificity conducive to genome-wide association studies. In addition, gender differences, differences between ethnic groups, and the need to recruit a large homogeneous sample of individuals make it virtually impossible to obtain phenotypic information and DNA samples from enough subjects to surpass a genome-wide multiple testing threshold for statistical significance. These daunting challenges have corralled the majority of human genetics studies on aggression into a search for the lost key under the streetlight, as they continue to focus mostly on a small number of well-established candidate genes related to bioamine function and metabolism. Advances in understanding the complex genetic architecture of aggressive behavior will benefit from the recruitment of model organisms in which the phenotype can be accurately quantified and the genetic background and environmental conditions can be precisely controlled. Comparative genomic studies can discover evolutionarily conserved principles and orthologous genes and genetic networks that are associated with aggression across phyla.

INSIGHTS INTO THE GENETICS OF AGGRESSION FROM MODEL ORGANISMS

Genetics of Aggression in Mice

Aggressive behaviors in mice can be readily quantified by the number of attacks and attack duration when mice are placed in various situations of social confrontation (76). In the resident-intruder assay, a resident male defends his territory (i.e., home cage) by attacking an intruder. Maternal aggression of a female with pups against a strange male can also be readily recorded. Aggression in mice is mediated via neural pathways similar to those in people (**Figure 3**) (82). Furthermore, similar neurotransmitters are implicated in the manifestation of aggressive behavior (82). Mice with a deletion of the *MAOA* gene show enhanced intermale aggression as adults (90, 101). Serotonin suppresses aggressive behavior (20, 74), whereas dopaminergic (94, 117) and noradrenergic (72) mechanisms enhance aggression. Modulation of aggression by serotonin is, however, dependent on the type of serotonin receptor that is activated (47, 82). Male mice that lack the 5-HT1B receptor show

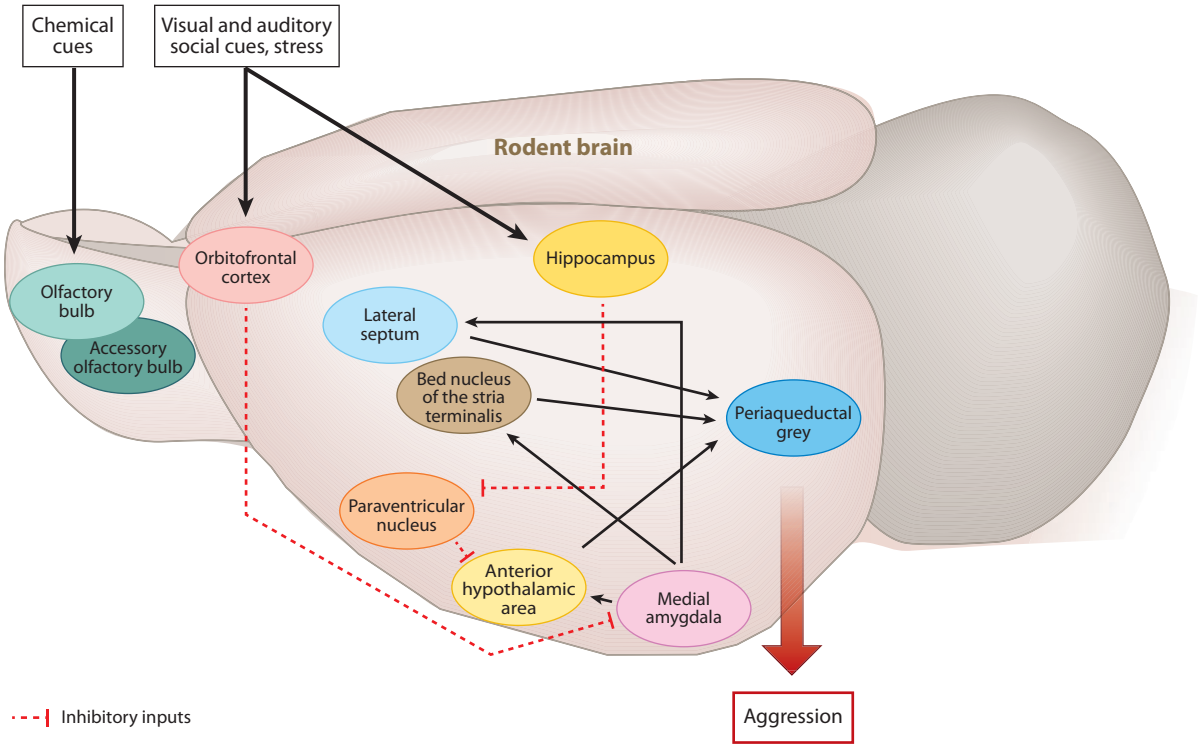


Figure 3

Brain regions implicated in aggressive behavior in rodents. Chemosensory inputs can trigger aggression through the olfactory and accessory olfactory systems, whereas visual and acoustic input, adverse social interactions, and stress generally elicit aggression in humans. In rodents, chemosensory signals activate the medial amygdala, which sends projections to the lateral septum, the bed nucleus of the stria terminalis, and the anterior hypothalamus. Projections from these regions to the periaqueductal gray result in the expression of aggression. Inhibitory inputs (*dashed lines*) can modulate aggression. Projections from the orbitofrontal cortex to the amygdala inhibit aggression. Optogenetic stimulation of neurons in the ventrolateral subdivision of the ventromedial hypothalamus elicits indiscriminate aggressive behavior in male mice (66). Patients with orbitofrontal cortical lesions are prone to uninhibited aggressive behaviors (for a more detailed description of neural mechanisms of aggression, see Reference 82).

Homologous recombination: breakage and reunion between homologous lengths of DNA, used in knockout mice to generate defective alleles of the target gene

high levels of aggression (100), whereas mice treated with a 5-HT1A receptor antagonist show reduced aggression (8). Raising synaptic serotonin levels by deleting the serotonin reuptake transporter via homologous recombination also lowers aggressive behavior (52, 65). The diverse effects of serotonin implicate multiple neural circuits that orchestrate aggressive behavior.

Although internal hormonal factors influence the propensity for aggression, environmental triggers can elicit acute expression of aggressive behaviors through activation of the endocrine system. These environmental signals are primarily chemical cues that are recognized by pheromone receptors in the vomeronasal organ, a chemosensory organ located above the palate that mediates the reception of chemical signals that provide social information about kinship or gender or information about the presence of predators (58, 78, 108). V1R pheromone receptors are G-protein-coupled receptors that signal via G_{i2}, are expressed by apical vomeronasal neurons and are activated by small molecules found in urine (63). V2R receptors constitute a class of pheromone receptors related to metabotropic glutamate receptors and are expressed by neurons in the basal layer of the vomeronasal organ. These receptors signal via G_0 and recognize peptide ligands, including breakdown products of major histocompatibility antigens (17). Mice in which the TRP2 channel, a central signal transduction component of vomeronasal neurons, was removed by homologous recombination were no longer aggressive in the resident-intruder assay and had lost gender discrimination, showing similar mating behavior toward males and females (104). Furthermore, removal of a large cluster of 16 genes that encode vomeronasal V1R receptors resulted in reduced maternal aggression, indicating that one or more of the deleted receptors interact with chemical cues that elicit maternal aggression (27). V2R receptors have been implicated in male-male aggression triggered via major urinary proteins (17). Tissue-specific deletion of the $G_{\alpha 0}$ subunit through homologous recombination prevents signaling via V2R receptors and results in increased aggression (16).

The genetic analysis of aggression in mice has employed two approaches: QTL mapping and analysis of single gene mutations. In the former approach, an aggressive strain of mice (e.g., BALB/cJ) is crossed with a nonaggressive strain (e.g., A/J), and linkage analysis is performed in an F2 population. Such a study identified chromosomal regions that harbor genes with allelic variants associated with variation in aggression between the parental strains (31). However, these studies are limitedin that the mapping population is derived from only two parental strains and thus captures a relatively limited sample of natural phenotypic variation. It has been difficult to identify causal alleles within large chromosomal QTL regions in mice. Testing the effect of knockouts of every candidate gene within such a region is generally not feasible. The Collaborative Cross, which consists of inbred lines derived from an advanced intercross population of eight different mouse strains and is being generated as a community resource for high-resolution QTL mapping (22), holds great promise for the identification of narrower QTL regions (6) but may still fall short of identifying causal alleles (73).

In studies complementary to QTL mapping approaches, several single-gene mutations have been identified that result in hyperaggression. A spontaneous mutation that lacks a functional gene for the nuclear receptor NR2E1, known as the fierce mutant, showed extreme hyperaggression (125), which could be suppressed by introduction of a functional human homolog of the *NR2E1* gene (1). Similarly, a knockout mutation of the neuronal nitric oxide synthetase gene resulted in mice that relentlessly attacked even opponents who had surrendered or displayed no interest in fighting and that killed their cage mates (81). Interestingly, maternal aggression against male intruders was reduced in these mice (46), whereas neuronal nitric oxide synthetase expression was increased in mice selected for high maternal aggression (45). It has been suggested that abnormal behaviors in the neuronal nitric oxide synthetase knockout

mice may be due to compromised serotonergic neurotransmission (20). Subsequently, a human association study, which included 1,308 patients with personality disorder, familial or adult attention deficit/hyperactivity disorder (ADHD), suicide attempters, and criminal offenders along with 1,954 controls, revealed a promoter polymorphism in the *NOS-I* gene associated with impulsivity, including hyperactive and aggressive behaviors (93). This study confirmed earlier studies with smaller sample sizes, which implicated polymorphisms and haplotypes in the *NOS-I* and *NOS-III* genes in suicidal behavior in Caucasians (97) and a polymorphism in the 3′-UTR of the *NOS-I* gene with completed suicides of Japanese men (26).

As is often found with single-gene mutations, the effects of both the null mutation in neuronal nitric oxide synthetase and the fierce mutation were influenced by genetic background. The hyperaggressive phenotype of the neuronal nitric oxide synthetase knockout allele was abolished after backcrossing from the C57BL/6J-129 background to the pure C57BL/6J genetic background (64). Similarly, the hyperaggressive phenotype of the fierce mutation was attenuated in the B6129F1 genetic background compared with C57BL/6J (125). These findings illustrate that genes that contribute to aggression are subject to modulation by modifiers in different genetic backgrounds, underscoring the notion that aggressive behavior is a phenotype that emerges from a genetic architecture that consists of ensembles of interacting genes. Disruption of certain components within such an ensemble can either unleash a hyperaggressive phenotype or can be buffered by other members of the gene ensemble to attenuate the mutational effect. Studies on *Drosophila melanogaster* mutants in controlled wild-derived genetic backgrounds have shown that epistatic modifiers that segregate in nature tend to reduce the magnitude of newly arising mutations (105, 122). The identification of genetic networks that underlie complex behavioral traits and the identification of epistatic interactions on a genome-wide scale are currently still impractical in people and in mice but can be accomplished in the powerful *D. melanogaster* genetic model.

Aggression in Flies: Enabling Systems Genetics

Drosophila presents a powerful model for the dissection of complex traits, including aggression, because large numbers of individuals of the same genotype can be reared rapidly under controlled environmental conditions and without regulatory restrictions. The first documentation of aggression in flies and its relation to mating behavior was reported in 1975 by Dow & von Schilcher (32), but it took more than 25 years before *Drosophila* materialized as a model organism for the systematic genetic dissection of aggressive behavior (21). This required the development of quantitative measures of aggression by either videotaping (28) or direct observations of flies fighting in the presence of a decapitated female (21) or a food droplet after a period of food deprivation (37). Aggressive behavior involved kicking, chasing, wing threat, and, in extreme cases, boxing, in which flies would tussle while holding one another with the front legs. Ethograms documenting these behaviors showed sexual dimorphism in fighting styles between males and females (84) in accordance with behavioral dimorphism between the sexes observed for virtually every other behavior (4) and most quantitative traits. Male-male aggressive interactions can be modulated by previous experience and social context (89, 126), and subgroups of neurons of the sexually dimorphic neuronal circuit that expresses the male-specific form of the sex determination gene *fruitless* have been implicated as one component of the nervous system essential for mediating intermale aggression (15, 18, 116). Similar to studies in people, serotonin has been implicated as a neurotransmitter that mediates aggression in *Drosophila* (2, 30). In addition, octopamine, the fly counterpart of norepinephrine expressed in a subgroup of neurons that project to the subesophageal ganglion (15, 55, 127), and neuropeptide F (30) modulate aggression in flies.

Attention deficit/hyperactivity disorder (ADHD): a family of neurological disorders that prevent an individual from regulating activity level, inhibiting behavior, and focusing on tasks

Ethogram: a diagrammatic depiction of a behavioral repertoire

False discovery rate: the expected proportion of false positives among all significant results

Artificial selection for increased aggression implicated a cytochrome P450, Cyp6a20, in mediating aggressive behavior (29). Further experiments showed that social experience modulates expression levels of *Cyp6a20* and that *Cyp6a20* expression levels are inversely related to levels of aggressive behavior (119). Expression of *Cyp6a20* in support cells of olfactory sensilla suggested that the activity of Cyp6a20 might be related to regulating pheromone sensitivity that triggers intermale aggression (119). The pheromone 11-*cis*-vaccenylacetate elicits male-male aggression via the Lush odorant-binding protein and the Or67d odorant receptor (118), and the effects of 11-*cis*-vaccenylacetate are modulated by olfactory neurons expressing the Or65a receptor (68). Furthermore, masculinization of females by expressing transgenes that are components of the sex determination pathway in pheromone-producing oenocytes elicited attacks rather than courtship from males (43). These observations show that pheromonal signals can trigger aggressive behavior, analogous to pheromone signaling in the mouse vomeronasal organ.

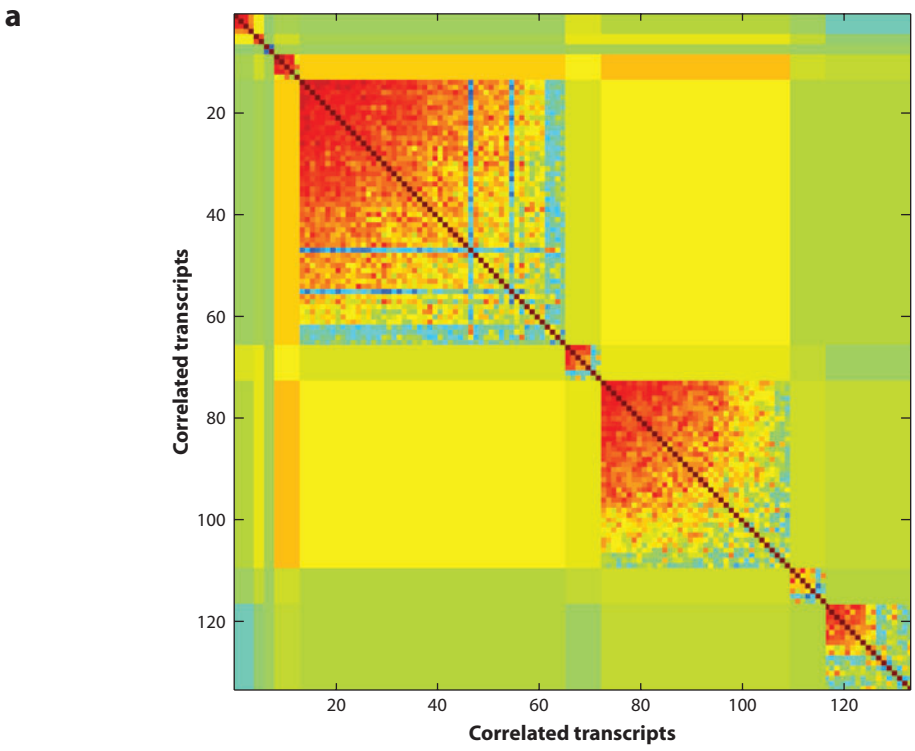
The artificial selection experiment that led to the identification of *Cyp6a20* had limited power because it employed a base population with low heterogeneity composed of a mixture of different Canton S laboratory strains and a single selected replicate (29). In contrast, bidirectional selection from a heterogeneous base population followed by microarray expression analyses contrasting duplicate high and low aggression lines revealed differential expression of as many as 1,539 transcripts at a stringent false discovery rate (>0.001), providing a glimpse of the genetic complexity that underlies aggressive behavior (37). The large number of genes implicated in aggression is consistent with the large mutational target evident from a study in which a screen of 170 co-isogenic *P*-element insertion lines identified 57 genes (~35%) with aberrant aggressive behavior compared with the control (38). These studies indicate that a large segment of the genome contributes to the manifestation of aggressive behavior, an observation that is likely to apply across phyla, and underscores the importance of defining how individual loci interact in genetic networks to gain an understanding of the complex genetic underpinnings of aggression.

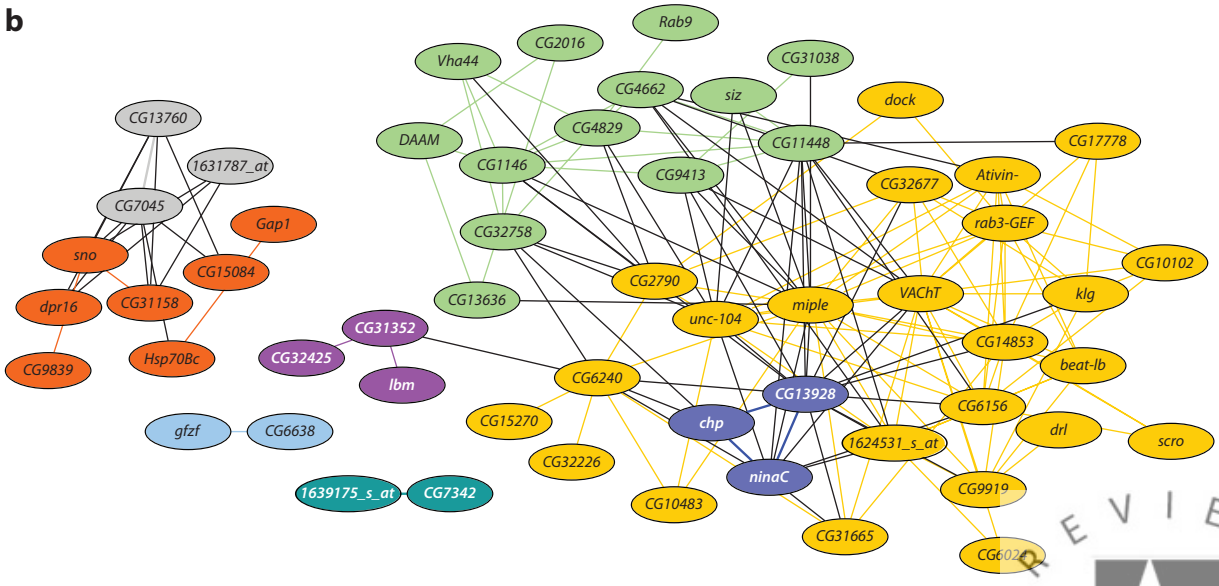
Two approaches have been used to identify ensembles of genes that mediate aggression, one based on covariant expression of transcripts and the other based on epistatic interactions. Microarray expression analysis of 40 inbred wild-derived *Drosophila melanogaster* lines showed that the transcriptome is highly genetically intercorrelated and organized as 241 biologically meaningful modules of covariant transcripts (7). The lines are vastly divergent for aggressive behavior, and regression of phenotypic values and transcript abundance levels followed by clustering analysis of the residuals revealed a genomic signature of modules containing 266 novel transcripts associated with aggression (**Figure 4**) (34). As with differentially expressed transcripts among low- and high-aggression selection lines, covariant transcripts span a wide variety of biological processes, and many are the products of computationally predicted genes of yet unknown functions (34).

The first evidence for extensive epistasis in the genetic architecture for aggression in *Drosophila* came from gene mapping studies

Figure 4

Modules of correlated transcripts associated with aggressive behavior among genome-wide transcriptional profiles from 40 inbred wild-derived *Drosophila melanogaster* strains. (*a*) Heat map of correlated probe sets organized as modules, using modulated modularity clustering (MMC) (103). Phenotypic values were regressed on transcript abundance levels, and the residuals of significant regressions were clustered into modules so that members within each module are more highly correlated with each other than with those outside the module. The diagram shows pairwise correlations between 133 transcripts and the strength of correlations within the modules decreases down the diagonal. Highly correlated modules appear in red. (*b*) A network view of the most highly correlated modules, in which the edges represent correlated transcripts and the color codes of the nodes represent the different modules in panel a. Figure used with permission from Reference 34.





Mushroom bodies: higher-order integrative structures in the insect brain

Pleiotropy: the condition in which a gene product affects multiple phenotypes

on recombinant inbred lines (36). Subsequent studies revealed epistatic interactions among six co-isogenic *P*-element insertion mutants that were hyperaggressive (129). Transcriptional profiling studies on heads from double heterozygotes derived from these *P*-element insertion lines showed widespread transcriptional epistasis. Assessment of the correlations between variation in gene expression, variation in aggressive behavior, and variation in morphological parameters of the mushroom body and central complex identified 1,197 transcripts that were associated with both aggressive behavior and the length of the α lobes of the mushroom body (129). The complexity of the neurogenomic architecture of aggression was also underscored by an analysis of the coordinated actions of transcription factors in the honeybee brain, which resulted in the construction of a transcriptional regulatory network model that predicted gene expression changes of more than 2,000 genes related with different behaviors, including aggression (19).

Fighting involves learning because social defeat reduces subsequent aggressive behavior (89). Thus, it is perhaps not surprising that mutations that reduce or enhance aggression show correlated effects on the structure of the mushroom bodies, which are integrative brain centers implicated in learning (95, 129). However, epistatic networks for brain morphology were largely distinct from the network observed for aggressive behavior, showing that these phenotypes, although dependent on an overlapping array of pleiotropic transcripts, are influenced differently by the mêlée of enhancer and suppressor effects among them (129).

Widespread epistatic interactions at the level of the transcriptome are in line with previous documentation of ripple effects through the transcriptome caused by single *P*-element insertions affecting olfactory behavior (3). These observations have significant impact on the interpretation of human genome-wide association studies in which epistatic interactions cannot be readily resolved because of lack of statistical power. Nevertheless, the widespread epistasis evident from studies on model organisms likely is a conserved property of genetic architectures across phyla. Thus, it is reasonable to assume that a polymorphic site associated with disease status or trait variation can exert its effects either directly or indirectly through its effects on the regulation of contextually related transcripts.

Genes implicated in aggressive behavior through analysis of covariant transcript abundance among inbred wild-derived lines and transcripts implicated from analyses of epistasis in double heterozygous *P*-element insertion lines show only moderate concordance, indicating that both approaches capture different aspects of the genetic architecture of aggressive behavior and bearing further testimony to the complex genetic underpinnings of this behavioral trait. Thus, aggression arises as an emergent property of a vast segment of the genome. Because of the combination of extensive epistasis and pleiotropy, disruptions in genes not primarily associated with aggression per se may indirectly influence aggressive behavior. The challenge posed to behavioral geneticists is to disentangle direct and indirect effects and to identify the most critical hub genes with the largest phenotypic effects.

CONCLUDING REMARKS: A GRAND CHALLENGE FOR THE FUTURE

Understanding the genetic architecture of aggression requires a systems-level approach with the following objectives: (*a*) achieving a quantitative interpretation of the effects of DNA variants in terms of variation in transcriptional networks and the proteome as well as analysis of causality of transcript abundance variation with phenotypic variation; (*b*) understanding the relationship between DNA variants and variation in neural circuitry and dissecting those components of the neural circuit, if possible, that are causally associated with aggression; (*c*) understanding the effects of gene-environment and gene-gene interactions on the genetic architecture of aggressive

behavior; (*d*) understanding how experience and endocrine factors influence variation in genome-wide transcript expression to the extent that we will be able to predict the consequences on the behavioral phenotype; and (*e*) developing methods to disentangle aggressive aspects from other aspects of neurological conditions in human populations. These are daunting endeavors that cannot be accomplished by human genetics studies alone but will require comparative genomics studies on genetically tractable model organisms. The recent generation of a panel of 192 inbred wild-derived *D. melanogaster* lines with sequenced genomes enables for the first time association analyses in this powerful model, empowering comprehensive systems genetics studies of complex behaviors (69). Systems genetics studies on model organisms hold great promise because they can identify not only evolutionarily conserved genes but also orthologous networks of genes that can be applied across phyla and provide novel insights into the genetic architecture of aggression in people that will go well beyond the few candidate genes that have been studied to date. If we can obtain a comprehensive understanding of the complex genetic underpinnings of aggressive behavior, we will, indeed, have gained a profound insight into human nature itself.

SUMMARY POINTS

1. Aggression is a quantitative trait, the manifestation of which is attributable to multiple segregating genes that are sensitive to the environment; aggression is under stabilizing selection.
2. The high heritability of aggressive behavior presents a favorable scenario for linkage mapping and association analyses.
3. Abnormal expression of aggressive behavior is a common consequence of traumatic brain injury, neuropsychiatric disorders, alcohol and substance abuse, and neurodegenerative diseases; however, interpretations of studies on polymorphisms in candidate genes for neuropathology-related aggression are complex, as it is difficult to discriminate correlations with disease status from causality on the aggressive phenotype.
4. Bioamine signaling plays a critical role in modulating aggressive behavior across phyla; in mice and *Drosophila*, pheromones can trigger aggressive behavior.
5. Genotype-by-environment interactions, together with genetic background effects, present major complicating factors for genome-wide association studies in human populations.
6. Aggression in mice can be readily quantified and is mediated via neural pathways similar to those in people; several mutations have been identified that result in hyperaggression.
7. Studies on mice and *Drosophila* have shown that genes that contribute to aggression are subject to modulation by modifiers in different genetic backgrounds, underscoring the notion that aggressive behavior is a phenotype that emerges from a genetic architecture that consists of ensembles of interacting genes.
8. Aggression arises as an emergent property of a large portion of the genome. Because of the combination of extensive epistasis and pleiotropy, disruptions in genes not primarily associated with aggression may indirectly influence aggressive behavior.

FUTURE ISSUES

1. Systems genetics studies on model organisms will be able to identify not only evolutionarily conserved genes but also orthologous networks of genes that can be traced across phyla and provide insights into the genetic architecture of aggression in people that will go well beyond the few candidate genes that have been studied to date.
2. Studies on possible epigenetic contributions to plasticity in aggressive behavior will be valuable to complement systems genetics approaches.
3. Future comparative genomic studies will be able to provide insights at the molecular genetic level into the evolutionary forces that modulate aggressive behavior and drive the establishment of social hierarchies.
4. The development of novel methods that will allow quantitative monitoring of the dynamics of genome-wide transcript levels in behaving animals in real time in defined neuronal circuits will enable deeper insights into the dynamic relationships between gene expression and neural activity during the initiation and execution of aggressive behavior.
5. Further studies in a systems genetics context are needed to examine how environmental challenges, in particular social or physical stress, modify aggressive behavior.
6. The development of new experimental designs and statistical methods will be needed to disentangle confounding factors of disease status and aggressive behavior in people to ultimately predict in early stages of the manifestation of neurological symptoms to what extent the affected individual is likely to develop agitated and aggressive behavior; such anticipatory information will facilitate advance preparation of better management and/or identify novel therapeutic targets for amelioration of behavioral symptoms.

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