ELSEVIER

Natural selection on the *Drosophila* antimicrobial immune system
Brian P Lazzaro

The evolutionary dynamics of immune defenses have long attracted interest because of the special role the immune system plays in mediating the antagonistic interaction between hosts and pathogens. The antimicrobial immune system of the fruit fly *Drosophila melanogaster* is genetically well characterized and serves as a valuable model for studying insect and human innate immune defenses. I review here evolutionary and comparative genomic analyses of insect antimicrobial immune genes, with an emphasis on *Drosophila*. Core signal transduction pathways in the immune system are orthologously conserved across long evolutionary distances, but genes in these pathways evolve rapidly and adaptively at the amino acid sequence level. By contrast, families of genes encoding antimicrobial peptides are remarkably dynamic in genomic duplication and deletion, yet individual genes show little indication of adaptive sequence evolution. Pattern recognition receptors that trigger humoral immunity are evolutionarily rather static, but receptors required for phagocytosis show considerable genomic rearrangement and adaptive sequence divergence. The distinct evolutionary patterns exhibited by these various classes of immune system genes can be logically connected to the functions of the proteins they encode.

Address
Department of Entomology, Cornell University, Ithaca, NY 14853, USA

Corresponding author: Lazzaro, Brian P (bplazzaro@cornell.edu)

Introduction
Natural selection may act strongly on immune systems as hosts adapt to novel, diverse, and coevolving pathogens. Any effective host defense system must have the capacity to first, recognize potentially pathogenic infection; second, signal activation of the immune response; and third, kill the infectious agent. From a pathogen's perspective, surviving the immune defense is essential. This places an evolutionary premium on mechanisms to evade, resist, or suppress host immunity. Every successful advance by the pathogen, however, creates selective pressure on the host

to evolve reestablished immunity. The stage is thus set for coevolutionary interactions wherein hosts and pathogens reciprocally adapt to each other even though no major shifts in pathology are necessarily achieved [1,2]. It is well established that genes in the immune systems of vertebrates and *Drosophila* exhibit significantly elevated rates of amino acid evolution relative to nonimmune genes (e.g. [3–5,6**]), indicative of molecular adaptation [7–9]. Not all immune response genes evolve equivalently, though, and we can draw inference regarding the nature of pathogen interaction with different components of the immune system through the distinct evolutionary histories and trajectories of various immune genes.

Insect antimicrobial immune responses consist primarily of defensive phagocytosis and extracellular circulation of potent antibiotic peptides. The fruit fly *Drosophila melanogaster* emerged as a valuable genetic model system for studying innate immunity in the mid-1990s (well reviewed in [10**]). The first evolutionary genetic analyses of *Drosophila* immunity genes quickly followed [11–13] and population genetic data on various components of the system have steadily accumulated since then. Complete genome sequencing of *D. melanogaster* and several other insects over the past few years, culminating last year with the landmark sequencing of 12 species of *Drosophila*, have now made comparative genomic analyses of insect immune systems possible [6**,14,15*,16*,17**]. In this review, I synthesize that literature to describe the molecular evolution of the *Drosophila* antimicrobial immune defense, relating gene function to evolutionary history and likely host–pathogen interactions.

Natural selection on the humoral immune response
The *D. melanogaster* humoral antimicrobial defense [10**] is regulated by pattern recognition receptors (PRRs) such as peptidoglycan recognition proteins (PGRPs) and Gram-negative binding proteins (GNBPs). These PRRs are exquisitely sensitive to ubiquitous and highly conserved microbial cell wall components such as bacterial peptidoglycans and fungal glucans. *Drosophila* humoral immune responses are initiated when activated PRRs trigger two primary signaling cascades, the Imd and Toll pathways, which ultimately drive the production of short, extracellularly secreted antimicrobial peptides (AMPs). Other PRRs play negative regulatory roles. Septic injury in *Drosophila* additionally activates wound healing through the JNK pathway, which bifurcates from the Imd pathway, and stress responses via the JAK/STAT signaling pathway. The *D. melanogaster* Toll pathway is

also involved in embryonic development, hematopoesis and possibly resistance to viruses and parasitoids. The genetic architecture of the *D. melanogaster* defense is stereotypical of insects, and homologous Imd, Toll, JAK/STAT, and JNK signaling pathways are found even in vertebrates.

Intuition might suggest that PRRs and AMPs should experience strong natural selective pressure because these proteins come into direct contact with invading microorganisms. Conversely, intracellular signaling genes might be expected to show little indication of adaptive evolution. In surprising defiance of this intuition, empirical data have consistently shown that signaling proteins evolve rapidly while PRRs and AMPs show little indication of adaptation at the amino acid sequence level. AMP and PRR genes show higher rates of genomic duplication and deletion than do signaling genes. These observations are illustrated in Figure 1 and discussed in detail below.

The genomic complement of antimicrobial peptides varies widely among insects, with many AMP gene families found in only a few closely related species. For example, *D. melanogaster* produces 8 described classes of AMPs encoded by 28 distinct genes, but genes encoding only three of these peptide classes (defensins, cecropins, and lysozymes) can be found in the genomes of bees, mosquitoes, and beetles [14,15[•],16[•],17[••]]. These other insects

Figure 1
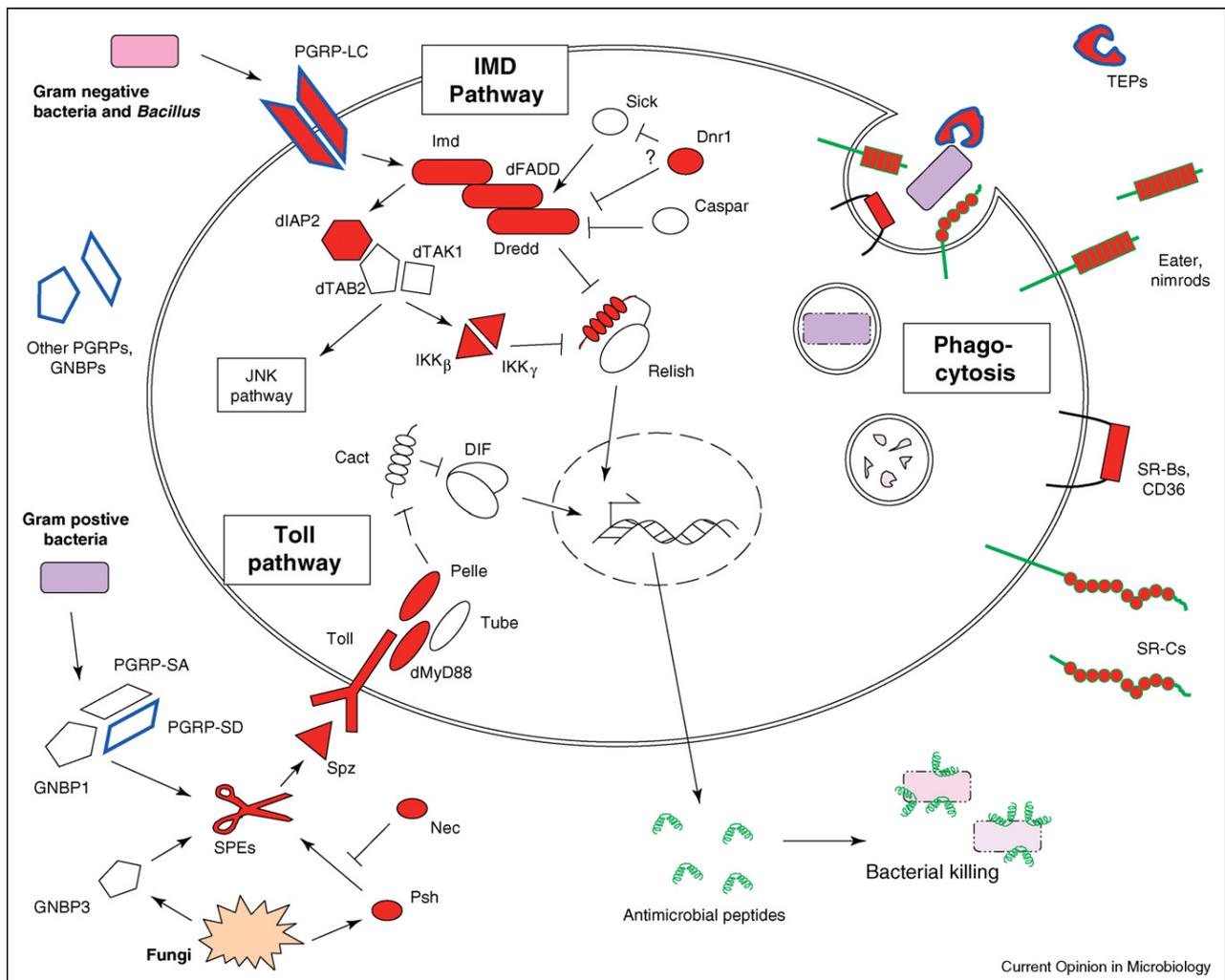
A schematic illustration of an idealized *D. melanogaster* immune responsive cell illustrating prominent proteins required for the activation of a humoral immune response and receptors involved in defensive phagocytosis. Proteins whose gene families have experienced considerable genomic turnover among *Drosophila*, *Anopheles*, *Aedes*, *Apis*, and *Tribolium* are outlined in heavy blue [6[••],14,15[•],16[•],17[••]]. Proteins whose gene families have experienced considerable genomic turnover within the genus *Drosophila* as well as between *Drosophila* and the other insects are outlined in heavy green [6[••]]. Red-shaded proteins have been implicated as evolving adaptively at the amino acid sequence level in *D. melanogaster* and/or *D. simulans* [4,6[••],33[•],41].

each produce their own unique AMPs. Genomic re-arrangements of AMP gene families and acquisitions of novel AMPs are apparently frequent and rapid in insects, and such events occur even within the genus *Drosophila* [6**,12,13,18–20] at a rate much higher than what is typical of *Drosophila* gene families [6**].

Despite their rapid genomic turnover, extensive study has revealed no evidence of adaptive amino acid diversification in *Drosophila* AMPs [6**,11,12,18,21,22], implying that minor adjustments in peptide function through altered amino acid sequence are not mediating host–pathogen coevolution. Most AMPs have simple and non-specific modes of antibiotic action, such as driving pathogen lysis through membrane disruption [23]. Even when microbes may be capable of evolving resistance to individual AMPs, the evolution of global resistance *in vivo* is probably limited by host employment of multiple AMPs with distinct activities. This strategy is analogous to the human attempts to forestall evolution of antibiotic resistance in clinical settings though simultaneous application of multiple antibiotics. The lack of adaptive sequence evolution in *Drosophila* AMPs contrasts with the observation that AMP gene family radiation is frequently associated with amino acid diversification in vertebrates [24•]. A mammalian defensin has recently been shown to have unexpected involvement in hair pigmentation [25], suggesting that mammalian AMPs may play roles outside immunity that place them under different selective pressure. Adaptive sequence radiation of a termite AMP has been observed, and the selection pressure was attributed to a major shift in host ecology [26].

PRRs are evolutionarily more stable across insects, with all of the insect genomes sequenced to date containing PGRP and GNBP multigene families. These show little indication of genomic rearrangement within the genus *Drosophila* [6**] or between the mosquitoes *Aedes aegypti* and *Anopheles gambiae* [17**]. PGRP and GNBP gene families have experienced considerable flux on the deeper lineages that separate flies, mosquitoes, bees, and beetles, though, with numerous independent expansions and contractions in family size evident [6**,15•,16•,17**]. Whether these genomic fluctuations mark adaptation to the distinct spectra of microbes faced by each of these insects remains to be determined.

PRRs could plausibly be expected to coevolve with pathogens if pathogens escape host recognition through evolutionary modification of their cell walls, but neither population genetic nor comparative genomic analyses have revealed substantial evidence for adaptive evolution in *Drosophila* PGRP or GNBP amino acid sequences [4,6**,27,28]. One exception is a two amino acid insertion in the recognition site of the PGRP-LCa splice variant of *Drosophila* species in the *melanogaster* subgroup. The

insertion is predicted to alter the peptidoglycan-binding properties of this PGRP-LCa isoform and appears to have been, along with several compensatory mutations, strongly favored by natural selection [6**]. The observation that PRRs show little evidence of adaptive sequence evolution, however, seems to be generalizable across invertebrates including *Nasutitermes* termites [29], *Anopheles* mosquitoes [30], and cladoceran arthropods in the genus *Daphnia* [31]. Evasion of host recognition by massive cell wall modification is possible, as evidenced by the vertically transmitted endoparasitic bacterium *Spiroplasma poulsonii* [32], but the relative rarity of adaptive amino acid evolution in PRRs would seem to suggest that dispensing with or substantially modifying structural components of the cell wall may not be a viable evolutionary strategy for most insect microbial pathogens.

There is striking genomic conservation across insects in core immune signaling pathways. Nearly every gene in the Toll, Imd, JAK/STAT, and JNK signaling cascades is found in perfect orthology between species of *Drosophila* [6**], mosquitoes [14,17**], the honey bee [15•], and the red flour beetle [16•]. In spite of this remarkable maintenance of orthology, signaling proteins in these pathways are rapidly and adaptively diverging at the amino acid sequence level [4,6**,17**,33•]. The Relish cleavage complex of the Imd signaling pathway provides a particularly striking example. Relish, an NF-κB family transcription factor, is cytoplasmically bound by an autoinhibitory domain in the absence of infection. Upon immune stimulation, a caspase-mediated cleavage complex degrades the inhibitory domain and the activated transcription factor is translocated to the nucleus. Several proteins in the cleavage complex (Dredd, dFADD, IKK$_\beta$, and Relish itself) show strong evidence adaptive evolution [4,6**,33•,34]. The putatively adaptive amino acid substitutions are systematically overrepresented in the *Relish* autoinhibitory domain and cleaved linker, the *Dredd* caspase domain, the dFADD death domain, and the IKK$_\beta$ kinase domain [6**,33•,34]. There thus is compelling evidence for adaptive evolution in the Relish cleavage complex as a whole, with adaptive substitutions found in protein domains functionally important for releasing the active Relish transcription factor. This adaptive evolution appears to be restricted to taxa in the *melanogaster* species group and is not characteristic of all species in the genus *Drosophila* [6**,35], but in an interesting convergence, *Relish* also shows credible evidence of adaptive evolution in *Nasutitermes* termites, again with putatively adaptive substitutions localized in and around the caspase cleavage site and the linker [29]. The direct agent of selection on the Relish cleavage complex has not been determined in either *Drosophila* or *Nasutitermes*, but one hypothesis is that the complex is engaged in a coevolutionary arms race with pathogens capable of interfering with host immune signaling [34], such as bacteria that inject immunomodulatory molecules into host cells via Type III Secretion

Systems [36,37[•]], immunosuppressive fungi [38], and parasitoid mutualistic polydnaviruses [39]. The requisite physical interactions among proteins in a signaling cascade may mean that host mutations to escape pathogen interference are fixed in conjunction with compensatory mutations in the same or other proteins, accelerating amino acid divergence and enhancing the signature of selection in the entire pathway [17[••],40].

The cellular immune response

The *Drosophila* cellular immune response to bacterial infection consists primarily of defensive phagocytosis by circulating stationary hemocytes [10[••]]. Bacteria to be phagocytosed are captured by membrane surface receptors such as those in the Eater/Nimrod and scavenger receptor families and then internalized in a membrane-enveloped phagosome where they are killed. Phagocytosis may be facilitated by extracellularly secreted opsonins, such as insect thioester containing proteins (TEPs), which are hypothesized to bind to both microbial and eukaryotic pathogens and enhance phagocytosis.

Phagocytosis receptors show considerable genomic diversification across the genus *Drosophila*. For instance, *eater* and *nimC1* have each independently expanded in multiple *Drosophila* species lineages and the *nimrod*-related gene *hemese* is unique to the *melanogaster* species group [6[••]]. Likewise, class C scavenger receptors have expanded from one gene in basal *Drosophila* species to four in the *melanogaster* group [6[••],41], although class B scavenger receptors have retained orthology between *Drosophila* and mosquitoes [6[••],17[••]]. Class C scavenger receptors also show exceptionally high rates of amino acid divergence within the *melanogaster* group [41], and several *nimrod*-related genes and scavenger receptors have been shown to evolve under recent adaptive evolution at the amino acid level [6[••],33[•],41]. The *TEP* family of opsonin genes has also undergone frequent genomic turnover in copy number between *Drosophila* and mosquitoes, honey bees, and *Tribolium* [14,15[•],16[•],17[••]]. Like phagocytic receptors, *TEP* genes frequently show evidence of adaptive sequence evolution in *Drosophila* [6[••],28], *Anopheles* [30], and *Daphnia* [31], with selected sites predominantly found in and around a domain that is proteolytically cleaved to activate the TEP protein. It is unknown whether the proteases that perform TEP cleavage are produced by the host or by microbes, so it is difficult to infer whether *TEP* adaptation is a response to diversity in pathogen proteases or to pathogen interference with *TEP* function. Overall, however, the receptors and opsonins mediating phagocytosis are evolutionarily remarkably dynamic with considerable evidence of ongoing positive selection. Phagocytic receptors may bind to diverse or evolutionarily labile pathogen molecules, in contrast to evolutionarily static PRRs which recognize highly conserved microbial compounds, and may be subject to interference by pathogen proteins.

The evolutionary properties of the *Drosophila* cellular response have not been studied beyond pathogen recognition. It is well known that many bacteria are capable of manipulating proteins involved in cytoskeletal rearrangement either to inhibit phagocytosis (e.g. [42,43]) or to promote bacterial invasion of host cells (e.g. [44]). To my knowledge, however, no studies have examined the evolutionary dynamics of insect genes involved in intracellular aspects of phagocytosis and bacterial killing. Neither have any studies addressed natural selection on *DSCAM*, a hypervariable phagocytic receptor gene potentially capable of generating through alternative splicing tens of thousands of isoforms that may be either secreted or membrane bound. Exposure of insect cells to bacteria specifically enhances the production of some isoforms, and silencing of *DSCAM* by RNAi reduces the efficiency of phagocytosis in *Drosophila* and mosquitoes [45,46]. Like the vertebrate *major histocompatibility complex* (*MHC*) [7], *DSCAM* may prove to be both evolutionarily and somatically diverse.

Conclusions

The recent wealth of population genetic and comparative genomic data allow us to draw some comprehensive conclusions regarding the evolution of the antimicrobial immune response in *Drosophila* and other insects. In the humoral antimicrobial defense, PRRs and AMPs show little evidence of adaptive amino acid diversification, although the independent AMP gene family radiations in several insect lineages may reflect adaptation to distinct pathogen suites. By contrast, however, adaptive sequence evolution is pervasive in orthologously conserved intracellular signaling molecules, suggesting that pathogen interference with host immune induction may drive coevolution between insects and pathogenic microbes. This model seems plausible if it is evolutionarily difficult for pathogens to evade detection by PRRs or to resist killing by AMPs. Disruption of signaling obviates the need for evasion or resistance, and the orthologous maintenance of core signaling pathways across distantly related insects may make these pathways attractive targets for pathogen interference. Pathogen receptors utilized in phagocytosis are much more diverse and adaptively evolving, suggesting that the recognition properties of these proteins may be quite distinct from those of PRRs. The evolutionary properties of intracellular proteins required for phagocytosis and phagosomal killing remain to be explored.

Critically, most studies till date have focused on long-term evolutionary properties of immune system genes. There has been far too little emphasis on short-term evolutionary dynamics, including recent adaptation to local pathogens or environmental conditions (but see

[47]). Even when data are suggestive of adaptive evolution, we are critically impaired in our ability to infer the proximal agent of selection because we know so little about the epidemiology of infection in natural *Drosophila* populations. Not only do we have little information about the diversity of microbes that infect *Drosophila* in the field, we know precious little about virulence mechanisms that natural pathogens employ, or how natural pathogens interact with the host immune system. Biologists interested in the evolution of insect immune system genes have a strong mandate to identify and characterize ecologically relevant pathogens. At the same time, there is pressing need to study the molecular evolution of immune systems in insects whose ecologies are better characterized than and are distinct from that of *D. melanogaster*.

Despite its shortcomings as an ecological system, the *Drosophila* model has proven fantastic for establishing the basic rules governing the functional and evolutionary genetics of insect immune systems. A series of careful and comprehensive population genetic studies combined with thoughtful whole genome comparisons both within the genus *Drosophila* and between *Drosophila* and other insects have given insight into the evolutionary dynamics of innate immunity that is unparalleled in other physiological systems or organisms.

Acknowledgements

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Dawkins R, Krebs JR: **Arms races between and within species**. *Proc R Soc Lond B* 1979, **205**:489-511.

2. van Valen L: **A new evolutionary law**. *Evol Theor* 1973, **1**: 1-30.

3. Murphy PM: **Molecular mimicry and the generation of host defense protein diversity**. *Cell* 1991, **72**:823-826.

4. Schlenke TA, Begun DJ: **Natural selection drives *Drosophila* immune system evolution**. *Genetics* 2003, **164**:1471-1480.

5. Nielsen R, Bustamante C, Clark AG, Glanowski S, Sackton TB, Hubisz MJ, Fledel-Alon A, Tanenbaum DM, Civello D, White TJ *et al.*: **A scan for positively selected genes in the genomes of humans and chimpanzees**. *PLoS Biol* 2005, **3**:e170.

6. Sackton TB, Lazzaro BP, Schlenke TA, Evans JD, Hultmark D,
•• Clark AG: **Dynamic evolution of the innate immune system in *Drosophila***. *Nat Genet* 2007, **39**:1461-1468.
The authors annotate immune response genes in the complete genome sequences of 12 species of *Drosophila*, then determine rates of genomic duplication and deletion among species and infer adaptive evolution based on nucleotide substitution patterns. This paper provides the first comparative genomic analysis of immune system evolution across closely related species.

7. Hughes AL, Nei M: **Pattern of nucleotide substitution at major histocompatibility complex class I loci reveals overdominant selection**. *Nature* 1988, **335**:167-170.

8. McDonald JH, Kreitman M: **Adaptive protein evolution at the Adh locus in *Drosophila***. *Nature* 1991, **351**:652-654.

9. Nielsen R, Yang Z: **Likelihood models for detecting positively selected amino acid sites and applications to the HIV-1 envelope gene**. *Genetics* 1998, **148**:929-936.

10. Lemaitre B, Hoffmann J: **The host defense of *Drosophila*
•• *melanogaster***. *Annu Rev Immunol* 2007, **25**:697-743.
This is a clear, comprehensive, and up-to-date review of functional studies of the *Drosophila melanogaster* immune system.

11. Clark AG, Wang L: **Molecular population genetics of *Drosophila* immune system genes**. *Genetics* 1997, **147**:713-724.

12. Ramos-Onsins S, Aguadé M: **Molecular evolution of the *Cecropin* multigene family in *Drosophila* functional genes vs. pseudogenes**. *Genetics* 1998, **150**:157-171.

13. Date A, Satta Y, Takahata N, Chigusa SI: **Evolutionary history and mechanism of the *Drosophila cecropin* gene family**. *Immunogenetics* 1998, **47**:417-429.

14. Christophides GK, Zdobnov E, Barillas-Mury C, Birney E, Blandin S, Blass C, Brey PT, Collins FH, Danielli A, Dimopoulos G *et al.*: **Immunity-related genes and gene families in *Anopheles gambiae***. *Science* 2002, **298**:159-165.

15. Evans JD, Aronstein K, Chen YP, Hetru C, Imler JL, Jiang H,
• Kanost M, Thompson GJ, Zou Z, Hultmark D: **Immune pathways and defence mechanisms in honey bees *Apis mellifera***. *Insect Mol Biol* 2006, **15**:645-656.
The authors examine the complete genome sequence of the honey bee and identify homologs of immune response genes characterized in *D. melanogaster* and other insects.

16. Zou Z, Evans JD, Lu Z, Zhao P, Williams M, Sumathipala N,
• Hetru C, Hultmark D, Jiang H: **Comparative genomic analysis of the *Tribolium* immune system**. *Genome Biol* 2007, **8**:R177.
The authors examine the complete genome sequence of the red flour beetle and identify homologs of immune response genes characterized in *D. melanogaster* and other insects.

17. Waterhouse RM, Kriventseva EV, Meister S, Xi Z, Alvarez KS,
•• Bartholomay LC, Barillas-Mury C, Bian G, Blandin S, Christensen BM *et al.*: **Evolutionary dynamics of immune-related genes and pathways in disease-vector mosquitoes**. *Science* 2007, **316**:1738-1743.
This paper compares the complete genome sequences of the distantly related mosquitoes *Aedes aegypti* and *Anopheles gambiae* to that of *D. melanogaster* in order to classify immune response genes exhibiting high and low rates of genomic turnover. The comparison among three species is more powerful than any pairwise comparison because it allows phylogenetic placement of many evolutionary events.

18. Jiggins FM, Kim KW: **The evolution of antifungal peptides in *Drosophila***. *Genetics* 2005, **171**:1847-1859.

19. Quesada H, Ramos-Onsins SE, Aguadé M: **Birth-and-death evolution of the *Cecropin* multigene family in *Drosophila***. *J Mol Evol* 2005, **60**:1-11.

20. Zhou X, Nguyen T, Kimbrell DA: **Identification and characterization of the *Cecropin* antibacterial protein gene locus in *Drosophila virilis***. *J Mol Evol* 1997, **44**:272-281.

21. Lazzaro BP, Clark AG: **Evidence for recurrent paralogous gene conversion and exceptional allelic divergence in the *Attacin* genes of *Drosophila melanogaster***. *Genetics* 2001, **159**:659-671.

22. Lazzaro BP, Clark AG: **Molecular population genetics of inducible antibacterial peptide genes in *Drosophila melanogaster***. *Mol Biol Evol* 2003, **20**:914-923.

23. Imler JL, Bulet P: **Antimicrobial peptides in Drosophila: structures, activities and gene regulation**. *Chem Immunol Allergy* 2005, **86**:1-21.

24. Tennessen JA: **Molecular evolution of animal antimicrobial
• peptides: widespread moderate positive selection**. *J Evol Biol* 2005, **18**:1387-1394.

This review article compares and contrasts the evolution of antimicrobial peptide genes in many species, including vertebrates and invertebrates.

25. Candille SI, Kaelin CB, Cattanach BM, Yu B, Thompson DA, Nix MA, Kerns JA, Schmutz SM, Millhauser GL, Barsh GS: **A β-defensin mutation causes black coat color in domestic dogs**. *Science* 2007, **318**:1418-1423.

26. Bulmer MS, Crozier RH: **Duplication and diversifying selection among termite antifungal peptides**. *Mol Biol Evol* 2004, **21**:2256-2264.

27. Jiggins FM, Hurst GD: **The evolution of parasite recognition genes in the innate immune system: purifying selection on *Drosophila melanogaster* peptidoglycan recognition proteins**. *J Mol Evol* 2003, **57**:598-605.

28. Jiggins FM, Kim KW: **Contrasting evolutionary patterns in *Drosophila* immune receptors**. *J Mol Evol* 2006, **63**:769-780.

29. Bulmer MS, Crozier RH: **Variation in positive selection in termite GNBPs and Relish**. *Mol Biol Evol* 2006, **23**:317-326.

30. Little TJ, Cobbe N: **The evolution of immune-related genes from disease carrying mosquitoes: diversity in a peptidoglycan- and a thioester-recognizing protein**. *Insect Mol Biol* 2005, **14**:599-605.

31. Little TJ, Colbourne JK, Crease TJ: **Molecular evolution of daphnia immunity genes: polymorphism in a *gram-negative binding protein* gene and an *alpha-2-macroglobulin* gene**. *J Mol Evol* 2004, **59**:498-506.

32. Hurst GD, Anbutsu H, Kutsukake M, Fukatsu T: **Hidden from the host: *Spiroplasma* bacteria infecting *Drosophila* do not cause an immune response, but are suppressed by ectopic immune activation**. *Insect Mol Biol* 2003, **12**:93-97.

33. Jiggins FM, Kim KW: **A screen for immunity genes evolving**
• **under positive selection in *Drosophila***. *J Evol Biol* 2007, **20**:965-970.
The authors screen the *D. melanogaster* genome for adaptively evolving immune response genes using a novel two-step approach. Candidate genes are first identified by virtue of high nonsynonymous genetic divergence between *D. melanogaster* and *D. yakuba* and *D. erecta*, then population genetic analyses are employed to determine whether highly divergent candidate genes evolve under positive selection in *D. melanogaster* and *D. simulans*.

34. Begun DJ, Whitley P: **Adaptive evolution of *relish*, a *Drosophila* NF-kappaB/IkappaB protein**. *Genetics* 2000, **154**:1231-1238.

35. Levine MT, Begun DJ: **Comparative population genetics of the immunity gene, *Relish*: is adaptive evolution idiosyncratic?** *PLoS One* 2007, **2**:e442.

36. Hueck CJ: **Type III protein secretion systems in bacterial pathogens of animals and plants**. *Microbiol Mol Biol Rev* 1998, **62**:379-433.

37. Apidianakis Y, Mindrinos MN, Xiao W, Lau GW, Baldini RL,
• Davis RW, Rahme LG: **Profiling early infection responses: *Pseudomonas aeruginosa* eludes host defenses by suppressing antimicrobial peptide gene expression**. *Proc Natl Acad Sci U S A* 2005, **102**:2573-2578.
The authors elegantly use gene expression microarrays to demonstrate that the pathogenic bacterium *P. aeruginosa* is capable of suppressing the *D. melanogaster* humoral immune response. Avirulent mutant *P. aeruginosa* lack this ability.

38. Pal S, St Leger RJ, Wu LP: **Fungal peptide Destruxin A plays a specific role in suppressing the innate immune response in *Drosophila melanogaster***. *J Biol Chem* 2007, **282**:8969-8977.

39. Thoetkiattikul H, Beck MH, Strand MR: **Inhibitor kappaB-like proteins from a polydnavirus inhibit NF-kappaB activation and suppress the insect immune response**. *Proc Natl Acad Sci U S A* 2005, **102**:11426-11431.

40. DePristo MA, Weinreich DM, Hartl DL: **Missense meanderings in sequence space: a biophysical view of protein evolution**. *Nat Rev Genet* 2005, **6**:678-687.

41. Lazzaro BP: **Elevated polymorphism and divergence in the class C scavenger receptors of *Drosophila melanogaster* and *D. simulans***. *Genetics* 2005, **169**:2023-2034.

42. Au C, Dean P, Reynolds SE, ffrench-Constant RH: **Effect of the insect pathogenic bacterium Photorhabdus on insect phagocytes**. *Cell Microbiol* 2004, **6**:89-95.

43. Avet-Rochex A, Bergeret E, Attree I, Meister M, Fauvarque MO: **Suppression of *Drosophila* cellular immunity by directed expression of the ExoS toxin GAP domain of *Pseudomonas aeruginosa***. *Cell Microbiol* 2005, **7**:799-810.

44. Pizarro-Cerdá J, Cossart P: **Bacterial adhesion and entry into host cells**. *Cell* 2006, **124**:715-727.

45. Watson FL, Püttmann-Holgado R, Thomas F, Lamar DL, Hughes M, Kondo M, Rebel VI, Schmucker D: **Extensive diversity of Ig-superfamily proteins in the immune system of insects**. *Science* 2005, **309**:1874-1878.

46. Dong Y, Taylor HE, Dimopoulos G: **AgDscam, a hypervariable immunoglobulin domain-containing receptor of the *Anopheles gambiae* innate immune system**. *PLoS Biol* 2006, **4**:e229.

47. Schlenke TA, Begun DJ: **Linkage disequilibrium and recent selection at three immunity receptor loci in *Drosophila simulans***. *Genetics* 2005, **169**:2013-2022.