Imunologie hmyzu – buněčné a humorální reakce

1. Úvod – nodulace, enkapsulace, koagulace, melanizace atd.

2. Nodulace bakterií – injikace bakteriální suspenze do larev VII. instaru zavíječe voskového *Galleria mellonella*, pozorování dříve injikovaných larev – fotodokumentace vytvořených nodulí (Olympus BX43 + kamera Infinity 2, Quick Photo Micro software).

3. Septické x aseptické poranění, nylon wool enkapsulace. Larvy potemníka moučného *Tenebrio molitor*, larvy VII. instaru *Galleria mellonella*, larvy zlatohlávka *Coelorrhina hornimani*.

4. Enkapsulace parazitů – entomopatogenní hlístice.

5. Melanizace - odběr hemolymfy larev VII. instaru zavíječe voskového *Galleria mellonella* a larev III. instaru *Drosophila melanogaster*, na filtračním papíru, s a bez fenylthiomočoviny fotodokumentace (Olympus SZX9 + Nikon D3100). Popis spektrofotometrického stanovení aktivity fenoloxidázy.

6. Koagulace - odběr hemolymfy larev VII. instaru zavíječe voskového *Galleria mellonella* – kapka na sklíčko, draw out methoda.

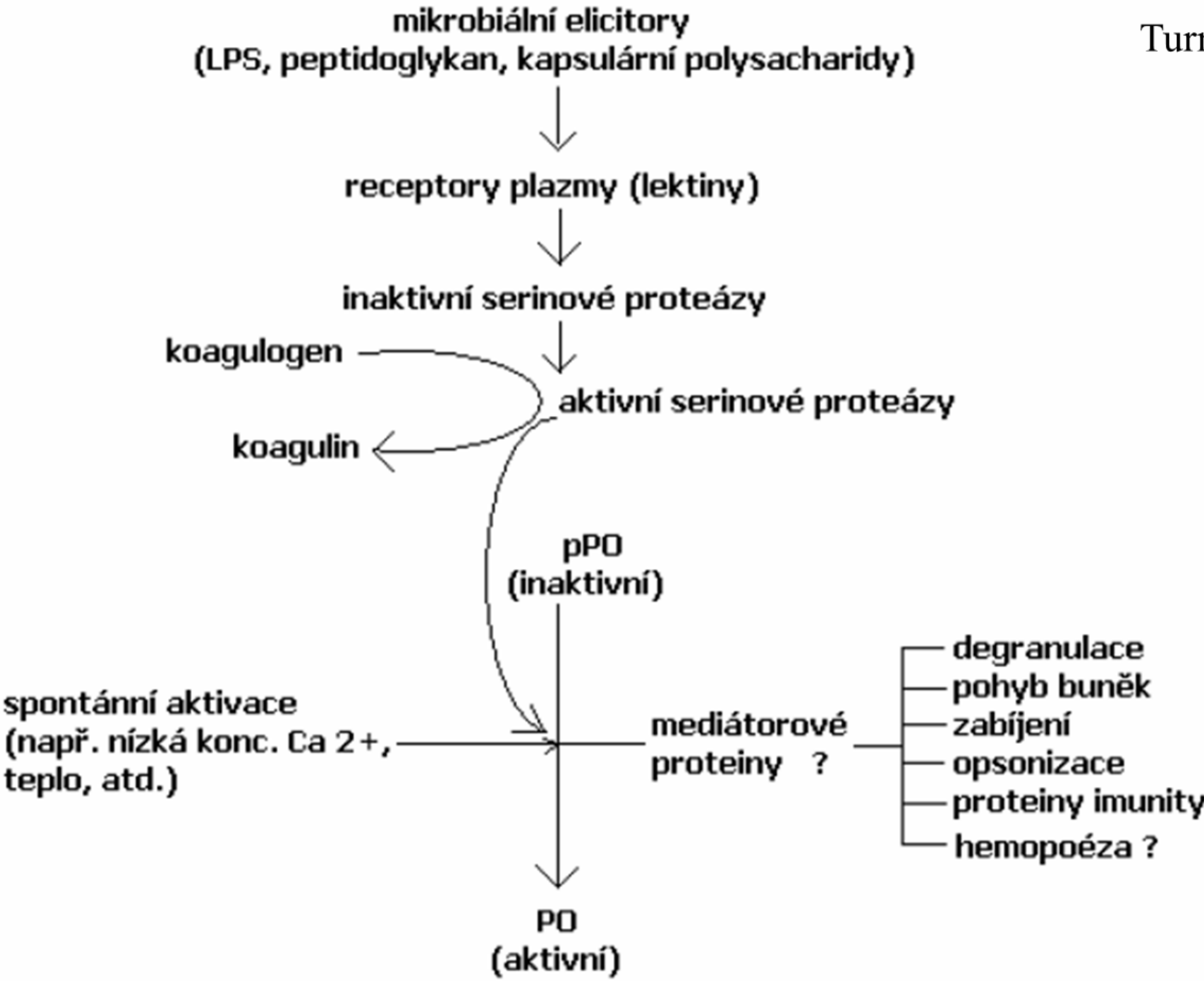
7. Hanging drop – metoda pro studium koagulace, larvy VII. instaru zavíječe voskového *Galleria mellonella.*

8. Melanizace v místě poranění – ukázka melanizovaných skvrn na několika druzích hmyzu (regenerace končetin - pakobylka *Pharnacia ponderosa*, larva zlatohlávka *Coelorrhina hornimani*.).

9. Behaviorální imunita – ukázka strašilek *Peruphasma schultei* – výstražný postoj, chemická obrana.

10. Indukce GFP signálu v místě poranění: *Drosophila melanogaster* DDC-GFP (Olympus SZX9 + UV adaptér + Nikon D3100).

Turner 1994



mikrobiální elicitory
(LPS, peptidoglykan, kapsulární polysacharidy)
receptory plazmy (lektiny)
inaktivní serinové proteázy
koagulogen
aktivní serinové proteázy
koagulin
pPO
(inaktivní)
spontánní aktivace
(např. nízká konc. Ca 2+,
teplo, atd.)
mediátorové
proteiny ?
degranulace
pohyb buněk
zabíjení
opsonizace
proteiny imunity
hemopoéza ?
PO
(aktivní)

Melanin
Polymerization
Quinones
Oxidation
L-DOPA
Hydroxylation
Tyrosine
PO

Box 2. Melanisation

Melanisation is the result of the oxidation of mono- and/or diphenols and it is an important reaction in most multicellular organisms, both animals and plants. In vertebrates, melanin provides pigmentation and protection and is important for the development of the central nervous system and the eyes, among others, but the pigment is also associated with melanomas. In most invertebrates, a redox enzyme, commonly called phenoloxidase, catalyses the reaction. This enzyme is often produced by blood cells and released to the plasma upon immunostimulation. By contrast, in vertebrates the corresponding enzyme, tyrosinase, which also is a redox enzyme with activity identical with that of phenoloxidase, is membrane-bound and found in a specialised organelle, the melanosome.

Cerenius et al. 2010

A Melanization from *S. carpocapsae* infection

Pena, Hallem, 2015

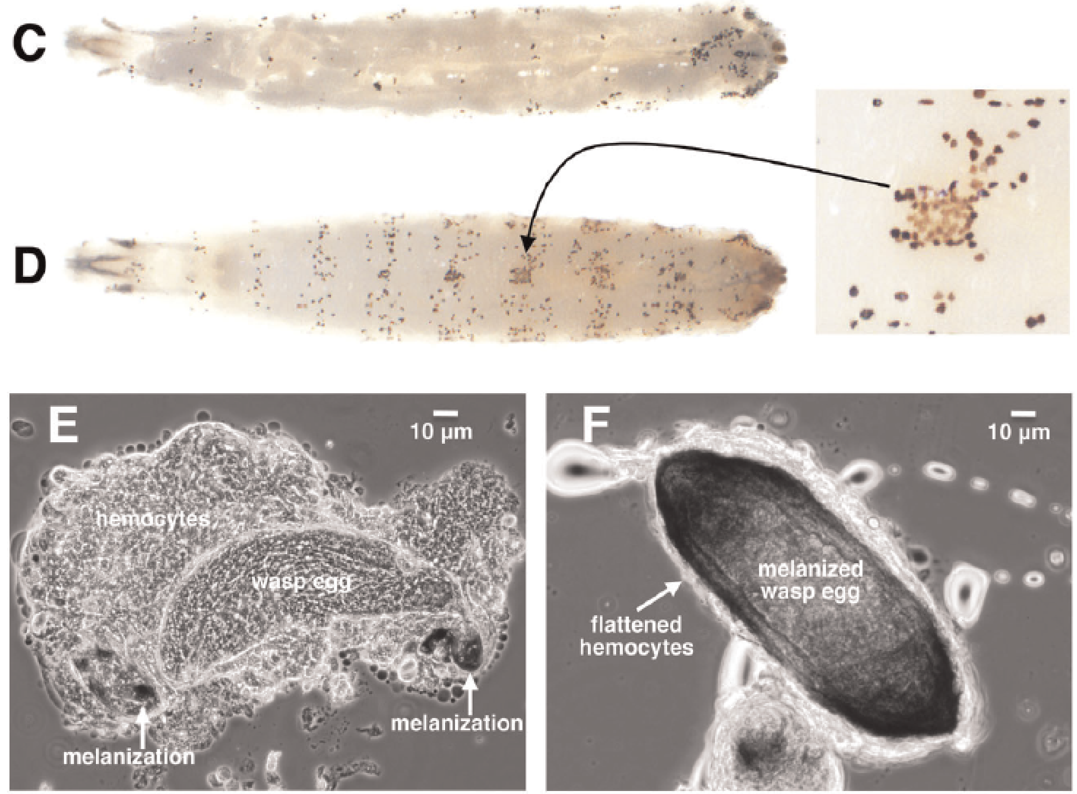
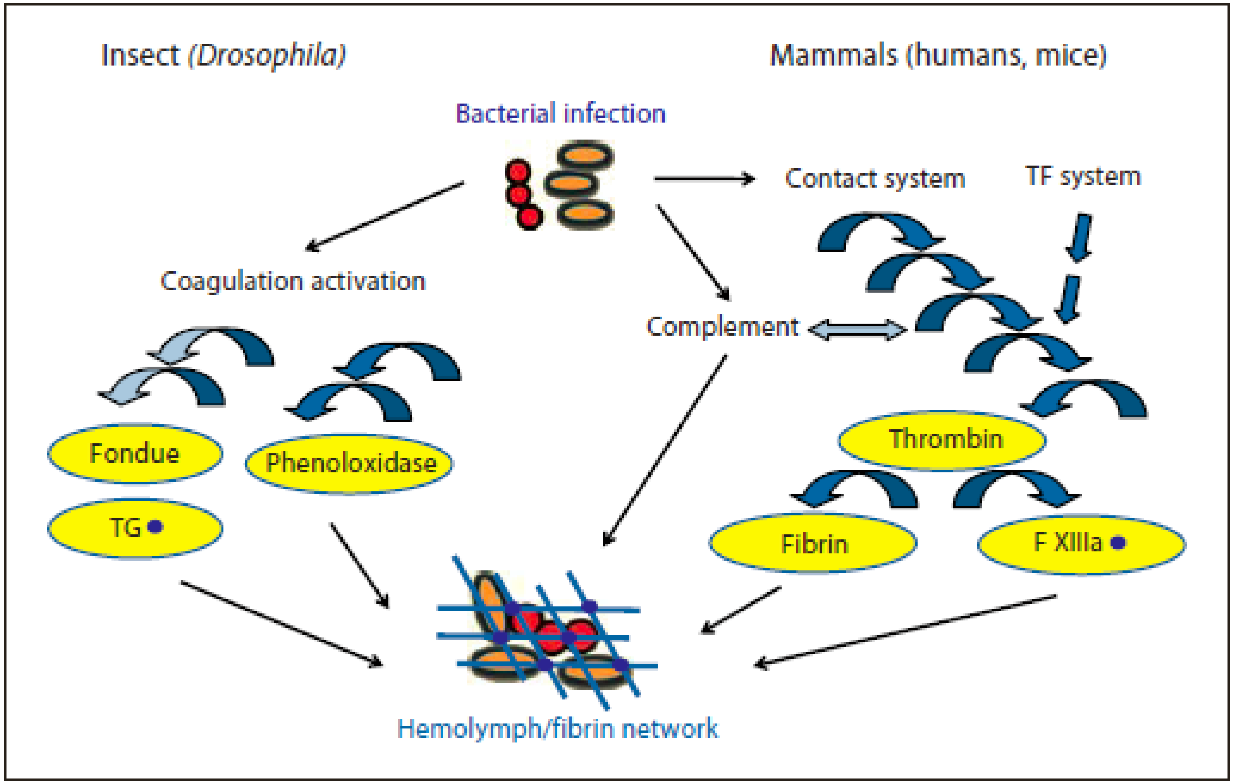


Figure 3. *D. suzukii* hemocytes and encapsulation of wasp eggs. (A) A 0.25×0.25×0.1 mm hemocytometer field from normal *D. suzukii* larvae showing abundant plasmatocytes; (B) hemocytometer field from *D. suzukii* larvae 12 hours after infection by wasp strain LbG486 showing increased podocyte and lamellocyte numbers; (C) control *D. melanogaster* larva with melanized crystal cells; (D) control *D. suzukii* larva with melanized crystal cells, showing color variation in inset; (E) initiation of encapsulation of LbG486 egg by *D. suzukii* showing loose hemocyte aggregation and melanization at anterior and posterior tips of egg; (F) LbG486 egg melanotically encapsulated by *D. suzukii*, showing surrounding layer of tightly spread hemocytes.
doi:10.1371/journal.pone.0034721.g003

Fig. 1. Clotting in insects and mammals: clotting in mammals involves the contact and the tissue factor (TF) system. For clarity only the final cleaved products are shown. There is increasing evidence for crosstalk between coagulation and the complement system [36]. In insects, coagulation involves the parallel activation of hemolymph proteins, such as Fondue and TG, as well as the prophenoloxidase-activating system. It is presently unknown whether a proteolytic cascade is involved in TG activation (arrows in light blue), although a protease is activated during clotting [39]. In both insects and vertebrates, clot formation leads to the TG/F XIIIa-dependent immobilization of bacteria limiting their further dissemination [32].



Loof et al., 2011

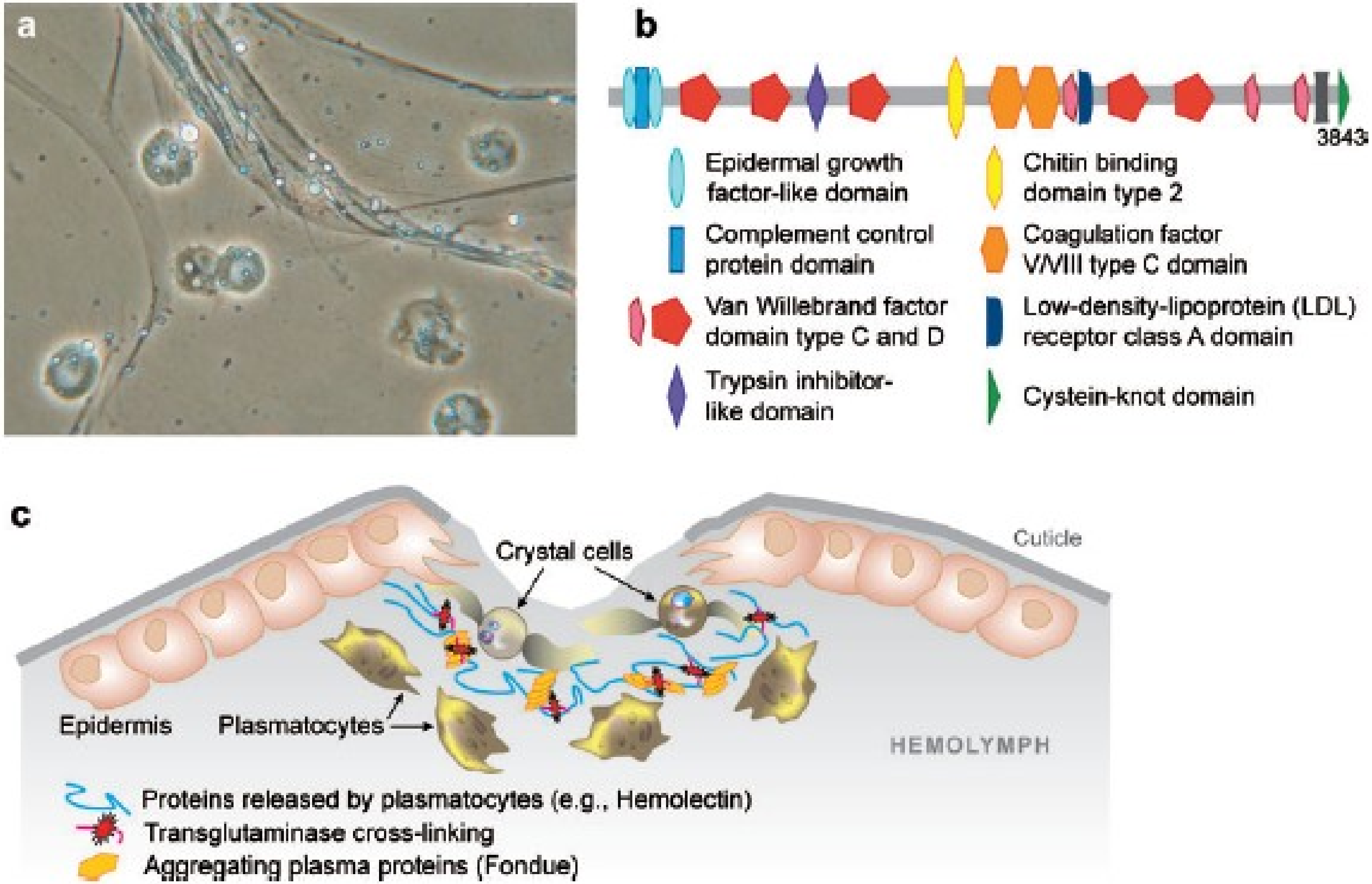
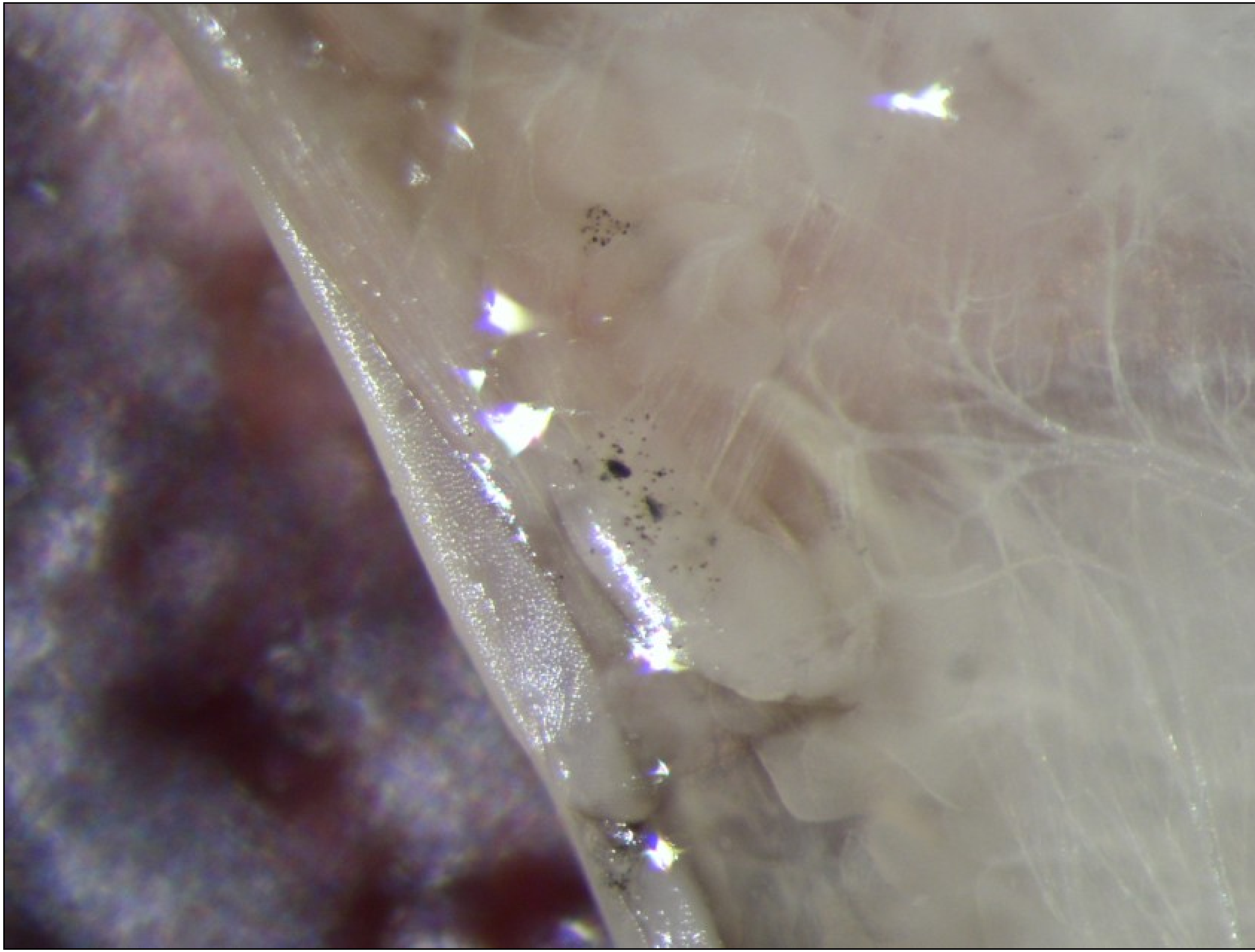


Figure 7

Clotting reaction in *Drosophila*. (*a*) A *Drosophila* clot with fibers and incorporated plasmatocytes. (Courtesy of Ulrich Theopold, Stockholm University.) (*b*) Structure of Hemolectin. Hemolectin is a large protein with domains that are also present in other clotting factors (197). Hemolectin is a major component of *Drosophila* clotting fibers. This figure was done using the SMART bioinformatic tool (EMBL, Heidelberg) for identifications and positions of domains. (*c*) A model for clot formation at an injury site. Upon injury, plasmatocytes immediately release Hemolectin and other proteins that form clot fibers. Cross-linkage of these fibers occurs with the help of proteins such as Fondue, transglutaminase, and proPO, the latter being released by crystal cells.

Lemaitre and Hoffmann 2007



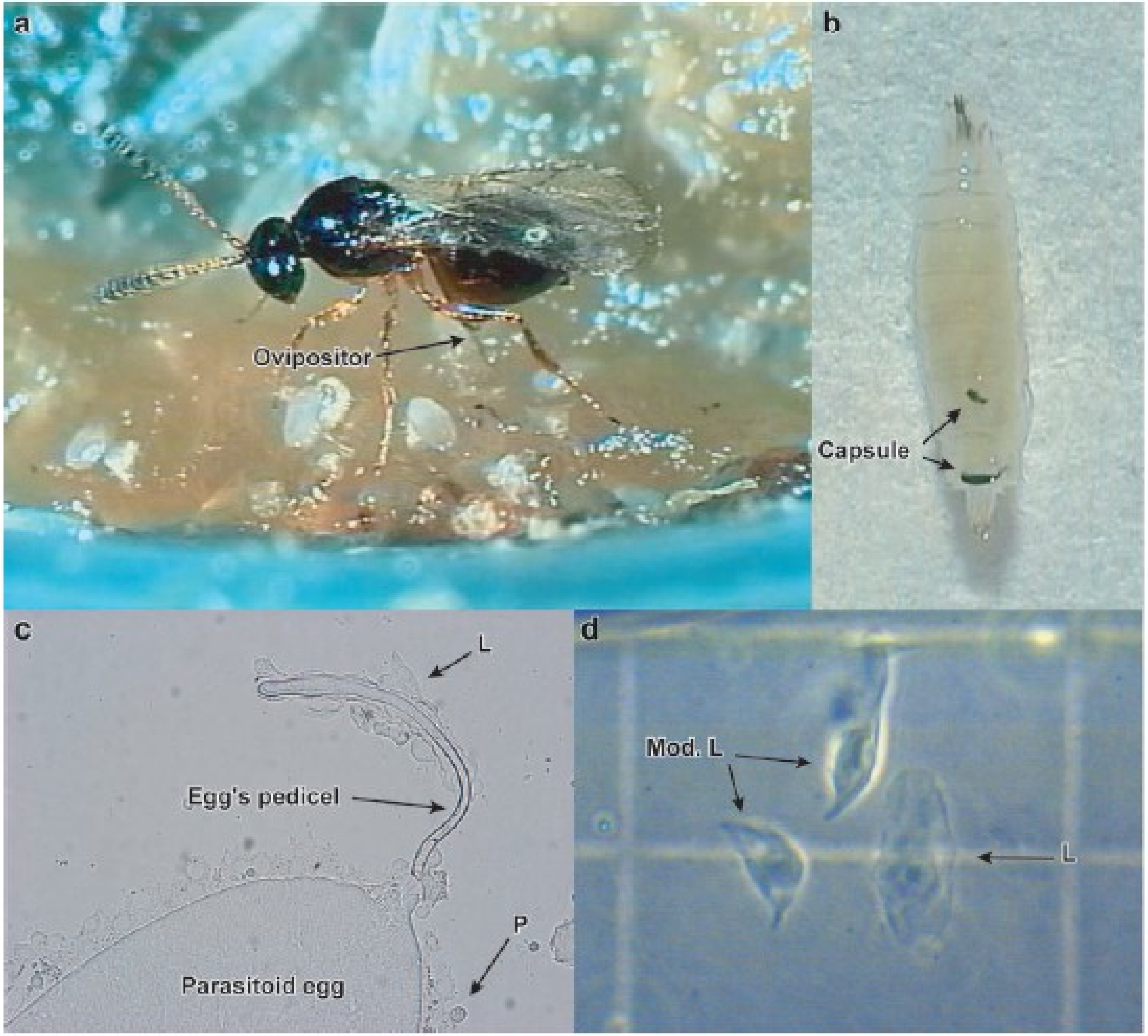
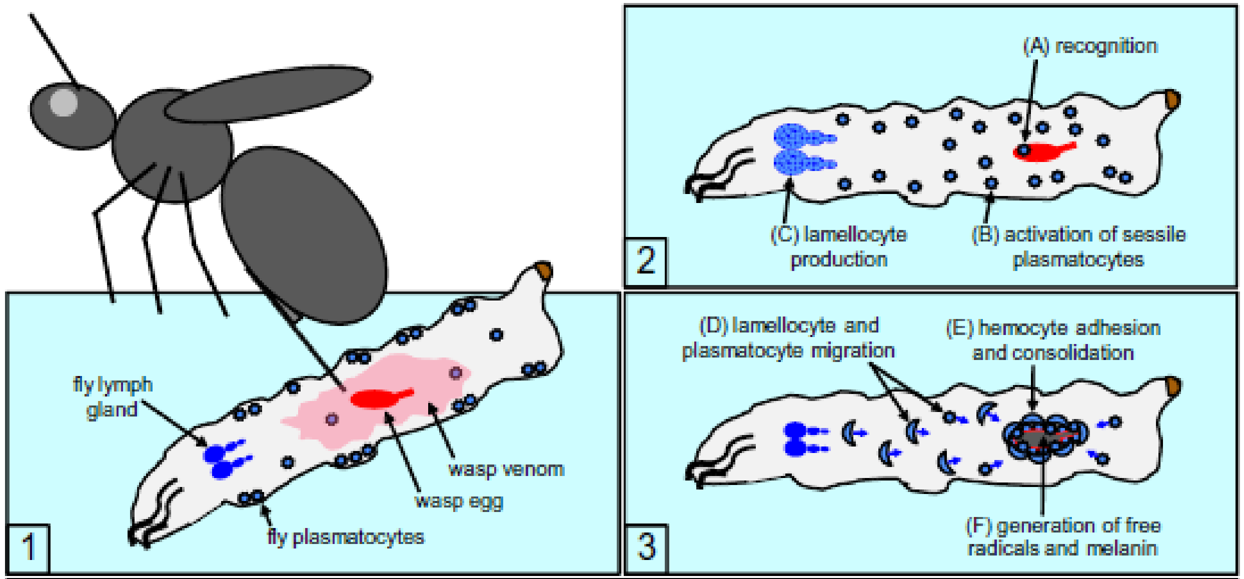


Figure 8

Drosophila infection by parasitoid wasp. (*a*) The parasitic wasp *Leptopilina boulardi* infects *Drosophila* second instar larvae (L2) and deposits eggs within the body cavity. (*b*, *c*) Encapsulation of the egg is the major host defense against parasitoid infection. The black capsules are visible through the larval cuticle (*b*). P, plasmatocyte. (*d*) *L. boulardi* can suppress the *Drosophila* encapsulation response by altering lamellocyte (L) morphology from a discoidal to a bipolar shape. L, normal lamellocyte; mod. L, bipolar-shaped lamellocyte. Courtesy of Aurore Dubuffet (*a*–*c*) and Corinne Labrosse (*d*), University of Tours, France.

Lemaitre and Hoffmann 2007



<u>Fly Encapsulation Steps That Wasp Venoms Are Known To Suppress</u>

(B) *Ganaspis sp.1* venom SERCA suppresses plasmatocyte calcium burst, preventing activation

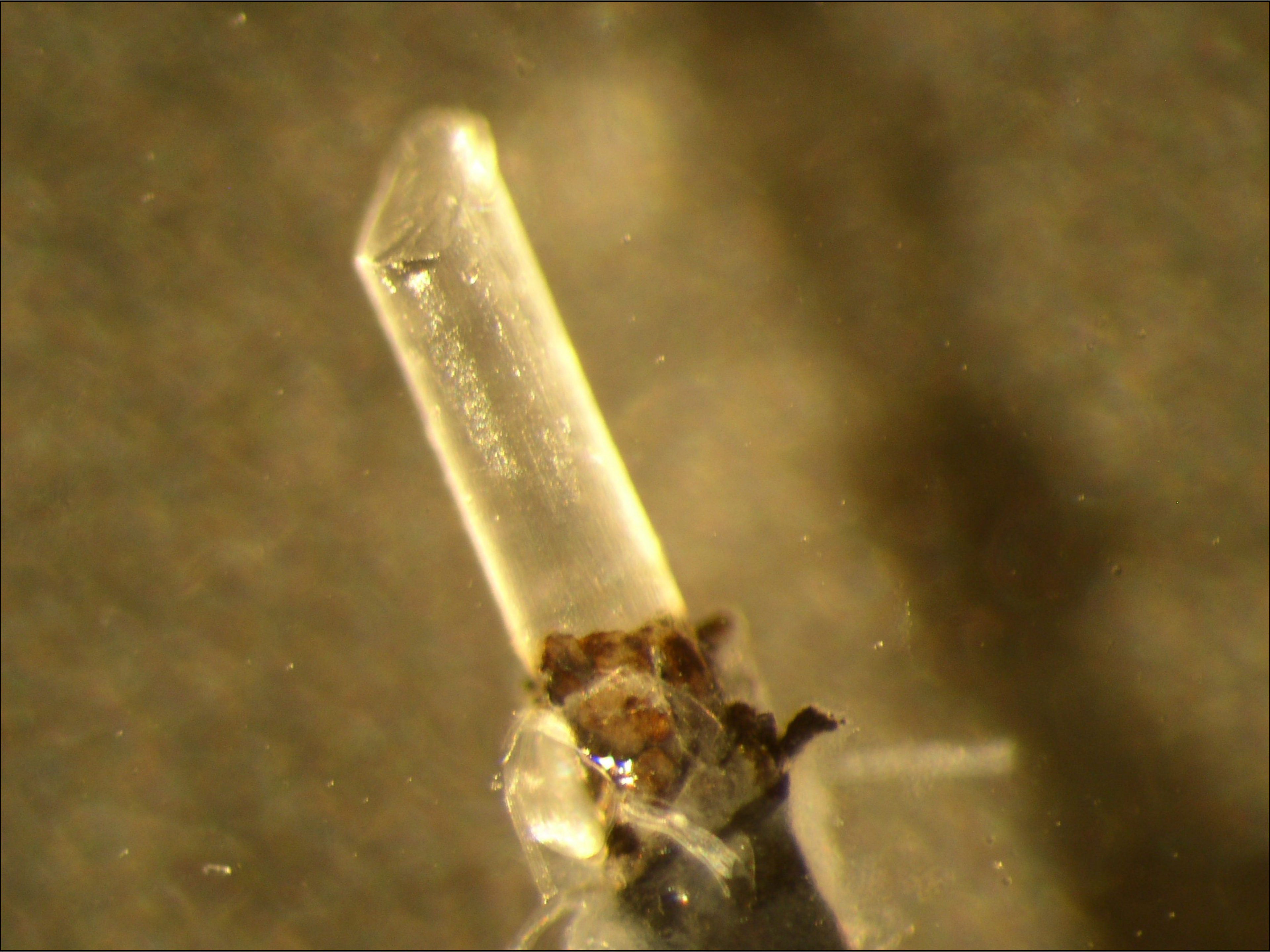
(E) *L. boulardi* venom RhoGap alters lamellocyte cytoskeletal structure, preventing adhesion

(E) *L. victoriae* unknown venom protein prevents lamellocyte surface protein N-glycosylation, preventing consolidation

(F) *L. boulardi* venom serpin suppresses phenoloxidase cascade, preventing melanization

(F) *L. boulardi* extracellular venom SOD suppresses phenoloxidase cascade, preventing melanization

Fig. 4. Interactions between Drosophila and endoparasitoid wasps. Wasps inject an egg and venom into the body cavity of a fly larva, and the fly recognizes the egg as foreign and mounts a melanotic encapsulation response. However, wasps evolve venom proteins that have specific ways of suppressing this fly immune response.



Encapsulation in *G. mellonella* after natural invasion of *H.bacteriophora*

Department of Molecular Biology & Functional Genomics

- Home
- Courses
- Research
- Seminars
- Staff
- Dissertations
- PhD Training
- Positions available
- News & Events
- Links
- Public Archive

- Intranet (only staff)

No PhD positions available now.
Next possible date for advertising PhD positions will be April 2, 2008

| Last updated 080225 |
| Webmasters |

and contribute to a more complete picture of the function and evolution of innate immunity. A net-like structure, which helps vertebrate neutrophils to catch and kill bacteria has recently been described. These neutrophil extracellular networks (NETs) show significant structural and functional similarity to insect clot networks but our results show that they are composed of different components providing an example for convergent evolution in innate immunity.

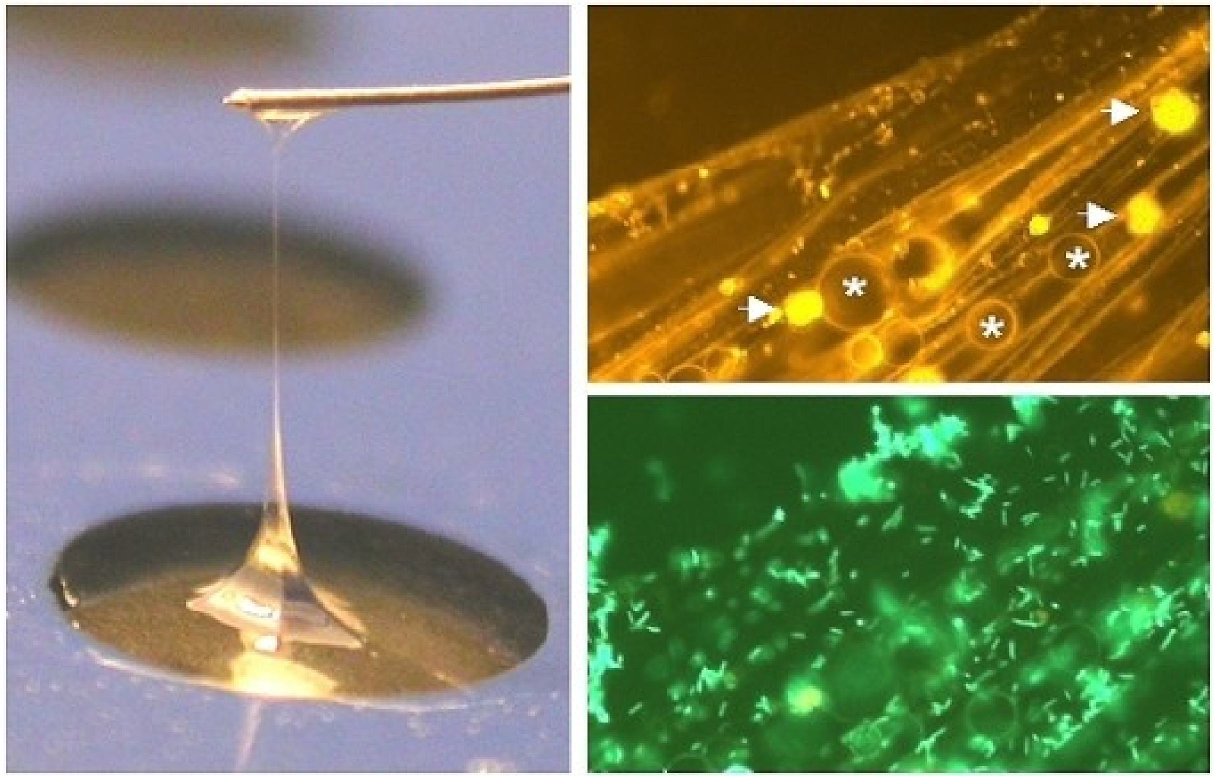


Fig.1: left: a *Drosophila* hemolymph sample turns viscous after bleeding. right: in the hemolymph clot, fibers can be stained with lectins (upper part) in which bacteria are captured (lower part, the bacteria are labeled with green fluorescent protein).

Insect clotting proteins –a source for novel biopolymers?