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ENCAPSULATION OF PARASITOID EGGS IN PHENOLOXIDASE-DEFICIENT MUTANTS OF *DROSOPHILA MELANOGASTER*

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Abstract—Eggs of the parasitoid *Leptopilina boulardi*, strain L104, are routinely encapsulated by haemocytes in *Drosophila melanogaster* larvae, and the capsules subsequently melanize. In *D. melanogaster* mutant strains deficient for phenoloxidase activity, L104 eggs are encapsulated by host haemocytes but the cellular capsules do not melanize and harden. These observations suggest that phenoloxidases are not essential for recognition of nonself and encapsulation of foreign objects in *D. melanogaster*, but they are required for blackening and hardening of haemocytic capsules.

Key Word Index: Phenoloxidase; encapsulation; parasitoid; *Leptopilina boulardi*; *Drosophila melanogaster*

INTRODUCTION

The common defence reaction against parasites and other large foreign objects that invade the insect haemocoel is encapsulation by haemocytes. Cellular capsules formed by the haemocytes in this process usually darken and harden (Salt, 1970). Although the chemical nature of the dark pigment within the capsules has not been established, it has generally been assumed that this substance is melanin resulting from the conversion of phenols to *o*-quinones. The phenoloxidases involved in the biochemical pathways leading to melanin production occur as proenzymes that are converted to active forms in a cascade of reactions involving at least six steps (Seybold *et al.*, 1975). Prophenoloxidases in the haemocoel are localized in haemocytes in some insects (Rizki, 1957; Pye and Yendol, 1972; Monpeyssin and Beaulaton, 1977; Rowley *et al.*, 1986) and in the haemolymph of other insects (Ashida *et al.*, 1983; Saul *et al.*, 1987).

The presence of phenoloxidases in the insect haemocoel agrees with the suggestion that these enzymes are involved in cellular defence reactions. However, it has been difficult to demonstrate the role this enzyme system plays in the defence reactions. Crosslinking of proteins takes place during melanization and sclerotization, so it is likely that phenoloxidases serve to strengthen cellular capsule walls formed around foreign bodies (Rizki and Rizki, 1984b). Stoltz and Cook (1983) provided evidence that phenoloxidases are involved in insect defence by demonstrating that parasites suppress phenoloxidase activity of a host insect. Since parasites must evade or disrupt host defence reactions to survive, the phenoloxidase system would be a promising target for attack by a parasite if the system is vital to the encapsulation process. Evidence that phenoloxidases function in the recognition of nonself has also been obtained by demonstrating that activators of prophenoloxidases enhance phagocytosis by insect blood cells *in vitro* (Ratcliffe *et al.*, 1984: Leonard *et al.*, 1985).

We adopted a different strategy to evaluate the contribution of phenoloxidases to the insect cellular defence reaction of encapsulation. This approach argues that a cellular defence response dependent on phenoloxidase activity cannot be mounted in a phenoloxidase-negative insect. Two mutant genes in *Drosophila melanogaster* that are not structural genes for prophenoloxidases cause the loss of this enzyme activity. Males hemizygous for the sex-linked gene *lozenge*^{rfg} (*lz*^{rfg}) lack phenoloxidase activity (Warner *et al.*, 1974; Rizki *et al.*, 1985). These mutant larvae do not have crystal cells, the haemocytes with paracrystalline inclusions that contain prophenoloxidases in normal *Drosophila* larvae (Rizki and Rizki, 1985).

The other mutation affecting the phenoloxidase activity of the crystal cells is *Black cell* (*Bc*). *Bc* is a dominant mutation of a gene located on the right arm of the second chromosome (Rizki *et al.*, 1980). *Bc*/*Bc* larvae have black cells in their lymph glands and haemocoel instead of crystal cells with paracrystalline inclusions. These mutant haemocytes result from atypical activation of prophenoloxidases within the crystal cells. Since the crystal cells are the sole source of haemolymph prophenoloxidases in *Drosophila* larvae, this source of proenzyme is destroyed in *Bc*/*Bc* larvae due to the crosslinking of intracellular proteins of the crystal cells. As a result, no prophenoloxidases are available in the haemolymph of *Bc*/*Bc* larvae.

That phenoloxidases are involved in wound healing (Lai-Fook, 1966) is confirmed by the fact that injured cuticle of *Bc*/*Bc* and *lz*^{rfg} larvae does not heal properly. A black crust is not formed when the cuticle of these mutant larvae is punctured by a glass needle (Rizki and Rizki, 1984b). To determine whether the recognition of nonself that precedes the encapsulation response by insect haemocytes requires phenoloxidase activity, a habitual parasitoid of *Drosophila* was employed as the nonself entity. Eggs of the L104 strain of the cynipid wasp *Leptopilina boulardi* are routinely encapsulated by lamellocytes in

normal *Drosophila* strains with phenoloxidase activity (Carton *et al.*, 1986; Rizki *et al.*, 1990). Lamellocytes are the discoidal haemocytes that layer around foreign objects to form capsules in the *Drosophila* haemocoel (Rizki and Rizki, 1984a). This type of haemocyte as well as the plasmatocytes of *Bc/Bc* and $lz^{r/g}$ larvae are normal despite the marked changes in the crystal cells of *Bc/Bc* larvae and the absence of crystal cells in $lz^{r/g}$ larvae. Therefore, the two mutant strains lacking phenoloxidases can be exploited to test the hypothesis that these enzymes are required for recognition of nonself in *Drosophila*.

MATERIALS AND METHODS

Larvae from four *D. melanogaster* strains were used as hosts in this study. The *Ore-R* wild type strain and a temperature-sensitive *melanotic tumor* strain ($tu\text{-}Sz^{ts}$) have crystal cells and phenoloxidase activity (Rizki *et al.*, 1985). The genotypes of the phenoloxidase-deficient strains were: (a) *Bc fj wt* and (b) ♂ $lz^{r/g}/Y$; ♀ *C(1)Dx ywf*. For explanation of symbols, see Lindsley and Grell (1968). In the latter stock, the attached-X female larvae have yellow mouthparts, and the triplo-X female and $lz^{r/g}$ male larvae have black mouthparts. Newly-emerged, first-instar larvae with black mouthparts in this stock were transferred to separate feeding dishes in which they were maintained until they were exposed to parasitoid females. The parasitized $lz^{r/g}$ males were separated from the triplo-X females in the late third instar on the basis of gonad size. Gonads of male larvae are three times the size of gonads in female larvae at this age (Roberts, 1986).

Drosophila larvae were grown at 25 or 27°C on cream of wheat/molasses medium seeded with live Fleischmann's yeast. At 46–48 h old they were removed from feeding chambers, rinsed with distilled water, and placed on filter paper strips moistened with 0.2% glucose solution (Rizki and Rizki, 1984a). The papers were transferred to plastic vials which contained parasitoids. After 2 h the parasitoids were removed from the vials and the host larvae were returned to fresh feeding chambers with cream of wheat medium. *Drosophila* hosts were dissected in Ringer solution to locate parasitoid eggs 24, 48, and 72 h after infection. Hosts at 72 h had pupariated.

The L104 strain of *Leptopilina boulardi* was kindly provided by Dr Y. Carton. The stock was bred on the Brazzaville strain of *D. melanogaster* also provided by Dr Carton. Adult parasitoids were maintained on 50% honey solution at 18°C. Female parasitoids that had not been allowed to oviposit previously were used for the experiments to assure adequate egg laying within a short time. Multiparasitism is common among *Leptopilina* species but only one larva survives and completes development in a *Drosophila* host (Carton *et al.*, 1986). Under the conditions used in this study, most hosts were infected by more than one parasitoid egg.

Phenoloxidase activity of haemocytes and parasitoids was demonstrated using the methods employed previously for proenzymes in polyacrylamide gels and in $tu\text{-}Sz^{ts}$ crystal cells (Rizki *et al.*, 1985). Haemocytes were collected in *Drosophila* Ringer solution and allowed to settle for 2–3 min on acid-cleaned microscope slides. The sample was then flooded with 3.7% paraformaldehyde solution in phosphate buffered saline at pH 7.2. After fixation for 20 min, the cells were rinsed in phosphate-buffered saline and transferred to 0.1 M potassium phosphate buffer at pH 6.3 (Mitchell and Weber, 1965). Prophenoloxidases were activated by a 20-min treatment with 50% 2-propanol in potassium phosphate buffer (Batterham and McKechnie, 1980). Following a 10-min rinse in the buffer, the samples were incubated in L-3,4-dihydroxyphenylalanine (DOPA) in potassium phosphate buffer for 1 h. They were than examined for blackening. After examination the specimens were stored in an 18°C incubator and reexamined 16 h later. This same procedure was used to test for phenoloxidase activity in L104 embryos and larvae removed from *Drosophila* hosts in three experiments.

RESULTS

The data on encapsulation and melanization of parasitoid eggs are given in Table 1. Parasitoid eggs recovered from $tu\text{-}Sz^{ts}$ and *Ore-R* larvae within 24 h after egg laying were encapsulated by lamellocytes, but melanization of the capsules was not apparent. Therefore, hosts of each group were allowed to continue development for an additional one or two days before they were dissected to retrieve the parasitoids. Melanized capsules were recovered from *Ore-R* and $tu\text{-}Sz^{ts}$ hosts on the second and third days after infection. By the second day, some parasitoid larvae had emerged. Melanization of the capsules did not prevent the parasitoid larvae from emerging

Table 1. Encapsulation and melanization of L104 in *Drosophila*

Day after oviposition	Host Strain	No.	Parasitoids: Encapsulated Melanized	Encapsulated Not melanized	Not encapsulated
1	*Ore-R*	7	0	18	4
	$tu\text{-}Sz^{ts}$	7	0	22	0
	Bc/Bc	7	0	30	0
	$lz^{r/g}$	7	0	31	1
2	*Ore-R*	8	25	6	0
	$tu\text{-}Sz^{ts}$	8	36	5	0
	Bc/Bc	8	0	35	2
	$lz^{r/g}$	8	0	39	3
3	*Ore-R*	3	11	0	0
	$tu\text{-}Sz^{ts}$	2	7	0	0
	Bc/Bc	5	0	21	0
	$lz^{r/g}$	3	0	13	0

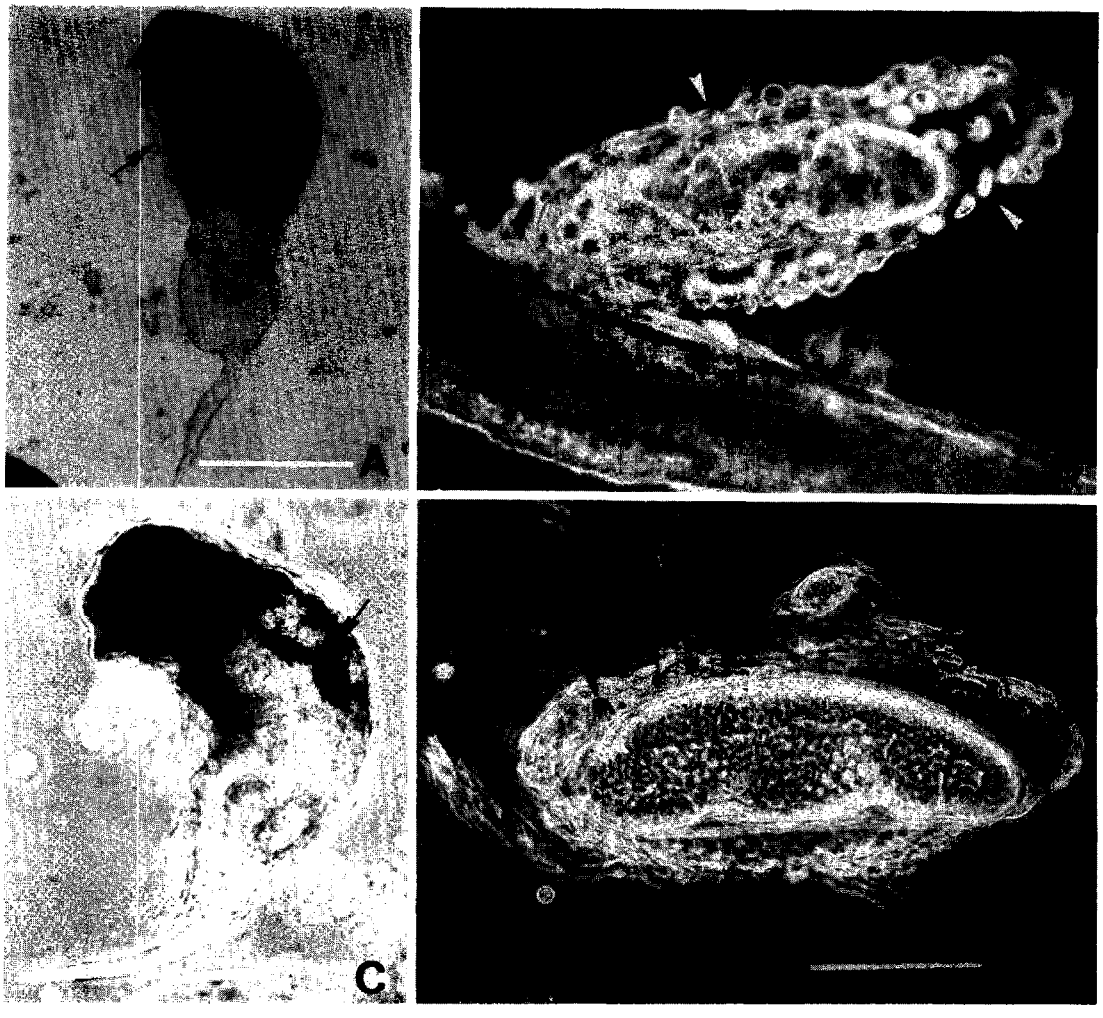

Fig. 1. *L. boulardi* eggs and larvae removed from *D. melanogaster* hosts. (A) Live, motile parasitoid larva emerging from a melanized capsule formed in an *Ore-R* larva. Part of the parasitoid is also visible beneath the melanized capsule (arrow). (B) A parasitoid egg attached to the gut (g) of a lz^{rlg} larva is surrounded by haemocytes (arrowheads). (C) A dead supernumerary parasitoid larva that is partially blackened with a necrotic gut (arrow). This parasitoid removed from a *Bc/Bc* host is surrounded by lamellocytes. (D) Encapsulation of a developing parasitoid egg by lamellocytes of a *Bc/Bc* host larva. Three black cells (arrows) are embedded in the capsule. Bar = 100 μm.

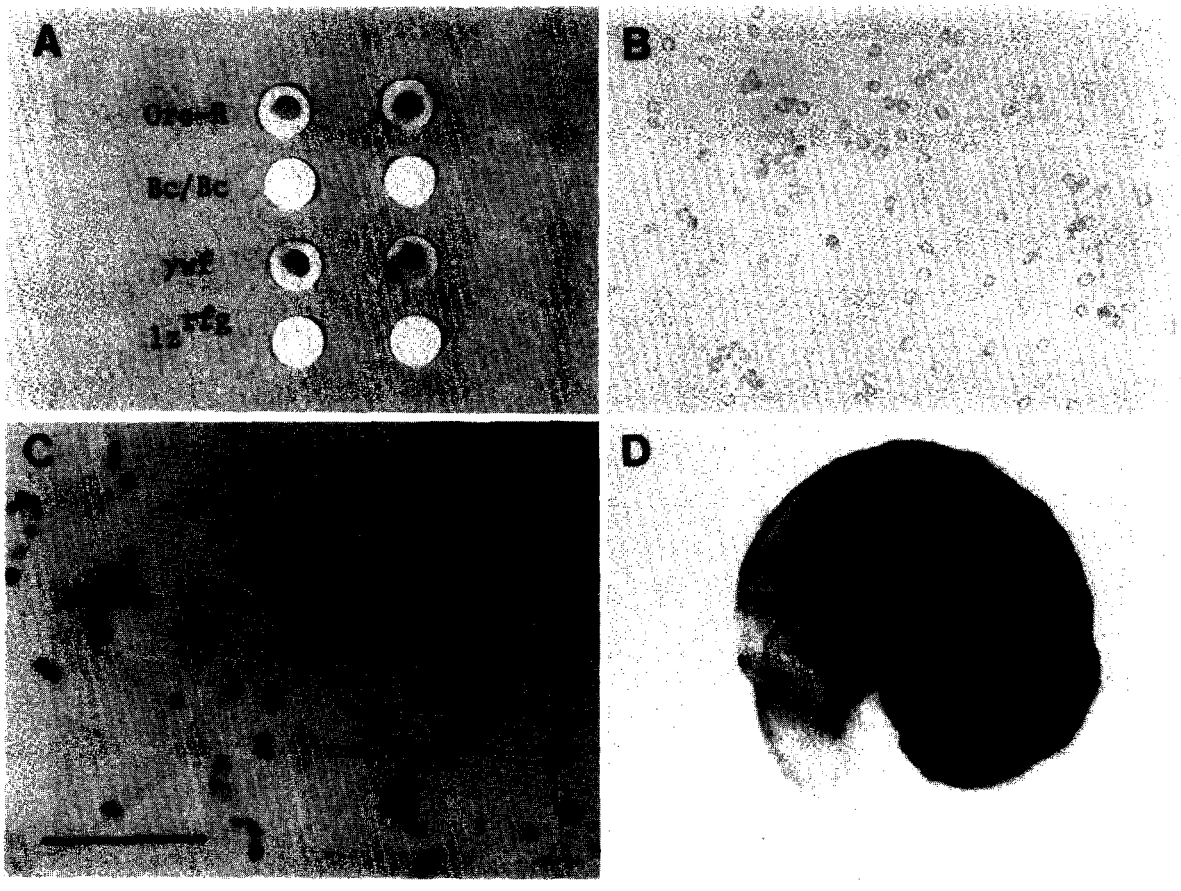

Fig. 2. (A) Haemolymph samples from different genotypes of 94 h-old *Drosophila* larvae were placed on buffer-soaked filter paper disks and photographed after 30 min. For each genotype, the left disk has haemolymph from two larvae and the right disk contains haemolymph from four specimens. Haemolymph blackening is apparent within 5 min and the maximum is attained within 30 min when the disks are maintained in a moist chamber. The *Bc/Bc* and lz^{rlg} samples did not darken. (B) Haemocyte sample from a parasitized lz^{rlg} larva incubated in DOPA solution. There was no sign of darkening after overnight incubation. Contrast to visualize the cells was maximized by adjusting the substage condenser diaphragm. (C) Control haemocyte sample from a *tu-Sz*^{ts} larva incubated in DOPA solution for 18 h and photographed in transmitted light. Dark pigment from the crystal cells has spread to plasmatocytes and a lamellocyte (arrow). (D) Parasitoid larva removed from the lz^{rlg} host sampled in (B). After incubation in DOPA for 18 h, the larva is melanized due to its endogenous phenoloxidase activity.

[Fig. 1(A)]. As described previously, *L. boulardi* eggs adhere tightly to host tissue surfaces and lamellocytes are unable to cover the portion of the egg that is attached to the host tissue (Rizki *et al.*, 1990). Since L104 eggs on host surfaces are never fully enclosed by lamellocytes, the parasitoid larvae can emerge using the unencapsulated surface as the route of escape.

In lz^{rfg} host larvae, lamellocytes were clearly discernible on the parasitoid egg surfaces by 24 h [Fig. 1(B)]. Eggs that were unhatched in lz^{rfg} hosts on the third day following parasitization were encapsulated but melanization of the capsules had not taken place. Wasp larvae had hatched in the hosts by the second day and as many as 12 eggs and larvae were found in a single host. Some eggs did not show any signs of embryonic development. In one host, two wasp larvae showed movement and one did not. This same host had six eggs, four of which were encased in haemocytic capsules but blood cells could not be seen on the surfaces of two other parasitoid eggs. Whether the presence of unencapsulated and encapsulated eggs in the same *Drosophila* larva does occur or this observation resulted from experimental manipulations that dislodged eggs and freed them of surrounding capsules cannot be resolved on the basis of these observations. None of the capsules in lz^{rfg} hosts appeared to be tightly formed. Blackened regions were visible in some encapsulated immobile wasp larvae in lz^{rfg} hosts [Fig. 1(C)]. Presumably, this darkening was a sign of necrosis in the supernumerary parasitoids.

Encapsulated parasitoid eggs were also found in *Bc/Bc* larvae. Capsules in these hosts were not melanized, but a few black cells were embedded within the layers of the lamellocytes forming the walls of the capsules [Fig. 1(D)]. In *Bc/Bc* larvae, circulating black cells are often surrounded by lamellocytes (Rizki *et al.*, 1980) and these lamellocytes with bound black cells are trapped between other lamellocytes during capsule formation (Rizki and Rizki, 1986).

The presence of dark pigment in the supernumerary parasitoids and the absence of pigment in the hemocytic capsules in the *Bc/Bc* and lz^{rfg} hosts that lack phenoloxidase activity suggests that the pigment is a product of the parasitoid and not host haemocytes. If the darkening results from phenoloxidase activity, then the source of the phenoloxidase must be the parasitoid larva and not the *Drosophila* host. Prior to determining the source of the phenoloxidase activity in the parasitoids removed from infected *Drosophila* hosts, haemolymph samples from lz^{rfg} and *Bc/Bc* larvae were reexamined to confirm that they do not have phenoloxidase activity. This was done by the method used previously to illustrate that haemolymph from *Drosophila* larvae that have phenoloxidase activity darkens when exposed to the air whereas haemolymph samples from mutant larvae lacking this enzyme activity do not blacken under these conditions (Rizki *et al.*, 1980). Haemolymph samples from *Ore-R* larvae and *ywf* female sibs of the lz^{rfg} males served as control groups for the survey [Fig. 2(A)].

To determine whether L104 wasps have phenoloxidases and, at the same time, demonstrate that the haemocytes of lz^{rfg} hosts do not, haemocyte samples from parasitized lz^{rfg} hosts were collected and parasitoids which did not show any signs of pigment were recovered from these same specimens. Haemocytes were also collected from $tu\text{-}Sz^{ts}$ larvae to serve as control samples. After activation in 2-propanol and incubation in DOPA for 1 h, the parasitoids and haemocytes were examined for darkening. Dark pigment was present in the crystal cells from $tu\text{-}Sz^{ts}$ larvae and in the parasitoid larvae removed from lz^{rfg} hosts. However, there was no darkening in the haemocytes from the lz^{rfg} parasitized or nonparasitized hosts. Sixteen hours later, the pigment from the crystal cells had diffused to nearby haemocytes in the $tu\text{-}Sz^{ts}$ sample as described in an earlier study (Rizki *et al.*, 1980). However, there was no pigment in the haemocytes from the parasitized lz^{rfg} larvae even after this lengthy incubation. The darkening within the parasitoid larvae was intense at this time [Fig. 2(B)–(D)].

DISCUSSION

In the absence of haemolymph phenoloxidase activity, the larval haemocytes of two *Drosophila* mutant strains encapsulate parasitoid eggs. These observations indicate that phenoloxidase activity is not necessary for the recognition of foreignness in *Drosophila* larvae. Cellular capsules in *Drosophila* larvae consist of layers of lamellocytes which are discoidal cells with sticky surfaces (Rizki, 1961; Rizki and Rizki, 1983). The stickiness of the lamellocytes must operate independently of phenoloxidase activity because lamellocytes of the phenoloxidase-deficient mutants are capable of adhering to form capsules around parasitoid eggs as well as dead supernumerary parasitoid larvae. Nappi (1973) found that encapsulation of *L. heterotoma* (formerly *Pseudeucoila bochei*) eggs was significantly reduced when phenylthiourea, an inhibitor of melanin formation, was administered to *Drosophila algonquin* larvae, and concluded that the phenoloxidase system plays an important role in insect immunity against parasites. Salt (1956) found that phenylthiourea did not prevent haemocyte envelopment of *Nemeritis canescens* eggs injected into *Carausius morosus* but it did interfere with melanization of the parasite eggs. Excessive doses of the inhibitor that resulted in poor health of the hosts decreased the haemocyte accumulation around the parasite eggs. The presence of the latter effect on the haemocytes was undoubtedly prompted by a loss of physiological homeostasis, so Salt's study clearly demonstrates that evaluation of haemocyte functions can be clouded if the experimental conditions are not controlled properly.

The cellular capsules formed in lz^{rfg} and *Bc/Bc* larvae did not melanize and harden whereas cellular capsules in *Drosophila* strains with phenoloxidase activity did darken and harden. Therefore, phenoloxidase activity must be involved at a later stage of the encapsulation process to provide melanin precursors and assure the structural stability of the capsule walls that is conferred by the phenolic crosslinking of proteins (Rizki and Rizki, 1984b). Whether the crystal cells participate in the formation of cellular capsules is not clear. The presence of black cells in the capsule walls around parasitoid larvae might be taken as evidence that they do. Black cells were also found

in the endogenous tumour masses in the double mutant strain of the *Bc* gene and a melanotic tumour gene, *tu-W* (Rizki and Rizki, 1984b). In *Bc* larvae, the black cells are usually surrounded by lamellocytes (Rizki *et al.*, 1980), so the possibility that black cells are incorporated in capsule walls when the lamellocytes surrounding them participate in capsule formation cannot be excluded. On the other hand, haemocytic capsules in normal *Drosophila* routinely melanize, so it seems reasonable to consider that the source of this melanin is the crystal cells.

Unlike the plasmatocytes and lamellocytes of *Drosophila* larvae, the crystal cells are extremely fragile (Rizki, 1957). When haemolymph is collected in saline solution, the crystal cells often swell and rupture and the paracrystalline inclusions in the cells dissociate. To demonstrate that the paracrystalline inclusions of the crystal cells contain prophenoloxidases requires that the haemocytes be adequately fixed prior to processing for enzyme activity (Rizki *et al.*, 1985). When *Drosophila* haemocyte samples are treated with activators for prophenoloxidases and then incubated in DOPA *in vitro*, the darkening reaction appears first in the paracrystalline inclusions of the crystal cells and then disperses throughout the crystal cells. Confirmation that the paracrystalline inclusions contain prophenoloxidase was recently obtained using an antibody to *Drosophila* proenzyme (Deng, 1988; Deng and Rizki, unpublished). With artificial activator the darkening subsequently spreads to neighbouring plasmatocytes and lamellocytes, but this diffusion does not occur when natural activator is used (Rizki *et al.*, 1985). The confinement of black pigment to the crystal cells when natural activator is used *in vitro* resembles the restriction of black pigment to the mutant crystal cells in *Bc/Bc* larvae. If the intense blackening that characterizes *Drosophila* capsules *in vitro* results from the release and diffusion of melanin precursors from crystal cells incorporated in capsule walls, then transport from the crystal cells must be controlled by specific conditions (Brunet, 1980).

The phenoloxidase system of *Drosophila* consists of three proenzymes (A_1, A_2, A_3) originally described by Mitchell and Weber (1965) and at least five other proteins involved in a cascade of reactions together with the A_1 protein to yield active enzyme *in vitro* (Seybold *et al.*, 1975). Acceptance of the monophenoloxidase PHOX as an additional proenzyme (Batterham and McKechnie, 1980) was based on the fact that it is a dimer and the A_1 component, which is also a monophenoloxidase, was reported to be a monomer (Seybold *et al.*, 1975). Recent evidence indicates that A_1 is a dimer (Deng, 1988; Deng and Rizki, unpublished) so PHOX and A_1 may be the same proenzyme. The A_2 and A_3 proteins which are diphenoloxidases are not essential in the minimal system described by Seybold *et al.* (1975). The three A proenzymes are encoded by different genes (Rizki *et al.*, 1985; Pentz *et al.*, 1986; Deng, 1988; Deng and Rizki, unpublished), so it is evident that the genetics of the *Drosophila* system is also complex. It is important to note that the mutation that affects the electrophoretic mobility of the A_3 component does not alter the mobility of the A_2 and A_1 components (Rizki *et al.*, 1985). Likewise, a genetic variant affecting the A_2 component does not alter the A_1 and A_3 proenzymes (Pentz *et al.*, 1986). Interspecific hybrids of *Drosophila* species whose A_1 proenzymes differ in electrophoretic mobility (slow and fast) have a slow, a fast and an intermediate A_1 dimeric band, but the electrophoretic mobilities of the A_2 and A_3 components in the interspecific hybrids remain unchanged (Deng, 1988; Deng and Rizki, unpublished). Therefore, the three proenzymes in *Drosophila* are distinct and do not result from polypeptide aggregation as generated in *Bombyx mori* (Ashida and Dohke, 1980) and *Calliphora erythrocephala* (Munn and Bufton, 1973).

How the complex phenoloxidase system in *Drosophila* is regulated during development and how activation is achieved when required for cellular defence reactions remain intriguing problems. The phenotype of the *Bc* mutant suggests that the Bc^+ gene may be involved in the activation of the proenzyme within the crystal cells, either as a regulator of the activator or the activator itself. The role that the *lz* locus plays in the phenoloxidase system must be confined to the differentiation of the cells that carry components of the phenoloxidase complex. Some *lz* alleles lack haemocytes with paracrystalline inclusions and are phenoloxidase negative whereas other *lz* alleles have normal crystal cells and are phenoloxidase positive (Rizki and Rizki, 1985). In double mutant combinations, the *lz* alleles that are phenoloxidase negative suppress the *Bc* phenotype whereas those *lz* alleles having this enzyme activity and normal crystal cells do not. Therefore, the differentiation of a functional crystal cell is necessary for the expression of the *Bc* mutant phenotype. The *lz* locus is on the X chromosome whereas the genes for the A proenzymes as well as the *Bc* gene are on the second chromosome. The *Bc* gene and the gene for the A_1 proenzyme are closely linked but non-allelic, so *Bc* gene product is distinct from A_1 protein (Deng, 1988).

The use of genetic variants to analyse the functions of individual components of the insect cellular defence system provides information untainted by experimental pitfalls. The role of prophenoloxidase in insect cellular defence mechanisms would be ideally assessed by null mutations for the structural genes coding the enzymes. Thus far, null mutations for the *Drosophila* proenzymes have not been uncovered, and the *Bc* and *lz* mutants are the only strains known to lack phenoloxidase activity. Information from the examination of these strains suggests caution in assigning a pivotal role to phenoloxidases in the recognition of nonself from self.

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REFERENCES

Ashida M. and Dohke K. (1980) Activation of prophenoloxidase by the activating enzyme of the silkworm, *Bombyx mori*. *Insect Biochem.* **10**, 37–47.

Ashida M., Ishizaki Y. and Iwahana H. (1983) Activation of prophenoloxidase by bacterial cell walls or β-1, 3 glucans in plasma of the silkworm *Bombyx mori*. *Biochem. Biophys. Res. Commun.* **113**, 562–568.

Batterham P. and McKechnie S. W. (1980) A phenol oxidase polymorphism in *Drosophila melanogaster*. *Genetika* **54,** 121–126.

Brunet P. C. J. (1980) The metabolism of the aromatic amino acids concerned in the cross-linking of insect cuticle. *Insect Biochem.* **10,** 467–500.

Carton Y., Bouletreau M., Van Alphen J. J. M. and Van Lenteren J. C. (1986) The *Drosophila* parasitic wasps. In *The Genetics and Biology of Drosophila* (Edited by Ashburner M. and Thompson J.), Vol. 3C, pp. 347–394. Academic Press, London.

Deng Y. (1988) Genetics and development of the A_1 component of the phenoloxidase system of *Drosophila melanogaster*. Ph.D. dissertation, The University of Michigan.

Lai-Fook J. (1966) The repair of wounds in the integument of insects. *J. Insect Physiol.* **12,** 195–226.

Leonard C., Ratcliffe N. A. and Rowley A. F. (1985) The role of prophenoloxidase activation in non-self recognition and phagocytosis by insect blood cells. *J. Insect Physiol.* **31,** 789–799.

Lindsley D. L. and Grell E. H. (1968) *Genetic Variations of Drosophila melanogaster*. Carnegie Institution of Washington Publication No. 627.

Mitchell H. K. and Weber U. M. (1965) *Drosophila* phenol oxidases. *Science* **148,** 964–965.

Monpeyssin M. and Beaulaton J. (1977) Donnees sur la localisation ultrastructurale d'une activite phenoloxidasique dans les hemocytes circulants de *Antheraea pernyi* au dernier age larvaire. *J. Insect Physiol.* **23,** 939–943.

Munn E. A. and Bufton S. F. (1973) Purification and properties of a phenol oxidase from the blowfly *Calliphora erythrocephla*. *Eur. J. Biochem.* **35,** 3–10.

Nappi A. J. (1973) The role of melanization in the immune reaction of larvae of *Drosophila algonquin* against *Pseudocoila bochei*. *Parasitology* **66,** 23–32.

Pentz E. S., Black B. C. and Wright T. R. F. (1986) A diphenol oxidase gene is part of a cluster of genes involved in catecholamine metabolism and sclerotization in *Drosophila* I. Identification of the biochemical defect in Dox-A2 [1(2)37Bf] mutant. *Genetics* **112,** 823–841.

Pye A. E. and Yendol W. G. (1972) Hemocytes containing polyphenoloxidase in *Galleria* larvae after injections of bacteria. *J. Invert. Path.* **19,** 166–170.

Ratcliffe N. A., Leonard C. and Rowley A. F. (1984) Prophenoloxidase activation: nonself recognition and cell cooperation in insect immunity. *Science* **226,** 557–559.

Rizki T. M. (1957) Alterations in the haemocyte population of *Drosophila melanogaster*. *J. Morph.* **100,** 437–458.

Rizki T. M. (1961) The influence of glucosamine-hydrochloride on cellular adhesiveness in *Drosophila melanogaster*. *Exptl Cell Res.* **24,** 111–119.

Rizki T. M. and Rizki R. M. (1983) Blood cell surface changes in *Drosophila* mutants with melanotic tumors. *Science* **220,** 73–75.

Rizki R. M. and Rizki T. M. (1984a) Selective destruction of a host blood cell type by a parasitoid wasp. *Proc. natn. Acad. Sci. U.S.A.* **81,** 6154–6158.

Rizki T. M. and Rizki R. M. (1984b) The cellular defence system of *Drosophila melanogaster*. In *Insect Ultrastructure* (Edited by King R. C. and Akai R.), Vol. 2. Plenum Press, New York.

Rizki T. M. and Rizki R. M. (1985) Paracrystalline inclusions of *Drosophila melanogaster* hemocytes have prophenoloxidases. *Genetics* **110,** s98.

Rizki T. M. and Rizki R. M. (1986) Surface changes on hemocytes during encapsulation in *Drosophila melanogaster*. In *Hemocytic and Humoral Immunity in Arthropods* (Edited by Gupta A. P.), pp. 157–190. Wiley, New York.

Rizki T. M., Rizki R. M. and Belloti R. A. (1985) Genetics of a *Drosophila* phenoloxidase. *Mol. gen. Genet.* **201,** 7–13.

Rizki T. M., Rizki R. M. and Carton Y. (1990) *Leptopilina heterotoma* and *L. Boulardi*: strategies to avoid cellular defense responses of *Drosophila melanogaster*. *Exp. Parasit.* **70,** 466–475.

Rizki T. M., Rizki R. M. and Grell E. H. (1980) A mutant affecting crystal cells in *Drosophila melanogaster*. *Wilhelm Roux's Archs Dev. Biol.* **188,** 91–99.

Roberts D. B. (1986) *Drosophila: A Practical Approach*. IRL Press, Oxford

Rowley A. F., Ratcliffe N. A., Leonard C. M., Richards E. H. and Renwrantz L. (1986) Humoral recognition factors in insects, with particular reference to agglutinins and the prophenoloxidase system. In *Hemocytic and Humoral Immunity in Arthropods* (Edited by Gupta A. P.). Wiley, New York.

Salt G. (1956) Experimental studies in insect parasitism IX. The reactions of a stick insect to an alien parasite. *Proc. R. Soc. Lond. Ser. B* **146,** 93–108.

Salt G. (1970) *The Cellular Defense Reactions of Insects*. Cambridge University Press, Cambridge.

Saul S. J., Bin L. and Sugumaran M. (1987) The majority of prophenoloxidase in insect haemolymph is present in the plasma and not in the haemocytes. *Dev. comp. Immunol.* **11,** 479–485.

Seybold W. D., Meltzer P. S. and Mitchell H. K. (1975) Phenol oxidase activation in *Drosophila*: a cascade of reactions. *Biochem. Genet.* **13,** 85–108.

Stoltz D. B. and Cook D. I. (1983) Inhibition of host phenoloxidase activity by parasitoid hymenoptera. *Experientia* **39,** 1022–1024.

Warner C. K., Grell E. H. and Jacobson K. B. (1974) Phenol oxidase activity and the *lozenge* locus of *Drosophila melanogaster*. *Biochem. Genet.* **11,** 359–365.