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# Mapping candidate genes for *Drosophila melanogaster* resistance to the parasitoid wasp *Leptopilina boulardi*

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**Summary**

*Drosophila melanogaster* resistance against the parasitoid wasp *Leptopilina boulardi* is under the control of a single gene (*Rlb*), with two alleles, the resistant one being dominant. Using strains bearing deletions, we previously demonstrated that the 55E2–E6; 55F3 region on chromosome 2R is involved in the resistance phenomenon. In this paper, we first restricted the *Rlb* containing region by mapping at the molecular level the breakpoints of the *Df*(*2R*)*Pc66*, *Df*(*2R*)*P34* and *Df*(*2R*)*Pc4* deficiencies, using both chromosomal *in situ* hybridization and Southern analyses. The resistance gene was localized in a 100 kb fragment, predicted to contain about 10 different genes. Male recombination genetic experiments were then performed, leading to identification of two possible candidates for the *Rlb* gene. Potential involvement of one of this genes, *edl*/*mae*, is discussed.

## 1. Introduction

Tremendous advances have been made during the last decade in understanding the antibacterial and antifungal immune response in insects (Hoffmann, 2003; Lemaitre, 2004), and defence mechanisms against parasites are now in the limelight. The anti-parasite response involves mainly the cellular part of the innate immune system, which was previously somehow neglected, leading to fast advances in understanding proliferation and differentiation of different categories of haemocytes (Russo *et al.*, 2001; Crozatier *et al.*, 2004; Meister, 2004). However, nothing is known about how insects recognize a parasite and which factors underlie the specificity of their response. Obtaining and characterizing specific host resistance genes would greatly help to achieve this goal and would provide a different way of analysing host–parasite interactions (Carton *et al.*, 2005).

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*Drosophila melanogaster* is parasitized by several hymenopteran wasps, including the larval parasitoid *Leptopilina boulardi* (Figitidae). Infested host larvae can react against the parasitoid egg by surrounding it with a cellular, melanized capsule (Carton & Nappi, 1997, 2001). Melanization of the capsule results from activation of the pro-phenoloxidase system whose components are enclosed in specific haemocyte cells, the cristal cells (Carton & Nappi, 1997; Meister & Lagueux, 2003). The main actors of the encapsulation process are the lamellocytes, large haemocytes that are mobilized to the surface of the parasite and form the multilayered capsule (Carton & Nappi, 1997).In addition, a strong increase in the number of circulating haemocytes, as well as a specific production of lamellocytes, are observed following infestation by parasitoid wasps (Russo *et al.*, 2001; Meister & Lagueux, 2003; Labrosse *et al.*, 2005). The components of the melanotic encapsulation response which kill the parasite have not been clearly identified, even though killing molecules such as quinones and reactive intermediates of oxygen and nitrogen are probably involved (Nappi *et al.*, 1995), but the presence of a capsule indicates the failure of parasitism.

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Using a well-characterized avirulent strain of *L. boulardi*, two *Drosophila melanogaster* isogenic strains with opposite encapsulation abilities have been obtained – a resistant (R), and a susceptible (S) strain – from a natural African population (Carton *et al*., 1992). The Drosophila S strain can not be considered as immune-incompetent since it is perfectly able to encapsulate the eggs of another parasitoid species, *Asobara tabida* (Vass *et al*., 1993). Resistance in the *Drosophila* R strain is thus highly specific, allowing recognition and destruction of *Leptopilina boulardi* eggs only. Our aim is to clone the *Rlb* gene, the first insect resistance gene to a parasitoid wasp analysed at the molecular level, and to determine its function.

The genetics of the resistance phenomenon are simple, with a single major segregating locus, *Rlb* (Resistance to *Leptopilina boulardi*) and two alleles, the resistant allele being dominant (Carton *et al*., 1992). Using classical genetic studies, *Rlb* has been localized on the second chromosome, between the *brown* and *lightoid* markers (Poirié *et al*., 2000). Strains bearing deletions in this region were used to further locate this gene, since the resistant phenotype is disturbed when the resistant allele faces a deletion. Results obtained with the deficiencies *Df*(*2R*)*Pc66*, *Df*(*2R*)*P34* and *Df*(*2R*)*Pc4* demonstrated that a region in 55E2; 55F3 had a great impact on *Drosophila melanogaster* ability to recognize and encapsulate *Leptopilina boulardi* eggs. This 300 kb region was recovered using cosmid and P1 phage clones and a complete restriction map was obtained (Hita *et al*., 1999).

The first localization of the *Rlb* gene was thus obtained using FlyBase data regarding the size and limits of *Df*(*2R*) deficiencies. However, some of these data were modified after our experiments and the size of the region was probably overestimated because of the possible discrepancy between the localization of the clones and the estimation of the deletion breakpoints. In the present work, in order to further determine the location of the resistance gene, we precisely localized the breakpoints of the deletions *Df*(*2R*)*Pc66*, *Df*(*2R*)*P34* and *Df*(*2R*)*Pc4* onto the restriction map of the region. For this purpose, we combined two different approaches: *in situ* hybridization with P1 and cosmid clones, and Southern analyses. A main region of 100 kb, containing about 10 predicted genes, was found to be involved in *D. melanogaster* specific resistance to parasitism by *L. boulardi*. In a second step, we performed male recombination experiments using a P-element inserted in the *Rlb*-containing region, more precisely in the 5′UTR of the *edl*/*mae* gene sequence (Preston *et al*., 1996; Chen *et al*., 1998; Baker *et al*., 2001). The results suggest that the *Rlb* gene is close to the P-element, leading to identification of two possible candidates for the resistance gene to *L. boulardi*: the *ed*/*mae* gene itself and CG33136 of unknown function.

## 2. Materials and methods

### (i) Drosophila *stocks*

For cytogenetic experiments, the UM-46442 (*Df*(*2R*) *Pc66*), BL-757 (*Df*(*2R*)*P34*), and UM-42689 (*Df*(*2R*) *Pc4*) fly strains, obtained from Umea (UM) or Bloomington (BL), were used. In these strains, chromosomes containing structural rearrangements of the 55A; 56C region are maintained as balanced stocks with the second chromosome balancer $ln(2LR)O,\ Cy^1\ dp^{lvI}\ pr^1\ cn^2$, designated as $Cy^O$ (Lindsley & Zimm, 1992). The wild strain Hikone was used as a standard stock. Stocks were routinely maintained at 25 °C, on standard yeast medium supplemented with dry active yeast.

For *in situ* hybridization experiments, analyses of polytene chromosomes were performed on F1 larvae originating from crosses between 12 females of a given balanced stock and males of the Hikone strain. These crosses were kept at 17–19 °C on the same medium.

For male recombination experiments, the following stocks were used:

The wild strain Rlb (1088) = $Rlb^+/Rlb^+$, resistant to *L. boulardi* (Carton *et al*., 1992). This strain is isogenic for chromosome 2.

The standard strains (non-resistant to *L. boulardi*) obtained from the Bloomington fly stock centre: BL-2535: $wg^{Sp\text{-}1}/Cy^O$; *Delta*(*2-3*) *Sb*/*TM6*, as a transposase stock; BL-10633: *y w*; $P(w^+)/Cy^O = y^1$ $w^{67c23}$, $P(w^{+mC} = lac\ W)\ edl^{k06602}/Cy^O$, a strain homozygous lethal which has the P-element $P(w^{+mC} = lac\ W)/l(2)k06602$ inserted in the 55E6; 55E9 region, in the 5′UTR of the *edl*/*mae* gene sequence.

Two multiple marker strains constructed for the experiments: *y w; dp bw sp* = *y w;* $dp^{ovl}$ *bw sp*, and *y w;* $dp^{ovl}$ $P(w^+)$ *bw sp*/$Cy^O$. This last strain, issued from the 10633 stock, contains the P-element inserted in the 55E6; 55E9 region.

The BL-10633 stock was obtained in a genomic insertion screen experiment, using the original target stock y[1] w[67c23]; +/+; +/+ (Török *et al*., 1993; Bier *et al*., 1989). Like most laboratory lines, the y[1] w[67c23]; +/+; +/+ stock (BL-6599) is completely susceptible (0 encapsulation; 71 larvae) to *L. boulardi*. The P-element of the BL-10633 line was thus inserted in a susceptible background.

### (ii) *Parasitoid stocks and encapsulation assays*

The origin of the avirulent strain of *L. boulardi* (Gif/Yvette, stock number 486) has been previously described in details (Dupas *et al*., 1998). Briefly, the G486 strain was obtained from an isofemale strain

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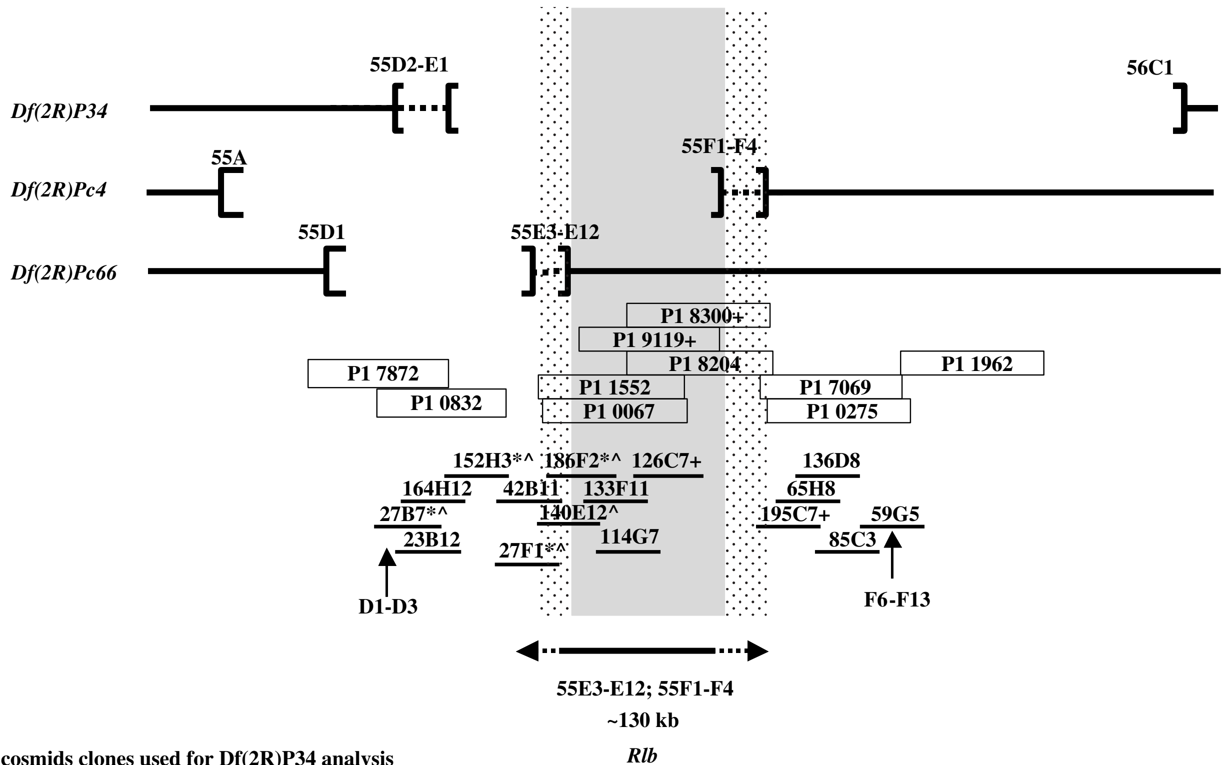


Fig. 1. Localization of *Df*(*2R*)*Pc66*, *Df*(*2R*)*P34* and *Df*(*2R*)*Pc4* deletion breakpoints using chromosomal data and *in situ* hybridization experiments. A region deleted in the *Df*(*2R*)*P34* and *Df*(*2R*)*Pc4* deficiencies but not in *Df*(*2R*)*Pc66* is known to contain the *D. melanogaster Rlb* resistance gene. The deletion breakpoints (right breakpoints of *Df*(*2R*)*Pc66* and *Df*(*2R*)*Pc4*, left breakpoint of *Df*(*2R*)*P34*) were inferred from chromosome observation and *in situ* hybridization with several clones. The cosmid 27F1 would constitute the right limit of *Df*(*2R*)*Pc66*, the P1 clone 8300 contains the right breakpoint of the *Df*(*2R*)*Pc4* deficiency and the cosmid 152H3 corresponds to the left limit of *Df*(*2R*)*P34*. Therefore the *Rlb* region is covered by a contig formed by the 1552 P1 phage clone and the main part of the 8300 P1 phage clone, corresponding to approximately 130 kb. This region is represented in grey with dotted parts indicating that the precise limits of the deficiencies remain to be defined. Thick lines and empty boxes represent cosmid and P1 phage clones that cover the region. Chromosomal data are reported for each deficiency and the corresponding region, contained between brackets, corresponds to a more precise localization inferred from *in situ* hybridization.

selected from a population collected in Brazzaville (Congo). To estimate the encapsulation rate, groups of 50 *D. melanogaster* second instar larvae (L2) were submitted to parasitism during 4 h by three *L. boulardi* G486 females. The larvae were dissected 2 days later and the number of encapsulated and non-encapsulated eggs was recorded. In all experiments, the encapsulation rate (ER) was calculated as the number of host larvae containing only encapsulated eggs (no parasitoid larva) divided by the total number of parasitized host larvae.

### (iii) *Cosmids and P1 phages*

Cosmids and P1 phage clones known to cover the 55D–55F region were previously described in Hita *et al*. (1999). Cosmids were provided by I. Siden-Kiamos and originated from a library made of Oregon R adult DNA (Siden-Kiamos *et al*., 1990). P1 phages were provided by C. MacKimmie and M. Ashburner and originated from a library made of *y, cn bw sp* strain adults (Smoller *et al*., 1991). The 27B7 and 59G5 cosmids are localized in 55D1–D3 and 55F6–F13, respectively (Hita *et al*., 1999 and FlyBase data). The localization of the clones is reported in Fig. 1.

### (iv) In situ *hybridization*

All Df(2R) strains have the second chromosome balanced with $Cy^O$, which was not suitable for chromosomal analyses. We then used F1 larvae from (Df(2R) × Hikone) crosses for chromosomal observations. In polytene chromosome preparations, the standard chromosome forms a loop in the region facing the deletion. We then performed *in situ* hybridization experiments onto (Df(2R) × Hikone) polytene chromosomes to determine which cosmid or

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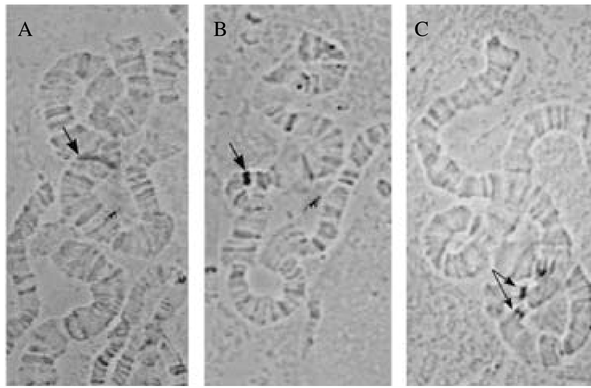


Fig. 2. Localization of *Df(2R)Pc66*, *Df(2R)P34* and *Df(2R)Pc4* breakpoints using *in situ* hybridization experiments. P1 and cosmid clones located in the 55D–55F region were used as probes onto polytene chromosomes of (*Df(2R)* × Hikone) F1 larvae. Arrowheads mark the hybridization signals. Three examples are shown. (*A*) Hybridization onto *Df(2R)P34*/Hikone chromosomes with the 27B7 cosmid as a probe. The signal is detected on both chromosomes. (*B*) Hybridization onto *Df(2R)Pc4*/Hikone chromosomes with the 126C7 cosmid clone. The signal is detected on the standard chromosome only. (*C*) Hybridization onto *Df(2R)Pc4*/Hikone polytene chromosomes using the 8300 P1 phage as a probe. This clone covers the deletion breakpoint: one signal is detected onto the complete chromosome and a thinner signal appears on the deleted chromosome.

P1 phage clone included the breakpoints of the deletions *Df(2R)Pc66*, *Df(2R)P34* and *Df(2R)Pc4*. Several clones mapped in 55 E2; 55F3 (Hita *et al.*, 1999) were used as probes: the 27B7, 152H3, 27F1, 186F2, 140E12, 126C7 and 195C7 cosmid clones, as well as the 9119 and 8300 P1 clones (Fig. 1). Three hybridization patterns could be observed depending of the location of the probe: (a) using a clone located outside the deletion, a large spot indicating hybridization onto both homologous chromosomes (Fig. 2*A*); (b) in the case of a clone located inside the deletion, a spot corresponding to hybridization onto the standard chromosome only, located in the loop region (Fig. 2*B*); (c) finally, with a clone covering the breakpoint of the deletion, one spot onto the standard chromosome and a thinner signal onto the deleted chromosome (Fig. 2*C*).

Dissection and fixation of salivary glands as well as chromosome squashes were performed as reported in Engels *et al.* (1986). The localization of the deficiencies breakpoints was confirmed using squashes of third-instar larval salivary glands prepared for visualization by a standard method (Lefevre, 1976). *In situ* hybridization onto polytene chromosomes was carried out as described by De Frutos *et al.* (1990) with the following modifications. Prior to pre-treatment of the chromosomes, the slides were placed in a 200 mM HCl bath for 20 min. The squashes were pre-treated for 15 min at 65 °C in a 2× SSC solution and denatured in freshly prepared 70% ethanol/0·07 M NaOH for 5 min (Pardue & Gall, 1975). The probes were labelled with digoxigenin-dUTP by random priming using the DIG DNA Labelling and Detection Kit supplied by Boehringer Mannheim. Cosmids and P1 phages, previously mapped in the 55D-F region (Hita *et al.*, 1999), were used as probes onto polytene chromosomes of F1 larvae *Df(2R)* × Hikone.

### (v) *Southern blot experiments*

Genomic DNA was extracted from adults flies of the Hikone strain, the F1 *Df(2R)Pc66* × Hikone and the F1 *Df(2R)Pc4* × Hikone. Standard techniques were used for DNA digestion with restriction enzymes (3 $\mu$g of DNA in each digestion), gel electrophoresis (0·8% Seakem agarose) and Southern blotting onto Nylon+ membranes (ICN products). The DNA fragments used as probes were obtained from digested cosmid and P1 clones using standard extraction procedures (Ausubel *et al.*, 1994). Obviously, when a fragment located inside a deletion is used as a probe, the intensity of the hybridization signal obtained with the DNA of the corresponding (*Df(2R)* × Hikone) strain is about half that of the signal detected with Hikone DNA. If the fragment is located outside the deletion, we expect the same signal intensity in the different strains. Finally, if the fragment covers the deficiency breakpoint, the intensity of the signal corresponding to the predicted restriction fragment will be reduced by half in (*Df(2R)* × Hikone) strains and another restriction fragment might be detected depending of the position of the next restriction site on the deleted chromosome. Two fragments located by our experiments outside the region of interest were used as controls: a 270 bp fragment corresponding to part of the gene *enabled*, localized in 56B, and a 5·3 kb fragment (A fragment) obtained from a *Bst*EII digestion of the 8300 P1 clone (position 65253–70591).

The probes were random-primed labelled with [$\alpha$-$^{32}$P]dATP (ICN Products) using the Klenow fragment of DNA polymerase I (Promega) and used at a concentration of $10^6$ cpm/ml of hybridizing solution. Hybridization was carried out at 65 °C in $Na_2HPO_4$–$NaH_2PO_4$ 0·5 M pH 7·2, SDS 7%, EDTA 1 mM. Final washing was done in 0·2× SSC, 0·1% SDS at 65 °C. Hybridized filters were autoradiographed with Fuji RX films at −80 °C. Quantification of DNA fragments in the strains was obtained using a phospho-imager (Packard). Experiments and counting were repeated at least three times.

### (vi) *Sequence analysis*

Sequences of the P1 clones 8300 and 1552 were obtained from the Berkeley Drosophila Genome Project (Kimmerly *et al.*, 1996; Adams *et al.*, 2000) and

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analysed for the presence of restriction sites using the Infobiogen Bisance programs (Dessen *et al*., 1990). Sequence data were analysed using ORFfinder and GeneFinder softwares and compared with databases using classical Blastp programs. Results were compared with FlyBase data.

### (vii) *Crosses for male recombination experiments*

Male recombination experiments can be used to map a gene of interest to the right or the left of a P-element insertion, using specific crosses (Preston *et al*., 1996; Chen *et al*., 1998). The general method consists in producing P-element-induced recombinations and determining whether a given allele of a gene of interest segregates with proximal or distal markers. In order to restrict the *Rlb*-containing region and define candidate genes, we performed male recombination experiments using a *Drosophila* stock with a P-element inserted roughly in the middle of the 55E6; 55E9 region (in the 5′UTR of the *edl* gene). Fig. 4 presents the general scheme of the crosses.

In a first step, the cross between $Rlb^+/Rlb^+$ males and $wg/Cy^O$; *Delta*(*2-3*) *Sb*/*TM6* females allowed the selection of F1 males of [$Cy^O/Sb$] phenotype which were $Rlb^+/Cy^O$; *Delta*(*2-3*) *Sb*/+. These males were crossed with females *y w*/*y w; dp* $P(w^+)$ *bw sp*/$Cy^O$. Among their progeny, the [$w^+$; *Sb*] males, possessing a P-element (with the mini white$^+$ sequence which restores an orange-eye phenotype), were retained. Their genotype is *y w*/*Y*; $Rlb^+$/dp $P(w^+)$ *bw sp*; *Delta*(*2-3*) *Sb*/+, with the two markers *dp* and *sp* flanking the P-element inserted on chromosome 2, and the chromosome 2 in *trans* possessing the $Rlb^+$ allele. The transposase source (*Delta2-3*) is provided by chromosome 3. In such male flies, P-element-induced recombination events result in the $Rlb^+$ allele co-segregating with either *dp* or *sp*, depending on the relative position of *Rlb* and the P-element inserted. These males were then crossed with females *y w*/*y w, dp bw sp*/*dp bw sp* (Fig. 4). In the next generation, four main phenotypic categories were observed, with a majority of non-recombinant individuals and few individuals arising from single recombination events (retaining *dp* or *sp*). Very rare individuals were produced by double recombination events or excision of the P-element. Male progeny which conserved the P-element (orange eyes) but suffered a single recombination (identified by the presence of either the *dp* or *sp* phenotypes), and had lost the *Delta*(*2-3*) *Sb* source of transposase chromosome, were retained. They were individually crossed with females of the 10633 strain in order to create isolated lines balanced over $Cy^O$, thus avoiding future recombination events. Therefore, as the $Cy^O$ balancer chromosome carries the *dp* and $sp^+$ alleles, two types of recombination lines phenotypically [*dp*] or [$sp^+$] were obtained. Assessing the resistance phenotype of these lines, we thus expected to determine whether $Rlb^+$ co-segregated with *dp* or *sp*, and to infer whether *Rlb* is proximal or distal to the P-element insertion. The resistance of each line was measured using a standard encapsulation test. If *Rlb* was proximal to the P-element, [*dp*] lines were expected to show a low rate of encapsulation compared with [$sp^+$] lines, and the reverse if *Rlb* was distal to the P-element.

## 3. Results

A region covered by the *Df*(*2R*)*P34* and *Df*(*2R*)*Pc4* deletions but not by the *Df*(*2R*)*Pc66* deletion is known to be involved in *D. melanogaster* resistance to *L. boulardi* (Hita *et al*., 1999; Poirié *et al*., 2000; Fig. 1). The localization of the left breakpoint of *Df*(*2R*)*P34* and the right breakpoints of *Df*(*2R*)*Pc4* and *Df*(*2R*)*Pc66* was performed to obtain a better characterization of this region.

### (i) *Limits of the deletions defined by* in situ *hybridization*

All Df(2R) strains have the second chromosome balanced with $Cy^O$, which was not suitable for chromosomal analyses. We then used F1 larvae from (Df(2R) × Hikone) crosses for chromosomal observations. Our observations regarding the limits of the deletions were as follow: the breakpoints of the *Df*(*2R*)*Pc66*, *Df*(*2R*)*P34* and *Df*(*2R*)*Pc4* deletions were found in (55D1; 55E8–E12), (55D2–E1; 56C1) and (55A; 55F3–F4), respectively. These results were similar to previous data from Doane & Dumapias (1987) with the exception of the right limits of *Df*(*2R*) *Pc66* (55E3–E4) and *Df*(*2R*)*Pc4* (55F). Chromosomal analysis of *Df*(*2R*)*Pc66* is difficult since the deleted fragment is rather small and a puff occurs in the corresponding region (55E; Ashburner, 1989). Its right limit is now reported in 56B2 (FlyBase data) but the deleted region is clearly smaller. Combining all these data, we considered the following breakpoint limits: *Df*(*2R*)*Pc66* (55D1; 55E3–E12), *Df*(*2R*)*P34* (55D2–E1; 56C1), *Df*(*2R*)*Pc4* (55A; 55F1–F4). *Rlb* was thus predicted to be located inside the 55E3–E12; 55F1–F4 region, which approximately corresponded to 300 kb.

We then performed *in situ* hybridization experiments onto polytene chromosomes to determine which cosmid or P1 phage clone included the breakpoints of the deletions *Df*(*2R*)*Pc66*, *Df*(*2R*)*P34* and *Df*(*2R*)*Pc4* (Figs 1, 2).

#### (a) *Right limit of* Df(2R)Pc66

We used cosmid clones mapped in 55 DE, namely 27B7, 152H3, 27F1, 140E12 and 186F2, as

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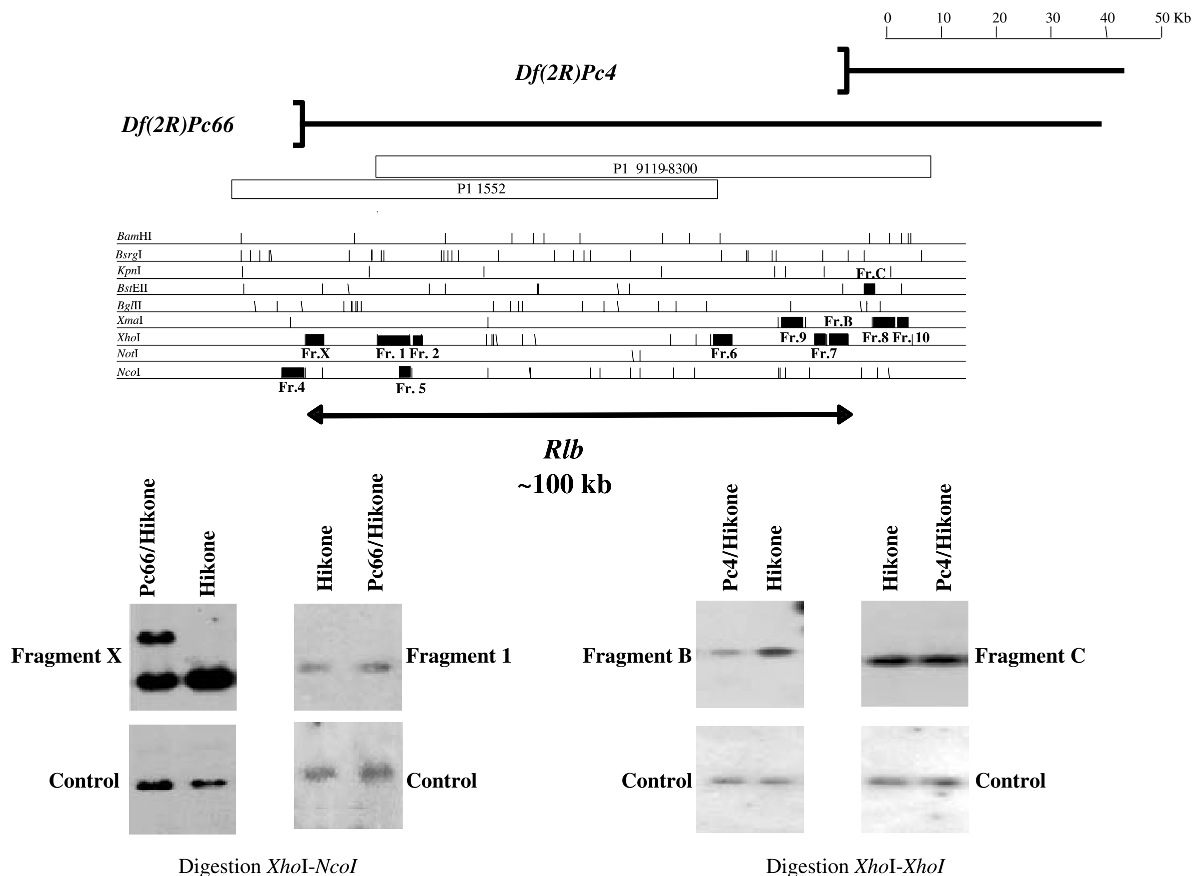


Fig. 3. Localization of the right breakpoints of *Df(2R)Pc66* and *Df(2R)Pc4* deficiencies using Southern blot experiments. A restriction map of the 1552/9119-8300 P1 phage contig is shown with the fragments used as probes represented as black boxes. Examples of hybridizations of these fragments on Southern blots containing *Df(2R)*/Hikone and Hikone DNA digested by *Xho*I and *Nco*I (left part) or *Xho*I (right part) are shown below. Control experiments were performed with fragments located outside the region in order to compare DNA quantities in *Df(2R)*/Hikone and Hikone lines. The *Xho*I/*Nco*I X fragment covers the limit of the *Df(2R)P66* deletion. The *Xho*I/*Xho*I B fragment constitutes the limit of the *Df(2R)Pc4* deletion. The *Rlb* region thus corresponds to the 100 kb DNA sequence located between the X fragment of the 1552 clone and the B fragment of the 8300 clone.

probes for *in situ* hybridization experiments onto (*Df(2R)Pc66* × Hikone) F1 polytene chromosomes (Fig. 1). The results showed that 27B7, 152H3 and the largest part of 27F1 were located inside the deleted region while 140E12 and the main part of 186F2 were outside the deleted region. The right limit of the deletion is thus contained in a region covered by the right part of 27F1 and the left part of 186F2. It corresponds approximately to the left limit of the P1 clone 1552.

### (b) *Left limit of* Df(2R)P34

Using several cosmid clones mapped in the 55DE region as probes onto (*Df(2R)P34* × Hikone) F1 polytene chromosomes, we demonstrated that the 27B7 clone was located outside the deletion but close to the left breakpoint. Using this clone as a probe, a large spot was observed on polytene chromosomes, outside the loop (Fig. 2*A*). On the contrary, the 152H3, 27F1 and 186F2 cosmid clones (Fig. 1) were clearly included inside the deleted region.

### (c) *Right limit of* Df(2R)Pc4

The 126C7 and 195C7 cosmids as well as the overlapping 9119 and 8300 phage P1 clones were used as probes onto (*Df(2R)Pc4* × Hikone) F1 polytene chromosomes. Hybridization results indicated that the 9119 P1 clone is included inside the deletion as well as 126C7 and a large part of the 8300 clone (Figs 1, 2*B* and *C*). The cosmid 195C7 is clearly located outside the deletion. As a consequence, the breakpoint of the deletion was assigned to the region of the 8300 clone that does not overlap the 9119 clone.

These data confirmed that part of the region covered by the *Df(2R)P34* deletion was also covered by *Df(2R)Pc66*. The *Rl*-containing region was then enclosed between the right limit of *Df(2R)Pc66* and the right limit of *Df(2R)Pc4*, corresponding to the

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Table 1. *Localization of the right breakpoints of Df(2R)Pc66 and Df(2R)Pc4 deletions using Southern blot experiments and radioactivity counts*

(*A*) Right limit of the *Df*(*2R*)*Pc66* deficiency

| | Pc66/H | Hikone | Second spot | Ratio |
|---|---|---|---|---|
| **Fr. 1** | 3828 | 3996 | | 0·96 |
| Fr. A | 2042 | 2145 | | 0·95 |
| **Fr. X** | 7544 | 12536 | 4318 | 0·60 |
| Fr. ena | 4572 | 3797 | | 1·20 |

(*B*) Right limit of the *Df*(*2R*)*Pc4* deficiency

| | Pc4/H | Hikone | Ratio |
|---|---|---|---|
| **Fr. B** | 268 | 425 | 0·63 |
| Fr. ena (C) | 4047 | 4255 | 0·95 |
| **Fr. C** | 8748 | 9108 | 0·96 |
| Fr. ena (C) | 8435 | 6884 | 1·22 |

Hybridization signals were obtained with restriction fragments of the 1552 and 8300 P1 clones and control fragments used as probes, on Southern blots containing DNA from Hikone (H) or F1 *Df*(*2R*)/Hikone individuals. Signals intensities (estimated in cpm) were obtained using a phosphoimager. Radioactive counts obtained for *Df*(*2R*)/Hikone and Hikone lines were used to obtain a ratio [*Df*(*2R*)/Hikone divided by Hikone] that was compared with the ratio calculated for control fragments.

Pc66/H, signal intensity with Pc66/Hikone DNA; Pc4/H, signal intensity with Pc4/Hikone DNA; Hikone, signal intensity with Hikone DNA. The fragments A and ena were used as controls.

1552 clone and the main part of the 8300 P1 phage clone (Fig. 1), a region of approximately 130 kb.

### (ii) *Limits of the deletions defined by Southern blot analyses*

This approach aimed to confirm *in situ* hybridization data and to localize the right breakpoint of *Df*(*2R*)*Pc66* and the right breakpoint of *Df*(*2R*)*Pc4* on the restriction map of the 55E–55F region. *Bam*HI, *Bsrg*I, *Kpn*I, *Bst*EII, *Bgl*II, *Sma*I, *Xho*I and *Nco*I restriction sites were determined both from the sequence obtained from the Berkeley Drosophila Genome Project and from our physical map of the region (Hita *et al*., 1999). Several restriction fragments were used as probes on Southern blots containing digested DNA from the Hikone strain and from (*Df*(*2R*)*Pc66* × Hikone) and (*Df*(*2R*)*Pc4* × Hikone) F1 adults.

#### (a) *Right limit of* Df(2R)Pc66

Five *Xho*I–*Xho*I, *Nco*–*Nco*I or *Xho*I–*Nco*I restriction fragments, distributed in a 38 kb region in the left part of the P1 clone 1552, were used as probes onto Southern blots of genomic DNA from (*Df*(*2R*)*Pc66* × Hikone) and Hikone strains. Our results indicated that a 3 kb *Xho*I–*Nco*I fragment (fragment X) in the 11983–15018 region contained the right limit of the *Df*(*2R*)*Pc66* deficiency (Fig. 3; Table 1). The hybridization signal corresponding to this restriction fragment was reduced by half in (*Df*(*2R*)*Pc66* × Hikone) compared with Hikone, and a new restriction fragment was detected. Hybridization with other fragments was in agreement with this conclusion. The fragments 1 (25094–31081) and 4 (7471–11495) were respectively located outside and inside the deletion.

#### (b) *Right limit of* Df(2R)Pc4

Seven restriction fragments scattered in 35 kb corresponding to the right part of the P1 clone 8300 were selected and used as probes on Southern blots containing *Df*(*2R*)*Pc4*/Hikone and Hikone DNA digested with several enzymes. The results showed that a 3·8 kb *Xho*I–*Xho*I fragment (fragment B) located between 57451 and 61243 on the 8300 P1 clone corresponded to the right limit of the *Df*(*2R*)*Pc4* deletion. When this fragment was used as a probe, the hybridization signal was reduced by half in *Df*(*2R*)*Pc4*/Hikone compared with Hikone. On the other hand, the *Bst*EII–*Bst*EII fragment (fragment C) in the 64294–65253 position hybridized with a *Xho*I–*Xho*I fragment corresponding to the 61243–72835 region and the same signal intensity was observed with the different strains (Fig. 3, Table 1). No other restriction fragment was detected using the B fragment as a probe, which suggested that the next *Xho*I restriction site on the deleted chromosome was either very close or very distant. The localization of the *Df*(*2R*)*Pc4* limit was in agreement with results obtained using other restriction fragments (data not shown).

These data allowed restriction of the *Rlb*-containing region to approximately 100 kb, predicted to contain 10 different genes (ORFfinder and Genefinder analyses, in agreement with FlyBase data).

### (iii) *Results of male recombination experiments*

Considering the fact that the resistance gene was still located in a rather large 100 kb region, we used the P-element-induced site-specific male recombination method to map it more precisely (Chen *et al*., 1998; Fig. 4). We obtained 32 recombinant lines, each occurring from a single recombination event, over a total of 30000 observed individuals. Many lines were poorly fertile or viable and, finally, 10 [*dp*] lines and 4 [$sp^+$] lines could be tested for their encapsulation rate (Table 2). Unexpectedly, both categories of recombinant lines showed low encapsulation abilities and were classified as totally susceptible to

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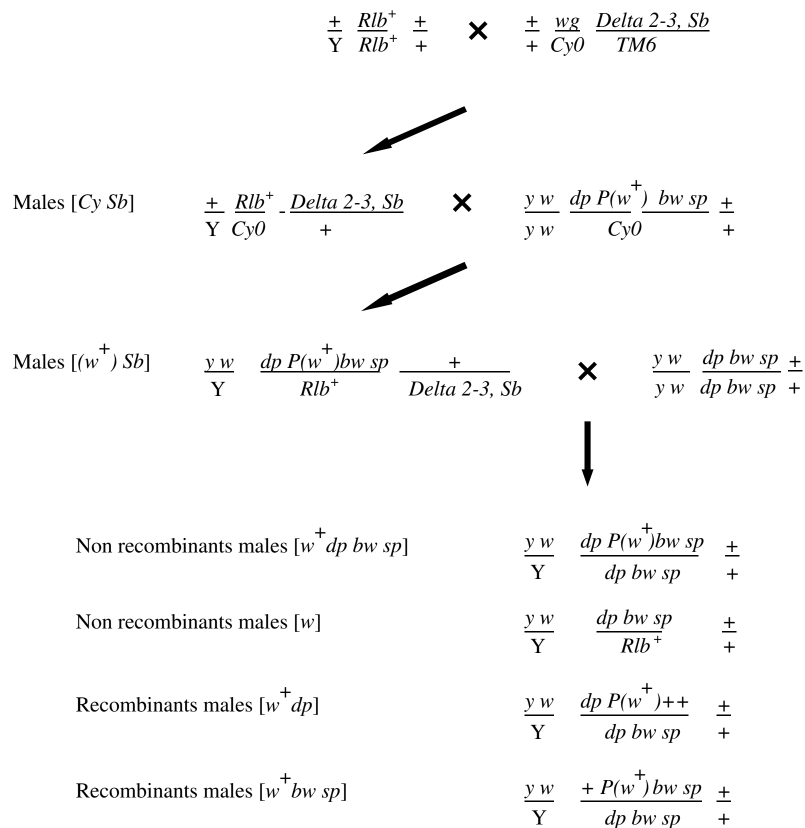


Fig. 4. Breeding scheme for mapping the *Rlb* gene using P-element-induced male recombination. *dp* and *sp* are used as proximal and distal markers flanking the P-element on chromosome 2, and a chromosome containing $Rlb^+$ is put in *trans* to the chromosome containing the P-element. The transposase source (*Delta 2-3*) is provided by chromosome 3. Recombination events are induced at the vicinity of the P-element in male flies in the presence of transposase. In the next generation, most flies are of parental phenotype; recombinants are identified by the presence of only the *dp* or the *sp* visible markers. Male recombinants are then crossed to females from a $Cy^O$ balancer stock to construct recombinant lines carrying one of the two types of recombinant chromosomes. Individuals from both types of lines are submitted to encapsulation assays to test whether the $Rlb^+$ allele is present or absent on the recombinant chromosome.

*L. boulardi*. As a control, we also tested F1 individuals from crosses between the $Rlb^+$ train and the transposase stock (non-resistant strain) and between the $Rlb^+$ strain and the 10633 stock (non-resistant). These F1 individuals had high encapsulation rates (86·2% and 86·3%, respectively), indicating that dominance of the $Rlb^+$ allele is not affected by the presence *in trans* of the P-element or the $Cy^O$ balancer.

## 4. Discussion

In a previous paper (Hita *et al.*, 1999), we reported the localization of the *Rlb* gene, a *Drosophila melanogaster* resistance gene to the parasitoid wasp *Leptopilina boulardi*, in a 300 kb region, in 55E2; 55F3. This localization was obtained using three strains bearing deletions on the second chromosome. The present study was designed to more precisely locate the resistance gene and define a small number of candidates. To restrict the *Rlb*-containing region, we first used combined chromosomal and molecular approaches, *in situ* hybridization and Southern blot experiments. The results were congruent and allowed better localization of the breakpoints of three deficiencies at the molecular level. All chromosomal experiments indicated that the region containing the 1552 P1 clone (right limit of *Df*(*2R*)*Pc66*) and the major part of the 8300 P1 clone (right limit of *Df*(*2R*)*Pc4*) was involved in resistance to *L. boulardi*. This region covered approximately 130 kb compared with the 300 kb region predicted using FlyBase data on *Df*(*2R*) breakpoints. A more precise localization of the right breakpoints of *Df*(*2R*)*Pc66* and *Df*(*2R*)*Pc4* was achieved using Southern blot experiments. Several restriction fragments from the 1552 and 8300 P1 phage clones were used as probes onto blots containing *Df*(*2R*)/Hikone and Hikone DNA, control fragments being used to compare DNA quantities in the different lines. The right limits of *Df*(*2R*)*Pc66* and *Df*(*2R*)*Pc4* were localized in two restriction fragments, thus restricting the region containing the *D. melanogaster* resistance gene to 100 kb. Analyses of the *Drosophila* genome sequence predicted the occurrence of 10 genes in this 100 kb *Rlb*-containing

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Table 2. *Male recombination mapping of the Rlb gene. Encapsulation rates of* L. boulardi *parasitoid eggs by the recombinant lines between the dp and sp loci*

| Recombinant line | No. of larvae | Encapsulation rate (%) |
|---|---|---|
| [dp +] recombinant chromosome | | |
| dp1 | 59 | 0·0 |
| dp2 | 40 | 2·5 |
| dp8 | 31 | 9·6 |
| dp9 | 85 | 2·3 |
| dp10 | 58 | 0·0 |
| dp14 | 51 | 0·0 |
| dp19 | 53 | 0·0 |
| dp24 | 45 | 0·0 |
| dp25 | 72 | 0·0 |
| dp27 | 33 | 12·1 |
| [+ sp] recombinant chromosome | | |
| sp17 | 46 | 2·1 |
| sp22 | 44 | 11·3 |
| sp24 | 80 | 6·3 |
| sp32 | 36 | 0·0 |
| Total [dp+] recombinants | 527 | $1·9 \pm 1·1$ |
| Total [+sp] recombinants | 206 | $5·3 \pm 3·1$ |
| Control Rlb strain | 153 | $86·3 \pm 6·0$ |

region (see FlyBase data, http://flybase.bio.indiana.edu). Among these genes, only four had at least a predicted molecular function: CG5473 (SP2637) encodes a protein with N-term asparagine hydrolase activity, CG15085 (*edl/mae*) has protein binding properties and is involved in the regulation of signal transduction, CG15072 (EMK/KIAA0999) has a protein serine/threonine kinase activity, and CG15 073, which contains a zinc finger domain, would act as a transcription regulator, potentially involved in cell proliferation.

Most of the predicted genes are possible candidates for the *Rlb* gene and we then performed male recombination experiments in order to further restrict the region of interest. The best tool for localizing the *Rlb* gene was the 10633 stock, a well-characterized one-insert line, having a P-element inserted inside roughly in the middle of the region of interest (in E6–E9). The procedure to map *Rlb* relative to the P-element insertion was based on the assumption that resistance to *L. boulardi* is monogenic (Carton *et al*., 1992) and the expectation that the resistance gene would segregate with either the proximal or the distal morphological marker. Under this assumption, only one of the two types of recombinant lines ought to present the resistance phenotype, measured by the encapsulation rate.

The analysis of recombinant lines led to unexpected results, both types of recombinants being susceptible to *L. boulardi*, regardless of their phenotype. Two explanations can be proposed to interpret these data. The resistance phenotype might be determined by closely linked genes, located at two loci, one on each side of the P-element. Resistance would thus necessitate the presence of two different genes co-acting in *cis*, the association of which would have been disrupted in recombinant lines. This hypothesis can not be rejected but seems rather unlikely. The other explanation retains the hypothesis of the one-locus model, locating the *Rlb* resistance gene very close of the P-element. Indeed, in male recombination experiments, more than 85% of recombinations occur within a 4 kb region around the P-element and they are associated with deletion/duplication events, with only half of the deletions extending over more than a 4 kb region (Preston & Engels, 1996; Preston *et al*., 1996). P-element-induced recombinations are thus an efficient way of producing knockout mutations of nearby genes. In our lines, the normal function of the closely linked *Rlb* gene would have been disrupted, leading to non-resistant recombinant lines, independently of the adjacent phenotypic marker. In addition, transfection effects occur at the *Rlb* locus, which indicates that somatic pairing of chromosomes have a role in the expression of this gene. Deletion/duplication events might have prevented short-range pairing between *Rlb* alleles.

The insertion of the P{lacW} element in the 10633 insert line has been mapped into the 5′UTR of the *edl/mae* transcription unit, 800 bp upstream of the initiation codon (Baker *et al*., 2001). This insertion itself cannot be solely responsible for the susceptibility of strain 10633 to *L. boulardi* as it occurred in a strain that was already susceptible. Close proximal predicted genes are CG33136 (about 4 kb distant) and CG5469 (about 8 kb distant), and the closest distal gene is CG15086 (about 9 kb distant); the functions of these three genes are currently unknown. The fact that we did not recover any resistant recombinant line strongly suggests that *edl/mae* itself or possibly CG33136 might be responsible for resistance to *L. boulardi*.

Under our assumptions, the *edl* gene, located in 55E6 (known in FlyBase under different synonyms: CG15085, l(2)k06602, *mae* (modulator of the activity of Ets) and *edl* (Ets-domain lacking)), is the more likely candidate for *Rlb*. The insertion of a P-element in the 5′UTR region of this gene has occurred in a susceptible background and is homozygous lethal at the larval or pupal stage due to disruption of expression (Baker *et al*., 2001). This insertion is thus clearly not responsible for susceptibility to *L. boulardi*. Besides, resistance is polymorphic in natural populations (Dupas *et al*., 2003), which suggests that resistant and susceptible alleles would rather differ slightly in their amino acid sequence or in their expression profiles. Only one homogeneous

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resistant strain is available at present and the sequence of the *edl* gene is polymorphic among susceptible strains, so that no significant correlation could be obtained between resistance/susceptibility and amino acid sequence differences. Regarding expression profiles, *edl/mae* is known to be precisely modulated to ensure appropriate transcriptional responses to receptor tyrosine kinase (RTK) signalling and to be finely regulated by feedback loops (Tootle *et al.*, 2003; Vivekanand *et al.*, 2004). To compare expression levels between resistant and susceptible strains, specific conditions will have to be defined, including for instance different times following infestation by the parasitoid wasp.

The *edl/mae* gene encodes a protein with an ETS-specific Pointed domain (SAM domain) but not an ETS DNA-binding domain and acts as a signalling intermediate that directly links the RTK/RAS/MAPK signalling pathway to its downstream transcription factor targets (Baker *et al.*, 2001). *edl/mae* mediates MAPK phosphorylation of the Ets transcription factors y*an/aop* and *Pointed P2*, thus modulating the balance between y*an*-mediated repression and *Pointed*-mediated activation of target genes (Vivekanand *et al.*, 2004). *yan/aop* and *edl/mae* have been implicated in a number of developmental processes in *Drosophila* but not so far in haematopoiesis or haemocyte function. However, RTK signalling leads to cell proliferation or differentiation and *yan/aop* is involved in cell choice between these two outcomes (Rogge *et al.*, 1995). As encapsulation of parasitoid eggs requires haemocyte proliferation and specific differentiation of lamellocytes in *Drosophila* larvae, *edl/mae* and *yan/aop* might be important components of the signalling cascade leading to these processes. The fact that ectopic expression of *yan/aop*$^{ACT}$, a *yan/aop* constitutively active allele, stimulates both proliferation of haemocytes and formation of lamellocytes in *Drosophila* larvae (Zettervall *et al.*, 2004) supports this hypothesis and the possible involvement of *edl/mae* in resistance to *L. boulardi*.

The implication of *edl/mae* or possibly of the closest gene CG33136 (of unknown function) in *Drosophila* resistance to the parasitoid wasp *L. boulardi* remains of course to be assessed using transgenese experiments, and the role of *Rlb* to be clearly demonstrated.

In recent years, parallel studies conducted in mammals and flies have emphasized the existence of common mechanisms regulating the vertebrate and invertebrate innate immune systems. This culminated in the discovery of the central role of the Toll pathway in *Drosophila* immunity against microorganisms and in the implication of Toll-like receptors in the mammalian innate immune response. Recent findings on the mechanisms underlying specification of the lamellocytes suggest new parallels in cellular immunity between *Drosophila* and vertebrates (Crozatier *et al.*, 2004). Genetic and molecular characterization of insect resistance genes to parasitoids will help to test this hypothesis. Finally, understanding the mechanisms of naturally occurring, specific resistances against parasitoid wasps, widely used in biological control, is of high agronomic interest.

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## References

Adams, M. D., Celniker, S. E., Holt, R. A., Evans, C. A., Gocayne, J. D., *et al.* (2000). The genome sequence of *Drosophila melanogaster*. *Science* **287**, 2185–2195.

Ashburner, M. (1989). *Drosophila: A Laboratory Handbook*. New York: Cold Spring Harbor Laboratory Press.

Ausubel, F. M., Brent, R., Kingston, R. R., Moore, D. D., Seidman, J. G., *et al.* (1994). *Current Protocols in Molecular Biology*. New York: Wiley.

Baker, D. A., Mille-Baker, B., Wainwright, S. M., Ish-Horowicz, D. & Dibb, N. J. (2001). Mae mediates MAP kinase phosphorylation of Ets transcription factors in *Drosophila*. *Nature* **411**, 330–334.

Bier, E., Vaessin, H., Shepherd, S., Lee, K., McCall, K., *et al.* (1989). Searching for pattern and mutation in the *Drosophila* genome with a P-lacZ vector. *Genes and Development* **3**, 1273–1287.

Carton, Y. & Nappi, A. J. (1997). *Drosophila* cellular immunity against parasitoids. *Parasitology Today* **13**, 218–227.

Carton, Y. & Nappi, A. (2001). Immunogenetics aspects of the cellular immune response of *Drosophila* against parasitoids. *Immunogenetics* **52**, 157–164.

Carton, Y., Frey, F. & Nappi, A. (1992). Genetic determinism of the cellular immune reaction in *Drosophila melanogaster*. *Heredity* **69**, 393–399.

Carton, Y., Nappi, A. J. & Poirié, M. (2005). Genetics of anti-parasite resistance in invertebrates. *Developmental and Comparative Immunology* **29**, 9–32.

Chen, B., Chu, T., Harms, E., Gergen, J. P. & Strickland, S. (1998). Mapping of *Drosophila* mutations using site-specific male recombination. *Genetics* **149**, 157–163.

Crozatier, M., Ubeda, J.-M., Vincent, A. & Meister, M. (2004). Cellular immune response to parasitization in *Drosophila* requires the EBF orthologue Collier. *PLoS Biology* **2**(8), e196.

De Frutos, R., Kimura, K. & Peterson, K. (1990). *In situ* hybridization of *Drosophila* polytene chromosome with digoxigenin-dUTP labeled probes. *Methods in Molecular Cellular Biology* **2**, 32–36.

Dessen, P., Fondrat, C., Valencien, C. & Mugnier, C. (1990). BISANCE: a French service for access to biomolecular databases. *Computer Applications in the Biosciences* **6**, 355–356.

Doane, W. W. & Dumapias, F. E. (1987). Deletion limits of the adp, map and nu loci in *D. melanogaster*. *Drosophila Information Service* **66**, 49.

https://doi.org/10.1017/S001667230600841X Published online by Cambridge University Press

Dupas, S., Frey, F. & Carton, Y. (1998). A single parasitoid segregating factor controls immune suppression in *Drosophila*. *Journal of Heredity* **89**, 306–311.

Dupas, S., Carton, Y. & Poirié, M. (2003). Genetic dimension of the coevolution of virulence-resistance in *Drosophila*: parasitoid wasps relationships. *Heredity* **90**, 84–89.

Engels, W. R., Preston, C. R., Thompson, P. & Eggleston, W. B. (1986). *In situ* hybridization to *Drosophila* salivary chromosomes with biotinylated DNA probes and alkaline phosphatase. *Focus* **8**, 6–8.

Hita, M. T., Poirié, M., Leblanc, N., Lemeunier, F., Lutcher, F., *et al*. (1999). Genetic localization of a *Drosophila melanogaster* resistance gene to a parasitoid wasp and physical mapping of the region. *Genome Research* **9**, 471–481.

Hoffmann, J. A. (2003). The immune response of *Drosophila*. *Nature* **426**, 33–38.

Kimmerly, W., Stultz, K., Lewis, S., Lewis, K., Lustre, V., *et al*. (1996). A P1-based physical map of the *Drosophila* euchromatic genome. *Genome Research* **6**, 414–430.

Labrosse, C., Eslin, P., Doury, G., Drezen, J. M. & Poirié, M. (2005). Haemocyte changes in *D. melanogaster* in response to long gland components of the parasitoid wasp *Leptopilina boulardi*: a Rho-GAP protein as an important factor. *Journal of Insect Physiology* **51**, 161–170.

Lefevre, G. (1976). A photographic representation and interpretation of the polytene chromosome of *Drosophila melanogaster* salivary glands. In *The Genetics and Biology of Drosophila* (ed. M. Ashburner & E. Novitski), pp. 32–66. New York: Academic Press.

Lemaitre, B. (2004). The road to Toll. *Nature Review in Immunology* **4**, 521–527.

Lindsley, D. L. & Zimm, G. G. (1992). *The Genome of* Drosophila melanogaster. San Diego: Academic Press.

Meister, M. (2004). Blood cells of *Drosophila*: cell lineages and role in host defence. *Current Opinion in Immunology* **16**, 10–15.

Meister, M. & Lagueux, M. (2003). Drosophila blood cells. *Cell Microbiology* **5**, 573–580.

Nappi, A. J., Vass, E., Frey, F. & Carton, Y. (1995). Superoxide anion generation in *Drosophila* during melanotic encapsulation of parasites. *European Journal of Cell Biology* **68**, 450–456.

Pardue, M. L. & Gall, J. G. (1975). Nucleic acid hybridization to DNA of cytological preparations. *Methods in Cell Biology* **10**, 1–16.

Poirié, M., Frey, F., Hita, M., Huguet, E., Lemeunier, F., *et al*. (2000). *Drosophila* resistance genes to parasitoids: chromosomal location and linkage analysis. *Proceedings of the Royal Society of London, Series B* **267**, 1417–1421.

Preston, C. R. & Engels, W. R. (1996). P-element-induced male recombination and gene conversion in *Drosophila*. *Genetics* **144**, 1611–1622.

Preston, C. R., Sved, J. A. & Engels, W. R. (1996). Flanking duplications and deletions associated with P-induced male recombination in *Drosophila*. *Genetics* **144**, 1623–1638.

Rogge, R., Green, P. J., Urano, J., Horn-Saban, S., Mlodzik, M., *et al*. (1995). The role of yan in mediating the choice between cell division and differentiation. *Development* **121**, 3947–3958.

Russo, J., Brehelin, M. & Carton, Y. (2001). Haemocyte changes in resistant and susceptible strains of *D. melanogaster* caused by virulent and avirulent strains of the parasitic wasp *Leptopilina boulardi*. *Journal of Insect Physiology* **47**, 167–172.

Siden-Kiamos, I., Saunders, R. D. C., Spanos, L., Majerus, T., Trenear, J., *et al*. (1990). Towards a physical map of the *Drosophila melanogaster* genome: mapping of cosmid clones within defined genomic divisions. *Nucleic Acids Research* **18**, 6261–6270.

Smoller, D. A., Petrov, D. & Hartl, D. L. (1991). Characterization of bacteriophage P1 library containing inserts of Drosophila DNA of 75–100 kilobase pairs. *Chromosoma* **100**, 487–494.

Tootle, T. L., Lee, P. S. & Rebay, I. (2003). CRM1-mediated nuclear export and regulated activity of the Receptor Tyrosine Kinase antagonist YAN require specific interactions with MAE. *Development* **130**, 845–857.

Török, T., Tick, G., Alvarado, M. & Kiss, I. (1993). P-lacW insertional mutagenesis on the second chromosome of *Drosophila melanogaster*: isolation of lethals with different overgrowth phenotypes. *Genetics* **135**, 71–80.

Vass, E., Nappi, A. J. & Carton, Y. (1993). Comparative study of immune competence and host susceptibility in *Drosophila melanogaster* parasitized by *Leptopilina boulardi* and *Asobara tabida*. *Journal of Parasitology* **79**, 106–112.

Vivekanand, P., Tootle, T. L. & Rebay, I. (2004). MAE, a dual regulator of the EGFR signaling pathway, is a target of the Ets transcription factors PNT and YAN. *Mechanisms of Development* **121**, 1469–1479.

Zettervall, C. J., Anderl, I., Williams, M. J., Palmer, R., Kurucz, E., *et al*. (2004). A directed screen for genes involved in Drosophila blood cell activation. *Proceedings of the National Academy of Sciences of the USA* **101**(39), 14 192–14 197.

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