Resistance to plague of Mus spretus SEG/Pas mice requires the combined action of at least four genetic factors

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ORIGINAL ARTICLE

Resistance to plague of *Mus spretus* SEG/Pas mice requires the combined action of at least four genetic factors.

Running title : Genetic architecture of mouse resistance to plague

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ABSTRACT

We have previously described SEG/Pas as the first mouse inbred strain able to survive subcutaneous injection of virulent *Yersinia pestis*, the agent of plague, and we identified *Yprl1*, *Yprl2* and *Yprl3* as three QTLs controlling this exceptional phenotype in females from a backcross between SEG/Pas and C57BL/6 strains. We have now developed congenic strains to further characterize the extent and effect of these genomic regions. In the present study, we confirm the importance of two of these regions, both in males and females, while the third one may well be a spurious association. We show that no genomic region alone is able to increase the survival of C57BL/6 mice but that C57BL/6 mice carrying both *Yprl2* and *Yprl3* exhibit intermediate resistance. Each of these two QTLs contains at least two sub-regions which are required to increase survival. Finally, through the analysis of congenic strains in an F1 background, we establish the mode of inheritance of the SEG-derived resistance alleles. Altogether, this study has clarified and enhanced our understanding of the genetic architecture of resistance to plague in SEG/Pas mice.

Keywords : Plague; mouse; resistance; *Yersinia pestis*; congenic strain

Introduction

Plague is an acute bacterial infection caused by the Gram-negative bacterium *Yersinia pestis*, which can develop in several forms, bubonic, septicaemic, or pulmonary. The rapid multiplication of the bacteria from the entry point and the extremely efficient mechanisms by which *Y. pestis* can escape host innate immune responses are responsible for very high mortality rate in the absence of effective antibiotherapy.[1-3]

However the existence of host genetic factors controlling variable levels of susceptibility is strongly suggested by at least three lines of evidence. First, during past plague pandemics, a fraction of the individuals who developed bubonic plague were able to survive. Second, some mammalian species, such as rodents, cats, camels or monkeys are susceptible, while others, such as dogs, cows and horses are naturally resistant.[4] The third line of evidence was provided by our recent report describing for the first time a mouse inbred strain demonstrating a high level of resistance to virulent strains of *Y. pestis*, while all mouse strains tested so far are susceptible.[5]

Taking advantage of a collection of mouse inbred strains sampling the genetic diversity found in the *Mus* genus, we evaluated their susceptibility to subcutaneous inoculation of 100 colony-forming units (CFU) of the virulent CO92 *Y. pestis* strain. While all classical laboratory strains, including C57BL/6 (B6), as well as strains derived from *M. musculus musculus*, *M. m. domesticus* or *M. m. castaneus* progenitors showed 0 to 23% survival, 75% of the males and 90% of the female mice of the *Mus spretus* derived SEG/Pas (SEG) strain were able to survive *Y. pestis* infection.[5]

Starting from this observation, we developed a forward genetics approach[6] to identify the genetic factors underlying the resistant phenotype. QTL analysis of a large (B6 × SEG)F1 × B6 (BSB) backcross cohort of 322 females detected three genomic regions, on chromosomes 3, 4 and 6, significantly associated with differential survival.[5] In the mixed B6-SEG background of

the backcross population, individuals heterozygous (B6/SEG) for either of the three QTLs showed ~20% increase in survival compared to individuals homozygous for the B6 allele at the same QTL. Interestingly, we demonstrated that the three QTLs, named *Yprl1* to *Yprl3* acted in an additive manner, and that mice heterozygous at the three QTLs showed a survival rate similar to that of SEG mice. Altogether, we concluded that the resistance to *Y. pestis* of SEG female mice was under the control of at least three dominant, SEG-derived, alleles accounting for most of the difference in susceptibility between the two parental strains.[5]

In this report, we used congenic strains for each of the three *Yprl* regions to confirm the existence of these QTL regions, to better describe the genetic architecture of resistance to *Y. pestis* in the mouse, and to refine the confidence intervals of underlying genes. We show that none of the three QTLs alone is able to increase survival in the susceptible B6 background, but that 25% of the males and 42% of the females carrying both *Yprl2* and *Yprl3* survived an inoculation of *Y. pestis*. We further demonstrate that each QTL region contains two distinct sub-regions, and that increased resistance is observed only in mice carrying all four sub-regions. Finally, we show that the (B6 × SEG)F1 background may provide the proper genomic environment to reveal the effect on survival of individual QTLs, as exemplified with *Yprl3*. Altogether, our data provide strong evidence that resistance to plague in SEG mice is conferred by the combined action of at least four genes, with different modes of inheritance.

Results

The resistance locus Yprl3 *is detected in BSB males, but neither* Yprl1 *nor* Yprl2

Males (n=217) from the BSB backcross described previously were inoculated with 100 CFU of *Y. pestis* CO92 and genotyped for the same 721 SNP markers. One hundred and forty mice survived the infection, giving a survival rate of 64%. QTL analysis of survival as a binary trait, revealed only one significant peak above the 0.05 threshold ($p = 1.9\times10^{-5}$; LOD = 4.136; Figure 1). This peak is located at 93Mb on chromosome 6, at a position very close to that of *Yprl3* (95 Mb), with a 95% confidence interval spanning from 73 to 108 Mb. At this position, survival was 48.5% in *B/B* homozygous males, while it rose to 76.7% in *B/S* heterozygotes, a level similar to that of SEG males (Supplementary Figure 1). This 28.2% difference in the survival of BSB males is greater than the 20.4% difference observed in females,[5] suggesting a stronger effect of *Yprl3* QTL in males.

No other peak reached the 0.1 significance level. In particular, no association was found in BSB males between survival and markers on chromosomes 3 and 4, where resistance loci *Yprl1* and *Yprl2* have been mapped in females. The search for interacting loci using the 'scantwo' feature of J/qtl software in BSB males detected no additional loci significantly associated with survival. These results suggest that two of the three *Yprl* QTLs could be female-specific.

Homozygous Yprl3 *congenic male mice showed delayed mortality*

In order to confirm the existence and to characterize the effect of QTLs in a pure inbred background, we developed a series of B6.SEG-*Yprl1*, B6.SEG-*Yprl2* and B6.SEG-*Yprl3* congenic strains by introgressing large regions of chromosomes 3, 4 and 6, encompassing the confidence intervals of *Yprl1* to *3*, from SEG in the susceptible B6 background. Figure 2 shows, for each QTL, the genomic segment transferred in the congenic strains with reference to the

QTL peak and confidence interval. The congenic strains for the QTL regions of chromosome 3, 4 and 6 (*Yprl1*, *2* and *3* respectively) are thereafter designated as cg1, cg2 and cg3, respectively, with the haplotype in superscript. For example, mice heterozygous for the *Yprl1* congenic segment are designated as cg1$^{B/S}$, while homozygotes are named cg1$^{S/S}$.

We produced cg3$^{B/S}$ heterozygous and cg3$^{S/S}$ homozygous male mice and assessed their resistance to plague by comparison with B6 (Figure 3). The survival curves were analyzed by logrank tests, to take into account both the final survival rate and the mortality time. Neither heterozygous cg3$^{B/S}$ nor homozygous cg3$^{S/S}$ males survived at a higher rate than B6, which indicates that the intrinsic effect of this genomic region is too weak to promote survival in a fully susceptible background. However, cg3$^{S/S}$ males died significantly later than both B6 and cg3$^{B/S}$ males (log-rank, $P < 0.0001$). This result suggests that the *Yprl3*^{SEG} resistance allele acts recessively on time to death in the congenic cg3 strain, while it was detected as a dominant survival-associated allele in the BSB population.

None of the Yprl *loci are self-sufficient to increase resistance in congenic females*

Females of the three congenic strains were inoculated with *Y. pestis.* As for males, both cg3$^{B/S}$ and cg3$^{S/S}$ mice were tested. However, we failed to breed cg1 and cg2 congenic mice to homozygosity because of sterility of homozygotes in at least in one sex. This is most likely due to genomic incompatibility reasons which are frequent in B6 × SEG crosses[7]. Thus, only heterozygous cg1$^{B/S}$ and cg2$^{B/S}$ females could be tested.

The survival rate and mortality time of *Y. pestis*-infected cg1$^{B/S}$ and cg2$^{B/S}$ females did not differ from that of B6 females (Figure 4). Moreover, unlike cg3$^{S/S}$ males, cg3$^{S/S}$ females did not show any difference with either B6 or cg3$^{B/S}$ females. These results could alternatively suggest that (1) one or more of these resistance loci was spuriously detected in the QTL analysis of the BSB cross; (2) each QTL alone did not have a strong enough effect to increase resistance

of susceptible B6 mice; (3) the detection of QTLs effects would require a genetic background exhibiting a resistance intermediate between that of the B6 and SEG parental strains. Further crosses were performed to discriminate these hypotheses.

Yprl2 *and* Yprl3 *together confer increased survival in bi-congenic females*

To address the first two hypotheses, we crossed the three congenic strains to produce cg1$^{B/S}$-cg2$^{B/S}$, cg1$^{B/S}$-cg3$^{B/S}$ and cg2$^{B/S}$-cg3$^{B/S}$ bi-congenic females. We also produced cg1$^{B/S}$-cg2$^{B/S}$-cg3$^{B/S}$ tri-congenic females. Groups of mice carrying these compound genotypes were assessed for their resistance to *Y. pestis* (Figure 5A).

First, we observed that 41.6% (10/24) of cg2$^{B/S}$-cg3$^{B/S}$ bi-congenic females survived the infection (versus 1/60 in B6, $P = 6.5 \times 10^{-6}$). This survival rate is much higher than that found in cg2$^{B/S}$ and cg3$^{B/S}$ congenic mice, which demonstrates that both *Yprl2* and *Yprl3* genomic regions contain at least one resistance locus each, and that these two QTL must be present simultaneously to overcome the susceptible B6 background.

On the other hand, neither cg1$^{B/S}$-cg2$^{B/S}$, nor cg1$^{B/S}$-cg3$^{B/S}$ bi-congenic mice were able to resist significantly better than B6 or congenic strains (cg1$^{B/S}$-cg2$^{B/S}$: 2/12 = 16.7%; cg1$^{B/S}$-cg3$^{B/S}$: 0/15 = 0%), which suggests either that the *Yprl1* region does not contain any resistance gene, or that *Yprl1* has a weaker effect compared to *Yprl2* and *Yprl3*.

Despite the difficulties of producing mice heterozygous for three large genomic regions,[7, 8] we produced a small group of tri-congenic mice ($n = 9$). These mice showed a resistance level lower but not significantly different from that of cg2$^{B/S}$-cg3$^{B/S}$ mice (2/9 = 22.2%; $P = 0.43$). Taken together with the cg1$^{B/S}$-cg2$^{B/S}$ and cg1$^{B/S}$-cg3$^{B/S}$ data, these results led us to consider that *Yprl1* could be a spurious QTL and to discard it from further analysis.

Yprl2 *participates in resistance to* Y. pestis *also in males*

We further tested whether *Yprl2* was indeed not involved in male resistance, or whether it had been missed in the analysis of BSB males. To this aim, we infected cg2$^{B/S}$, cg3$^{B/S}$ (same as above, Figure 3) and cg2$^{B/S}$-cg3$^{B/S}$ males. Neither cg3$^{B/S}$ (as previously reported), nor cg2$^{B/S}$ males exhibited increased resistance compared with B6 males (1/18 = 5.5% and 2/51 = 3.9%, respectively; $P = 1$). By contrast, bi-congenic cg2$^{B/S}$-cg3$^{B/S}$ males survived to a higher level than B6 males (11/44 = 25% and 2/51 = 3.9%, respectively; $P = 0.0053$; Figure 5B), indicating that, although no association was found between survival and chromosome 4 markers in BSB males, *Yprl2* was able to increase the effect of *Yprl3*. We concluded that *Yprl2* participates in resistance to *Y. pestis* in both sexes.

Yprl2 *and* Yprl3 *dissection identifies at least four loci required for resistance*

As both cg2$^{B/S}$ and cg3$^{B/S}$ congenic strains carry large genomic regions (approximately 73 and 123 Mb, respectively), we selected recombinants and bred them to produce sub-congenic strains carrying either the proximal or the distal part of *Yprl2* and *Yprl3* (see Figure 2 for detailed composition). The resulting sub-congenics were intercrossed to produce combinations of the four sub-regions. All mice were heterozygous at the sub-regions. Data from males and females were pooled since both sexes gave similar results.

As anticipated from the result of cg2$^{B/S}$ and cg3$^{B/S}$ congenic strains, mice carrying only the proximal or distal region of either QTL did not show any difference from B6 in their susceptibility to *Y. pestis* (data not shown). In both males and females, mice carrying the full region at one QTL and either the proximal or the distal sub-region at the other QTL did not show increased survival (Figure 6). However, cg2.RecP$^{B/S}$-cg3$^{B/S}$ males and females died later than B6 ($P < 0.0001$, Figure 7). We concluded that each of the four sub-regions contains a

resistance locus necessary to confer the survival rate observed in cg2$^{B/S}$-cg3$^{B/S}$ bi-congenic mice.

Yprl3 *increases survival of (B6 × SEG)F1 mice*

Our results from congenic and bi-congenic strains suggested that resistance is dependent upon the combined effect of multiple SEG-derived alleles at the *Yprl2* and *Yprl3* loci. Moreover, in the BSB backcross population, we observed that mice homozygous for the B6 allele at the three QTLs still resist at a higher level than B6 (27%, $P = 0.0017$),[5] suggesting that even a low proportion (25%) of SEG alleles dispersed throughout the genome is able to confer minimal level of resistance to *Y. pestis*. We hypothesized that studying single QTLs on a mixed B6-SEG background could increase the power to detect small effects.

We have previously reported that (B6 × SEG)F1 mice show intermediate resistance between B6 and SEG.[5] This hybrid background is easy to obtain and is very reproducible. Therefore, we set up crosses between heterozygous congenic females and SEG males in order to obtain both *Yprl*$^{B/S}$ heterozygotes and *Yprl*$^{S/S}$ homozygotes on the (B6 × SEG)F1 background.

In a preliminary experiment, we infected SEG and F1 females. Although F1 mice resisted at a lower level than SEG mice, the difference was not statistically significant, which was not a favorable condition to detect an increase in survival possibly conferred by a resistance QTL. The difference was larger in males and we therefore investigated the effect of *Yprl2* and *Yprl3* in the F1 background. F1-cg3 (*Yprl3*$^{S/S}$) males resisted at a much higher level than F1 (*Yprl3*$^{B/S}$) males (11/21 = 52.4% and 6/28 = 21.4%, respectively; $P = 0.035$; Figure 8), which clearly demonstrates that an effect of the *Yprl3* locus on survival could be detected, provided an appropriate genetic background is used. It implies that the *Yprl3*^{SEG} allele is inherited co-dominantly since *Yprl3*$^{S/S}$ and *Yprl3*$^{B/S}$ showed different levels of resistance on the same genetic background. We further tested F1-cg3.RecP$^{S/S}$ mice which are homozygous for the SEG

haplotype in the proximal part of the *Yprl3* QTL, and found that they were much more resistant than F1 males (20/32 = 62.5% and 6/28 = 21.4%, respectively; $P = 0.0018$; Figure 8), and did not differ in survival from F1-cg3$^{S/S}$ mice. This result indicates that this proximal region is sufficient to recapitulate the increased resistance observed in F1-cg3$^{S/S}$ mice. We also tested F1-cg3.RecD$^{S/S}$ which are homozygous for the distal part of the *Yprl3* region. Although only a small number of mice could be produced due to low fertility, F1-cg3.RecD$^{S/S}$ survived to the same level as F1 mice (2/8 = 25% and 6/28 = 21.4%, respectively). These results suggest that the SEG allele at the proximal locus of the *Yprl3* interval is co-dominant (since F1-cg3.RecP$^{S/S}$ survived better than F1), while the SEG allele at the distal locus is fully dominant (since F1-cg3.RecP$^{S/S}$ and F1-cg3$^{S/S}$ survive similarly and F1-cg3.RecD$^{S/S}$ and F1 also survived similarly).

Conversely, we detected no difference in survival between F1-cg2$^{S/S}$ (homozygous for *Yprl2*) and F1 males (6/16 = 37.5% and 6/29 = 20.6%, respectively; $P = 0.29$; Figure 8), which may result from *Yprl2* SEG allele(s) acting in a fully dominant manner.

Discussion

Y. pestis is one of the most virulent bacteria for humans and cause for extremely acute infections. Many rodents, including mouse laboratory strains are highly susceptible to *Y. pestis*.[5, 9, 10] We previously reported that almost all inbred strain tested succumbed in less than a week following experimental subcutaneous inoculation of 100 CFU of a virulent strain such as CO92. The only outstanding exception was the *Mus spretus*-derived SEG/Pas strain which showed high survival rate under these conditions. Through a forward genetics approach, we identified three candidate genomic regions controlling survival in BSB females. In each region, SEG alleles act dominantly to increase survival by 20%, and BSB mice heterozygous for the three regions were found to resist to the same level as SEG mice.[5] In parallel, we explored a number of clinical and immunological parameters in search for pathophysiological mechanisms associated with resistance to plague.[11]

The present study aimed at confirming the existence of these QTLs and at refining our understanding of the genetic architecture of resistance to *Y. pestis* in mice. First, we analyzed males from the same BSB backcross used in our initial study. We found a major QTL at a position very close to that of *Yprl3*, suggesting that *Yprl3* is efficient in both sexes. Notably, its effect on survival was stronger in males than in females (28.2% and 20.4%, respectively). No other suggestive or significant QTL was found in males. In particular, no association was found at the *Yprl1* and *Yprl2* genomic locations. This could have at least three reasons. First, the number of males analyzed in the backcross population was one third smaller than the number of females (217 and 322, respectively), resulting in reduced power for QTL detection. However, if *Yprl1* and *Yprl2* were efficient in males, they would likely show at least weak association with survival, which was not observed. Second, we reported that the difference in survival rate between SEG and B6 was greater in females than in males, which led us analyze only females in the first study. *Yprl1* and *Yprl2* genes could indeed have no effect in males, resulting in the

reduced survival observed in SEG males compared to females. The third hypothesis is that one or both QTLs were spuriously detected in females and not replicated in males. This is plausible as their LOD score was just above the significance threshold.

Congenic strains are used in mouse complex traits to confirm and further characterize QTLs identified by association in segregating crosses.[12] We therefore developed congenic strains by introgressing putative SEG-derived resistance alleles in the susceptible B6 background. Since the confidence intervals observed in the BSB cross were quite large, suggesting the presence of several linked genes, we transferred genomic regions encompassing 73 to 123 Mb. We could obtain homozygous congenic mice only for *Yprl3*, probably because of interspecific allelic incompatibilities which are common in B6 × SEG crosses.[7, 8]

The phenotyping of congenic and bi-congenic strains led to important and unexpected conclusions. First of all, none of the QTLs identified in females was able to increase the very low level of resistance of B6 mice. Although this was surprising considering the 20% increase in survival that each of them was able to induce in BSB mice, this is not an uncommon observation when a late and general phenotype such as death is considered. For example, in their dissection of the genetic control of systemic lupus erythematosus, Morel and colleagues identified four genomic regions associated with the development of glomerulonephritis, a late complication of auto-immunity.[13] When they developed congenic strains on the resistant background, they observed the development of mild kidney lesions in only one of them, while the three other strains remained unaffected.[14]

Our second observation was that cg2$^{B/S}$-cg3$^{B/S}$ bi-congenic mice showed a survival rate approximately half that of SEG mice. Unexpectedly, the same observation was made in males and females, although the *Yprl2* locus had not been identified in BSB males. Congenic strains provide stronger experimental evidence than association studies in segregating populations since genetic composition is controlled and homogeneous between groups and within groups.

Therefore, we can conclude that the previously described *Yprl2* and *Yprl3* are both true QTLs controlling resistance to *Y. pestis*.

On the other hand, congenic strains have failed to provide confirmation of the existence of *Yprl1* identified in BSB females. This locus was associated with the same level of resistance as the other two, with a LOD score just above the genome-wide threshold for significance. It is therefore possible that it was a spurious QTL. In fact, literature reports many examples of QTL described in a segregating population and not replicated in congenic strains.[15] Another hypothesis could be that this locus acts epistatically with another locus. However, we did not detect any such interaction in the analysis of BSB females. Therefore, we decided not to pursue the analysis of *Yprl1*.

Congenic strains are also invaluable in refining the location of QTLs, through the fragmentation of the congenic interval.[16-20] Because the *Yprl2* and *Yprl3* peaks were quite broad, cg2 and cg3 encompass 50 to 80% of the entire chromosome length. As an initial step towards QTL fine mapping, we split each congenic strain in two sub-regions. However, removing any of the four sub-regions abolished the survival observed with $cg2^{B/S}$-$cg3^{B/S}$ bi-congenic mice. This indicates that each of these four regions contains at least one gene critical to induce a detectable level of resistance in the B6 background. Moreover, SEG alleles at these four regions act under a dominant (or semi-dominant) mode of inheritance.

The comparison of the BSB cross with congenic strains, led us to postulate that the genetic background as a whole was crucial in revealing the effect of individual QTLs. Each QTL likely provides a small increase in the capacity to overcome bacterial multiplication and dissemination. QTL effects on survival could be detected in the BSB cross since mice lacking the QTLs (but yet having inherited 25% of their genome from SEG) survived at an intermediate rate of 30%.[5] Under these conditions, even mild QTL effects on resistance mechanisms could induce a detectable variation of survival rate. Conversely, on a pure, highly susceptible B6

background, the small increase in resistance controlled by QTLs would have no detectable impact on the ability to survive. This led us to compare mice differing for the QTLs, in an F1 background. This experiment was set up by crossing congenic females with SEG males. We could not use this approach in females because of the high resistance level of F1s. It was successful in males with *Yprl3*. Our results suggest that such crosses between a congenic strain and its donor strain could be very helpful in unraveling the effect of QTL regions which are missed in the recipient background.

Assessing the mode of inheritance of QTLs is important as an indication of their mode of action. According to the BSB data (in which mice are either *B/B* or *B/S*), *Yprl2* and *Yprl3* must be inherited dominantly or semi-dominantly. Due to the difficulties inherent to interspecific crosses, cg3 is the only congenic strain that could be bred to homozygosity. In males, we showed that, although they did not survive better than B6 and cg3$^{B/S}$ mice, cg3$^{S/S}$ mice died later, which suggests of a semi-dominant mode of inheritance. Moreover, in F1 crosses, we found that F1-cg3.RecP$^{S/S}$ mice, which are homozygous for the SEG haplotype in the proximal part of the *Yprl3* region, did not differ in survival from F1-cg3$^{S/S}$ mice and survived better than F1 mice. Altogether, these results suggest that SEG alleles are co-dominant at the proximal locus of *Yprl3* and dominant at the distal locus. Conversely the absence of difference between F1-cg2$^{S/S}$ and F1 males suggests that SEG alleles at both regions of *Yprl2* are fully dominant.

In conclusion, this study has clarified and enhanced our understanding of the genetic architecture of resistance to plague in SEG/Pas mice. We have definitely confirmed the existence of two complex genomic regions, acting in both males and females. Although neither of them is able by itself to promote survival, mice carrying both regions resist at an intermediate level. These two QTLs encompass at least four genes. For three of them, the dominant inheritance of SEG alleles suggests that they are gain-of-function variants. The last one is semi-dominantly inherited and could be a quantitative variant. There must be other resistance genes

since *Yprl2* and *3* together fail to provide the same resistance level as SEG. Finally, the use of a hybrid background exhibiting an intermediate level of resistance was capable of revealing *Yprl3* effect on survival and will be instrumental in the fine mapping of the cg3.RecP region.

The identification of the genes underlying the effects of the four chromosomal regions will require a combination of approaches including the fine genetic mapping of each region, the identification of more subtle and earlier phenotypes than death to compare the progression of the disease in congenic and sub-congenic strains, and whole-genome expression studies to detect pathways that are differentially regulated in response to *Y. pestis* exposure.

Materials and methods

Mice and crosses. The SEG/Pas strain was developed as previously described[21] and is maintained at the Institut Pasteur. C57BL/6 (B6) mice were purchased at 6 weeks of age from Charles River Laboratory (L'Arbresle, France). They were maintained for at least three weeks in the Institut Pasteur animal facilities prior to inoculation. Male B6×(SEG×B6)F1 backcross mice (BSB) were part of the same cross described in our previous study.[5]

Congenic strains were developed by backcrossing 10 times SEG onto B6. Specifically, SEG males were mated with B6 females. F1 females were then backcrossed to B6 males. To overcome hybrid sterility which affect 7/8 backcross males,[7] the resulting BC1 (or N2) females that had retained either of the QTL regions (see genotyping below) were mated with B6 males. This cross was repeated once more. For further generation, males heterozygous for the genomic regions of interest were crossed with B6 females. After 10 generations of backcrossing (N10), attempts were made to establish homozygous congenic strains. However, most congenic strains had to be maintained by continuously backcrossing heterozygous carriers. To facilitate reading, a simplified nomenclature is used throughout the article. B6.SEG-*Yprl1* to B6.SEG-*Yprl3* are referred to as cg1 to cg3. The haplotype is added in superscript (e.g. $cg1^{B/S}$ describes a mouse heterozygous for the congenic segment).

Sub-congenic strains were produced by selecting recombinants from the progeny of N10 (or more) congenic mice. RecP and RecD are used to designate the sub-congenic strains carrying the proximal and the distal region of the original congenic region, respectively (e.g. cg2.RecD).

Bi-congenic individuals were obtained by intercrossing heterozygous mice of congenic strains (e.g. $cg1^{B/S}$ and $cg2^{B/S}$). Tri-congenic individuals were obtained by intercrossing $cg1^{B/S}$ males with $cg2^{B/S}$-$cg3^{B/S}$ bi-congenic females. Mice homozygous for *Yprl2* (or *Yprl3*) on a

(B6 × SEG)F1 background were produced by crossing SEG males with heterozygous cg2$^{B/S}$ (or cg3$^{B/S}$) females and were named F1-cg2$^{S/S}$ (or F1-cg3$^{S/S}$).

Notably, the continuous backcrossing of congenic strains to B6 (beyond 10 generations), and the genotypic selection of bi- and tri-congenic mice in the segregating progeny of crosses between congenic strains precluded the potential effect of residual SEG alleles.

All animal experiments were approved and conducted in accordance with the Institut Pasteur Biosafety Committee, and in compliance with French and European regulation on protection of animals used for scientific purposes. Mice were kept in isolators after inoculation. They were given food and water *ad libitum* and were kept at 22±2°C with alternating 12h periods of light and dark.

Culture of *Yersinia pestis* strains and experimental infections of mice. Experimental infections were performed using the virulent *Y. pestis* strain CO92.[22] Cultures were carried out at 28°C for 48h on LB agar medium supplemented with 0.002% (W/v) hemin (LBH). Bacteria were collected and suspended in saline. Bacterial concentration was estimated by spectrometry at 600 nm (1 O.D. ~10^9 CFU/ml) and confirmed by colony-forming units (CFU) counts on LBH agar plates. Mice were injected s.c. between 8 and 12 weeks of age, in the ventral region, with a 100 µl inoculum, and were then monitored daily for 14 days. For practical reasons, congenic strains were tested over multiple infection experiments. To control for consistency and allow merging data, B6 and SEG control mice ($n \geq 5$) were included in every experiment.

Genotyping. For congenic strains, mice were genotyped using DNA prepared from tail biopsies collected at weaning age. Microsatellite markers were genotyped according to standard PCR protocols, and using 4% agarose gels. DNA preparation and genotyping of BSB mice was previously described.[5] The markers used for genotyping congenic mice are listed in Supplementary Table 1. The interval between neighbor markers varied between 3 and 40 Mb

(~2-20 cM), which precluded missing double crossing-overs since interference is known to encompass 30-40 cM in the mouse.[23]

Statistical and QTL analysis. Survival rates were compared by Fisher's exact test and survival curves were compared by a logrank (Mantel-Cox) test using StatView 5.0 (SAS Institute Inc, Cary NC) or Prism 4.0 statistical software (GraphPad, La Jolla CA). QTL analysis was performed by using the J/qtl software version 1.3.1. The survival rate was analyzed as a binary trait. Significance thresholds of LOD scores were estimated by 1,000 permutations of experimental data, and were 2.77 and 2.43 for 5% and 10% thresholds, respectively. Ninety-five percent confidence intervals were estimated in J/qtl using the Bayesian Credible Interval function.

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Supplementary information is available at Genes and Immunity's website.

Conflict of interest

The authors declare no conflict of interest.

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FIGURE LEGENDS

Figure 1 QTL mapping of survival to a *Y. pestis* challenge in 218 BSB males, showing a single significant QTL on chromosome 6 (*Yprl3*). Dashed lines denote thresholds of significance ($P = 0.1$ and $P = 0.05$, permutation test).

Figure 2 LOD score curves illustrating the position of each QTL peak (vertical dashed line), the 95% confidence interval (vertical lines), and the genomic segment transferred in congenic and sub-congenic strains (black boxes) on (A) chromosome 3 (females), (B) chromosome 4 (females) and (C) chromosome 6 (upper graph: females; lower graph: males). Hatched boxes depict the undefined boundaries of the congenic regions inherited from SEG. Positions are indicated in Mb.

Figure 3 Survival over a 14-day period of B6 ($n = 49$), heterozygous cg3$^{B/S}$ ($n = 29$) and homozygous cg3$^{S/S}$ ($n = 31$) male mice following s.c. inoculation of 10^2 CFU of strain CO92 *Y. pestis*. Mortality of cg3$^{S/S}$ males was delayed compared to B6 and cg3$^{B/S}$ males (log-rank, $P < 0.0001$).

Figure 4 Survival curves of B6 ($n = 59$), heterozygous cg1$^{B/S}$ ($n = 15$), cg2$^{B/S}$ ($n = 18$) and cg3$^{B/S}$ ($n = 23$) females and homozygous cg3$^{S/S}$ females ($n = 10$) to s.c. inoculation of 10^2 CFU. None of the congenic strains was found to be more resistant than B6.

Figure 5 Survival curves of bi- and tri-congenic females and congenic and bi-congenic males. (A) Females: B6 ($n = 50$), heterozygous bi-congenic cg1$^{B/S}$-cg2$^{B/S}$ ($n = 12$), cg1$^{B/S}$-cg3$^{B/S}$ ($n = 15$) and cg2$^{B/S}$-cg3$^{B/S}$ ($n = 24$) and heterozygous tri-congenic females cg1$^{B/S}$-

cg2$^{B/S}$-cg3$^{B/S}$ (n = 9). cg1$^{B/S}$-cg2$^{B/S}$ and cg1$^{B/S}$-cg3$^{B/S}$ were not different from B6, while the survival rate of cg2$^{B/S}$-cg3$^{B/S}$ (10/24 = 41.6%) was significantly higher than that of B6 (1/60 = 1.6%, P =6.5×10^{-6}). cg1$^{B/S}$-cg2$^{B/S}$-cg3$^{B/S}$ mice did not survive in higher percentage than B6 (2/9 = 22.2% and 1/60 = 1.6%, respectively; P = 0.059), but died significantly later (log-rank, P = 0.0057). cg2$^{B/S}$-cg3$^{B/S}$ and cg1$^{B/S}$-cg2$^{B/S}$-cg3$^{B/S}$ did not significantly differ in their survival rate. (B) Males: B6 (n = 51), heterozygous cg2$^{B/S}$ (n = 18), cg3$^{B/S}$ (n = 29, same as on Figure 3) and heterozygous bi-congenic cg2$^{B/S}$-cg3$^{B/S}$ (n = 44). Survival rate of cg2$^{B/S}$-cg3$^{B/S}$ (11/44 = 25.0%) was higher than that of B6 and cg3$^{B/S}$ (2/51 = 3.9%, P = 0.0053 and 1/18 = 5.5%, P = 0.0019, respectively).

Figure 6 Survival rate of male and female B6 (n = 110), cg2$^{B/S}$ (n = 36), cg3$^{B/S}$ (n = 55), sub-congenics of cg2 and cg3: cg2.RecP$^{B/S}$-cg3$^{B/S}$ (n = 33), cg2.RecD$^{B/S}$-cg3$^{B/S}$ (n = 39), cg2$^{B/S}$-cg3.RecP$^{B/S}$ (n = 33) and cg2$^{B/S}$-cg3.RecD$^{B/S}$ (n = 31), cg2$^{B/S}$-cg3$^{B/S}$ (n = 68) and SEG (n = 122). The top table gives the genotype of each group for the proximal and distal interval of *Yprl2* and *Yprl3*. White box: *B/B*; hatched box: *B/S*; black box: *S/S*. The graph shows the survival rate of each group (with error bars indicating one standard deviation). Only cg2$^{B/S}$-cg3$^{B/S}$ mice survived at a significantly higher rate than B6 (21/68 = 30.8% and 3/110 = 2.7%, respectively; P = 1.32×10^{-7}).

Figure 7 Survival curves of males and females sub-congenic for *Yprl2* and *Yprl3*. (A) Survival curves of females: B6 (n = 59), cg2.RecP$^{B/S}$-cg3$^{B/S}$ (n = 20), cg2.RecD$^{B/S}$-cg3$^{B/S}$ (n = 21), cg2$^{B/S}$-cg3.RecP$^{B/S}$ (n = 18), cg2$^{B/S}$-cg3.RecD$^{B/S}$ (n = 13) and cg2$^{B/S}$-cg3$^{B/S}$ (n = 24). Only cg2$^{B/S}$-cg3$^{B/S}$ survived to a significantly higher rate than B6 (10/24 = 41.6% and 1/60 = 1.6%, respectively; P = 6.5×10^{-6}). Females cg2.RecP$^{B/S}$-cg3$^{B/S}$ died significantly later than B6 (P < 0.0001). (B) Survival curves of males: B6 (n = 51), cg2.RecP$^{B/S}$-cg3$^{B/S}$ (n = 13),

cg2.RecD$^{B/S}$-cg3$^{B/S}$ (n = 18), cg2$^{B/S}$-cg3.RecP$^{B/S}$ (n = 15), cg2$^{B/S}$-cg3.RecD$^{B/S}$ (n = 18) and cg2$^{B/S}$-cg3$^{B/S}$ (n = 44). Only cg2$^{B/S}$-cg3$^{B/S}$ survived to a significantly higher rate than B6 (11/44 = 25.0% and 2/51 = 3.9%, respectively; P = 0.0053). Males cg2.RecP$^{B/S}$-cg3$^{B/S}$ died significantly later than B6, (log-rank, $P < 0.0001$).

Figure 8 Survival curves of male F1 (n = 28), F1-cg2$^{S/S}$ (n = 16), F1-cg3$^{S/S}$ (n = 21) and F1-cg3.RecP$^{S/S}$ (n = 32) mice. F1-cg3$^{S/S}$ and F1-cg3.RecP$^{S/S}$ mice survived at a higher rate than F1 (11/21 = 52.4%, 20/32 = 62.5% and 6/28 = 21.4%, respectively; P = 0.035 and P = 0.0018, respectively). The difference between F1-cg2$^{S/S}$ and F1 was not significant.

Supplementary Figure 1 Survival rate of B6, BSB and SEG males according to their haplotype at *Yprl3* and their genetic background. The top table gives the genotype of each group at *Yprl3* and the genetic background. White box: B/B; hatched box: B/S; black box: S/S. Split boxes depict loci for which fully genotyped BSB animals were either B/B or B/S. The graph shows the survival rate (with error bars indicating one standard deviation) of each group. The difference between the two left groups reflects the effect of unidentified QTLs. BSB mice heterozygous for *Yprl3* show the same survival rate as the SEG strain.

Supplementary Table 1 Microsatellites markers used for selection of congenic mice. Positions are given in megabases.

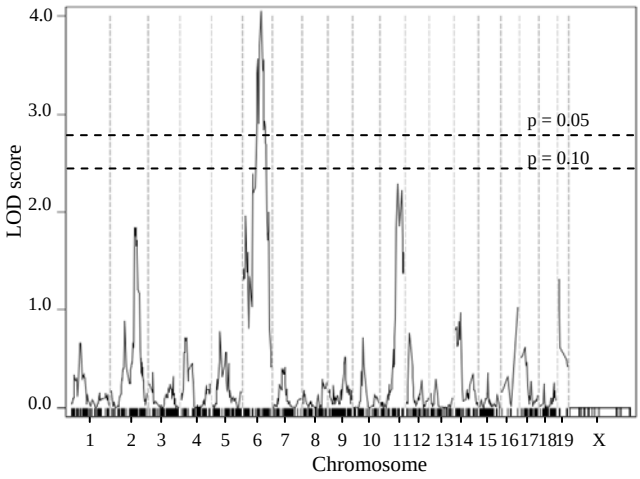

4.0
3.0
2.0
1.0
0.0
LOD score
p = 0.05
p = 0.10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X
Chromosome

Figure 1.

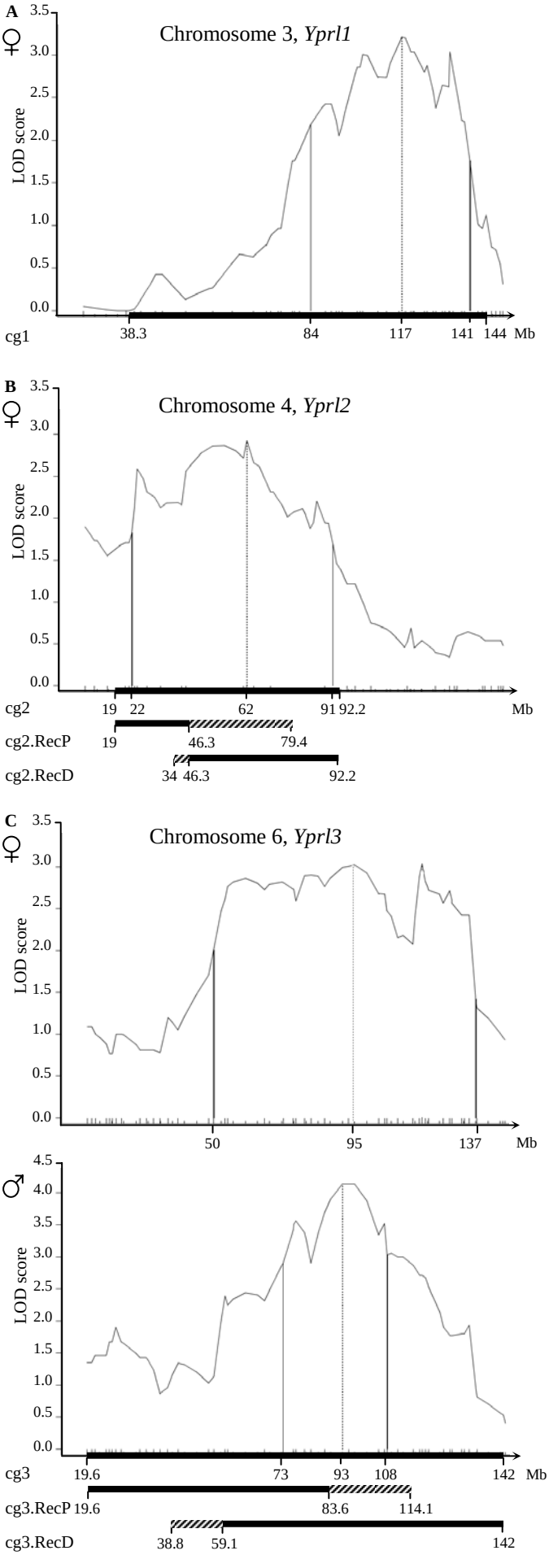

A
Chromosome 3, Yprl1
LOD score
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
cg1
38.3
84
117
141
144
Mb
B
Chromosome 4, Yprl2
LOD score
cg2
19
22
62
91
92.2
Mb
cg2.RecP
19
46.3
79.4
cg2.RecD
34
46.3
92.2
C
Chromosome 6, Yprl3
LOD score
50
95
137
Mb
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
cg3
19.6
73
93
108
142
Mb
cg3.RecP
19.6
83.6
114.1
cg3.RecD
38.8
59.1
142

Figure 2.

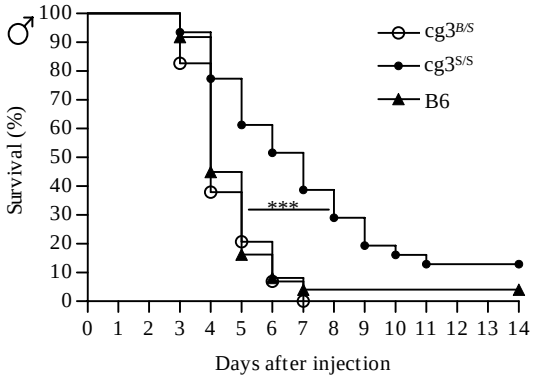

♂
100
90
80
70
60
50
40
30
20
10
0
Survival (%)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days after injection
***
cg3B/S
cg3S/S
B6

Figure 3.

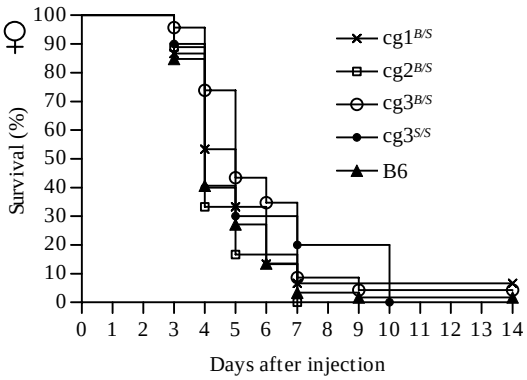

♀
Survival (%)
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days after injection
cg1B/S
cg2B/S
cg3B/S
cg3S/S
B6

Figure 4.

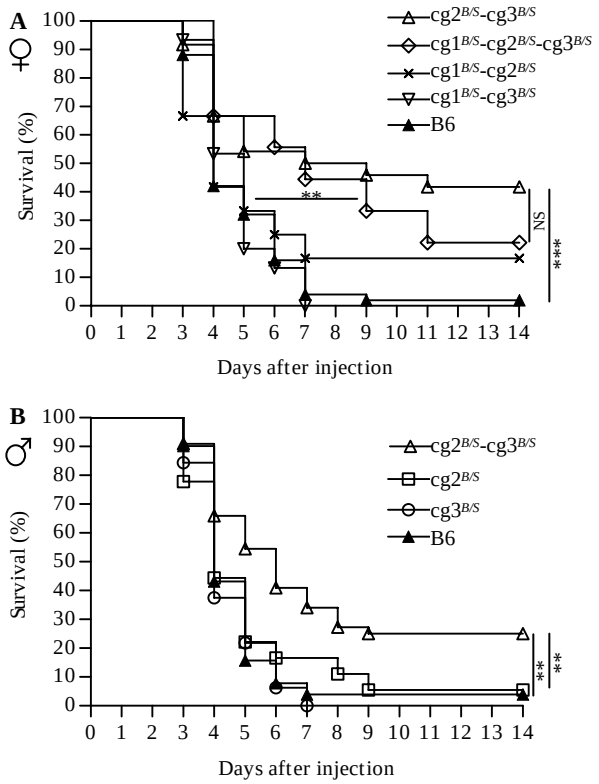

A
♀
Survival (%)
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days after injection
cg2B/S-cg3B/S
cg1B/S-cg2B/S-cg3B/S
cg1B/S-cg2B/S
cg1B/S-cg3B/S
B6
**
NS
***
B
♂
Survival (%)
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days after injection
cg2B/S-cg3B/S
cg2B/S
cg3B/S
B6
**
**

Figure 5.

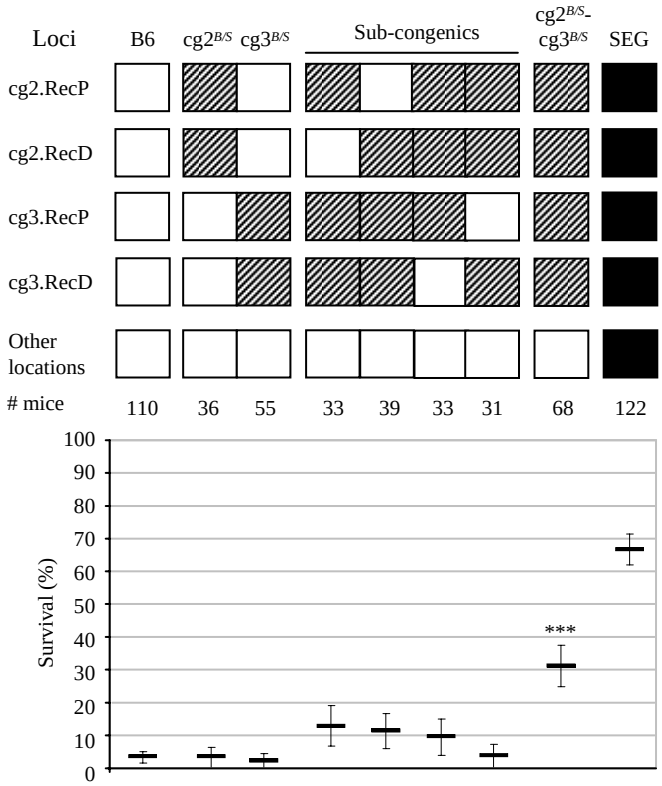

Loci
B6
cg2B/S
cg3B/S
Sub-congenics
cg2B/S-cg3B/S
SEG
cg2.RecP
cg2.RecD
cg3.RecP
cg3.RecD
Other locations
mice
110
36
55
33
39
33
31
68
122
Survival (%)
100
90
80
70
60
50
40
30
20
10
0
***

Figure 6.

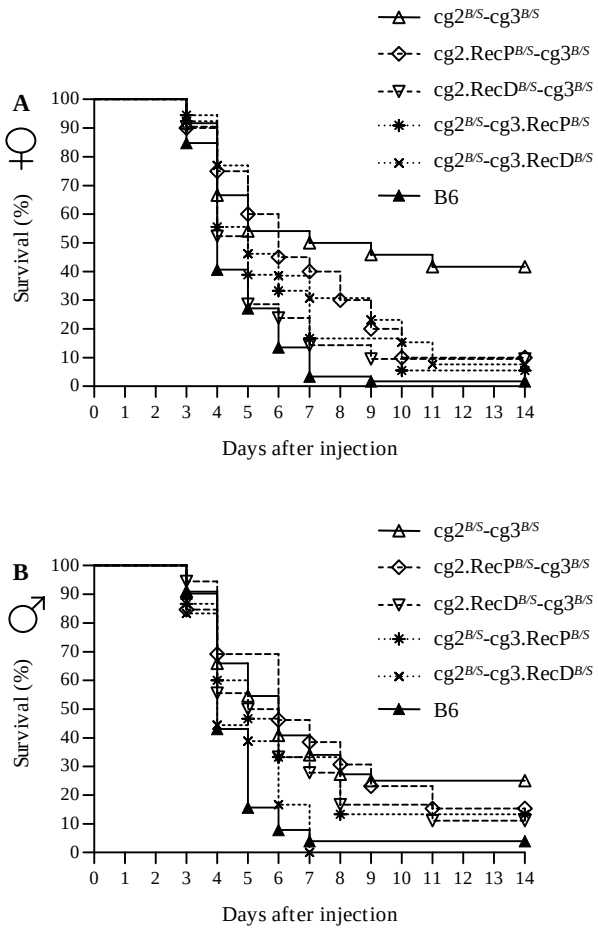

A
♀
Survival (%)
Days after injection
cg2B/S-cg3B/S
cg2.RecPB/S-cg3B/S
cg2.RecDB/S-cg3B/S
cg2B/S-cg3.RecPB/S
cg2B/S-cg3.RecDB/S
B6
B
♂
Survival (%)
Days after injection
cg2B/S-cg3B/S
cg2.RecPB/S-cg3B/S
cg2.RecDB/S-cg3B/S
cg2B/S-cg3.RecPB/S
cg2B/S-cg3.RecDB/S
B6

Figure 7.

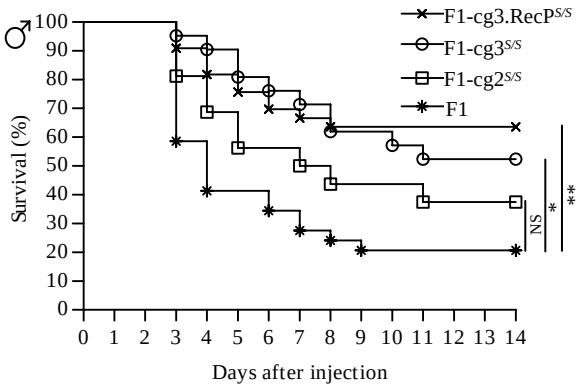
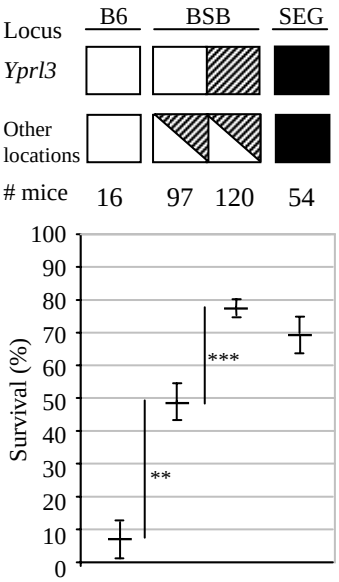

♂
Survival (%)
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days after injection
F1-cg3.RecP S/S
F1-cg3 S/S
F1-cg2 S/S
F1
NS
*
**

Figure 8.



Supplementary Figure 1.

Chromosome	*QTL*	*Marker name*	*Position (Mb)*
3	*Yprl1*	*D3Mit169*	38.0
		D3Mit242	79.4
		D3Mit107	114.1
		D3Mit14	131.6
		D3Mit197	143.9
4	*Yprl2*	*D4Mit260*	18.9
		D4Mit93	33.9
		D4Mit89	46.3
		D4Mit114	79.6
		D4Mit301	88.7
		D4Mit144	92.2
6	*Yprl3*	*D6Mit346*	19.6
		D6Mit222	38.9
		D6Mit184	53.2
		D6Mit8	83.6
		D6Mit383	114.0
		D6Mit254	125.3
		D6Mit194	128.1
		D6Mit293	142.0

Supplementary Table 1.