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QTL MAPPING FOR *CAENORHABDITIS ELEGANS* SURVIVORSHIP IN RESPONSE TO *ESCHERICHIA COLI* AND *STENOTROPHOMONAS MALTOPHILIA*

by

ZIYI WANG

B.Sc.(Agr.), China Agricultural University, 2006

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Approved by:

Major Professor
Dr. Michael A. Herman

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ZIYI WANG

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Abstract

Caenorhabditis elegans are free-living bacterivorous nematodes that naturally consume bacteria as food source. As an excellent genetic model, *C. elegans* has proven to be a successful system to study innate immune responses to human pathogens, which resulted in identification of many evolutionarily conserved defense pathways. Most of these studies examined innate immune pathway mutants in a single genetic background in response to monoculture of human pathogens that worms might not necessarily encounter in the wild. While this has led to the successful genetic dissection of these defense pathways, in order to fully understand their biological functions, the relevant ecological and evolutionary context needs to be taken into account. The bacterial environment *C. elegans* naturally encounter is likely to be highly heterogeneous. While many bacteria are mainly considered as dietary resource for worms, some could be potential pathogens. Worms thus constantly face the challenge to defend against the pathogens mixed in the food. *Stenotrophomonas maltophilia* is one such bacterium. *S. maltophilia* is a ubiquitous bacterium that has been found associated with native nematodes. But it can also cause nosocomial infections in human, especially in immune-compromised individuals. Due to its natural resistance to multiple antibiotics, it has been emerging as an opportunistic human pathogen. Our lab isolated a *S. maltophilia* strain, JCMS, which was found being pathogenic to *C. elegans*. Both *C. elegans* strains, N2 (Bristol, England) and CB4856 (Hawaii), showed decreased survivorship when fed on *S. maltophilia* JCMS compared to *E. coli* OP50. However, more interestingly, the specific responses towards bacteria are different between strains. This indicates that survivorship of *C. elegans* is determined by not only genetic and environmental factors, but also genotype by environment (G×E) interactions (GEI). In order to identify the underlying genetic basis, we mapped quantitative trait loci (QTL) in a N2×CB4856 recombinant inbred panel for the survivorship in response to *E. coli* OP50 and *S. maltophilia* JCMS.

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Introduction

Caenorhabditis elegans are free-living bacterivorous nematodes that naturally consume bacteria as food. It has proven to be an excellent system to study innate immune responses to human pathogens, which resulted in successful identification and genetic dissection of many evolutionarily conserved defense pathways (reviewed by Kim and Ewbank, 2015). The PMK-1 p38 MAPK (mitogen-activated protein kinase) pathway, for example, was found to be required for defense to many bacterial pathogens, such as *Pseudomonas aeruginosa* (Kim *et al*., 2002), *Salmonella enterica* (Aballay *et al*., 2003), *Bacillus thuringiensis* (Huffman *et al*., 2004), and many others (Montalvo-Katz *et al*., 2013). Another conserved pathway, DBL-1/TGF-Beta signaling was shown to contribute to immune response toward *Serratia marcescens* (Mallo *et al*., 2002; Mochii *et al*., 1999), and later many other pathogens (reviewed by Gumienny and Savage-Dunn, 2013). The DAF-2/DAF-16 insulin signaling pathway, which had long been found to be involved in dauer development and longevity, was later found also involved in antibacterial defense (Garsin *et al.,* 2003*).* However, these studies almost always examined defense pathway mutants developed in a single genetic background with monoculture of human pathogens that worms may not necessarily encounter in the wild. In contrast to the success of genetic dissection of these pathways, our understanding about ecology of *C. elegans* and natural bacteria associated with *C. elegans* is still relatively limited. Without the knowledge of the ecological and evolutionary context, the significance of the biological roles of these pathways for survival of *C. elegans* in the wild would be difficult to comprehend.

Recent efforts to characterize its natural habitats indicate that instead of a soil nematode thought by many for long, *C. elegans* are colonizers of bacteria-rich habitats, especially those sites associated with human activity, such as compost heaps, and on rotten stems and fruits at orchards and gardens (Félix and Braendle, 2010). In addition, *C. elegans* were found in the wild predominantly as dauers, an alternative diapause developmental stage, and being associated with diverse invertebrate dispersal vectors such as snails, isopods, and myriapods (Barrière and Félix, 2005). Thus dauer larvae, being more resistant to environmental stress, were thought to be a dispersal morph (Baird *et al*., 1994; Baird, 1999; Kiontke, 2002). Proliferating populations, however, were evident, especially in decaying vegetation (Barrière and Félix, 2005). Yet, these

do not seem like sustainable sites that can support the population growth in the long run. After all, resources are finite and will eventually become exhausted. Thus, it has been since suggested that *C. elegans* lives a 'boom-and-bust' lifestyle, where the population thrives rapidly when the resource is ample and once the food sources become scarce and limited, worms develop into dauers and disperse by hitchhiking on invertebrate carriers in order to relocate and seek for new resource (Félix and Braendle, 2010).

Clearly, the resource of bacterial food supply would heavily affect *C. elegans* survival in the wild. Given that the composition of these bacteria is heterogeneous, where the diversity and abundance might be subject to temporal and spacial variation, it is highly likely that despite potential nutritional values, some of these natural bacteria could also be pathogenic to *C. elegans*. One such potential pathogen is *Stenotrophomonas maltophilia*, a ubiquitous bacterium that has been isolated in association with nematodes *Pristionchus pacificus* (Rae *et al*., 2008) but also found from natural environment of *C. elegans* (B Samuel and MA Félix, personal communication). The local isolate, *S. maltophilia* JCMS, significantly reduces lifespan of *C. elegans* in comparison to those feeding on standard lab food *E. coli* OP50, indicating a possible pathogenic role. Surprisingly, *daf-2* mutants that are otherwise long-lived on any other bacteria tested to date show similar reduced lifespan as wildtype animals (White *et al*., 2016). More interestingly, we found *C. elegans* strains N2 (Bristol, England) and CB4856 (Hawaii) respond to *E. coli* OP50 and *S. maltophilia* JCMS differently and observed a significant genotype by environment (G×E) interaction (GEI), which has been thought to be a major mechanism that maintains genetic variation in natural populations.

Genetic variation is the basic material for evolution. Although natural selection works at the level of phenotype, the underlying genetic variation is required because evolution only occurs when heritable variation is present. Genetic variation can be identified by directly sequencing the gene of interest involved in the biological process or by a mapping approach. One mapping approach, Quantitative Trait Loci (QTL) mapping has been carried out in *C. elegans* to study a variety of complex traits from longevity, to gene expression and behavior (Ayyadevara *et al*., 2001; Li *et al*., 2006; McGrath *et al*., 2009; and reviewed by Gaertner and Phillips, 2010). Several recent studies have been particularly successful to pinpoint polymorphisms in causal

genes for the variation of behavioral traits that possibly facilitate *C. elegans* adaptation to bacteria (Reddy *et al*., 2009; Chang *et al*., 2011; Bendesky *et al*., 2011).

The *C. elegans* – bacteria system is an excellent model to study the ecological and the evolutionary impact of host-microbe interactions. To identify the genetic basis underlying variation of survivorship in response to bacteria, we chose to take a quantitative genetic approach to map QTL in a N2×CB4856 recombinant inbred panel for survivorship in response to *E. coli* OP50 and *S. maltophilia* JCMS. This approach provides us with an unbiased tool to identify the responsible genomic regions that are not restrained to any presumption of the functions of the possible candidate genes.

Materials and Methods

Nematode strains and maintenance

All *C. elegans* strains were obtained from Kammenga lab (Wageningen University, Wagningen, The Netherlands) unless otherwise specified, where N2 (Bristol, England) and CB4856 (Hawaii), were originally obtained from Caenorhabditis Genetics Center (CGC). Upon arrival, all strains were allowed to propagate and frozen (Stiernagle, 2006). Worms using for experiments were freshly thawed from frozen stock and maintained under standard conditions (Brenner 1974; Sulston and Hodgkin 1988).

Recombinant Inbred Lines (RILs) were originally described in Li *et al.* (2006). Briefly, the RILs were generated by crossing N2 and CB4856, followed by inbreeding for 20 generations. Among approximately 1500 RILs, 80, referred as the core set, were chosen randomly and genotyped with 121 SNP makers evenly distributed across the genome (Li *et al*., 2006); another 120 randomly chosen RILs were genotyped with 96 SNP markers. From these 200 RILs, 154 strains were used in the QTL mapping experiments. Eighty strains were from Li *et al*. (2006), 19 strains from Elvin *et al*. (2011), 16 strains from Rodriguez *et al*. (2012) and additional 39 strains for this study.

Introgression lines (ILs) were developed by back-crossing a subset of RILs with N2 followed by selfing and genotyping as described in Doroszuk *et al.* (2009). In this study, 36 ILs, each with a unique single homozygous introgression segment of CB4856 in an N2 genomic background, on chromosome I (ewIR01-ewIR18) and IV (ewIR45 – ewIR63 w/o ewIR57) were used for the further confirmation of QTL mapping.

Bacteria strains and cultures

Escherichia coli strain OP50 and *Stenotrophomonas maltophilia* strain JCMS were used to feed *C. elegans* in this study. Pure bacterial culture is maintained by isolation streak on an LB agar plate.

E. coli OP50 was obtained from lab stock that was originally obtained from CGC. *S. maltophilia* JCMS was isolated from a mixed culture of natural bacteria associated with native nematode of *Mesorhabditis sp.* initially isolated from a soil sample of Konza Prairie, near Manhattan, Kansas. Nonetheless, it is possible that *S. maltophilia* JCMS is not truly associated with native nematode but only presents ubiquitously in soil or even could be a laboratory contamination introduced later during the process of isolation (White *et al.* 2016). However, *S. maltophilia* are often found or co-isolated from the natural environment of *C. elegans* (B Samuel and MA Félix, personal communication), thus it is safe to say at least chance is high for *C. elegans* to encounter *S. maltophilia* in its natural habitat.

Survivorship Measurement

All strains were age-synchronized by bleaching (Emmons *et al*., 1979), during the process of which, the isolated eggs were suspended in 1mL fresh M9 buffer and allowed to hatch overnight in 20°C incubator on a rotator. Newly hatched age-synchronized L1 larvae were then transferred to an NGM plate seeded with OP50 and allowed to grow to young adults. Ten young adults from each strain were picked onto experimental plate seeded with each bacterium respectively in triplicate.

Worms were kept at 25°C during the experiment except when survivorship was monitored daily by counting at room temperature. Worms that did not response to gentle touch by a platinum wire were considered to be dead. Carcasses of dead worms were removed upon discovery. Worms that burrowed into agar, or dehydrated due to crawling onto the plastic wall of the petri dish or displayed unnatural sickness such as bagging or germline protrusion and etc. were removed from experiment upon discovery. Note that the day on which the experiment was initiated was day 1 instead of day 0. For example, worms that were scored with a survivorship of 7 days had been exposed to bacteria for 6 days.

Experimental plates were prepared by spotting each 5cm NGM plate with 100µl *E. coli* OP50 or *S. maltophilia* JCMS liquid culture that was freshly grown overnight (12-16 hours) in a 30°C shaker from a single colony inoculation into liquid LB media, respectively. The plates were left for 16-24 hours in room temperature for the bacterial culture to grow. FUDR (5-Fluoro-2'-

deoxyuridine, SIGMA-AUDRICH®) treatment was used to eliminate the germline, making the worms sterile, which allowed us to more easily follow survivorship simultaneously in multiple strains without the interference from progeny. Briefly, 110µl of 10mg/ml FUDR solution was sterilized by filtration (WhatmanTM PuradiscTM 25mm 0.2µm polyethersulfone membrane) and applied around seeded bacterial spot in the center of the 5cm plate containing 11ml of NGM agar (final FUDR concentration 0.1mg/ml) and allowed to soak in for at least 12 hours before used for experiment.

QTL mapping

QTL mapping for survivorship was performed using model six for Composite Interval Mapping in WinQTLCart2.5 (Wang S., C. J. Basten, and Z.-B. Zeng (2012). Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm). Specifically, forward and backward regression method was implemented to control for genetic background, with probabilities for selecting into and out of the model as 0.1, respectively. Window size was set as default of 10cM to block a genomic region between nearby markers for testing site. Walking speed was set as 1cM. Threshold was generated by 1000 permutations on phenotypic data for significance level < 0.05 for each trait.

In total, 154 RILs were examined for survivorship in response to two different bacteria, *E. coli* OP50 and *S. maltophilia* JCMS, respectively. The overall mean of all individual animals was used as trait value for a specific RIL strain to map QTL, for *C. elegans* survivorship on *E. coli* OP50 and *S. maltophilia* JCMS, respectively. Differential survivorship was calculated by subtracting the average survivorship on *S. maltophilia* JCMS from that on *E. coli* OP50 and was used as trait value, which is intended to identify the genetic structure underlying G×E interactions. For feasible experimental measurement of survivorship, RIL strains were examined as several independent batches on different dates, thus a batch effect should be accounted. Briefly, we set "other trait" as "date of experiment" in the QTL mapping model, which means that date was used as a categorical co-factor for the regression analysis of the quantitative trait, where the batch effect was "regressed out" and the residuals were used for the actually analysis. Polymorphic markers and genetic map were as described in Li *et al*., 2006.

Quantitative genetic analysis with ILs

Survivorship of ILs was measured on each bacteria, *E. coli* OP50 and *S. maltophilia* JCMS, as described above. Two sets of 18 introgression lines (ILs) from chromosome I and IV, respectively, along with N2 and CB4856, were examined, in two rounds of experiments. Strain, round, and the interaction effects were determined by analysis of variance for the whole data set of ILs for a specific chromosome. A step-wise approach was applied to assess individual strain effect. First, for each individual IL vs. N2 comparison, analysis of variance with a full model (survivorship = strain + round + strain × round) was performed to determine the significant terms. Significant round and/or interaction term(s) then were incorporated into the otherwise simple generalized linear model (survivorship = strain) to assess the individual strain effect. It was then followed by a second step of multiple comparison correction for P-value by the "BY" (Benjamini and Yekutieli, 2001) method to control family-wise Type-I error rate. This can be done as all the ILs on the same chromosome were examined with both parental lines in the same set of experiments. BY-adjusted P-value p0.05 was used as threshold for significant detection.

Examination of G×E interaction in ILs

To further dissect the QTL of differential survivorship, ILs were examined individually by comparison with N2. Analysis of variance was performed for each pairwise comparison by model: survivorship = strain + bacterium + strain × bacterium + rnd. The significant "strain × bacterium" term was interpreted as a significant G×E interaction. Reaction norms of the ILs showing significant GEI were plotted to facilitate the visualization of specific source of GEI.

Statistical Analysis

Broad sense heritability (BSH, denoted as H^2) for simple survivorship trait was calculated by $H^2 = V_G / V_P$, where V_G was the variance among 154 RILs and V_P was the total phenotypic variation as sum of within and among line variation.

Additive allelic effect was calculated by [μ(N2) – μ(CB4856)]/2, where μ(N2) was the mean phenotypic value of all RILs that carry N2 allele at a specific genomic position, while μ(CB4856) was the mean phenotypic value of all RILs that carry CB4856 allele. RIL strains that were missing genotype data were excluded from the calculation.

Results

Survivorship in the response to bacterial diet reveals variation in a *C. elegans* mapping population

Survivorship in response to *E. coli* OP50 and *S. maltophilia* JCMS, respectively, was measured for 154 Recombinant Inbred Lines (RILs) as well as two parental strains, N2 (Bristol, England) and CB4856 (Hawaii). There was substantial variation in survivorship among the RILs, regardless of bacterial food source. When fed on *E. coli* OP50, survivorship among RIL strains spanned over 9.48 days, ranging from 9.96 days (WN37) to 19.44 days (WN11), with 13.15 days as the median (Figure 1A). For response to *S. maltophilia* JCMS, survivorship among RILs spanned over 7.38 days, ranging from 5.39 days (WN96) to 12.77 days (WN73), with 8.43 days as the median (Figure 1B). CB4856 outlived N2 on both bacteria (Figure 1). The difference in survivorship between N2 and CB4856 was significant when fed on *E. coli* OP50 (N2 = 13.57±1.07 days, CB4856 = 15.67±1.06 days, $t = 2.7385$, $df = 34$, $p=0.0098$, Figure 1A) and only marginally significant when fed on *S. maltophilia* JCMS (N2 = 8.66±1.02 days, CB4856 = 10.04±0.95 days, $t = 1.9522$, $df = 34$, $p = 0.0592$, Figure 1B). The range of variation among RILs was much wider than that between two parental strains. However, the phenotypic values of all 154 RILs fell in between the range of two standard deviations of parental means, thus there was no evidence of transgressive segregation, although it is a common phenomenon observed in hybrid segregating populations (Rieseberg *et al*., 2003).

The majority of the RILs displayed reduced survivorship as compared to the two parental strains on both bacteria. Yet median survivorships of RILs are very close to N2, regardless of the bacterial food. In response to *E. coli* OP50, 45 RILs displayed significantly decreased survivorship as compared to N2, the parent with lower survivorship, while there were only five RILs that displayed significantly increased survivorship than CB4856, the parent with higher survivorship (Figure 1A). In response to *S. maltophilia* JCMS, 51 strains displayed significantly decreased survivorship as compared to N2 while eight strains displayed significantly increased survivorship as compared to CB4856 (Figure 1B).

In addition, there was a significant strain effect for survivorship on each bacterium (F = 20.323, p-value < 2.2e-16 for survivorship on *E. coli* OP50; and F = 20.5655, p-value < 2.2e-16 for survivorship on *S. maltophilia* JCMS; one-way ANOVA, not assuming equal variances). Broad sense heritability is calculated by $H^2 = V_G / V_P$, where V_G is the among line variance, V_P is the total variance calculated as the sum of variances of among lines and within line. For survivorship on *E. coli* OP50, the broad sense heritability is 34% whereas for survivorship on *S. maltophilia* JCMS, it is 37%. These results demonstrate that the variation for survivorship in response to bacterial diet has a strong genetic basis and is highly heritable.

QTL mapping for *C. elegans* survivorship on *E. coli* OP50

Three significant QTL for *C. elegans* survivorship in response to *E. coli* OP50 were detected, *SE1*, *SE2* and *SE3* (Figure 2, Table 1). All three QTL are autosomal with *SE1* mapping on chromosome I, and *SE2* and *SE3* on chromosome IV. We did not detect epistasis between these QTL.

SE1 peaked at marker C1M17 with a LOD score of 7.6 on the right arm of the chromosome I. The 95% confidence interval (CI) approximated by 1-LOD reduction from the maximum LOD value of the QTL peak (Lander and Botstein, 1989), gives roughly a 1.5Mb (6.2cM) genomic interval that contains 401 genes. The additive effect, calculated as (N2-CB4856)/2, was -0.7. Thus for this QTL, the Hawaiian allele increases survivorship in response to *E. coli* OP50 by approximately 1.4 days (Table 1).

The QTL *SE2* and *SE3* mapped close to each other on chromosome IV and were joined into a large region that was above the significance threshold. *SE2* peaked on C4M3 with a LOD score of 6.05 and *SE3* peaked on C4M6 with a LOD score of 3.98. The confidence intervals of these two QTL, however, can be separated by using 1-LOD reduction method (Table 1). *SE2* had a roughly 0.75Mb (8.7cM) CI that contains 135 genes while *SE3* had a 1.6Mb (3.3cM) CI that contains 1390 genes. Clusters of piRNAs/21U-RNAs genes are responsible for the large number of genes within *SE3* interval. 21U-RNAs crowd two broad regions of chromosome IV (Ruby *et al*., 2006) and count for more than 900 genes within the *SE3* confidence interval. The contracted map distance for *SE3* could be due to reduced recombination rate in the center of the

chromosome, which has been observed in many mapping crosses (Rockman and Kruglyak, 2009). For both QTL, the Hawaiian allele reduced the survivorship on *E. coli* OP50 by roughly 1.2 and 1 days respectively (Table 1).

Examination of ILs for *C. elegans* survivorship on *E. coli* OP50

Two sets of 18 introgression lines (ILs) from chromosome I and IV, respectively, were used to confirm and further dissect the defined QTL. Each IL carries a specific and distinct introgression of CB4856 genomic region in an otherwise N2 genomic background. Each set of 18 ILs along with N2 were examined for survivorship in response to *E. coli* OP50, respectively, for two independent rounds of experiments.

Analysis of variance indicated that there were significant strain and round effects, as well as the interaction between the two, in each dataset (Table 2). Therefore, we took a step-wise approach to assess the strain effect. Briefly, each individual IL was initially compared with N2 in a strain specific generalized linear model that contains specific significant terms for that strain. It was then followed by multiple comparison correction for P-value by "BY" method to control family-wise Type-I error rate.

Six of the chromosome I IL strains showed significantly different survivorship from N2. Four strains, ewIR10, ewIR11, ewIR12 and ewIR16 showed significantly increased survivorship that was consistent with the QTL mapping results. The first three strains contain introgressions, respectively, which cover the entire *SE1* QTL whereas ewIR16 partially overlaps but may not necessarily cover the entire *SE1* interval. Among these four strains, survivorship of ewIR12 and ewIR16 was comparable to CB4856 ($p = 0.9689$, $p = 0.6041$, two-tailed t-test) while survivorship of ewIR10 and ewIR11 was significantly higher than CB44856 ($p = 0.0319$, $p = 0.0133$, two-tailed t-test). In addition, survivorship of ewIR14 and ewIR17 was significantly decreased in comparison to N2 (Figure 3A). These results confirm the *SE1* QTL and indicate the possibility of the existence of additional QTL.

Among the 18 Chromosome IV ILs, survivorship of eight strains was significantly decreased as compared to N2 (Figure 3B). Five out of these eight strains (ewIR48, ewIR49, ewIR51, ewIR52

and ewIR53) contain introgressions that cover completely or partially cover the *SE2* and *SE3* QTL intervals. The remaining three IL strains harbor introgressions that do not overlap with either *SE2* or *SE3*, indicating presence of additional QTL.

QTL mapping for *C. elegans* survivorship on *S. maltophilia* JCMS

Two significant QTL for *C. elegans* survivorship in response to *S. maltophilia* JCMS were detected, *SS1*and *SS2* (Figure 2, Table 1). Both QTL mapped to chromosome I, but on opposite arms. *SS1* peaked slightly to the right of C1M2 (-17.28cM) with a LOD score 6.8. The 95% confidence interval approximated by 1-LOD reduction from the maximum LOD value of the QTL peak (Lander and Botstein, 1989) resulted in a roughly 1.6Mb (5.1cM) genomic interval that contains 248 genes. *SS2* mapped on the right arm of the chromosome I and peaked slightly to the left of marker C1M18 (17.46) with a LOD score of 7.6. The 95% confidence interval of *SS2* spanned over a 2.4Mb (12.7cM) region that contains 591 genes. In addition, *SS2* partially overlaps *SE1*. No epistatic interaction between *SS1* and *SS2* was detected.

Additive effects for both QTL were negative, -0.7 for *SS1* and -0.6 for *SS2* respectively. Thus for both QTL, Hawaiian allele increases survivorship in response to *S. maltophilia* JCMS, which is consistent with the observation that CB4856 displayed higher survivorship than N2 when fed on *S. maltophilia* JCMS (Table 1).

Examination of ILs for *C. elegans* survivorship on *S. maltophilia* JCMS

Both sets of ILs, of chromosome I and IV respectively, were examined for survivorship in response to *S. maltophilia* JCMS. Due to significant effects for strain, round of experiment, and the interaction between the two, for both data sets (Table 3), the step-wise approach described previously was applied to assess the individual strain effect in comparison to N2.
Among the chromosome I ILs, ewIR01 carries introgression that overlaps with *SS1*. However, while survivorship of ewIR01 was significantly different from N2, it displayed an opposite allelic effect from *SS1*. Specifically, the QTL *SS1* suggests that the CB4856 allele would increase survivorship, whereas ewIR01 displayed decreased survivorship. For *SS2*, ewIR11 and ewIR12

contain the QTL interval in the introgressions respectively, and showed significantly increased survivorship. Yet several other ILs that also contain the *SS2* region did not show any significant effect. In addition, there were 10 ILs that displayed significantly decreased survivorship as compared to N2, which cannot be explained by any previously identified QTL. Therefore, this suggests the presence of additional QTL, yet their specific locations cannot be pinpointed by the current results (Figure 4A).

Despite that we did not detect QTL in response to *S. maltophilia* JCMS on chromosome IV in the previous mapping experiments with RILs, we still examined *C. elegans* survivorship in response to *S. maltophilia* JCMS in the set of chromosome IV ILs as it may potentially identify novel QTL. Among this set of ILs, seven strains displayed significantly decreased survivorship as compared to N2. Based on the common segment approach, two QTL were localized approximately to the interval between C4M3 and C4M4, and the region between C4M15 and C4M16, respectively (Figure 4B).

QTL mapping for differential survivorship between *E. coli* OP50 and *S. maltophilia* JCMS

Analysis of variance was performed on all 154 RILs for the survivorships in response to *E. coli* OP50 and *S. maltophilia* JCMS. Variation in survivorship was significantly affected not only by bacteria and strains but also there showed significant strain by bacteria interactions (Table 4). Reaction norms of all 154 RILs survivorships in response to both bacteria were plotted, which revealed a large GEI (Figure 5). There was clearly a bacterial effect, where *S. maltophilia* JCMS significantly reduced *C. elegans* survivorship in comparison to *E. coli* OP50. However, the degree of reduction varied from strain to stain (Figure 5). We then calculated differential survivorship for each strain by subtracting the survivorship on *S. maltophilia* JCMS from that on *E. coli* OP50, and used this as a trait value for each RIL strain to map QTL.

Two significant QTL for differential survivorship of *C. elegans* were detected, *DS1* and *DS2* (Figure 6, Table 5). *DS1* mapped on the left end of chromosome I and peaked at CIM2 (-17.28) with a LOD score of 5.4. *DS1* peaked on top of *SS1*. Its 95% confidence interval by 1-LOD

reduction gives an interval covering the entire genomic region left to 1.3Mb (14.7cM) on chromosome I, which contains 228 genes. *DS2* mapped to chromosome IV, and peaked at C4M3 (-8.5cM) with a LOD score of 2.6. The 95% confidence interval by 1-LOD reduction covers a 1.12Mb (6.6cM) region with 233 genes that is largely overlapped with and slightly narrower than *SE2* (Table 5).

Additive effects for *DS1* and *DS2* are 0.8 and 0.65, respectively. This suggests differential survivorship, i.e. the reduction or survivorship between *E. coli* OP50 and *S. maltophilia* JCMS, in strains carrying N2 alleles (1.6 days) is greater than those carrying CB4856 alleles (1.3 days) (Table 5).

Examination of ILs for G×E Interactions

Chromosome I and IV ILs were examined by individual pairwise comparison with N2 for survivorship in response to both bacteria, *E. coli* OP50 and *S. maltophilia* JCMS, respectively. Analysis of variance was performed to determine the significance of G×E, i.e. strain by bacteria, with model: survivorship = strain + bacteria + strain × bacteria + round. Reaction norms were plotted to visualize specific source of GEI for each strain.

For chromosome I ILs, 11 out of 18 strains showed significant GEI (Figure 7A). Among these 11 strains, 10 strains, ewIR01, ewIR03, ewIR04, ewIR05, ewIR07, ewIR08, ewIR09, ewIR10, ewIR13 and ewIR18, showed specific significant effect to one bacterium but not the other except for only ewIR11 showed significant difference from N2 in response to both bacteria (Figures 3A, 4A, 7A). Most of the GEI in those strains was due to significant difference in survivorships in response to *S. maltophilia* JCMS, except for ewIR10 that is due to the opposite (Figure 7B). ewIR11 was the only strain where the survivorship was significantly increased as compared with N2 for both bacteria, yet the differential survivorship was significantly larger in ewIR11 than in N2.

For chromosome IV ILs, seven strains showed significant GEI (Figure 8A). Among these seven strains, ewIR45, ewIR55 and ewIR56 showed significant effect to *E. coli* OP50 but not *S.*

maltophilia JCMS (Figures 3B, 4B and 7); ewIR54 showed significant effect to *S. maltophilia* JCMS but not *E. coli* OP50. Interestingly, while neither ewIR58 nor ewIR62 showed an effect on either bacterium (Figures 3B and 4B), they both displayed significant GEI and showed an opposite effect on both bacteria, respectively (Figure 8B). The only strain that displayed significant effect on both bacteria is ewIR51, where the reduction of survivorship between two bacteria for ewIR51 was significantly greater than that of N2 (Figure 8B).

Discussion

Phenotypic variation is one of the prerequisites for evolution by natural selection. According to Darwin, natural selection requires three necessary and sufficient conditions to act, namely variation in traits, differential fitness conferred by the traits, and heritability of the traits. While the latter two require proper analyses to infer and identify, phenotypic variation is easily observed in the nature even for untrained eyes. For survivorship in response to bacteria, we observed significant difference between two *C. elegans* parental lines, N2 and CB4856 yet only limited variation in response to *S. maltophilia* JCMS. Despite the limited phenotypic variation in two parental lines, we observed expanded variation in the hybrid segregation population, i.e. RILs, developed from the two parents and successfully mapped QTL for both traits. This suggests the variation of the traits being examined could be constrained by the genetic background and interaction with other genes. Once being shuffled and uncoupled by hybrid segregation, the phenotypic effect of the underlying genetic variation could become more distinct. Thus, significant trait differences in parental lines is not necessarily always a prerequisite for a successful QTL mapping, which has been proved an effective approach to identify the heritable genetic basis underlying such variable traits.

While many QTL have been previously reported for a variety of *C. elegans* traits, due to the specific experimental details and the nature of sensitivity to experimental conditions, it is usually difficult to directly compare such results. However, we found that *SE1* located very close to a previously identified longevity QTL on chromosome I by mapping using Bergerac-BO × RC301 recombinant inbred progeny (Ayyadevara *et al*., 2001) as well as CL2a × Bergerac-BO recombinant-inbred population (Ayyadevara *et al*., 2003). These earlier experiments were carried out at 20°C by an extreme phenotype selection design in survival liquid media with (Ayyadevara *et al*., 2001) and without (Ayyadevara *et al*., 2003) FUDR treatment. Our experiment design used bacterial seeded NGM agar plates with FUDR treatment and was carried out at 25°C. These results infer it is likely that there is a robust QTL in vicinity of this region controlling the variation of survivorship in response to *E. coli* OP50, regardless of the experimental temperature and design. According to WormBase release WS244, CB4856 has a natural deletion

(WBVar02121104) of 20kb near this region which might contribute to the observed QTL. Further investigation is needed however to pinpoint the causal genetic variation.

For the allelic effect, according to QTL mapping results, for *SE1*, the CB4856 allele increases survivorship and for *SE2* and *SE3*, the CB4856 allele decreases survivorship. Results with ILs are consistent with RILs results. For *SS1* and *SS2*, according to QTL mapping results, CB4856 allele increases survivorship. Two ILs, ewIR11 and ewIR12 that cover the QTL region of *SS2* also showed increased survivorship thus confirmed the QTL results. However, the majority of the remaining chromosome I ILs that carry distinct introgression regions and ewIR01 that covers *SS1* region, significantly decreased survivorship. We suspect that the flipped allelic effect of *SS1* could be due to a genetic background effect or interactions with other undetected genetic components. Additional repeats of the experiments may be needed to confirm this possibility.

When mapping with ILs for survivorship in response to both bacteria, we observed strains that carry introgressions distinct from the QTL region showed significant effects, which indicates the existence of QTL in addition to those identified from RIL mapping. For example, no QTL for survivorship in response to *S. maltophilia* JCMS was mapped on chromosome IV. Yet when we examined chromosome IV ILs, seven strains displayed significant effects in responsive to *S. maltophilia* JCMS. Four of these strains also showed significant response to *E. coli* OP50. IL strain ewIR51, which significantly reduced survivorship on both bacteria, has been found previously to carry a major QTL that is responsive for maternal hatching rate (Snoek *et al*., 2014; Stastna *et al*., 2015). It is likely that this QTL on chromosome IV is responsive for survivorship on both bacteria and indicates a possible link at the genetic level of survivorship and reproduction.

Overall, IL mapping results showed an elevated statistical power in detection of QTL in comparison to QTL mapping with RILs. This is likely due to a controlled genetic background effect. In addition, in our 154 RILs, besides the core set 80 RILs, the rest of RILs contain varying degrees of missing genotypic data that could also affect the power of detection for QTL mapping. Finally, the significant round effect could be a possible explanation for the

inconsistency in our ILs results, suggesting that survivorship is highly sensitive to environmental conditions.

Phenotypic plasticity refers to changing phenotype of an organism in response to different environments. Specifically, phenotypic plasticity is observed when the phenotypic variation of a single genotype is explained by environmental differences. In this study, the difference in survivorship in response to different bacterial food manifests the phenotypic plasticity. G×E interaction or GEI occurs when the direction and/or magnitude of such plastic response varies among different genotypes. Specifically, GEI is manifested as significant difference in survivorship responsive to *E. coli* OP50 as compared to *S. maltophilia* JCMS, among different strain of RILs. It is generally thought that environment-dependent alleles underlie phenotypic plasticity and G×E interaction. GEI can be detected when environment/treatment specific QTL are identified in mapping under multiple environments. This type of QTL study usually involves mapping simple effect QTL traits under multiple environments followed by additional statistical analysis to identify GEI. In this study, the QTL mapping results for simple effect survivorship in response to single bacterial food, either *E. coli* OP50 or *S. maltophilia* JCMS, suggest that *SS1*, *SE2* and *SE3* show GEI for that they are bacteria specific QTL (Figure 2). However, we adopted an alternative approach where we used a simple QTL model to directly map differential survivorship between two different bacterial environments and successfully identified environment specific QTL. *DS1* mapped on top of *SS1* and *DS2* on top of *SE2*. These results are expected and consistent with the environment-specific alleles as the underlying genetic basis for GEI.

Additive effect for *DS1* suggests the differential survivorship between two bacteria for N2 alleles is greater than CB4856 alleles, which translates into a steeper slope for strain carry N2 allele vs. strain with CB4856 allele in a reaction norm (Figure 7B). The inconsistency in the ILs results in comparison to QTL mapping with RILs is mainly due to the flipped allelic effect for *SS1*. For *DS2*, the results are consistent between RILs and ILs results and with the RILs and ILs results of simple effect QTL *SE2*.

For each identified QTL region, there are hundreds of genes within the interval, many of which do not have any previously characterized function or no previously shown functions directly involved in pathogen defense or general innate immunity. Further investigation is needed to examine genetic variation in these genes in order to identify and pinpoint the causal polymorphism.

In summary, our results demonstrate survivorship of *C. elegans* in response to bacteria has a strong genetic basis and is highly heritable. The variation in survivorship is determined by both genetic and environmental factors as well as genotype by environment interaction. We successfully mapped three QTL for survivorship in response to *E. coli* OP50 and two QTL for that in response to *S. maltophilia* JCMS. We also mapped two QTL in response to differential survivorship between two bacteria and demonstrated the effectiveness of this QTL approach by using computed trait value to detect treatment specific QTL and simplify the detection of GEI.

Figures and Tables

Figure 1. Variation of survivorship among RILs.

Survivorship of 154 RILs in response to A: *E. coli* OP50 and B: *S. maltophilia* JCMS. X-axis: RILs. Strains are ordered in ascending trait values from left to right. Y-axis: Survivorship (Lifespan, LSP) in days. Each gray dot represents a trait value of an individual animal with the colored dot as the mean survivorship of a specific recombinant inbred line. Red dashed horizontal line indicates the mean survivorship of N2 (13.57 days for *E. coli* OP50, 8.66 days for *S. maltophilia* JCMS), and green solid line the mean survivorship of CB4856 (15.67 days for *E. coli* OP50, 10.04 days for *S. maltophilia* JCMS). Error bars show the standard errors.

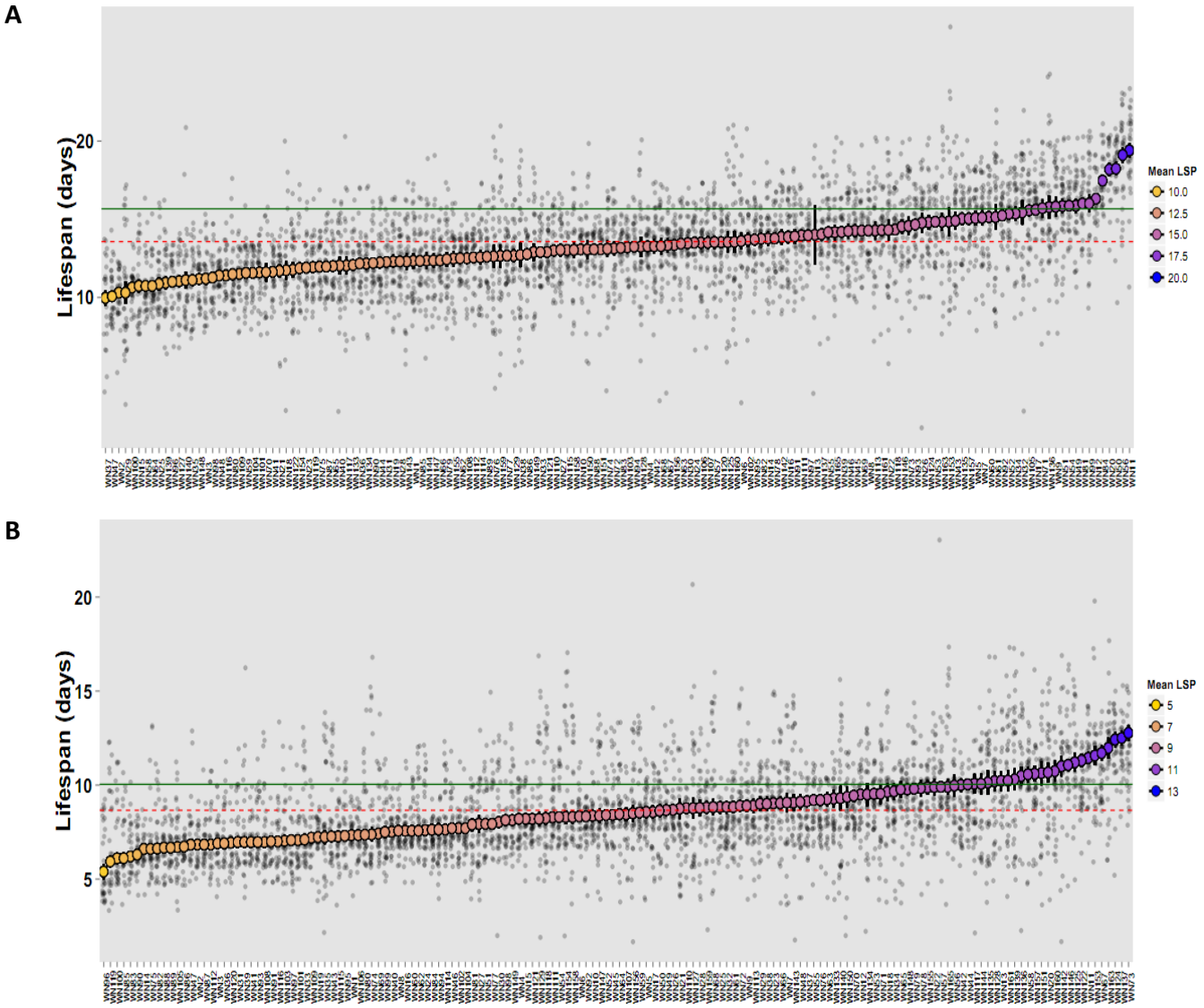


Figure 2. QTL mapping for survivorship in response to *E. coli* OP50 and *S. maltophilia* JCMS.

QTL was performed by WinQTLCart CIM Model 6, forward and backward regression method with probabilities for selecting into and out of the model as 0.1 respectively. Horizontal lines are for trait specific thresholds determined by 1000 permutations for a significance level of 0.05. For survivorship on *E. coli* OP50 (solid red line), LOD=2.5; for survivorship on *S. maltophilia* JCMS (dashed green line), LOD=2.6. Triangles along the x axis indicate the marker positions. Lower panel shows the corresponding additive effects.

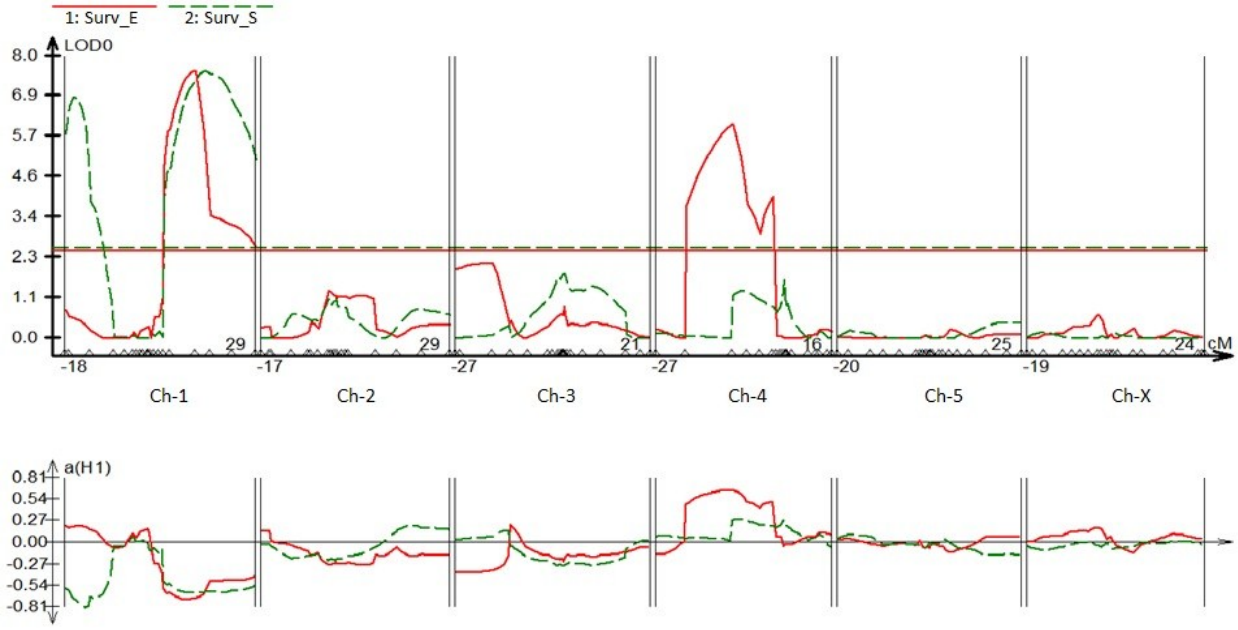
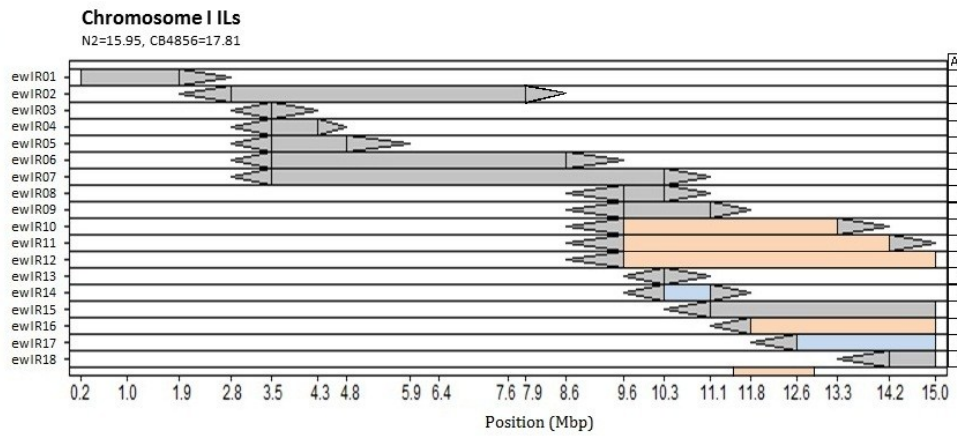


Figure 3. Examination of ILs for survivorship in response to *E. coli* OP50.

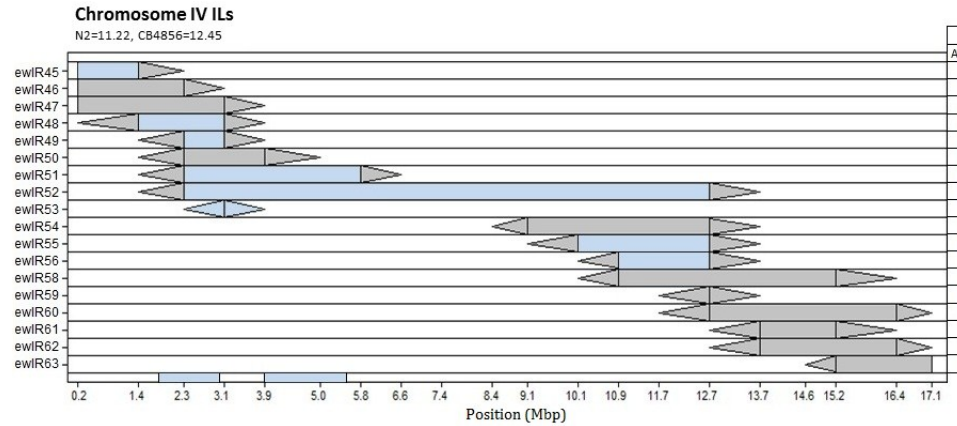
A. Chromosome I ILs; B. Chromosome IV ILs. Gray blocks indicate specific position of the introgression of each IL. Arrows at the ends indicate uncertain break point between two adjacent markers. Pink indicates significant positive CB4856 allelic effect; blue indicates significantly negative CB4856 allelic effect. Cells filled with gray indicate that a term is significant. QTL identified by RILs analysis are indicated at the bottom. X-axis illustrates the physical position of the markers.

A



	significant terms					
AvrSurvE	strain	rnd	strain:rnd	p	p.adjust
15.45				0.17600	1.00000
15.22				0.08450	0.59067
15.80				0.74000	1.00000
16.33				0.35500	1.00000
15.67				0.50000	1.00000
15.07				0.01860	0.16717
16.42				0.24000	1.00000
16.53				0.17100	1.00000
16.28				0.32500	1.00000
13.70				5.41E-10	1.70E-08
13.93				3.83E-12	2.41E-10
17.76				0.00019	0.00239
15.55				0.32800	1.00000
14.73				7.06E-07	1.48E-05
17.17				0.02410	0.18952
17.38				0.00403	0.04226
14.72				0.00052	0.00654
15.78				0.67100	1.00000

B

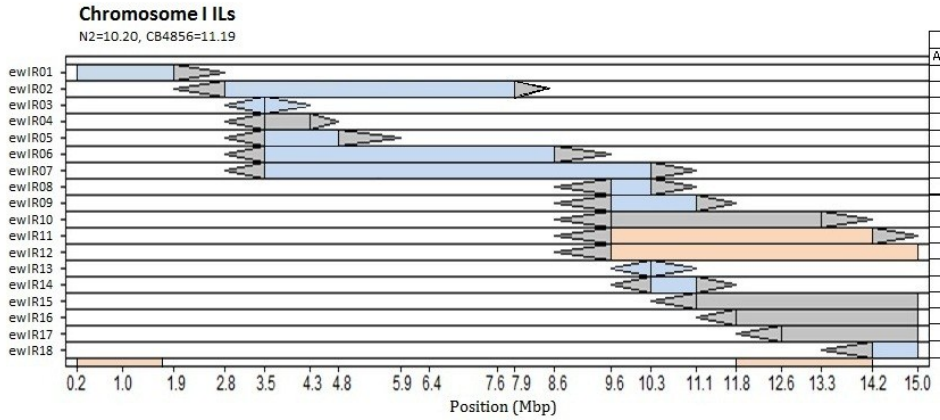


	significant terms				
AvrSurvE	strain	rnd	strain:rnd	p	p.adjust
9.28				9.00E-09	5.66E-07
10.48				0.02240	0.14092
10.73				0.10900	0.57145
10.28				0.00134	0.01686
10.42				0.00345	0.03101
10.12				0.94258	1.00000
8.58				0.00436	0.03429
9.85				0.00031	0.00650
10.20				0.00110	0.01686
11.35				0.74400	1.00000
9.68				8.08E-06	2.54E-04
10.12				0.00190	0.01992
10.47				0.01491	0.10422
11.05				0.58312	1.00000
11.48				0.39400	1.00000
11.57				0.34051	1.00000
11.75				0.07022	0.40163
10.87				0.23920	1.00000

Figure 4. Examination of ILs for survivorship in response to *S. maltophilia* JCMS.

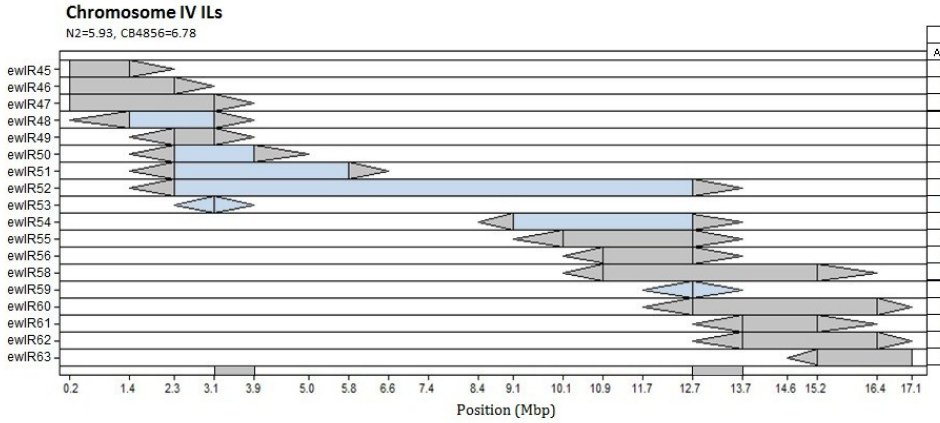
A. Chromosome I ILs; B. Chromosome IV ILs. Gray blocks indicate specific position of the introgression of each IL. Arrows at the ends indicate uncertain break point between two adjacent markers. Pink indicates significant positive CB4856 allelic effect; blue indicates significantly negative CB4856 allelic effect. Cells filled with gray indicate that a term is significant. QTL identified by RILs analysis are indicated at the bottom. X-axis illustrates the physical position of the markers.

A



	significant terms				
AvrSurvS	strain	rnd	strain:rnd	p	p.adjust
8.43				1.02E-07	8.02E-07
8.63				1.32E-07	9.23E-07
7.98				1.37E-14	8.62E-13
9.53				0.0856	0.33658
8.08				1.78E-08	2.96E-07
8.15				3.77E-08	3.95E-07
8.13				7.41E-08	6.66E-07
7.42				4.13E-11	1.30E-09
8.97				0.00472	0.02284
10.33				0.50650	1.00000
11.73				5.27E-04	2.76E-03
11.83				0.00052	0.00276
8.10				8.00E-07	5.03E-06
8.05				2.35E-08	2.96E-07
10.45				0.03370	0.15144
10.33				0.75900	1.00000
9.36				0.05100	0.21390
8.63				2.35E-08	2.96E-07

B



	significant terms				
AvrSurvS	strain	rnd	strain:rnd	p	p.adjust
5.53				0.01120	0.07046
5.92				0.04119	0.21595
5.92				0.90700	1.00000
4.92				3.65E-09	7.65E-08
5.48				0.00878	0.06137
4.71				4.36E-15	2.74E-13
4.75				1.00E-14	3.15E-13
5.12				2.15E-07	2.71E-06
5.08				3.42E-03	0.03074
5.17				8.93E-09	1.40E-07
5.58				0.02960	0.16929
6.20				0.34811	1.00000
6.50				0.00841	0.06137
5.28				6.08E-06	6.38E-05
5.93				0.05179	0.25063
5.77				0.15115	0.63394
5.53				0.11300	0.50779
6.12				0.37821	1.00000

Figure 5. G×E Interactions among RILs.

Reaction norms of 154 RILs were plotted for visualization of GEI. X axis indicates bacterial food. Y axis is survivorship in days. Green thick line represents CB4856, and red dashed line represents N2.

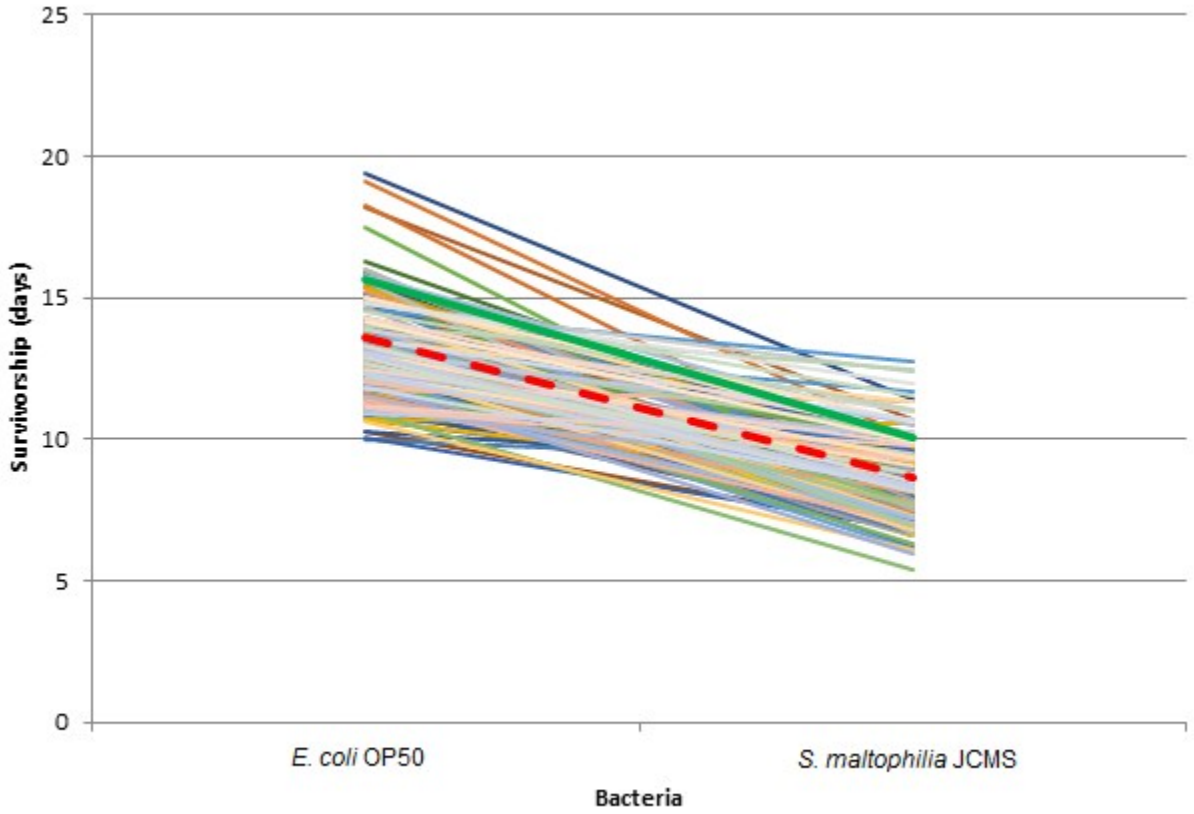


Figure 6. QTL mapping for differential survivorship between *E. coli* OP50 and *S. maltophilia* JCMS.

Differential survivorship, calculated by subtracting the average survivorship on *S. maltophilia* JCMS from that of *E. coli* OP50 for each RILs strain, was used as trait value. QTL mapping was performed by WinQTLCart CIM Model 6, forward and backward regression method with probabilities for selecting into and out of the model as 0.1 respectively. Horizontal lines are for trait specific thresholds determined by 1000 permutations for a significance level of 0.05. For differential survivorship (blue), LOD = 2.5. Triangles along the x axis indicate the marker positions. Lower panel shows the corresponding additive effects.

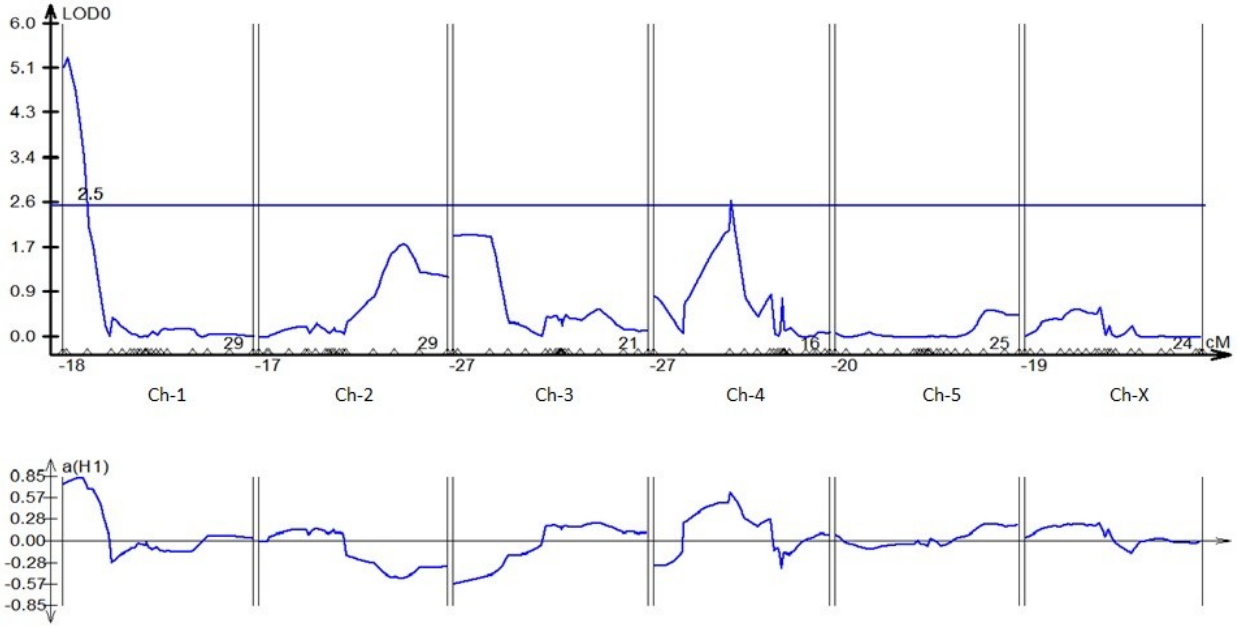


Figure 7. G×E interactions in Chromosome I ILs.

A. Analysis of Variance for each pair of individual Chromosome I ILs with N2.

ILs were examined individually by comparison with N2. Analysis of variance was performed for each pairwise comparison by model, survivorship = strain + bacterium + strain × bacterium + rnd. The significant "strain × bacterium" term was interpreted as a significant G×E interaction (GEI). Gray blocks indicate specific position of the introgression of each IL. Arrows at the ends indicate uncertain break point between two adjacent markers. Green highlight indicates strains that show significant GEI. X-axis illustrates the physical position of the markers.

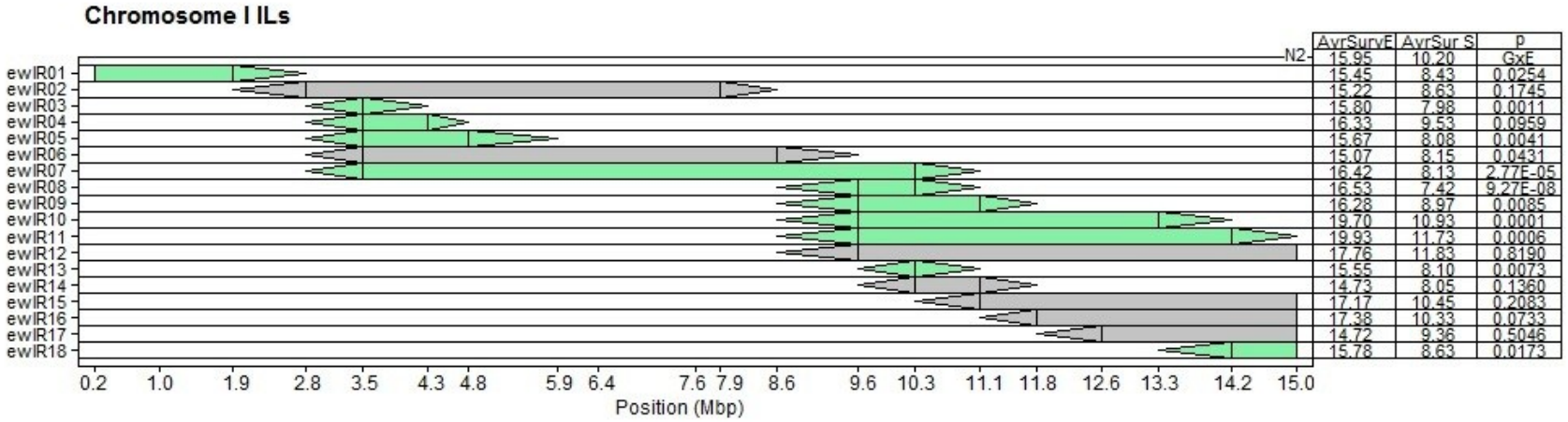
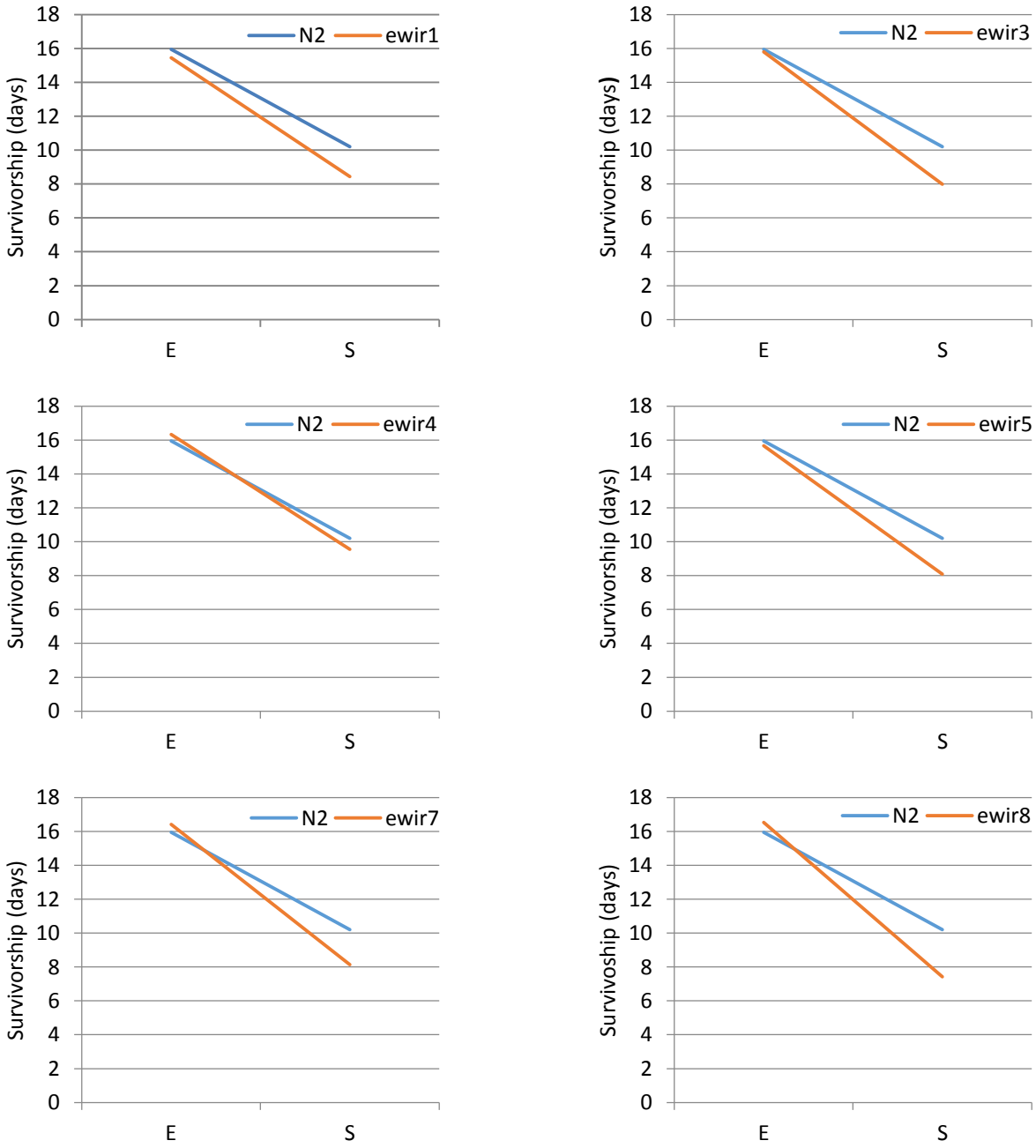
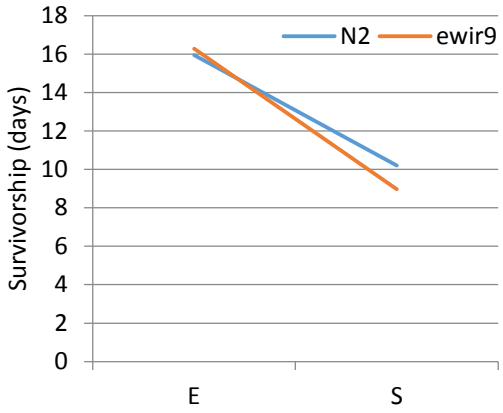


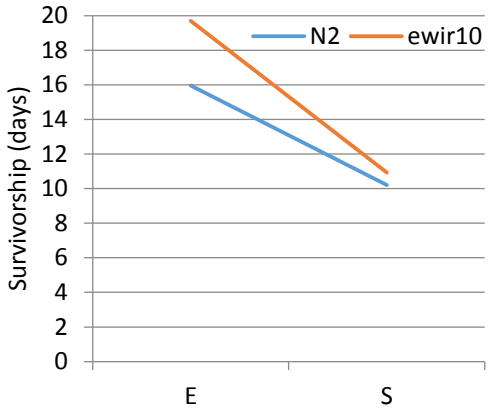
Figure 7. G×E interactions in Chromosome I ILs.

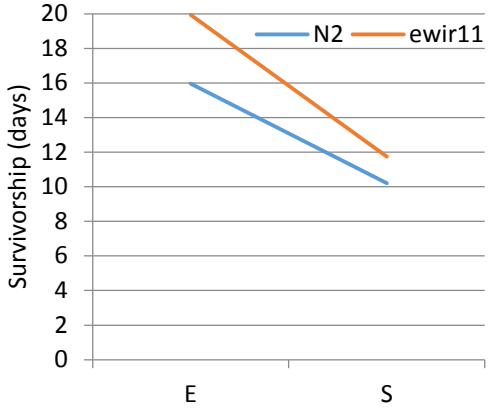
B. Reaction norms for Chromosome I ILs showing significant G×E.

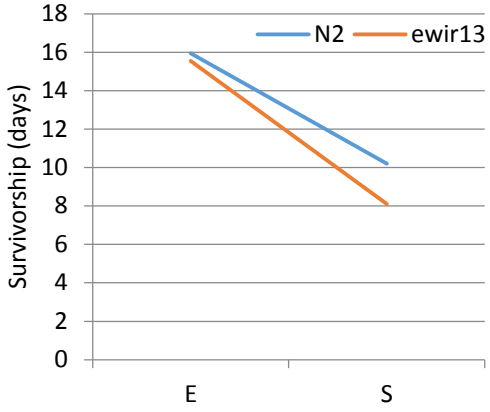
X axis indicates bacterial food. E represents *E.coli* OP50; and S represents *S. maltophilia* JCMS. Y axis is the survivorship in days.




Survivorship (days)
18
16
14
12
10
8
6
4
2
0
N2
ewir9
E
S


Survivorship (days)
20
18
16
14
12
10
8
6
4
2
0
N2
ewir10
E
S


Survivorship (days)
20
18
16
14
12
10
8
6
4
2
0
N2
ewir11
E
S


Survivorship (days)
18
16
14
12
10
8
6
4
2
0
N2
ewir13
E
S

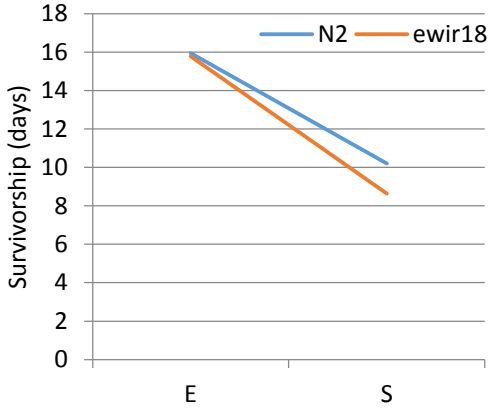
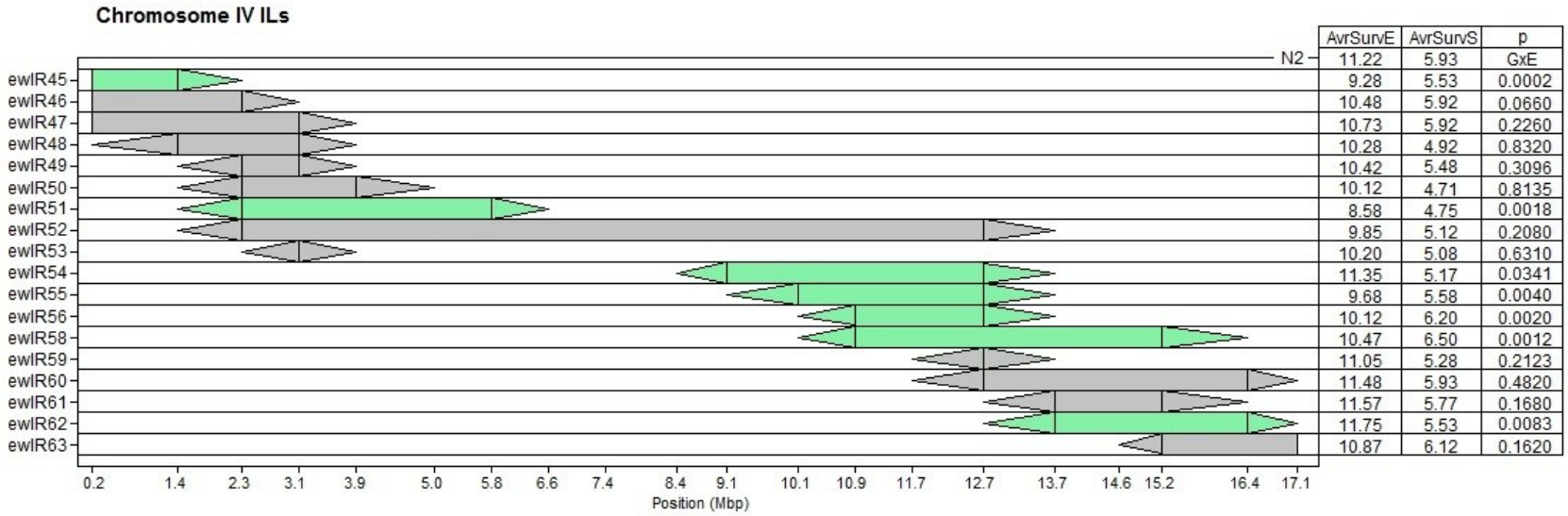

Survivorship (days)
18
16
14
12
10
8
6
4
2
0
N2
ewir18
E
S

Figure 8. G×E interactions in Chromosome IV ILs.

A. Analysis of Variance for each pair of individual Chromosome IV ILs with N2.

ILs were examined individually by comparison with N2. Analysis of variance was performed for each pairwise comparison by model, survivorship = strain + bacterium + strain × bacterium + rnd. The significant "strain × bacterium" term was interpreted as a significant G×E interaction (GEI). Gray blocks indicate specific position of the introgression of each IL. Arrows at the ends indicate uncertain break point between two adjacent markers. Green highlights strains that show significant GEI. X-axis illustrates the physical position of the markers.

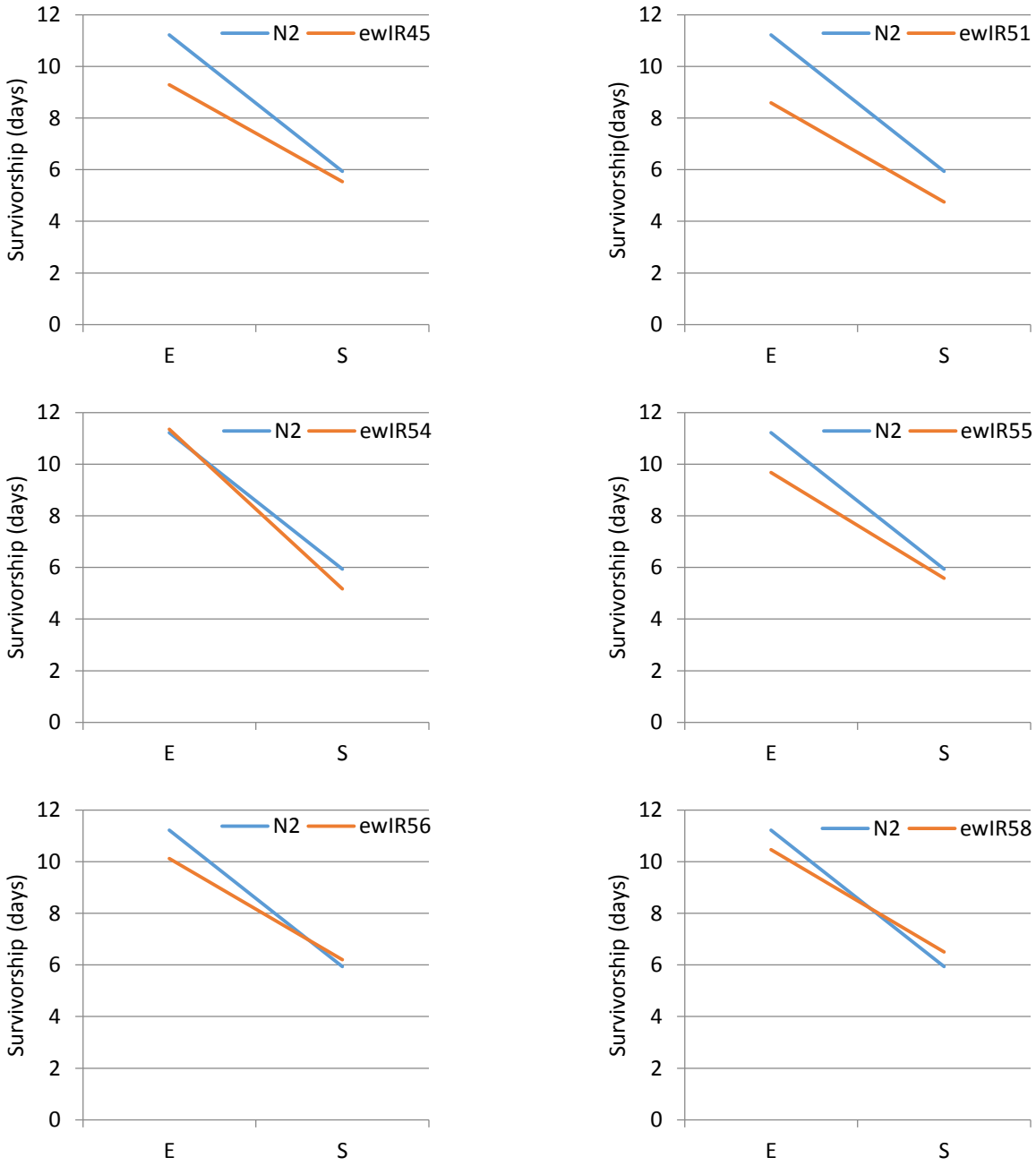


	AvrSurvE	AvrSurvS	p
N2	11.22	5.93	GxE
ewIR45	9.28	5.53	0.0002
ewIR46	10.48	5.92	0.0660
ewIR47	10.73	5.92	0.2260
ewIR48	10.28	4.92	0.8320
ewIR49	10.42	5.48	0.3096
ewIR50	10.12	4.71	0.8135
ewIR51	8.58	4.75	0.0018
ewIR52	9.85	5.12	0.2080
ewIR53	10.20	5.08	0.6310
ewIR54	11.35	5.17	0.0341
ewIR55	9.68	5.58	0.0040
ewIR56	10.12	6.20	0.0020
ewIR58	10.47	6.50	0.0012
ewIR59	11.05	5.28	0.2123
ewIR60	11.48	5.93	0.4820
ewIR61	11.57	5.77	0.1680
ewIR62	11.75	5.53	0.0083
ewIR63	10.87	6.12	0.1620

Figure 8. G×E interactions in Chromosome IV ILs.

B. Reaction norms for Chromosome IV ILs showing significant G×E.

X axis indicates bacterial food. E represents *E.coli* OP50; and S represents *S. maltophilia* JCMS. Y axis is the survivorship in days.



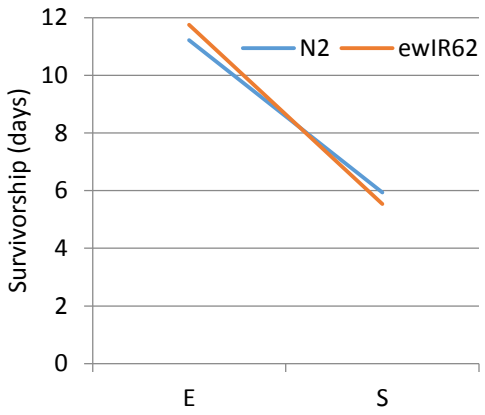

12
10
8
6
4
2
0
Survivorship (days)
N2
ewIR62
E
S

Table 1. Summary of QTL mapping results.

Name	Bac	Chr	Closest Maker	Peak position (cM)	Peak LOD	Add.	95% CI L ~ R (width cM) Genomic position (bp)	# of genes In CI
SE1	E	I	C1M17	13.87	7.6	-0.7	8.9 ~ 15.1 (6.2) I:11483000..12942300	399
SE2	E	IV	C4M3	-8.53	6.1	0.6	-15.1 ~ -6.4 (8.7) IV:1847900..3029000	236
SE3	E	IV	C4M6	1.45	4.0	0.5	-1.6 ~ 1.7 (3.3) IV:3926417..5480582	1386
SS1	S	I	C1M2> (-17.28)	-16.3	6.8	-0.7	-18.26 ~ -13.2 (5.1) I:169000..1741000	248
SS2	S	I	C1M18< (17.46)	16.04	7.6	-0.6	10.8 ~ 23.5 (12.7) I:11820000..14170000	591

Table 2. Summary of analysis of variance for ILs Survivorship on *E. coli* OP50.

		Df	Sum Sq	Mean Sq	F value	Pr(>F)
Chromosome I ILs	strain	18	2324	129.10	18.081	< 2e-16
	rnd	1	32	31.62	4.429	0.035566
	strain:rnd	18	329	18.26	2.557	0.000361
	residuals	1101	7861	7.14		
Chromosome IV ILs	strain	18	720	40.0	12.380	< 2e-16
	rnd	1	786	785.9	243.201	< 2e-16
	strain:rnd	18	239	13.3	4.109	1.99e-08
	residuals	1095	3538	3.2		

Table 3. Summary of analysis of variance for ILs Survivorship on *S. maltophilia* JCMS.

		Df	Sum Sq	Mean Sq	F value	Pr(>F)
Chromosome I ILs	strain	18	1938	107.7	30.669	< 2e-16
	rnd	1	2712	2712.4	772.758	< 2e-16
	strain:rnd	18	564	31.3	8.925	< 2e-16
	residuals	1100	3861	3.5		
Chromosome IV ILs	strain	18	276.0	15.34	17.09	< 2e-16
	rnd	1	192.3	192.26	214.30	< 2e-16
	strain:rnd	18	95.1	5.28	5.89	9.8e-14
	residuals	1096	983.3	0.90		

Table 4. Analysis of variance for 154 RILs shows significant effect for G×E Interactions.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
bac	1	51419	51419	11097.28	<2e-16
strain	153	14544	95	20.52	<2e-16
bac : strain	153	8723	57	12.30	<2e-16
Residuals	8753	40556	5		

Table 5 QTL mapping results for differential survivorship

Name	Chr	Closest Maker	Peak position (cM)	Peak LOD	Add.	95% CI L ~ R (width cM) Genomic position (bp)	# of genes In CI
DS1	I	C1M2	-17.28	5.4	0.8	Left of -14.7 I:0..1339000	228
DS2	IV	C4M3	-8.5	2.6	0.65	-13.2/-6.6 (6.6) IV:1902300..3023000	233

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