doi:10.1111/j.1420-9101.2009.01830.x

SHORT COMMUNICATION

The effect of pathogens on selection against deleterious mutations in *Drosophila melanogaster*

JADENE A.YOUNG, CHRISTOPHER P. YOURTH & ANEIL F. AGRAWAL

Department of Ecology & Evolutionary Biology, University of Toronto, Toronto, ON, Canada

Keywords:

deleterious mutations;
disease;
host–parasite interactions;
selection;
stress.

Abstract

In natural populations, fitness is reduced by both deleterious mutations and parasites. Few studies have examined interactions between these two factors, particularly at the level of individual genes. We examined how the presence of a bacterial pathogen, *Pseudomonas aeruginosa*, affected the selection against each of eight deleterious mutations in *Drosophila melanogaster*. We found that mutations tended to become more deleterious in the presence of disease. This increase in the average selection was primarily due to three genes with the remainder showing little evidence of change.

Introduction

It remains somewhat unclear why most organisms regularly shuffle their genomes through sex and recombination (Otto & Lenormand, 2002; Agrawal, 2006a). Much effort has been put into finding an explanation that is broadly applicable to diverse taxa. Because all natural populations suffer from both parasites and deleterious mutations, a large number of theoretical sex models have focused on either parasites (Hamilton *et al.*, 1990; Peters & Lively, 1999; Agrawal, 2006b) or mutations (Kondrashov, 1982; Agrawal & Chasnov, 2001; Keightley & Otto, 2006).

However, there has been comparatively little effort expended on investigating the joint action of parasites and deleterious mutations in selecting for sex. Indeed, the influential review of West *et al.* (1999) emphasized the need for more exploration of these combined forces. Theory by Howard & Lively (1994, 1998) demonstrated that parasites and deleterious mutations together can be more successful in selecting for sex than predicted by these factors individually. They observed this result despite assuming that mutations and infection have independent effects on fitness. If fitness effects interact, it is conceivable that parasites and mutations could more strongly favour sex. Indeed, a recent extension of the Howard and Lively model found that when infection and mutations interact synergistically, sexual populations are more resistant to invasion by asexual competitors (A. W. Park, J. Jokela & Y. Michalakis, unpublished data).

Interactions between parasites and mutations have been investigated in several systems using a variety of methods. Several authors have found that exposure to parasites magnifies inbreeding depression (Stevens *et al.*, 1997; Coltman *et al.*, 1999; Ilmonen *et al.*, 2008), though others have failed to find such an effect (Haag *et al.*, 2003; Salathe & Ebert, 2003). However, such tests are nonideal in the present context because it is unclear whether any observed effect reflects an interaction between disease agents and random deleterious alleles or rather a special interaction between disease and homozygosity at loci relevant to immunity such as the MHC locus.

Other authors have measured the effect of parasites on control genotypes vs. genotypes that have been loaded with deleterious alleles via mutagenesis. Peters (1999) found no evidence of interactions between *Pseudomonas* infections and mutation in *Arabidopsis thaliana*. Killick *et al.* (2006) found some evidence that the effects of mutation in *Daphnia magna* change in the presence of infectious bacteria, though only under some environmental conditions.

One disadvantage to using mutagenesis is that each 'mutant' genotype typically contains multiple mutations. This increases the power to measure the average interaction between parasites and mutations but reduces the

Correspondence: Aneil F. Agrawal, Department of Ecology & Evolutionary Biology, University of Toronto, Toronto, ON, Canada, M5S 3B2.
Tel.: +1 416 946 5563; fax: +1 416 978 5878;
e-mail: a.agrawal@utoronto.ca

ability to observe variation in interaction effects of parasites with individual mutations. An analogous issue has been raised with respect to the study of epistasis (mutation-by-mutation effects). On average, epistasis is often close to zero but only by studying individual mutations has it become clear that this result occurs because of a mix of positive and negative interactions cancelling each other out (de Visser & Elena, 2007).

We are aware of only a single experiment that has measured how parasites alter selection on individual mutations. Cooper *et al.* (2005) found that an intracellular parasite of *E. coli* tended to increase the selection on individual mutations. However, this average result belies a large amount of variation. Whereas parasites aggravated the effect of some mutations, parasites had no detectable effect on most mutations and alleviated selection against a small fraction of mutations.

Here we investigate the interaction between a multicellular eukaryote, *Drosophila melanogaster* and the pathogenic bacteria *Pseudomonas aeruginosa*. This bacterium is a common insect pathogen and has been found in wild populations of *D. melanogaster* (Corby-Harris *et al.*, 2007b). *Pseudomonas aeruginosa* is known to have severe effects on host survival (Erickson *et al.*, 2004; Corby-Harris *et al.*, 2007a). In the first experiment, we verify that a simple new method of exposing fly larvae to bacteria reduces larval viability, as expected if exposure causes infection. In the second experiment, we use this method of exposure to test whether the presence of the pathogen alters selection on each of eight individual mutations.

Methods and materials

Study system

Flies were obtained from a large outbred population, originally from Dahomey, West Africa. This population has been maintained at large size in the laboratory for about three decades and in the present laboratory for over 4 years. All stocks were maintained at 25 °C on standard yeast–sugar–agar food. The PA01 strain of *Pseudomonas aeruginosa* was used.

Experiment 1 – exposure to bacteria via food

Previous studies using this host–parasite system have infected adult flies by forcing them to consume *P. aeruginosa* by contaminating their food with quantities of bacteria as large as roughly 10^9 cells per fly (Chugani *et al.*, 2001; Erickson *et al.*, 2004). In the first experiment, we examine the effectiveness of an alternative, perhaps more realistic, method of exposure – larvae are provided with the bodies of dead infected adults as an additional food source.

Pseudomonas aeruginosa cultures were initiated in sterile King's B medium (KB) and allowed to grow overnight at 37 °C. The resulting cultures were diluted to an optical density (OD_{600}) of 1.0. Fifty adult wild-type virgin females were injected in the thorax with live *Pseudomonas aeruginosa* (PA01 strain) using a 0.15 mm minutien pin dipped in the live liquid culture of *P. aeruginosa*. As a control, 50 adult wild-type virgin females were injected in the thorax with sterile KB. The day after injection, flies were killed by decapitation and added to larval vials (below). To standardize the treatment, we only used adults that we killed by decapitation; flies that had died for other reasons (including infection) were not used. Most flies infected with PA01 by the above method die before internal bacterial counts reach approximately 10^7 cells (D'Argenio *et al.*, 2001; C. Yourth, unpublished data), two orders of magnitude fewer cells than used in previous adult feeding assays (Chugani *et al.*, 2001; Erickson *et al.*, 2004). It should be noted however that the bacteria are more concentrated within the adult carcasses.

Thirty first-instar wild-type larvae were placed into each of 50 vials containing standard media. The body of a single decapitated infected adult was added to each of 25 of these larval vials; the body of single decapitated uninfected adult was added to the remaining 25 vials (controls). Ten days later, flies surviving to adulthood were counted and discarded. A second count was done 3 days later to score any slow developing flies. Because these are count data, the data were analyzed using the generalized linear model procedure *glm* in *R* (R Development Core Team, 2008) with Poisson errors (Crawley, 2007).

Experiment 2 – selection against mutations

We measured larval viability selection against mutations at eight autosomal genes. Each of the mutations was backcrossed for a minimum of 14 generations into the Dahomey population. These mutations were originally obtained from the Bloomington Stock Center. All of the mutations have dominant visible effects on the adults allowing for scoring of a large number of individuals. The mutations used here had effects on eyes (Gl^1, Gla^1, R^1), effects on wings (Ly^1), effects on bristles (Sb^1, Ki^1), effects on body colour (Frd^1) and effects on antennae ($Antp^{Ns}$). None of these genes have any direct effect on immune function based on their descriptions in *FlyBase* (http://flybase.bio.indiana.edu). Larval viability selection was measured on each mutation independently.

For each mutation, we used the following procedure. On day 1, four heterozygous mutant males (*M*/+) and four 1–2 day-old wild-type virgin females (+/+) were placed into a single vial to reproduce (n = 200 vials per mutant type). On day 3, the parental flies were removed and discarded. To each vial, we added either the bodies of four infected decapitated adult females (n = 100 per mutant type) or the bodies of four uninfected decapitated

adult females (n = 100 per mutant type). These infected or uninfected 'food source' females were prepared as described in the first experiment. On day 11 and 14, the mutant and wild-type offspring that emerged from the vials were scored. Spot checking of vials after day 14 indicated that almost no offspring emerged after this period.

To analyze the total number of survivors, we used *glm* with mutation and infection treatment as fixed effects. Because of high residual variance, we used quasi-Poisson errors (Crawley, 2007). To analyze the frequency of mutants among the survivors, we used *glm* with mutation and infection treatment as fixed effects. Quasi-binomial errors were used. For both analyses, we used the Helmert contrasts option. Main and interaction effects were tested with F-tests using the *drop1* function.

For the estimates of selection shown in Fig. 1, we combined data from all replicates for each combination of gene and treatment to obtain the best point estimate of the frequency of mutants. In the absence of selection, the expected frequency of mutants is 50%. Using the simple fitness model $w_{+/+} = 1$ and $w_{M/+} = 1 - s$, the expected frequency of mutants is given by $f = 0.5(1 - s)/(1 - 0.5s)$, which can be rearranged to obtain an estimate of selection, $s_{est} = (1 - 2f_{obs})/(1 - f_{obs})$. Bootstrap 95% confidence intervals were obtained by re-sampling replicates with replacement and recalculating s_{est}. The estimates of selection are provided to help visualize the results; the statistical analysis was performed on the numbers of mutant and wild-type individuals per vial as described above.

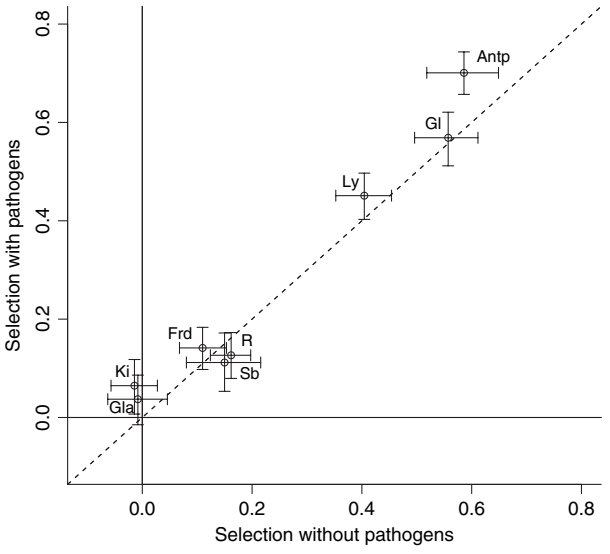

Fig. 1 Selection on mutations with and without pathogens. Error bars represent bootstrap 95% confidence intervals. Dashed line is the one to one line (equal selection).

Results

In Experiment 1, larvae were observed consuming the carcasses of dead adults. In control vials, which received the body of a decapitated uninfected female, the mean number of surviving offspring was 24.48 ± 0.76 (mean ± SE). This number is similar to what we have found in other experiments in which no decapitated body is used (A. Laffafian and A.F. Agrawal, unpublished data). In vials receiving the body of a decapitated infected female, the mean number of surviving offspring was 19.96 ± 0.90. Thus, exposure to *P. aeruginosa* reduced survival by ~18% and this effect was highly significant ($t_{48} = 3.84$, $P = 0.0003$).

Across all eight mutations studied in Experiment 2, over 164 000 flies were scored (on average, 103.0 per vial). Consistent with Experiment 1, fewer flies emerged from infected vials than uninfected vials (101.5 vs. 104.8), though the treatment effect was marginally nonsignificant with respect to the total number of survivors ($F_{1,1581} = 3.43$, $P = 0.06$). There was a significant effect of gene ($F_{7,1581} = 31.07$, $P < 10^{-15}$) but no treatment × gene interaction ($F_{7,1581} = 0.499$, $P = 0.84$).

However, our main interest was with respect to the frequency of mutants. In this regard, there was a significant effect of gene ($F_{7,1581} = 151.6$, $P < 10^{-15}$) indicating that the different mutations varied in their effect on viability. The treatment effect was also significant ($F_{1,1581} = 8.08$, $P = 0.0045$); the direction of this term indicated that mutants were less frequent in the infected vials, suggesting that pathogens increase the strength of selection. However, the treatment × gene interaction ($F_{7,1581} = 2.56$, $P = 0.013$) term was also significant, indicating that pathogens have variable effects on selection against different mutations. Figure 1 shows estimates of selection against each of the eight mutations. Five of the mutations experience very similar selection in both treatments. The other three genes (*Antp*, *Ki*, and *Ly*) all appear to experience stronger selection in the presence of the pathogen.

Discussion

Deleterious mutations are an unavoidable consequence of imperfect replication. Their presence in populations reduces mean fitness, resulting in a so-called 'mutation load' (Haldane, 1937; Muller, 1950). Parasites are also a ubiquitous feature of natural populations, imposing mortality and potentially serving as an important ecological source of selection. While each force has received considerable attention alone, further exploration of possible interactions between them is warranted (West *et al.*, 1999).

We found that parasites tend to increase the strength of selection against deleterious mutations. As described in the 'Introduction', this finding is consistent with a number of earlier results. As in the only other study to

examine individual gene effects (Cooper *et al.*, 2005), we found that not all genes were affected equally. Most of our genes showed little or no effect but a few genes experienced stronger selection when exposed to parasites.

Our results are also consistent with the idea that selection becomes stronger under stress (Parsons, 1987; Kondrashov & Houle, 1994; Jasnos *et al.*, 2008 but see Kishony & Leibler, 2003; Martin & Lenormand, 2006). This interpretation assumes that pathogens are stressful, i.e. pathogens cause a reduction in average absolute fitness. Experiment 1 clearly supports this claim; survival was 18% lower in infected vials. However, in Experiment 2 survival was only slightly lower in infected vials. This difference between the experiments is likely due to the differences in density. In Experiment 1, densities were low (30 larvae per vial). Because survival is typically very high under such conditions, the addition of a virulent pathogen caused an obvious decline in survivorship. In Experiment 2, larval densities were much higher (on average, over 100 offspring surviving to adulthood per vial) and we know from other experiments that larval mortality is typically much higher at such densities using this fly population and similar culture conditions (A. Laffafian & A. Agrawal, unpublished data). It is likely that at these higher densities, deaths caused by pathogens simply replaced deaths that would have otherwise occurred due to density dependence. Although the total number of survivors was similar in uninfected and infected vials, the frequency of mutants tended to be lower in infected vials. This suggests that pathogen-mediated mortality was more selective than regular density-dependent mortality. That is, nonselective ecological deaths were replaced by selective deaths when disease was present.

Why do parasites appear to affect selection on some genes but not others? Although all vials in Experiment 2 involved four virgin wild-type females laying eggs for 2 days, egg densities might have varied among gene treatments if mutant males vary in either their latency to mate or their ability to affect female egg-laying rate. It is possible that parasites only alter selection when density is particularly high (or low). Because we did not measure egg density, we cannot directly assess this possibility. However, inspection of the total number of surviving wild-type adults among the different gene treatments does not provide strong compelling evidence of for this explanation. The genes experiencing the greatest change in selection (*Antp*, *Ly* and *Ki*) have, on average, higher wild-type density than the other genes but not significantly so ($t_5 = 0.90$, $P = 0.41$). Moreover, the gene with the highest density of wild-type survivors (*Gl*) does not show any disease-mediated change in selection, despite being subject to fairly strong selection.

There are several ways selection could become stronger in the presence of disease. Mutants could be more likely to encounter pathogens, more easily infected by pathogens, or less tolerant of infection. Studies such as ours and others (Peters, 1999; Killick *et al.*, 2006) cannot distinguish between these possibilities. Other studies examine only one of these possibilities (Cooper *et al.*, 2005). The various ways in which mutants could be affected by disease likely have different implications for disease epidemiology and host–parasite coevolution. Ideally, future studies will provide more detailed insight into the extent to which disease alters the strength of selection on mutations as well as the route by which it does so.

Acknowledgments

This work was supported by the Natural Sciences and Engineering Research Council of Canada (USRA to JAY and Discovery Grant to AFA).

References

Agrawal, A.F. 2006a. Evolution of sex: why do organisms shuffle their genotypes? *Curr. Biol.* **16**: R696–R704.

Agrawal, A.F. 2006b. Similarity selection and the evolution of sex: revisiting the Red Queen. *PLoS Biol.* **4**: e265.

Agrawal, A.F. & Chasnov, J.R. 2001. Recessive mutations and the maintenance of sex in structured populations. *Genetics* **158**: 913–917.

Chugani, S.A., Whiteley, M., Lee, K.M., D'Argenio, D., Manoil, C. & Greenberg, E.P. 2001. *QscR*, a modulator of quorum-sensing signal synthesis and virulence in *Pseudomonas aeruginosa*. *Proc. Natl Acad. Sci. USA* **98**: 2752–2757.

Coltman, D.W., Pilkington, J.G., Smith, J.A. & Pemberton, J.M. 1999. Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution* **53**: 1259–1267.

Cooper, T.F., Lenski, R.E. & Elena, S.F. 2005. Parasites and mutational load: an experimental test of a pluralistic theory for the evolution of sex. *Proc. R. Soc. Lond. B* **272**: 311–317.

Corby-Harris, V., Habel, K.E., Ali, F.G. & Promislow, D.E.L. 2007a. Alternative measures of response to *Pseudomonas aeruginosa* infection in *Drosophila melanogaster*. *J. Evol. Biol.* **20**: 526–533.

Corby-Harris, V., Pontaroli, A.C., Shimkets, L.J., Bennetzen, J.L., Habel, K.E. & Promislow, D.E.L. 2007b. Geographical distribution and diversity of bacteria associated with natural populations of *Drosophila melanogaster*. *Appl. Environ. Microb.* **73**: 3470–3479.

Crawley, M.J. 2007. *The R Book*. John Wiley & Sons Ltd, West Sussex.

D'Argenio, D.A., Gallagher, L.A., Berg, C.A. & Manoil, C. 2001. *Drosophila* as a model host for *Pseudomonas aeruginosa* infection. *J. Bacteriol.* **183**: 1466–1471.

Erickson, D.L., Lines, J.L., Pesci, E.C., Venturi, V. & Storey, D.G. 2004. *Pseudomonas aeruginosa relA* contributes to virulence in *Drosophila melanogaster*. *Infect. Immun.* **72**: 5638–5645.

Haag, C.R., Sakwinska, O. & Ebert, D. 2003. Test of synergistic interaction between infection and inbreeding in *Daphnia magna*. *Evolution* **57**: 777–783.

Haldane, J.B.S. 1937. The effect of variation on fitness. *Am. Nat.* **71**: 337–349.

Hamilton, W.D., Axelrod, R. & Tanese, R. 1990. Sexual reproduction as an adaptation to resist parasites (a review). *Proc. Natl Acad. Sci. USA* **87**: 3566–3573.

Howard, R.S. & Lively, C.M. 1994. Parasitism, mutation accumulation and the maintenance of sex. *Nature* **367**: 554–557.

Howard, R.S. & Lively, C.M. 1998. The maintenance of sex by parasitism and mutation accumulation under epistatic fitness functions. *Evolution* **52**: 604–610.

Ilmonen, P., Penn, D.J., Damjanovich, K., Clarke, J., Lamborn, D., Morrison, L., Ghotbi, L. & Potts, W.K. 2008. Experimental infection magnifies inbreeding depression in house mice. *J. Evol. Biol.* **21**: 834–841.

Jasnos, L., Tomala, K., Paczesniak, D. & Korona, R. 2008. Interactions between stressful environment and gene deletions alleviate the expected average loss of fitness in yeast. *Genetics* **178**: 2105–2111.

Keightley, P.D. & Otto, S.P. 2006. Interference among deleterious mutations favours sex and recombination in finite populations. *Nature* **443**: 89–92.

Killick, S.C., Carlsson, A.M., West, S.A. & Little, T.J. 2006. Testing the pluralist approach to sex: the influence of environment on synergistic interactions between mutation load and parasitism in *Daphnia magna*. *J. Evol. Biol.* **19**: 1603–1611.

Kishony, R. & Leibler, S. 2003. Environmental stresses can alleviate the average deleterious effect of mutations. *J. Biol.* **2**: 14.

Kondrashov, A.S. 1982. Selection against harmful mutations in large sexual and asexual populations. *Genet. Res.* **40**: 325–332.

Kondrashov, A.S. & Houle, D. 1994. Genotype–environment interactions and the estimation of the genomic mutation rate in *Drosophila melanogaster*. *Proc. R. Soc. B* **258**: 221–227.

Martin, G. & Lenormand, T. 2006. The fitness effect of mutations across environments: A survey in light of fitness landscape models. *Evolution* **60**: 2413–2427.

Muller, H.J. 1950. Our load of mutations. *Am. J. Hum. Genet.* **2**: 111–176.

Otto, S.P. & Lenormand, T. 2002. Resolving the paradox of sex and recombination. *Nat. Rev. Genet.* **3**: 252–261.

Parsons, P.A. 1987. Evolutionary rates under environmental stress. *Evol. Biol.* **21**: 311–347.

Peters, A.D. 1999. The effects of pathogen infection and mutation on life history characteristics in *Arabidopsis thaliana*. *J. Evol. Biol.* **12**: 460–470.

Peters, A.D. & Lively, C.M. 1999. The red queen and fluctuating epistasis: a population genetic analysis of antagonistic coevolution. *Am. Nat.* **154**: 393–405.

R Development Core Team. (2008) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria, Vienna. http://www.R-project.org.

Salathe, P. & Ebert, D. 2003. The effects of parasitism and inbreeding on the competitive ability in *Daphnia magna*: evidence for synergistic epistasis. *J. Evol. Biol.* **16**: 976–985.

Stevens, L., Yan, G.Y. & Pray, L.A. 1997. Consequences of inbreeding on invertebrate host susceptibility to parasitic infection. *Evolution* **51**: 2032–2039.

de Visser, J.A.G.M. & Elena, S.F. 2007. The evolution of sex: empirical insights into the roles of epistasis and drift. *Nat. Rev. Genet.* **8**: 139–149.

West, S.A., Lively, C.M. & Read, A.F. 1999. A pluralist approach to sex and recombination. *J. Evol. Biol.* **12**: 1003–1012.

Received 26 May 2009; revised 20 July 2009; accepted 27 July 2009