chickens (*8*, *26*), but not with small-scale feather tip melanization across species of *Phylloscopus* warblers (*27*). In addition, snow geese, arctic skuas, and bananaquits show that *MC1R* frequently controls variation between eumelanin production and the absence of melanin, whereas in chickens and most mammals it controls relative amounts of eumelanin and red/yellow phaeomelanin (*8*, *9*, *26*).

Variation in plumage color in geese and skuas provides a rare example where the major molecular genetic determinant of a quantitative trait has been identified in wild populations. The association of *MC1R* variation with naturally occurring melanism in three divergent avian lineages (Anseriforms, snow geese; Charadriiforms, arctic skuas; and Passeriforms, bananaquits) reveals a conserved mechanism of plumage color evolution through many tens of millions of years of avian history (*28*). The repeated involvement of *MC1R* is surprising, because over 100 loci are known to affect pigmentation in vertebrates (*29*). This presumably reflects some combination of a high mutability to functionally novel *MC1R* alleles, a relative absence of deleterious pleiotropic effects of these alleles, and the visibility of dominant or codominant melanic *MC1R* alleles to natural selection. Our results provide strong support for the notion that, at least in the case of melanism in birds, evolution is driven by mutation rather than selection on existing standing genetic variation.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/303/5665/1870/DC1
Materials and Methods
Tables S1 and S2
References

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Genetic Basis of Natural Variation in *D. melanogaster* Antibacterial Immunity

Brian P. Lazzaro,[1]* Bonnielin K. Sceurman,[2] Andrew G. Clark[3]

Many genes involved in *Drosophila melanogaster* innate immune processes have been identified, but whether naturally occurring polymorphism in these genes leads to variation in immune competence among wild flies has not been tested. We report here substantial variability among wild-derived *D. melanogaster* in the ability to suppress infection by a Gram-negative entomopathogen, *Serratia marcescens*. Variability in immune competence was significantly associated with nucleotide polymorphism in 16 innate immunity genes, corresponding primarily to pathogen recognition and intracellular signaling loci, and substantial epistasis was detected between intracellular signaling and antimicrobial peptide genes. Variation in these genes, therefore, seems to drive variability in immunocompetence among wild *Drosophila*.

Efficacy of immune response is a critical determinant of fitness, and higher eukaryotes have accordingly evolved sophisticated mechanisms for suppressing bacterial infection. For invertebrates, this is mediated by generalized, or innate, immune pathways, which include phagocytosis by scavenging macrophages and the extracellular circulation of short antibiotic peptides (*1*, *2*). Artificially generated mutations that abolish the function of key genes have resulted in severe immune deficiencies [(*1*) and citations therein]. In addition, studies of population-level variation have suggested that *Drosophila* immunity genes evolve under positive natural selection (*3*–*5*). However, phenotypic effects of naturally occurring polymorphism in innate immune genes have not previously been studied in invertebrates. We sought to measure variability in immunocompetence among *Drosophila melanogaster* in wild populations with the aim of attributing that variation to gene candidates involved in immune function. We initially focused on loci encoded on the *D. melanogaster* second chromosome, examining 21 protein-coding genes of diverse function (Table 1). Seven candidate genes are hypothesized to be involved in microbial recognition: four class C scavenger receptors [*SR-CI*, *SR-CII*, *SR-CIII*, and *SR-CIV* (*6*)] and a three-gene cluster of putative peptidoglycan recognition proteins [*PGRP-SC1A*, *PGRP-SC1B*, and *PGRP-SC2* (*7*)]. Candidates involved in signal transduction are three Toll-like receptors (*Tehao*, *18-Wheeler*, and *Toll-4*), the rel transcription factor *Dif*, rel inhibitor *cactus*, and intracellular signaling genes *imd* and *IK2* (*2*). The remaining seven genes encode the secreted antibacterial peptides Defensin, Metchnikowin, Attacins A, B and C, and Diptericins A and B (*8*–*12*).

[1]Department of Entomology, 4138 Comstock Hall, Cornell University, Ithaca, NY 14853, USA. [2]Laboratory of Biochemistry and Genetics, NIDDK, National Institutes of Health, Building 8, Room 323, 8 Center Drive MSC 0840, Bethesda, MD 20892, USA. [3]Department of Molecular Biology and Genetics, 107 Biotechnology Building, Cornell University, Ithaca, NY 14853, USA.

*To whom correspondence should be addressed: bl89@cornell.edu

We generated 101 *D. melanogaster* lines, each homozygous for an independent chromosome 2 isolated directly from a wild North American population and crossed into a common genetic background (*13*). Consequently, all genetic variation among test lines could be attributed to naturally occurring polymorphism in chromosome 2 loci. Adult flies from each line were manually infected with *S. marcescens* at 4 to 8 days after eclosion, and viable pathogen load was measured by quantitative plating at prescribed times after infection (*13*). In total, more than 16,000 flies were infected, and colony counts were obtained from 6924 plates. Homogenates from all sham-infected flies failed to yield colonies.

Systemic mean loads of *S. marcescens* differed by only marginal significance at 7, 15, 26, and 39 hours after artificial infection [analysis of variance (ANOVA), $P = 0.02$], suggesting that bacterial density generally reached a stable plateau over that time. Because no statistically significant interaction occurred between the parameters of time after infection and genetic line (ANOVA, $P = 0.67$), data from different time points could be pooled. Genetic line was a significant determinant of bacterial load at all times after infection, and was highly significant when all times were pooled (ANOVA, $P < 10^{-4}$) with extreme lines differing by 10 phenotypic standard errors (Fig. 1). For technical convenience, flies assayed at 7 and 26 hours after infection had been infected in the morning, and flies assayed at 15 and 39 hours after infection had been infected in the evening. A strong effect of circadian period at time of infection was observed in bacterial loads (ANOVA, $P < 10^{-4}$), with higher bacterial loads occurring in PM-infected flies. However, the interaction of circadian time of infection with genetic line was not statistically significant (ANOVA, $P = 0.14$). This effect of circadian time is consistent with previous studies showing that expression of multiple *D. melanogaster* immune response genes cycle over the circadian day (*14*). In no case did we observe a significant effect of *Drosophila* sex on bacterial load, nor did we observe any significant interaction between sex and genetic line. After controlling for the effects of time after infection and circadian time of day, 47.2% of the variance in bacterial load could be attributed directly to genetic differences among lines. Due to the large statistical effect of the circadian time of day of infections, further association tests were done after attempting to control for this variable with analysis of covariance (ANCOVA) and after parsing the phenotype data into sex and circadian time categories.

Polymorphism was ascertained in each of the 21 candidate genes by sequencing 10 to 12 alleles chosen independently of prior phenotyp-

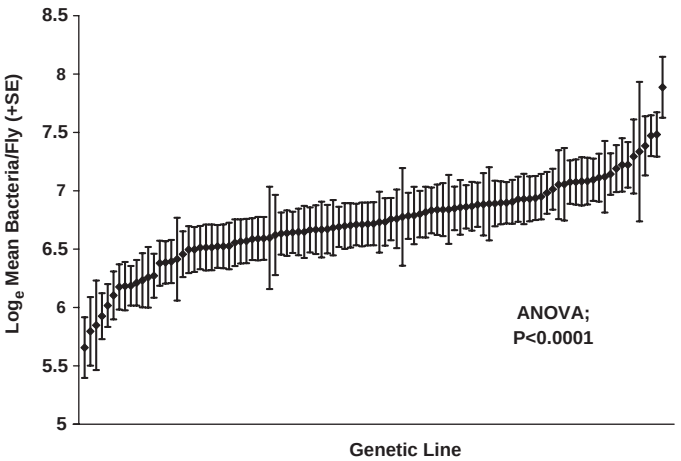

Fig. 1. Variability among *D. melanogaster* lines in the ability to suppress systemic growth of the Gram-negative entomopathogen *S. marcescens*. Bacterial load was estimated by counting viable *S. marcescens* colonies grown from flies homogenized at prescribed times after artificial infection (*13*). Genetic line was a highly significant determinant of bacterial load (ANOVA, $P < 10^{-4}$), with extreme lines differing by 10 phenotypic standard errors.

A Probability of Association with Phenotype by Chance

			Composite Probability	All times F	All times M	All times Pool	AM infected F	AM infected M	AM infected Pool	PM infected F	PM infected M	PM infected Pool
Pathogen Recognition	PGRP-SC1A	0.8 kb 3' of STOP (3771856)	0.050	0.978	0.040	0.204	0.482	0.157	0.929	0.079	0.402	0.264
	PGRP-SC1B	0.7 kb 3' of START (3775491)	0.001	0.827	0.001	0.020	0.653	0.050	0.604	0.615	0.001	0.013
	PGRP-SC2	1.1 kb 5' of START (3776631)	0.024	0.198	0.170	0.045	0.297	0.087	0.136	0.232	0.594	0.227
	SR-CI	exon 3 (4115944)	0.128	1.000	0.160	0.333	0.893	0.050	0.253	0.636	0.914	0.878
	SR-CII	intron 2 (7274899)	0.030	0.025	0.564	0.063	0.144	0.652	0.198	0.149	0.985	0.248
		425 bp 3' of STOP (7273320)	0.070	0.105	0.789	0.462	0.047	0.183	0.743	0.235	0.314	0.211
	SR-CIII	70 bp 3' of STOP (4114081)	0.006	0.226	0.097	0.109	0.023	0.033	0.001	0.752	0.893	0.970
	SR-CIV	intron 1 (3516282)	0.007	0.350	0.042	0.064	0.089	0.036	0.060	0.510	0.220	0.122
		exon 2 (3516084)	0.004	0.310	0.039	0.084	0.375	0.135	0.294	0.424	0.022	0.020
Toll-like	18-W	1.5 kb 5' of START (15174292)	0.023	0.103	0.333	0.133	0.217	0.644	0.638	0.109	0.206	0.047
		1.3 kb 5' of START (15174512)	0.134	0.127	1.000	0.770	0.049	0.311	0.981	0.555	0.700	0.461
	Tehao	1.0 kb 5' of START (13423707)	0.116	0.554	0.470	0.245	0.625	0.121	0.043	0.956	0.429	0.795
	Toll-4	2.0 kb 5' of START (9074348)	0.116	0.493	0.226	0.698	0.631	0.725	0.711	0.411	0.021	0.270
		1.8 kb 5' of START (9074527)	0.039	0.267	0.151	0.899	0.673	0.365	0.794	0.020	0.020	0.642
Intracellular Signaling	cactus	1.2 kb 5' of START (16304218)	0.001	0.002	0.557	0.064	0.007	0.780	0.119	0.006	0.431	0.076
		intron 2 (16302377)	0.077	0.096	0.437	0.754	0.019	0.469	0.505	0.208	0.429	0.779
		intron 4 (16294464)	0.006	0.277	0.071	0.109	0.517	0.868	0.968	0.198	0	0.002
	DIF	intron 1 (17399824)	0.001	0.093	0.080	0.015	0.026	0.343	0.030	0.835	0.097	0.249
		intron 1 (17398373)	0	0.411	0	0.013	0.987	0.008	0.073	0.196	0.048	0.066
		intron 1 (17399732)	0.001	0.093	0.080	0.015	0.026	0.343	0.030	0.835	0.097	0.249
	IK2	1.4 kb 5' of START (20644626)	0.004	0.202	0.074	0.058	0.128	0.213	0.037	0.471	0.113	0.126
		1.4 kb 5' of START (20644684)	0.002	0.126	0.034	0.029	0.079	0.194	0.035	0.473	0.047	0.074
		exon 3 (20647314)	0.035	0.121	0.167	0.064	0.339	0.703	0.640	0.501	0.043	0.170
	imd	1.4 kb 5' of START (13476537)	0	0.187	0.054	0.030	0.150	0.104	0.076	0.189	0.186	0.061
		410 bp 5' of START (13475539)	0.003	0.133	0.188	0.045	0.066	0.056	0.012	0.398	0.425	0.836
		310 bp 5' of START (13475440)	0.006	0.087	0.352	0.054	0.031	0.165	0.009	0.626	0.506	0.986
		150 bp 5' of START (13475280)	0.006	0.075	0.174	0.034	0.016	0.397	0.005	0.397	0.827	0.407
AMP	Attacin C	intron (8458816)	0.030	0.162	0.167	0.070	0.112	0.402	0.247	0.607	0.092	0.237
	Diptericin A	1.3 kb 5' of START (13928254)	0.062	0.313	0.393	0.332	0.042	0.869	0.053	0.174	0.385	0.699
		1.2 kb 5' of START (13928294)	0.045	0.053	0.221	0.563	0.181	0.799	0.802	0.146	0.154	0.632

B Percent of Phenotypic Variation Explained

	All times F	All times M	All times Pool	AM infected F	AM infected M	AM infected Pool	PM infected F	PM infected M	PM infected Pool
PGRP-SC1A		1.6%							
PGRP-SC1B		13.3%	4.8%		2.8%			7.0%	7.5%
PGRP-SC2			0.0%						
SR-CI					4.2%				
SR-CII	2.0%								
				0.0%					
SR-CIII				0.0%	3.6%	2.2%			
SR-CIV		0.3%			1.4%				
		1.0%						1.0%	5.2%
18-W									0.0%
				0.8%					
Tehao						4.5%			
Toll-4								5.0%	
							3.9%	1.4%	
cactus	1.9%			1.6%			4.5%		
				2.0%					
								5.6%	12.1%
DIF			0.0%	0.0%		0.0%			
		8.1%	4.9%		4.6%			2.8%	
			3.1%	2.1%		3.1%			
IK2						10.8%			
		0.3%	0.0%			0.3%		0.8%	
								4.8%	
imd			2.3%						
			0.1%			0.0%			
				10.4%		16.4%			
			1.4%	1.8%		1.4%			
Attacin C				0.0%					
Diptericin A									

Fig. 2. (**A**) Sites in candidate genes that are significantly associated with variation in bacterial growth suppression in males, females, or pooled-sex analyses from flies infected in circadian AM and/or PM, representing a total of nine phenotype constructions. Several sites have effects that are limited by sex or environment (circadian day). The composite probability is derived from a comprehensive assessment of the multiple combinations of sex and circadian time (*13*). Associations that have a comparison-wise significance between 0.05 and 0.01 are shaded in blue. Associations with a null probability smaller than 0.01 are shaded in red. Numbers in parentheses are the unique nucleotide coordinates from Release 3.1 of the *D. melanogaster* genome sequence assembly (*25*). More detail on each polymorphism is presented in fig. S1; disequilibrium relationships among sites are illustrated in fig. S2. (**B**) Percentage of the phenotypic variance explained by each site. Percentages shown are of the observed phenotypic variance attributable to genetic line. Because the statistical significance of an association is partially dependent on allele frequency, the rank orders of strength of association and percent of variance explained are not equivalent.

ic information (*12*, *13*). More than 100 kb of unique sequence encompassing coding regions, introns, and 1 to 2 kb of upstream putative regulatory sequence were surveyed for polymorphism, with 127 of the uncovered sites typed across all 101 test lines (*13*) (fig. S1). Linkage disequilibrium among typed markers was generally nonsignificant, with the exception of a small number of sites showing intralocus correlations (fig. S2). The effects of the 127 genotyped markers in the 21 candidate genes on mean bacterial load were tested for each of the 9 phenotypic constructions: male, female, or pooled-sex flies in each or both circadian periods (*13*). Thirty polymorphic sites were detected in 16 genes at which allelic state was significantly associated with variation in systemic *S. marcescens* load (Fig. 2A) in at least one phenotypic construction. Most of these markers explain less than 10% of the phenotypic variance observed (Fig. 2B), although that variance may be underestimated if the typed markers are in linkage disequilibrium with but are not themselves the sites causal to the polymorphism. The five genes that had no polymorphic sites associating with variability in resistance to *S. marcescens* all encoded antibacterial peptides. Many of the polymorphic markers that associated with variability in bacterial load showed effects that were limited by sex or circadian time of day, although this may partially stem from differences in statistical power when the data are subdivided. To generally assess the broader relationship between candidate gene markers and the immunity phenotypes observed, a composite probability was generated for each site based on the strength of association in the nine subphenotypes. These composite probabilities were similar to those calculated from the data set pooled across sexes and circadian times but were less sensitive to statistical effects resulting from sex- or circadian-limited genetic effects. Fifteen sites among the 127 immune gene polymorphisms had a composite *P* value smaller than 0.01, the vast majority of which were located in genes involved in pathogen recognition and intracellular signaling (Fig. 2A).

To detect potential epistatic interactions among sites and loci, associations between bacterial load and genotypes consisting of all observed pairs of sites were tested. Of 5815 pairwise tests conducted, 392 were significant at a nominal $P \leq 0.05$, compared with 291 that would be expected by chance (Fig. 3A). This suggested a partial nonadditive nature of the observed allelic effects. The preponderance of significant epistatic interactions were detected among intracellular signaling loci and between intracellular signaling loci and antimicrobial peptide genes (Fig. 3B). This contrasted with the single-site analyses, where polymorphism in antibacterial peptides was generally nonsignificant (Fig. 2A) and highlights the importance of considering genetic background when measuring genetic effects.

Several conclusions about the structure of naturally occurring variation in *D. melanogaster* innate immunity can be drawn from this work. First, the continuous and normally distributed phenotypic variation among lines (Fig. 1) and the generally small contributions of individual markers to the total genetic

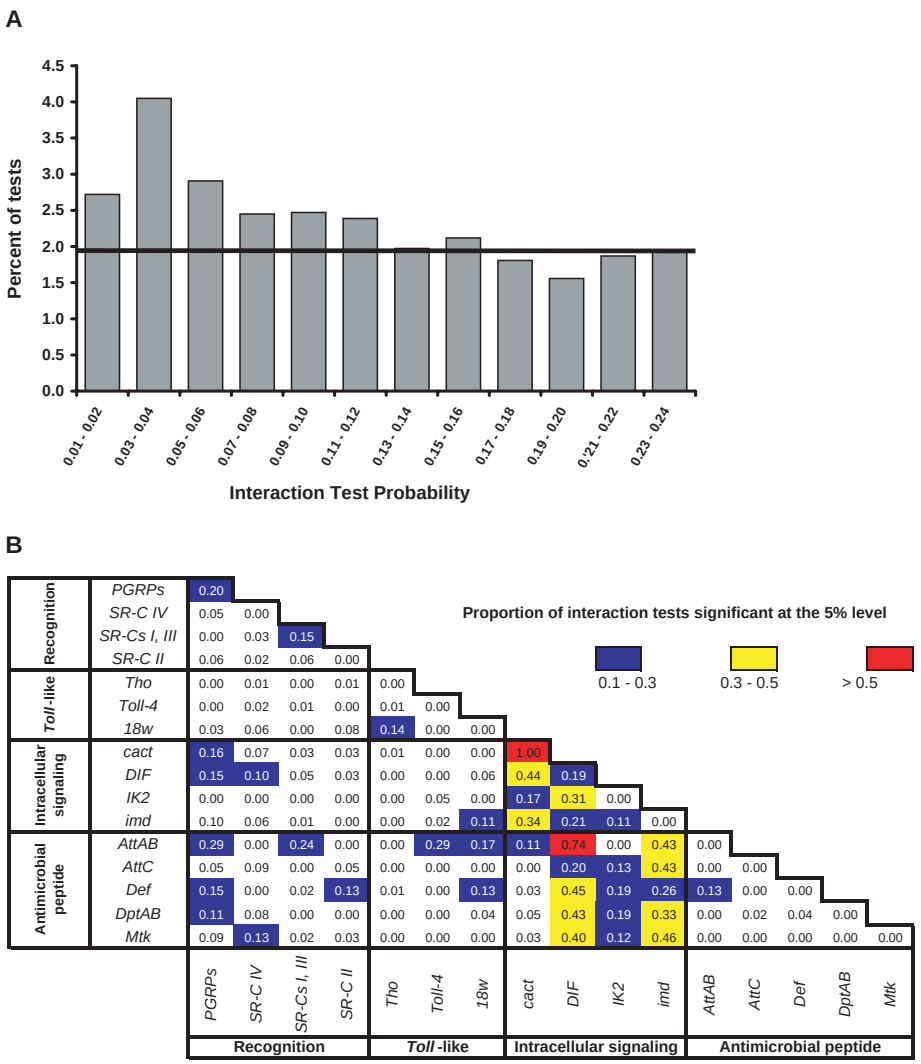

Fig. 3. (**A**) Distribution of tail *P* values for two-site interaction tests. The proportion of interaction tests in each probability class is plotted in the histogram. The horizontal line indicates the expected proportion of tests in each class under the null hypothesis of no biological interactions. (**B**) Distribution within and among the loci of two-site interaction tests significant at or below a nominal $P = 0.05$. Red boxes indicate that more than half of the two-site interaction tests performed across a pair of loci are significant at the 5% level. Locus pairs at which 30 to 50% of the tests were significant are shaded in yellow, and pairs at which 10 to 30% of the tests were significant are shaded in blue.

Table 1. Distribution of molecular markers among candidate loci. "Cyt. pos." is the cytological position of each locus. Length of the survey region is measured in kilobases. "Sites" is the number of polymorphic markers genotyped within each locus, without regard to linkage relationships among markers. Genic locations and site genotypes in each line are available in fig. S1. Disequilibrium relationships among markers are illustrated in fig. S2.

Locus	Cyt. pos.	Length (kb)	Sites
Pathogen recognition			
PGRP-SC1A, -SCIB, -SC2	44E	10.0	10
SR-CI, SR-CIII	24D	4.3	13
SR-CII	48E	4.4	7
SR-CIV	23F	4.6	7
Toll-like receptors			
Toll-4	30A	6.8	8
Tehao	34C	7.8	16
18-Wheeler	56F	7.6	6
Signal transduction			
DIF	36C	20.7	8
cactus	35F	14.8	7
IK2	38D	5.1	5
Immune deficiency	55C	4.0	7
Antibacterial peptides			
Attacin AB	51C	4.9	8
Attacin C	50A	3.1	7
Defensin	46D	1.4	5
Diptericin AB	55F	4.7	7
Metchnikowin	52A	1.8	6

variance imply that most naturally occurring variation in immunocompetence is derived from mutations of minor effect. However, mutations of larger effect (explaining 10 to 15% of the measured phenotypic variance) can be found segregating in intracellular signaling molecules (Fig. 2B). Second, the polymorphic sites most significantly associated with variance in suppression of *S. marcescens* are found in pathogen recognition and intracellular signaling molecules, with antibacterial peptide genes harboring markedly few polymorphisms that produce individually significant effects. The apparent strength of influence of recognition and signaling molecules may result from the regulatory control these proteins exert over a variety of immune-related cellular processes (*1*, *2*, *15*). In particular, because activation of *Drosophila* immune signaling pathways induces the expression of components of those pathways (*16*, *17*), small changes in expression or activity of signaling components may show an amplified effect on the phenotype. In contrast, because antimicrobial peptides are the most downstream targets of immune regulation and are partially redundant (*18*), these genes might be expected to make weaker contributions to phenotypic variability. Third, although polymorphic markers were typed in both transcribed and regulatory regions of candidate genes (fig. S1), many of the significantly associated sites are located upstream of protein-coding sequences. Because the markers may not themselves be the sites causing phenotypic variability, it is possible that upstream markers are in linkage disequilibrium with untyped amino acid variants in the coding region. However, it is also likely that variation in transcriptional regulation of immunity genes may influence a substantial proportion of observed phenotypic variation. This hypothesis is particularly attractive in light of the epistatic interactions observed between intracellular signaling molecules and antimicrobial peptide genes.

D. melanogaster immune competence and the phenotypic effects of genetic polymorphism were found to vary over the circadian day. Notably, a complex of sites upstream of the *imd* gene showed strong associations with bacterial load sustained by AM-infected flies, but not by PM-infected flies, with one site explaining 16.4% of the AM phenotypic variance. *imd* is an important regulator of the response to Gram-negative bacteria. Circadian cycling of the expression of immune-related genes, including *imd*, has been previously documented in the absence of immune challenge (*14*). The current results suggest that circadian cycling could have an important genetic influence on the immunocompetence of *D. melanogaster* in the wild. Interactions between genotype and sex or environment in *D. melanogaster* quantitative traits are not unprecedented, as studies of sensory bristle number, wing shape, and longevity have previously identified multiple quantitative trait loci that are either strongly influenced by or completely dependent on sex and/or environment (*19*–*22*).

It is not apparent why phenotypically strong mutations in a vital trait such as immune competence are allowed to persist in the population. Potentially, these may exist in mutation-selection-drift balance, as the result of other physiological trade-offs (*23*, *24*) or as polymorphisms balanced against multiple environmental pathogens. Further study of sequence level and phenotypic variability in innate immune systems is warranted to address this question.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/303/5665/1873/DC1
Materials and Methods
Figs. S1 and S2
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Impact of Nitrogen Deposition on the Species Richness of Grasslands

Carly J. Stevens,[1,2]* Nancy B. Dise,[1,3] J. Owen Mountford,[2] David J. Gowing[4]

A transect of 68 acid grasslands across Great Britain, covering the lower range of ambient annual nitrogen deposition in the industrialized world (5 to 35 kg N ha^{-1} $year^{-1}$), indicates that long-term, chronic nitrogen deposition has significantly reduced plant species richness. Species richness declines as a linear function of the rate of inorganic nitrogen deposition, with a reduction of one species per 4-m^2 quadrat for every 2.5 kg N ha^{-1} $year^{-1}$ of chronic nitrogen deposition. Species adapted to infertile conditions are systematically reduced at high nitrogen deposition. At the mean chronic nitrogen deposition rate of central Europe (17 kg N ha^{-1} $year^{-1}$), there is a 23% species reduction compared with grasslands receiving the lowest levels of nitrogen deposition.

Conservation of biodiversity underpins some of the largest and most ambitious environmental legislation in the world (*1*). Most of the focus of this legislation is on mitigating damage to ecological communities from direct environmental degradation such as land clearing. Although chronic "low-level" stresses such as air pollution are also considered to damage biodiversity, their actual importance is not well understood.

Because nitrogen (N) is the limiting nutrient for plant growth in many terrestrial ecosystems (*2*), atmospheric deposition of reactive N has the potential to reduce plant species richness (the number of species in a given area—an important component of biodiversity) through favoring species bet-

[1]Department of Earth Sciences, The Open University, Milton Keynes MK7 6AA, UK. [2]National Environment Research Council Centre for Ecology and Hydrology, Monks Wood, Huntingdon PE17 2LS, UK. [3]Department of Biology, Villanova University, Villanova, PA 19085, USA. [4]Department of Biological Sciences, The Open University, Milton Keynes MK7 6AA, UK.

*To whom correspondence should be addressed. E-mail: c.j.stevens@open.ac.uk

"Genetic Basis of Natural Variation in *D. melanogaster* Antibacterial Immunity"

Brian P. Lazzaro, Bonnielin K. Securman and Andrew G. Clark

MATERIALS AND METHODS

Drosophila and *Serratia* stocks

Each *Drosophila melanogaster* line is homozygous for an independent chromosome 2 isolated from a wild population in Pennsylvania, USA, and crossed into a common genetic background. The lines were established with distinct wild female *Drosophila melanogaster*, collected in State College, Pennsylvania, USA, in 1998 and 1999 and placed in individual vials to oviposit. F1 or F2 male progeny were mass-mated to females from a highly inbred *SM5/Pm; spa*^{pol} balancer stock. Individual male progeny from this cross were backcrossed to females from the same balancer stock, and the resulting offspring were selectively sib-mated to eliminate *SM5* and create a set of lines each homozygous for an independent, naturally occurring second chromosome *(S1)*. Males from 101 of these lines were recurrently backcrossed to females from the inbred *SM5/Pm; spa*^{pol} balancer stock for 5 generations to replace the background chromosomes X, 3, and 4. In a 6th backcross generation, virgin females from each line were crossed to males from the balancer stock to replace the Y chromosome. Progeny from these crosses were sib-mated, and flies carrying the *SM5* chromosome selectively eliminated in the following generation to restore the wild-derived chromosome to homozygous state. These lines continue to be maintained as homozygous *spa*^{pol} to a guard against contamination. Thus, the 101 lines share the same X, Y and 4th chromosomes, and

should, on average, be greater than 99% co-isogenic for the third chromosome. Any genetic variation segregating among the 101 experimental lines is attributable to second chromosome loci, with the background chromosomes contributed by the *SM5* balancer stock used in extraction.

Our *Serratia marcescens* strain is derived from ATCC Strain 13315, but we suspect that it has fixed a virulence attenuation mutation in our lab. Mortality of flies infected with a saturated overnight culture is approximately 80% over the course of a week, with negligible mortality within the first 48 hours (data not shown).

Determination of Systemic Pathogen Load

Flies were maintained at 24^{o}C under 12-hour light-dark cycles prior to and during infection experiments. Fresh inoculates of *S. marcescens* from a constant source culture were grown to saturation in 2 ml LB broth, then diluted with sterile LB broth to an optical density of $A_{600} = 1.0 \pm 0.1$ for each day of infecting. Adult flies were manually infected at four to eight days post-eclosion by piercing the thorax with a 0.1 mm dissecting pin coated in liquid *Serratia* culture. This procedure delivers approximately 10^3 bacteria to each fly (data not shown). Flies were anesthetized with CO_2 and kept under light CO_2 flow for 2-4 minutes during the infection procedure. Pathogen load was measured by homogenizing pairs of flies in 500 ul LB broth and quantitatively plating 25 ul homogenate on LB agar plates with an Autoplate 4000 spiral plater (Spiral Biotech, Bethesda, MD). Plates were grown at 37^{o}C until colonies were visible, then the plates were scanned and the density of colony forming units (CFU) in the homogenates was back-calculated with the Q-Count detection system (Spiral Biotech, Bethesda, MD).

Homogenates from flies sham-infected with a sterile needle always failed to yield colonies. In total, more than 16,000 flies were infected and colony counts were obtained from 6924 plates.

SNP Discovery and Genotyping

More than 100 kb of unique sequence encompassing coding regions, introns and 1-2 kb upstream of putative regulatory sequence were surveyed for polymorphism at each of the 21 candidate genes in 12 *D. melanogaster* lines previously described *(S1)*. Precise limits of survey regions and primer sequences are available upon request from BPL. 127 polymorphisms ascertained from sequencing were genotyped in the entire set of lines, primarily by fRFLP *(S2)* and pyrosequencing (Pyrosequencing AB, Uppsala, Sweden). Genotypes of all 101 lines at all typed polymorphisms, as well as phenotypic measures, are listed in Supplementary Figure 1.

Statistical analysis

Per-plate colony counts were $\log_e$-transformed and provided an adequate fit to a normal distribution. Mixed linear models were evaluated using SAS Stat software (SAS Institute, Cary, NC). Associations between bacterial load and genotypes at individual polymorphic sites were tested using the mean trait value of each line as a response variable in a series of linear models where the sole independent variable was the state at each polymorphic site. Critical values were determined by re-testing the linear models on 10,000 random permutations of the mean trait values against the marker genotypes. The single highest *F*-ratio from each permutation was retained at each site to generate a

comparisonwise null distribution to which the true data were compared *(S3)*. *F*-ratios and permutation tests were calculated independently for each of the 9 phenotypic combinations of sex and circadian day. A composite probability was generated for each site by summing the natural logs of the *F*-ratios from all 9 subphenotypes. Critical values for the composite probabilities were generated by permuting the *F*-ratios across sites and repeating the summing operation. For tests of epistasis, mean *Serratia* load was used as the response variable, with the independent variables being the two-site genotypes of all possible pairs of sites in the data. One thousand permutations were used to obtain critical values for the interaction terms.

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Supplemental Figure S1. Phenotypic measures and marker genotypes for all 101 2nd chromosome substitution lines at all typed polymorphisms. Phenotypic measures are least-square means obtained after correcting for incidental sources of variation. Marker positions are identified both by their genic positions in candidate loci and by their unique coordinates in the Release 3.1 assembly of the *D. melanogaster* genome sequence *(25)*. Missing data are indicated with a question mark.

Supplemental Figure S2. The statistical significance of linkage disequilibrium among genotyped marker pairs across all loci, calculated using Fisher's Exact test. Site pairs are shaded in blue if allelic states are correlated at an uncorrected $P < 0.05$, and in red if $P < 0.01$. Marker positions are identified both by their genic positions in candidate loci and by their unique coordinates in the Release 3.1 assembly of the *D. melanogaster* genome sequence *(25)*.

Microsoft Excel versions of the Supplemental Figures may be found online at http://www.entomology.cornell.edu/Faculty_Staff/Lazzaro