1 **Dominance reversal maintains large-effect resistance polymorphism in temporally varying**

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Abstract

A central challenge in evolutionary biology is to uncover mechanisms maintaining

functional genetic variation[1]. Theory suggests that dominance reversal, whereby alleles

subject to fluctuating selection are dominant when beneficial and recessive when

deleterious, can help stabilize large-effect functional variation in temporally varying

environments[2,3]. However, empirical evidence for dominance reversal is scarce because

testing requires both knowing the genetic architecture of relevant traits and measuring the

dominance effects on fitness in natural conditions[4]. Here, we show that large-effect,

insecticide-resistance alleles at the *Ace* locus in *Drosophila melanogaster*[5,6] persist

worldwide at intermediate frequencies and exhibit dominance reversal in fitness as a

function of the presence of an organophosphate insecticide. Specifically, we use laboratory

assays to show that the resistance benefits driven by these alleles are dominant, while the

associated costs are recessive (or codominant). Further, by tracking insecticide resistance

and genome-wide allele frequencies in field mesocosms, we find that resistance and

resistant alleles increase and then decrease rapidly in response to an insecticide pulse but

are maintained at low frequencies in the absence of pesticides. We argue that this pattern is

only consistent with beneficial reversal of dominance. We use Wright's theory of

dominance[7] to hypothesize that dominance reversal should be common in general if the

environmental shifts that make alleles deleterious also make them behave effectively as

loss-of-function and thus recessive alleles . We also use the known haplotype structure of

mesocosm populations to establish that the insecticide pulse generates chromosome-scale

genomic perturbations of allele frequencies at linked sites. Overall, our results provide

compelling evidence that this mechanism can maintain functional genetic variation and

enable rapid adaptation to environmental shifts that can impact patterns of genomic

variation at genome-wide scales via linked fluctuating selection.

Main text

The maintenance of functional genetic variation at large-effect loci for fitness-related traits in natural populations is a fundamental paradox[8,9]. Directional selection should in principle rapidly erode such variation. Yet many systems show persistence of such large effect loci at intermediate frequencies suggesting the action of some form of balancing selection[10,11]. A particularly striking example comes from studies of seasonal dynamics in *Drosophila*, which revealed the presence of large-effect seasonal polymorphisms[12,13] that rapidly respond to environmental fluctuations shifting between advantageous to deleterious multiple times during a single seasonal cycle[14,15]. Some of these seasonal alleles have strong enough selective effects that they can change frequencies by tens of percent per season, yet still remain polymorphic over hundreds of thousands or even millions of generations[12]. Polymorphisms of large effect responding to fluctuating selection have been observed in a wide range of other systems including other insects[16–19], fish[20,21], mammals[22,23], and plants[24]. In all these examples the alternative alleles are subject to strong antagonistic and fluctuating selection that could help maintain large-effect polymorphisms in the overall population by generating effective overdominance. Theoretical models suggest that fluctuating selection can indeed stabilize genetic variation in the face of temporally varying selection especially if combined with beneficial dominance reversal[2,3,25].

Under beneficial dominance reversal, alleles are partially or completely dominant when beneficial, but then become partially or completely recessive when deleterious[26,27]. This mechanism acts as a stabilizing force because it protects recessive deleterious alleles from selection and slows down their loss. At the same time, it allows a rapid response to selection

shifts, as adaptive alleles are always dominant and thus quickly respond to selection even when

rare. Even modest dominance reversals have been shown to protect variation under fluctuating

selection in single- or multiple-locus models[3]. However, it remains unknown how often the

beneficial dominance reversal occurs in nature for functional polymorphic large-effect loci and

whether it arises naturally within genetic systems. The scarcity of relevant adaptive traits with a

known genetic architecture, and the challenges of measuring fitness and dominance effects in

natural environments, make these questions difficult to address.

Importantly, models of fluctuating selection that incorporate beneficial dominance

reversal of large-effect alleles predict stable, large-amplitude fluctuations at the adaptive locus

and thus could drive significant effects on linked genomic variation[28,29]. These effects on linked

sites can extend across large genomic distances, with their magnitude directly proportional to

how rapidly the adaptive allele frequencies shift[30]. These long-range effects of strong fluctuating

selection have rarely been quantified, as the haplotype structure of adaptive alleles at a selected

locus is typically not known and the linked selection becomes virtually undetectable in standing

genetic data for old and common alleles present on multiple haplotypes.

Here we investigate the dominance of large-effect alleles conferring resistance to

organophosphate insecticides at the *Ace* locus in natural populations of *Drosophila melanogaster*

both in organophosphate-rich and -free environments. *Ace* encodes acetylcholinesterase (AChE),

a key enzyme in the insect nervous system responsible for breaking down acetylcholine[31].

Organophosphate insecticides, introduced in the 1950s and 1960s, act as irreversible inhibitors of

AChE, causing insect death by binding to its active site and preventing removal of acetylcholine

from the synapses upon firing[32]. These insecticides have imposed seasonally fluctuating selection

pressures on *D. melanogaster* populations, as seen in seasonal resistance fluctuations in field

studies[33,34]. Therefore, this case provides a compelling example of a large-effect polymorphism

subject to fluctuating selection, in which the causal locus, the mechanistic basis of selection, and

the conditions that favor alternative alleles are well understood.

To understand how selection maintains resistant *Ace* alleles in nature, we first analyzed

genomic data from worldwide *D. melanogaster* populations from the Drosophila Evolution Over

Space and Time (DEST) database[35] and country-level pesticide use data from the Food and

Agricultural Organization of the United Nations (FAO)[36] for the period of 2000-2022. Previous

studies identified resistant *Ace* alleles in *D. melanogaster* with mutations at four conserved sites

near the AChE active site (I161V, G265A, F330Y, G368A), with <u>V</u>G<u>F</u>G and <u>VA</u>Y<u>G</u> being the

most common resistance haplotypes[5,6] (inset, **Fig. 1a**). Our analysis of two panels of inbred lines

collected in an orchard in Pennsylvania (LNPA[14]) and a farmer's market in North Carolina

(DGRP[37]) confirmed these as the most common resistant haplotypes in resistant *Ace* alleles

(**Extended Data Fig. 1a, b**). The LNPA population also carries the <u>V</u>GF<u>A</u> haplotype at ~13%

frequency. For our analysis, we used the shared I161V resistance polymorphism as a marker for

all three resistant *Ace* alleles.

We found evidence for spatially and temporally varying selection on the I161V

polymorphism. Spatially, its frequency increases with pesticide use intensity across countries

(linear mixed-effect model, p = 0.006) (**Fig. 1a, b**) and shows stronger spatial variation

compared to control SNPs matched for recombination and average frequency (p = 0.005) (**Fig.**

1c). Temporally, analyzing seasonal frequency patterns at Linvilla Orchards, a non-organic farm

where samples were collected in spring and fall between 2009 and 2015, we found a consistent

decline in I161V frequency when comparing available fall samples paired to the following spring

(generalized linear model, p = 0.005), suggesting either fitness costs in the absence of

insecticides during winter or seasonal migration (**Fig. 1d**). In addition, we found that the I161V

polymorphism shows stronger temporal fluctuations than control SNPs (p = 0.053) (**Fig. 1e).**

Figure 1

Figure 1. Organophosphate-resistant *Ace* alleles persist at intermediate frequencies in natural *Drosophila melanogaster* populations and respond to spatial and seasonal insecticide selection. a, Allele frequency map of I161V (pie charts), a marker of organophosphate-resistant *Ace* alleles, overlaid on insecticide use intensity (shading). Inset shows common resistant *Ace* alleles with three different resistance haplotypes (R1: VGFG, R2: VGFA, R3: VAYG) in *D. melanogaster* (See also **Extended Data Fig. 1** for frequencies of resistant haplotypes in DGRP and LNPA panels of fully sequenced inbred lines). **b,** I161V frequency increases with increased pesticide use (linear mixed-effect model). **c,** I161V shows higher variance in frequency compared to matched control SNPs across populations (orange dashed line indicates I161V). **d,** Seasonal changes in I161V frequency at Linvilla Orchards showing decline from fall (dark blue) to spring (pink) samples during insecticide-free periods (turquoise, mid-fall to mid-spring) (generalized linear model, mean ± s.e.m.). **e,** Cumulative frequency changes at Linvilla Orchards samples show elevated allele frequency fluctuations of I161V compared to matched control SNPs (orange dashed line indicates I161V).

115   To test for the beneficial dominance reversal of the resistant *Ace* alleles, we used a

116 laboratory-based approach to measure key fitness-associated phenotypes in organophosphate-

117 rich and -free environments (**Fig. 2a,b**). Using the LNPA panel of inbred lines (**Extended Data**

118 **Fig. 1a,b**), we took a round robin approach to generate recombinant populations with

119 homozygous sensitive, homozygous resistant, and heterozygous genotypes for each resistant

120 allele (S: IGFG, R1: <u>V</u>GFG, R2: <u>V</u>GF<u>A</u>, R3: <u>VAY</u>G) (see **Methods for details**). This approach[38]

121 allowed us to control for the genotype at the *Ace* locus while allowing diversity in the rest of the

122 genome, thus making more likely that associated phenotypic effects across recombinant

123 populations are driven by the variation at *Ace* locus itself.

124   We first estimated resistance to organophosphates using malathion LD_{50} values (dose

125 causing 50% egg-to-adult mortality). The resistance mutations alter the shape of AChE's active

126 site, preventing malathion binding[5]. The R2 and R3 *Ace* alleles significantly increased malathion

127 LD_{50} by ~5.8x and ~7.8x respectively ($p < 0.001$ for both), and were strongly dominant over the

128 sensitive S *Ace* allele, with dominance *h* of 0.78 and 0.77 for R2 and R3, respectively ($p < 0.001$

129 for $h > 0.5$ in both cases). The R1 *Ace* allele showed no significant increase in malathion

130 resistance ($p = 0.646$) with inconclusive dominance effects (p 0.751) (**Fig. 2, Extended Data**

131 **Fig. 2, Extended Data Table 1**). We expected resistance to be dominant because malathion

132 covalently binds to and inactivates the sensitive S *Ace* allele rendering it effectively loss-of-

133 function, while the resistant *Ace* alleles remain functional. This pattern follows Wright's

134 physiological theory of dominance[7], which predicts that functional alleles are dominant over

135 loss-of-function alleles, as even one copy of the functional allele often provides sufficient gene

136 activity to carry out the required function.

While these mutations provide resistance benefits, they also impair AChE activity and

stability[39], likely rendering them deleterious in the absence of insecticides[40]. We assessed the

cost of resistance in the absence of organophosphates by quantifying two key fitness-associated

phenotypes affected by impaired gene function: viability (egg to adult survival) and fecundity

(number of eggs laid by gravid females). All homozygous resistant genotypes exhibited lower

phenotypic fitness values compared to the homozygous sensitive genotype, for either or both of

these phenotypes, with viability reductions ranging from 12.4% to 16.9% (all $p < 0.001$) and

fecundity reductions from 16.2% (R2R2, $p = 0.37$) to 46.8% (R3R3, $p < 0.001$). The costs driven

by the three resistant alleles were either significantly recessive for at least one of the phenotypes

for the strongest resistance alleles R2 and R3 (viability R2: $h = 0.17$, $p = 0.027$, fecundity R3: h

$= -0.02$, $p = 0.001$), or indistinguishable from codominance for R1 (**Fig. 2g-l, Extended Data**

Tables 2-3). Overall, the sensitive S *Ace* allele is beneficial and dominant/codominant and never

recessive towards its resistant counterparts in terms of the costs in viability and fecundity in the

absence of malathion.

These results are consistent with the beneficial reversal of dominance. They also suggest

that the response to organophosphates at the concentrations similar to the LD_{50} of the sensitive

homozygous genotype should induce a very rapid increase in the frequency of the resistant *Ace*

alleles and in the level of overall resistance, given that resistant *Ace* alleles are strongly

beneficial and dominant. We also expect that after a reversal in selection pressure (removal of

insecticides), the trajectory of resistant alleles should depend on their starting frequency. When

initially rare, they should decline slowly due to their recessive costs hidden in heterozygotes.

When initially common, they should decline more rapidly as these recessive costs become

evident in homozygotes.

Figure 2

Figure 2. Beneficial reversal of dominance of organophosphate-resistant *Ace* alleles for fitness-associated phenotypes relevant to malathion-rich and malathion-free environments. **a-b**, Under the beneficial dominance reversal, R alleles should be beneficial and partially or completely dominant in malathion-rich environments ($W_{SR} > (W_{SS}+W_{RR})/2$, orange), and deleterious and partially or completely recessive in malathion-free environments ($W_{SR} < (W_{SS}+W_{RR})/2$, blue). **c,** Selection (*s*) and dominance (*h*) coefficients for each fitness-associated phenotype are estimated based on the viability selection model. If s>1, the resistant R *Ace* allele is beneficial; if s<1, it is deleterious. If h=1, the resistant R *Ace* allele is dominant to the sensitive S allele; if h=0, the reverse is true. **d-l**, Comparison of malathion LD_{50} (**d,e,f**), egg-adult viability (n=10 technical replicates, 50 eggs/replicate) (**g,h,i**), and fecundity (n=8–10 technical replicates, 5 females/bottle/3 days) (**j,k,l**) among *Ace* genotypes. Malathion LD_{50} was calculated from a four-parameter logistic model fitted to egg-adult viability measurements on media with a range of malathion concentrations (n=2 technical replicates, 50 eggs/replicate, see **Extended Data Fig. 2**). Egg-adult viability and fecundity data were analyzed with logistic and linear regression models.

To test these predictions, we directly investigated the population dynamics at the *Ace*

locus in an ecologically realistic environment by evolving replicate *D. melanogaster* populations

in the presence and absence of seasonally fluctuating malathion in field mesocosms at an

experimental orchard in Pennsylvania (**Fig. 3a,** see **Methods**). We established 20 replicate

populations derived from a baseline population constructed from the LNPA panel of 76 inbred

lines used in our lab experiments (**Extended Data Fig. 1**). These lines were outbred for 4

generations in large indoor cages before ~1000 males and females were used to seed each

outdoor cage (see **Methods**). After ~4 weeks of untreated propagation, we divided the

populations into ten malathion-treated and ten untreated outdoor cages. To mimic natural

insecticide exposure, we applied stepwise increasing concentrations of commercial malathion in

the growth media of the treated populations (1.5-2.5 ppm from July 21 to August 24, 2021,

followed by 7.5 ppm until September 6, 2021). These doses exert strong selection for resistant

Ace alleles based on our lab LD_{50} measurements (**Extended Data Fig. 2**). Following a reversal

of selection pressure (removal of malathion), the experiment continued without insecticides in all

20 cages through December 22, 2021. Throughout the experiment, we tracked population

dynamics, malathion resistance, and genome-wide allele frequencies at eight timepoints (~every

one to two generations), focusing on trajectories of resistant *Ace* alleles and linked sites in

response to fluctuating malathion treatment (**Fig. 3a**).

Figure 3

Figure 3. Beneficial reversal of dominance of resistant *Ace* alleles for fitness in an ecologically realistic environment. a, Twenty field mesocosm cages were seeded with an outbred population of *D. melanogaster* generated via outcrossing for 4 generations 76 inbred lines carrying the sensitive S and resistant R1, R2, and R3 Ace alleles. Ten cages received a malathion pulse after the initial population establishment. Eight timepoints were sampled to track the evolution of malathion resistance and genome-wide allele in the treated and untreated populations. Malathion did not affect estimated adult fly population sizes (mean ± s.e.m.) in treated populations. **b,** The malathion pulse led to a significant increase and subsequent decline of resistance (LD_{50}, ppm) in the treated populations while resistance remained stable in the untreated populations. For LD_{50} calculations see **Methods** and **Extended Data Fig. 3**. Light lines/points show individual populations (orange, treated; blue, untreated), dark lines/points represent mean ± s.e.m. (n=10 populations per treatment group). **c-d,** Trajectories of *Ace* alleles in treated (**c**) and untreated (**d**) populations. In treated populations, the malathion pulse led to a significant increase in the frequency of the resistant R2 and R3 *Ace* alleles, followed by a decline in the frequency of R3. *Ace* allele frequencies remained stable in the untreated populations. Light lines/points show individual allele trajectories, dark lines/points represent the mean ± s.e.m. (n = 10 populations per treatment group). (**continued on next page**)

Figure 3 (continued). e, Inferred selection (s) and dominance (h) coefficients from changes in the resistant *Ace* allele frequency. The colored ranges show the selection coefficients corresponding to the 0.95 confidence intervals of the slope distribution, obtained by bootstrapping cages within each treatment group. The overlap between the orange (treated cages) and dark blue (untreated cages) restricts the possible values of *s* and *h*. Within that overlap, lower values of *h* better explain both sets of data, as shown by higher values of the joint likelihood calculated from the bootstrap distributions (see **Methods**). All results of statistical tests can be found in **Extended Data Tables 4-6**.

The first surprising result was that the seasonal population dynamics were qualitatively

similar in both malathion-treated and untreated cages (**Fig. 3a**). In all cages, population sizes

increased through the summer months until reaching maximum size and then slowly declined in

late Fall as abiotic conditions worsened. We interpret the unchanged seasonal population

dynamics as an indication that population size in all cages is primarily limited by high levels of

pre-adult mortality which masks the additional mortality imposed by malathion.

To characterize malathion resistance, we tracked egg to adult survival on diets with 0-15

ppm malathion and estimated LD_{50} at each timepoint per population (see **Methods** and

Extended Data Fig. 3). In malathion-treated cages, resistance increased rapidly from ~2.2 ppm

to ~12.6 ppm during treatment (t = 24.26, p < 0.001), followed by a significant decline to ~10.6

ppm after removal of malathion (t = -3.96, p = 0.001) (**Fig. 3b, Extended Data Table 4.1**). In

contrast, resistance levels in untreated cages remained stable at ~2.2 ppm throughout the

experiment (t = 1.07, p = 0.288) (**Fig. 3b, Extended Data Table 4.2**).

Using a haplotype-aware method to analyze pooled sequencing data[41], we inferred the

frequency dynamics of the sensitive and resistant *Ace* alleles (see **Methods**). In treated cages, the

resistant *Ace* alleles R2 and R3 increased significantly during malathion treatment (R2: t = 13.93,

p < 0.001; R3: t = 27.59, p < 0.001) with the speed of change consistent with the selection

coefficients (*hs*) on the order of 150-200% per generation, with R3 subsequently declining post-

treatment (t = -5.18, p < 0.001) (**Fig. 3c, Extended Data Table 5**). The R1 allele did not increase

significantly during the malathion phase, consistent with our lab data showing that it confers no

detectable malathion resistance (**Fig. 2c, Extended Data Table 5**). In untreated populations, all

resistant *Ace* allele frequencies did not show detectable change in frequency (**Fig. 3d, Extended**

Data Table 6).

To assess the contribution of the resistant *Ace* alleles to malathion resistance evolution in

the field mesocosms, we applied a viability selection model parametrized by lab-measured *Ace*

genotype viabilities (see **Methods**). This model closely recapitulated both the allele frequency

trajectories (**Extended Data Fig. 4a,b**) and the evolution of malathion resistance (**Extended**

Data Fig. 4c,d) observed in the field mesocosms. The agreement between model predictions and

experimental data provides compelling evidence that *Ace* is the major effect locus conferring

malathion resistance in the treated cages.

The results from the field mesocosms were qualitatively consistent with our expectations.

The *Ace* genetic system exhibited two key behaviors, rapid response to malathion treatment and

maintenance of resistance at low levels in its absence. In the malathion-treated cages, we

observed a significant decline of high-frequency resistant *Ace* alleles post malathion treatment,

indicating considerable fitness costs for resistant alleles when common. In contrast, untreated

cages showed fairly stable frequencies of low-frequency resistant alleles, suggesting low

selection coefficients (s) or dominance parameters (h) in the absence of malathion.

To obtain bounds on evolutionary parameters, we fit the allele frequency data of the

treated and untreated cages to a viability selection model where all resistant alleles share the

same fitness cost (see **Methods**). The model that fits both datasets best had low cost ($s \sim 2.5$ to

5%) and low dominance values ($h < 0.38$) (**Fig. 3e**). At higher values of h, there is no single

selection coefficient that can explain the patterns of allele frequency change for both treated and

untreated cages, and the likelihood of the data is higher the closer h is to zero. Overall, the data

are consistent with the fitness cost of organophosphate resistance being recessive and low,

allowing the resistant *Ace* alleles to persist at low frequencies for extended periods of time.

Such rapid frequency fluctuations of the *Ace* alleles are expected to affect variation at

linked genetic regions. The *Ace* genetic system presented us with a rare opportunity to study

these effects directly as the resistant alleles at the *Ace* locus are well-defined, the key resistant R2

and R3 *Ace* alleles increased in frequency during the malathion treatment and declined

afterwards, and the linkage patterns of genome-wide SNPs with the *Ace* alleles at the beginning

of the experiment are known from our inbred line genotypes. To characterize the extent of

forward and reverse sweeps driven by the R2 and R3 alleles during and after malathion

treatment, respectively, we used a two-step approach (see **Methods** for details). First, we

employed quasibinomial logistic regression to estimate genome-wide allele frequency changes

during malathion treatment (July 26 to September 21, 2021) and post-treatment (September 21 to

December 22, 2021). Next, we quantified changes in allele frequencies in SNPs tightly linked to

the sweeping R2 and R3 *Ace* alleles across the genome ($r^2 > 0.03$ and frequency > 60% in

resistant lines, *Ace*-linked) and those that were largely unlinked ($r^2 < 0.01$, control).

During the malathion treatment, we observed a virtually chromosome-wide forward

selective sweep driven by the R2 and R3 *Ace* resistant alleles in the malathion-treated

populations (**Fig. 4a**). This is consistent with the extremely rapid rise of the *Ace* alleles over 2-4

generations and the maximum recombination rate of ~5cM/Mbp in *D. melanogaster*, implying

the effects conservatively extending over a region of ~5-10 Mbp. The effect sizes of *Ace*-linked

sites were consistently elevated above the control sites across the chromosome arms 3L and 3R.

The sweep in the 3R chromosome was located from ~4Mbp to 17Mbp (~13Mbp wide), which

includes the *Ace* locus at 9.6Mb and extended to the 3L chromosome from ~5Mbp to 19Mbp

(~14Mbp wide). Note that, around position 19.5Mb on chromosome arm 3L control sites showed

a strong directional perturbation while *Ace*-linked sites uniformly decreased in frequency,

possibly suggesting the presence of an additional locus responding to malathion treatment that

interfered with the *Ace* sweep (**Fig. 4a,** asterisk**)**. Similarly, another putative locus was detected

on chromosome arm 2R, where both *Ace*-linked and control sites showed comparable frequency

perturbations (**Fig. 4a** asterisk). The study of these additional adaptive loci to malathion

treatment is beyond the scope of this work. As expected, in the untreated populations where the

frequencies of the R2 and R3 *Ace* alleles remained stable (**Fig. 3d**), *Ace*-linked sites showed no

elevated frequency changes compared to the control sites (**Fig. 4b**).

After the cessation of malathion treatment and the associated reversal of the insecticide

selection pressure, we detected an ~5Mbp reverse sweep driven by the R2 and R3 *Ace* alleles in

the malathion-treated populations (**Fig. 4c**). Individual SNPs within 1Mbp of the *Ace* locus

demonstrated a strong reversal effect, in which sites that increased in frequency during treatment

tended to decrease afterwards, with this reversal being significantly stronger for *Ace*-linked

compared to control sites (**Fig. 4e**). As expected, in the untreated populations, *Ace*-linked sites

showed neither significant median frequency changes (**Fig. 4d**) nor the reversal behavior (**Fig.**

4f). Of note, our ability to detect the reversal of individual SNPs is affected by the degradation of

the initial haplotype structure due to recombination through the course of the experiment, making

it possible that the extent of the reverse sweep is even longer than 5Mbp.

Figure 4

Figure 4. **Strong fluctuating selection of resistant *Ace* alleles caused chromosome-scale forward sweep during malathion treatment and reverse sweep after treatment**. **a-d**, Effect sizes (median allele frequency changes) for *Ace*-linked (orange/blue) and unlinked control (grey) SNPs in 1Mbp windows across the genome were estimated using quasibinomial logistic regression (see **Methods**). Changes during malathion treatment in treated (**a**) and untreated (**b**) populations. Changes after treatment cessation in treated (**c**) and untreated (**d**) populations. The brackets represent the inferred width of the sweep. Stars indicate additional adaptive loci to malathion treatment. **e-f**, Comparison of effect size for *Ace*-linked (orange/blue) and unlinked control (grey) SNPs during (y-axis) and after malathion treatment (x-axis) in malathion-treated (**e**) and untreated (**f**) populations within 1Mb from *Ace*. Each panel shows SNPs grouped by their distance from *Ace* (in Mb): [-1, -0.5], [-0.5, 0], [0, 0.5], and [0.5, 1]. In panel **e**, red stars indicate regions where the regression slopes significantly differ between *Ace*-linked and unlinked control SNPs.

266   Here we provide compelling evidence, both in the laboratory and in a highly replicated

267 field mesocosm experiment, for beneficial dominance reversal at a large-effect locus subject to

268 strong, temporally fluctuating selection. In the lab, we showed that resistant *Ace* alleles are

269 beneficial and dominant in the presence of organophosphates yet become deleterious and

270 recessive in their absence. Based on these results, we predicted the rapid increase in frequency of

271 the resistant *Ace* alleles in the presence of organophosphates and the relative stability in their

272 absence. The field mesocosm experiment together with modeling confirmed our predictions,

273 revealing that the cost of resistance is likely recessive, as only a recessive cost is consistent with

274 the observed stability when rare and the reduction in frequency when common. Critically, we

275 also demonstrate a way to quantify dominance in fitness in field mesocosms by measuring allele

276 trajectories under selection starting at different frequencies[4].

277   Our findings allow us to speculate about the likely mechanistic reasons and prevalence

278 for dominance reversals in fitness under temporally varying selection. In the presence of

279 organophosphates, the benefit of the resistant *Ace* allele should be dominant because the reduced

280 activity of the sensitive *Ace* allele will be compensated by the sufficient activity of the resistant

281 *Ace* allele. This follows Wright's physiological theory of dominance[7] which posits that reduction

282 in gene activity by one half should have only a modest fitness effect for large-effect loci due to a

283 concave mapping of gene activity to fitness. Similarly, in the absence of organophosphates, the

284 cost should be recessive because the sensitive *Ace* allele maintains normal function and the logic

285 of the physiological model of dominance applies again. This reversal of dominance therefore

286 extends Wright's model of dominance as the identity of the loss-of-function, deleterious and

287 recessive allele shifts in concert with the fluctuating environment. This mechanism is compatible

288 with the stochastic additive scale and concave fitness function (SAS-CFF) model which predicts

the maintenance of polymorphisms in variable environments[42]. We argue that such reversals of

dominance should be widespread for polymorphic large-effect loci under temporally varying

selection in nature. For example, large-effect loci involved in resistance to insecticides and host

plant toxins, both of which are subject to temporally varying selection, likely exhibit similar

dominance patterns[43,44].

An important implication of fluctuating selection affecting large-effect loci is the effect

on linked genomic variation. Our experimental evolution setup allowed us to quantify a

chromosome-wide forward sweep and a reverse sweep at the *Ace* locus in response to fluctuating

insecticide selection. These perturbations are very hard to detect without knowing the exact

linkage structure genome-wide, as such old alleles are present on diverse genetic backgrounds.

Given the data that *D. melanogaster* genome contains a fair number (tens to hundreds) of large-

effect alleles displaying large frequency shifts due to fluctuating selection[14,15] and the likelihood

that this is the case for many other species[18,19], fluctuating selection at large-effect loci could act

as a major force shaping levels of genetic variation in natural populations[28,29].

To conclude, our work demonstrates that beneficial reversal of dominance can operate at

large-effect loci, stabilizing a large effect functional polymorphism and enabling rapid responses

to environmental change. To test the generality of this mechanism, future studies should examine

other genetic systems, particularly large-effect loci with known genetic architecture responding

to temporally varying environments, such as those conferring resistance to host plant toxins and

pesticides. Determining the biochemical basis of dominance reversal in these systems would test

our proposed extension of Wright's physiological theory. Additionally, investigating the effects

of repeated fluctuating selection cycles could reveal how recurrent forward and reverse sweeps

shape genome-wide variation patterns, potentially illuminating a key evolutionary force driving

genomic variation in natural populations across taxa.

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analysis; A.H., CT, and Z.K.M. were responsible for investigation; P.S. and D.A.P. jointly

supervised the research. P.S. was responsible for conceptualization, investigation, formal

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conceptualization, investigation, formal analysis, writing-review and editing, and funding

acquisition.

Methods

Population genomic analysis for variation of organophosphate-resistant *Ace* alleles across

space and time

We first conducted a literature review to compile all known naturally segregating target site

resistance mutations in the acetylcholinesterase (*Ace* gene) protein of *D. melanogaster* that

confer resistance to organophosphates. Mutero and colleagues in 1994[45] first identified the

resistance mutations F115S, I199V, I199T, G303A and F368Y, and numbered them based on the

precursor acetylcholinesterase (AChE) protein. Menozzi and colleagues in 2004[5] identified

combinations of the resistance mutations I161(199)V, G265(303)A, F330(368)Y and

G368(406)A in worldwide organophosphate-resistant *D. melanogaster* strains and numbered

them based on the mature AChE protein (numbers in the brackets refer to the precursor AChE

protein). They did not identify the resistance mutation F77(115)S. At position 77, the mutation

TTC to T**C**C changes phenylalanine to serine (Chr3R: 9,070,101, v5.39). At position 161, the

mutation ATC to **G**TC changes the isoleucine to valine (Chr3R:9,069,721, v5.39). At position

265, the mutation GGC to G**C**C changes the glycine to alanine (Chr3R:9,069,408 , v5.39). At

position 330, the mutation TTT to T**A**T changes the phenylalanine to tyrosine (Chr3R:9,069,054

, v5.39). At position 368, the mutation GGC to G**C**C/G**CT** changes the glycine to alanine

(Chr3R: 9,063,921 , v5.39). The AChE protein's numbering changed between the studies after its

crystallization in 2000, which defined the N-terminal signal peptide and the C-terminal

sequence[46]. The mutation G368A was missanotated in the study of adaptation of *D.*

melanogaster to organophosphate insecticides by Karasov and colleagues[6].

Organophosphate-resistant *Ace* alleles in DGRP and LNPA inbred lines

We characterized the presence of organophosphate-resistant *Ace* alleles in two fully

sequenced inbred line panels, the Drosophila Genetic Reference Panel (DGRP) and our LNPA

panel of inbred lines. The DGRP panel consists of 205 inbred lines derived from wild-caught

females collected at the Raleigh, North Carolina Farmers Market in 2003[37]. The LNPA panel

consists of 76 inbred lines derived from wild-caught individuals collected in Spring and Fall

from Linvilla Orchards, Media PA USA[14]. Both panels contain the two common resistant *Ace*

alleles (Ace-R1: VGFG and Ace-R3: VAYG) at high frequency, the sensitive *Ace* allele (Ace-S:

IGFG), and other rare resistant alleles. The LNPA panel also contains the resistant *Ace* allele

Ace-R2: VGFA at high frequency. We used the LNPA panel for our lab and field mesocosm

experiments.

<u>Spatial and seasonal variation of organophosphate-resistant *Ace* alleles in natural *Drosophila*</u>

<u>*melanogaster* populations</u>

To examine the spatial variation of the I161V polymorphism, a marker of common

organophosphate-resistant *Ace* alleles (Ace-R1: VGFG, Ace-R2: VGFA, Ace-R3: VAYG), we

first analyzed the relationship between country-level pesticide use and I161V frequency in

Drosophila melanogaster populations. We obtained pesticide use data from the Food and

Agriculture Organization (FAO) FAOSTAT database[36], which provides country-level estimates

of pesticide use per area of cropland. For population genomic data, we used the Drosophila

Evolution over Space and Time (DEST) database, which contains pooled genome-wide allele

frequency estimates from 753 population samples of *D. melanogaster* collected worldwide. For

our analyses, we included pesticide use data and genomic data for the period of 2000-2022.

To visualize the spatial distribution of insecticide use and frequency of the I161V

polymorphism across locations, we created a choropleth map where countries are shaded based

on their pesticide use per cropland area, with pie charts indicating the frequency of the resistant

(I161V) and sensitive alleles at sampled locations. For each location, we calculated median allele

frequencies across available samples for the period 2000-2022. To test whether insecticide use

predicts the frequency of the resistance allele I161V, we fitted a linear mixed effects model using

the lmer() function in the R package lme4 (version 1.1.33). The model included I161V frequency

as the response variable and insecticide use per cropland area as a fixed effect. We included a

random effect for cities.

In addition, to further test whether I161V shows evidence of spatial selection rather than

drift, we compared its variance across samples collected in spring to matched control SNPs. We

selected control SNPs based on three criteria: (1) located within 2Mb but at least 100kb away

from *Ace* to avoid linked SNPs, (2) within the ln(3R)K cosmopolitan inversion to match

inversion status, and (3) average frequency across samples within 4% of the median frequency of

the I161V resistance mutation. We used the cumulative distribution function (CDF) to quantify

how extreme the I161V variance was compared to the control SNPs.

To examine the temporal variation of the I161V polymorphism, we first tested two

hypotheses: (1) whether the frequency of the resistant allele decreases during winter months

when insecticide pressure is relaxed, potentially due to fitness costs, and (2) whether the

frequency of the resistant allele increases during the growing seasons when insecticides are

applied. We analyzed time series data from Linvilla Orchards, a non-organic orchard in

Pennsylvania, USA. Samples were collected near apple trees from 2009 to 2015, with paired

spring and fall collections each year, allowing us to test both hypotheses. To account for

sampling variance in pooled sequencing data, we calculated the effective number of

chromosomes sampled (Nc) following Machado et al. (2016), where $Nc = (1/N + 1/R)^{\wedge}(-1)$, with

N being the number of chromosomes in the pool ($2 \times$ number of flies) and R the read depth at the

site. We then tested for systematic seasonal changes using generalized linear models (GLM) with

binomial error structure. The models used the number of resistant and susceptible alleles

(weighted by Nc) as the response variable and season as a categorical predictor. For the winter

decline hypothesis, we paired each fall sample with the following spring sample. For the summer

increase hypothesis, we paired each spring sample with the following fall sample from the same

year. This approach allowed us to directly test for consistent directional changes in allele

frequencies across seasons while accounting for both biological and technical sources of variance

in the data.

Finally, to test whether the temporal dynamics of I161V reflect selection rather than drift,

we compared its frequency fluctuations to those of matched control SNPs at Linvilla. To

quantify temporal variation, we calculated the cumulative absolute change in I161V allele

frequency over time as the sum of frequency differences between consecutive timepoints. This

metric captures the total magnitude of frequency changes, regardless of direction. We selected

control SNPs that had similar starting conditions as I161V, with initial frequencies matching the

I161V frequency (0.42) at the first Linvilla timepoint within ±4%. To ensure a valid comparison,

control SNPs were selected from within the same genomic region and inversion status as I161V,

while excluding potentially linked sites (same criteria as for the spatial analysis). We then used

the cumulative distribution function to assess whether I161V's temporal variation was

significantly greater than that observed in the matched control SNPs, which would suggest that

its frequency changes are driven by seasonal selection rather than drift.

Laboratory experiments to estimate the dominance of resistant *Ace* alleles for fitness-associated phenotypes in the presence and absence of malathion

To assess whether the dominance of each resistant *Ace* allele (Ace-R1: <u>V</u>GFG, Ace-R2: <u>V</u>GF<u>A</u>, Ace-R3: <u>VA</u>Y<u>G</u>) over their sensitive counterpart (Ace-S: IGFG) reverses for fitness-associated phenotypes that are primarily under selection in organophosphate-rich and -free environments, we first used our LNPA panel of inbred lines to generate recombinant populations with homozygous sensitive, homozygous resistant, and heterozygous *Ace* genotypes for each *Ace* allele. This step was meant to bring potential deleterious, recessive mutations in the inbred lines into heterozygosity. We then measured malathion resistance, viability and fecundity in homozygous and heterozygous populations for each *Ace* genotype.

<u>Generation of recombinant populations with homozygous sensitive, homozygous resistant, and heterozygous *Ace* genotypes</u>

To generate recombinant populations for each *Ace* allele, we first intercrossed 2-3 inbred lines with the same *Ace* allele using a round robin cross scheme (e.g., Line_1 SS x Line_2 SS, Line_2 SS x Line_3 SS, Line_3 SS x Line_1 SS) to bring all founders with the same *Ace* allele together (10 non-mated females x 10 males per round robin cross). We then combined 16 F1 flies (8 non-mated females and 8 males) from each of the round robin crosses per *Ace* allele to establish the next generation. Subsequently, we maintained the homozygous populations in vials and allowed them to recombine for four generations until we performed the phenotype measurements. We used lines 12LN6-70_(B33), 12LN6-92_(B3), LNPA-3 for the sensitive *Ace* allele (Ace-S: IGFG), lines 12LN6-86_(B29) and LNPA-53 for the R1 resistant *Ace* allele (Ace-

R1: <u>V</u>GFG), lines LNPA-14, LNPA-17 and LNPA-87 for the R2 resistant *Ace* allele (Ace-R2:

<u>V</u>GF<u>A</u>), and lines 12LN6-45_(B26), 12LN6-82_(B4), and 12LN6-94_(B19) for the R3 resistant

Ace allele (Ace-R3: <u>VAY</u>G).

<u>Measurements of fitness-associated phenotypes</u>

For all experiments, we first set up breeding cages with ~ 500 4-to-7-day-old flies of

homozygous or heterozygous populations for the sensitive and the resistant *Ace* alleles. To

generate the heterozygous recombinant populations, we crossed SS non-mated females with

homozygous males of each resistant allele (R1R1, R2R2, R3R3).

To compare the level of malathion resistance among the different alleles, we measured

egg-to-adult viability on diets that ranged in malathion concentration from 0 ppm to 30 ppm. For

this, we collected eggs of each population that were laid within a period of 24 hours and then

introduced 50 eggs of each population into narrow vials containing approximately 7 ml of

Drosophila growth media supplemented with the respective malathion concentration. We tested

each of the malathion concentrations in duplicate vials. We used commercial malathion of 50%

strength (Southern Ag Malathion 50% E.C.) and dissolved it in the *Drosophila* growth media.

This medium was kept overnight at 4°C and used the next day for the assay. We monitored adult

eclosion every evening over a period of 10 days until all adult flies had enclosed.

To compare viability among the different genotypes, we introduced 50 eggs of each

population into narrow vials containing ~7 ml of *Drosophila* growth media. Each of the

genotypes were tested in 10 replicate vials. We monitored adult eclosion every evening over a

period of 7 days until all adult flies had enclosed.

To compare fecundity among the different genotypes, we used density-controlled (~50

eggs/vial) adult female progeny of each population. We introduced five 3-day-old females of

each genotype in egg-laying bottles fitted with egg-laying caps filled with Drosophila growth

media. Each day over a period of 3 days we counted the number of eggs laid. Each of the

genotypes were tested in 8-10 replicate egg-laying bottles. The egg-laying caps were examined

under a stereoscope where we counted the eggs laid each day over the 3-day period.

<u>Statistical analysis of fitness-associated phenotypes</u>

We assessed fecundity, viability, and malathion resistance for the three genotypes (SS,

SR, RR) of each *Ace* allele (R1, R2, R3) using R (version 4.2.2). For fecundity and viability, we

fitted linear and logistic regression models, respectively, calculating estimated marginal means

and performing pairwise comparisons adjusted for multiple testing using the Benjamini-

Hochberg procedure. We then implemented a bootstrap procedure (1,000 resamples) to estimate

deviation from additivity, selection coefficient, and dominance coefficient. The deviation from

additivity was calculated as W[SR] - (W[SS] + W[RR]) / 2, where W represents fitness

(fecundity or viability) for each genotype. The selection coefficient s was computed as (W[RR] –

W[SS]) / W[SS], indicating the relative fitness of the resistant genotype compared to the

sensitive genotype. The dominance coefficient h was calculated as (W[SR] – W[SS]) / (W[RR] –

W[SS]), measuring the degree to which the heterozygote phenotype is closer to either

homozygote. Significance was assessed by testing whether results differed from zero (additivity

and selection) or 0.5 (dominance). For malathion resistance, we estimated LD_{50} values for each

genotype using four-parameter logistic dose-response models $f(x) = c + (d-c) / (1 + e^{\wedge}(b(x-e)))$,

where e is $LD_{50,}$ d (upper limit) = 100%, c (lower limit) = 0%, and b is the relative slope around

e. For this, we used the L.4() function in the drc package (version 3.0.1)[47] in R (version 4.2.2)

with egg to adult viability at the range of malathion concentrations as the response variable. We

then used a bootstrap procedure (1,000 resamples) to compare LD_{50} values between genotypes

and calculate the same coefficients, substituting LD_{50} values for W in the formulas. For all

analyses, bootstrap resampling was used to calculate confidence intervals and p-values, with

significance assessed against null hypotheses of 0 (additivity and selection) and 0.5 (dominance).

Field mesocosm experiments to track the evolution of malathion resistance and genome-

wide allele frequencies in the presence and absence of fluctuating malathion selection

Experimental orchard and field mesocosm cages

To mimic the natural surroundings and weather conditions that flies are exposed to in nature, we

conducted the study in an experimental orchard at the University of Pennsylvania in

Philadelphia. The orchard consisted of cleared land with walk-in mesocosm cages (2m x 2m x

2m) constructed of fine mesh and built around metal frames (BioQuip PO 1406C). We planted a

dwarf peach tree in each mesocosm cage to provide flies with shade. All peaches were removed

before ripening to prevent flies from feeding or laying eggs on them. To provide flies with a

feeding and breeding substrate, we added 1.5lb aluminum loaf pans with 400ml Drosophila

growth media every two days during the experiment. To avoid an overcrowded developmental

environment for the eggs, each loaf pan was covered with a screen mesh lid every two days

during the experiment, and eggs were allowed to develop until eclosion ceased, at which point

adult flies were released into the general population. The loaf pans were placed on a shelving

unit oriented away from direct sunlight to protect them from rain and direct sun.

Establishment of experimental *D. melanogaster* populations in field mesocosm cages

To construct the baseline *D. melanogaster* population for the experiment, we crossed the

76 fully sequenced LNPA inbred lines from Pennsylvania. We used inbred lines to facilitate the

use of haplotype inference to attain high effective sequencing coverage[41]. To initiate the baseline

population, we combined 10 females and 10 males from each line into a single breeding cage.

After 4 generations of mating and density-controlled rearing in laboratory conditions, we

introduced 500 females and 500 males of a single age cohort into each mesocosm cage on June

21, 2021.

Malathion treatment over the course of the experiment

A diagram of the experimental design is shown in **Fig. 3a**. In the first step (*Establishment*

phase: June 21 to July 20, 2021), we established 20 replicate populations in mesocosm cages and

allowed them to expand in the absence of interventions. After establishment, the populations

were split into ten malathion-treated and ten malathion-untreated populations. To mimic the

insecticide exposure of flies in nature, the malathion-treated populations were exposed to

malathion in the summer. In nature, insecticides are mainly applied during summer for pest

control and their application ceases in the fall[48]. During the malathion treatment phase of the

experiment, we sequentially exposed the populations to stepwise increasing concentrations of

malathion, from 1.5 (July 21 to July 27, 2021) to 2.5ppm (July 28 to August 24, 2021) and

7.5ppm (August 25 to September 6, 2021). We used commercially available malathion grade of

50% strength (Southern Ag Malathion 50% E.C.) and dissolved it in the *Drosophila* growth

media. We selected malathion doses based on the Malathion 50% E.C. label instructions to

control small insect pests in the field and a previous long-term selection experiment for

malathion resistance in *D. melanogaster*[49]. The medium supplemented with malathion was

cooked once per week and stored at 4˚C until deployed. After the malathion treatment, the

experiment lasted until early winter (September 7 to December 22, 2021). Over the course of the

experiment, we performed high-resolution temporal sampling (~ bi-weekly sampling during the

summer and monthly during the fall) to track evolution of malathion resistance and genome-wide

allele frequencies.

Tracking population size in the field mesocosms

To make relative comparisons of population sizes between the malathion-treated and untreated

cages, we photographed 4 equal-size quadrats of the ceiling in each cage at dusk (approximately

2.5% of the ceiling for each quadrat). To count the number of adult flies in each photograph, we

designed and validated an algorithm for semi-automated image processing. The number of adult

flies in each of the 4 photographs was multiplied by 40 to correct for total surface area and obtain

4 population size estimates per cage.

Statistical analysis of population data

To assess the effects of time and malathion treatment on population size, we fitted a linear

mixed-effect model with the estimated population size as the response variable. We defined our

model to investigate the quadratic relationship of time with population size and to account for the

random effects attributable to differences in cages. The regression analysis was performed in R

(version 4.2.2) using lme4 (version 1.1.33).

Tracking malathion resistance in the field mesocosms

To characterize the rate and direction of the evolution of malathion resistance in the malathion-

treated and untreated populations, we repeatedly assayed the evolving populations. Prior to the

malathion resistance assay, we collected F1 eggs from each mesocosm cage and transferred them

in 2 groups of 200 eggs to bottles with media (i.e., density controlled) in a constant common

garden environment (25˚C, 12L:12D, 50% humidity) in the laboratory to distinguish evolution

from phenotypic plasticity. We then performed the malathion resistance assay with density and

age-controlled individuals from each mesocosm cage in the F2 generation (25˚C, 12L:12D, 50%

humidity). The common garden experiment for assaying malathion resistance took place at

Stanford University with F1 eggs in bottles with media shipped overnight from the University of

Pennsylvania.

Malathion resistance measurements

To characterize the level of malathion resistance in the malathion-treated and untreated

populations, we tracked egg to adult survival in the F2 generation on diets that ranged in

malathion concentration from 0 ppm to 15 ppm over the course of the experiment. For the

collection of F2 eggs, we placed ~ 400 4-to-7-day-old F1 individuals in breeding cages with egg

lay caps for 5-7 hours. We then introduced 50 F2 eggs in a narrow vial containing ~7ml of

Drosophila growth media supplemented with malathion. We tested each of the malathion

concentrations in duplicate vials. We used commercial malathion of 50% strength (Southern Ag

Malathion 50% E.C.) and dissolved it in the *Drosophila* growth media. This medium was kept

overnight at 4ºC and used the next day for the assay. We monitored adult eclosion every evening

over a period of 10 days until all adult flies had eclosed.

<u>Statistical analysis of malathion resistance data</u>

To assess the temporal dynamics of malathion resistance in both malathion-treated and untreated

cages, we fitted separate linear mixed-effects models using the lmer() function in the R package

lme4 (version 1.1.33). The response variable in these models was malathion resistance, measured

as the LD_{50} value, the dose that causes 50% egg-to-adult mortality. For the malathion-treated

cages, we modeled resistance changes over two distinct time periods: (1) from July 26, 2021, to

September 21, 2021, when populations were evolving under malathion treatment, and (2) from

September 21, 2021, to December 22, 2021, after malathion treatment had ceased. In contrast,

for the malathion-untreated cages, we assessed resistance changes over the full experimental

period, from July 26, 2021, to December 22, 2021. To avoid biases, we excluded data from July

13, 2021, because the LD_{50} estimate from this time point was underestimated due to a technical

error. In each model, we included time point as fixed effect, while a random intercept was

included for each cage to account for between-cage variability. The main outcome measure of

malathion resistance for each cage at each time point was the mean of 1–2 technical replicate

LD_{50} measurements. To estimate LD_{50} for each technical replicate per cage per time point, we

fitted four-parameter logistic dose-response models using the L.4() function in the drc package

(version 3.0.1) in R (version 4.2.2) with egg to adult viability at the range of malathion

concentrations as the response variable.

Track genome-wide allele frequencies in the field mesocosms over time

To track the trajectories of the resistant and sensitive *Ace* alleles and characterize the extent of

the sweep caused by the *Ace* alleles, we tracked genome-wide allele frequencies of the evolving

populations across the experiment using the software tool HAF-pipe. HAF-pipe estimates

genome-wide allele frequencies in the evolving populations via inference of known founder

haplotypes from low-coverage pooled sequencing data [41]. As part of our experimental design, we

performed whole-genome, low-coverage pooled sequencing of 100 randomly selected F1

females from each of the 20 evolving populations at eight time points through the experiment. To

measure biological and technical variation, we performed whole-genome, low-coverage pooled

sequencing of different sets of 100 randomly selected F1 females from the 20 evolving

populations at two time points collected at Stanford (biological replicates) and of same samples

of 100 randomly selected F1 females but processed independently (technical replicates). Last, to

evaluate the accuracy of HAF-pipe, we performed whole-genome, high-coverage pooled

sequencing of four biological replicate samples from the baseline population and for the evolving

populations at an early and a late time point. The data for the untreated cages were also used for

a recent independent study from Bitter and colleagues [15].

Sample collection, DNA extraction, library preparation, and sequencing

We collected samples from each population approximately every two generations during

the summer phase and every generation during the fall phase. To collect the samples, we placed a

new loaf pan with Drosophila growth media in each mesocosm cage one day before collection.

The next day, we transferred five groups of 200 F1 eggs to bottles with media, where we

controlled the density and maintained them in a constant laboratory environment of 25°C,

12L:12D, and 50% humidity. Three bottles with F1 eggs were kept at UPenn and two bottles

with F1 eggs were shipped overnight to Stanford. At Stanford, F1 adults were allowed to lay

eggs, and 200 F2 eggs were placed in vials for density control. For both the F1 and F2

generation, we harvested 4-to-7-day-old individuals after eclosion and preserved them in 100%

ethanol in 1.5ml vials at -20°C until further processing.

For each sample, we prepared genomic DNA from pools of 100 ethanol-preserved

females. We first submerged the samples twice in a rehydration buffer (400 mM NaCl, 20 mM

Tris-HCl pH 8.0, 30 mM EDTA) for 15 min at room temperature and then isolated genomic

DNA using the Monarch Genomic DNA purification kit (Cat No. 3010L, New England Biolabs).

The manufacturer's protocol was modified to include a step for tissue homogenization with two

3mm glass beads (Cat No. 11-312C, Fisher Scientific) in a bead beater for five minutes to ensure

complete homogenization of the flies so that the genomic DNA from each fly was equally

represented in the genomic DNA pool. We quantified the genomic DNA using the Qubit dsDNA

BR (Broad-Range) Assay kit (Cat No. Q32853, Life Technologies) on a Qubit

spectrophotometer and estimated its purity on a NanoDrop spectrophotometer.

We then prepared sequencing libraries using the Illumina DNA prep, (M) Tagmentation

kit (Cat No. 20018705, Illumina Inc.) and the Illumina UD Indexes sets (Cat No 20027123,

20027214, 20042666, 20042667, Illumina Inc.) for unique dual indexing. We modified the

manufacturer's protocol by preparing libraries in 1/5 of the recommended reaction volume to

maximize kit efficiency and pooling equimolarly amplified libraries in sets of eight after the

library amplification step. After the cleanup, we quantified the pooled libraries using a Qubit

spectrophotometer and an Agilent Bioanalyzer. Finally, the pooled libraries were 150bp paired-

end sequenced on the NovaSeq 6000 S2 platform at the Sequencing Service Center at the

Stanford Genomics Center and on a NovaSeq 6000 SP platform at Admera. All sequences will

be deposited in SRA.

Processing of DNA sequencing data for genome-wide allele frequency estimation

We trimmed the raw sequencing reads of adapter sequences and bases with quality score

< 20. We then mapped the trimmed reads to the *D. melanogaster* reference genome (v5.39) using

bwa and default parameters [50], and aligned reads were deduplicated using Picard tools. We

randomly sampled reads from the aligned bams to obtain an equivalent, genome-wide coverage

of 8x across all samples. We computed haplotype-derived allele frequency (HAF) estimates

using HAF pipe. Briefly, after using an expectation-maximization algorithm to estimate

haplotype frequencies in overlapping genomic windows from mapped sequence reads (originally

developed by Kessner and colleagues [51]) at each site previously identified as polymorphic in the

inbred reference panel, we summed the estimated frequencies of haplotypes carrying the

alternate allele to obtain HAFs . We conducted haplotype inference in window sizes that varied

proportionally to the expected length of un-recombined haplotype blocks, which varied as a

function of the estimated number of generations since the original outbreeding. HAFs were

compiled to only include those sites with an average minor allele frequency (MAF) > 0.02 in the

baseline population, and present in at least one evolved sample at a MAF > 0.01.

674
675 **Track trajectories of sensitive and resistant *Ace* alleles in the evolving populations over**

676 **time**

677
We first estimated the frequency of each of the 76 founder haplotypes in the *Ace* region

(Chr3R:9,063,921-9,069,721) in each of the malathion-treated and malathion-untreated

populations at each time point, focusing on the founders containing the four known resistance

mutations . To obtain an accurate measure of the frequency of each haplotype, we calculated the

mean frequency across the 10 overlapping genomic windows that included each site within the

region of interest.

We then estimated the relative frequency of each *Ace* allele, defined by the combination

of the four known resistance mutations, in each population at each time point. Given our

knowledge of the resistance mutation combinations for each founder, we computed the relative

frequency of each *Ace* allele (Ace-S: IGFG, Ace-R1: VGFG, Ace-R2: VGFA, Ace-R3: VAYG,

Ace-R4:IGFA, Ace-R5:IGYG, Ace-R6:VAFG, Ace-R7: VAYA) by summing the frequencies of

the haplotype blocks from the 76 founders that shared the same *Ace* allele. For founders

heterozygous for the *Ace* alleles, we divided the contributions of their haplotype block evenly

between the two corresponding *Ace* allele frequencies. *Ace* alleles with a frequency < 0.01%

(Ace-R4:IGFA, Ace-R5:IGYG, Ace-R6:VAFG, Ace-R7: VAYA) in the evolving populations

were excluded from further analysis.

Statistical analysis of *Ace* allele frequency data

To assess the temporal dynamics of *Ace* allele frequencies (Ace-S: IGFG, Ace-R1:

VGFG, Ace-R2: VGFA, Ace-R3: VAYG), we fitted separate linear mixed-effects models using

the lmer() function in the R package lme4 (version 1.1.33). The response variables in these

models were the frequencies of each *Ace* allele. For the malathion-treated cages, we modeled *Ace*

allele frequency changes over two distinct time periods: (1) from July 26, 2021, to September 21,

2021, when populations were evolving under malathion treatment, and (2) from September 21,

2021, to December 22, 2021, after malathion treatment had ceased. In contrast, for the

malathion-untreated cages, we assessed *Ace* allele frequency changes from the beginning of the

treatment to the end of the experiment, from July 26, 2021 to December 22, 2021.

Model *Ace* allele frequency evolution

We simulated the evolution of *Ace* allele frequencies and malathion resistance in our mesocosm

experiments using a viability selection model[52]. Additionally, based on the changes in *Ace* allele

frequencies in malathion-free environments across both sets of cages, we estimated the possible

ranges for the fitness costs of and dominance of malathion resistance.

The impact of viability on *Ace* allele frequency dynamics and malathion resistance in the field

mesocosm experiment

We investigated whether selection acting on viability recapitulates the allele frequency

and malathion resistance dynamics observed in the field. We focused on viability as it is a key

fitness-related trait in both malathion-rich and malathion-free environments. Furthermore, we

had laboratory measurements of viabilities for *Ace* genotypes across ranges of malathion

concentrations, allowing us to parametrize our model and test its predictions.

Within our model, the population frequencies of the *Ace* alleles change only due to selection that

acts on viability. We assumed that the effects of other forces (mutation, recombination, and

genetic drift) were negligible in comparison over the timescales that we considered (~10

generations). Under these assumptions, the frequency dynamics of the sensitive (f_0) and resistant

(f_1, f_2, f_3) *Ace* alleles follow from

$$f_i(t+1) = \frac{f_i(t) \sum_j w_{ij} f_j(t)}{\overline{w}(t)},$$

where t is time measured in generations, w_{ij} is viability of individuals carrying both i and j

alleles, and $\underline{w}$ is the mean population viability defined as

$$\overline{w}(t) = \sum_{i,j} f_i(t) f_j(t) w_{ij}.$$

We used laboratory measurements to determine individual viabilities w_{ij} in the absence

and presence of malathion (at 2.5 and 7.5 ppm). We used the marginal mean values from the

linear model for viability for each genotype and the predicted viability values from the dose-

response analysis (see previous section on 'Statistical analysis of fitness-associated phenotypes').

Due to the lack of measurements for heterozygous resistant genotypes, we assumed that

combinations of resistant alleles are codominant, i.e. that *$w_{12} = 0.5 \cdot w_{11} + 0.5 \cdot w_{22}$; $w_{13} = 0.5 \cdot$*

$w_{11} + 0.5 \cdot w_{33}$; $w_{23} = 0.5 \cdot w_{22} + 0.5 \cdot w_{33}$. The initial *Ace* allele frequencies, *$f_i(0)$*, were

estimated as the median values from the first sampling time point (July 13, 2021) for each

treatment group.

To estimate *D. melanogaster* generation times at our eight sampling time points, we used

a degree-day model based on Behrman and Schmidt[53]. This model assumes *D. melanogaster*

requires 112 degree-days to complete one generation, with development occurring between 12°C

and 29°C. We calculated daily degree-days as [(daily max. temp. + daily min. temp.) / 2] - 12°C,

adjusting temperatures outside the 12-29°C range to these limits. Cumulative degree-days were

tracked, with each 112 degree-day accumulation marking a new generation. This allowed us to

estimate generations at each sampling time point throughout the experiment.

We then calculated the population resistance *R* as a weighted sum,

746
$$R(t) = \sum_{i,j} g_{ij}(t) w_{ij},$$

747   where $g_{ij}(t)$ are genotype frequencies and w_{ij} are the corresponding viabilities. The initial

748   genotype frequencies were estimated from the initial allele frequencies, assuming Hardy-

749   Weinberg equilibrium:

750
$$g_{ij}(0) = f_i(0) f_j(0).$$

751   The consequent genotype frequencies followed from the viability selection model,

752
$$g_{ij}(t) = \begin{cases} \dfrac{2f_i(t)f_j(t)w_{ij}}{\overline{w}}, i \neq j, \\ \dfrac{f_i(t)f_j(t)w_{ij}}{\overline{w}}, i = j. \end{cases}$$

754 <u>Inferring the ranges of possible fitness costs and dominance coefficients from empirical allele</u>

755 <u>frequency trajectories</u>

756   To estimate the fitness cost and dominance of malathion resistance in the malathion-free

environment, we analyzed the empirical frequency trajectories of these alleles in both the

untreated and treated populations after the removal of malathion. This approach allowed us to

constrain the range of possible parameters that could explain the observed frequencies of the

resistant alleles in both the treated and untreated populations. Assuming the resistant alleles share

the same selection (s) and dominance (h) coefficients, and further that the cost of resistance is

small (s<<1), the frequency dynamics follow

$$\partial_t f = s(f \cdot (1 - f) \cdot (f + h(1 - 2f))),$$

allowing us to find *s* that would be consistent with the observed slope $\partial_t f$ of the frequency

trajectory for a given h[54].

766   To do that, we first bootstrapped the combined frequency trajectories of resistant alleles

in individual cages within each treatment group 10,000 times. For each set of bootstrapped

trajectories, we used linear regression (LinearRegression().fit function from the scikit-learn

Python library[55] to infer their slope. For a range of $h \in [0,1]$, we then calculated the bounds on *s*

by finding the values that correspond to the lower and upper bound of the 95% confidence

interval of the bootstrap distribution. To further constrain the range of possible parameters, we

similarly inferred the full distribution of *s* for each set of cages, and evaluated their overlap by

computing the joint likelihood function,

774
$$\mathcal{L} = \prod p(s),$$

where $p(s)$ is the probability density corresponding to a fixed value of s. Larger values of L

therefore correspond to more likely combinations of h and s given the observed frequency

dynamics.

Characterize extent of forward and reverse sweep caused by *Ace* resistant alleles

To assess how fluctuating organophosphate selection on the R2 and R3 *Ace* resistant alleles

affected genome-wide frequency trajectories of linked SNPs during and post-malathion

treatment, we employed a two-step approach. First, we used quasibinomial logistic regression to

estimate allele frequency shifts for genome-wide SNPs during malathion treatment (July 26 to

September 21, 2022) and post-treatment (September 21 to December 22, 2021). Second, we

developed a linked SNP selection procedure using linkage information from the inbred lines to

identify SNPs linked (Ace-linked) and not linked (control) to the sweeping R2 and R3 *Ace*

alleles across the genome. We used these sets of SNPs to quantify the extent of both the forward

Ace sweep during malathion treatment and the reverse *Ace* sweep post-treatment.

<u>Quasi-binomial logistic regression to estimate allele frequency differences in malathion-treated</u>

<u>and untreated populations during and after the malathion treatment</u>

To quantify allele frequency changes during malathion treatment (July 26 to September

21, 2022) and post-treatment (September 21 to December 22, 2021) in both treated and untreated

populations, we used a quasibinomial logistic regression approach[14,56]. This method models the

number of reads mapped to the alternative allele 'α' (relative to the reference allele 'A') at each

biallelic SNP for each pooled sample. To account for both the expected accuracy of HAFs as

well as pseudo-replication due to a fixed number of individuals in each sample [57], we estimated

an effective read depth reads (at each biallelic SNP for each pooled sample per population using

799
$$n_{eff} = \frac{((2 \times N \times EC) - 1)}{2 \times N + EC}$$

800   where N is the number of individuals per sample (100) and EC is the expected effective read

801   coverage based on the accuracy of HAF-pipe.

802   We then used a logit link implemented in a quasi-binomial logistic regression to model the

803   counts of the alternative allele 'α' ($C_a = HAF * neff$) and reference allele 'A' ($C_A = (1 -$

804    $HAF) * neff$) as a function of time in the malathion-treated and -untreated populations

805
$$logit(p_a) = log\left(\frac{p_a}{1 - p_a}\right) = b_0 + b_1 \times time + e$$

806   where p_a is probability of observing the allele 'α', while the coefficients b_0 and b_1 correspond to

807   the intercept and the effect of time, respectively. We fitted the model using the glm() function in

808   R (version 4.2.2) with family = 'quasibinomial'. To compute the allele frequency change (effect

809   size) between two time points (x_1 and x_2), we calculated

810
$$\Delta p = p_2 - p_1 = \frac{e^{(b_0 + b_1 \times x_2)}}{1 + e^{(b_0 + b_1 \times x_2)}} - \frac{e^{(b_0 + b_1 \times x_1)}}{1 + e^{(b_0 + b_1 \times x_1)}}$$

<u>Analysis of allele frequency changes of Ace-linked SNPs</u>

To quantify the extent of the forward and reverse *Ace* sweeps, we identified SNPs linked

to the sweeping R2 and R3 *Ace* alleles (*Ace*-linked), as well as unlinked (control) SNPs, using

whole genome data from the 76 sequenced Pennsylvania inbred lines (LNPA). First, we labeled

R2 and R3 *Ace* alleles as "R" and the remaining alleles (R1 and S) as "S", creating a binary

"R"/"S" *Ace* locus across the inbred lines. Among the inbred lines, 54 were homozygous "S", 5

heterozygous "R/S", and 17 homozygous "R". To select linked SNPs, we used the inbred lines

data to compute r2 between the binary "R"/"S" *Ace* locus and every reported biallelic SNP in the

inbred LNPA lines as the squared correlation coefficient of allelic indicator variables[58]. We

initially retained SNPs with r2 > 0.03, a threshold frequency previously used to deem two loci

highly linked[15]. However, to avoid issues with low-frequency SNPs in finite populations, we

added an allele count condition. We selected SNPs with $r^2 > 0.03$ that also have an allele present

in ≥60% of "R/R" lines and in ≤40% of "S/S" and "S/R" lines. This ensures selection of high-

frequency, well-assorted SNPs between "R" and "S" lines, which are more informative for sweep

analysis. This approach yielded 5672 *Ace*-linked SNPs across chromosomes 2, 3, and X. The

60% threshold was chosen as the highest value providing enough *Ace*-linked SNPs to uniformly

cover the genome, with consistent results when varied. Additionally, for each *Ace*-linked SNP,

we selected an unlinked control SNP with $r^2 < 0.01$ with the binary "R"/"S" *Ace* locus. To ensure

comparable initial conditions, unlinked control SNPs were chosen within 500kb of their linked

counterpart and had an allele frequency within 0.05 of the *Ace*-linked SNP at the start of

malathion treatment in each cage. Finally, to ensure that we track the SNP alleles that are linked

specifically with the resistant lines, we transformed the alleles frequencies based on the sign of r

— the non-squared correlation coefficient of allelic indicator variables. If r between *Ace* and a

SNP is positive, then the alternative allele is assorted together with the resistant *Ace* alleles in the

inbred lines, if the sign of r is negative, then the reference allele is linked to resistant *Ace* alleles,

and in this case the alternative/reference labels were flipped, and the allele frequencies f_t were

transformed to 1-f_t. This process yielded 4793 matched unlinked control SNPs, fewer than the

linked SNPs due to the stringent matching criteria. To visualize the results, we aggregated SNPs

into 1Mb windows along the genome and used bootstrap resampling to compute median GLM-

estimated effect sizes and confidence intervals for *Ace*-linked and unlinked control SNPs

separately for both forward and reverse sweeps. We excluded the areas around the centromeres

and chromosome ends where less than 20 *Ace*-linked or control SNPs were observed. To

characterize the width of each sweep, we performed a two-sample Mann-Whitney U-test within

every 1Mb window across the chromosome and recorded the width of the contiguous range of

windows around *Ace* in which the median for *Ace*-linked SNPs is significantly greater than the

median for control SNPs at the 0.05 confidence level.

To quantify the reversal phenomenon during the *Ace* sweep post-malathion treatment, we

analyzed allele frequency changes for the same Ace-linked and unlinked control SNPs during the

post-treatment period (September 21 to December 22, 2021). We binned SNPs into

500Kb windows based on the genomic distance from the *Ace* locus on chromosome 3R and then

compared post-treatment to during-treatment frequency changes by plotting them against each

other within each bin. To quantify the reversal, we performed a joint linear regression for *Ace*-

linked and control SNPs and recorded which bins have shown a slope for *Ace*-linked SNPs

significantly more negative compared to control at the 0.05 confidence level.

Data and Code availability

The data and associated code for this study will become available after manuscript acceptance in

public repositories: Dryad (https://doi.org/10.5061/dryad.w0vt4b937) for the data and GitHub

(https://github.com/mkarageorgi/Beneficial_Dominance_Reversal) for the code. The sequences

of the LNPA inbred lines used to construct the baseline *D. melanogaster* population for the field

mesocosm experiment are available on NCBI under accession ID PRJNA722305. The raw

sequencing reads for pooled samples from the malathion-treated cages, collected during the field

mesocosm experiment, will be deposited in the SRA BioProject under accession number

PRJNA1031645. The raw sequencing reads for pooled samples from the untreated cages are

already available in the same SRA BioProject (PRJNA1031645).

Extended Data Figure 1

a

b

Ace haplotype	LNPA relative frequency (%)	DGRP relative frequency (%)
IGFG	63.16	54.15
VGFA	13.16	0.49
VAYG	12.50	30.73
VGFG	7.89	12.20
IGYG	1.32	0.98
IGFA	0.66	NA
VAFG	0.66	NA
VAYA	0.66	NA
IAFG	NA	1.46

Extended Data Figure 1. Sensitive and resistant *Ace* alleles in the panel of LNPA and DGRP inbred lines. a, Heatmap showing clustering of seventy-six *D. melanogaster* isogenic lines in the LNPA panel based on the sensitive or resistant *Ace* allele they carry (Ace-S: IGFG, Ace-R1: <u>V</u>GFG, Ace-R2: <u>V</u>GF<u>A</u>, Ace-R3: <u>VAY</u>G). The x-axis in the heatmap shows the genomic position on chromosome 3R around the *Ace* locus. Each row represents the *Ace* haplotype information of one isogenic line. Each column represents one biallelic exonic SNP at the *Ace* locus. Reference alleles are colored in dark grey, alternative alleles are colored in light grey, heterozygotes in medium grey. For the resistance mutations, alleles coding for the resistance mutations are colored in orange, alternative in grey, and heterozygotes in yellow. **b,** Table showing frequency of the sensitive and each of the resistant *Ace* alleles carrying different resistance haplotypes in the panels of LNPA and DGRP inbred lines.

Extended Data Figure 2

a

b

Dose response curve analysis for each Ace genotype

Genotype	LD50	std.error	statistic	p.value
SS	1.39	0.18	7.79	3.7768e-12
R1R1	1.56	0.20	7.68	6.3407e-12
R2R2	8.16	0.40	20.41	7.7563e-40
R3R3	10.81	0.45	23.96	3.3625e-46
SR1	2.34	0.19	12.49	3.0000e-23
SR2	6.80	0.26	25.89	2.1374e-49
SR3	8.84	0.30	29.95	1.3764e-55

Extended Data Figure 2. Comparison of malathion resistance among the *Ace* genotypes. a, Lab measurements of egg to adult viability in recombinant populations carrying different *Ace* genotypes on standard Drosophila media with a range of malathion concentrations (n= 2 technical replicates per genotype for each malathion doses, 50 eggs per replicate). Four-parameter logistic models fitted to data of each *Ace* genotype. The shaded areas highlight viability of each genotype at malathion doses (2.5ppm and 7.5 ppm) applied in the field mesocosm experiment. **b,** Table with estimated LD$_{50}$ values for each *Ace* genotype from four-parameter logistic dose-response model (see also **Methods**).

Extended Data Figure 3

Extended Data Figure 3. **Tracking evolution of malathion resistance in the field mesocosm experiment.** Egg-adult survival on diets with a range of malathion concentrations as measured after two generations of common garden rearing throughout the field mesocosm experiment. Light lines and points show the mean egg-adult survival for individual malathion-treated (orange) and untreated (blue) populations (n = 2 technical replicates, 50 eggs per replicate). Dark lines and points represent the mean ± s.e.m. from all populations for each treatment group (n = 10 populations per treatment group). Further information on experimental design can be found in **Methods**.

Extended Data Figure 4

Extended Data Figure 4. Modeling the impact of viability selection on *Ace* allele frequency dynamics and malathion resistance in the field mesocosm experiment. **a-b,** Comparison between the observed *Ace* allele frequency trajectories over 10 generations in the malathion-treated (**a**) and untreated (**b**) populations from the field mesocosm experiment (mean ± s.e.m.) and the expected trajectories from simulations based on a viability selection model using laboratory-derived parameters. See **Methods** for details about the viability selection model. **c-d,** Comparison between the observed egg-to-adult survival across a range of malathion concentrations (**c**) and the evolution of malathion resistance (LD_{50}, ppm) (**d**) over 10 generations in the field mesocosm experiment (mean ± s.e.m.), and the expected results from the same model simulations. Dashed lines represent the expected values from the viability selection model, while solid lines represent the observed data from the field experiment. Generations were estimated based on a degree-day model starting from the first sampling point (July 13, 2021). In all plots, points correspond to each generation and estimated time point, and are connected by lines for visualization.

Extendend Data Table 1

Effect estimates from linear model analysis for LD50

Parameter	LD50	Std.Error	P.value
SS mean	1.39	0.19	-
SR1 mean	2.34	0.13	-
R1R1 mean	1.56	0.14	-
SS vs. SR1	-0.93	0.34	0.0063
SS vs. R1R1	-0.17	0.37	0.646
SR1 vs. R1R1	0.76	0.39	0.0516
SR1 - (SS + R1R1)/2 (additivity)	0.76	0.32	1e-16
(R1R1 - SS) / R1R1 (selection, s)	-0.25	0.29	0.751
(SR1 - SS) / (R1R1 - SS) (dominance, h)	-1.29	5.10	0.751
SR2 mean	6.80	0.24	-
R2R2 mean	8.16	0.36	-
SS vs. SR2	-5.38	0.36	0
SS vs. R2R2	-6.72	0.42	0
SR2 vs. R2R2	-1.34	0.45	0.003
SR2 - (SS + R2R2)/2 (additivity)	1.87	0.35	1e-16
(R2R2 - SS) / R2R2 (selection, s)	3.83	1.05	1e-16
(SR2 - SS) / (R2R2 - SS) (dominance, h)	0.78	0.06	1e-16
SR3 mean	8.84	0.32	-
R3R3 mean	10.81	0.49	-
SS vs. SR3	-7.41	0.36	0
SS vs. R3R3	-9.37	0.79	0
SR3 vs. R3R3	-1.96	0.80	0.0145
SR3 - (SS + R3R3)/2 (additivity)	2.59	0.48	1e-16
(R3R3 - SS) / R3R3 (selection, s)	5.64	1.45	1e-16
(SR3 - SS) / (R3R3 - SS) (dominance, h)	0.77	0.07	1e-16

Extendend Data Table 2

Effect estimates from logistic model analysis for viability

Parameter	Viability	Std.Error	Lower.CI	Upper.CI	P.value
SS mean	0.89	0.01	-	-	-
SR1 mean	0.86	0.02	-	-	-
R1R1 mean	0.76	0.02	-	-	-
SS vs. SR1	1.42	0.27	-	-	0.0702
SS vs. R1R1	2.61	0.47	-	-	<.0001
SR1 vs. R1R1	1.84	0.30	-	-	4e-04
SR1 - (SS + R1R1)/2 (additivity)	0.03	0.02	-0.02	0.07	0.214
(R1R1 - SS) / R1R1 (selection, s)	-0.15	0.03	-0.2	-0.09	0.001
(SR1 - SS) / (R1R1 - SS) (dominance, h)	0.29	0.20	-0.15	0.64	0.214
SR2 mean	0.87	0.02	-	-	-
R2R2 mean	0.74	0.02	-	-	-
SS vs. SR2	1.28	0.25	-	-	0.205
SS vs. R2R2	2.96	0.53	-	-	<.0001
SR2 vs. R2R2	2.31	0.39	-	-	<.0001
SR2 - (SS + R2R2)/2 (additivity)	0.05	0.02	0.01	0.1	0.027
(R2R2 - SS) / R2R2 (selection, s)	-0.17	0.05	-0.28	-0.09	0.001
(SR2 - SS) / (R2R2 - SS) (dominance, h)	0.17	0.15	-0.15	0.45	0.027
SR3 mean	0.86	0.02	-	-	-
R3R3 mean	0.78	0.02	-	-	-
SS vs. SR3	1.42	0.27	-	-	0.0702
SS vs. R3R3	2.43	0.44	-	-	<.0001
SR3 vs. R3R3	1.72	0.29	-	-	0.0018
SR3 - (SS + R3R3)/2 (additivity)	0.02	0.02	-0.02	0.06	0.286
(R3R3 - SS) / R3R3 (selection, s)	-0.13	0.02	-0.18	-0.08	0.001
(SR3 - SS) / (R3R3 - SS) (dominance, h)	0.32	0.19	-0.15	0.67	0.286

Extendend Data Table 3

Effect estimates from linear model analysis for fecundity

Parameter	Fecundity	Std.Error	Lower.CI	Upper.CI	P.value
SS mean	200.22	11.99	-	-	-
SR1 mean	165.33	14.01	-	-	-
R1R1 mean	136.50	14.86	-	-	-
SS vs. SR1	34.89	19.81	-	-	0.14
SS vs. R1R1	63.72	20.42	-	-	0.01
SR1 vs. R1R1	28.83	20.42	-	-	0.17
SR1 - (SS + R1R1)/2 (additivity)	-3.03	17.26	-36.62	31.59	0.879
(R1R1 - SS) / R1R1 (selection, s)	-0.32	0.09	-0.48	-0.12	0.003
(SR1 - SS) / (R1R1 - SS) (dominance, h)	0.55	0.40	-0.07	1.32	0.881
SR2 mean	187.60	13.64	-	-	-
R2R2 mean	167.78	14.37	-	-	-
SS vs. SR2	12.62	19.81	-	-	0.53
SS vs. R2R2	32.44	20.33	-	-	0.37
SR2 vs. R2R2	19.82	19.81	-	-	0.49
SR2 - (SS + R2R2)/2 (additivity)	3.60	18.42	-33.15	43.16	0.834
(R2R2 - SS) / R2R2 (selection, s)	-0.16	0.09	-0.33	0.03	0.093
(SR2 - SS) / (R2R2 - SS) (dominance, h)	0.39	4.09	-2.3	3.23	0.842
SR3 mean	202.00	12.71	-	-	-
R3R3 mean	106.44	11.99	-	-	-
SS vs. SR3	-1.78	17.47	-	-	0.92
SS vs. R3R3	93.78	16.95	-	-	<.0001
SR3 vs. R3R3	95.56	17.47	-	-	<.0001
SR3 - (SS + R3R3)/2 (additivity)	48.67	12.84	22.41	73.56	1e-16
(R3R3 - SS) / R3R3 (selection, s)	-0.47	0.07	-0.59	-0.31	0.001
(SR3 - SS) / (R3R3 - SS) (dominance, h)	-0.02	0.19	-0.48	0.29	0.001

Extendend Data Table 4

Extended Data Table 4.1 Effect of time on malathion resistance (LD50) in treated cages

Predictors	During treatment				After treatment			
	Estimates	std. Error	Statistic	p	Estimates	std. Error	Statistic	p
Intercept	-3.02 ***	0.46	-6.58	**<0.001**	15.56 ***	1.09	14.29	**<0.001**
Time	2.48 ***	0.10	24.26	**<0.001**	-0.57 ***	0.14	-3.96	**0.001**
Random Effects								
σ^2		0.91				0.98		
τ_{00}		0.00 Treatment_Cage				0.24 Treatment_Cage		
ICC						0.20		
N		10 Treatment_Cage				10 Treatment_Cage		
Observations		40				30		
Marginal R^2 / Conditional R^2		0.938 / NA				0.302 / 0.441		
AIC		122.513				101.066		

* p<0.05   ** p<0.01   *** p<0.001

Extended Data Table 4.2 Effect of time on malathion resistance (LD50) in untreated cages

Predictors	Beginning of treatment to end of experiment			
	Estimates	std. Error	Statistic	p
Intercept	2.27 ***	0.15	14.91	**<0.001**
Time	0.03	0.02	1.07	0.288
Random Effects				
σ^2		0.16		
τ_{00} Treatment_Cage		0.03		
ICC		0.15		
N Treatment_Cage		10		
Observations		59		
Marginal R^2 / Conditional R^2		0.017 / 0.166		
AIC		83.651		

* p<0.05   ** p<0.01   *** p<0.001

Extendend Data Table 5

Extended Data Table 5.1 Effect of time on S (IGFG) frequency in treated cages

Predictors	During treatment				After treatment			
	Estimates	std. Error	Statistic	p	Estimates	std. Error	Statistic	p
Intercept	0.88 ***	0.02	49.15	**<0.001**	0.04	0.05	0.74	0.468
Time	-0.13 ***	0.00	-30.81	**<0.001**	0.02 *	0.01	2.64	**0.014**
Random Effects								
σ^2	0.00				0.00			
τ_{00}	0.00 cage				0.00 cage			
N	10 cage				10 cage			
Observations	50				30			
Marginal R^2 / Conditional R^2	0.951 / NA				0.194 / NA			
AIC	-148.876				-97.774			

*p<0.05 ** p<0.01 *** p<0.001*

Extended Data Table 5.2 Effect of time on R1 (VGFG) frequency in treated cages

Predictors	During treatment				After treatment			
	Estimates	std. Error	Statistic	p	Estimates	std. Error	Statistic	p
Intercept	0.08 ***	0.00	16.13	**<0.001**	0.04 **	0.01	3.09	**0.005**
Time	-0.00 ***	0.00	-4.05	**<0.001**	0.00	0.00	0.46	0.651
Random Effects								
σ^2	0.00				0.00			
τ_{00}	0.00 cage				0.00 cage			
ICC					0.25			
N	10 cage				10 cage			
Observations	50				30			
Marginal R^2 / Conditional R^2	0.251 / NA				0.005 / 0.250			
AIC	-276.043				-162.492			

*p<0.05 ** p<0.01 *** p<0.001*

Extended Data Table 5.3 Effect of time on R2 (VGFA) frequency in treated cages

Predictors	During treatment				After treatment			
	Estimates	std. Error	Statistic	p	Estimates	std. Error	Statistic	p
Intercept	0.01	0.01	0.93	0.356	0.14 **	0.04	3.39	**0.002**
Time	0.04 ***	0.00	13.93	**<0.001**	0.02 **	0.01	3.03	**0.006**
Random Effects								
σ^2	0.00				0.00			
τ_{00}	0.00 cage				0.00 cage			
N	10 cage				10 cage			
Observations	50				30			
Marginal R^2 / Conditional R^2	0.798 / NA				0.240 / NA			
AIC	-178.169				-107.043			

*p<0.05 ** p<0.01 *** p<0.001*

Extended Data Table 5.4 Effect of time on R3 (VAYG) frequency in treated cages

Predictors	During treatment				After treatment			
	Estimates	std. Error	Statistic	p	Estimates	std. Error	Statistic	p
Intercept	0.03	0.01	1.88	0.067	0.78 ***	0.05	15.18	**<0.001**
Time	0.09 ***	0.00	27.59	**<0.001**	-0.04 ***	0.01	-5.18	**<0.001**
Random Effects								
σ^2	0.00				0.00			
τ_{00}	0.00 cage				0.00 cage			
ICC	0.04							
N	10 cage				10 cage			
Observations	50				30			
Marginal R^2 / Conditional R^2	0.937 / 0.940				0.481 / NA			
AIC	-170.172				-96.128			

*p<0.05 ** p<0.01 *** p<0.001*

Extendend Data Table 6

Extended Data Table 6.1 Effect of time on S (IGFG) haplotype frequency in untreated cages

Predictors	Beginning of treatment to end of experiment			
	Estimates	std. Error	Statistic	p
Intercept	0.63 ***	0.02	39.49	**<0.001**
Time	0.00	0.00	0.21	0.838
Random Effects				
σ^2		0.00		
$\tau_{00\ cage}$		0.00		
ICC		0.03		
N_{cage}		10		
Observations		69		
Marginal R^2 / Conditional R^2		0.001 / 0.032		
AIC		-196.762		

*p<0.05   ** p<0.01   *** p<0.001*

Extended Data Table 6.2 Effect of time on R1 (VGFG) haplotype frequency in untreated cages

Predictors	Beginning of treatment to end of experiment			
	Estimates	std. Error	Statistic	p
Intercept	0.06 ***	0.00	14.68	**<0.001**
Time	0.00	0.00	1.91	0.061
Random Effects				
σ^2		0.00		
$\tau_{00\ cage}$		0.00		
N_{cage}		10		
Observations		69		
Marginal R^2 / Conditional R^2		0.051 / NA		
AIC		-370.061		

*p<0.05   ** p<0.01   *** p<0.001*

Extended Data Table 6.3 Effect of time on R2 (VGFA) haplotype frequency in untreated cages

Predictors	Beginning of treatment to end of experiment			
	Estimates	std. Error	Statistic	p
Intercept	0.07 ***	0.01	9.08	**<0.001**
Time	-0.00	0.00	-0.02	0.985
Random Effects				
σ^2		0.00		
$\tau_{00\ cage}$		0.00		
ICC		0.02		
N_{cage}		10		
Observations		69		
Marginal R^2 / Conditional R^2		0.000 / 0.024		
AIC		-285.686		

*p<0.05   ** p<0.01   *** p<0.001*

Extended Data Table 6.4 Effect of time on R3 (VAYG) haplotype frequency in untreated cages

Predictors	Beginning of treatment to end of experiment			
	Estimates	std. Error	Statistic	p
Intercept	0.24 ***	0.01	17.99	**<0.001**
Time	-0.00	0.00	-0.86	0.392
Random Effects				
σ^2		0.00		
$\tau_{00\ cage}$		0.00		
ICC		0.05		
N_{cage}		10		
Observations		69		
Marginal R^2 / Conditional R^2		0.010 / 0.064		
AIC		-222.940		

*p<0.05   ** p<0.01   *** p<0.001*

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