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Intralocus sexual conflict and insecticide resistance

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The *BA* allele of the *Drosophila* cytochrome P450 gene *Cyp6g1* confers resistance to a range of insecticides. It is also subject to intralocus sexual conflict when introgressed into the *Canton-S* background, whose collection predates the widespread use of insecticides. In this genetic background, the allele confers a pleiotropic fitness benefit to females but a cost to males, and exhibits little sexual dimorphism in conferred insecticide resistance. It is unclear whether these sexually antagonistic effects also exist in current populations that have naturally evolved with insecticides, where genetic modifiers that offset male costs might be expected to evolve. Here, we explore these issues using *Drosophila melanogaster* caught recently from an Australian population in which the *BA* allele naturally segregates. While we find increased fecundity in insecticide-resistant *BA* females and no consistent evidence of fitness costs in males, experimental evolution indicates balancing selection at the locus. We suggest that this apparent discrepancy may be due to reduced investment in reproduction in resistant males. Our results at the population level are consistent with previous work, and suggest that individual-level fitness assays do not always capture sexually antagonistic fitness effects that emerge in a population context.

1. Introduction

Explaining how genetic variation can be maintained in the face of selection is a major goal of evolutionary biology. One mechanism that can maintain variation is balancing selection through intralocus sexual conflict (IASC). IASC occurs when one or both sexes are constrained from reaching sex-specific phenotypic optima by a shared genetic architecture underlying traits subject to sexually antagonistic selection [1–3]. When such a constraint occurs, any improvement in the fitness of one sex will be counterbalanced by a fitness reduction in the other [1]. The result is a 'tug-of-war' [4] of allelic replacement between males and females driven by sexually antagonistic selection at the same loci, dragging the two sexes' trait values to and fro between their sex-specific phenotypic optima [3]. Thus, in order to reach fixation, any sexually antagonistic allele must provide a net selective advantage when averaged across both sexes [5]. When this is not the case, sexually antagonistic alleles can be maintained at stable equilibrium frequencies by balancing selection [6–8]. Cases in which precise loci currently or previously subject to IASC have been identified are exceedingly rare. One such case is insecticide resistance at the *Cyp6g1* locus in the fruit fly *Drosophila melanogaster* [8–10].

Upregulation of the cytochrome P450 gene *Cyp6g1* in *D. melanogaster* can confer resistance to a broad range of insecticides including neonicotinoids and the organochloride dichlorodiphenyltrichloroethane (DDT) [11–14]. The *Cyp6g1* locus in *D. melanogaster* is the site of a series of at least four DDT resistance alleles (and an ancestral susceptible allele) featuring a tandem duplication of the gene and several transposable element (TE) insertions, where progressively higher levels of resistance are conferred across the evolutionary series [15]. At least some of these TEs contain novel cis-regulatory sequences responsible for upregulating *Cyp6g1* expression, namely the long terminal repeat

of a degenerate *Accord* TE inserted 291 bp upstream of *Cyp6g1* [16,17]. The *Cyp6g1*-associated *Accord* insertion is near or at fixation in most populations outside of Eastern Africa, with low levels of microsatellite variation flanking the gene consistent with a recent selective sweep—most probably due to the onset of DDT use in the mid-twentieth century [18].

Recent work has shown that one of the alleles in the series, the *BA* allele, is subject to sexually antagonistic selection when introgressed into the *Canton-S* strain (relative to the susceptible *M* allele) in the absence of insecticide. *Canton-S* is a naturally DDT-susceptible stock (carrying the *M* allele) collected in the early twentieth century before the widespread use of DDT began, and is thus naive to selection imposed by DDT. Resistant $BA^{Canton\text{-}S}$ females are more fecund, have higher egg and larval viability, and shorter egg and larval development times than susceptible $M^{Canton\text{-}S}$ females [9]. By contrast, resistant $BA^{Canton\text{-}S}$ males have lower mating success when in direct competition with susceptible $M^{Canton\text{-}S}$ males [10], and insecticide resistance exhibits low levels of sexual dimorphism in *Canton-S* consistent with these sexually antagonistic fitness effects. This sexual conflict is able to explain frequencies of the *BA* allele during its recent evolutionary history, including the maintenance of variation at the locus [8]. Thus, the *BA* allele is a rare case of IASC where the precise locus of conflict is known and the allele is segregating in wild populations.

It remains to be seen whether conflict at the *Cyp6g1* locus occurs in contemporary populations of *D. melanogaster* (i.e. those that carry the *BA* allele and have evolved with selection imposed by widespread insecticide use), as theory predicts genetic modifiers should emerge that offset any costs of resistance by allowing sex-specific trait expression [19–21]. However, the strong selection imposed by insecticides may mask sexual antagonism and weaken selection for these modifiers. Indeed, Smith *et al*. [10] found that the *BA* allele did not carry a cost to males when introgressed into a recently caught isogenic line, demonstrating that the pleiotropic effects of the *BA* allele can be mediated by epistasis. Additionally, as previous work has investigated the *BA* allele in isogenic backgrounds we do not know how generalizable this sexual antagonism is. It may be the case that the particular genetic variation fixed in the *Canton-S* strain generates sexual antagonism that would not be seen in more genetically heterogeneous populations.

Here, using homozygous resistant and susceptible populations of *D. melanogaster* produced from multiple isofemale lines caught recently from the wild, we assay fitness in both sexes and show that the previously detected female fitness benefit of the *BA* allele exists in a contemporary population, but the effect on males is less clear. Additionally, because IASC may be resolved by sex-limited gene expression, we quantify sex-specific relative *Cyp6g1* expression as well as resistance to DDT and find significant sexual dimorphism in both alleles. Nevertheless, when estimating the dynamics of the *BA* and *M* alleles in populations allowed to evolve experimentally, we find evidence of balancing selection that is not predicted by a mathematical model of IASC parametrized with fitness estimates we obtain in more simplistic settings. We suggest that this is due to emergent effects at the population level not being reflected by standard fitness assays, and present a hypothesis for a possible cause of this apparent discrepancy.

2. Material and methods

(a) Isofemale lines and populations

Genetically heterogeneous populations homozygous for either the susceptible *M* allele or resistant *BA* allele were created from *D. melanogaster* isofemale lines (hereafter isolines, [22]; $n = 15$). These isolines were collected from Margaret River, Australia, in 2013 and were polymorphic at the *Cyp6g1* locus with both the susceptible *M* and resistant *BA* allele segregating within them (diagnostic polymerase chain reaction (PCR) as per Schmidt *et al*. [15]). Virgin adults were crossed within each isoline and genotyped, with offspring from homozygote crosses retained to produce a homozygous *M* and homozygous *BA* version of each isoline. Twenty males and females from each resistant and susceptible isoline were then used to found homozygous *BA* and *M* populations, respectively. Isolines capture and maintain snapshots of the genetic variation and covariation segregating within a source population, and this procedure maximizes the genetic variation within our populations while minimizing the genetic variation between them. While it is unlikely for *Cyp6g1* to be the only locus to differ between the populations, this procedure affords a greater level of control over the genetic background than the use of different isolines to establish each population. However, we cannot definitively rule out any effects we report being due to the contribution of segregating alleles in linkage with either *Cyp6g1* allele. As the starting N_e was equal between the populations, we do not expect differential inbreeding to influence the results presented here.

(b) Experimental animals

Focal experimental animals were collected from the homozygous *BA* or *M* populations described above as first instar larvae, and were all 3–5 day old virgin adults during experimentation. Where specified, *sepia* competitors and mates were collected from a population of *D. melanogaster* homozygous for the recessive *sepia* mutation (obtained from the Bloomington Drosophila Stock Center).

(c) Male fitness assays

(i) Pre-copulatory competitive ability

To test for effects of the *BA* allele on pre-copulatory male fitness, we estimated the mating success of *BA* and *M* males in direct competition. One resistant and one susceptible male were simultaneously introduced into a vial containing one *sepia* female. Males were marked with pink or blue powder paint to allow identification, with half of the males of each genotype marked with each colour [10,23]. Once either male successfully initiated copulation, the unsuccessful male was removed from the vial. We recorded the latency to mate, copulation duration, and genotype of the successful male. Assaying mating success in a competitive trial integrates both success in male–male competition and female mate preference [24]. Females were subsequently left to oviposit for 5 days. Seventeen days after the start of oviposition, we counted the number of adult offspring that had eclosed. Vials in which neither male secured a mating were discarded (47% of trials, final $n = 160$ in two experimental blocks).

(ii) Post-copulatory competitive ability

To test for effects of the *BA* allele on post-copulatory male fitness, we estimated the fertilization success of *BA* and *M* males in competition with a standardized *sepia* competitor. Sperm competition [25,26] assays were performed for both sperm defence (P1) and sperm offence (P2). In the P1 assay, resistant and susceptible males were mated to *sepia* females as per the pre-copulatory competition assay with the exception that only one male was

included in each trial (total $n = 70$). Mated *sepia* females were then given the opportunity to remate with a virgin *sepia* male for 6 h every 24 h until remating occurred. Once remated, females were left to oviposit for 5 days as per the pre-copulatory competition assay. We assigned offspring to their sire based on phenotype (*sepia* or wild-type). We recorded copulation duration for both the initial mating and remating, as well as relative male size and the number of offspring produced before remating. The P2 assay was conducted in the same manner other than the order of *sepia* and focal males was reversed (total $n = 66$).

(d) Female fitness assays

(i) Offspring production

To test for effects of the *BA* allele on female fitness, we estimated the fecundity of both *BA* and *M* females. Half of each female genotype was mated to resistant males and the other half to susceptible males in a fully factorial design to examine any effect of male genotype on offspring production. Females were allowed to oviposit for 5 days following a single mating, with offspring counted 17 days after the start of oviposition in each vial. This measure integrates egg viability, larval viability, and egg production. Females that produced no offspring were removed from analyses ($n = 25$) as it was not possible to determine the source of the mating failure [27], leaving a total sample size of $n = 147$ in two experimental blocks.

(ii) Egg production and offspring viability

To determine whether differences in offspring production could be attributed directly to maternal fitness rather than indirectly through offspring fitness, we estimated egg production, egg–larvae viability and larvae–adult viability. Resistant and susceptible females were housed with either resistant or susceptible males and allowed to oviposit freely for 18 h (before eggs would begin to hatch [28]). Eggs were then counted, and a maximum of 10 eggs per female were haphazardly selected and transferred to fresh media at a density of five eggs per vial (two females did not lay any eggs and were excluded from analyses). Twenty-four hours later, the number of unhatched eggs was counted, and 17 days after the start of oviposition, the number of successfully eclosed offspring was counted. Females that laid fewer than five eggs were excluded from egg to adult viability calculations (3% of females, final $n = 95$).

(e) Mathematical model and experimental evolution

To estimate the evolutionary dynamics of the *BA* and *M* alleles in a more realistic context, replicate populations were founded with the *BA* allele in competition with the *M* allele at either a low initial frequency (0.1, $n = 3$) or a high initial frequency (0.9, $n = 3$) and these were allowed to evolve for seven non-overlapping generations. Populations were founded with 200 flies at an equal sex ratio within each genotype. Each generation, populations were given access to food for 72 h to allow oviposition of the next generation. Ninety-six individuals were haphazardly selected and genotyped to score allele frequencies in each generation (diagnostic PCR as per Schmidt *et al.* [15]). The next generation was then founded by all offspring collected across 5 days from the date offspring began eclosing. To generate predictions at the population level from our individual-level fitness estimates, a single-locus nonlinear recursion model of IASC presented by Rostant *et al.* [8] (summary in the electronic supplementary material) was parametrized with data from the male and female fitness assays and used to predict allele frequencies which were then compared with the empirical allele frequency data.

(f) Dichlorodiphenyltrichloroethane resistance

To test for the presence of sexual dimorphism in insecticide resistance, we estimated the sex-specific LD50 (the concentration at which, on average, 50% of individuals die) of DDT for both alleles. The inside of glass vials was laced with DDT by pipetting 500 µl of an acetone–DDT solution into the vial and rolling the vial until all acetone had evaporated (vials were left for a further 24 h to remove any residual acetone vapours). Adult flies were introduced to the DDT-laced vials (10 per vial) and mortality was scored after 24 h. Each genotype and sex combination was exposed to a range of 12 concentrations of DDT (each replicated three times) that spanned the LD50 value, the extremes of which were as close to zero and total mortality as practical, and a pure acetone control.

(g) *Cyp6g1* expression

To test for the presence of sexual dimorphism in *Cyp6g1* expression, we estimated sex-specific relative *Cyp6g1* transcript abundance for both alleles using quantitative real-time PCR (qRT-PCR). RNA was extracted from pooled samples of 10 whole adult individuals for both genotypes of both sexes homogenized in RNAlater (Fisher) using the Purelink RNA Mini Kit (Ambion) standard protocol (three biological replicates per genotype-sex combination). qRT-PCR was performed using Brilliant III Ultra-Fast SYBR kit (Agilent) on a Stratagene Mx3000P in technical triplicates. *Cyp6g1* transcript abundance was quantified relative to a pooled master sample of all samples using the efficiency-calibrated method of Pfaffl [29], normalized by the reference gene *Rpl32*. Baseline fluorescence-corrected efficiencies and Cq values were estimated using LinRegPCR version 2016.0 [30] (mean efficiencies; *Cyp6g1* 1.97 ± 0.09, *Rpl32* 1.91 ± 0.08).

(h) Statistical analyses

All statistical analyses were performed in R v. 3.1.2 [31]. General/Generalized Linear Mixed Models (GLMMs) were implemented in *lme4* [32]. Overdispersed models were fitted with quasi-error structures in the case of General/Generalized Linear Models (GLMs) or with an observation level random effect in the case of GLMMs [33]. Maximal models were fitted with all explanatory variables and their interactions, and then simplified via stepwise term deletion (for brevity, we report only significant interactions, but full model tables are available in the electronic supplementary material). *Cyp6g1* genotype was always retained as a main effect as it is the primary variable under investigation.

The proportion of mating trials won by resistant males was compared to 0.5 (the expected proportion under equal competitiveness) using a two-tailed Fisher's exact binomial test. To examine the effect of male genotype on the remaining fitness measurements, we implemented a multivariate analysis of covariance (MANCOVA) with log copulation latency, copulation duration, and number of offspring as response variables; successful male genotype as an explanatory variable; and relative male size and female size as covariates. Forty-eight individuals were excluded from this analysis due to incomplete data (final $n = 112$).

Male P1 and P2 were compared between genotypes using quasi-binomial GLMs with male genotype, relative copulation duration, and the number of offspring produced before remating as explanatory variables.

Female offspring production was compared between genotypes using a Poisson GLMM. Egg production, egg–larvae viability and larvae–adult viability were compared between genotypes using GLMs fitted with quasi-Poisson (egg production) and quasi-binomial (both viability measures) error structures. Full models were fitted with female genotype, male genotype, female size, with block and unique ID as random

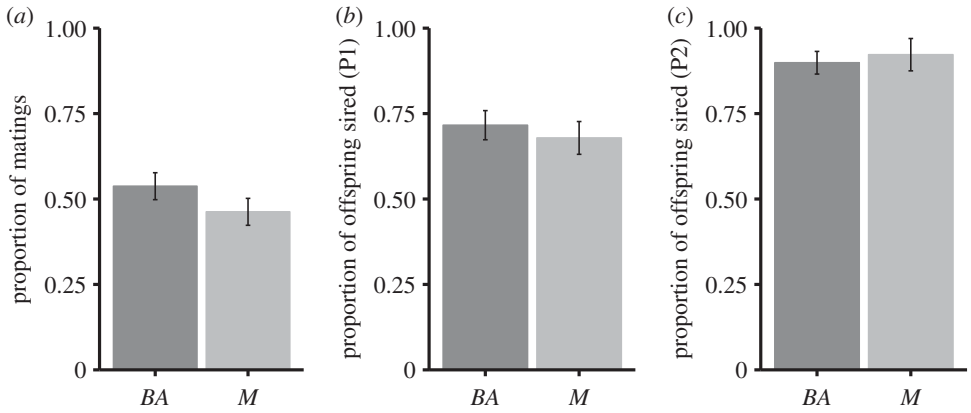

Figure 1. Competitive performance (±standard error (s.e.)) of resistant *BA* and susceptible *M* males in the (*a*) pre-copulatory competitive assay, (*b*) sperm defence assay, and (*c*) sperm offence assay.

effects for offspring production. Interactions were omitted from the offspring production GLMM as their inclusion prevented model convergence.

Changes in the frequency of the *BA* allele in the experimental evolution populations were assessed using GLMMs with *BA* frequency as the response variable, generation as a fixed effect, and replicate population as a random effect.

Sex-specific LD50 values and associated 95% confidence intervals (CI) were estimated using *drc* [34]. LD50 values were considered significantly different when their 95% CIs did not overlap [35].

Cyp6g1 expression was compared between genotypes and sexes using a GLM with log relative fold change (as per Pfaffl [29]) as the response variable with sex and genotype as explanatory variables.

3. Results

(a) Male fitness assays

(i) Pre-copulatory competitive ability

Overall, resistant and susceptible males did not differ in their proportion of obtained matings (exact binomial test, $Bin_{0.5}$, number of resistant matings 86 of 160 trials, $p = 0.38$, figure 1). However, there was variation between the two experimental blocks with resistant males securing a significantly lower proportion of matings than susceptible males in block 1 (exact binomial test, $Bin_{0.5}$, number of resistant matings 13 of 43 trials, $p = 0.01$; figure 1), but a significantly higher proportion of matings in block 2 (exact binomial test, $Bin_{0.5}$, number of resistant matings 73 of 117 trials, $p = 0.009$; figure 1).

Female size had a significant effect on the multivariate combination of log copulation latency, copulation duration, and offspring number because of a large and positive significant univariate effect on copulation duration (table 1). Univariate tests revealed that copulation duration was significantly shorter for resistant males than susceptible males, but this was not great enough to drive a significant effect in the multivariate test (table 1).

(ii) Sperm competitive ability

P1 was not significantly influenced by male genotype (GLM, $F_{1,67} = 0.08$, $p = 0.77$) or relative copulation duration (GLM, $F_{1,66} = 3.54$, $p = 0.06$), but decreased significantly as the number of offspring produced before remating increased (GLM, $F_{1,68} = 4.89$, $p = 0.03$). P2 was not significantly influenced by male genotype (GLM, $F_{1,63} = 0.15$, $p = 0.7$), relative copulation duration (GLM, $F_{1,64} = 0.75$, $p = 0.39$), or the number of offspring produced before remating (GLM, $F_{1,62} = 0.22$, $p = 0.64$).

(b) Female fitness assays

(i) Offspring production

Resistant females produced significantly more offspring than susceptible females (GLMM, $\chi^2_5 = 6.50$, $p = 0.01$; figure 2). Larger females also produced significantly more offspring (GLMM, $\chi^2_5 = 7.97$, $p < 0.01$). Male genotype had no significant effect on offspring production ($\chi^2_6 = 0.88$, $p = 0.35$).

(ii) Egg production and offspring viability

Resistant females produced significantly more eggs than susceptible females (GLM, $F_{1,89} = 10.88$, $p < 0.01$; figure 2), whereas male genotype (GLM, $F_{1,88} = 0.51$, $p = 0.48$) and female size (GLM, $F_{1,87} = 0.22$, $p = 0.63$) had no effect. Egg–larvae viability was not significantly influenced by female genotype (GLM, $F_{1,88} = 0.16$, $p = 0.69$), male genotype (GLM, $F_{1,87} = 2.89$, $p = 0.09$), or female size (GLM, $F_{1,86} = 0.02$, $p = 0.89$) (figure 2). Larval–adult viability was not significantly influenced by female genotype (GLM, $F_{1,86} = 0.01$, $p = 0.34$), male genotype (GLM, $F_{1,84} = 0.04$, $p = 0.85$), or female size (GLM, $F_{1,85} = 0.21$, $p = 0.65$; figure 2).

(c) Mathematical model and experimental evolution

The IASC model of Rostant *et al*. [8] parametrized with our data (relative resistant male mating success, $m = 1$; relative resistant female fecundity, $F = 1.56$) predicted that across seven generations *BA* frequency should increase marginally to 0.908 from an initial frequency of 0.9, and increase to 0.26 from an initial frequency of 0.1 (figure 3). These predictions were not met in the experimental populations. The frequency of the *BA* allele decreased significantly across generations in populations where the allele was at the high initial frequency (GLMM, $F_{1,3} = 18.56$, $p < 0.001$) and increased significantly across generations from the low initial frequency (GLMM, $F_{1,3} = 17.68$, $p < 0.001$), both converging towards an intermediate frequency (figure 3).

(d) Dichlorodiphenyltrichloroethane resistance

Resistant males had an 8.2-fold higher DDT resistance than susceptible males and resistant females had a 14.4-fold

Table 1. MANOVA and univariate ANOVA of male pre-copulatory competitive ability. Significant *p*-values are in italics.

	MANOVA			
	Pillai's trace		**$F_{3,108}$**	***p*-value**
male genotype	0.059		2.208	0.091
male size difference	0.035		1.277	0.286
female size	0.119		4.801	*0.004*
	male genotype (mean ± s.e.)		**univariate ANOVAs**	
	resistant	**susceptible**	**$F_{1,108}$**	***p*-value**
log copulation latency (min)	3.897 ± 0.123	3.819 ± 0.152	0.162	0.688
copulation duration (min)	17.646 ± 0.792	20.762 ± 0.967	6.349	*0.013*
no. of offspring	65.484 ± 1.946	64.813 ± 3.138	0.034	0.854
	male size difference β		**$F_{1,108}$**	***p*-value**
log copulation latency (min)	−0.324		0.079	0.78
copulation duration (min)	−14.439		3.226	0.075
no. of offspring	21.742		0.609	0.437
	female size β		**$F_{1,108}$**	***p*-value**
log copulation latency (min)	2.239		0.608	0.437
copulation duration (min)	68.392		14.059	*0.0003*
no. of offspring	−29.268		0.295	0.588

higher resistance than susceptible females (figure 4). Additionally, susceptible females had a 5.5-fold higher resistance than susceptible males, and resistant females had a 9.7-fold higher resistance than resistant males (figure 4). All differences were considered statistically significant as there were no CI overlaps (Bonferroni corrected *t*-tests gave identical results).

(e) *Cyp6g1* expression

The relative expression of *Cyp6g1* was significantly higher in females than males (GLM, $F_{1,9} = 229.48$, $p < 0.001$), and significantly higher in resistant flies than susceptible flies (GLM, $F_{1,9} = 186.99$, $p < 0.001$; figure 5).

4. Discussion

Recent work has shown that the DDT-resistant *BA* allele of the *D. melanogaster Cyp6g1* gene is subject to sexually antagonistic selection in the absence of insecticide when introgressed into the *Canton-S* background [8–10]. Here, we find that the previously detected female benefit of the *BA* allele [9] exists in a population recently sampled from the wild. By contrast, we find no overall cost of the *BA* allele in males in the relatively simple individual assay environment, but some evidence of variation in the *BA* allele effect on male fitness, as well as reduced reproductive investment by *BA* males. Additionally, we find sexual dimorphism in DDT resistance and *Cyp6g1* expression that help explain the lack of a detectable male mating cost in individual-level assays. A mathematical model of IASC parametrized with our empirical fitness estimates predicted that the *BA* allele should go to fixation. However, using experimental evolution we find evidence of balancing selection as the *BA* allele was maintained at intermediate frequencies, which matches previous findings [8].

In order to assay the pleiotropic fitness effects of the *BA* allele, both McCart *et al.* [9] and Smith *et al.* [10] introgressed the allele into the naturally susceptible *Canton-S* strain. While introgressing a resistance allele into a common genetic background is a powerful approach to quantify its fitness effects relative to a susceptible allele, we know that epistatic interactions can influence the pleiotropic fitness effects of resistance [10]. Indeed, IASC is itself the result of sex-specific epistasis. As the experimental populations used in this study were founded by 15 isolines, each with higher between-line genetic variation than within-line genetic variation, our fitness measures include the contribution of within-population epistatic interactions (albeit a subset of those that would be present in the wild) and are arguably more generalizable than assaying in any single genetic background. Additionally, even though quantifying the fitness effects of a resistance allele in naive genetic backgrounds may yield insight into the original effects of the allele before potential compensatory evolution could ameliorate any costs, it does not inform us of the effects present in a contemporary population that has coevolved with the resistance allele. By using recently caught flies from a population where the *BA* allele is naturally segregating, our fitness measures also include the contribution of any cost-ameliorating genetic architecture that has coevolved with the *BA* allele.

Overall, we found no significant difference between resistant and susceptible males in their pre-copulatory or post-copulatory competitive ability when assayed in paired competitions (figure 1). This is consistent with the results of Smith *et al.* [10], who introgressed the *BA* allele into another recently caught genetic background and found no cost to

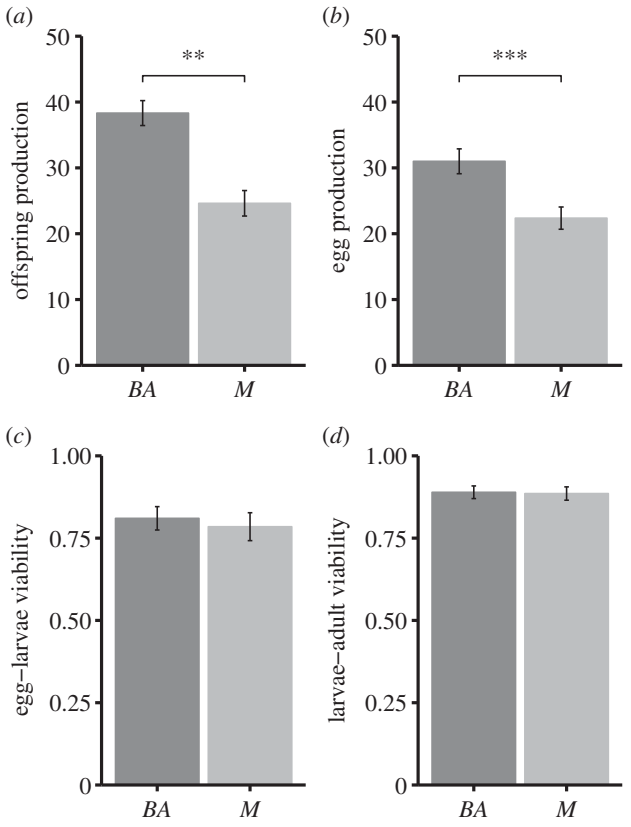

Figure 2. Means (±s.e.) of resistant *BA* and susceptible *M* females for (*a*) offspring production, (*b*) egg production, (*c*) egg to larvae viability, and (*d*) larvae to adult viability (**$p = 0.01$, ***$p < 0.01$).

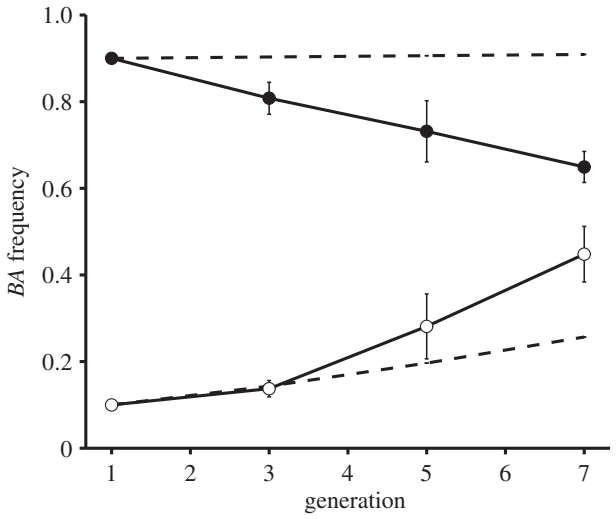

Figure 3. Mean frequency (±s.e.) of the resistant *BA* allele across seven generations of experimental evolution from an initially high frequency (closed circles) and an initially low frequency (open circles). Black dashed lines indicate model predictions based on fitness assay data.

males in the same assay. While there was no significant overall effect, there was an interaction between male mating success and experimental block that demonstrated variation in pre-copulatory competitive ability. The ability to secure matings is a key determinant of male fitness [36,37]. This interaction could be epistatic or genotype-by-environment [38], or alternatively may simply be a statistical artefact of forcing competitions to have simple binomial outcomes as this potentially increases the standard error of the mean.

We found that resistant females exhibited a qualitatively similar increase in fecundity to that seen in *Canton-S* by McCart *et al.* [9] (figure 2). The increase in offspring production can be apportioned directly to increased egg production in resistant females and not greater egg or larval viability. This contrasts with the findings of McCart *et al.* [9] findings where resistant offspring were more viable. Larger females were also significantly more fecund than smaller females—a general trend in insects [39]. Male genotype did not have any significant effect on female offspring production, which is consistent with previous evidence that the female benefit may be a maternal effect [40].

The DDT-resistance assay confirms that *BA* individuals of both sexes are indeed more resistant to DDT than their *M* counterparts. Additionally, there is significant sexual dimorphism in the relative level of resistance conferred by both alleles. Schmidt *et al.* [15] introgressed the *BA* allele into the *Canton-S* background and reported no sexual dimorphism in resistance conferred by the *M* allele and an approximately 1.3-fold increase in resistance conferred by the *BA* allele in females relative to males. Here, we show that *M* females are 5.5-fold more resistant than *M* males, and resistant *BA* females are 9.7-fold more resistant than *BA* males. This level of sexual dimorphism hints at the presence of genetic modifiers that have coevolved with the *Cyp6g1* locus allowing sex-specific gene expression in this population. Consistent with this, we found sexual dimorphism in relative *Cyp6g1* expression across both alleles. This sexual dimorphism may explain the lack of a detectable mating disadvantage for resistant males compared to the *Canton-S* strain, in which sexual dimorphism in resistance is less pronounced. IASC can be resolved by the evolution of the genetic architecture underlying the conflict whereby the genetic constraints preventing sexual dimorphism are removed (e.g. [4,41]). However, as most (if not all) phenotypic traits are genetically correlated with other traits, it is likely that networks of genetic correlation may to some extent always retain a certain level of constraint and IASC [4]. When modifiers do evolve that ameliorate the costs of IASC they are expected to have a greater effect on the sex under stronger sexual selection [42], but it is impossible to express from our data whether the sex-specific expression of *Cyp6g1* we report here has any sex-linkage.

Our individual-level fitness assays suggest that the *BA* allele is not sexually antagonistic in this recently sampled population, where it appears to be selectively neutral in males but beneficial in females. Consistent with this, phenotypic and qRT-PCR assays revealed sex-specific resistance regulation. These data support the hypothesis that genomic modifiers offsetting at least some of the male resistance costs exist in this population.

We sought to validate our individual-level fitness estimates in a more realistic context by measuring the frequency dynamics of the *BA* and *M* alleles in experimentally evolving populations that started at either an initially high or low frequency of the *BA* allele. We first parametrized the model of Rostant *et al.* [8] with our fitness estimates to obtain quantitative predictions. The parametrized model predicted that the *BA* allele should rapidly increase in frequency from a low initial frequency and increase marginally from a high initial frequency after seven generations (dashed black lines, figure 3). While the trajectory of our initially low-frequency populations qualitatively fits this pattern, we found a decrease in frequency across seven

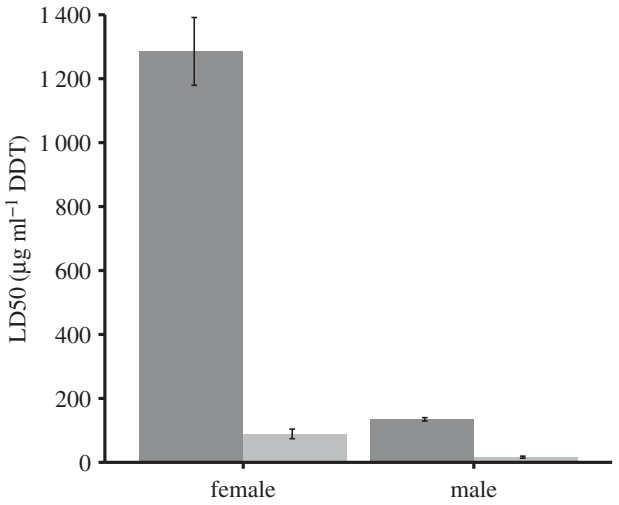

Figure 4. DDT resistance (LD50 ± 95% CI) in males and females carrying the resistant *BA* allele (dark grey bars) and the susceptible *M* allele (light grey bars).

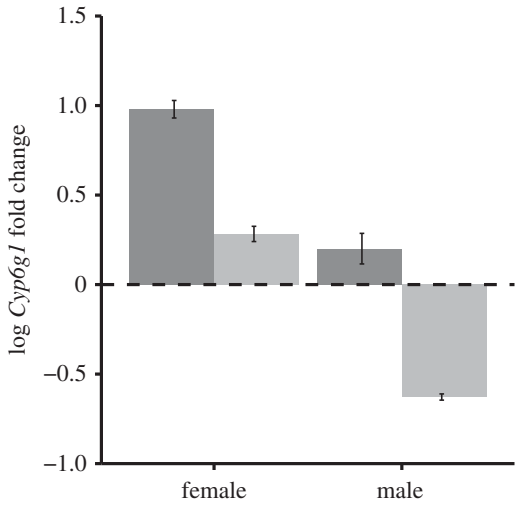

Figure 5. Relative *Cyp6g1* expression (± s.e.) in males and females carrying the resistant *BA* allele (dark grey bars) and the susceptible *M* allele (light grey bars).

generations in the initially high-frequency populations that the model did not predict. These results indicate that there is balancing selection at the *Cyp6g1* locus. This is consistent with Rostant *et al*. [8] who, when empirically testing the model parametrized with fitness estimates for *Canton-S* flies, also found that *BA* frequency converged to a stable intermediate value. As noted however, our population-level findings are inconsistent with the data from individual-level fitness assays.

How can we explain this apparent discrepancy? One explanation is that individual-level assays do not fully reflect fitness in more natural population settings. In a review of the literature investigating costs of resistance to *Bt* toxins in insects, Gassmann *et al*. [43] found that trait-based approaches detected costs in 34% of experiments. By contrast, population-based approaches (those that track the frequency of resistance alleles in a population over time) detected fitness costs in 62% of experiments. Thus, costs to resistance could emerge in a population context that would not be detected in individual-based fitness assays. This may in part be due to the fact that male mating success can be dependent on social context [44]. It may also be the case that other mechanisms (e.g. overdominance) are responsible for the maintenance of the *M* allele in our experimental evolution populations. However, since our findings at the population level mirror those of Rostant *et al*. [8] IASC seems the more likely explanation. The presence of polymorphism at the *Cyp6g1* locus in the original isolines themselves suggests this balancing selection may also be present in the source population. Our experimental evolution data are consistent with this idea, but further work is needed to clarify this possibility.

One potential proximate explanation for the apparent discrepancy between our individual-level and population-level data is that mating pairs remained *in copula* for longer when males carried the susceptible *M* allele. As copulation duration can be used as a proxy for reproductive investment in males [45,46], the effect of male genotype on copulation duration could represent lower reproductive investment by resistant *BA* males. Sperm is transferred relatively rapidly in *D. melanogaster*, and extended copulation duration probably achieves increased semen transfer and associated accessory gland protein effects (e.g. reduced female remating rate) [47,48]. Shorter copulation duration in resistant males could thus reduce the ability of these males to delay female remating, which would decrease resistant male fitness, but would not be detectable in the design of our fitness assays. It may, however, help explain the balancing selection observed in our experimental evolution populations where females had the opportunity to remate constantly across several days.

5. Conclusion

Using recently sampled *D. melanogaster*, we show that an insecticide-resistance allele at the *Cyp6g1* locus confers a pleiotropic fitness benefit to females in the form of increased reproductive output, and while having no direct detectable effect on male fitness, it does reduce male reproductive investment. Additionally, we find some sexual dimorphism in DDT resistance and relative *Cyp6g1* expression that could explain the lack of detectable sexual antagonism at the individual level. These individual-level data suggest the presence of genetic modifiers that at least partially ameliorate previously reported male mating costs. Nonetheless, we find evidence of balancing selection at the *Cyp6g1* locus in experimentally evolving populations that were not theoretically predicted. These population-level data suggest that, despite sexual dimorphism in resistance and gene expression, some sexual antagonism remains. Taken together, our results suggest that individual-level fitness assays may not capture sexually antagonistic fitness effects that emerge at the population level, and such effects can maintain resistance at the *Cyp6g1* locus in the absence of insecticide.

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material.

Authors' contributions. M.F.H., N.W., and D.J.H. designed the study and wrote the manuscript; M.F.H., C.E.G., E.C.R.T., and M.R.C. conducted the experiments; M.F.H. analysed the data.

Competing interests. We have no competing interests.

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