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Behavioral response to combined insecticide and temperature stress in natural populations of *Drosophila melanogaster*

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Running Title

Response to DDT and temperature in *D. melanogaster*

Data Archival

Data archive will be available on DRYAD pending acceptance of the article. Now available at: https://www.dropbox.com/s/0e80vmrmfajqoq0/Fournier-Leveletal2015_DataArchive.zip?dl=0

Abstract

Insecticide resistance evolves extremely rapidly, providing an illuminating model for the study of adaptation. With climate change reshaping species distribution, pest and disease vector control needs rethinking to include the effects of environmental variation and insect stress physiology. Here we assessed how both long term adaptation of populations to temperature and immediate temperature variation affects the genetic architecture of DDT insecticide response in Drosophila melanogaster. Mortality assays and behavioral assays based on continuous activity monitoring were used to assess the interaction between DDT and temperature on three field-derived populations from climate extremes (Raleigh for warm temperate, Tasmania for cold oceanic and Queensland for hot tropical). The Raleigh population showed the highest mortality to DDT whereas the Queensland population, epicentre for derived alleles of the resistance gene Cyp6g1, showed the lowest. Interaction between insecticide and temperature strongly affected mortality, particularly for the Tasmanian population. Activity profiles analyzed using self-organizing maps show the insecticide promoted an early response while elevated temperature promoted a later response. These distinctive early or later activity phases revealed similar responses to temperature and DDT dose alone but with more or less genetic variance depending on the population. This change in genetic variance among populations suggests that selection particularly depleted genetic variance for DDT response in the Queensland population. Finally, despite similar (co)variation between traits in benign conditions, the genetic responses across population differed under stressful conditions. This showed how stress-responsive genetic variation only reveals itself in specific conditions and thereby escapes potential trade-offs in benign environments.

Keywords

DDT, Cyp6g1, genetic variation, activity monitoring, self-organizing map, G-matrix

Introduction

As climate change is shifting the distribution of pest and disease vectors, renewed efforts in pest management strategies are required. However, pest species have often evolved some degree of resistance when confronted to lethal dose of insecticides, proving the limits of current strategies. In times of major uncertainty about the climate and the emergence of new pests, understanding the interplay between resistance and adaptation to climate is a pressing question in evolutionary biology.

Lessons from the past offer valuable opportunity to improve strategies for tomorrow: over the past sixty years, massive amounts of the insecticide DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) were applied worldwide to control pests and disease vectors. This widespread and non-specific selection pressure has driven the emergence and diffusion of resistance alleles, making insecticide exposure the best example of how insect populations adapt to stressful environments (Crow, 1966; Merrell, 1994). Despite being banned in most countries, DDT is still in use in some parts of the world and remains a persistent pollutant in natural ecosystems because of its environmental stability, acute toxicity and low water solubility (World Health Organization, 1989; Eskenazi et al., 2009). DDT thus provides an unmatched opportunity to investigate how a systematic and well defined selective pressure with global, albeit uneven distribution, has shaped insects' natural populations. Moreover, since geographically dispersed populations of insects have come in contact with DDT, we can ask to which extent adaptation to climate alters the genetic architecture of insecticide resistance.

The fruit fly Drosophila melanogaster has a cosmopolitan distribution, adapting to diverse climates from tropical to temperate, and has colonized human-modified environments, therefore being exposed to high amounts of insecticides. With respect to climate adaptation, the distribution of genetic variation in D. melanogaster has been extensively studied along the North American and East Australian coasts (Weeks et al. 2002; Turner et al. 2008; Paaby et al. 2010; Fabian et al. 2012; Reinhardt et al. 2014; Adrion et al. 2015). Despite independent colonization and demographic histories, some degree of parallel evolution across these continents was observed for the loci believed to increase thermotolerance, with allele frequencies changing with latitude (Rako et al. 2007; van Heerwaarden and Sgrò 2011). This strong genetic differentiation demonstrates the importance of thermal adaptation and allows sampling populations specifically preadapted to different temperature regimes. In addition to genetic adaptation, a population may also respond to environmental stress through phenotypic plasticity, i.e. the expression of an altered phenotype for a given genotype across different environments (Merilä and Hendry 2014). In Drosophila simulans high plasticity in thermal tolerance

was shown to bear a significant fitness cost and to be under significant genetic constraint (Bubliy et al., 2013), however it is unclear whether plasticity is a ubiquitous solution to a changing environment. A powerful way to disentangle adaptation from plasticity is to investigate populations originating from diverse regions where they have adapted to particular climates and to assess them in multiple environments. The adaptive hypothesis can be thus assessed by testing populations of different origin in a common environment and the plasticity hypothesis by testing a specific population across multiple environments.

Resistance to DDT was observed as early as 1947 (Brown, 1958), and attributed to the metabolism of DDT into non-toxic DDE (Perry, 1960). In Drosophila melanogaster, the Cytochrome P450 encoding gene Cyp6g1 contributes to DDT resistance in many populations (Daborn et al., 2002). Longstanding exposure to DDT has triggered the gradual evolution of the Cyp6g1 locus from the ancestral and susceptible M haplotype to highly resistant AA, BA and BP haplotypes that have emerged via gene duplication and multiple transposable element insertions (Accord, HMS-Beagle and P-elements), increasing gene expression (Schmidt et al., 2010). Interestingly, the resistant haplotypes have not reached global fixation and the Accord retrotransposon was found to follow a pattern of latitudinal differentiation in the USA and in Australia (Catania et al., 2004; Turner et al., 2008). Temperature variation is therefore a potential explanation for this heterogeneous distribution of resistance alleles. More generally, temperature has frequently shown to modulate insecticide mortality, with a surprisingly inconsistent effect across taxa and insecticides (Amarasekare and Edelson 2004; Muturi et al. 2011; Janssens et al. 2014). However, this modulating effect has never been investigated for multiple populations of a single species.

Here, we investigate the response of three populations of D. melanogaster sampled from different climatic regions, to gradients of DDT and temperature. The sample represents populations from a hot tropical climate in North Queensland (Australia), from a warm temperate climate in Raleigh, North Carolina (USA) and a cool oceanic climate in Tasmania (Australia). We first measured the effect of temperature on DDT mortality to test the extent of plasticity within and across populations of different climatic origins. Then we used a more sensitive assay that monitored the activity of flies continuously over the 24 hours of the exposure to specific DDT and temperature combinations. Substantial changes in activity regime were observed over time, leading to the identification of two temporally distinct phases of response over the course of the stress exposure. These phases were further analyzed as function-valued traits in order to estimate the genetic component of the variance for all DDT and

temperature combinations in a unified model. Finally, the values of the functions for activity metrics and mortality under specific regimes of temperature and DDT dose were used to determine the level of genetic constraint within population using genetic variance-covariance matrices (G-matrices) analysis. Altogether, our study reported extensive interaction between DDT and temperature and showed how increasing tolerance to DDT across populations was correlated with reducing genetic variance for activity traits. Despite similar trait variation in benign conditions, the response to stress was heterogeneous across populations, probably due to differences in the intensity of selection for stress resistance each population has faced.

Materials and Methods

Drosophila melanogaster source populations, breeding and maintenance

To study the behavioral effect of DDT combined with temperature stress, three naturally sourced populations were used: a set of inbred lines from the Drosophila Reference Genetic Panel (DGRP, Mackay et al., 2012, Suppl. Table S1) originally sourced from Raleigh (North Carolina, USA); a cool climate population from Hobart (Tasmania, Australia); and a hot climate population from Innisfail (Queensland, Australia). To be able to compare the Australian field-caught populations (which had been maintained for 12 generations as mass bred populations) to the highly inbred DGRP, these were inbred by single pair brother-sister mating for 10 generations to increase homozygosity. All flies were bred in constant 25ºC in 250mL plastic bottles under permanent light to remove the influence of circadian rhythm. Insecticide susceptibility and the activity of insecticide degrading enzymes exhibit circadian rhythms (Hooven et al. 2009; Beaver et al. 2010). However since circadian rhythm varies between lines (Low et al. 2012; and personal observations of DGRP lines) and since it is credible that it is also affected by temperature - a key variable of our experimentation (Hamby et al. 2013), we decided to minimize its influence by maintaining flies in constant light (Hooven et al. 2009). The culture media was based on Bloomington corn media. The founder populations represented an initial 192 genotypes for the Raleigh (DGRP) population, 30 for the Tasmania and 30 for the Queensland population, respectively. Unless stated otherwise, all assays were performed using 10 non-virgin female flies aged between 5 to 12 days with at least 4 replicates for each dose/temperature/genotype combination. Flies were left 24 hours to recover in holding vials after being sorted on a CO_2 gas plate.

Population sampling and selection of a reference middle DDT dose

To compare the populations with minimal sampling bias, reference subsets of 12 genotypes were carefully chosen for each population. These subsets included the genotypes having a maximally different response to DDT within each population. This was based on two phenotypes measured in the same experiment: proportion of flies knock-downed after three hours of exposure to DDT (3hKD) and proportion of mortality after 24 hours (24hDT). Assays were performed using 10mL glass vials coated with a solution of DDT diluted in 200µL acetone, stoppered with a cotton ball soaked in 5% sucrose and placed at constant 25ºC.

Due to dramatic differences in DDT dose response, a reference middle DDT dose was first determined for each population to approximate the Lethal Dose for 50% of the population (LD_{50}). This was measured in a 24h and 25ºC assay with each population tested as bulk of 50 randomly chosen females for three different doses (30, 120 and 480µg per 10mL vials). For the Raleigh and Tasmania populations, we identified 60 and 120µg of DDT as the round doses the closest to the LD_{50}, respectively. For the Queensland population, due to extremely high resistance, no mortality above 40% was observed when exposed to 480µg, which led us to use 480µg of DDT as low dose in subsequent assays. For the Raleigh, Tasmania and Queensland populations, we measured 3hKD and 24hDT when exposed to 60, 120 and 480µg of DDT, respectively. At these doses, variance in 24hDT was equal among populations (Bartlett's K-squared=0.72567, p-value=0.6957). However variance in 3hKD was greater in the initial Queensland population (Bartlett's K-squared = 24.951, p-value = 3.819e-06) but no difference in 3hKD was seen between the Raleigh and Tasmania populations (Bartlett's K-squared = 1.0983, p-value = 0.2946). Finally, 12 genotypes maximizing variability for the two traits were selected for each population (Suppl. Fig. S1).

Molecular Characterisation of neutral RFLP markers and the Cyp6g1 haplotype

PCR based RFLP assays were performed to characterize the level of neutral molecular diversity present in the samples. Genomic DNA was extracted from ~10 adult flies following a CTAB Phenol-Chloroform extraction protocol. 13 different sets of primers corresponding to markers 2L1, 2L3, 2L5, 2R2, 2R4, 2R6, 3L1, 3L3, 3L5, 3R1, 3R3, X3 and X5 from Mackay et al. (2012) were used to amplify DNA fragments, restriction-digested and visualized by agarose gel electrophoresis. In addition, Cyp6g1 shown to be a major DDT resistance gene in Drosophila (Daborn et al., 2002), was genotyped. Cyp6g1 encodes a P450 oxidase whose expression level has been associated with variation in resistance (Daborn et al., 2002; Festucci-Buselli et al., 2005; Chung et al., 2007). The Cyp6g1 gene exhibits copy

number variation and the resistance haplotypes are marked by various transposable elements. The ancestral susceptible haplotype has a single gene copy and has no insertion (referred to as the M or 'minus' haplotype) and then the successive insertion of the Accord LTR element, the HMS-Beagle retrotransposon and a partial P-element steadily increased the Cyp6g1 expression level and give rise to the 'Accord-Accord' (AA), 'Beagle-Accord' (BA) and 'Beagle-P-element' (BP) haplotypes all of which have at least one copy of Cyp6g1 (Schmidt et al. 2010). To specifically test the effect of the Cyp6g1 haplotype on the activity response to DDT, the presence of Accord/Beagle/P-element was genotyped in the Tasmanian and Queensland population based on the three PCR assays of (Schmidt et al. 2010; primers HI, LI, JK) and retrieved from the DGRP database for the Raleigh population. The amplicon typing matched those described in Schmidt et al. (2010) with the exception of a novel haplotype found once in the Tasmanian and once in the Queensland population. This novel haplotype had at least two copies of Cyp6g1 (primers JK) and an HMS-Beagle insertion upstream of at least one of these copies (LI), however the HI primers which bridge the Accord transposable element insertion sites failed to produce an amplicon, perhaps because of primer binding site polymorphism or because of further indel events. We therefore refer to this new haplotype as the BX haplotype.

Activity monitoring assays under changing temperature and DDT concentration

Fly activity was measured using the Drosophila Activity Monitoring system (DAM6, Trikinetics, Waltham, MA, USA). The DAM6 system consists of a Plexiglass support where tubes carrying flies are inserted so that infrared beams record any crossing of the tube over a given time as a proxy for activity. Each 10mL vial containing 10 females and a specific dose of DDT was inserted in the DAM6, the flies were allowed to recover from manipulation stress for an hour after which the number of crossings was recorded every two minutes over 23 hours. Assays were performed at three different temperatures (20ºC, 25ºC and 30ºC) and four population-specific doses approximately corresponding to control, half the population LD_{50} (low dose), LD_{50} (medium dose, but see previous sections for the Queensland population) and twice the LD_{50} (high dose). These doses corresponded to 0, 30, 60 and 120µg of DDT in 10mL vials for the Raleigh population; 0, 60, 120 and 240µg of DDT for the Tasmania population; and 0, 480, 960 and 1920µg of DDT for the Queensland population. For any combination of temperature and genotype, all different doses were run side-by-side and replicated twice in one assay and twice at a later date to avoid potential batch effects. After 24 hours, the tubes were pulled out of the DAM6 unit and mortality was scored (Fig. 1).

Self-Organizing Map Analysis

Log-transformed activity counts over time were analyzed using Kohonen's Self-Organising Map method (SOM, Kohonen, 2001) as implemented in the R\kohonen package (Wehrens & Buydens, 2007). Activity over time profiles were projected over a 6 x 7 hexagonal non-toroidal map (the opposite edges of the map are not connected) with a run length of 10000, defining 42 units, each representing a consensus profile between activity readings sharing a similar pattern. This SOM size was determined by minimizing the distance between each map unit and the observed activity profiles mapped to this unit (the smaller the better), penalized by the overall size of the map. After map units were defined through SOM as presented in Fig. 2a, the units were clustered into groups. These groups were defined through hierarchical clustering weighted by the count (frequency of observing a specific behavior type, Fig. 2b) and the mortality associated with the profiles supporting each unit (Fig. 2 c,d), using the "flat" and most frequently observed profile of the experiment as reference (Group 1).

The effects of the population of origin, temperature and DDT dose nested in the population effect and Cyp6g1 promoter type on the belonging to a behavioral group were tested through multinomial logistic regression using the multinom() function of the R\nnet package in the following model:

$$log(\frac{Pr(Y_i = 2)}{Pr(Y_i = 1)}) = \alpha_2 Pop + \beta_2 Cyp6g1 + \gamma_2 Temp * Pop + \delta_2 Dose * Pop$$

$$log(\frac{Pr(Y_i = k)}{Pr(Y_i = 1)}) = \alpha_k Pop + \beta_k Cyp6g1 + \gamma_k Temp * Pop + \delta_k Dose * Pop$$

for the k groups, where α, β, γ and δ are the predictors of the log-odd ratio of behaving like Group {2,...,k} instead of reference group 1. The significance of the logistic regression coefficients was assessed using z-tests.

Modeling of Early and Later Activity and Mortality

23 hours activity profiles were divided into two phases: first an early activity phase followed by a later activity phase. This is justified because mortality was significantly greater in Group 5, corresponding to a profile with intense early activity which sharply decreased afterwards. Early and later activity levels were computed as the sum of the activity counts from the beginning of the experiment to the breakpoint time and from the breakpoint time to the end of the experiment, respectively. The breakpoint time

between the two phases was determined as the time for which the early activity was maximally correlated with mortality and the early and later activity minimally correlated using Pearson's moment. Finally, to analyze the overall response to temperature and DDT in the different populations and to determine how much variation was present within population, the early activity, later activity and mortality were analyzed independently for each population using Bayesian general linear mixed-models implemented in the R\MCMCglmm package (Hadfield, 2010) as:

$$Y_{ij} = \alpha Temp + \beta Dose + \gamma Temp^2 + \delta Dose^2 + \eta Temp * Dose + \kappa_i Geno + \lambda_i Geno * Temp + \xi_i Geno * Dose + \varepsilon_{ij}$$

where α, β, γ, δ and η are the predictors of the fixed effects of temperature and dose; κ_i, λ_i and ζ_i are the predictors of the random genotype effects and ε_{ij} is the residual term. The MCMC sampling was run 25000 times with a burn-in period of 5000 iteration and a thinning interval of 20. The models were subsequently adjusted by removing non-significant fixed effects or random effect based on deviance information criteria (DIC) to optimize the model fit.

Measuring genetic constraint in the genetic variance/covariance structure across populations

Matrices of variance-covariance (G-matrices) between traits for each population were extracted from the solution of the adjusted mixed-linear models described previously for five subsets of experimental conditions, centered on the population mean, and each subset of traits was compared across populations using the indices and tests suggested in Charmantier et al. (2014). The five subsets of traits corresponded to 1- all traits in all temperature and DDT dose conditions, 2- traits for all temperature in absence of DDT (control), 3- traits for all temperature in presence of DDT (dose>0), 4- traits for the 25°C temperature for all DDT conditions (control and all DDT doses) and 5- traits for the low and high temperature conditions (20°C and 30°C) for all DDT conditions. We first computed the sum of all eigenvalues as an index of the total genetic variance available and the maximum variance along the first eigenvector relative to all eigenvalues (Kirkpatrick, 2009). Modified Mantel test was then used to test proportionality between G-matrices and Random Skewers test was used to test similarity in response to perturbation of the G-matrices based on the R functions provided in Roff et al. (2012). Finally, the properties of the common subspace between two G-matrices and the angle between the first eigenvector of these G-matrices were measured using Krzanowski common subspace test (Krzanowski, 1979) as implemented in the MCMCglmm\Krzanowski.test() function. Significance levels for all tests

241 described here were assessed by comparing the observed test statistic to the null distribution obtained
242 through randomisation (randomly reallocating individuals to one of the two populations compared).
243 Random draws were performed by permuting 1000 times the lines of the two G-matrices compared in a
244 given test. For the Random Skewers test, 100 skewers were drawn from a uniform U(-1,1) distribution.
245 To obtain robust estimates, the solution of the MCMCglmm models were computed five times
246 independently and for each set of solutions, the 1000 randomisation procedure was run five times, this
247 ensured stable sampling of p-values for all the tests described previously.
248
249 **Results**
250 Molecular diversity in sampled populations
251 To represent contrasted climatic origins, three populations were sampled: a warm temperate reference
252 population in Raleigh (North Carolina, USA), a hot tropical population in Innisfail (Queensland,
253 Australia) and a cool oceanic population in Hobart (Tasmania, Australia). For each population, 12
254 inbred lines were selected from larger initial populations to jointly maximize the diversity in response
255 to DDT for 3 hour knock-down and 24 hour mortality phenotypes (Suppl. Fig. S1). We first tested that
256 the sampling scheme lead to an even representation of neutral genetic diversity across populations
257 using 13 RFLP markers scattered over the genome. All 13 makers were polymorphic in each of the
258 three population samples (Suppl. Table S1) with a Nei diversity index (Nei, 1978) computed from these
259 markers of 0.35 for the Raleigh population, 0.41 for the Tasmania population and 0.41 for the
260 Queensland population. These similar Nei index values suggested no dramatic difference in levels of
261 neutral molecular diversity between populations, allowing further cross-population comparison.
262 The haplotype structure of the Cyp6g1 resistance locus was also examined with molecular markers and
263 showed within and among population diversity (Suppl. Table S1). The Raleigh population was the only
264 population bearing the DDT susceptible ancestral M haplotype (frequency=0.1), with most individuals
265 having the moderately resistant Accord-Accord (AA, frequency=0.7) and Beagle-Accord (BA,
266 frequency=0.2) haplotypes. The Tasmanian population consisted in mostly AA haplotype
267 (frequency=0.8) with one BA and one Beagle-uncharacterized allele (BX) haplotype (frequency=0.1
268 and 0.1, respectively). Finally, BA haplotype was less frequent in the Queensland population
269 (frequency=0.5) than in the other population (t-test, p-value<0.01) with a single BX haplotype
270 (frequency=0.08), while the highly resistant Beagle-P-element haplotype (BP, frequency=0.42) was
271 more frequent than in the other populations (t-test, p-value<0.01).

272

273 Temperature-modulated mortality to DDT

274 The mortality of lines exposed to DDT was very different across populations (Fig. 1). The Raleigh
275 population was the most susceptible (LD_{50} for all three temperatures = 52µg), the Tasmanian was
276 intermediary (LD_{50}=301µg) and the Queensland was the most resistant (the extrapolated
277 LD_{50}=405924µg). Given this dramatic difference in DDT sensitivity, we selected a dose for each
278 population such that mortality across populations was similar at the reference temperature of 25°C
279 (60µg per 10mL vials for the Raleigh population; 120µg for the Tasmania population; and 960µg for
280 the Queensland population; ANOVA, $F_{2,89}$=1.216, p-value=0.301). These doses were assigned to the
281 middle doses where each population had a separate series of three doses such that low dose = middle
282 dose/2 and high dose= 2x middle dose. Despite some discrepancies in the Raleigh/25°C and the
283 Queensland/20°C assays, increasing DDT dose increased mortality (GLM, $F_{3,1304}$=148.79, p-
284 value<0.001) among the other seven population-by-temperature combinations.
285 Once the DDT dose gradient was determined, we tested whether the mortality to DDT was modulated
286 by the temperature. Indeed, temperature variation (20°C, 25°C or 30°C) significantly affected mortality
287 (GLM, $F_{2,1305}$=39.23, p-value<0.01). The populations responded differently to the temperature
288 gradient; the Tasmania population showed high plasticity with decreasing mortality at higher
289 temperatures, the Queensland population was only moderately effected by temperature, whereas the
290 Raleigh population showed lower mortality at 25°C (Fig 1). T-tests on the linear-models across all
291 populations show that 20°C and 30°C led to higher mortality and thus could be considered more
292 stressful conditions ($t_{20°C\ vs\ 25°C}$= -5.78, p-value<0.001 and $t_{25°C\ vs\ 30°C}$=1.38, p-value<0.001). Altogether,
293 the extensive variation in mortality rate across experimental conditions reflected how temperature
294 variation modulated the level of mortality to a gradient of DDT dose, promoting different levels of
295 stress response within and across populations.
296

297 Activity patterns associated with exposure to DDT and temperature variation

298 To sensitively examine the stress response over the course of the exposure to DDT and temperature, the
299 activity of flies was monitored in real time over 23 hours. The activity profiles ranged from
300 continuously low activity to dramatic activity change over time. To identify discrete patterns in these
301 data, activity profiles were classified using a Self-Organizing Map (SOM, Kohonen 2001). The 1732
302 activity readings (36 lines from 3 populations, 3 doses plus control, 3 temperatures and 4 to 6

replicates) were classified into 42 activity profile types arrayed within a SOM (Fig. 2a). The most common activity profile corresponded to monotonous and low activity in the top-left corner of the map and was considered as the reference in further analyses. The map mainly distinguished two features of the activity profiles: the profiles with an early activity peak on the bottom-left corner and with a later increase in activity in the top-right corner. In addition, the top-left corner corresponded to low activity for both early and late phases of the assay period, in opposition to the bottom-right corner having high activity for both early and late phases. The 42 activity profiles were further clustered into five groups (Materials and Methods), resulting in: Group 1 including solely the reference activity profile; Group 2 including activity profiles deviating only slightly from the Group 1; Group 3 including profiles with later activity peak and overall low mortality; Group 4 including profiles with both high early and later activity; and Group 5 including profiles with early activity peak and high mortality. Details of the count for each population and condition are reported in Suppl. Fig S2a-c.

The temperature, DDT dose and population of origin had a significant influence in determining the activity profile of flies (multinomial logistic regression, Table 1). Increase in temperature particularly triggered a Group 3 type of activity response for all three populations (eg. From Table 1 we can see that for every additional Celsius degree the Raleigh population exhibits exp(0.60)=1.82 more chance to show a Group 3 than a Group 1 activity profile); Group 4 was less affected by temperature. In contrast, an increase in DDT dose generally increased the likeliness of a Group 5 response (in the Raleigh population: increasing the DDT dose from low to middle or from middle to high increases the chance of observing a Group 5 activity profile by exp(0.94)=2.56) but was highly unlikely to promote Group 3 or 4 responses. The exception to this was the Tasmania population, which was more likely to exhibit a Group 5 response irrespective of the temperature or DDT dose. In addition, the presence of Cyp6g1 BA (n=283 observations with this haplotype in the whole dataset) or BP (n=392) resistant haplotypes reduced the likeliness of observing a Group 5 response, strengthening the proposition that Group 5 reflects a DDT susceptible profile. The estimates for the BX and M haplotypes effects have to be interpreted with caution due to their low frequency in the data.

The exploratory 42 SOM profiles were further analyzed as function-valued trait so that the DDT and temperature responses could be modeled over a continuous gradient. Two quantitative traits was derived to describe the key changes in activity regime over the course of the stress exposure. As the most distinct phases revealed by the SOM analysis of the activity profiles was the presence of a distinctive early and later phase, the transition between the two phases was determined to occur at a

breakpoint time of 10 hours and 40 minutes (Materials and Methods and Suppl. Fig. S3). The early and later activity were defined as two continuous traits by summing the activity counts over the time each phase lasted, and those traits were subsequently analyzed in a quantitative genetics framework.

Within and across population response to temperature and DDT

To determine the extent of genetic variation involved in the insecticide response within populations, the sum of early and later phase activity as well as mortality observed after 24 hours were analyzed using mixed-linear models (Table 2). The solutions of the models are presented in Figure 3, with the fixed effects of the abiotic factors (temperature and DDT dose) presented as population-specific response surfaces and the superimposed random effect of within population genetic variation presented as heat maps. Altogether, these mixed-linear models explained a very substantial proportion of phenotype variation with global R^2 of 0.53 for early activity, 0.69 for later activity and 0.72 for mortality. Among all fixed effects (temperature^2, temperature, dose^2, dose and temperature-by-dose), the interaction between temperature and DDT was the only fixed effect significant for all traits (early activity, later activity and mortality) in all three populations. Despite quantitative differences in the magnitude of the fixed effects, for each trait, the shape of the response at the whole population level (not including the genetic differences within population) was fairly consistent across populations.

In all models, all three random effects (genotype, genotype-by-temperature and genotype-by-dose) improved the models fit based on Deviance Information Criterion (DIC). For all traits and populations, genetic effects had the highest variance when exposed to high temperature and to low DDT dose. However, levels of within population genetic variance differed dramatically across populations. The Raleigh population showed the overall highest genetic variance with less than 32% of the total phenotypic variance left as residual (for early activity, Table 2). Furthermore, the variance of the genotype-by-temperature effect for early activity and mortality was greater than the one of the genotype-by-dose effect, showing stronger temperature related genetic plasticity. Tasmania showed an intermediate level of overall genetic variation (Figure 3) but with stronger temperature plasticity (variance of the genotype-by-temperature effect always greater than that of the genotype-by-dose effect, Table 2). The Queensland population showed altogether much less genetic variance with between 51 and 84% of residual variance. Furthermore, in contrast to the other populations, the variance of the genotype-by-dose effect was greater than any other genetic variance, perhaps reflecting the fact that this is the only population sample with the highly resistant BP haplotypes.

Genetic constraint in the response to temperature and DDT gradient

The level of genetic correlation among traits based on the solution from the mixed-models differed substantially across the different conditions. Increasing the temperature significantly increased the correlation among traits, suggesting increased constraint (Fig. 4). In the contrary, increasing the insecticide dose reduced this correlation, suggesting that DDT stress induced more diverse trait combinations among genotypes. To determine whether the genetic constraint in trait variation within population was different between benign and stressful conditions (ie temperature extremes and presence of DDT), trait genetic variance and covariance matrices (G-matrices) for particular temperature and DDT dose conditions were extracted from the mixed-linear models. Each populations' G-matrices were defined to specifically contrast the traits response to DDT (control vs. Dose) and to temperature stresses (25°C vs. stressful temperatures).

The eigenanalysis of these matrices showed the Raleigh population as bearing the greatest amount of genetic variation for all subsets of conditions followed by the Tasmania and finally the Queensland population (Table 3a). Furthermore, the variation along the first eigenvector of each G-matrix or effective dimension, corresponding to a measure of genetic variation most readily available to selection, represented at least 49% of the total genetic variation in any population for any set of conditions (effective dimension $\max\lambda/\Sigma\lambda < 2$).

The tests performed on G-matrices for each subset of condition showed differences in matrices structure across populations (Table 3b). Both the Mantel (correlation) and Random Skewer tests (response to perturbation) showed Queensland as the most differentiated population while Raleigh and Tasmanian populations were more similar. Strikingly, the G-matrices in the most stressful set of conditions (with DDT or for temperatures of 20°C or 30°C) were systematically less similar than in more benign conditions (no DDT or 25°C). This suggests that stress tends to promote different reactions for populations that have different resistance levels.

We next tested the presence of a common subspace between matrices which indicates that the pair of populations compared can evolve the same trait values. The G-matrices measured in absence of DDT overlapped the most while those measured in the presence of DDT had a reduced common subspace (a greater p(<rand.) in Table 3b indicates that the observed value is smaller than in the randomized data and thus the overlap is smaller than expected by chance). The analysis of the angle between the first eigenvectors of these G-matrices show the same general pattern as for the volume of the common

subspace. For the tested range of condition, these results showed that the presence or absence of DDT led to strong differences in how traits respond within population but far less so temperature. When comparing populations, the G-matrices for the Raleigh and the Queensland populations always showed the greatest angle, further emphasizing the difference in the nature of the genetic variation available to respond environmental stress in these two populations. Overall, these results show the populations tested reacted differently under stressful conditions induced by DDT or temperature, even with similar trait covariation structure in benign conditions.

Discussion

The Physiological response to DDT and temperature across populations

This study shows that insect activity measures through Drosophila Activity Monitors captures highly heritable traits that are sensitive enough to provide novel insights into insecticide biology (Denecke et al. 2015). Both DDT and temperature stress trigger increased activity. However, the response to DDT exposure leads to an early reaction whereas temperature leads to a later one. This is coherent with the mode of action of DDT, which causes nervous system misfiring, while the response to temperature may first involve activity reduction before triggering escape past a time threshold (Hoffmann and Parsons 1997; Dillon et al. 2009; Ma and Ma 2012).

Interestingly, we detected a significant interaction between the DDT dose and the temperature for all traits analyzed in all three populations (Table 2). Such a modulating effect of the temperature on insecticide action has been reported for five out of seven insecticides tested in the larvae of the grasshopper Melanoplus differentialis (Thomas) (Amarasekare & Edelson, 2004) and for an organophosphate insecticide in the damsel fly Ishnura elegans (van der Linden) (Janssens et al., 2014). A temperature effect is also illustrated by the reduced efficiency of malathion on the mosquitos Culex restuans (Theobald) and Aedes albopictus (Skuse) at 20°C compared to 25°C and 30°C (Muturi et al., 2011). These mosquito results contrasts with our Drosophila results to the extent that mortality was highest at 20°C in our study. This discrepancy may be due to the respective mode of action of DDT and malathion or the way the different species have evolved to overcome them. Malathion is an insecticide blocking acetylcholine esterase activity, and resistance in field populations is often associated with target site resistance. Conversely, DDT acts on sodium channels and resistance in D. melanogaster is mostly mediated by P450 detoxification and so higher temperature may increase the insect metabolic rate and thus the detoxification efficiency. Altogether, our finding joins the body of literature reporting

that the insect response to insecticides depends on temperature and reinforces the possibility that different genetic architectures may arise from insecticide pressures in different environments.

Heterogeneous selection for resistance across populations

The three populations examined in this study exhibited substantially different resistance levels; from the more sensitive Raleigh population to the highly resistant Queensland population. They differ in the degree of interaction between DDT and temperature; with the Tasmanian population being the most plastic. They also differ with respect to genetic variance for the traits examined; with the Queensland flies exhibiting less genetic variance for all of them. Although the molecular variation present in the natural populations of origin might have been different, this is striking considering the initial population from which the Queensland population was sampled exhibited high variation for the 3h knock-down phenotype (see materials and methods). Given that the Queensland flies in this study, like those sampled in a prior study by Schmidt et al. (2010) have a higher frequency of the DDT resistance alleles at the Cyp6g1 locus, it is our contention that all these population differences can be attributed to selection for insecticide resistance depleting variation at resistance loci and possibly on other correlated traits. Given the equivalent levels of neutral standing variation present in all three populations, a bottleneck or founder effect of the resistant genotype alone (Piiroinen et al. 2013) is unlikely to have generated the pattern of genetic variation observed. So we propose that there has been an indirect shift in the genetic architecture of traits such as has been reported in the case of the resistance of the moth Choristoneura rosaceana (Harris) to various classes of insecticides wherein multiple life history traits were affected (Carriere and Roff 1995).

The Queensland population shows extreme levels of resistance and limited temperature plasticity, and also has a much higher frequency of the most resistant Cyp6g1 haplotypes. In the activity assays reported here, all the lines bearing the most derived Cyp6g1 alleles (i.e. BP and BA haplotypes) show predominantly "flat" activity profiles (Gr.1 and 2 in Fig 2a) which differ from more susceptible fly lines that exhibited an early burst of activity (Gr. 5 in Fig. 2a; Table 1). While this study reaffirms that large effect alleles at the Cyp6g1 locus contribute to DDT resistance, it also implies that other loci contribute to DDT resistance and the associated temperature plasticity. Specifically, the Tasmania and Raleigh populations have similar Cyp6g1 allele frequencies (here and in other studies: Catania et al. 2004; Schlenke and Begun 2004) yet the Tasmania population exhibits twice the resistance of the Raleigh population and is much more plastic. In addition, since our study specifically used female bred

458 under constant light, we only investigated a subset of the potential factors known to modulate the
459 genetic basis of insecticide response (Mackay 2001; Beaver et al. 2010; Hamby et al. 2013). In this
460 context, our report is probably still underestimating the true extent of plasticity in relation to stress
461 response in natural population of D. melanogaster.
462
463 Strong genetic constraint in the behavioral response to DDT
464 Our results showed no dramatic difference in the way the three populations responded to the
465 temperature gradient alone (Figure S2a-c, Control column). All populations, no matter the level of
466 exposure to DDT, altered their activity in response to temperature in a similar fashion: from mostly low
467 activity profiles at 20°C (Gr. 1) to continuously high profiles at 25°C (Gr. 4) and finally exhibiting
468 stress at 30°C with an escape behavior through increased later activity (Gr. 3). There is thus no
469 ubiquitously altered response to temperature induced by DDT resistance. However, when traits were
470 clustered together based on the conditions they were measured in, differences in genetic correlations
471 between traits emerged (Table 3a and 3b). Strikingly, these differences were only observed in the
472 stressful set of conditions, in coherence with the hypothesis that enhanced level of additive genetic
473 variation is favored by environmental stress (Hoffmann & Parsons, 1997). Insecticide bioassays are
474 usually conducted under standard environmental conditions and insecticide resistance has relatively
475 rarely been associated with a fitness cost. However, we report strong levels of genotype-by-
476 environment interaction, with activity traits showing a stronger environmental influence of both
477 temperature and DDT dose and mortality being more influenced by genetic factors (Table 2). Under
478 this type of genetic determinism, insecticide resistance may not have an immediate fitness consequence
479 under benign conditions but could in return become more pronounced under stressful conditions.
480 Genotype-by-environment interaction could therefore represent a potential mechanism for mitigating
481 the deleterious effects (i.e. cost) of insecticide resistance as cost would not be ubiquitous but only be
482 revealed under specific stress. This may also suggest that in spite of being found in various taxa and
483 insecticide classes (Kliot & Ghanim, 2012), identifying trade-offs requires testing a broad range of
484 conditions and may have been underestimated in the literature.
485 The G-matrices analysis suggested a common genetic structure for traits across populations (Table 3a)
486 but the stress response did not necessarily sit in the same space (Krzanowski test, Table 3b). In theory,
487 if selection was to act solely on standing variation, the dimension of the common subspace between the
488 G-matrices of the populations compared should have a volume roughly equivalent to the one of the

smallest G-matrix considered (Aguirre et al., 2014). In the analysis reported, significantly non-overlapping spaces were found between the most and least resistant population when exposed to DDT. This result can be interpreted by considering that mutations of strong effect are capable of changing the space in which the genetic variation lies in a population (Jones et al., 2003). Such a mutational shift is expected in the context of resistance mutation and would lead to defining a new space for evolution of traits. Therefore, the Cyp6g1-BP haplotype specific to the Queensland population potentially reflects such a mutational shift. In this scenario, the dramatic effect of BP is constraining the variation for activity traits through a conditionally pleiotropic effect, only revealed in stressful conditions.

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References

Adrion, J. R., M. W. Hahn, and B. S. Cooper. 2015. Revisiting classic clines in Drosophila melanogaster in the age of genomics. Trends Genet. 31:434–444.

Aguirre, J. D., E. Hine, K. McGuigan, and M. W. Blows. 2014. Comparing G: multivariate analysis of genetic variation in multiple populations. Heredity (Edinb). 112:21–9. Nature Publishing Group.

Amarasekare, K. G., and J. V Edelson. 2004. Effect of temperature on efficacy of insecticides to differential grasshopper (Orthoptera: Acrididae). J. Econ. Entomol. 97:1595–602.

Beaver, L. M., L. A. Hooven, S. M. Butcher, N. Krishnan, K. A. Sherman, E. S.-Y. Chow, and J. M. Giebultowicz. 2010. Circadian clock regulates response to pesticides in Drosophila via conserved Pdp1 pathway. Toxicol. Sci. 115:513–20.

Brown, A. W. 1958. The insecticide-resistance problem: a review of developments in 1956 and 1957. Bull. World Health Organ. 18:309–21.

Bubliy, O. A., T. N. Kristensen, and V. Loeschcke. 2013. Stress-induced plastic responses in

519 Drosophila simulans following exposure to combinations of temperature and humidity levels. J. Exp.
520 Biol. 216:4601–7.

521 Carriere, Y., and D. A. Roff. 1995. Change in genetic architecture resulting from the evolution of
522 insecticide resistance : a theoretical and empirical analysis. Heredity (Edinb). 75:618–629.

523 Catania, F., M. O. Kauer, P. J. Daborn, J. L. Yen, R. H. Ffrench-Constant, and C. Schlotterer. 2004.
524 World-wide survey of an Accord insertion and its association with DDT resistance in Drosophila
525 melanogaster. Mol. Ecol. 13:2491–504.

526 Charmantier, A., D. Garant, and L. E. B. Kruuk. 2014. Quantitative Genetics in the Wild. OUP Oxford.

527 Chung, H., M. R. Bogwitz, C. McCart, A. Andrianopoulos, R. H. Ffrench-Constant, P. Batterham, and
528 P. J. Daborn. 2007. Cis-regulatory elements in the Accord retrotransposon result in tissue-specific
529 expression of the Drosophila melanogaster insecticide resistance gene Cyp6g1. Genetics 175:1071–7.

530 Crow, J. F. 1966. Evolution of resistance in hosts and pests. Pp. 263–275 in Scientific Aspects of Pest
531 Control. National Research Council Publication No. 1402, Washington, D.C.

532 Daborn, P. J., J. L. Yen, M. R. Bogwitz, G. Le Goff, E. Feil, S. Jeffers, N. Tijet, T. Perry, D. Heckel, P.
533 Batterham, R. Feyereisen, T. G. Wilson, and R. H. Ffrench-Constant. 2002. A single p450 allele
534 associated with insecticide resistance in Drosophila. Science 297:2253–6.

535 Denecke, S., C. J. Nowell, A. Fournier-Level, T. Perry, and P. Batterham. 2015. The Wiggle Index: An
536 Open Source Bioassay to Assess Sub-Lethal Insecticide Response in Drosophila melanogaster. PLoS
537 One 10:e0145051. Public Library of Science.

538 Dillon, M. E., G. Wang, P. A. Garrity, and R. B. Huey. 2009. Review: Thermal preference in
539 Drosophila. J. Therm. Biol. 34:109–119.

540 Eskenazi, B., J. Chevrier, L. G. Rosas, H. A. Anderson, M. S. Bornman, H. Bouwman, A. Chen, B. A.
541 Cohn, C. de Jager, D. S. Henshel, F. Leipzig, J. S. Leipzig, E. C. Lorenz, S. M. Snedeker, and D.
542 Stapleton. 2009. The Pine River statement: human health consequences of DDT use. Environ. Health
543 Perspect. 117:1359–67.

544 Fabian, D. K., M. Kapun, V. Nolte, R. Kofler, P. S. Schmidt, C. Schlötterer, and T. Flatt. 2012.
545 Genome-wide patterns of latitudinal differentiation among populations of Drosophila melanogaster
546 from North America. Mol. Ecol. 21:4748–69.

547 Festucci-Buselli, R. A., A. S. Carvalho-Dias, M. de Oliveira-Andrade, C. Caixeta-Nunes, H.-M. Li, J.
548 J. Stuart, W. Muir, M. E. Scharf, and B. R. Pittendrigh. 2005. Expression of Cyp6g1 and Cyp12d1 in
549 DDT resistant and susceptible strains of Drosophila melanogaster. Insect Mol. Biol. 14:69–77.

550 Hadfield, J. D. 2010. MCMC Methods for Multi-Response Generalized Linear Mixed Models: The
551 MCMCglmm R Package. J. Stat. Softw. 33.

552 Hamby, K. A., R. S. Kwok, F. G. Zalom, and J. C. Chiu. 2013. Integrating circadian activity and gene
553 expression profiles to predict chronotoxicity of Drosophila suzukii response to insecticides. PLoS One
554 8:e68472. Public Library of Science.

555 Hoffmann, A. A., and P. A. Parsons. 1997. Consistent heritability changes under poor growth
556 conditions. Trends Ecol. & Evol. 12:460–461.

557 Hooven, L. A., K. A. Sherman, S. Butcher, and J. M. Giebultowicz. 2009. Does the clock make the
558 poison? Circadian variation in response to pesticides. PLoS One 4:e6469. Public Library of Science.

559 Janssens, L., K. Dinh Van, and R. Stoks. 2014. Extreme temperatures in the adult stage shape delayed
560 effects of larval pesticide stress: A comparison between latitudes. Aquat. Toxicol. 148:74–82.

561 Jones, A. G., S. J. Arnold, and R. Bürger. 2003. Stability of the G-matrix in a population experiencing
562 pleiotropic mutation, stabilizing selection, and genetic drift. Evolution 57:1747–60.

563 Kirkpatrick, M. 2009. Patterns of quantitative genetic variation in multiple dimensions. Genetica
564 136:271–84.

565 Kliot, A., and M. Ghanim. 2012. Fitness costs associated with insecticide resistance. Pest Manag. Sci.
566 68:1431–7.

567 Kohonen, T. 2001. Self-Organizing Maps. 3rd ed. Springer Berlin / Heidelberg, Berlin, Heidelberg.

568 Krzanowski, W. J. 1979. Between-Groups Comparison of Principal Components. J. Am. Stat. Assoc.
569 74:703–707.

570 Low, K. H., W.-F. Chen, E. Yildirim, and I. Edery. 2012. Natural Variation in the Drosophila
571 melanogaster Clock Gene Period Modulates Splicing of Its 3′-Terminal Intron and Mid-Day Siesta.
572 PLoS One 7:e49536. Public Library of Science.

573 Ma, G., and C.-S. Ma. 2012. Effect of acclimation on heat-escape temperatures of two aphid species:

574 Implications for estimating behavioral response of insects to climate warming. J. Insect Physiol.
575 58:303–9.

576 Mackay, T. F. C. 2001. Quantitative trait loci in Drosophila. Nat Rev Genet 2:11–20.

577 Mackay, T. F. C., S. Richards, E. A. Stone, A. Barbadilla, J. F. Ayroles, D. Zhu, S. Casillas, Y. Han,
578 M. M. Magwire, J. M. Cridland, M. F. Richardson, R. R. H. Anholt, M. Barron, C. Bess, K. P.
579 Blankenburg, M. A. Carbone, D. Castellano, L. Chaboub, L. Duncan, Z. Harris, M. Javaid, J. C.
580 Jayaseelan, S. N. Jhangiani, K. W. Jordan, F. Lara, F. Lawrence, S. L. Lee, P. Librado, R. S. Linheiro,
581 R. F. Lyman, A. J. Mackey, M. Munidasa, D. M. Muzny, L. Nazareth, I. Newsham, L. Perales, L.-L.
582 Pu, C. Qu, M. Ramia, J. G. Reid, S. M. Rollmann, J. Rozas, N. Saada, L. Turlapati, K. C. Worley, Y.-
583 Q. Wu, A. Yamamoto, Y. Zhu, C. M. Bergman, K. R. Thornton, D. Mittelman, and R. A. Gibbs. 2012.
584 The Drosophila melanogaster Genetic Reference Panel. Nature 482:173–178. Nature Publishing Group,
585 a division of Macmillan Publishers Limited. All Rights Reserved.

586 Merilä, J., and A. P. Hendry. 2014. Climate change, adaptation, and phenotypic plasticity: the problem
587 and the evidence. Evol. Appl. 7:1–14.

588 Merrell, D. J. 1994. The Adaptive Seascape: The Mechanism of Evolution. U of Minnesota Press.

589 Muturi, E. J., R. Lampman, K. Costanzo, and B. W. Alto. 2011. Effect of temperature and insecticide
590 stress on life-history traits of Culex restuans and Aedes albopictus (Diptera: Culicidae). J. Med.
591 Entomol. 48:243–50.

592 Nei, M. 1978. Estimation of Average Heterozygosity and Genetic Distance from a Small Number of
593 Individuals. Genetics 89:583–590.

594 Paaby, A. B., M. J. Blacket, A. A. Hoffmann, and P. S. Schmidt. 2010. Identification of a candidate
595 adaptive polymorphism for Drosophila life history by parallel independent clines on two continents.
596 Mol. Ecol. 19:760–74.

597 Perry, A. S. 1960. Metabolism of Insecticides by Various Insect Species. J. Agric. Food Chem. 8:266–
598 272. American Chemical Society.

599 Piiroinen, S., L. Lindström, A. Lyytinen, J. Mappes, Y. H. Chen, V. Izzo, and A. Grapputo. 2013. Pre-
600 invasion history and demography shape the genetic variation in the insecticide resistance-related
601 acetylcholinesterase 2 gene in the invasive Colorado potato beetle. BMC Evol. Biol. 13:13.

602 Rako, L., M. J. Blacket, S. W. McKechnie, and A. A. Hoffmann. 2007. Candidate genes and thermal
603 phenotypes: identifying ecologically important genetic variation for thermotolerance in the Australian
604 Drosophila melanogaster cline. Mol. Ecol. 16:2948–57.

605 Reinhardt, J. A., B. Kolaczkowski, C. D. Jones, D. J. Begun, and A. D. Kern. 2014. Parallel geographic
606 variation in Drosophila melanogaster. Genetics 197:361–73.

607 Roff, D. A., J. M. Prokkola, I. Krams, and M. J. Rantala. 2012. There is more than one way to skin a G
608 matrix. J. Evol. Biol. 25:1113–26.

609 Schlenke, T. A., and D. J. Begun. 2004. Strong selective sweep associated with a transposon insertion
610 in Drosophila simulans. Proc. Natl. Acad. Sci. U. S. A. 101:1626–31.

611 Schmidt, J. M., R. T. Good, B. Appleton, J. Sherrard, G. C. Raymant, M. R. Bogwitz, J. Martin, P. J.
612 Daborn, M. E. Goddard, P. Batterham, and C. Robin. 2010. Copy Number Variation and Transposable
613 Elements Feature in Recent, Ongoing Adaptation at the <italic>Cyp6g1</italic> Locus. PLoS Genet
614 6:e1000998. Public Library of Science.

615 Turner, T. L., M. T. Levine, M. L. Eckert, and D. J. Begun. 2008. Genomic analysis of adaptive
616 differentiation in Drosophila melanogaster. Genetics 179:455–73.

617 van Heerwaarden, B., and C. M. Sgrò. 2011. The effect of developmental temperature on the genetic
618 architecture underlying size and thermal clines in Drosophila melanogaster and D. simulans from the
619 east coast of Australia. Evolution 65:1048–67.

620 Weeks, A. R., S. W. McKechnie, and A. A. Hoffmann. 2002. Dissecting adaptive clinal variation:
621 markers, inversions and size/stress associations in Drosophila melanogaster from a central field
622 population. Ecol. Lett. 5:756–763.

623 Wehrens, R., and L. M. C. Buydens. 2007. Self- and Super-organizing Maps in R: The kohonen
624 Package. J. Stat. Softw. 21.

625 World Health Organization. 1989. DDT and Its Derivatives: Environmental Aspects, Environmental
626 Health Criteria monograph No. 83. Geneva.

Figures captions

Fig. 1: Mortality to DDT for a gradient of temperature for 12 genotypes of each of the three populations. A specific DDT dose range was used for each population, expressed in µg per 10mL vials bearing 10 females. For Raleigh: Control=0µg, Low=30µg, Mid=60µg, High=120µg; for Tasmania: Control=0µg, Low=60µg, Mid=120µg, High=240µg; and for Queensland: Control=0µg, Low=480µg, Mid=960µg, High=1920µg. The dose range for each population was chosen so mortality was similar across population for the mid dose at 25°C (ANOVA, p-val>0.3).

Fig. 2: Self-Organizing Map of the activity profiles over 23h. Activity is measured as the number of cross of an infrared beam sitting in the middle of the 10mL vial per individual fly (10 female per vial) on the DAM6 system (Trikinetics, MA, USA). a) The 42 units of the maps were defined to best represent the profiles in the 1752 observations. The groups (Gr.) correspond to clusters of similar profiles based on hierarchical clustering weighted by the count and mortality observed (see Materials and Methods). b-f) Count plots reporting the number times a given unit was observed in the data for specific sets of conditions. N: number of observation used in each analysis. X: units not observed in the specific set of conditions.

Fig. 3: Response landscape for the models reported in Table 2 for the three populations. The 3-dimensional surfaces represent the variation for the gradient of fixed effects (DDT dose and temperature) and the heatmaps, the within population genetic variation as random effects (genotype, genotype-by-DDT dose and genotype-by-temperature). For each combination of trait and population, the x- and y-axis of the surface and heatmap are represented on the same scale. The z-axis of the surface for each trait is identical across populations.

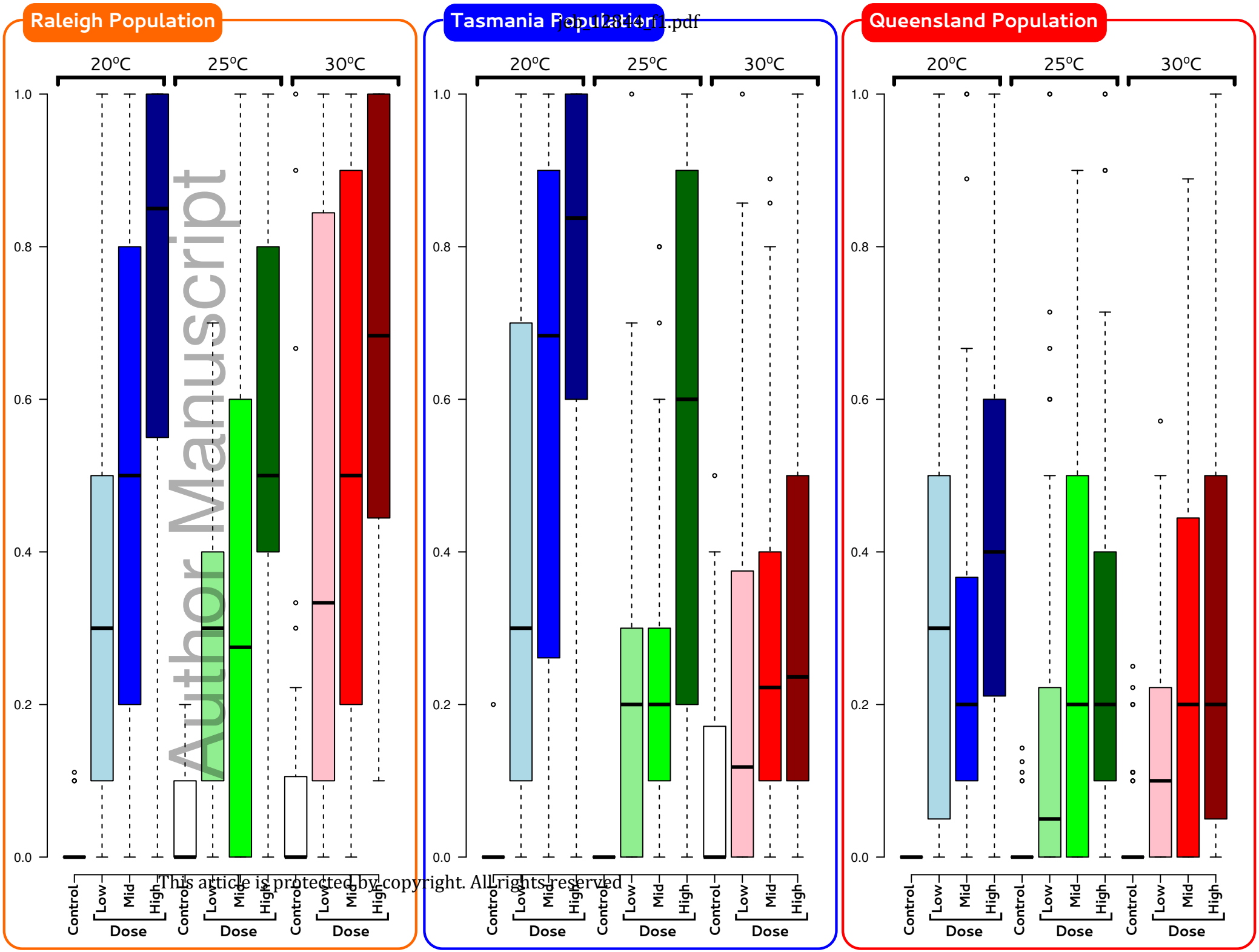
Fig.4: Mean genetic correlation between traits (early activity, later activity and mortality) along the temperature gradient (left panel) and the DDT dose gradient (right panel). The correlation were computed using Pearson's moment correlation using the solution of the mixed-linear model for each genotype. For each temperature, all traits measured for all DDT dose at this specific temperature were combined; for each DDT dose, all traits for all temperatures at this specific dose were combined.

Fig. S1: Scatterplot of the proportion of knock-down after 3 hours and the proportion of mortality after

24 hours for the three populations (mean of 3 replicates). Doses are expressed in µg of DDT per 10mL vials. The solid dots represent all the genotypes tested and the colored triangles, the 12 genotypes chosen within each populations to maximize the diversity for both traits.

Fig. S2a-c: Count plots reporting the percentage of times a given unit was observed in the data for each of the three population in each set of conditions.

Fig. S3: Correlation coefficients between each pair of quantitative traits (Early Activity, Later Activity and Mortality) for a range of putative breakpoint in time. The vertical red line represents the optimal breakpoint, jointly minimizing the correlation between activity traits and maximizing their correlation with mortality.



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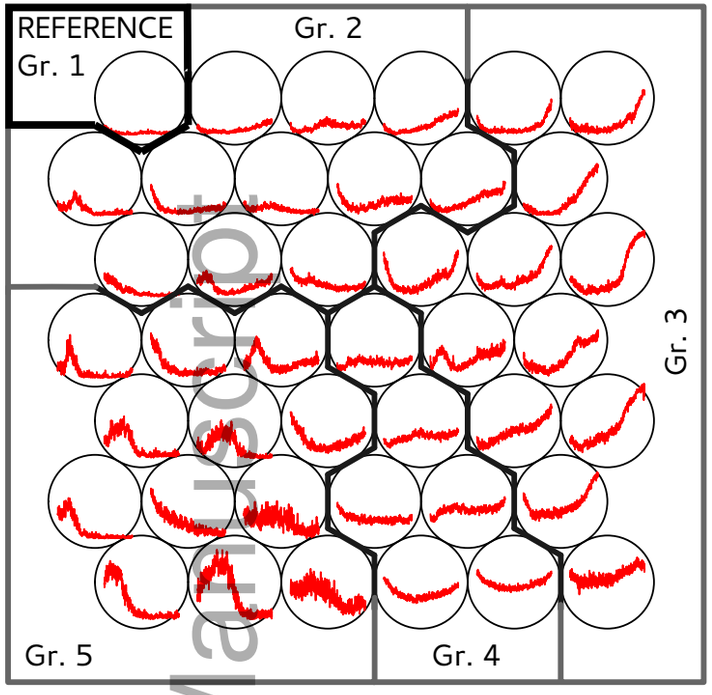
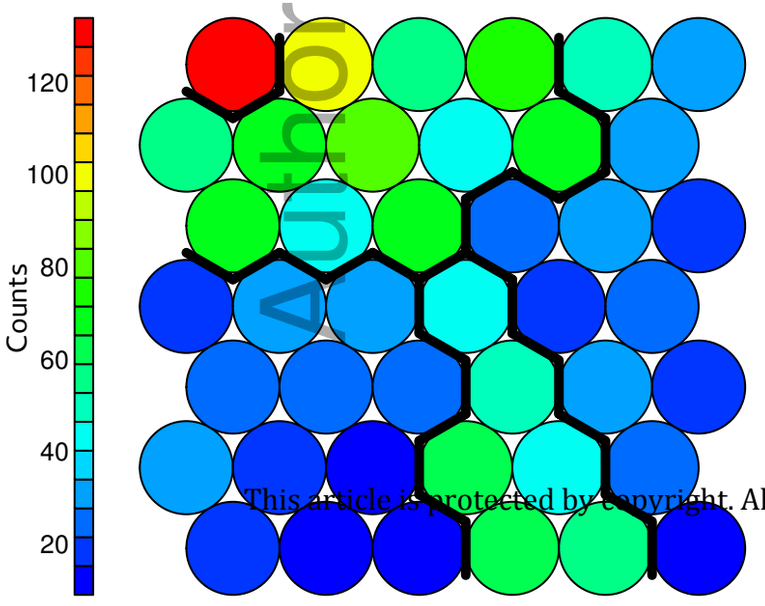
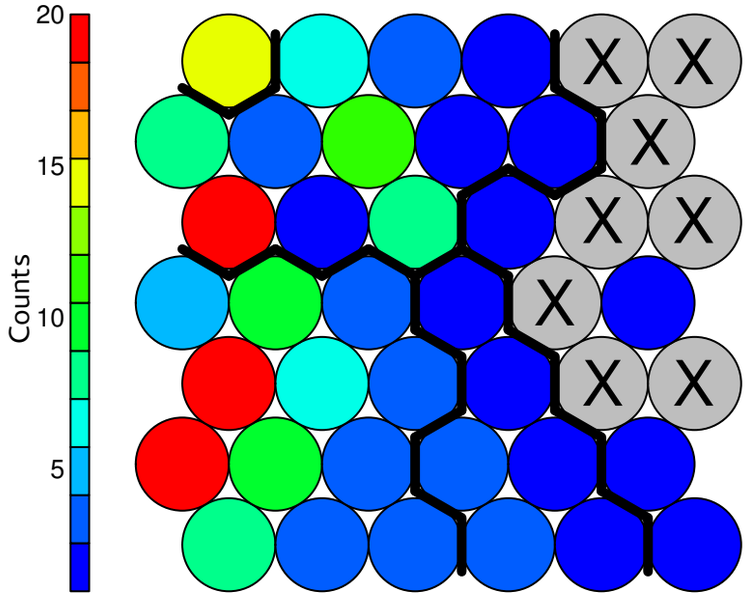
a) Self Organizing Maps Units



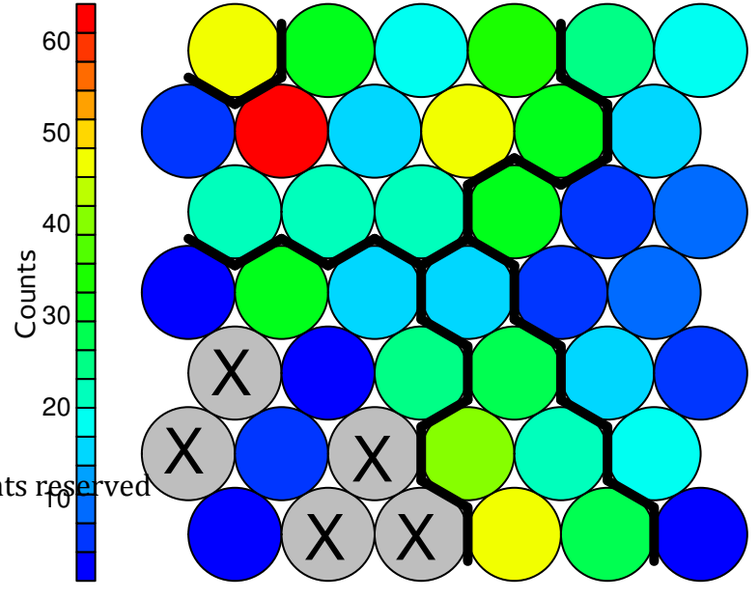
b) All Observations (N=1752)



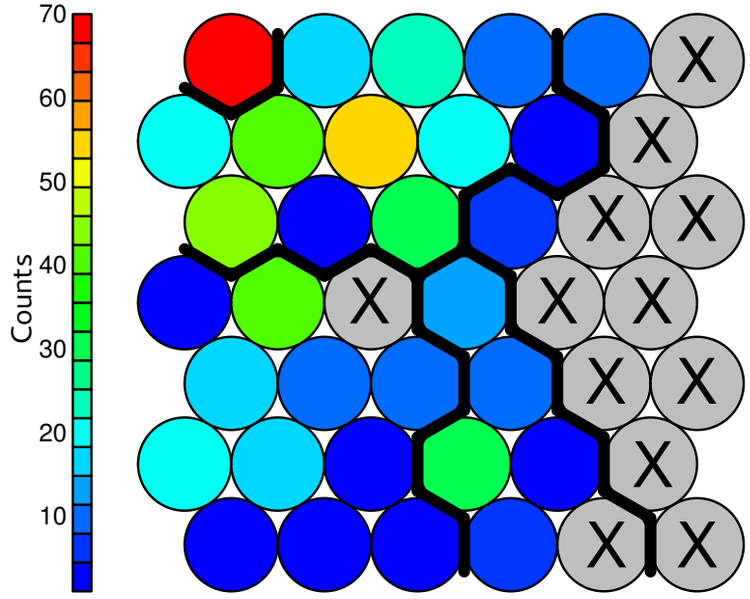
c) High Mortality – >70% (N=193)



d) Low Mortality – <30% (N=777)



e) Low Temperature – 20C (N=539)



f) Control – No DDT (N=435)