Altruism during extra-corporeal detoxification in insects

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Abstract

Altruism is common in eusocial insects. Here, we report on a yet unexplored altruistic extra-corporeal detoxification of insecticides in the non-eusocial Drosophila melanogaster. Wild-type flies incubated with DDT, a contact insecticide, in a closed environment die as expected. However, incubation of a second cohort in the same environment after removal of the dead flies was not lethal. Consistent to the kin selection theory, the effect is significantly lower if un-related wild-type flies are used in the assay. This indicates kin selection. Incubation assays with Chlorpyrifos, another contact insecticide, yielded identical results, while incubation assays with Chlorantraniliprole, again a contact insecticide, was toxic for the second cohort of flies. Consequently, following individuals might be saved from intoxication and therefore, this phenomenon may serve as an example of non-eusocial insect altruism. This novel program is, however, not omnipotent as it targets certain xenobiotics while others remain active. The molecular and genetic mechanisms await identification and characterization.

Altruism during extra-corporeal detoxification in insects

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Abstract

Altruism is common in eusocial insects. Here, we report on a yet unexplored altruistic extra-corporeal detoxification of insecticides in the non-eusocial *Drosophila melanogaster*. Wild-type flies incubated with DDT, a contact insecticide, in a closed environment die as expected. However, incubation of a second cohort in the same environment after removal of the dead flies was not lethal. Consistent to the kin selection theory, the effect is significantly lower if un-related wild-type flies are used in the assay. This indicates kin selection. Incubation assays with Chlorpyrifos, another contact insecticide, yielded identical results, while incubation assays with Chlorantraniliprole, again a contact insecticide, was toxic for the second cohort of flies. Consequently, following individuals might be saved from intoxication and therefore, this phenomenon may serve as an example of non-eusocial insect altruism. This novel program is, however, not omnipotent as it targets certain xenobiotics while others remain active. The molecular and genetic mechanisms await identification and characterization.

Keywords: Altruism; xenobiotic, insecticide, detoxification, *Drosophila*.

Introduction

According to W. D. Hamilton's inclusive fitness theory (kin selection), a trait or behaviour is altruistic when the fitness cost of the actor is lower than the fitness benefit of the recipient which is directly proportional to the genetic relatedness between actor and recipient (*rb>c*; r=relatedness, b=benefit for recipient, c=cost for actor; (West *et al.* 2007)). In insects, usually eusocial species such as ants, bees and termites are considered to show altruistic behaviour. This extends to the point that "an animal acting on this principle would sacrifice its life if it could thereby save more than two brothers, but not for less" (Hamilton 1963). Here, we report on our observations during exposure of the non-eusocial fruit flies (*Drosophila melanogaster*) to insecticides arguing that first visitors of a contaminated site are able to detoxify the site to the benefit of the second visitors while they die.

Xenobiotics including plant secondary metabolites and insecticides challenge insects in their daily life as they may perturb cell, tissue and organ physiology at worst causing death. For survival, hence, they have developed elaborate structural and molecular defence mechanisms to escape or disarm xenobiotic toxicity (Gao *et al.* 2022a). First, the cuticle that covers the body and the endings of the digestive system serves as a barrier to some extent preventing xenobiotics penetration. If xenobiotics overcome the cuticle barrier, potent genetic and molecular programs are elicited for detoxification. The molecular players of the detoxification response have been studied extensively in various insect species. They act in concert in different internal tissues such as the fat body and the midgut. A key entry site of xenobiotics are the ends of the legs, the tarsa. These body parts are designed to sense the substratum with gustatory sensilla and need to have a cuticle with adapted higher permeability (Ling *et al.* 2014; Dinges *et al.* 2021) and flexibility. A subtype of these sensilla, in addition, may have pores permitting uptake of small molecules. Thickening of the tarsal cuticle in response to continuous exposure to insecticides has been reported in mosquitos

(Balabanidou *et al.* 2019). Thus, the tarsa are dynamic cuticular structures communicating with the proximal environment. Our finding suggest that an extra-corporeal detoxification mechanism may exist in insects that protects insects against their proximal environment. As protection extends to insects visiting the site of the toxic micro-environment after the first visit of their relatives, we consider this behaviour as altruistic.

Results & discussion

Exposure of wild-type flies to different amounts of the contact insecticide DDT (Gao *et al.* 2022b) in an incubation vial caused paralysis and death with an efficiency that depended on the insecticide amounts (Fig. 1A). After removal of the dead flies, exposure of a second cohort of flies in the same incubation vial did not compromise survival even at the highest DDT amounts (Fig. 1B). We speculated that the first cohort of flies actively modified and detoxified DDT raising the chance of the second cohort to survive. Alternatively, the first cohort flies might have passively improved survival of the second cohort by adsorption of DDT to their surface. Following this argument, removal of the corpses of the first cohort may cause a depletion of DDT amounts that are not lethal to the second cohort flies. To test this possibility, we added silica beads to vials containing a high DDT amount prior to the incubation with flies (Fig. 1C, D). These flies died. Moreover, flies incubated with these beads removed from the DDT-vial and deposited in a clean vial died as well. Thus, physical contact with DDT depletes the effective amounts of DDT, which, however, remains toxic to the second cohort. This observation indicates that DDT had not decayed due to prolonged usage when the second cohort was exposed to it. Candidate molecules that may interfere with DDT toxicity are cuticular hydrocarbons at the fly body surface. Addition of fly surface wash solutions or vegetable oil (mimicking surface lipids) did not detoxify DDT exposed to the first cohort flies (Fig. 1E). Thus, lipids are probably not involved in DDT detoxification. An alternative mode of DDT detoxification is the contact of the substrate with the proboscis. To study this possibility, we removed the proboscis of the first-cohort flies prior to incubation with DDT. After successful wound-healing, flies without proboscis died upon contact with DDT (Fig. 1F). The second cohort, however, by the majority survived the assay. This indicates that oral DDT mitigation is irrelevant. Next, we sought to reduce the residual toxicity of DDT after incubation with the first cohort. In a simple scenario, we reckoned that cuticular chitin my adsorb DDT and thereby reduce its adverse effects (Fig. 1G). Second-cohort flies were, therefore, added to the vial supplemented with chitin. Against our hypothesis, addition of chitin to the vial after removal of the first-cohort flies reduced survival of the second-cohort flies. Possibly, this effect is due to remobilization of DDT by chitin. Although the mode of function of chitin on DDT is enigmatic, we can draw an important conclusion from this experiment as it demonstrates that in the initial trials without chitin DDT is present but chemically or physically masked or detoxified when the second cohort flies are incubated in the vial after the first cohort. In other words, the first cohort flies do actively, but reversibly, modify the substratum.

According to the kin selection theory, the beneficial effects of a behaviour are more pronounced when the actor and recipient are related. To test whether this applies to our system, we incubated a different wild-type population as a second cohort (Fig. 1H). The survival rate of the second cohort was lower when the wild-type populations differed in the two vials than when the same population was incubated in the consecutive vials.

Next, we addressed the possibility that other insect species than *D. melanogaster* might have an identical effect on DDT toxicity. For this purpose, we incubated a honeybee (*Apis mellifera*) worker in a vial containing different amounts of DDT (Fig 1I). This incubation was lethal to the honeybee. After removal of the dead honeybee, a cohort of wild-type *D. melanogaster* was incubated in the same vial. These flies survived this treatment. We conclude that insects, along with their internal detoxification responses, may possess a detoxification mechanism that acts outside their body.

We wondered if this extra-corporeal detoxification response may modify the efficiency of other, unrelated xenobiotics, we repeated the two-cohort experiments with the insecticides Chlorpyrifos and Chlorantraniliprole (Fig. 1J,K). While Chlorpyrifos was detoxified in these assays, Chlorantraniliprole retrained its toxicity. Thus, whereas some chemically different xenobiotics are

detoxified by the extra-corporeal detoxification response, some others are not targeted by this process. In conclusion, along with the internal detoxification response, insects have developed an extra-corporeal detoxification mechanism that, in contrast to the former, does not only protect the individual that launches it but the population of insects in the niche (Fig. 2). The altruistic notion comes into play considering that in the field, *D. melanogaster* flies tend to cluster in their micro-habitat (Soto-Yeber *et al.* 2018).

We reckon that this altruistic process involves the tarsa. Consistent with recently published findings (4), the insect tarsa appear to be molecularly and genetically autonomous organs involved in xenobiotic defence. One may even speculate that bacteria that colonise especially the tarsa might participate to this detoxification program (Hong *et al.* 2022). The genetic, molecular and cellular mechanisms of the underlying program await identification and characterization. Indeed, the model insect *D. melanogaster* is a perfect system to advance in ecological genetics in this direction as understanding this problem will have a considerable impact on insect ecology and pest science.

Materials and Methods

Ten Tübingen and Dijon wild-type and 91R flies were incubated with the contact insecticides DDT (Dichlorodiphenyltrichloroethane) and chlorpyriphos and Chlorantraniliprole in glass vials (first cohort). As in the following experiments, the number of knockdown flies was recorded every hour for four hours at room temperature. Knockdown occurred when flies showed paralysis. After incubation of the first cohort flies, the vial was emptied and a second cohort of male or female flies was added to the vial. In the honeybee experiment, a single *Apis mellifera* worker (from Pforzheim, Germany) was incubated in the vial instead of the first cohort of flies. Second cohort flies were added to the vial after four hours of incubation when the honeybee was dead. For proboscis removal experiments, flies without proboscis served as the first cohort flies. In the silica beads experiment, ten silica beads were added to a vial without flies. After removal of the beads,10 flies were incubated in the same vial. Also, ten flies were exposed to the removed silica beads to test for DDT adhesion to the beads. Ten wild-type females were added to the vial containing rapeseed oil and DDT. Chitin was added to a second cohort of 10 wild-type females. Detail protocols are provided as supporting information. All raw data are available upon request.

Author Contributions: JY performed the experiments. YW supervised, designed and analysed the experiments. BM supervised, designed and analysed the experiments and wrote the manuscript.

Competing Interest Statement: We declare no competing interests.

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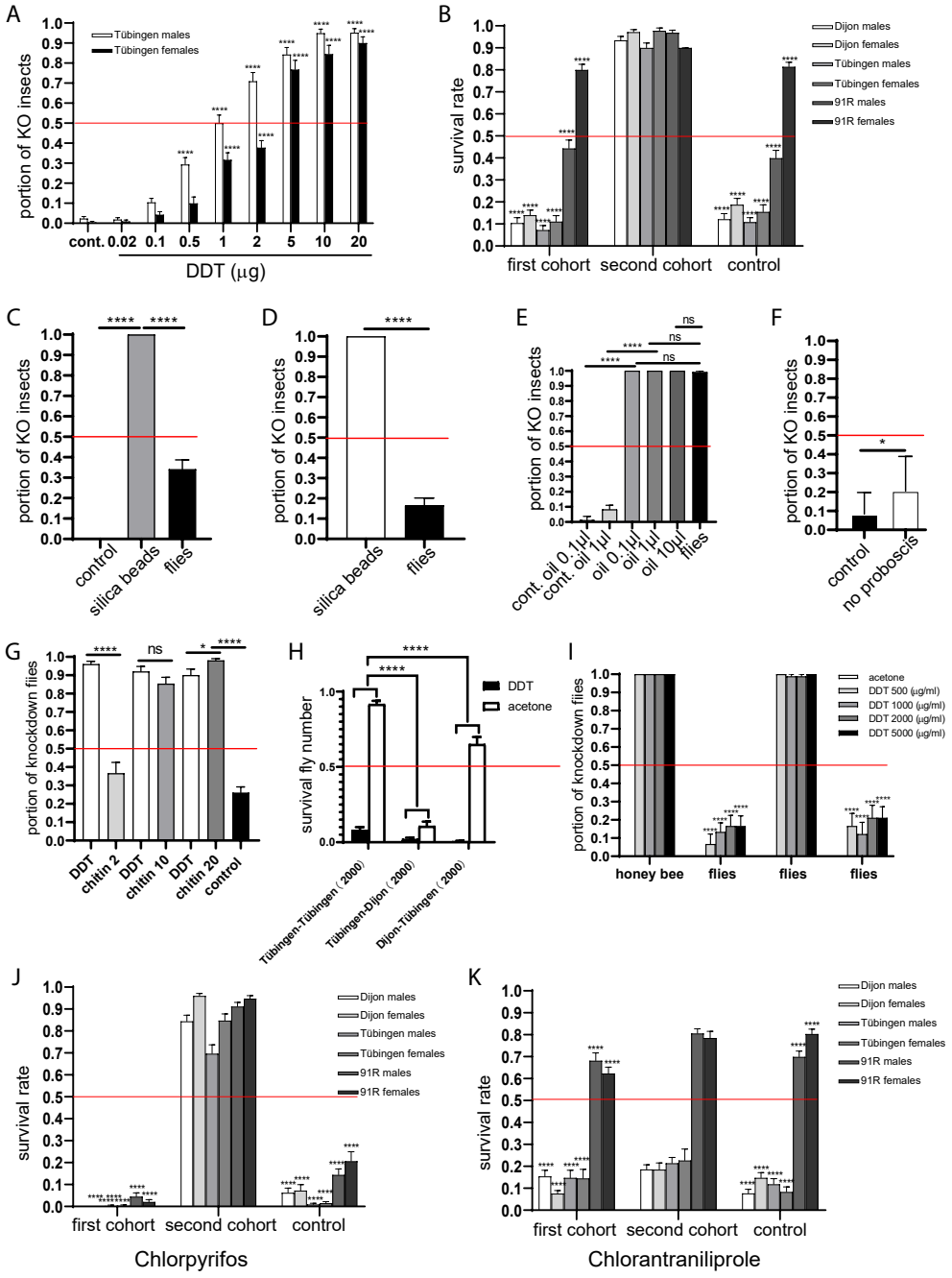
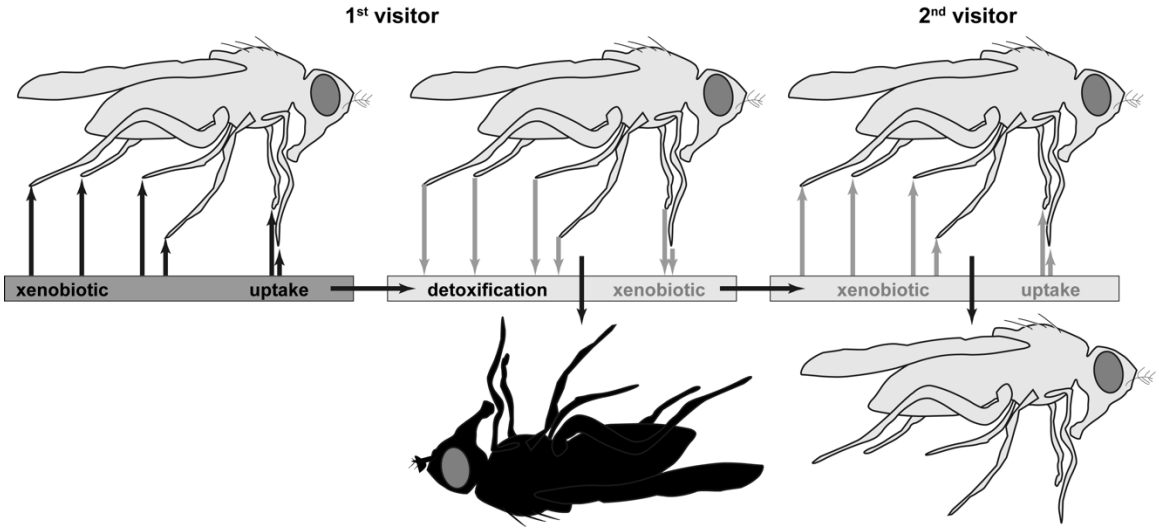
Figures

Figure 1. DDT toxicity declined after contact with flies.



Wild-type flies were incubated with different DDT amounts (A). First cohort wild-type flies were incubated in DDT-vials; after removal of these flies, a second cohort of wild-type flies was incubated in the same vial (B). As a control, flies were incubated with unused DDT-vials. Instead of first cohort flies, a honeybee worker was incubated in a DDT-vial before addition of a second cohort of flies (B). Silica beads were incubated in a DDT-vial prior to the addition of the second cohort flies (C). Flies were exposed to silica beads after contact with DDT (D). Flies were exposed to DDT or DDT with various amounts of oil (E). First cohort females without proboscis were exposed to DDT before second cohort flies (F). Second cohort flies were incubated in DDT-vials with various amounts of chitin (G). The first and second cohort flies derived from different wild-type populations (H). Exposure of first and second cohort flies to Chlorpyriphos (J) or Chlorantraniliprole (K). Data (n=9-42) were evaluated by Student's t-test. Asterisks indicate significant differences (*, $p < 0.05$; ****, $p < 0.0001$).

183 **Figure 2.** Model.



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185 Insects contacting xenobiotics including insecticides or plant secondary metabolites in their proximal
186 environment are able to modify it with their tarsa. In the field, this may be sufficient to ensure
187 survival. Even if they do not survive the contact, this process potentially protects the following
188 visitors.