

Correspondences

# DDT resistance in flies carries no cost

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Mutations that confer pesticide resistance are predicted to carry a cost in the absence of pesticide and consequently not to spread to fixation [1,2]. However, DDT resistance in *Drosophila melanogaster* (*DDT-R*) is approaching fixation globally, long after withdrawal of DDT [3]. There are two possible explanations for this. First, other insecticides, to which *DDT-R* confers cross-resistance [4], may be continuing selection. Second, *DDT-R* may not carry the expected fitness cost. Here we look at the fitness of *DDT-R* in the absence of insecticide. Surprisingly, when inherited via the female, the *DDT-R* locus actually *increases* both adult fecundity and the viability of eggs and larvae, as well as speeding

both larval and pupal development.

Despite the ability to select for several different cytochrome P450 genes conferring resistance to DDT in the laboratory [5-8], resistance in all current field strains is associated with over-expression of a single allele of a single gene, *Cyp6g1*, carrying an *Accord* transposon in its 5' end [3,9]. This single allele was found in all resistant strains from a global survey of ~600 current field strains, and global frequencies of *DDT-R*, in non-African *D. melanogaster* populations, are approaching fixation [3]. In an outstanding example of parallel evolution, insertion of a different transposable element in the 5' end of the *Cyp6g1* homolog in *D. simulans* was also shown to be associated with insecticide resistance [10]. *DDT-R* is therefore a widespread and representative mechanism of insecticide resistance.

To look for costs associated with *DDT-R* we chose the historically important strain Hikone-R [11], which contains exactly the same allele as that present in all current resistant field strains [9]. We backcrossed the Hikone-R resistant strain five

times to a standard susceptible strain Canton-S, to reduce the effect of differing genetic backgrounds. We also cured both strains of *Wolbachia* (see the supplemental data available with this article online), to eliminate any potential alteration of fecundity by this intracellular bacterium [12,13]. We then compared egg, larval and pupal viability, as well as the total number of eggs laid by adults, at two different temperatures, in a full life history analysis of the backcrossed resistant strain versus the original susceptible strain. We also compared heterozygous flies where resistance was inherited from the female (*RS*) with flies where resistance was inherited from the male (*SR*).

Surprisingly, *RS* flies significantly outperform their *SR* counterparts in counts of egg and larval viability at both 20°C and 25°C (Figure 1A,B); however, this advantage disappears during the pupal stage (Figure 1C). This disappearance of the *RS* advantage in the pupal stage is similar to phenotypes conferred by other genes showing a maternal contribution in flies [14]. Further at 20°C, *RS* genotypes again developed faster than their

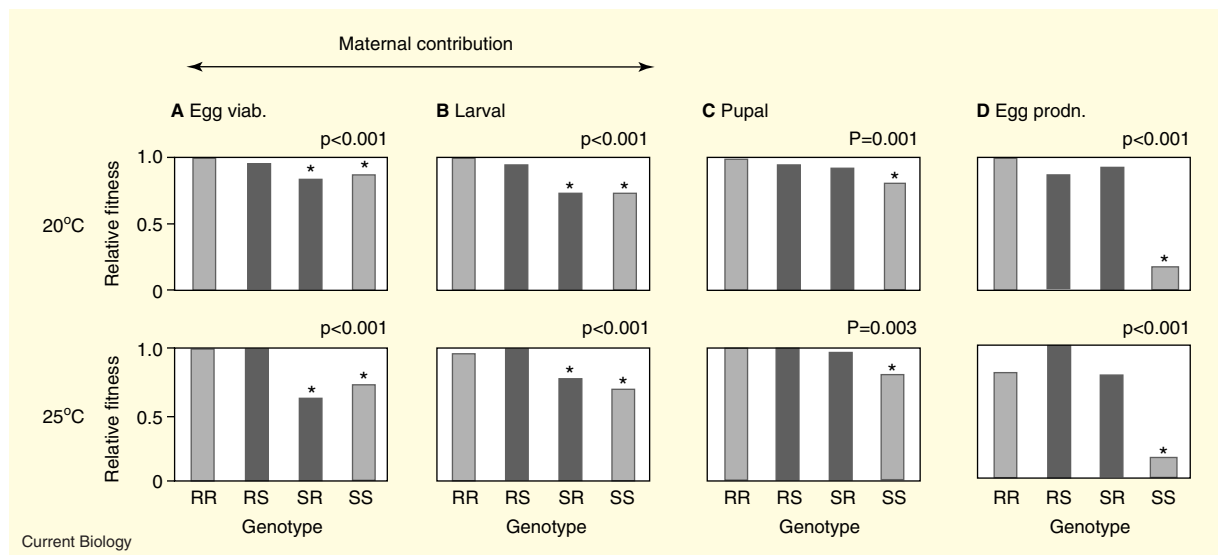


Figure 1. Life history analysis of the different *DDT-R* genotypes.

Note that the viability of *RS* eggs and larvae, where resistance comes from the female, is superior to that of *SR* eggs and larvae, where resistance comes from the male, at both 20°C and 25°C. Note also that this advantage disappears during the pupal stage and adult egg production, as expected for a trait passed on as a maternal contribution (arrow). The fitness of each genotype is plotted relative to the most fit which is given a value of 1.0; an asterisk indicates that the differences between the genotypes are significant at the 5% level (ANOVA, with Tukey post-hoc pairwise comparisons) and individual *P* values are given above each histogram.

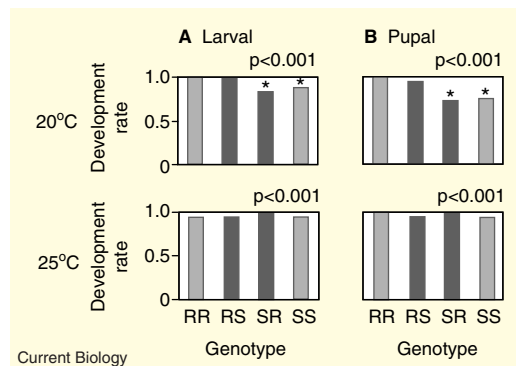


Figure 2. Differences in the development rate of larvae and pupae of the *DDT-R* genotypes.

Note that, as for viability, both the larval and pupal development of the *RS* genotype is significantly faster than that of *SR* at 20°C, however this advantage is obscured during faster development at 25°C.

*SR* counterparts, an effect obscured by faster development of both genotypes at 25°C (Figure 2). Therefore, both the viability and development rate of both larvae and pupae is improved when resistance is inherited via the female.

Following five generations of backcrossing, we have replaced ~98% of the genome of Hikone-R with the susceptible Canton-S background. Thus, although the female linked fitness advantage could formally be associated with a closely linked gene, the simplest explanation is that the advantage is associated with *DDT-R* itself. It is not possible to explain our results based on simple heterozygote advantage obtained on outcrossing an inbred line, as the effect is female limited and does not occur in both *RS* and *SR* heterozygotes.

Previous microarray-based studies of strains from a single *D. melanogaster* population differing in male reproductive success under competitive conditions suggested that low *Cyp6g1* transcription was linked to high male reproductive success [15]. This alternative hypothesis, of a potential fitness cost of *Cyp6g1* over-transcription in males [15], was not, however, investigated with a full life history analysis, and it is therefore not clear if the correlation with mating competitiveness was accidental, due to the inclusion of a *DDT-R* parent line.

Our study demonstrates that the single *DDT-R* allele, or a closely linked modifier, confers a female linked advantage in the absence of insecticide selection.

Although fitness modifiers have been documented [16,17], no fitness advantage for an insecticide resistance locus has been conclusively demonstrated. This, together with the continued use of other insecticides to which *DDT-R* confers cross-resistance [4,9], may therefore help explain the fact that *DDT-R* is approaching global fixation [3], long after the discontinued use of DDT itself. Interestingly, although the insertion of transposable elements is usually assumed to have a negative fitness cost [18], rare multiple insertions have been correlated with a dramatic increase in fitness [19]. The *Accord/Doc* insertion point may therefore correspond to one such 'hot-spot'. Finally, although we are unsure if the *Accord* insertion pre-dates or post-dates the introduction of DDT, the recent spread of this advantageous allele is more consistent with a recent mutational event.

Together, these results suggest that other xenobiotic resistance mechanisms, or indeed other adaptive traits, may not incur significant fitness costs in the absence of the selective agent, as predicted by population genetic theory.

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#### Supplemental data

Supplemental data including

Experimental Procedures are available at <http://www.current-biology.com/cgi/content/full/15/15/R587/DC1/>

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## Evolution of the extinct Sabretooths and the American cheetah-like cat

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The sabretooths (*Smilodon* and *Homotherium*) and the American cheetah-like cat (*Miracinonyx*) were the top predators in Late Pleistocene America, but became extinct about 13 thousand years ago [1]. As the evolutionary history of these taxa remains poorly understood [1,2], we analysed their phylogenetic relationship to extant felids. In contrast to previous molecular studies [3], our results show that the sabretooths diverge early and are not closely related to any living cats. This supports their morphological placement in a separate subfamily (Machairodontinae) [1]. Despite its remarkable morphological similarity to the African cheetah (*Acinonyx jubatus*), *Miracinonyx* appears to have evolved from a puma-like ancestor, presumably in response to similar ecological pressures [4,5].

Even though the evolutionary relationships within the cat family (Felidae) have been analysed using morphological, molecular and palaeontological data [1], many proposed relationships remain contentious, including those of recently extinct species. For example, the sabretooth cats are known from plentiful Late Pleistocene fossil material and have been placed, based on morphological criteria, outside of all extant cats (subfamily Felinae) as a separate subfamily, the Machairodontinae [1]. In contrast, an early ancient DNA study tentatively suggested that *Smilodon* was a member of the Felinae and thus part of the modern cat radiation [3].

The relationships of the American cheetah-like cat,

*Miracinonyx trumani*, are similarly confused as it had previously been mistaken for an ancestral or modern puma (e.g. *Puma trumani*). It was only recognised as a truly distinct group after abundant post-cranial fossil material became available [6]. Morphologically, *M. trumani* strongly resembles the modern African cheetah (*Acinonyx jubatus*) [4], which has led to uncertainty about whether it is more closely related to the Old World cheetah or the New World puma [2,4,6,7].

To address these questions, we used ancient DNA techniques, including independent replication, to generate mitochondrial DNA sequences from several specimens of *Smilodon populator* from Patagonia and a *M. trumani* specimen from Wyoming. In total, 1302 bp of *cytochrome b* (*cytb*), *NADH5*, *ATP8*, as well as 12S and 16S ribosomal RNA genes were recovered (Supplemental Data). We also obtained a short fragment of *cytb* from a Yukon scimitar-toothed cat (*Homotherium serum*), another recent member of the Machairodontinae.

Phylogenetic analyses (Figure 1; Supplemental Data) show that *Smilodon* and *Homotherium* fall outside the extant cats, the Felinae. This is in agreement with their traditional morphological placement [1]. The deep genetic split between the two sabretooth taxa is also consistent with morphological analyses [1].

These phylogenetic analyses also reveal that *M. trumani* is the sister taxon to the puma, rather than the African cheetah. Another endemic American cat, the jaguarundi, groups with *Miracinonyx* and the puma. This result corroborates the first morphological studies using complete specimens of *Miracinonyx* [6,7], but also raises questions about the anatomical structures used to classify *Miracinonyx* as a cheetah [4]. Re-analysis of these characters [2,7] has emphasised that many of them are associated with a highly cursorial lifestyle, such as elongated limbs and enlarged nares [2,4], which increase running speed and air-intake efficiency, respectively. In contrast, other limb