openings as the antorbital region of the skull becomes increasingly longer (*10*). Associated with this change is the gradual emargination of the frontals and parietals along the edges of the upper temporal fenestrae for the increase in the attachment areas for the adductor jaw musculature. The quadrate also shows a gradual relative increase in height, possibly related to the increase in the adductor jaw muscles. Finally, as in other vertebrates, the relative size of the orbit decreases throughout ontogeny. Despite these changes, the embryo maintains the diagnostic skull width-to-height ratio of *Massospondylus*.

The proportionately enormous skull; the long, horizontally held neck; the proportionately long forelimbs; and the small caudals with weakly developed transverse processes and hemal spines all indicate that the hatchlings of *Massospondylus* were obligate quadrupeds (Fig. 3). This is in contrast to the body proportions of adult *Massospondylus*, which are characterized by a small head, short forelimbs, and robust caudals with large transverse processes and hemal arches. As in most prosauropods, these adult proportions indicate at least facultatively bipedal locomotion (*14*, *15*). The changes from a sauropodlike condition with long forelimbs (*16*) and quadrupedal posture in early ontogenetic stages of *Massospondylus* to the plesiomorphic condition of short forelimbs and bipedal posture in the adult suggest that the quadrupedal posture of sauropods (*17*) may have evolved through paedomorphosis, the retention of early ontogenetic features in the adult, as first suggested by Bonaparte and Vince (*18*).

Modification of the sauropodomorph neck may have been correlated with the evolution of quadrupedality in this clade. The reconstructed quadrupedal posture for the embryo is at least in part related to the structure of the neck. As in most sauropodomorphs (*19*), the neck of *Massospondylus* was held more or less horizontally. This condition represents an autapomorphy for Sauropodomorpha and differs from the plesiomorphic condition of an S-curved neck in other dinosaurs and their immediate outgroups among Ornithodira (*20*). The S-curved neck is associated with their bipedal posture and the need for bringing the head closer to the center of gravity of the body. The presence of a horizontally held neck in the prehatching stages of basal sauropodomorphs like *Massospondylus* may have constrained them to a quadrupedal posture, one that required long forelimbs.

The combination of the body proportions and poorly developed dentition suggest that the hatchlings may have required parental care (*21*). The diminutive ventral elements of the pelvic girdle, small caudal vertebrae, and relatively enormous head of the *Massospondylus* embryos suggest it would have been difficult for the hatchlings to move around efficiently. The virtual absence of teeth in these embryos is another indicator of altricial behavior. Only a single possible tooth fragment is preserved in the two skulls, whereas other delicate, loosely attached elements were preserved largely undisturbed. Even if most of the teeth were poorly mineralized or lost postmortem, they were not well suited for feeding. If this interpretation is correct, these embryos provide early evidence of altricial behavior in a nonavian dinosaur.

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Supporting Online Material

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Pesticide Resistance via Transposition-Mediated Adaptive Gene Truncation in *Drosophila*

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To study adaptation, it is essential to identify multiple adaptive mutations and to characterize their molecular, phenotypic, selective, and ecological consequences. Here we describe a genomic screen for adaptive insertions of transposable elements in *Drosophila*. Using a pilot application of this screen, we have identified an adaptive transposable element insertion, which truncates a gene and apparently generates a functional protein in the process. The insertion of this transposable element confers increased resistance to an organophosphate pesticide and has spread in *D. melanogaster* recently.

The *Drosophila* genome contains a large number of active transposable element (TE) families that generate new TE insertions (*1*, *2*). Many such TEs are deleterious at least partly because ectopic recombination among them scrambles chromosomes (*3*, *4*). Thus, many new TE insertions cannot reach high population frequencies unless they either recombine infrequently (*4*) or they lead to a sufficiently beneficial change to overcome the disadvantage of ectopic recombination.

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To search for unusually frequent and therefore putatively adaptive TE insertions, we conducted a population survey of all of the 16 identified insertions of long interspersed element (LINE)–like *Doc* TEs (*5*) located in regions of high recombination in the sequenced *D. melanogaster* genome (*4*). All *Doc* elements except one (*Doc1420*) appeared to be subject to strong purifying selection. *Doc1420* occurred unusually frequently (*4*) despite being neither unusually short nor unusually divergent, which suggests that it either generates or is closely linked to an adaptive mutation.

Doc1420 is very frequent worldwide (~80%) and is much more rare (*G* test; $P = 0.0001$) in putatively ancestral African pop-

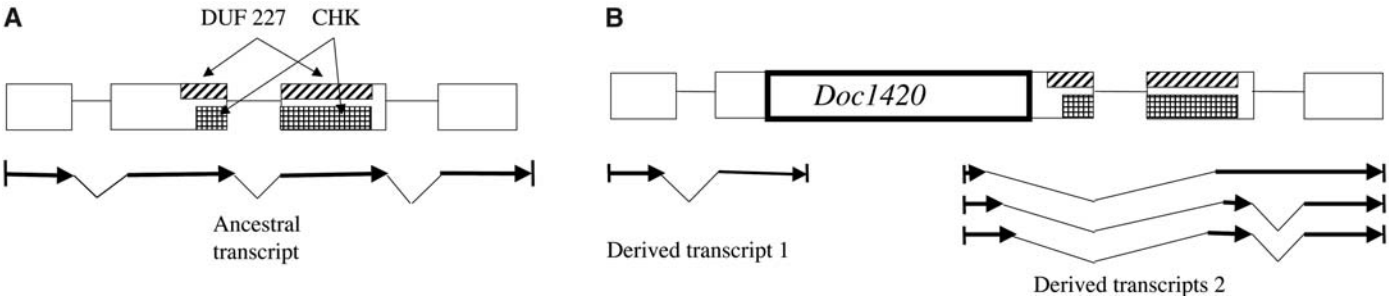

Fig. 1. Structure of *CHKov1*. (**A**) The structure of the ancestral transcript (before the insertion of *Doc1420*). The four exons are represented by rectangles. The transcript is depicted with black arrows. The striped and crosshatched areas represent PFAM domain DUF227 and SMART domain CLK, respectively. (**B**) Display of the altered transcripts in the strain possessing *Doc1420*. The first altered transcript contains the first exon, part of the second exon on the 5′ side of *Doc1420*, and ends 391 to 394 bp inside the *Doc1420* sequence. Identified transcripts in the second set start 3465 bp inside *Doc1420*; contain a short region of variable length (21, 38, and 77 bp in the three sequenced cases); they splice out, respectively, 1555, 1685, and 1549 bp regions containing part of the second exon on the 3′ side of *Doc1420* and part of the third exon; and they proceed until the end of the gene. In addition, one of the sequenced transcripts fails to splice out the intron between the third and fourth exons.

ulations of *Drosophila* in Zimbabwe and Malawi (table S1). This pattern is consistent with a genetic linkage between *Doc1420* and an adaptation associated with global expansion of *D. melanogaster* out of Africa.

To investigate the possible adaptive effects of *Doc1420*, we examined its effects on neighboring genes. *Doc1420* interrupts a predicted gene, *CG10618* (which we named *CHKov1*). *CHKov1* contains four exons [of length 175, 494, 367, and 197 base pairs (bp)], and *Doc1420* has inserted into the second exon (*6*). *CHKov1* appears to be functional; the *D. yakuba* ortholog of *CHKov1* [*D. yakuba* diverged from *D. melanogaster* ~5 million years ago (*7*)] shows conservation of all six intron junctions and of the predicted open reading frame. Moreover, there is a significant lack of amino acid substitutions (K_a), compared with the number of synonymous substitutions (K_s) between these two sequences (K_a/K_s = 0.1332; 95% confidence interval is 0.0486 to 0.3648), indicating that purifying selection has been preserving the sequence of the *CHKov1* protein for the past ~10 million years.

The *Doc1420* insertion in *CHKov1* generates two sets of altered transcripts (Fig. 1B) (*6*). In the presence of *Doc1420*, the transcript containing all four exons (and thus the original protein) appears to be absent. *CHKov1* and its paralogs share the PFAM domain DUF227 (amino acids 94 to 321), whose function is unknown (*8*), and a SMART domain CHK (amino acids 130 to 321), with a putative choline kinase function (*9*). The developmental profiles (*10*) of several of the closest paralogs of *CHKov1* have a consistent pattern of expression: high levels of expression in larvae and adults and low levels of expression in the embryo and pupae (fig. S2). This pattern is consistent with involvement of the paralogs of *CHKov1* (and by implication, with *CHKov1* itself) in digestion or detoxification. The altered transcripts (Fig. 1B) lack most of the conserved PFAM and SMART domains, which suggests that the original enzymatic function of *CHKov1* is likely to be lost in the presence of *Doc1420*.

If *Doc1420* is adaptive (or linked to an adaptive mutation), we expect to find the signature of an incomplete selective sweep: a sharp reduction of variability among the alleles linked to *Doc1420*. We tested this prediction by sequencing two regions [1.9 kilobases (kb) in the 5′ direction and 1.5 kb in the 3′ direction] immediately adjacent to *Doc1420* in an unstratified sample of 43 isofemale North American strains and seven isofemale Zimbabwe strains. We also sequenced this region in a single isofemale strain of *D. simulans*.

The haplotype structure near *Doc1420* (Fig. 2) strongly supports the hypothesis of a recent incomplete selective sweep. We found only six distinct haplotypes among the 34 alleles containing the *Doc1420* insertion, whereas each one of the 23 alleles without *Doc1420* is a unique haplotype. We conducted a series of coalescent simulations (*6*) to demonstrate that this structure is unexpected under neutrality. In 10^5 simulations, we failed to generate any samples with fewer than 18 distinct haplotypes (compared with the observed six distinct haplotypes) linked to a polymorphism as frequent as *Doc1420* [$P < 0.00001$, assuming recombination rate of 3.3 cM/Mbp (*11*); $P = 0.0009$, assuming no recombination].

If an incomplete selective sweep is associated with *Doc1420,* its signature should decay at increasing distances from *Doc1420*. Indeed, at a distance of ~18 kb in both the 5′ and 3′ directions from *Doc1420* (fig. S1), neutrality cannot be rejected, whereas at the two closer regions (15 kb in the 5′ direction and 11 kb in the 3′ direction from *Doc1420*), we found a modest signature of positive selection ($P < 0.05$ in each case). These results suggest that the incomplete selective sweep is centered at or very near to *Doc1420*.

We can use the rate of decay of the haplotype structure to estimate (*12*) that the latest incomplete selective sweep in this region occurred ~500 to 2400 generations ago. If one assumes 10 to 20 generations per year, this translates into ~25 to 240 years ago. The spread of *Doc1420* in the worldwide population of *D. melanogaster* appears at about the same time that drastic anthropogenic changes in the environment occurred, and possibly concurrently with the worldwide expansion of *D. melanogaster* out of Africa.

What is the adaptive effect that might be responsible for this recent expansion of the *Doc1420*-containing allele? We deduced that changes in *CHKov1* (a putative choline kinase) might affect choline metabolism in general and possibly the function of acetylcholine esterase. Given that acetylcholine esterase is the target of several types of pesticides, including organophosphates, we hypothesized that *Doc1420* insertion into *CHKov1* might confer resistance to organophosphates.

To test this possibility, we used repeated backcrosses to generate two *D. melanogaster* strains, with largely identical genetic backgrounds except for that at *CHKov1*, where one strain [*Doc+* (intro)] carried the insertion of *Doc1420* and the other [*Doc–* (intro)] lacked it (*6*). We assayed these two strains and the two parental strains for resistance to a commonly used organophosphate pesticide [azinphos-methylphosphate (AZM)]. The results (Fig. 3) indicate that the presence of *Doc1420* is associated with increased resistance to organophosphates in both the parental and the introgression strains. *Doc+* (introgression) has substantially lower mortality in this assay (20%) than does *Doc–* (intro) (68%) ($P < 0.001$).

To understand the history of this locus more completely, we investigated the molecular evolution of *CHKov1* and *CHKov2* (a paralog of *CHKov1* located immediately on the 5′ side, also known as *CG10675*). We specifically asked whether the *Doc1420*-containing alleles have evolved in a pattern consistent with a complete knockout of *CHKov1* or whether there is evidence of functionality of any of the resulting transcripts. We used the McDonald-Kreitman test (*13*), which tests the equality of

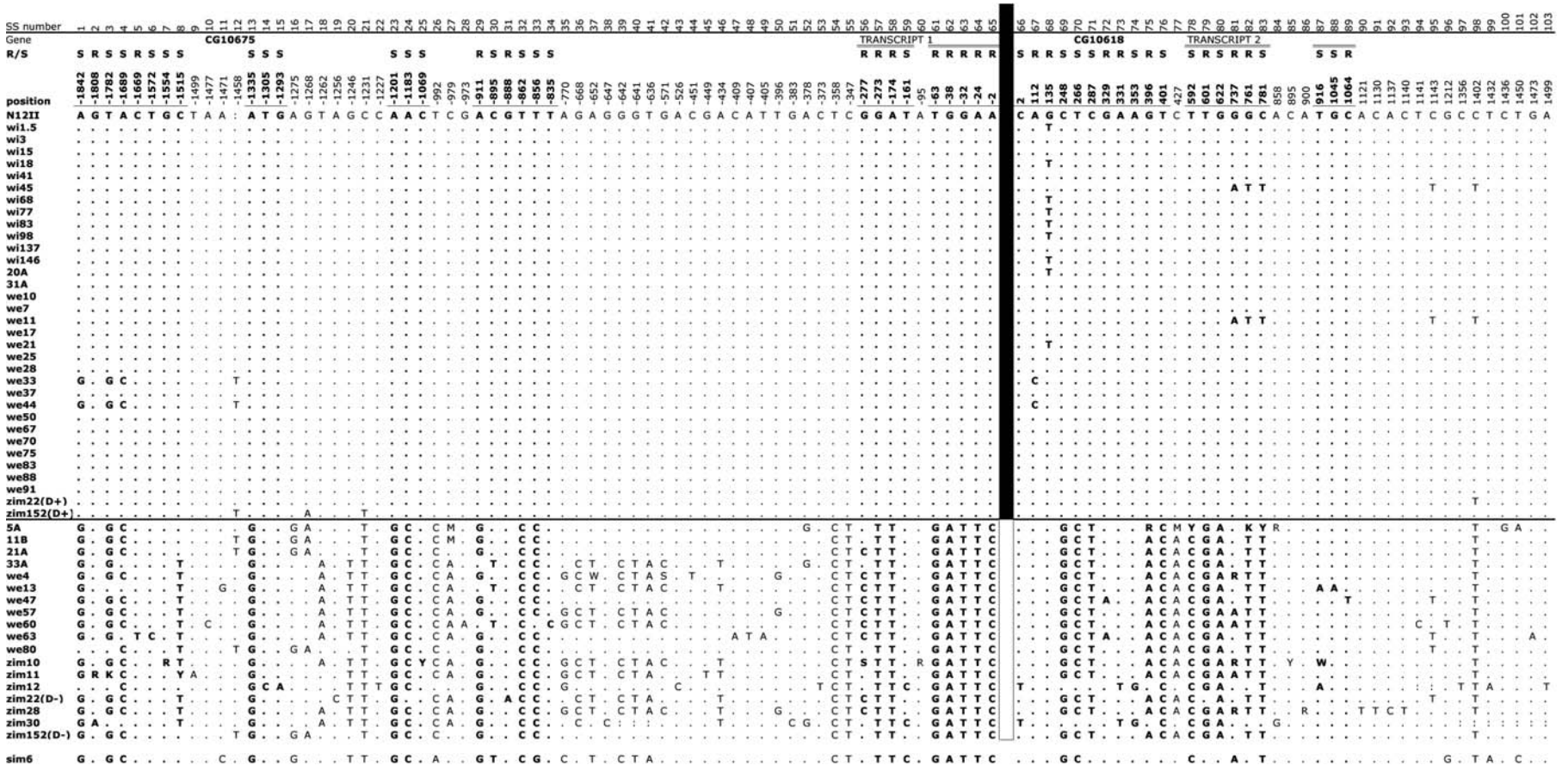

Fig. 2. Sequence of the 3.4-kb region flanking *Doc1420*. The figure shows the segregating sites (SS) within the 3.4-kb region flanking the *Doc1420* insertion. The SS number and the distance from *Doc1420* are at the top. The SS within coding regions are in bold and are identified as replacement (R) or synonymous (S) polymorphisms. The SS within *CHKov1* map to either derived transcript 1 (TRANSCRIPT 1) or to derived transcript 2 (TRANSCRIPT 2). *Doc1420* is shown as a black rectangle; the absence of Doc1420 is shown as an empty rectangle. The state for all of the segregating sites in the *D. simulans* sequence is shown at the bottom.

the ratio of amino acid and synonymous polymorphisms to the ratio of the number of amino acid and synonymous substitutions expected under a neutral model of evolution. This test (Table 1) revealed no violations of neutral expectations either for *CHKov2* or for the haplotypes of *CHKov1* lacking *Doc1420*. In contrast, haplotypes of *CHKov1* bearing *Doc1420* exhibited excess amino acid replacement polymorphism (Table 1), but only within the first and not the second set of derived transcripts (Fig. 1B). This excess is largely due to seven apparently derived amino acid polymorphisms (segregating sites 57, 58, 61, 62, 63, 64, and 65 in Fig. 2). Moreover, at these sites the derived states are fixed within the alleles containing *Doc1420*, whereas the ancestral states are fixed within the alleles lacking *Doc1420*. Additional sequencing of the transcript-1 region of *CHKov1* in 32 strains from Asia, Europe, Australia, and South America (table S3) confirmed this pattern. Thus, it appears that these amino acid changes have been newly generated within an allele containing *Doc1420*.

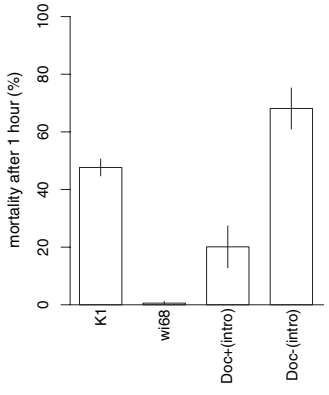

Fig. 3. Pesticide sensitivity assays. The average mortality in the presence of AZM for the four studied strains averaged over three independent experiments. The time of exposure and the dosage of AZM were chosen to achieve ~50% mortality of the more susceptible parental strain (*K1*). The error bars represent standard errors.

These seven new amino acid changes cluster together at the C terminus of the putative protein derived from derived transcript 1 (Fig. 1B and Fig. 2). Overall, they exchange primarily hydrophobic with primarily hydrophilic amino acids. In six out of seven cases, the changes are to either arginine or asparagines. No pattern of adaptive evolution is evident in the sequences on the 3′ side of *Doc1420*. These observations strongly suggest that positive natural selection has acted at the coding level of the new truncated polypeptide generated by the truncation of *CHKov1* by *Doc1420* (Fig. 1B; derived transcript 1). By implication, this new protein is likely to be functional.

Although the spread of the *Doc1420*-containing allele apparently took place 25 to 240 years ago, this allele contains eight independent changes, which suggests that it is much older and had undergone substantial evolution before its recent expansion. Indeed, the divergence of *Doc1420* from the consensus *Doc* sequence at 11 positions implies that *Doc1420* inserted ~90,000 years ago and certainly more than 240 years ago [G test, $P = 0.0004$; assuming 3×10^{-8} substitutions per bp per year (*14*)]. Further substantiating this claim, the pairwise divergence per nucleotide between the *Doc1420*-containing and *Doc1420*-lacking alleles (0.012 bp^{-1}) is more than twice as large ($P < 0.05$, as determined by bootstrapping) as the divergence among the alleles lacking *Doc1420* (0.005 bp^{-1}). The *Doc1420*-containing allele appears unusually old, even if the analysis is limited only to synonymous sites in *CHKov1* and *CHKov2* (table S2).

We propose that at some point in the past, one allele of *CHKov1* went through a number of drastic changes: first the insertion of *Doc1420* generated a functional truncated protein, which subsequently underwent rapid amino acid evolution. The final allele expanded 25 to 240 years ago in the worldwide population of *D. melanogaster*, with the aid of positive natural selection apparently by resistance to organophosphates. We speculate that the *Doc1420*-containing allele has been evolving in an isolated population of *D. melanogaster* for a substantial length of time, possibly ~90,000 years. Thus, although the recent expansion of the *Doc1420*-containing allele might have been caused by organophosphate resistance, the original reasons for its fast evolution and persistence must be related to some other phenotypic effect. To evaluate these possibilities, we need to understand the function of the truncated version of *CHKov1* and the phenotypic effects of the loss of its original function.

Recently, resistance to some *Bacillus thuringiensis* (Bt) toxins in *Heliothis virescens* was mapped to the TE-induced loss of Bt-toxin receptors (*15*). Moreover, resistance to dichlorodiphenyl-trichloroethane (DDT) in *D. melanogaster* (*16*) and possibly in *D. simulans* (*17*) is largely caused by the TE-induced up-regulation of a *cytp450* gene. Our research suggests another mechanism of pesticide resistance, which is mediated by either the loss of a nontarget gene or by the generation of a new protein. These cases underscore the importance of TEs to the evolution of pesticide resistance in particular and to adaptive evolution in general. The screen for adaptive TEs described in this work should help us understand the process and frequency of TE-generated adaptive mutations.

Table 1. The McDonald-Kreitman test of selection acting on *CHKov1*. Divergence is calculated in comparison with the sequence of *D. simulans*. The numbers in parentheses refer to the number of segregating sites in the haplotypes lacking *Doc1420*.

Gene region	Coding effect	Divergence	Polymorphism	P value
CHKov2	Synonymous	31	16 (13)	0.96 (0.80)
	Replacement	8	4 (4)	
CHKov1	Synonymous	36 (38)	12 (10)	0.03 (0.45)
	Replacement	19 (19)	17 (8)	
Transcript 1*	Synonymous	7	1	0.007
	Replacement	3	8	
Transcript 2*	Synonymous	16	5	0.66
	Replacement	9	4	

*Results for the positions within the first derived transcript or the second set of transcripts (assuming correct splicing of the third intron).

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