23. Sundari, R., Vikineswary, S., Yusoff, M. and Jones, E. B. G., *Bot. Mar.*, 1996, **39**, 327–333.
24. Hyde, K. D., in *The Biology of Marine Fungi* (ed. Moss, S. T.), Cambridge University Press, Cambridge, 1986, pp. 311–322.
25. Hyde, K. D., *Hydrobiologia*, 1989, **178**, 199–208.
26. Volkmann-Kohlmeyer, B. and Kohlmeyer, J., *Mycologia*, 1993, **85**, 337–346.
27. Farrent, C., Hyde, K. D. and Jones, E. B. G., *Trans. Br. Mycol. Soc.*, 1985, **85**, 164–167.
28. Nakagiri, A., *Inst. Ferment. Osaka Res. Commun.*, 1989, **14**, 52–79.
29. Steinke, T. D. and Jones, E. B. G., *S. Afr. J. Bot.*, 1993, **59**, 385–390.
30. Kohlmeyer, J., *Aust. J. Bot. Suppl. Ser.*, 1983, **10**, 67–76.
31. Nakagiri, A. and Tokura, R., *Trans. Mycol. Soc. Jpn.*, 1987, **28**, 413–436.
32. Koch, J. and Petersen, K. R. L., *Mycotaxon*, 1996, **60**, 397–414.
33. Strongman, D, Miller, J. D. and Whitney, N. J., *Proc. N. S. Inst. Sci.*, 1985, **35**, 99–105.
34. Haythorn, J. M., Jones, E. B. G. and Harrison, J. L., *Trans. Br. Mycol. Soc.*, 1980, **74**, 615–623.
35. Crumlish, B. and Curran, P., *Mycologist*, 1994, **8**, 83–84.
36. Kohlmeyer, J. and Volkmann-Kohlmeyer, B., *Mycol. Res.*, 1989, **92**, 410–421.
37. Koch, J., *Friesia*, 1974, **10**, 209–250.
38. Kohlmeyer, J., *Trans. Br. Mycol. Soc.*, 1971, **57**, 473–492.
39. Schmidt, I., *Hohere. Meerespilze der Ostsee. Biol. Rundsch.*, 1974, **12**, 96–112.
40. Tokura, R., *Bot. Mar.*, 1984, **27**, 567–569.
41. Schmidt, I., *Nat. Natursch. Meckl.*, 1969, **7**, 5–14.
42. Rees, G., Johnson, R. G. and Jones, E. B. G., *Trans. Br. Mycol. Soc.*, 1979, **72**, 99–106.
43. Jones, E. B. G., Moss, S. T. and Koch, J., *Trans. Br. Mycol. Soc.*, 1980, **74**, 625–631.
44. Koch, J. and Jones, E. B. G., *Can. J. Bot.*, 1989, **67**, 1183–1197.
45. Koch, J., *Nor. J. Bot.*, 1982, **2**, 163–169.
46. Volkmann-Kohlmeyer, B. and Kohlmeyer, J., *Can. J. Bot.*, 1993, **71**, 992–995.

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Remobilizing P elements out of the *stambh A* locus of *Drosophila melanogaster*

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P elements from two P-tagged alleles, *stm* A^{P1} and *stm* A^{P4}, of the temperature-sensitive paralytic mutant *stambh A* (*stm A*) were remobilized to derive wild-type revertants and P excision lethals. *stm* A^{P1} remutated to lethality, while *stm* A^{P4} reverted to the wild type. The P excision lethals are not amorphic mutations, since they were weaker in paralytic phenotype (i) than the parental P-tagged allele and (ii) for deficiencies for *stm A*. The lethals collectively affected all stages of the life cycle, demonstrating that *stm* A^{+} is needed throughout fly development.

P transposon elements serve as excellent tools for genetic analyses due to the ease with which mutations can be created at high frequencies, by way of insertions and excisions. Excision of P elements from an original insertion site can either be precise, resulting in reversion of the original mutation to wild type or imprecise (transposon leaves part of its sequence or takes away flanking nucleotides of the host gene), resulting in secondary mutations. Excision events involving more than one transposon often result in chromosomal rearrangements such as duplications, deletions, inversions and translocations[1]. Frequencies of precise excisions of P elements leading to wild-type revertants in *Drosophila melanogaster* are reported to vary widely from 4×10^{-13} at the *white* locus[2,3] to 3.5×10^{-1} at the *singed* locus [4].

stambh A (*stm A*) was first identified through a recessive, reversible, temperature-sensitive (ts) paralytic mutation mapping to 56.8 cM on the second chromosome[5]. EMS-induced homozygous viable alleles, *stm* A^{1} and *stm* A^{2} paralyse at 38°C in 3–4 min and recover to normality at 23–24°C in 5–6 min. Later, isolated unconditional embryonic lethal alleles, *stm* A^{7} and *stm* A^{12} (also EMS-induced), showed hypotrophy of the anterior embryonic dorsal cuticle overlying the brain with a concomitant hypertrophy of the anterior dorsal neurogenic region, the brain[6]. The *stm* A^{7} and *stm* A^{12} alleles were weaker in their paralytic phenotype when heterozygous over *stm* A^{1} and *stm* A^{2}. The time required for paralysis of 50% of flies (at 38°C) of *stm* A^{1} and *stm* A^{2} was 2.4 and 1.4 min respectively, while that for *trans*-heterozygote viable/lethal combinations of *stm* A^{1} (or *stm* A^{2})/*stm* A^{12} (or *stm* A^{7}) ranged between 4 and 7 min[6]. *stm* A^{1}/+ and *stm* A^{2}/+ heterozygotes also show weak paralysis at 39°C, a temperature at which wild-type flies do not paralyse[6]. Based on these observations it was proposed that homozygous viable ts paralytic alleles *stm* A^{1} and *stm* A^{2}, are not simple hypomorphs, but are semi-dominant gain of function neomorphs. Embryonic lethal alleles, *stm* A^{7} and *stm* A^{12} on the other hand, are extreme or complete loss

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of function amorphs[6]. To further characterize the *stm A* locus, five recessive P transposon-tagged alleles, *stm* A^{P1} to *stm* A^{P5}, were generated[7] using non-autonomous native P elements from a *Birm-2* donor that contains ~ 17 P elements on its second chromosome[8]. While employing a multiple P element source such as *Birm-2*, there is every likelihood that multiple P insertions will result on all the chromosomes. In the *stm* A^{P1} to *stm* A^{P5} lines, extraneous P elements in the genome were 'removed' by recombination or independent assortment. Briefly, the X and 3rd chromosomes, of P-tagged lines were replaced by P-element-free wild-type X and 3rd chromosomes, by independent assortment. Extraneous P elements on chromosome 2 were 'removed' by recombination with a *al dp b c px sp*-marked P-free second chromosome, thereby replacing the chromosomal region proximal and distal to *stm A* with the marked chromosomal regions. This process almost made sure that the *stm* A^{P} strain carried only a single P element.

In this report, a study of secondary mutations generated by remobilizing P elements from the two P-tagged alleles, *stm* A^{P1} and *stm* A^{P4} is presented.

The genetic reversion test was conducted by crossing homozygous *stm* A^{P1} and *stm* A^{P4} to 'Jumpstarter' *Cyo/Gla*; Δ2–3 *Sb/TM6 Ubx*. Dysgenic male F_1 progeny of the genotype *stm* A^{P}*/CyO* (or *Gla*); Δ2–3 *Sb*/+ were test-crossed to *b stm* A^{1}/*b stm* A^{1}; +/+ females. F_2 *b stm* A^{1}/+ *stm* $A^{P??}$ male progeny were tested for paralysis at 38°C and flies that did not paralyse for up to 10 min were selected. Individual + *stm* $A^{P??}$ chromosomes were extracted and revertants were confirmed after retesting. Recessive P Excision Lethal (PEL) events were scored by crossing another set of dysgenic male F_1 flies (from the same original parental cross) to *CyO/Pm* females. F_2 *CyO* (or *Pm*)/*stm* $A^{P??}$ males were individually mated to *stm* A^{12}*/Pm* virgins. The F_3 progeny of each cross were scored for absence of *stm* $A^{P??}$*/stm* A^{12} flies.

The consequences of P remobilization from *stm* A^{P1} and *stm* A^{P4} were very different (Table 1). Wild-type revertants were recovered at a frequency of 1.1×10^{-3} from *stm* A^{P4} among 3637 chromosomes tested, while no revertants from *stm* A^{P1} were recovered among 3414 chromosomes that were screened. No PELs were recovered from *stm* A^{P4} among the 200 chromosomes tested, while PELs were recovered at a frequency of 1.07×10^{-4} (6 PELs from 557 single-pair matings) from *stm* A^{P1}. Both reversion and lethal remutation frequencies fall well within the reported biological range of P excision[2–4]. The distinct difference in the unstable behaviour of the two alleles strongly suggests that the two P insertions were at two different sites, with excisions from *stm* A^{P1} leading to lethality and those from *stm* A^{P4} leading to wild-type

Table 1. Wild-type revertants and P Excision Lethals (PELs) recovered by remobilizing the P insert from *stm* A^{P1} and *stm* A^{P4}

	Wild-type reversions		P Excision Lethals	
stm A allele	Total no. scored	No. (frequency) of revertants	Total no. scored	No. (frequency of PELs)
stm A^{P1}	3414	0	557	6 (1.07×10^{-4})
stm A^{P4}	3637	4 (1.1×10^{-3})	200	0

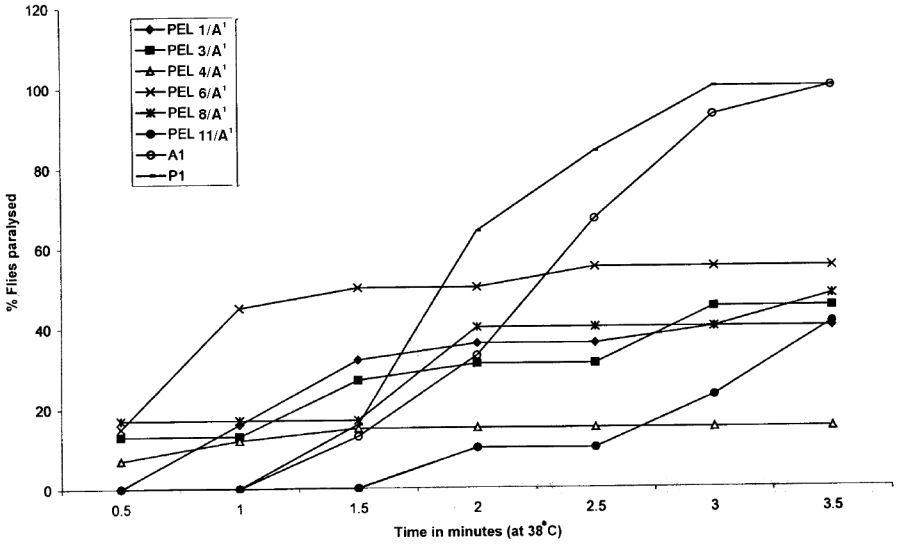

Figure 1. Paralysis kinetics of *stm* A^{PEL}/*stm* A^{1} adult males.

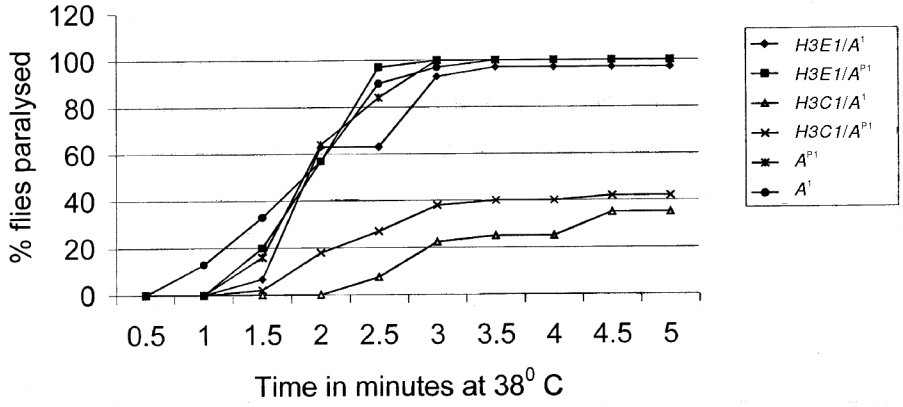

Figure 2. Paralysis kinetics of *stm A*/deficiency adult males at 38°C.

reversions. These results on transposase-driven remutation nevertheless constitute ample genetic proof that the *stm* A^{P1} and *stm* A^{P4} alleles were indeed P element-induced.

Male adults of the six *stm* A^{PEL}/*stm* A^{1} heterozygotes showed a weaker paralytic phenotype (took longer time to paralyse) than homozygous adults of *stm* A^{P1} and *stm* A^{1} (Figure 1). *stm* A^{1} and *stm* A^{P1} flies take 3.5 min for 100% paralysis at 38°C. Only 40–55% of the heterozygous PEL/*stm* A^{1} adults of PELs 1, 3, 6, 8 and 11 lines paralysed in 3.5 min. PEL 4/*stm* A^{1} showed the weakest phenotype, with less than 20% flies paralysing. Differences in terms of paralysis time and kinetics among the 6 PELs demonstrated that each PEL represented an independent remutation event.

The paralysis kinetics of flies heterozygous for *stm A* and two overlapping deficiencies *Df(2R)H3C1*; 43F–44D3–8 and *Df(2R)H3E1*; 44D1–4 to 44F12 that uncover *stm A* were determined. *Df(2R)H3C1/stm A* flies had a weak paralytic phenotype, with only 40% flies showing paralysis. It is likely that *Df(2R)*H3C1 does not eliminate *stm A* completely. *Df(2R)H3E1/stm* A^{P1} (and *stm* A^{1}) (Figure 2, -■- and -♦-) had strong paralytic phenotypes that were nearly identical to *stm* A^{1} and *stm* A^{P1} homozygotes (Figure 2, -Δ- and -×-). In neither case did the *Df(2R)/stm A* show a stronger paralytic phenotype than the respective homozygotes. The paralysis kinetics of *stm* A^{PEL}/*stm* A^{1} heterozygotes are weaker than those of *DfH3E1/stm* A^{1} (compare Figures 1 and 2), suggesting that the PELs are weak mutations that do not completely eliminate *stm A* function. In the final analysis, neither *Df/stm A* nor PEL/*stm A* flies have a stronger paralytic phenotype than their respective *stm A* homozygotes. This strongly supports our earlier conclusion[6] that mutations at *stm A*, leading to paralysis in homozygous condition, are not simple hypomorphs but are semi-dominant neomorphs that increase or alter in some way the *stm A* function.

The six *stm* A^{PEL} lines were completely lethal when homozygous. PEL 3, 4 and 6 were however weakly viable (0.5–9%) when heterozygous over the *stm* A^{12} lethal allele (Table 2). A study of the lethal phases of development of *stm* A^{PEL} lines was carried out by scoring stage-specific lethality of individuals from *stm* A^{PEL}/+ ♀ × *stm* A^{PEL}/+ ♂ intermatings. If the PELs were strictly recessive lethals, then only 25% of individuals from the above cross are expected to be lethal. Lethality in all the six PELs exceeded the 25% level (Figure 3) and were classified as semi-dominant lethals. Lethal phases in the six PEL mutations affected different stages of fly development. PEL 1 was predominantly embryonic lethal, while PELs 3, 4, 6, 8 and 11 were predominantly larval lethals (Figure 3). The observation that the PELs affect embryonic, larval and pupal stages implies that *stm* A^{+} function is necessary throughout the development of *D. melanogaster*. Cleared unhatched embryos of PEL 1, 4, 6 and 11 appeared like wild-type embryos and did not show any cuticle loss associated with the *stm* A^{7} and *stm* A^{12} embryos, demonstrating that not all embryonic lethal *stm A* alleles show a neurogenic embryonic cuticle-loss phenotype.

Polytene chromosomes of the six PELs did not show any chromosomal aberrations. In the absence of any cytologically visible rearrangements, the only possible events leading to lethality could be (i) sub-microscopic deletions[9] or (ii) a lethal P reinsertion to a site within or outside *stm A*. Multiple P insertions when mobilized, are

Table 2. Viability of *stm* A^{PEL}/*stm* A^{12} flies determined from *stm* A^{12}/*Pm* × *stm* A^{PEL}/*Pm* crosses

PEL line	No. of flies A^{12}/*PEL*	No. of flies Total	Expected no. of A^{12}/*PEL*	Per cent viability* of A^{12}/*PEL*
1	0	815	271.7	0
3	42	1415	471.7	8.9
4	5	1272	424.0	1.2
6	1	604	210.3	0.5
8	0	751	250.3	0
11	0	831	277.0	0

*Calculated as observed no. of *stm* A^{PEL}/*stm* A^{12} ÷ Expected no. of *stm* A^{PEL}/*stm* A^{12} × 100)

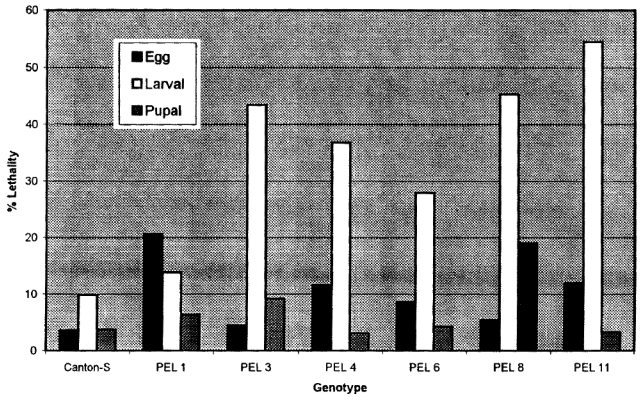

Figure 3. Developmental lethality in *stm* A^{PEL} alleles.

reported to produce complex rearrangements by random and often imprecise rejoining of breaks at or near P excision sites[10]. The absence of chromosomal rearrangements suggests that our P lines do not carry multiple inserts.

stm A is a developmentally interesting gene with mutant alleles affecting behaviour, embryonic neurogenesis and susceptibility to neuropoisons[5–7]. In this report on a study of P excision lethals derived from two independent P insertion alleles of *stm A*, it has been demonstrated that (i) *stm A* function is needed throughout development, (ii) homozygous viable ts paralytic alleles of *stm A* are not simple hypomorphs, but are very likely semi-dominant neomorphs, and (iii) not all lethal *stm A* alleles show embryonic neural hypertrophy. In this post-genomic sequence era, it is imperative to characterize *stm A* at the molecular level, to learn what the genetic limits of *stm A* are, what are its products and their effects on fly development. The *stm* A^{P1} and *stm* A^{P4} lines are being used for isolating genomic DNA flanking the P insert, by inverse PCR or by conversion of the Birm-2 insert to a lacZ plasmid rescuable enhancer trap line by targetted transposition[11].

1. Roiha, H., Rubin, G. M. and O'Hare, K., *Genetics*, 1988, **119**, 75–83.
2. Rubin, G. M., Kidwell, M. G. and Bingham, P. M., *Cell*, 1982, **29**, 987–994.
3. O'Hare, K. and Rubin, G. M., *Cell*, 1983, **34**, 25–35.
4. Engels, W. R., *Genet. Res.*, 1979, **33**, 219–236.
5. Shyngle J. and Sharma, R. P., *Indian J. Exp. Biol.*, 1985, **23**, 235–240.
6. Chandrashekaran, S. and Sarla, N., *Genetica*, 1993, **90**, 61–72.
7. Chandrashekaran, S., *Curr. Sci.*, 1993, **65**, 80–82.
8. Robertson, H. M., Preston, C. R., Phillis, R. W., Johnson-Schilz, D., Benz, W. K. and Engels, W. R., *Genetics*, 1988, **118**, 461–470.
9. Engels, W. R., *Mobile DNA* (eds Berg, D. E. and Howe, H. M.), American Society for Microbiology, Washington DC, 1989, pp. 1–102.
10. Engels, W. R. and Preston, C. R., *Genetics*, 1984, **107**, 657–678.
11. Sepp, K. J. and Auld, V. J., *Genetics*, 1999, **151**, 1081–1091.

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New evidence for plant-eating in a Miocene mustelid

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A new species of *Leptarctus* is described on the basis of a partial skull from the North American Miocene. The new species, *Leptarctus desuii*, has typical leptarctine characteristics, including heavy zygomatic arches, well-developed hypocones on the fourth upper premolars, wide muzzle and broad skull. The third upper premolar differs from those of other known *Leptarctus* in having double cusps and a cingulum on the lingual side. Cranial and dental morphology suggests that *Leptarctus* had a less carnivorous diet than any other mustelid.

LEPTARCTUS is one of the rarest of the known fossil carnivores. The genus ranges through the Miocene of North America and Inner Mongolia[1,2]. Characters diagnosing *Leptarctus* as a mustelid include absence of M^2 and absence of the notch between the blades of the upper carnassial. Though *Leptarctus* is a mustelid, the teeth bear many similarities to the teeth of the procyonids, *Procyon lotor* and *Nasua nasua*[3]. Unlike other mustelids, *Leptarctus* has prominent double sagittal crests, heavy zygomatic arches, a prominent occipital crest, a well-developed hypocone on P^4, grooved lower canines, raccoon-like mandibles and unique bony projections on the tympanic bullae. Its unusual anatomy invites comparison with a unique herbivorous marsupial, the koala bear. Geologically, species of *Leptarctus* range from the basal Hemingfordian (Early Miocene) to the top of the Hemphillian (Late Miocene).

Systematic paleontology

Class Mammalia Linnaeus, 1758
Order Carnivora Bowditch, 1821
Family Mustelidae Fischer von Waldheim, 1817
Subfamily Leptarctinae Gazin, 1936
Genus *Leptarctus* Leidy, 1856
Leptarctus desuii sp. nov.

Holotype: BHI (Black Hills Institute of Geological Research) 1571 (Figure 1), an anterior portion of skull with left P^2–M^1, right P^3–M^2.

Type locality: Carlson Quarry, SW1/4, Sec. 14, T. 1N, R. 32W, Hitchcock County, Nebraska, USA.

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